Application des micro-organismes halophiles au traitement des effluents industriels hypersalins

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APPLICATION DES MICRO-ORGANISMES HALOPHILES AU TRAITEMENT DES EFFLUENTS INDUSTRIELS HYPERSALINS

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I want to dedicate this book to the People of India for the lesson of life and tolerance.

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Liste des publications associées à cette thèse

Articles publiés dans des revues à comité de lecture

- **Lefebvre, O**., Habouzit, F., Bru, V., Delgenes, J.P., Godon, J.J., Moletta, R., 2004. Treatment of hypersaline industrial wastewater by a microbial consortium in a sequencing batch reactor. Environmental Technology. 25(5), 543-553.

- **Lefebvre, O**., Vasudevan, N., Torrijos, M., Thanasekaran, K., Moletta, R., 2005. Halophilic biological treatment of tannery soak liquor in a sequencing batch reactor. Water Research. 39(8), 1471-1480.

- **Lefebvre, O**., Vasudevan, N., Torrijos, M., Thanasekaran, K., Moletta, R., 2006. Anaerobic digestion of tannery soak liquor with an aerobic post-treatment. Water Research. 40(7), 1492-1500.

Articles soumis

- **Lefebvre, O**., Quentin, S., Torrijos, M., Godon, J.J., Delgenès, J.P., Moletta, R. Impact of increasing NaCl concentrations on the efficiency and microbiology of anaerobic digestion.

- **Lefebvre, O**., Vasudevan, N., Thanasekaran, K., Moletta, R., Godon, J.J. Microbial diversity of hypersaline wastewater: the example of tanneries.

- **Lefebvre, O**., Moletta, R. Treatment of industrial saline wastewater : a literature review.

Communications à des congrès internationaux

- **Lefebvre, O**., Vasudevan, N., Thanasekaran, K., Moletta, R. Biological treatment of tannery soak liquor in a bioreactor using halophilic bacteria. In: International Conference on Coastal and Freshwater Issues, December 8-10, 2003, Anna University, Chennai, Inde, pp.40-41. *Communication orale.*

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Avant-propos

Cette thèse a été effectuée à part égale au Laboratoire de Biotechnologie de l'Environnement (INRA, Narbonne, France) et au Centre for Environmental Studies (Anna University, Chennai, Inde). L'INRA et Anna University se sont associés dans le cadre d'une cellule franco-indienne des bioprocédés de l'environnement (CEFIBE) pour étudier les enjeux de la dépollution des effluents salés en Inde et en France et la faisabilité du traitement de ces effluents par voie microbienne.

En Inde, le Tamil Nadu souffre d'un déficit chronique en eau et, de plus, l'eau est très fréquemment polluée par le sel. Ce phénomène est aggravé par le nombre des industries génératrices de pollution saline dans ce pays, notamment dans le secteur textile et celui du cuir. L'état du Tamil Nadu est le cœur de l'industrie du cuir indienne avec plus de 60% de l'activité de tannage du pays, et environ 50% de toutes les exportations de produits en cuir. Du procédé de tannage résultent de grandes quantités d'effluents de caractéristiques différentes selon la source et, notamment, l'effluent issu du rinçage des peaux (préservées par le sel lors du trajet entre l'abattoir et la tannerie) est particulièrement chargé en matière organique et en sel (NaCl) et traité par évaporation, ce qui engendre des nuisances (surface requise pour l'évaporation, pollution des sols et des nappes phréatiques par infiltration, odeurs, etc.). Le recours à des modes de traitement alternatifs limitant l'impact environnemental de cet effluent devrait être envisagé et la voie biologique est à ce titre particulièrement intéressante.

Le problème des pollutions salines n'est pas limité à l'Inde, et la France aussi dispose d'industries générant des rejets salés. Un exemple sera étudié dans cette thèse à travers une industrie tartrique (Faure S.A.) dont la capacité d'expansion est limitée faute de traitement adéquat des effluents salins qu'elle émet.

Chapitre 1. Introduction

1.1 Contexte

La salinité d'un liquide se définit comme la somme des cations et des anions qu'il contient. Le principal sel, présent en quantité quasi inépuisable dans les mers, océans, lacs salés et aussi dans les gisements de sel gemme, est le chlorure de sodium (NaCl). L'importance économique du sel (NaCl) a longtemps permis l'existence de taxes et de monopoles gouvernementaux sur le sel. Même le terme de « salaire » trouve son origine dans le latin *salarium*, et fait référence aux paiements attribués aux soldats romains pour l'achat de sel. En 1995, la consommation de sel de l'Union Européenne a atteint 34,2 millions de tonnes. Le sel est en effet un produit de première nécessité, utilisé pour la conservation des aliments autant que pour la fabrication de produits chimiques tels que l'acide chlorhydrique, l'hydroxyde de sodium (soude caustique), le bicarbonate de sodium, etc. Les secteurs industriels de l'agro-alimentaire, de la chimie/pharmacie, du cuir, du textile et du pétrole sont de grands consommateurs de sel mais aussi d'importants générateurs de pollution saline.

La production d'effluents salins (également ou moins concentrés en sel que l'eau de mer, c'est à dire contenant une concentration en sels ≤ 35 g l^{-1}) et hypersalins (plus concentrés en sel que l'eau de mer, soit contenant une concentration en sels > 35 g l^{-1}) représente environ 5% des effluents totaux à traiter dans le monde. De plus, les pays en développement sont particulièrement confrontés à ce problème, puisque les déchets industriels et urbains de ces pays sont souvent rejetés dans des zones dépressionnaires salines à hypersalines. La dégradation des effluents salés est donc une problématique qui touche à la fois le milieu industrialisé en général et l'environnement périurbain de nombreux pays en développement.

En ce qui concerne la dimension écologique du problème de la salinisation, la directive 2000/60/CE [67] du Parlement européen et du Conseil du 23 octobre 2000 établissant un cadre pour une politique communautaire dans le domaine de l'eau exige que des mesures soient prises pour prévenir les effets nocifs de la salinisation. Quelle que soit la masse d'eau douce considérée, la salinité est l'un des paramètres à prendre en compte, et les Etats membres doivent définir des normes propres à assurer le bon fonctionnement de l'écosystème correspondant et l'apparition de communautés biologiques ne s'écartant que légèrement de celles qui sont généralement associées à l'écotype en conditions normales. En vertu de l'article 11 de la directive-cadre, les Etats membres doivent alors arrêter des mesures visant à garantir le respect de ces normes. Pour les eaux souterraines, l'Annexe V de la directive 2000/60/CE [67] instaure le suivi de la conductivité afin d'éviter l'invasion d'eau salée ou autre altération anthropique des

concentrations en sel de la masse d'eau. Ces dispositions doivent assurer la protection de toutes les masses d'eau contre la pollution saline.

S'agissant du contrôle des sources ponctuelles de pollution saline, la directive 96/61/CE [66] du Conseil relative à la prévention et à la réduction intégrées de la pollution en provenance d'activités industrielles exige que les Etats membres introduisent un système intégré d'autorisation pour différentes installations industrielles dont les capacités de production excèdent des seuils donnés. Les autorisations doivent comporter, pour tous les polluants visés, des valeurs limites d'émission établies sur la base des meilleures techniques disponibles (MTD).

En vertu de la directive 2000/60/CE, les seuils de rejet en France sont fixés à 200 mg Cl^{-} l^{-1} dans le cas d'un rejet vers le milieu naturel. Dans le cas d'un rejet vers une station d'épuration, la concentration maximale doit être définie avec l'exploitant de la station.

En raison du renforcement de la législation, le traitement des effluents salins est une problématique d'actualité. Une simple recherche par mots-clés sur le « Web of Science » permet d'appréhender l'intérêt actuel pour la dépollution de ces effluents. La Figure 1.1 illustre ainsi cette mouvance caractérisée par une production scientifique encore peu abondante mais en forte progression depuis le début des années 2000.

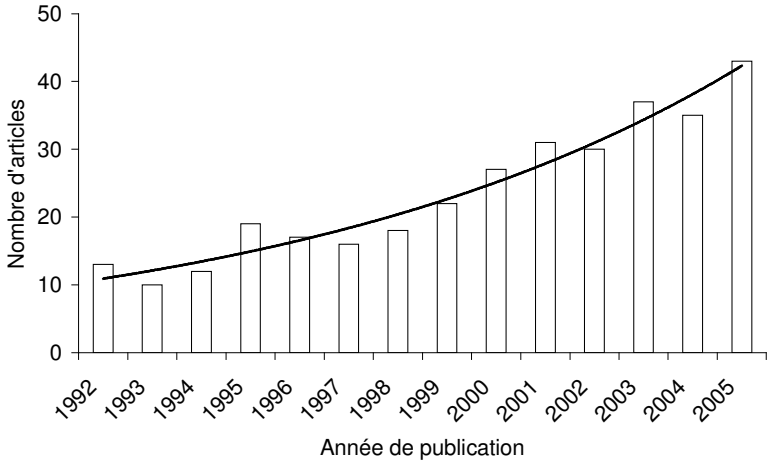

Figure 1.1 Evolution du nombre d'articles scientifiques se rapportant au traitement des effluents salins et hypersalins au cours du temps.

Le traitement du sel s'effectue par voie physico-chimique, mais la voie biologique utilisée pour le traitement de la matière organique des effluents salins pourrait permettre de réduire le coût de la finition physico-chimique. L'épuration biologique de tels effluents ne peut s'effectuer qu'au moyen de micro-organismes tolérant les fortes concentrations en sel (halotolérants), voire les requérant (halophiles). Ces micro-organismes de

l'extrême sont présents dans les océans mais aussi dans des environnements hypersalins, tels que les marais salants ou les lacs alcalins. Leur survie et leur diversité dans les effluents industriels hypersalins ainsi que leur capacité à dégrader la pollution organique carbonée, azotée et phosphorée de ces effluents est l'objet de cette thèse.

1.2 Plan de la thèse

Le **chapitre 2** fait le point sur les connaissances bibliographiques relatives à :

- La description des micro-organismes halophiles et halotolérants, ainsi que la biodiversité des environnements salins et hypersalins
- La description des principales activités industrielles génératrices d'effluents salins et la caractérisation de la pollution engendrée par ces activités
- L'état de l'art des traitements appliqués aux effluents salins

Il permet de conclure sur les enjeux scientifiques de la thèse.

Le **chapitre 3** établit les principes généraux des matériels et méthodes utilisés, les spécifications techniques étant, quant à elles, précisées dans la section matériels et méthodes de chaque article ultérieurement présenté.

Les **chapitres 4, 5 et 6** présentent les résultats obtenus sous la forme d'articles scientifiques.

Le **chapitre 4** décrit la mise en œuvre du procédé discontinu sequencing batch reactor (SBR) appliqué au traitement aérobie de deux effluents industriels hypersalins. Le premier type d'effluent traité est généré lors de la production de tartrate de calcium à partir de solutions alcalines de détartrage chimique des cuves à vin par l'entreprise Faure S.A. Le second type d'effluent traité est un effluent de tannerie résultant du lavage initial des peaux. Ces deux effluents industriels sont très différents mais présentent cependant des caractéristiques communes, à savoir une forte salinité et une teneur élevée en matière organique. L'évolution dans le temps des performances des procédés de traitement de ces effluents est détaillée dans ce chapitre.

Le **chapitre 5** décrit dans un premier temps la mise en œuvre de procédés anaérobies de traitement de l'effluent de tannerie traité au chapitre 4. Les procédés employés sont un digesteur à biomasse libre : upflow anaerobic sludge blanket (UASB) et un digesteur à biomasse fixée sur un support mobile : le réacteur à lit mobile. Le chapitre 5 établit

ensuite un comparatif entre les performances obtenues en aérobiose et en anaérobiose sur le même type d'effluent. Une dernière partie vise alors à mieux comprendre les phénomènes d'inhibition limitant la digestion anaérobie des effluents salés en soumettant deux réacteurs SBR anaérobies à un stress salé, croissant par paliers. Un premier réacteur a été alimenté avec un effluent complexe vinicole : la vinasse, tandis qu'un second réacteur a été alimenté avec un substrat simple : l'éthanol. La nature simple ou complexe du substrat utilisé est susceptible d'induire une réponse différente en terme de nature et diversité de la biomasse impliquée dans l'épuration et en terme d'inhibition du procédé par le sel.

Enfin le **chapitre 6** établit un inventaire microbien de boues halophiles prélevées dans les bioréacteurs précédemment décrits. Le but de ce chapitre est d'explorer la biodiversité microbienne, encore largement inconnue, des boues activées hypersalines. Il s'agit également dans ce chapitre d'établir si ces milieux offrent un potentiel de diversité comparable aux boues activées « conventionnelles », avec toutes les conséquences qui peuvent en découler sur l'efficacité et la stabilité des performances du traitement biologique.

Cette thèse se conclut par un résumé et une discussion générale des résultats obtenus (**chapitre 7**).

Chapitre 2. Etude bibliographique

2.1 Le sel et les micro-organismes halophiles

2.1.1 L'extrêmophilie : le cas de l'halophilie

L'extrêmophilie désigne l'aptitude de certains organismes à se développer dans des conditions physiques et chimiques défavorables pour la plupart des organismes vivants. Parmi les domaines les plus étudiés de l'extrêmophilie se trouvent les hautes températures (thermophilie), mais aussi les fortes salinités (halophilie).

On différencie les bactéries halophiles des bactéries halotolérantes. En effet, le terme « halophile » désigne les micro-organismes nécessitant la présence de sel (NaCl) dans le milieu pour leur croissance. En revanche, le terme « halotolérant » signifie que les micro-organismes tolèrent différentes concentrations en sel durant leur croissance. Les bactéries nécessitant moins de 1% de sel pour une croissance optimale ne sont pas considérées comme halophiles. En 1962, Larsen a défini 3 catégories de bactéries halophiles selon la concentration en sels qui amène à une croissance optimale des micro-organismes [125]. Cette concentration peut varier de 1 à 6% pour les bactéries faiblement halophiles, de 3 à 15% pour les bactéries halophiles modérées et jusqu'à 15-30% pour les bactéries halophiles extrêmes. Par ailleurs, les bactéries halotolérantes acceptent des concentrations modérées de sels mais non obligatoires pour leur croissance. Les domaines de croissance de ces 3 catégories d'organismes halophiles sont représentés schématiquement sur la Figure 2.1 [21].

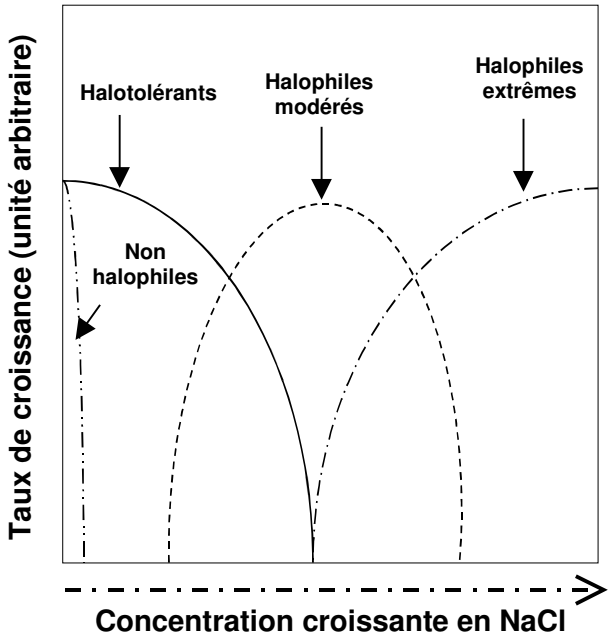

Figure 2.1 Les différents groupes de micro-organismes halophiles.

Ces gammes de concentrations en sel (NaCl) ont été affinées depuis et s'étendent de 3 à 10% pour les procaryotes halotolérants, de 9 à 25% pour les procaryotes halophiles modérés et de 22 à plus de 40% pour les Archaea halophiles. Les différentes catégories de micro-organisme halophiles sont représentées dans le Tableau 2.1 [123]. Dans certains marais salants, la concentration en sel (NaCl) peut atteindre 700 g l^{-1}. Dans ces conditions, les sels de lithium précipitent après des cycles de deux ans et ces milieux semblent abiotiques au vu des connaissances actuelles [169].

Tableau 2.1 Catégories de micro-organisme halophiles.

Catégorie	NaCl (M)		NaCl (g l^{-1})	
	Gamme	Optimum	Gamme	Optimum
Non halophile	0–1,0	<0,2	0–60	<10
Faiblement halophile	0,2–2,0	0,2–0,5	10–115	10–30
Halophile modéré	0,4–3,5	0,5–2,0	25–200	30–115
Halophile extrême "Borderline"	1,4–4,0	2,0–3,0	80–230	115–175
Halophile extrême	2,0–5,2	>3,0	115–300	>175
Halotolérant	0–>1,0	<0,2	0–>60	<10
Haloversatile	0–>3,0	0,2–0,5	0–>175	10–30

2.1.2 Les mécanismes d'adaptation à la vie en milieu hypersalin

L'intégrité des bactéries halophiles en milieu salin exige le maintien d'un équilibre osmotique entre le cytoplasme et le milieu environnant. Les micro-organismes halophiles ont développé des mécanismes spéciaux afin de s'adapter à l'environnement hypersalin.

Régulation de la pression osmotique

A faible concentration le sel (NaCl) est indispensable au fonctionnement cellulaire, mais à forte dose, il entraîne la mort cellulaire par sortie d'eau. Pour compenser la pression osmotique du milieu hypersalin environnant la plupart des micro-organismes halophiles et halotolérants accumulent essentiellement du chlorure de potassium (KCl) chez les Archaea, ou des composés organiques dissous ayant un potentiel osmotique, comme les sucres, les polyols ou les acides aminés, chez les Bacteria [98]. A titre d'exemple, *Chromatium glycolicum* utilise le glycolate [36], *Desulfovibrio halophilus* le tréhalose et la glycine bétaïne [232], *Haloanaerobacter salinarius* la glycine bétaïne [155].

Adaptation des protéines à l'hypersalinité

En accumulant dans leur cytoplasme des quantités de sel proche de la saturation, les bactéries halophiles empêchent effectivement la sortie d'eau mais se soumettent à un nouveau type de stress cellulaire : le stress salin. Avec de telles concentrations en KCl, des protéines « normales » deviennent insolubles et précipitent. Or les organismes halophiles ne semblent pas connaître ce stress cellulaire : leurs protéines sont non seulement solubles et fonctionnelles à de fortes concentrations en KCl, mais elles se dénaturent lorsque la concentration en sel diminue. Pour ce faire, les protéines « halophiles » concentrent fortement le sel près de leur surface [63] et utilisent ses capacités hygroscopiques pour capturer les molécules d'eau nécessaires à leur repliement, leur stabilisation et leur solubilité. Ce phénomène est rendu possible par une abondance d'acides aminés « acides », connus pour interagir fortement avec les molécules d'eau et les cations tels que K^+ [134].

Si personne ne peut affirmer si les protéines halophiles sont le résultat d'une adaptation à un environnement extrême ou bien si elles représentent la survivance de conditions de vie primitive « salées », des études récentes suggèrent que les océans primitifs avaient une salinité particulièrement élevée et que, par ailleurs, les propriétés des protéines halophiles étendent considérablement la gamme de concentrations en sel

dans lesquelles elles restent stables, alors que les protéines non halophiles fonctionnent dans des conditions plus restreintes [25]. On peut donc supposer que les organismes halophiles ont réussi à survivre mieux que les autres dans des milieux fluctuants en salinité et que, au contraire, ce sont les organismes non halophiles qui ont dû s'adapter pour survivre dans des environnements de faible salinité [134].

2.1.3 Diversité moléculaire des halophiles

Depuis le début des années 90, l'essor des méthodes de phylogénie moléculaire basées sur le séquençage de l'ADNr 16S a révolutionné l'écologie microbienne. Les méthodes analytiques basées sur le séquençage du génome ont en effet révélé la grande majorité non cultivable des espèces microbiennes longtemps inconnues puisque non cultivables. Un chiffre marquant : aujourd'hui, seuls 26 des 52 principaux phyla identifiés dans le domaine des Bacteria comprennent des représentants cultivables [178]. Depuis 1997, le nombre de nouveaux clones environnementaux séquencés par ces méthodes moléculaires puis ajoutés à la base de données Genbank dépasse celui des nouveaux micro-organismes cultivés.

Diversité phylogénétique des halophiles

Les halophiles et halotolérants présentent une grande diversité phylogénétique. On les trouve parmi les trois grands domaines du vivant : Archaea, Eukaryota et Bacteria [164].

Au sein des Archaea, la famille des Halobacteriaceae comprend la plupart des halophiles aérobies, répartis au sein de 14 genres, incluant notamment *Halobacterium*, *Halococcus*, *Haloarcula*, *Haloferax*, *Halorubrum*, *Halobaculum*, *Natrialba*, *Natromonas*, *Natronobacterium* et *Natronococcus* [108]. On les trouve dans la Mer Morte, les marais salants et les lacs alcalins hypersalins. Egalement, la branche méthanogène des Euryarchaeota contient des halophiles dont l'activité méthanogène est possible à des seuils proches de la saturation en NaCl : *Methanohalophilus*, *Methanohalobium*, *Methanospirillum* [108; 164].

Dans le domaine des Eukaryota, les halophiles sont plus rares. Le principal représentant des halophiles est l'algue verte *Dunaliella* qui est davantage halotolérante que strictement halophile et tolère une large gamme de salinité. On peut aussi citer le crustacé *Artemia* [164]. Enfin, parmi les eucaryotes, des levures osmotolérantes (*Rhodotorula mucilaginosa* et *Pichia guilliermondii*) isolées de bassins d'évaporation d'effluents pharmaceutiques en Israël croissent à 15% de sel et même au-delà [124]. La

survie de levures à de fortes salinités a été confirmée par Dan *et al.* qui ont montré qu'une culture de levures croissait plus rapidement à forte salinité qu'une culture bactérienne, cette dernière étant inhibée par le sel [48].

Le domaine des Bacteria regroupe la plus grande diversité des halophiles, la plupart étant halophiles modérées plutôt qu'extrêmes. En passant en revue les principaux phyla, tels qu'ils ont été définis par Rappé et Giovannoni [178], il en ressort que les halophiles sont ubiquistes et présents dans un grand nombre de groupes phylogénétiques (cf.Tableau 2.2).

Tableau 2.2 Représentants des halophiles dans le domaine des Bacteria.

Phylum	Environnement	Références
Proteobacteria	Lacs alcalins	[15; 137; 180; 205-207; 237]
	Marais salants	[18; 50; 94; 105; 154]
	Sédiments marins	[14; 238; 239]
	Environnement marin	[16; 73; 139; 140; 175; 186; 221; 222]
	Grandes Plaines Salées, Oklahoma	[35]
Firmicutes	Salins	[38; 154]
	Lac alcalin	[104]
	Environnement marin	[32; 222]
	Mer Morte	[10]
Bacteroidetes	Environnement marin	[158; 222]
	Marais salants	[154; 167]
	Grandes Plaines Salées, Oklahoma	[35]
Chlorobi	Environnement marin	[83; 222]
Planctomycetes	Environnement marin	[17; 32; 141]
Chloroflexi	Environnement marin	[16; 77; 78; 222]
	Tapis microbien hypersalin	[117]

Phylum	Environnement	Références
Verrucomicrobia	Environnement marin	[141; 231]
	Salins	[33]
Gemmatimonadetes	Sédiments marins	[240]
Cyanobacteria	Lacs alcalins	[75; 85]
	Lagons hypersalins	[201; 241]
	Marais salants	[18; 37; 94]
	Sédiments hypersalins	[30]
	Environnement marin	[29; 162; 199; 202; 222]
Spirochaetes	Environnement marin	[29; 222]
	Marais salants	[154]
	Source chaude côtière	[198]
Actinobacteria	Environnement marin	[119; 129; 179; 196; 222; 228]
	Marais salant	[5; 8; 42]
	Sol salin	[130]
Chlamidiae	Environnement marin	[29]
Nitrospira	Bioréacteur salé	[41]
OP3	Environnement marin	[141]
OP8	Environnement marin	[141]
OP9	Environnement marin	[32]
OP10	Environnement marin	[32]
OP11	Sédiments marins	[129; 234]
NKB19	Sédiments marins	[129]
Marine group A	Environnement marin	[16; 61; 71; 78; 133; 141; 179; 213]
	Sédiments marins	[28]
ABY1	Sédiments marins	[216]
Guaymas1	Sédiments marins hydrothermaux	[214]
Acidobacteria	Environnement marin	[32]

Du Tableau 2.2, il ressort que les principaux types d'environnements où se retrouvent les micro-organismes halophiles sont la mer, les marais salants et les lacs alcalins. La diversité microbienne de ces milieux salins est par conséquent explorée dans la suite de cette étude bibliographique.

Diversité moléculaire des milieux marins

Il a déjà été expliqué que le boom des méthodes moléculaires basées sur le séquençage de l'ADNr 16S a permis au nombre des nouveaux clones environnementaux de dépasser celui des nouveaux micro-organismes cultivés dans la base de données Genbank. Et très rapidement ce sont les micro-organismes marins qui ont le plus alimenté cette base de données, devançant les micro-organismes du sol [178]. Il était en effet, pour des raisons techniques, plus facile d'extraire de l'ADN microbien de l'eau de mer que du sol. Il en résulte une bonne connaissance des micro-organismes marins, principalement halotolérants et halophiles faibles à modérés. Il en ressort ainsi que la plupart des Bacteria identifiées par les méthodes moléculaires appartiennent aux divisions des Proteobacteria (67%) et Cytophagales (25%) [70], ce dernier groupe faisant partie du plus vaste phylum des Bacteroidetes. Mais les micro-organismes marins sont présents aussi dans tous les autres phyla recensés dans le Tableau 2.2.

Diversité moléculaire des marais salants

Les marais salants, dans lesquels l'énergie solaire est employée afin d'évaporer l'eau de mer et de récolter le sel, représentent un écosystème passionnant pour l'étude des halophiles. En effet la salinité dans les marais salants recouvre toute la gamme de salinités, depuis celle de l'eau de mer jusqu'à la saturation en NaCl et parfois au-delà, dans les cas où l'on cherche à précipiter les chlorures de potassium, magnésium, lithium et bore.

La diversité moléculaire des marais salants, déterminée par le séquençage de l'ADNr 16S, a ainsi pu donner une image de l'écologie microbienne en milieu hypersalin. Benlloch *et al.* ont, les premiers, décrit par les méthodes moléculaires les communautés microbiennes d'un bassin de cristallisation espagnol, dévoilant un petit groupe homogène de Bacteria appartenant au phylum des Proteobacteria, proches de *Rhodopseudomonas marina* [19]. Les Archaea aussi constituaient un groupe homogène avec deux clones d'un genre inconnu reliés de façon distante au genre *Haloferax*.

Par la suite, d'autres études ont montré que la biodiversité des Bacteria diminuait lorsque la salinité augmentait et que les communautés bactériennes des bassins de moindre densité (<14% de sel) n'avaient que 30% de similarité avec celles des bassins

de concentration (21% de sel), les communautés de ces derniers ne présentant aucune similarité avec celles des bassins de cristallisation (30% de sel) [147]. Cependant ces remarques sont à nuancer au vu des résultats sur les communautés Archaea qui sont hautement similaires entre les bassins de concentration et les bassins de cristallisation, et qui par ailleurs se révèlent stables dans le temps. Par ailleurs, aucune Archaea n'a pu être amplifiée à des salinités inférieures à 13% de NaCl et les communautés microbiennes ne présentent que 50% de similarité entre le bassin de cristallisation espagnol en question et un autre situé en Israël [184]. Dans les bassins de cristallisation, la diversité des Archaea apparaît très faible, avec un clone Archaea non cultivable représentant près de 100% des clones amplifiés [7; 184]. Anton *et al.* ont par la suite identifié un nouveau genre d'halophile extrême dénommé *Salinibacter* et pouvant constituer jusqu'à 25% de la biomasse des bassins les plus concentrés des marais salants [8]. Au regard du Tableau 2.2, les halophiles des marais salants se retrouvent dans un certain nombre de phyla du domaine des Bacteria : Actinobacteria, Spirochaetes, Cyanobacteria, Verrucomicrobia, Bacteroidetes, Firmicutes, Proteobacteria.

Diversité moléculaire des lacs alcalins

Les lacs alcalins ont aussi fait l'objet d'analyses multiples. Il s'agit d'un type particulier de lac salé, contenant entre autres sels une grande quantité de carbonates et bicarbonates de sodium [206]. Duckworth *et al.* ont ainsi mis en évidence des Gamma Proteobacteria du genre *Halomonas*, ainsi que des Actinobacteria et Firmicutes au sein de six lacs alcalins situés au Kenya et en Tanzanie [59]. Des Archaea haloalcalophiles appartenant aux genres *Natronococcus* et *Natronobacterium* ont aussi été mises en évidence dans ces mêmes lacs. Hollibaugh *et al.*, en étudiant le Mono Lake alcalin de Californie, ont observé des Bacteria proches de bactéries connues, preuve d'une vaste distribution de ces groupes de part le monde, et ont aussi fait le constat d'une biodiversité inférieure à l'eau de mer et d'une relative stabilité de la communauté bactérienne de ce lac au cours du temps [96]. Une autre étude dans ce même lac n'a pas permis de caractériser Archaea [132]. Par contre des groupes de Beta Proteobacteria ont été détectés dans ce même lac [229].

Récemment, Sorokin et Kuenen ont isolé en culture pure une centaine d'isolats à partir d'échantillons provenant de multiples lacs alcalins de différents continents [206]. Ces isolats appartenaient en majorité à trois nouveaux genres de bactéries sulfo-oxydatrices du phylum des Gamma Proteobacteria : *Thioalkalimicrobium*, *Thioalkalivibrio* et *Thioalkalispira*. Cette faible diversité peut s'expliquer par la difficulté d'adaptation des ces bactéries à la double contrainte du sel et du pH alcalin. Au regard du Tableau 2.2, c'est

dans trois phyla que se retrouvent majoritairement les Bacteria des lacs alcalins : Proteobacteria, Firmicutes et Cyanobacteria.

Autres milieux hypersalins

Eder *et al.* ont étudié l'écologie microbienne de la Mer Rouge dans une zone spécifique où la salinité augmente brutalement de 4 à 26% sur une frange de 3m, à 1500m de profondeur [60]. L'analyse moléculaire de l'ADNr 16S a mis en évidence une biodiversité faible exclusivement constituée d'organismes anaérobies halophiles appartenant aux genres *Halanaerobium*, *Propionibacterium* et *Clostridium*. Le Solar Lake égyptien, d'une salinité comprise entre 10 et 20% a aussi fait l'objet de nombreuses études. Cytryn *et al.* y ont observé un groupe d'Archaea similaires à celles que l'on retrouve dans les marais salants, ainsi que deux groupes de méthanogènes dans des régions du lac enrichies en méthane et enfin un groupe proche de *Thermoplasma* [46]. Enfin, l'écologie microbienne des produits agro-alimentaires salés a révélé une forte similarité avec les bactéries marines. C'est notamment le cas de la microflore constituant la croûte de certains fromages, constituée à plus de 10% de bactéries marines probablement apportées par l'usage de sel de mer [69]. Quant aux produits à base de viande marinée dans le sel et l'acide, très prisés dans les pays nordiques, ils sont parfois souillés par des bactéries acido-lactiques anaérobies et psychrotrophes [22].

Conclusions

- Les bactéries halophiles présentent une très vaste diversité particulièrement dans le milieu marin.
- La biodiversité et la composition des communautés halophiles dépendent de la salinité.
- Les communautés des milieux hypersalins apparaissent relativement stables dans le temps.

2.2 Les pollutions salines dans l'industrie : études de cas

2.2.1 L'industrie agro-alimentaire

L'utilisation du sel dans l'industrie agro-alimentaire

Le sel destiné à la consommation humaine a un double rôle de nutrition et de conservation des aliments. La teneur en sel des aliments est très variable, allant de quelques pour cent pour les plats préparés, le pain, les fromages, à plus de 40% dans le cas de préparations très concentrées comme les bouillons en sachets. Par ailleurs, le sel agit comme un agent dépresseur de l'activité de l'eau dans les aliments, assurant ainsi leur stabilité microbiologique. En effet, la disponibilité de l'eau présente dans l'atmosphère ou dans une substance favorise la croissance bactérienne. Or l'activité de l'eau (Aw) est inversement proportionnelle à la pression osmotique d'un composé. Ainsi, elle est affectée par la présence plus ou moins importante de sels ou de sucres dissous dans l'eau. Enfin, selon sa concentration en sel, l'eau salée autorise le développement de certains micro-organismes au détriment d'autres qui sont soit détruits soit inactivés. Ainsi, le sel agit comme antimicrobien sélectif ou agent bactériostatique. Le rôle d'inhibiteur-retardateur du sel est mis à profit en conserverie, notamment des viandes et du poisson (salaisons), tandis que son rôle de régulateur-orientateur sur le développement des micro-organismes sert, par exemple, au processus de fabrication puis d'affinage des fromages [134]. De nombreux secteurs agro-alimentaires sont concernés par l'utilisation de sel et les rejets de pollution saline.

La production de conserves de viandes, charcuterie, salaisons (saucisses, saucissons, jambons, poitrine, etc.) et produits nécessitant l'utilisation de boyaux

On distingue généralement les « salaisons vraies » dans lesquelles le sel est réparti uniformément dans la viande à un taux supérieur à 5%, assurant ainsi une conservation de plusieurs mois, des « produits salés » dans lesquels le sel est réparti parfois de façon hétérogène et à des taux de 1 à 2% au maximum, servant alors davantage à accroître la qualité organoleptique du produit qu'à en assurer sa conservation [134]. Le sel est aussi largement utilisé dans les produits marinés très populaires en Europe du Nord. Les marinades sont typiquement des émulsions d'eau et d'huile contenant du sel, du sucre et des acides. Le double effet du sel et du pH acide agit alors comme un exhausteur de goût en même temps qu'un agent antimicrobien. En réalité, il semblerait que le pouvoir

tampon de la viande neutralise l'acidité de la marinade, rendant son action antimicrobienne inopérante [22].

La confiserie ou conserverie de légumes saumurés (olives, etc.), la fabrication de bouillons et potages

Dans l'industrie de la conserve, le sel agit par osmose, c'est à dire qu'il pénètre dans les aliments au fur et à mesure que ceux-ci se vident de leur eau de constitution. Par ailleurs, alors qu'il pénètre peu à peu à l'intérieur des aliments, le sel inhibe la croissance des micro-organismes qu'ils contiennent, retardant leur décomposition. Le sel se retrouve généralement à un taux de 2% dans les conserves de légumes [134].

L'industrie laitière : fabrication de beurre et de fromages (principalement l'affinage des fromages à croûte morgée)

La concentration moyenne en sel dans le fromage est de l'ordre de 1 à 2%. Dans les fromages bleus et dans certains fromages de chèvre, elle peut toutefois atteindre 3 à 4%. Dans le cycle de fabrication des fromages, le salage s'effectue après l'égouttage et avant l'affinage. Il sert à protéger les fromages contre les micro-organismes indésirables (protection d'autant plus nécessaire que le fromage est humide), mais aussi à sélectionner des micro-organismes spécifiques nécessaires à la maturation du produit (développement du goût et de la fleur du fromage). Ainsi le sel permet la sélection de souches microbiennes productrices d'enzymes utiles à la protéolyse et à la lipolyse du fromage et conditionne ainsi son affinage. En effet, la transformation du lait en fromage se fait sous l'influence de différents micro-organismes ou produits sécrétés par des êtres vivants. Jusqu'à l'affinage et la maturation, le sel sélectionne certains micro-organismes producteurs d'enzymes spécifiques indispensables à la protéolyse du caillé et autorise le développement d'espèces utiles telles que celles responsables de la pousse du bleu dans la masse de certains fromages ou celles formant la croûte fleurie en blanc des camemberts, ou encore la flore du rouge des munsters et des pont-l'évêque [134].

En ce qui concerne le beurre, le taux de sel n'excède pas 5% du poids total du beurre demi-sel et 10% du beurre salé, en respect des normes françaises. Le salage permet d'inhiber partiellement le développement microbien, notamment celui des ferments lactiques [134].

La boulangerie, biscuiterie et biscotterie

Dans la fabrication du pain, le sel agit comme exhausteur de goût et retarde la fermentation de la levure. Dans la pratique, le boulanger utilise une quantité de sel qui varie entre 1,5 et 2,25% du poids de la farine. Dans les industries de biscotterie, biscuiterie et pâtisserie, le sel est utilisé en quantité moindre, généralement inférieure à 1%. Les pâtes sont en effet sucrées (autre agent dépresseur de l'activité de l'eau) et le sel ne sert qu'à réguler la fermentation et à soutenir le goût du sucre [134].

La pêche et l'industrie de la pêche

Les poissons habituellement traités par salage sont l'anchois, la sardine et la sardinelle, le thon et la bonite, le maquereau, la morue et le hareng, ainsi que certains œufs de poisson (caviar et succédanés). Le sel agit alors en tant qu'agent déshydratant de la chair du poisson. En enlevant l'eau de constitution, il entraîne l'inhibition de la flore psychrotrophe non halophile. Par ailleurs, le sel coagule les albumines des matières azotées, ce qui a pour effet de « colmater » les cellules et ainsi de les mettre à l'abri du contact de l'air et des souillures [134].

La production d'effluents salins par l'industrie agro-alimentaire

Les sources de pollutions salines dans les entreprises agro-alimentaires proviennent essentiellement :

- De l'utilisation de produits conservés dans une solution de saumure
- De la fabrication de saumure pour la production du produit fini (frottage des fromages, salage des jambons par injection, etc.)
- De l'utilisation de sel « sec » (chlorure de sodium, sel nitrité, etc.)

Les légumes saumurés

Les sources de pollution saline liées à la production de légumes saumurés concernent l'utilisation de saumure pour la conserverie et la confiserie de légumes, les rejets de bains de saumure et les pertes de saumure se retrouvant dans les eaux de lavage. Les huileries d'olive rejettent notamment de grandes quantités de déchets solides (peaux et noyaux) et liquides salés [225]. Les effluents des procédés de fabrication d'huile d'olive, de couleur noire, sont de plus caractérisés par la présence de sucres, acides organiques,

tannins, polyphénols, polyalcools, pectines et lipides [166; 215], induisant une charge organique de 0,1 à 0,3 g l^{-1} de demande chimique en oxygène (DCO) [115].

Les fromages

Les problèmes de pollutions salines en fromagerie concernent principalement les fromages à croûte frottée. Les sources de pollution sont liées à l'utilisation de saumure dans la morge (solution utilisée pour le frottage des fromages), l'utilisation de sel sec pour saler les fromages dans certains cas et les eaux de lavage pouvant contenir des sels. Les effluents de fromagerie s'accompagnent également d'une quantité élevée de matières en suspension (0,5 à 1 g l^{-1} de MES) et de demande biochimique en oxygène (1,5 à 2,5 g l^{-1} de DBO_5) dans la morge récupérée après le frottage des meules [52].

La charcuterie, les salaisons

Les sources de pollution saline en charcuterie sont liées à l'utilisation de boyaux conservés dans des fûts de saumure, au salage des viandes (jambons, etc.) par injection d'une saumure et aux pertes se retrouvant dans les eaux de lavage. Par ailleurs, la DCO moyenne de ces effluents avoisine 30 g kg^{-1} de carcasse et les rejets, notamment des injecteurs, sont fortement souillés par des graisses et des MES [52].

L'industrie de la pêche

Les sources de pollution saline concernent dans un premier temps le déchargement des poissons hors du bateau, accompagnés d'eau de mer. Cet effluent d'une salinité similaire à l'eau de mer est caractérisé par une DCO de 13 à 39 g kg^{-1} de poissons déchargés [12]. L'industrie agro-alimentaire de transformation des produits de la pêche génère par la suite des effluents riches en azote protéique, en matière organique et en sels [6; 146]. Il contient également des quantités non négligeables de graisses (0,25 g l^{-1}, en moyenne) [145] et de sulfates qui limitent leur biodégradabilité anaérobie par les Archaea méthanogènes [218].

2.2.2 L'industrie chimique et l'industrie pharmaceutique

L'industrie chimique est le principal utilisateur de sel en Europe, particulièrement le secteur du chlore et de la soude (44 % du total des ventes en 1994). Le déclin de la production des substances chimiques à base de chlore, qui résulte notamment de l'arrêt de la production des chlorofluorocarbones (CFC) et d'une plus faible demande de

blanchiment de pulpe et de papier, est compensée par la demande croissante de matières plastiques en chlorure de polyvinyle (PVC), ainsi que par la demande de soude caustique, coproduit de l'électrolyse de la saumure.

La fabrication de produits chimiques mais aussi pharmaceutiques génère par conséquent, en plus des déchets solides et des émissions de gaz, des effluents liquides fréquemment concentrés en sels. La difficulté de traitement de ces effluents provient en grande partie de leur variabilité non seulement d'une usine à l'autre mais aussi dans le temps au sein d'une même unité de production, en fonction des produits synthétisés. Les effluents pharmaceutiques sont par ailleurs généralement riches en matières solubles (sulfates notamment) et en suspension, ainsi qu'en substances organiques toxiques et réfractaires au traitement biologique, notamment des composés aromatiques [72]. Les effluents pharmaceutiques sont souvent caractérisés par une forte DCO et un ratio DCO/DBO_5 élevé [177].

Dans le cadre de cette revue bibliographique, nous pouvons détailler ici un cas particulier qui a fait l'objet de recherches dans cette thèse. L'entreprise Faure S.A. produit du tartrate de calcium à partir de solutions alcalines de détartrage chimique des cuves à vin. Ces solutions sont composées d'eau, de sel de seignette (tartre, sodium et potassium), de soude résiduelle et de matières organiques résiduelles du vin (composés aromatiques, notamment). La précipitation du tartrate de calcium se fait par acidification (acide chlorhydrique, HCl) et par ajout d'un sel de Calcium (chlorure de calcium, $CaCl_2$). Les cristaux de tartrate de calcium sont collectés puis utilisés comme matière première avant d'être vendus principalement dans les secteurs de l'œnologie et de l'agro-alimentaire. Auparavant, les cristaux sont essorés et lavés pour éliminer l'excès de chlorure, ce qui engendre des effluents hypersalins. Les effluents issus de la phase d'essorage sont recyclés en tête du procédé et après un certain nombre de recyclages, ils sont envoyés dans des bassins d'évaporation. Les effluents issus de la phase de lavage sont directement envoyés sur les bassins d'évaporation. L'effluent à traiter contient en moyenne 9 g l^{-1} de DCO, 4,5 g l^{-1} de DBO_5, 500 mg l^{-1} de MES, de 50 à 250 mg l^{-1} d'azote (N) et de 5 à 40 mg l^{-1} de phosphore (P). Son pH est de 6,7. Il est particulièrement riche en composés aromatiques résiduels des cuves de vinification.

2.2.3 L'industrie pétrolière

Le pétrole brut est un mélange complexe qui contient des hydrocarbures principalement aliphatiques, alicycliques et aromatiques. Il peut être classé selon sa densité spécifique et d'après son degré API (American Petroleum Institute) en condensé

naturel, léger, moyen, lourd et extra-lourd [181]. Le processus de raffinage du pétrole fait appel à des produits dé-émulsifiants et les effluents générés par la décantation de l'émulsion pétrole-eau sont appelés eaux de production. Ces dernières présentent une large gamme de salinité allant de l'eau douce jusqu'à trois fois la salinité de l'eau de mer et au-delà [56]. Elles contiennent par ailleurs des hydrocarbures et composés phénoliques [52].

2.2.4 L'industrie textile

Utilisation du sel dans l'industrie textile

Le sel est utilisé dans l'industrie textile lors du procédé de teinture, afin d'améliorer la fixation des encres sur les fibres. En fait, le rendement de fixation de l'encre sur la fibre dépend de la concentration en sel utilisée lors du procédé et la couleur recherchée : les nuances les plus sombres requièrent davantage de sel [194].

Impact environnemental de l'industrie textile

Les différentes opérations intervenant dans les procédés de l'industrie textile présentent des impacts conséquents au niveau de l'environnement. Les différentes étapes du procédé ainsi que les effluents générés sont représentées sur la Figure 2.2 [194].

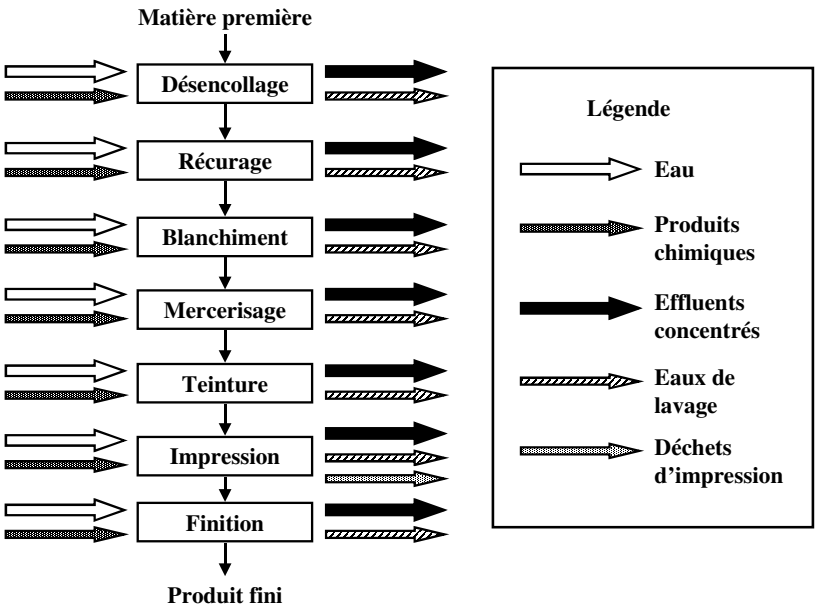

Figure 2.2 Les différentes étapes du procédé de fabrication de textile et effluents associés.

Après avoir passé les différents prétraitements (désencollage, récurage, blanchiment) puis le mercerisage, la matière première est teinte, ce qui génère un effluent caractérisé par un pH alcalin, principalement dû à l'ajout de soude et de savon, par une forte concentration en sel (NaCl), nécessaire à la fixation de la teinture, et par une quantité élevée de DCO provenant des composés des auxiliaires de teinture. Par ailleurs, l'effluent est particulièrement riche en composés organiques aromatiques résiduels (phénols) qui le rendent difficilement biodégradable et la couleur de l'effluent est non seulement esthétiquement inacceptable, mais, de plus, endommage gravement les écosystèmes aquatiques en retardant la pénétration de la lumière dans l'eau, entravant ainsi l'activité photosynthétique des algues [194]

2.2.5 L'industrie du cuir

Cette thèse traite en grande partie des possibilités de traitement des effluents de lavage des peaux générés par les tanneries indiennes. Il est par conséquent utile d'analyser plus avant les enjeux liés à cette industrie dans un contexte bien particulier, celui de l'Inde.

Article : Options for the Treatment of Tannery Soak Liquor

Résumé

L'industrie du cuir représente l'un des secteurs majeurs de l'industrie indienne. Cette industrie s'est modernisée selon un modèle de développement durable basé sur la mise en place de technologies propres et de stations d'épuration des effluents, de façon à permettre à l'industrie de gagner en valeur ajoutée. Le procédé de tannage requiert de grandes quantités d'eau et génère par conséquent d'important volumes d'effluents. De nos jours, le traitement des effluents de tannerie est obligatoire en Inde et c'est généralement sous la forme d'un effluent composite, constitué du mélange de tous les effluents produits par la tannerie, que ce traitement a lieu. Pourtant, certains effluents possèdent des caractéristiques particulières et gagneraient à être séparés et traités séparément.

Abstract

Nowadays the leather industry represents a strong sector of the Indian industry. The sustainable model of development of this industry was based on the promotion of cleaner technologies and effluent treatment processes, in order to make it possible for the industry to climb the value-added chain. The tanning process is almost wholly a wet process that generates high amounts of wastewater. Hence the treatment of tannery wastewater is always required and is practised as a composite effluent treatment in India. Yet, some streams have highly specific characteristics and should be segregated and treated separately.

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Importance of the leather sector and leather trade worldwide

Leather is a skin of animal undergoing tanning and intended to be transformed into an object. The majority of the world production of leather results from the skin of slaughtered animals such as cows, bulls, horses, sheep, calves and goats. The skin of kangaroos, deers, various reptiles (lizards, snakes), birds (ostrich) and fishes (salmon) can also be used.

Globally, approximately 18 billion square feet of leather are processed yearly [101]. There is an increasing trend in this industry with a heavy shift from developed countries to developing countries, such as China and India, where production costs are lower and environmental regulations less stringent [107]. The main leather producing countries in the world - accounting for almost two thirds of the finished leather produced - are shown in Table 2.3 [101].

Table 2.3 Leather production of the main leather producing countries.

Country	Million square feet equivalent
China	3,000
Italy	1,820
India	1,375
Korea	1,000
Brazil	725
USA	700
Former Soviet Union	650
Spain	500
Turkey	450
Mexico	450
Pakistan	370
Argentina	320

Source: International Council of Tanners

The leather industry is very much an international industry, with raw hides and skins, part processed leather, finished leather, leather components and leather products widely imported and exported. Consequently, the international trade of all leather products

averages 40 billion US$ per year, which is 2.4 times as much as the value generated by the trade of the meat of the same animals [101]. Developing countries became net exporters of bovine hides and skins, leather and leather products in the 1990s and their share of the global value of exports has risen from 20% to over 40% between 1980 and 1995 [156].

The Indian leather industry: history of a sustainable development

Nowadays the leather sector represents a strong and fast growing sector of the Indian industry. Its potential is enormous with regard to employment and exports. 2.5 million People, including 30% of women, work in this sector, with a large part (nearly 60-65%) of the production in the small/cottage sector [45]. The state of Tamil Nadu is the largest tanning centre in India accounting for more than 60% of the tanning in the country, and about 50% of the total exports of leather and leather products [89]. The annual production value is about 4 billion US$ and the annual export value reaches 2 billion US$ (about 2% of the world trade), which puts India amongst top 10 of export earners, as can be seen on Table 2.4 [45].

Table 2.4 Export gains of the main leather exporting countries.

Exporting Country	Value in thousands US$ in 2002
China	16,160,702
Italy	12,942,283
Germany	2,916,415
Spain	2,806,026
France	2,777,324
Brazil	2,563,985
Belgium	2,045,030
India	1,877,787
Korea Rep.	1,828,856
USA	1,578,202

Source: Council for Leather Exports

The growth of the sector is impressive, with export gains passing from 765 million US$ in 1990-91 to 2,094 million US$ in 2003-04 as can be seen Figure 2.3 [45].

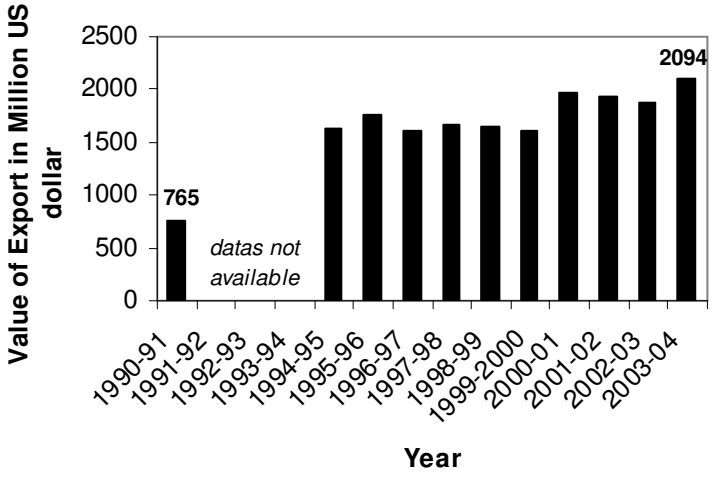

Figure 2.3 Export gains of Indian leather sector in the past fifteen years.

The success of the Indian leather industry can be explained by the abundance of the resources available (India ranks first among the major livestock-holding countries in the world, with 57% of buffalo, 16% of cattle, 20% of goats and 4% of sheep [156]), as well as by effective strategic choices and an important political support.

In the early 1970s, India selected the leather industry as an export thrust sector and a mean of increasing the country's foreign exchange and strengthening national employment [156]. At that time, the environmental matters were not of prior concern, even if the first individual effluent treatment plant applied to an Indian tannery was operated in 1979 in Tamil Nadu.

In 1985, two major events affected the Indian leather industry. First, the competition of China affected the Indian leather sector, and, consequently, the export of wet blue leather (i.e. the semi-manufactured stage of production) was banned by the Indian Government in order to boost the export of more valuable leather products. Second, the Indian Pollution Control Board became stricter on the treatment of tannery effluents, but had to face the hostility of the small scale tanneries, arguing that the lack of money, qualified experts, surface and discharge facilities were as many obstacles to the construction of treatment plants. Furthermore, tannery effluents were considered as untreatable at that time, due to more than 170 chemical products used during the process. Consequently, the number of tanneries treating their effluents in Tamil Nadu only increased from 13 in 1985 to 22 in 1989. At the same time the total number of tanneries in Tamil Nadu decreased from 975 in 1985 to 858 in 1989, as can be seen on Figure 2.4 [65].

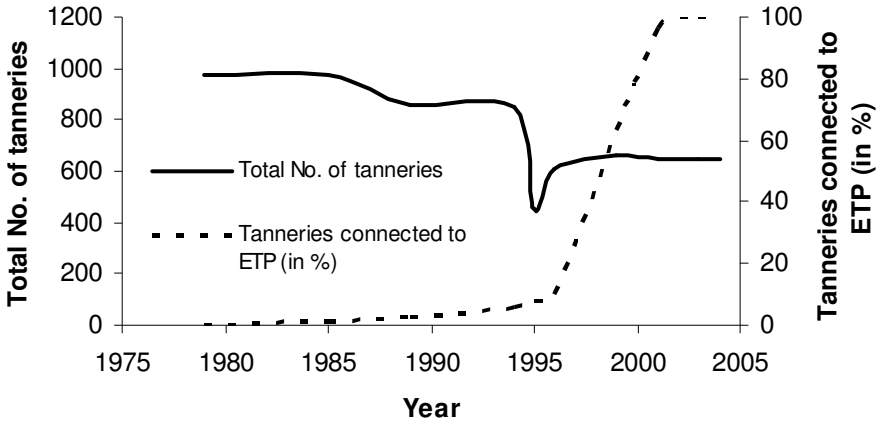

Figure 2.4 Connection of Indian tanneries to effluent treatment plants in the last two decades.

The 1990s started well with India's economic liberalisation policies which made the investment climate conducive for foreign and domestic investors [89]. Consequently, the export of leather and leather products increased from 765 million US dollar in 1990-91 to 1,762 million US dollar in 1995-96, as can be seen on Figure 2.3.

In 1991, a subsidy/grant policy was adopted to encourage the building of common effluent treatment plants (CETPs) in tannery clusters: 25% of the cost of the CETP would be paid by the Central Government, 25% by the State Government, 40% through a soft loan and the final 10% as a share between the tanners. The first CETP was completed in Vaniyambadi, Tamil Nadu, in 1992. Two other CETPs were achieved in 1995 in Pallavaram, Tamil Nadu and in Ranipet, Tamil Nadu. In addition, 42 tanneries in Tamil Nadu were equipped with individual ETPs in 1995 [65].

The second half of the 1990s were characterised by a deceleration of global trade which affected the leather industry on the same basis as the others. They were also marked by an environmental revolution in the Indian leather sector. Indeed, the year 1995 was marked by a historical decision of the Supreme Court of India. In the case entitled Velore Citizens' Forum Vs Tanners of North Arcot District, the court decreed that all tanners in the State of Tamil Nadu without their own effluent treatment plants or connection to a CETP must immediately close down and shall not reopen until they have access to treatment facilities [89]. The judgement also noted that tanners must be made to pay for the damage caused to the environment in the past due to discharge of untreated effluent. More than 400 tanneries had to close down, as indicated on Figure 2.4. Following this judgement, more than 12 CETPs were completed within a short time,

connected to more than 614 tanneries. In addition, more than 120 tanneries set up their own treatment systems. Today, 14 CETPs are operated in Tamil Nadu and there is not a single tannery in Tamil Nadu without access to an effluent treatment facility [89].

Thus, India, thanks to strategic economic choices, such as the decision of exporting only finished leather products, could increase the added value of its exported products, without neglecting employment. Another key of success was the investment in cleaner production technologies. The environmental concern did not have as a single objective to attract green credits but also to increase the quality of the finished product [156]. Nowadays, environmental concern is required for the sustainable development of any industrial sector, especially when it is turned towards export, and the Indian leather industry proved to be able to evolve in this direction. The consequence is the growth of this sector in the last fifteen years as can be stated from Figure 2.3.

There are still unresolved issues concerning the treatment of tannery wastewater. This concerns mainly the total dissolved solids (TDS) content of the effluents. In a region like Tamil Nadu, which suffers from chronic water scarcity, the contamination of ground water by TDS is of prior concern. In this state, a maximum limit of 2,100 mg TDS l^{-1} has been imposed by the Government and the Pollution Control Board is responsible for the application of this norm and promotes the use of reverse osmosis techniques for the recovery of good water from saline effluents. In addition, the creation of a pipeline to carry treated effluent still high in TDS to the sea as a permanent solution to the problem is at an advanced stage of discussion [89].

Environmental impact of the leather industry

Tanning Process

The tanning process, which will turn raw hides and skins into a finished leather product, is a lengthy process that takes place in several steps. Before reaching the tannery, raw hides and skins must be preserved from deterioration using different methods of preservation including salting, chilling, freezing and the use of biocides.

After reaching the tannery, the salted skins are soaked in pure water to humidify them and to eliminate salt, blood and dirt. After the skins are soaked for a period of two hours to seven days, the flesh is withdrawn mechanically from the inner part of the skin. To remove the hair, the skins are immersed during one to nine days in a solution of water and lime containing a small quantity of sodium sulphide. All the residues of meat and hair are then scraped, generally manually with a blunted knife.

The following operation consists in deliming the skins by soaking them in a diluted solution of acid, which reduces the swelling caused by lime. The majority of the skins are treated simultaneously with enzymes during the bating step. The skin gets softened, relaxed, clean and ready for pickling and tanning. During the pickling step, weak acid and salt solutions are used to bring the pelt to the weakly acid state required for most tanning processes. Stronger pickling solutions are used to preserve pelts so that they can be stored or transported in a stable form over periods of several months.

The next step, called tanning, converts the protein of the raw hide or skin into a stable material, which will not putrefy and is suitable for a wide variety of purposes. Every type of skin can undergo several types of tanning. The process is selected according to the use reserved for leather. The two principal processes of tanning are the mineral tanning, like the chromium salts tanning, and the vegetable tanning. The chromium tanning is often carried out in one day, whereas the vegetable tanning requires several weeks or several months. The latter makes it possible to obtain firmer leather, more elastic and resistant to water. Leather which after chrome tanning has not been further processed and is sold in the wet condition is called wet blue but the added value of the product will be increased through the finishing operations in order to level the colour, cover grain defects, control the gloss and provide a protective surface with good resistance to water, chemical attack and abrasion [4].

Pollution Impact

The potential environmental impacts of tanning are significant. The tanning process is almost wholly a wet process that generates high amounts of wastewater, as shown in Table 2.5. The characteristics of the different streams of wastewater generated during the process are indicated in Table 2.5 as well.

Table 2.5 Characteristics of tannery wastewater.

Parameter	Soaking	Beam house operation (liming, reliming, fleshing, deliming)	Pickling & chrome tanning	Wet finish – rechroming, dyeing & fat liquor	Composite (including washings)
Volume of the effluent (m^3 t^{-1} of hides or skins)	6-9	6-10	1.5-3	3-5	30-40
pH	7.5-8	8-12	2.2-4	3.5-4.5	7-9
BOD_5 (g l^{-1})	1.1-2.5	2-8	0.4-0.8	1-2	1-3
COD (g l^{-1})	3-6	3-15	1-3	2-7	2-8
Sulphide (mg S l^{-1})	-	50-200	-	-	30-150
Total solids (g l^{-1})	35-55	6-20	30-60	4-10	15-25
Dissolved solids (g l^{-1})	32-48	5-15	29-58	3.4-9	13-20
Suspended solids (g l^{-1})	3-7	3-15	1-2	0.6-1	2-5
Chlorides (g Cl l^{-1})	15-30	3-6	15-25	0.5-1	6-9.5
Total chromium (g Cr l^{-1})	-	-	1.5-3	0.03-0.06	0.08-0.2

Note: The volume of wastewater applicable is for hides (cow & buffalo) and goatskins and not for wool sheepskins

Source: Central Leather Research Institute, Chennai, India

In addition, significant volumes of solid wastes are produced, including trimmings, degraded hides and hair from the beamhouse processes. The solid wastes can represent up to 70% of the wet weight of the original hides [107]. Large quantities of sludge are also generated. Decaying organic material produces strong odours. Hydrogen sulphide is released during dehairing and ammonia is released in deliming.

Treatment of Tannery Wastewater

Commonly applied tannery effluent treatment systems are shown in Figure 2.5. The soak liquor, produced by the soaking of hides and skins, can be segregated from the other streams and sent to solar evaporation pans (SEPs) because of its high salt content. The other streams are mixed to form the composite wastewater, which is screened, equalised, physically and chemically treated, and then biologically treated. 3 biological options are usually available: conventional lagoon system, fully aerobic system or combined treatment with upflow anaerobic sludge blanket (UASB).

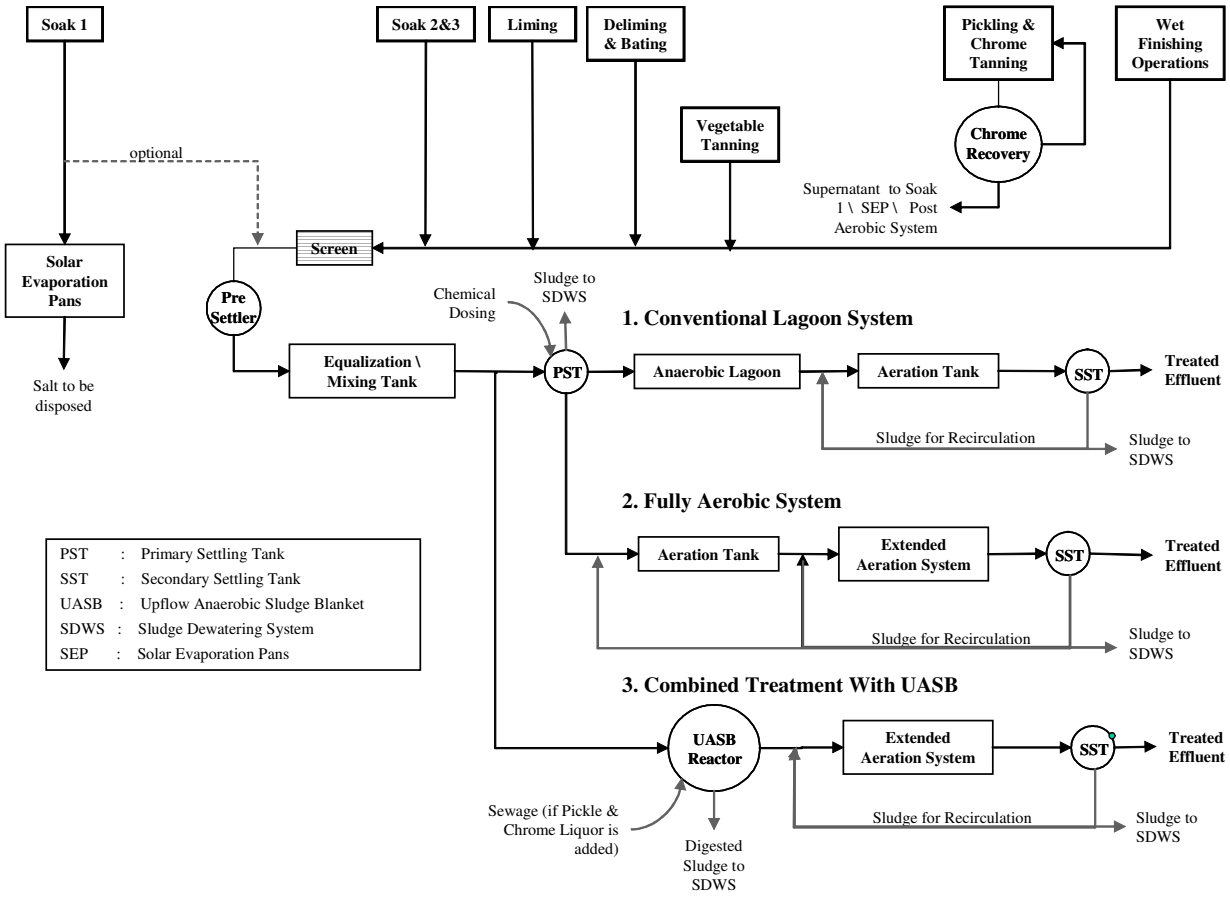

Figure 2.5 Tannery Effluent Treatment Processes
Source: Central Leather Research Institute, Chennai, India

Options for a better management of tannery soak liquor

It has been shown that the treatment of tannery wastewater is always required and is nowadays always treated in Tamil Nadu and India, usually as a composite effluent, made of all effluent streams produced in the tannery and mixed together. Yet, some streams have highly specific characteristics and should be segregated and treated separately. For instance, the lime liquor is rich in sulphide and the chrome wastewater rich in chromium.

In a context of water scarcity and intrusion of salt in freshwater in Tamil Nadu, the management of the soak liquor, which is characterised by high organic load, high suspended solids (sand, lime, hair, flesh, dung, etc.) and high salinity, is of prior concern. Because of its high specificity, the soak liquor should be segregated and evaporated whenever it is possible. In case of not enough space to evaporate it, it should be treated separately before being mixed with the composite wastewater. In both cases, there is an interest in treating the soak liquor through biological methods, using halophilic micro-organisms, to reduce the amount of organic matter in the effluent.

Acknowledgments

This study was conducted in the Centre for Environmental Studies, Anna University, Chennai (India), thanks to a cooperation between Anna University and the French National Institute for Agronomic Research (Institut National de la Recherche Agronomique, INRA), under the framework of the Indo-French Cell for Bioprocesses on Environment (IFCBE). The authors want to acknowledge Mr K.V. Emmanuel for sharing his knowledge of the Indian leather sector.

2.3 Etat de l'art des traitements appliqués aux effluents hypersalins

Cette partie de l'étude bibliographique a fait l'objet d'un article de revue bibliographique qui est présenté en Annexe I à ce document.

2.3.1 Technologies propres – Réduction à la source

Différents aménagements devraient être systématiquement mis en œuvre dans l'industrie afin de limiter la génération de pollutions salines dans le cadre des technologies propres :

- Elimination/réduction de la quantité de sel utilisé dans le procédé
- Ségrégation des solutions de saumure par l'aménagement d'un bac de récupération de la saumure. Ce bac permettrait d'optimiser le temps de séjour de la saumure rejetée et d'améliorer la décantation du sel solide, mais aussi d'éviter le départ de sel solide vers l'égout, lisser les rejets chargés en sel et éliminer l'effet de choc sur une station d'épuration ou sur le milieu naturel

- Déstockage maîtrisé – étalement des rejets dans le but d'éviter les à-coups de pollution lors des vidanges de bains de saumure
- Recyclage direct ou après régénération pour l'élimination des MES et graisses
- Valorisation des rejets salins

Quelques exemples d'application des technologies propres appliquées aux effluents salins et de leur intérêt économique se trouvent dans l'industrie de la pêche. Afin de diminuer les rejets lors du déchargement des poissons, Roeckel *et al.* proposent ainsi d'introduire une étape de recirculation de l'eau après dégrillage, floculation, centrifugation des effluents et recyclage des MES ainsi séparées dans l'eau utilisée pour le déchargement. Cette étape permettrait de réduire de 91,6% la DCO de l'effluent produit et d'améliorer de 7% productivité de l'industrie [185]. Dans l'industrie de transformation des produits de la pêche, une étude de cas a montré qu'il était possible de réduire la consommation d'eau de 31,8% par la ségrégation des effluents en fonction de leur débit et de leur charge organique. Ce procédé permettrait de recycler la matière organique des effluents les plus chargés, ce qui accroîtrait de 5,5 t j^{-1} la quantité de produit commercialisable dans le cas étudié [183].

2.3.2 Traitements physico-chimiques des effluents hypersalins

Les effluents hypersalins sont souvent récalcitrants au traitement biologique. C'est d'autant plus vrai lorsque les effluents contiennent par ailleurs des composés toxiques. C'est pourquoi les procédés physico-chimiques sont souvent employés pour le traitement de la matière organique mais aussi des sels contenus dans ces effluents. Les principales techniques appliquées aux effluents salins sont l'évaporation, la coagulation-floculation, les techniques membranaires et les procédés d'oxydation avancée. Ces techniques demandent souvent un investissement et des coûts opérationnels plus élevés que pour un traitement biologique et sont donc adaptées aux volumes importants.

Les techniques thermiques

L'évaporation solaire est le procédé le plus commun de traitement des effluents salés dans les pays chauds en développement. Dans l'industrie en cuir, notamment, les effluents générés lors du rinçage des peaux sont souvent isolés des autres rejets et envoyés dans des bassins d'évaporation solaire en raison de leur teneur élevée en sel. Cependant, la réutilisation du sel obtenu ainsi est impossible à cause de son degré

d'impureté. Le taux d'évaporation peut être amélioré en utilisant les évaporateurs sous vide ou le séchage par air chaud.

La coagulation-floculation

La coagulation-floculation est fréquemment utilisée en traitement primaire dans les stations d'épuration, assurant notamment le traitement de la DCO colloïdale. Elle utilise généralement des polymères sous forme de produits commerciaux. Appliqué aux effluents salins, ce procédé peut également assurer l'élimination des sels (sulfates, notamment). Le Tableau 2.6 indique quelques essais de coagulation recensés dans la littérature.

Tableau 2.6 Essais de coagulation-floculation d'effluents salins.

Substrat	Sel ($g\ l^{-1}$)	Coagulant	pH	Temps de contact (min)	Dose ($g\ g^{-1}$ de comp. Élim.)	Composé éliminé	Rdt épur. (%)	Réf
Effluent pharmaceutique	29	$Ca(OH)_2$	8	30	4,8	DCO	46	[177]
Effluent pharmaceutique	29	$Ca(OH)_2$	8	30	6,7	SO_4^{2-}	32	[177]
Effluent de transformation du poisson	35	$FeCl_3$	4,3	-	0,4	Protéines	40-50	[145]

Il apparaît ainsi que les effluents pharmaceutiques peuvent être traités par coagulation pour épuration partielle de la matière organique et des sulfates [177]. Concernant les effluents de l'industrie de transformation des produits de la mer, la floculation au chlorure ferrique permet l'élimination de 40 à 50% des protéines [145]. Plus récemment, Ellouze *et al.* ont aussi étudié la coagulation des colloïdes des effluents de transformation industrielle de seiche au moyen de sulfate d'aluminium (165,5 mg l^{-1}) suivie d'une étape de floculation au moyen d'oxyde de magnésium (750 mg l^{-1}) et chlorure d'ammonium poly-diméthylé (35 mg l^{-1}). La coagulation-floculation de cet effluent a permis de réduire la turbidité de l'effluent de 7 unités de turbidité néphélométrique (UTN) et la DCO de 90% [64].

Les techniques membranaires

Les techniques membranaires assurent le transfert de molécules sélectionnées sous l'effet d'un gradient de concentration, d'un gradient de pression, ou d'un champ électrique. Ainsi, l'ultrafiltration peut assurer l'élimination des MES et de la DCO colloïdale des effluents salins. Cette utilisation a notamment été notée dans l'industrie de transformation du poisson où elle permet de concentrer et de recycler dans le process industriel les protéines de poissons contenues dans ces effluents, permettant ainsi d'accroître la productivité de l'usine [2]. La combinaison d'une étape de centrifugation et d'ultrafiltration des effluents salins générés par la production d'huile d'olive permet également l'élimination de 90% de la DCO et la séparation des graisses [215].

Par ailleurs, l'osmose inverse permet l'élimination des sels et est le procédé le plus couramment employé en désalinisation. Toujours concernant le traitement des effluents de la production d'huile d'olive, l'osmose inverse permet ainsi d'assurer l'élimination de 99,4% des sels et 98,2% de la DCO, ainsi que l'élimination complète de la couleur et de la DBO_5, à une pression trans-membranaire de 55,2 bars et un flux de 52,5 l m^{-2} h^{-1} [211].

Les résines échangeuses d'ions

Les techniques d'échange ionique sont adaptées aux solutions ioniques « propres », donc peu envisagées pour le traitement des effluents, sauf en traitement tertiaire. En effet, la présence élevée de graisses et de MES peut induire un colmatage des résines. Elles permettent par contre l'élimination des sels.

L'ozonation

L'ozone (O_3), parfois combiné aux rayons ultraviolets (UV), permet d'abaisser les niveaux de DCO/DBO, et de détruire les composés organiques et inorganiques oxydables. Ces procédés permettent d'oxyder totalement les composés organiques (sous forme de dioxyde de carbone, CO_2 et d'eau, H_2O), même s'il n'est généralement pas nécessaire d'opérer à un tel niveau de traitement. L'ozone est particulièrement approprié pour le traitement des effluents contenant des composés récalcitrants, toxiques ou non biodégradables. En revanche, ils ne permettent pas l'élimination des sels. L'ozonation est donc particulièrement utilisée pour traiter les effluents hypersalins de l'industrie textile, assurant la décoloration et une réduction de la DCO [157].

2.3.3 Traitement biologique aérobie des effluents hypersalins

Généralités sur le traitement aérobie des effluents

Le traitement biologique aérobie des effluents consiste principalement en l'oxydation de la matière organique par des micro-organismes hétérotrophes aérobies qui l'utilisent pour synthétiser de la biomasse (boues activées) en présence d'oxygène (cf. Figure 2.6). La composition des cellules ainsi formées se traduit généralement par la formule $C_{60}H_{87}N_{12}O_{23}P$ ou $C_5H_7NO_2$, si le phosphore n'est pas pris en compte. Un autre phénomène appelé respiration endogène est caractérisé par une autoconsommation des micro-organismes. Les procédés à aération prolongée favorisent la respiration endogène pour réduire la quantité de boue (considérée comme un déchet secondaire) générée par le procédé de traitement.

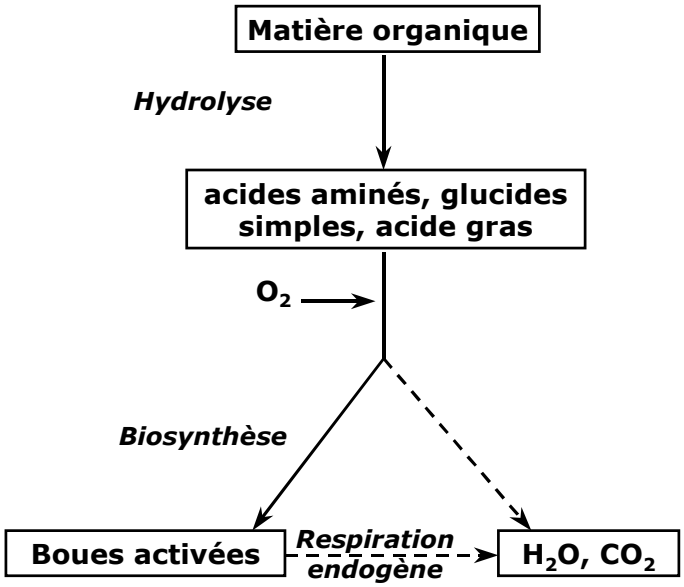

Figure 2.6 Schéma du principe de l'élimination de la pollution carbonée dans les conditions aérobies.

Effet du sel sur le traitement aérobie des effluents

Les teneurs élevées en sel compromettent le bon fonctionnement d'un système conventionnel aérobie de traitement des eaux usées [136]. Woolard et Irvine ont recensé en 1995 les principaux dysfonctionnements causés par le sel [236]: les micro-organismes conventionnels sont sensibles aux changements ioniques brusques et ne supportent pas des concentrations en sel supérieures à 50 g l^{-1}. Par ailleurs, les fortes teneurs en sel perturbent le métabolisme microbien, ce qui entraîne une baisse des taux d'épuration des matières organiques, la réaction de nitrification entre autres, étant particulièrement

inhibée. De plus, le sel a tendance à faire augmenter le taux de MES dans l'effluent, en raison de la lyse de nombreux organismes par le sel (protozoaires, entre autres) et de par une perturbation de la floculation. Par ailleurs, l'acclimatation des bactéries au sel se perd vite si la salinité vient à baisser. Enfin, la concentration en oxygène diminue lorsque la salinité augmente.

Pourtant, dès les années 1940, le suivi pendant 8 ans d'une station d'épuration à boues activées fonctionnant avec de l'eau de mer en Inde a montré que cette dernière épurait aussi bien les effluents domestiques qu'une station équivalente fonctionnant avec de l'eau douce [173].

Applications du traitement aérobie aux effluents salins

La dégradation de la matière organique par les micro-organismes halophiles en aérobiose a suscité un intérêt croissant depuis le milieu des années 1990. Le Tableau 2.7 recense les principaux réacteurs aérobie qui ont été utilisés pour le traitement des effluents salins.

Tableau 2.7 Traitement biologique aérobie d'effluents synthétiques et industriels salins.

(cma : charge massique appliquée ; cva : charge volumique appliquée ; ETPM : effluent de transformation de produits de la mer ; MVS : matières volatiles en suspension ; SB(B)R : sequencing batch (biofilm) reactor ; TSH : temps de séjour hydraulique ; V : volume)

Substrat	Inoculum halophile	Salinité ($g\ l^{-1}$)	Procédé	V (l)	DCO alim ($g\ l^{-1}$)	TSH (h)	cva ($kg\ DCO\ m^{-3}\ j^{-1}$)	MVS ($g\ l^{-1}$)	cma ($kg\ DCO\ kg^{-1}$ de MVS j^{-1})	Rdt DCO (%)	Ref
Synthétique (mélasse)	Non	20	Réacteur fed-batch	15	5	16	7,5	1,1	6,82	80	[111]
Synthétique (mélasse)	Non	50	Réacteur fed-batch	15	5	13	9,3	1	9,23	59	[111]
Synthétique (mélasse)	Oui	50	Biodisques en rotation	10	5	4	30	29	1,03	85	[58]
Synthétique (mélasse)	Oui	100	Biodisques en rotation	10	5	4	30	28	1,07	60	[58]
Synthétique (Phénol)	Oui	150	SBBR	1	0,29	48	0,15	3	0,05	99	[235]
Synthétique (Phénol)	Oui	150	SBR	1	0,25	24	0,25	1	0,25	99,5	[236]
Synthétique (glucose, acétate)	Non	60	SBR	5	1,2	6	4,8	-	-	32	[219]
Synthétique (≈ETPM)	Oui	32	Bioréacteur à membrane	8	5	36	3,4	11,2	0,30	85	[49]

Substrat	Inoculum halophile	Salinité ($g\ l^{-1}$)	Procédé	V (l)	DCO alim ($g\ l^{-1}$)	TSH (h)	cva (kg DCO $m^{-3}\ j^{-1}$)	MVS ($g\ l^{-1}$)	cma (kg DCO kg^{-1} de MVS j^{-1})	Rdt DCO (%)	Ref
Synthétique (≈ETPM)	Oui	32	Bioréacteur à membrane	3,6	1,2	13,7	2,1	11	0,19	91	[49]
Synthétique (≈ETPM)	Oui	10	SBR	10	0,55	20	0,7	4,1	0,17	87,9	[152]
Effluent de saumurage	Oui	30-60	Boues activées	Labo	4,6	35	3,2	4,9	0,64	96	[112]
Effluent de saumurage	Oui	150	Boues activées	5000	120	168	17	-	-	60-70	[120]
ETPM	Oui	74	Lit fixe	1,5	2,7	72	1	8	0,11	60	[76]
ETPM	Oui	20	Boue activée	5	2,7	72	0,9	2,8	0,32	88	[116]

Il ressort du Tableau 2.7 que la gamme de salinité des effluents traités par la voie aérobie s'étend de 10 à 150 g l^{-1}. Par ailleurs, la plupart de ces études sont basées sur des effluents synthétiques. Le traitement a généralement été possible sur ces effluents synthétiques à des charges volumique et massique appliquées plus élevées que celles généralement utilisées sur substrat industriel.

Au cours des dix dernières années, la contribution de Kargi et son équipe au traitement biologique aérobie est considérable, principalement sur substrats synthétiques (mélasse). En 1996, Kargi et Dinçer se sont d'abord intéressés à l'effet de la concentration en sel sur le traitement biologique d'un effluent salin synthétique dans les conditions aérobies en utilisant un réacteur biologique en mode fedbatch [109-111]. L'effluent synthétique, caractérisé par un ratio DCO/N/P de 100/10/1 et composé de mélasses diluées, urée, KH_2PO_4 et dont la concentration en sel a varié entre 1 et 5% de NaCl, a été épuré par des boues activées. Kargi et Dinçer ont ainsi observé que le rendement d'épuration de la DCO de l'effluent a chuté de 85 à 59% quand la salinité a augmenté de 0 à 5% [111]. Par la suite, Dinçer et Kargi ont testé des procédés d'épuration innovants en conditions salines, comme, par exemple, un procédé biologique aérobie constitué de disques en rotation, dont le nombre et la surface étaient modulables [58]. Ce réacteur a servi à épurer un effluent synthétique dans des conditions croissantes de salinité (0-10%) et a permis de dépasser 80% d'épuration de la DCO de l'effluent, tant que la concentration en sel restait inférieure à 50 g l^{-1}.

Il ressort aussi du Tableau 2.7 que l'utilisation d'un inoculum halophile est une pratique courante pour le traitement biologique aérobie des effluents, dans le but d'optimiser les performances. Ainsi, l'ajout d'une souche halotolérante du genre *Halobacter* dans un système de boues activées a permis à Kargi et Dinçer d'améliorer significativement les performances de boues activées [110]. Selon le même principe, *Halobacter halobium* a par la suite été ajouté à une culture de boues activées au sein d'un biofiltre aérobie dans lequel les cellules étaient immobilisées sur des particules de céramique [113]. Le procédé fonctionnait en continu à différentes concentrations en sel. L'ajout d'*Halobacter halobium* dans les boues activées a encore permis d'améliorer les performances du réacteur, notamment aux plus fortes concentrations en sel. L'enrichissement par le même *Halobacter halobium* de boues activées traitant un effluent industriel de saumurage a enfin permis de dépasser 95% d'élimination de la DCO [112]. Le même principe (enrichissement de boues activées par les bactéries halotolérantes *Staphylococcus* sp. et *Bacillus cereus*) appliqué à un effluent agro-industriel hypersalin généré par la production de prunes saumurées a là encore permis l'épuration de 90% de la DCO de cet effluent dans un réacteur pilote séquentiel [120]. Enfin, le traitement d'effluents industriels générés par la transformation de seiches, pieuvres et crevettes a

révélé les difficultés du traitement biologique aérobie des effluents industriels (non synthétiques). En utilisant un réacteur à boues activées classique [116] ou un lit fixe [76], les charges appliquées volumique (de l'ordre de 1 kg DCO m^{-3} j^{-1}) et massique (< 0.3 kg DCO kg^{-1} de MVS j^{-1}) sont bien inférieures à celles obtenues sur effluents synthétiques et ce malgré l'utilisation d'un inoculum adapté aux fortes salinités.

Parmi les procédés de traitement des effluents, le sequencing batch reactor (SBR) est connu de longue date pour être particulièrement robuste et supporter des conditions extrêmes [92]. Il n'est par conséquent pas surprenant que ce procédé ait été employé à plusieurs reprises pour le traitement des effluents hypersalins (cf. Tableau 2.7). Parmi les premiers, Woolard et Irvine, en 1994, ont inoculé un réacteur SBBR (sequencing batch biofilm reactor) avec des bactéries halophiles modérées, prélevées dans le Grand Lac Salé, afin de traiter un effluent synthétique contenant 150 g l^{-1} de sel. Les rendements épuratoires mesurés sur le phénol dépassaient en moyenne 99% [235]. Ils ont renouvelé l'expérience en 1995 avec un SBR à culture libre, atteignant cette fois des rendements moyens de 99,5% [236]. Plus récemment, Uygur et Kargi ont aussi eu recours au SBR pour dépolluer un effluent salin synthétique [219]. Ils ont observé une baisse du rendement épuratoire de la DCO de 90 à 32%, lorsque la salinité augmentait de 0 à 6%.

Il existe d'autres applications potentielles du traitement des effluents hypersalins comme, par exemple, le recyclage d'un milieu de culture de micro-algues (*Dunaliella salina*) produisant du beta carotène, après traitement biologique [192]. Dans cette expérience, cet effluent riche en matière organique (glycérol) contenait entre 17 et 25% de sel et l'épuration du glycérol n'était possible qu'en supplémentant les boues avec de l'azote, du phosphore, du potassium et du magnésium.

Conclusion

- La plupart des études sur le traitement biologique aérobie des effluents salins se basent sur des effluents synthétiques
- Le traitement biologique aérobie est plus efficace sur substrat synthétique que sur substrat industriel
- L'ajout de bactéries halotolérantes ou halophiles peut, dans certains cas, améliorer les performances du système
- Le SBR apparaît très populaire pour le traitement aérobie des effluents hypersalins
- Peu de recherches ont dépassé le stade laboratoire

2.3.4 Digestion anaérobie des effluents hypersalins

Généralités sur la digestion anaérobie

Parallèlement aux recherches sur le traitement biologique aérobie des effluents salins et hypersalins, les possibilités de digestion anaérobie de ces mêmes effluents ont également été explorées. Le traitement anaérobie des effluents permet la transformation de la pollution carbonée en méthane (CH_4) et dioxyde de carbone (CO_2). La digestion anaérobie se déroule selon trois étapes successives : hydrolyse des macromolécules, acidification de la matière organique en hydrogène gazeux (H_2), CO_2 et acides gras volatils (AGV) puis conversion de ces intermédiaires réactionnels en biogaz constitué principalement de CH_4 et CO_2 (cf. Figure 2.7). Ces procédés requièrent une association symbiotique de micro-organismes. Dans un premier temps, les micro-organismes acidogènes utilisent l'oxygène dissous et lié aux molécules de l'effluent pour produire des AGV. Ils créent ainsi les conditions idéales au développement des Archaea méthanogènes (conditions anaérobies avec des composés de faible poids moléculaire). Dans un second temps, les méthanogènes consomment ces AGV, maintenant des conditions de pH favorables aux acidogènes.

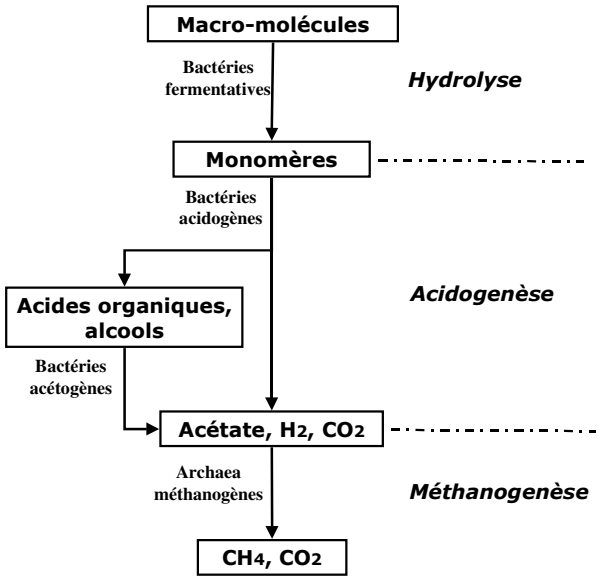

Figure 2.7 Principales voies métaboliques et natures des populations microbiennes de la digestion anaérobie.

Effet du sel sur le traitement anaérobie des effluents

Les teneurs élevées en sel sont cependant connues pour avoir un effet inhibiteur sur la digestion anaérobie, principalement dû aux cations. Il a ainsi été déjà rapporté qu'une concentration en sodium excédant 10 g l^{-1} bloque la méthanogenèse [84; 121; 182]. Cependant, Omil *et al.* n'ont montré aucun effet toxique clair d'un effluent d'industrie de transformation du poisson (d'une salinité proche à l'eau de mer) sur une installation pilote anaérobie proche du contact anaérobie [163]. Ils ont ainsi montré que l'adaptation d'une biomasse méthanogène active à la salinité de l'effluent était possible et ont conclu que l'efficacité d'un tel procédé dépendait d'une stratégie adéquate d'adaptation de la biomasse aux fortes salinités. Feijoo *et al.* ont indiqué par ailleurs que la toxicité du sodium sur les boues dépendait de plusieurs facteurs tels que les effets d'antagonisme dus à d'autres ions à des concentrations adéquates, à la nature et à l'adaptation progressive des boues au sel, ainsi qu'au substrat méthanogène utilisé [68].

Applications du traitement anaérobie aux effluents salins

Les capacités épuratoires des halophiles anaérobies sont connues de longue date. Ainsi, la décomposition en anaérobiose de la cellulose dans des conditions de salinité extrême (25% de NaCl) a été étudiée dans le lac Sivash et les lagunes hypersalines d'Arabat [200]. Dans le lac Sivash, la décomposition de la cellulose s'est faite à une vitesse maximale à une concentration en NaCl de 5%, tandis que la flore microbienne de la lagune a décomposé la cellulose le plus rapidement à une concentration en sel de 15%.

Pourtant, les applications de la digestion anaérobie aux effluents salés en bioréacteurs sont relativement rares ; elles sont compilées dans le Tableau 2.8.

Tableau 2.8 Traitement biologique anaérobie d'effluents synthétiques et industriels salins.
(AF : anaerobic filter ; AFFR : anaerobic fixed film reactor ; CSTR : completely stirred tank reactor ; DFAFBR : down-flow anaerobic fixed bed reactor ; ETPM : effluent de transformation de produits de la mer ; UAF : upflow anaerobic filter ; UASB : upflow anaerobic sludge blanket ; USBF : upflow sludge bed-filter)

Substrat	Inoculum halophile	Salinité ($g\ l^{-1}$)	Procédé	V (l)	DCO alim ($g\ l^{-1}$)	TSH (h)	cva (kg DCO $m^{-3}\ j^{-1}$)	MVS ($g\ l^{-1}$)	cma (kg DCO kg^{-1} de MVS j^{-1}	Rdt DCO (%)	Réf
Effluent d'inuline	Non	10	UASB	1 100 000	7,9	6-8	23-32	18-31	0,7-1,8	65-80	[87]
Fumier	Non	15	DFAFBR	1,4	1,9	96	0,5	-	-	90	[188]
Effluent pharmaceutique	Non	20	AFFR	9,4	25	60	10	35,7	0,28	67	[72]
Effluent de pisciculture	Non	35	CSTR	15	70,1	660	2,5	25	0,10	55	[74]
Effluent de pêcherie	Oui	40	CSTR	1,5	6	72	2	-	-	50	[12]
ETPM	Non	15	UAF	1,1	34	288	2,8	57,1	0,05	83	[86]
ETPM	Non	7,7-26,3	UASB	1	1,7	3	13,6	27,2	0,50	77	[23]
ETPM	Non	13,6-33,7	Contact anaérobie	15 000	10-60	180-240	1-8	-	-	70-90	[163]
ETPM	Oui	-	USBF hybride	2,3	1-1,5	18	1,5-2	7,4	0,2-0,3	70-90	[153]
ETPM	Oui	30	Filtre anaérobie	2,5	5,5	9,2	14,3	-	-	70	[224]

Il ressort du Tableau 2.8 que le traitement biologique anaérobie a été testé sur un certain nombre d'effluents industriels (principalement en relation avec l'industrie de transformation du poisson), dans une gamme de salinité de 10 à 40 g l^{-1}, donc moins étendue que pour le traitement aérobie.

Les possibilités de dégradation anaérobie des effluents de transformation des produits de la mer ont été très étudiées au cours des dix dernières années : une équipe chilienne s'est notamment intéressée à la digestion anaérobie des effluents de pêcherie, principalement ceux générés lors du déchargement des poissons. Après recyclage et traitement primaire pour éliminer les protéines et les graisses, Aspé *et al.* ont montré que l'effluent, contenant 4-6 kg DCO m^{-3}, 1.85 kg SO_4^{2-} m^{-3} et 16.2 kg Cl^- m^{-3}, peut être traité par la voie anaérobie, en utilisant un inoculum marin, et générer une activité méthanogène spécifique à 37°C de 0.065 kg de DCO-CH_4 kg^{-1} de MVS j^{-1} [12]. Aspé *et al.* ont également modélisé les phénomènes d'inhibition par l'ammoniac de la digestion anaérobie sur ce type d'effluent, concluant que la méthanogenèse était l'étape la plus inhibée [11]. Par la suite, le traitement d'effluents générés par la transformation industrielle du poisson a été étudié par différents procédés tels que le filtre anaérobie [86; 153; 224], l'UASB [23] et le contact anaérobie [163]. Les rendements épuratoires observés sur ce genre de substrat ont généralement permis d'éliminer entre 70 et 90% de la DCO, à une charge volumique appliqué (cva) comprise entre 1 et 15 kg DCO m^{-3} j^{-1} et une charge massique appliquée (cma) faible inférieure à 0,5 kg DCO kg^{-1} de MVS j^{-1} (cf. Tableau 2.8).

Parmi les autres types d'effluents industriels étudiés, Rovirosa *et al*. se sont intéressés à la digestion anaérobie d'un effluent de porcherie dilué dans une eau saline synthétique (15 g l^{-1} de sel). Le rendement d'épuration de la DCO excédait 90% pour un temps de séjour hydraulique (TSH) de 96 h et 68% pour un TSH de 12 h [188]. Gangagni Rao *et al.* ont, quant à eux, traité un effluent pharmaceutique salé à l'aide d'un lit fixe anaérobie. Dans les conditions stables de fonctionnement et à une cva de 10 kg m^{-3} j^{-1} de DCO, les rendements épuratoires se sont stabilisés entre 60 et 70% de la DCO et entre 80 et 90% de la DBO_5 [72].

Les essais de traitement anaérobie des effluents de l'industrie textile se sont généralement limités à une décoloration de l'effluent accompagnée d'une réduction très modeste de sa charge organique. Ceci est lié à la nature aromatique complexe des encres qui leur confère un caractère toxique difficilement biodégradable. Isik et Sponza ont traité un effluent synthétique simulant un effluent textile (i.e. contenant des sels, des encres, etc.) en UASB. La décoloration de l'effluent a été assurée à plus de 90% même quand le TSH a été réduit de 100 à 6h. Par contre, les rendements épuratoires ont chuté

de 80 à 29% de la DCO dans le même temps [103]. L'influence de la concentration en sel a aussi été testée en UASB sur le même type d'effluent conduisant à des résultats similaires, à savoir bonne décoloration de l'effluent (proche de 100%) mais forte inhibition de la méthanogenèse lorsque la salinité augmente. Ainsi en passant de 0 à 128 g l^{-1} de sel et à TSH et cva constants, le rendement d'épuration de la DCO a chuté de 80 à 18% et la chute la plus forte s'observe pour les salinités supérieures à 30 g l^{-1}. Par ailleurs, la déstabilisation des méthanogènes est aussi caractérisée par une augmentation de la concentration en AGV et une diminution du pH [102]. En se basant sur la production de méthane maximale, Manu et Chaudhari ont fixé la limite maximale de la concentration en encres tolérée avant inhibition de la méthanogenèse à 400 mg l^{-1} [144]. Ainsi, certaines bactéries sont capables de cliver les composés aromatiques des encres en anaérobiose, mais le produit de clivage n'est pas biodégradable dans les conditions anaérobies, d'où une décoloration mais peu de diminution de la DCO.

Enfin, une équipe danoise a travaillé au sein d'une usine produisant fructose et inuline, et dont les effluents de production sont salés en raison de la présence d'une unité de déminéralisation [87]. Le traitement avait lieu au moyen d'un réacteur de 1100 m^3 constitué de deux réacteurs UASB superposés, l'un fonctionnant à forte charge et l'autre à faible charge. En entrée du réacteur, la DCO avoisinait 8 g l^{-1} et la concentration en Cl^- 4 g l^{-1}. A une charge massique appliquée (cma) de 1 kg DCO kg^{-1} MVS j^{-1} et une charge volumique appliquée (cva) de 30 kg DCO m^{-3} j^{-1}, le fonctionnement du réacteur s'est révélé stable sur une période de trois mois. Les rendements d'épuration étaient généralement supérieurs à 70% pour la DCO et 90% pour les AGV. Il s'agit là d'une des rares applications à l'échelle industrielle de digestion anaérobie d'un effluent salé.

Conclusion

- La dégradation anaérobie des effluents industriels a fait l'objet d'études, notamment sur effluents de transformation industrielle du poisson et sur effluents de l'industrie textile
- La faible charge massique applicable sur effluents hypersalins a souvent été compensée par l'utilisation de réacteurs permettant l'accumulation d'une biomasse importante
- De même que pour le traitement aérobie, peu de recherches ont dépassé le stade laboratoire

2.3.5 Traitement combiné anaérobie / aérobie des effluents hypersalins

Application du traitement combiné anaérobie / aérobie pour la dépollution carbonée des effluents hypersalins

Devant les performances modestes de l'épuration aérobie et anaérobie, des effluents salins, le couplage de ces deux modes de traitement a été envisagé par la suite, dans le but d'améliorer les performances épuratoires des procédés de traitement des effluents fortement concentrés en sel. Panswad et Anan ont notamment tenté d'apporter une finition aérobie après le traitement anaérobie en appliquant un processus anaérobie/anoxique/aérobie sur un effluent synthétique contenant 3% de sel, à condition que l'inoculum soit acclimaté aux conditions élevées de salinité [168]. Les rendements épuratoires de la DCO proches de 71% obtenus sont cependant décevants.

Le traitement combiné anaérobie / aérobie peut par contre s'avérer très utile pour le traitement des effluents hypersalins de l'industrie textile. En effet, les produits du clivage anaérobie des encres sont parfois biodégradables en conditions aérobies ce qui ouvre la porte à des procédés anaérobie/aérobie afin de cliver/biodégrader les encres de ces effluents. Ainsi, l'expérience la plus concluante à ce jour a eu recours à un procédé continu anaérobie/aérobie, qui a conduit à 84% de réduction de la DCO, 86% de la couleur et 52,2% des amines aromatiques à un TSH de 2,9 j et une cva globale de 1,17 kg DCO m^{-3} j^{-1} [208]. Les amines aromatiques produites en anaérobiose étaient en effet dégradées par la suite en aérobiose. Des tests de toxicité sur des daphnies ont confirmé la diminution de la toxicité de l'effluent ainsi traité [209].

Application du traitement combiné aérobie / anaérobie pour la dépollution azotée des effluents hypersalins

L'association de procédés aérobie et anaérobie permet également le traitement de l'azote dans les effluents salins. Le traitement biologique de l'azote s'effectue classiquement par nitrification / dénitrification. La nitrification consiste en l'oxydation enzymatique de l'ammonium (NH_4^+) en nitrite (NO_2^-) puis nitrate (NO_3^-) sous l'influence de bactéries nitrifiantes autotrophes aérobies, comme le montre l'équation *(i)*.

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2\ H^+ + H_2O \qquad (i)$$

La dénitrification est la réduction enzymatique du NO_3^- en azote gazeux (N_2) sous l'influence de bactéries hétérotrophes, comme le montre l'équation *(ii)*. C'est un procédé anoxique (qui a lieu en l'absence d'oxygène mais en présence de forme oxydée de l'azote).

$$NO_3^- + 6H^+ + 5e^- \rightarrow 0{,}5\,N_2 + 3\,H_2O \qquad (ii)$$

- Dénitrification des effluents salins

La capacité des halophiles à utiliser des oxyanions comme accepteurs finaux d'électrons est connue de longue date chez les halophiles. Dès le milieu des années 1980, des bactéries halophiles extrêmes ont été isolées depuis des sites variés et cultivées en anaérobiose et en présence de nitrate [95]. La croissance de nombre de ces isolats s'est accompagnée de production de nitrite, oxyde nitreux et azote gazeux. Ces résultats ont confirmé l'existence de bactéries dénitrifiantes halophiles extrêmes et leur présence au sein d'une grande variété de milieux hypersalins. Mancinelli et Hochstein ont notamment montré la capacité de *Halobacterium vallismortis*, *Halobacterium mediterranei* et *Halobacterium marismortui* à croître en anaérobiose en présence de nitrate seulement et à le dénitrifier [143]. La même propriété a été observée depuis chez de nombreuses autres bactéries aérobies halophiles modérées [223]. Par la suite, Shieh et Liu ont montré l'existence de bactéries halophiles capables à la fois de dénitrification et de fermentation [197]. D'après leurs travaux, une souche non identifiée gram- et anaérobie facultative s'est montrée capable de croître en anaérobiose en dénitrifiant les nitrates, nitrites ou oxydes nitreux, ou, alternativement, en effectuant la fermentation du glucose ou du mannose. Enfin, plus récemment, la cinétique de réduction du nitrate par *Halomonas campisalis* à pH 9 et 12,5% de NaCl a été suivie, avec des taux de dénitrification supérieurs dans le cas de cultures enrichies en acétate [171]. Cependant l'application de ces propriétés au traitement de l'azote se heurte à des difficultés : Dinçer et Kargi ont ainsi montré que les concentrations en sel supérieures à 2% sont responsables de diminutions significatives des performances de nitrification et de dénitrification des réacteurs biologiques et que la réaction de dénitrification est plus affectée par le sel que la réaction de nitrification [57]. Dinçer et Kargi entrent sur ce point en contradiction avec Panswad et Anan qui ont conclu à de meilleures capacités de tolérance et d'adaptation aux fortes salinités des bactéries dénitrifiantes par rapport aux bactéries nitrifiantes, elles-mêmes s'adaptant mieux que les bactéries hétérotrophes responsables de la dépollution carbonée [168]. Ces derniers ont ainsi pu acclimater une communauté dénitrifiante en conditions salines et maintenir un taux de réduction du nitrate de 2 mg $N\text{-}NO_3^-$ g^{-1} de MES h^{-1} à 30 g NaCl l^{-1}.

- Nitrification des effluents salins

L'inhibition des bactéries nitrifiantes par le sel est connue de longue date [136]. Mais ce n'est que récemment que ces résultats ont été précisés par de multiples études. Chen et Wong ont montré que l'adaptation progressive au sel d'une culture continue de boues activées nitrifiantes donnait de meilleurs résultats qu'une autre culture fonctionnant à une concentration en sel fixe [41]. Cependant, au-delà de 18 g Cl l^{-1}, le processus de nitrification devenait instable. En dessous de 10 g Cl l^{-1}, les bactéries dominantes oxydant l'ammoniac étaient *Nitrosomonas europaea*, *Nitrosomonas eutropha*, *Nitrosomonas halophila*, and *Nitrosococcus mobilis*, tandis que les bactéries dominantes oxydant le nitrite appartenaient au genre *Nitrobacter*. A 18 g Cl l^{-1}, seul *Nitrosococcus mobilis* survivait tandis que les bactéries oxydant le nitrite disparaissaient. Chen et Wong ont conclu à une concentration critique de 18 g Cl l^{-1} permettant le maintien d'une population nitrifiante. Ils rejoignent ainsi les conclusions de Dahl *et al.* qui ont montré que la nitrification pouvait avoir lieu dans des conditions opérationnelles jusqu'à 20 g Cl l^{-1}, avec un taux de nitrification maximal de 2 mg N g MVS^{-1} h^{-1} [47]. Dahl *et al.* ont par ailleurs montré qu'une augmentation brutale de la concentration en chlorure avait une action inhibitrice sur les bactéries nitrifiantes. Par la suite, Vredengbregt *et al.* ont montré que la nitrification était possible jusqu'à 34 g Cl l^{-1} au moins, dans un lit fluidisé, à condition que la charge ammoniacale soit maintenue à 15 mg NH_3 l^{-1} h^{-1} [227]. En 1999, Panswad et Anan n'ont observé qu'une diminution modérée de l'activité nitrifiante de 4 à 3 mg N-NH_3 oxydé g MES^{-1} h^{-1} suite à une augmentation de la salinité de 5 à 30 g l^{-1}, à condition d'utiliser des boues acclimatées au sel [168]. Par ailleurs les capacités de récupération des bactéries nitrifiantes suite à un choc de 70 g l^{-1} de sel se sont avérées excellentes. Enfin, Campos *et al.* ont mis en évidence un phénomène d'inhibition combinée de la nitrification par le sel et l'ammoniac : l'accumulation d'ammoniac a commencé à une charge ammoniacale de 3 g N-NH_3 l^{-1} j^{-1} et à une concentration en sel de 525 mM (13,7 g NaCl l^{-1}, 19,9 g $NaNO_3$ l^{-1} et 8,3 g Na_2SO_4 l^{-1}) [34].

Application du traitement combiné aérobie / anaérobie pour la dépollution phosphorée des effluents hypersalins

L'association de procédés aérobie et anaérobie permet également le traitement du phosphore dans les effluents salins. La déphosphatation biologique consiste en l'accumulation du phosphore de l'eau brute dans la biomasse cellulaire sous l'influence de micro-organismes présentant la caractéristique de concentrer le phosphore sous forme de granules de polyphosphates lorsqu'elles sont soumises à des alternances de conditions anaérobie et aérobie.

Uygur et Kargi ont ainsi eu recours à un réacteur SBR alternant des phases anaérobies, oxiques, anoxiques et oxiques à différentes concentrations en sel (0-6% de NaCl) dans le but de déterminer les effets de l'inhibition par le sel sur l'épuration de l'azote et du phosphore dans un effluent synthétique [219]. Ils ont alors observé que les performances d'épuration de la DCO, du $N\text{-}NH_4^+$ et du $P\text{-}PO_4^{3-}$ diminuaient lorsque la concentration en sel augmentait. Le rendement épuratoire de la DCO a ainsi diminué de 96 à 32% quand la salinité a augmenté de 0 à 6%. Dans le même temps, le rendement épuratoire du $N\text{-}NH_4^+$ a diminué de 96 à 39% et le rendement épuratoire du $P\text{-}PO_4^{3-}$ de 84 à 22%. Une autre conséquence a été l'augmentation de l'indice de boue (sludge volume index, SVI). L'ajout d'*Halobacter* a alors permis d'améliorer les performances du réacteur de façon considérable, particulièrement aux salinités supérieures à 2% [114]. Les rendements épuratoires sur la DCO, le $N\text{-}NH_4$ et le $P\text{-}PO_4^{3-}$ ont ainsi atteint 73%, 51% et 31% respectivement à une salinité de 5%, contre 47%, 36% et 21% sans l'ajout d'*Halobacter*.

2.3.6 Impact du sel sur la turbidité des effluents et la sédimentation des boues

Les problèmes de décantation et donc de forte turbidité dans les milieux hypersalins ont fréquemment été observés et de multiples raisons ont été données à ce phénomène. Tout d'abord la densité de l'eau salée est supérieure à l'eau douce et offre donc une résistance à la décantation. L'hypersalinité diminue également la quantité de bactéries filamenteuses qui participent à l'intégrité mécanique et à la structure des flocs [217]. Enfin le manque de protozoaires pourrait aussi influer sur la turbidité de l'effluent car ils se nourrissent des micro-organismes, limitant ainsi leur abondance [236]. Or il a été montré que les protozoaires supportent mal les chocs de salinité et ne survivent normalement pas plus de 24 h à un choc de NaCl supérieures à 40 g l^{-1} [191]. Cet état de fait peut expliquer la rareté des protozoaires dans les boues activées hypersalines. Pourtant, les observations plus anciennes de Pillai et Rajagopalan ont montré que, dans des conditions stables, l'eau de mer n'est pas un obstacle aux développement de protozoaires ciliés et que les performances d'une station d'épuration fonctionnant avec de l'eau de mer étaient dépendante du nombre de ces protozoaires [173]. Holubar *et al.* ont démontré cette influence des protozoaires sur la turbidité des effluents salins [97]. Ils ont adapté des protozoaires à un effluent domestique salé contaminé par des hydrocarbures pétroliers. En culture continue, inoculée par une culture mixte de protozoaires provenant de boues activées, une seule espèce de protozoaire ciliée, *Uronema nigricans*, a pu se développer. La turbidité de l'effluent s'est révélée être

inversement dépendante de la quantité d'*Uronema nigricans*. De plus, le rendement épuratoire de la DCO a atteint 45,8%, contre 35,4% dans le cas de boues activées sans protozoaires.

Malgré les effets négatifs du sel sur la turbidité des effluents et la sédimentation, il semble qu'il ne soit pas un obstacle à la croissance de la biomasse microbienne. Ainsi, Panswad et Anan n'ont observé aucun effet d'une forte salinité (30 g NaCl l^{-1}) sur la concentration en MES dans un bioréacteur, à condition que les boues soient préalablement acclimatées [168]. Kubo *et al.* ont également obtenu une croissance microbienne normale à une salinité de 15% [120]. Par ailleurs, Panswad et Anan n'ont pas observé d'effets des fortes salinités sur la décantabilité des boues [168]. Plus récemment, les études de Campos *et al.* vont également dans le même sens et ont montré que les fortes salinités n'ont pas d'effet à long terme sur les propriétés physiques des boues [34]. Ils ont ainsi pu maintenir une concentration de 20 g MVS l^{-1} dans un réacteur à boues activées, grâce à un indice de boue (IB) de 11 ml g^{-1} de MVS. Et si Uygur et Kargi ont trouvé que l'IB augmentait avec la salinité, les valeurs d'IB (97 ml g^{-1}) qu'ils ont obtenues à 6% de sel dans un SBR traduisent la bonne décantabilité des boues, même aux fortes salinités [219]. Enfin, Raj et Anjaneyulu ont eux aussi montré que forte salinité et bonne décantabilité des boues étaient compatibles [177]. Un effluent pharmaceutique contenant 16 g l^{-1} de sel traité par un procédé aérobie à boues activées n'a pas empêché la croissance de la biomasse (mesurée en terme de MVS), d'autant plus rapide que la charge appliquée et le TSH étaient élevés. Par ailleurs, les valeurs d'IB n'excédaient 100 que dans le cas où la cva excédait 1 kg DCO m^{-3} j^{-1}.

Face aux problèmes de sédimentation des effluents salins, l'utilisation de bioréacteurs à membrane pourrait être envisagée. Quelques études font état de telles applications pour le traitement des eaux vannes des bateaux de croisière [118] et aussi pour empêcher le lessivage d'une culture pure d'une bactérie sulfato-réductrice halotolérante *Desulfobacter halotolerans* [220]. Dan *et al.* ont comparé l'efficacité d'un bioréacteur à membrane contenant des levures (yeast membrane bioreactor, YMBR) à un autre contenant des bactéries (bacterial membrane bioreactor, BMBR), pour le traitement d'un effluent contenant 5 g DCO l^{-1} et 32 g NaCl l^{-1} [49]. En augmentant progressivement la charge appliquée de 3,4 à 16,3 kg DCO m^{-3} j^{-1}, les rendements d'épuration de la DCO par le YMBR ont chuté de 85 à 60%. En augmentant progressivement la charge appliquée de 2,1 à 7,9 kg DCO m^{-3} j^{-1}, les rendements d'épuration de la DCO par le BMBR ont chuté de 91 à 76%. Le taux de dégradation maximal de la DCO a atteint 0,93 g DCO g MES^{-1} j^{-1} pour le YMBR avec une cma de 1,5 g DCO g MES^{-1} j^{-1} et 0,32 g DCO g MES^{-1} j^{-1} pour le BMBR avec une cma de 0,4 g DCO g MES^{-1} j^{-1}. Ils en ont conclu que le BMBR était recommandé pour les faibles cma, tandis que le YMBR devient plus avantageux quand la

cma augmente. Par ailleurs, Dan *et al.* ont remarqué que les membranes se colmatent moins dans le cas du YMBR.

2.3.7 Biodégradation des hydrocarbures par les bactéries halophiles

Devant la menace des marées noires, l'intérêt de trouver des bactéries éliminant les hydrocarbures en conditions salines remonte aux années 1970. De nombreuses études concernent donc la dégradation de ces composés organique aliphatiques ou aromatiques par les bactéries halophiles. Or ces recherches peuvent également déboucher sur des applications au traitement des effluents hypersalins, car de nombreux secteurs industriels génèrent des effluents salins riches en composés organiques aliphatiques de longue chaîne (résidus pétroliers) mais aussi aromatiques (industries chimique, pharmaceutique, textile). Cette partie propose donc une synthèse des connaissances de la biodégradation des hydrocarbures en conditions salines.

Application des bactéries halophiles à la biodégradation des hydrocarbures aliphatiques

Les premiers à avoir essayé de traiter les hydrocarbures en utilisant des bactéries halophiles non identifiées et issues du Grand Lac Salé (Utah, USA) sont Ward et Brock [230]. Ils ont montré que le taux de dégradation de l'hexadécane diminuait quand la salinité augmentait. Au-delà de 20% de sel, l'hexadécane n'était plus du tout transformé, d'où leur conclusion que les salinités extrêmes constituaient un obstacle à la biotransformation des hydrocarbures. Par la suite, Bertrand *et al.* ont isolé une Archaea halophile extrême capable de dégrader un certain nombre d'hydrocarbures (tétradécane, hexadécane, eicosane, heneicosane et pristane) à 22% de sel mais incapable de croître à moins de 10%, ce qui met en évidence la capacité de certains consortia à dégrader les hydrocarbures uniquement dans une gamme limitée de salinité [20]. Kulichevskaya *et al.* ont aussi isolé une Archaea halophile (*Halobacterium* sp.) capable de dégrader des alcanes à longue chaîne (C10-C30) en milieu hypersalin (15-30% de NaCl) [122]. Les Bacteria halophiles s'avèrent aussi aptes à dégrader les hydrocarbures à très haute salinité. Par exemple, *Marinobacter hydrocarbonoclasticus* dégrade les hydrocarbures à des salinités de l'ordre de 20% [73]. Il existe enfin d'importantes communautés bactériennes halophiles modérées dans l'antarctique qui s'attaquent efficacement aux hydrocarbures [148]. Un actinomycète, appartenant au genre *Rhodococcus*, isolé du port de Mumbai (Inde), pollué de façon chronique par les hydrocarbures, s'est avéré capable

de dégrader la fraction aliphatique et aromatique de trois résidus pétroliers. Il s'avère ainsi que les contaminations chroniques par les hydrocarbures favorisent l'émergence d'une microflore capable de dégrader ces hydrocarbures [196]. Plus récemment, un consortium bactérien isolé d'une mangrove s'est avéré apte à traiter divers hydrocarbures à une salinité comprise entre 0 et 18%. L'efficacité du traitement était meilleure dans le cas où les cellules étaient immobilisées sur des fibres de polypropylène que quand elles étaient libres [56].

Application des bactéries halophiles à la biodégradation des hydrocarbures aromatiques

Les composés aromatiques se retrouvent dans nombres d'effluents salins, allant de l'industrie agro-alimentaire (effluents oléicoles) à l'industrie pétrolière, en passant par les industries chimique, pharmaceutique et textile. C'est la raison pour laquelle les essais de dégradation de ces composés en conditions salines abondent dans la littérature, en commençant par le phénol (représentant des monomères aromatiques non acides) : Woolard et Irvine, en 1994, ont inoculé un réacteur SBR avec des bactéries halophiles modérées, prélevées dans le Grand Lac Salé, afin de traiter un effluent synthétique contenant du phénol et 150 g l^{-1} de sel. Les rendements épuratoires dépassaient en moyenne 99% [235]. Ils ont renouvelé l'expérience en 1995 avec un SBR à culture libre, atteignant cette fois des rendements moyens de 99,5% [236]. Plus tard, la biodégradation du phénol a été étudiée avec plus de détails en halophilie en utilisant un micro-organisme identifié comme étant *Pseudomonas halodurans (*basonyme *Halomonas halodurans)*, isolé du Grand Lac Salé, qui croît entre 1 et 14% de sel et dont l'optimum de croissance se situe à 5% de sel [93]. En batch, cette espèce est capable de dégrader, comme seule source de carbone et d'énergie, 0,1 g l^{-1} de phénol. Dans des conditions de croissance optimale (entre 30 et 50 g l^{-1} de NaCl), la dégradation de 0,1 g l^{-1} de phénol est complète au bout de 13 h pour la seule souche étudiée jusqu'à présent. Pour des concentrations salines plus importantes, la minéralisation complète du phénol est plus lente, allant jusqu'à 100 h pour 140 g l^{-1} de NaCl [93]. La biodégradation complète du phénol a également été observée à 10% de sel et pH neutre en utilisant des cultures mixtes halophiles provenant de divers lacs salés américains : Peyton *et al.* ont observé une diminution du taux spécifique de croissance des micro-organismes lorsque la concentration initiale en phénol augmentait de 50 à 320 mg l^{-1}, ce qui suggère un phénomène d'inhibition par le substrat [172]. Récemment, une équipe s'est intéressée à la biodégradation aérobie du benzène en conditions hypersalines, en utilisant une culture mixte issue de sols pollués dans les Great Salt Plains, Oklahoma [160; 161]. Cette culture s'est avérée capable de dégrader complètement le benzène, toluène,

ethylbenzène et xylène en une à deux semaines. L'analyse de la communauté bactérienne a montré que le genre dominant de la culture était *Marinobacter*.

L'application des halophiles à la biodégradation des composés aromatiques ne se limite pas au phénol mais peut s'étendre aux hydrocarbures aromatiques polycycliques. Ainsi, *Halomonas halodurans* dégrade non seulement le phénol [93], mais est par ailleurs capable de cliver les composés aromatiques complexes, tels que le benzoate [53; 187]. Parmi les Archaea, la souche EH4 parvient à dégrader l'acénaphtène, le phénantrène, l'anthracène ainsi que le 9-méthylanthracène [20]. Dans le domaine des Bacteria, des isolats appartenant aux genres *Rhodococcus*, *Arthrobacter*, *Bacillus* et *Pseudomonas* se sont avérés capables de croître sur naphtalène comme seule source de carbone entre 6 et 9% de sel [174]. Des bactéries isolées de l'Antarctique sont également capables de dégrader le phénantrène [148]. Enfin, parmi les Eucaryotes, des levures osmotolérantes (*Rhodotorula mucilaginosa* et *Pichia guilliermondii*) isolées de bassins d'évaporation d'effluents pharmaceutiques en Israël croissent à 15% de sel et même au-delà. Ces deux souches tolèrent par ailleurs des valeurs extrêmes de pH, comprises entre 2 et 10. *Rhodotorula* dégrade l'anthracène, le phénantrène et l'alcool de benzyle. L'activité respiratoire de *Pichia* est quant à elle stimulée par la présence d'acide benzoïque, d'anthracène, de bisphénol-A, d'acide brobenzoïque et de 2-fluorophénol [124].

La capacité des halophiles à dégrader des composés aromatiques halogénés fréquents dans les pesticides est aussi connue. La déchloration des chlorophénols et chlorophénoxyphénols a ainsi été observée en anaérobiose dans les eaux souterraines hypersalines jouxtant un lac alcalin aux USA [26]. Une souche haloalcalophile appartenant aux Halomonadaceae et isolée de l'Alkali Lake (Oregon, USA), contaminé par le 2, 4 dichlorophénoxyacétate (2,4-D) est par ailleurs capable de dégrader jusqu'à 200 mg l^{-1} de 2,4-D en 3 jours à pH 9,5 et 6% de NaCl [142]. Les Archaea halophiles *Haloarcula*, *Halobacterium* et *Haloferax* dégradent également les hydrocarbures halogénés tels les trichlorophénols et les insecticides lindane et DDT. La réduction du nitrobenzène, nitrophénol, nitroaniline, 2,4-dinitrophénol et 2,4-dinitroaniline en amines correspondantes par les Eubacteria modérément halophiles et anaérobies exclusives *Halanaerobium praevalens* et *Sporohalobacter marismortui* a également été observée [165].

Enfin, certains composés récalcitrants comme les pesticides organophosphorés (diisopropyl fluorophosphate, nitrophénylméthyl-phosphinate et nitrophénylethyl-phosphinate ont pu être dégradés par un micro-organisme isolé du Grand Lac salé (Utah, USA) appartenant au genre *Alteromonas* et croissant entre 2 et 24% de sel [51]. Ce dernier possède en effet une forte activité enzymatique, optimale à pH 8,5 et 50°C,

contre de nombreux composés organophosphorés fortement toxiques. Plus récemment, un autre halophile phylogénétiquement proche de *Pseudomonas beijerinckii* et *Chromohalobacter marismortui* ont utilisé du phosphonoacétate, 2-aminoéthyl-, 3-aminopropyl-, 4-aminobutyl-, méthyl- et ethyl-phosphonate comme source de phosphore pour leur croissance à 10% de NaCl [90].

2.3.8 Conclusions

La présente étude bibliographique a mis en évidence la complexité du traitement des effluents fortement concentrés en sel. Différentes techniques physico-chimiques ou biologiques peuvent être appliquées, chacune présentant des avantages et des inconvénients listés dans le Tableau 2.9.

Tableau 2.9 Avantages et inconvénients de différents procédés de traitement des effluents salés [52].
(+ avantage du procédé; – inconvénient du procédé ; Evapo : évaporation ; Co./Fl. : Coagulation/Floculation ; O.I. : osmose inverse ; Bio. Aéro. : biologique aérobie ; Bio. Ana. : biologique anaérobie).

Paramètres considérés	Evapo.	Co./Fl.	U.F.	O.I.	Ré-sine	Ozone	Bio. Aéro.	Bio. Ana.
Investissement	+	+	−	−	−	−	+	+
Coût opérationnel	++	−	−	−	−	−	+	++
Durée de vie	+	+	−	−	−	+	+	+
Surface requise	−	+	+	+	+	+	+	+
Odeurs	−−	+	+	+	+	+	−	−
Déchets secondaires	−−−	−−	−	−	−	++	−−	+
Simplicité d'utilisation	++	+	−−	−−	−−	−−	−	−
Automatisation/personnel	+	+	+	+	+	+	−	−
Risque/sécurité	+	+	+	+	+	−	+	+
Sensibilité aux chocs	+	+	+	+	+	+	−	−
Elimination MES+DCO colloïdale	+	+	+	+	−	−	−	−
Elimination DCOs dure	+	+	−	+	−	+	−	−
Elimination sels	+	+	−	+	+	−	−	−
Elimination métaux	+	+	−	+	−	−	−	−

Il ressort du Tableau 2.9 que les procédés biologiques constituent le moyen le moins coûteux de traiter les effluents hypersalins. Cependant, en raison des limites inhérentes à ces procédés en milieu salé (faible élimination des MES et incapacité à épurer le sel), leur combinaison avec des procédés physico-chimiques est inévitable. Une séquence « générique » combinant traitement biologique (secondaire) et physico-chimique (tertiaire) pour le traitement des effluents salins est ainsi présentée en Figure 2.8. Il est évident que le choix du procédé sera guidé par la nature de l'effluent à traiter (nature des sels, de la matière organique). Par exemple, un effluent riche en sulfates ne pourra

que difficilement être traité par la voie anaérobie, en raison des phénomènes de compétition entre bactéries sulfato-réductrices et méthanogènes.

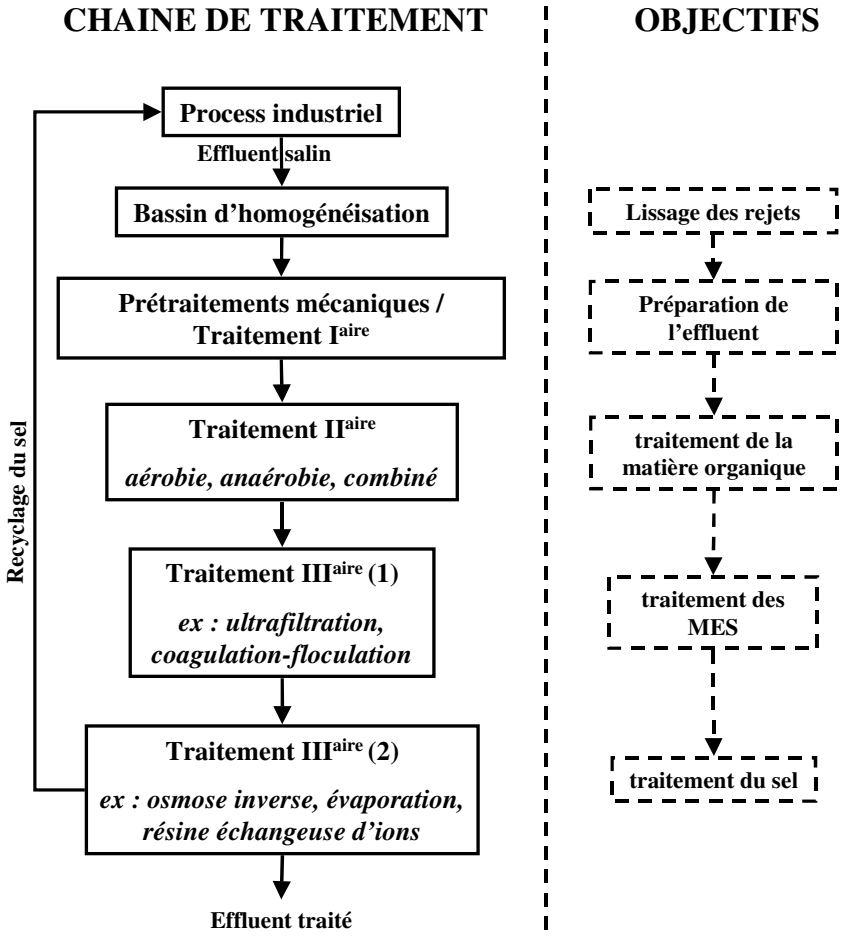

Figure 2.8 Séquence "générique" simplifiée du traitement d'un effluent fortement concentré en sel.

Tout d'abord, le premier point sensible dans le traitement des effluents salins concerne la réduction maximale de la quantité de sel introduite dans le process industriel. La construction d'un bassin d'homogénéisation est également indispensable afin de lisser les rejets d'effluents salés et de limiter l'effet de choc (sel et charge organique) sur le traitement biologique. Après prétraitements mécaniques et traitement primaire éventuel, le traitement secondaire (biologique aérobie, anaérobie ou combinant les deux) pourrait assurer l'élimination d'une part importante de la pollution carbonée, mais aussi azotée et phosphorée. Le traitement tertiaire serait alors préconisé pour l'élimination (1) des MES (problème récurrent dans les effluents salins) puis (2) du sel, selon les méthodes préconisées par la littérature [52]. Si la qualité du sel ainsi récupéré le permet, son recyclage en tête du process industriel devrait alors être considéré en vertu des principes

des technologies propres. Evidemment, ce schéma « générique » a vocation à être adapté au type d'effluent à traiter.

2.4 Conclusion générale de l'étude bibliographique et objectifs de la thèse

Dans la section 2.1, il a été montré que de vastes connaissances ont été accumulées sur la physiologie et la diversité des micro-organismes halophiles, probablement en raison de l'attrait particulier des extrêmophiles sur la communauté scientifique. Globalement, les méthodes moléculaires ont montré que les halophiles présentent une vaste diversité au sein du domaine des Bacteria. On les retrouve principalement dans les mers et océans, mais aussi dans des environnements hypersalins tels que les marais salants ou les lacs alcalins.

Puisque les micro-organismes halophiles sont largement présents dans tous les types de milieux salins et hypersalins, leur application au traitement des effluents industriels hypersalins apparaît comme une évidence. Ces effluents sont générés par des secteurs industriels variés, comprenant notamment l'industrie chimique et l'industrie du cuir (section 2.2).

Jusqu'à présent, la plupart des effluents hypersalins sont traités par la voie physico-chimique, caractérisée par un coût plus élevé que le traitement biologique. Afin de réduire les coûts, il serait utile de combiner traitement physico-chimique (pour l'élimination des sels, notamment) et biologique (pour l'épuration de la matière organique). Les recherches dans ce domaine sont encore rares et principalement basées sur des effluents synthétiques (section 2.3). Les rares effluents industriels hypersalins ayant fait l'objet de tentatives de traitement biologique sont liés à l'industrie de la pêche et de transformation des produits de la mer. Les connaissances concernant le traitement biologique des effluents industriels sont donc encore lacunaires, bien que quelques tendances se dégagent :

- Efficacité inférieure sur effluent industriel que sur substrat synthétique
- Amélioration des performances par l'enrichissement en bactéries halophiles ou halotolérantes
- Charges appliquées faibles

Au vu des lacunes et des questions soulevées par la bibliographie, les objectifs scientifiques de cette thèse s'agencent autour de deux axes principaux. Le premier axe est opérationnel et appliqué et consiste à tester et à comparer la biodégradabilité aérobie et anaérobie de quelques effluents industriels hypersalins inédits représentant un enjeu environnemental majeur, tant dans les pays développés que dans les pays en développement. Ces tests peuvent être effectués en utilisant différents procédés aérobies et anaérobies. L'utilisation d'un procédé à biomasse fixée peut notamment permettre d'évaluer les capacités de fixation de la biomasse sur support dans les conditions salines et de comparer l'efficacité des procédés à biomasse libre et à biomasse fixée.

Du point de vue plus fondamental de l'écologie microbienne, on peut s'interroger sur la faculté des micro-organismes halotolérants à s'adapter à l'augmentation de salinité lors du démarrage d'un bioréacteur avec inoculum non halophile. De plus, il y a urgence à décrire la biodiversité des environnements hypersalins que sont les boues d'épuration fonctionnant dans les conditions salines. En effet cette diversité peut permettre d'estimer le potentiel de biodégradation de ces boues halophiles, en partant du postulat qu'une biodiversité élevée assure un meilleur fonctionnement et une meilleure stabilité des boues.

Chapitre 3. Matériels et méthodes

Il a été choisi ici de s'intéresser principalement aux principes des principales méthodes utilisées au cours de ce travail. Les caractéristiques techniques assurant la reproductibilité des expériences sont précisées dans la partie matériels et méthodes propre à chaque article présenté ultérieurement.

3.1 Substrats

Les différents substrats utilisés au cours de cette thèse pour l'alimentation de bioréacteurs en conditions salines sont :

- Un effluent d'acide tartrique (section 4.1) dont les caractéristiques sont présentées dans le Tableau 4.1.
- Un effluent de tannerie (sections 4.2, 5.1 et 5.2) dont les caractéristiques sont présentées dans le Tableau 4.4., le Tableau 5.1. et le Tableau 5.4.
- Un effluent de vinasse de distillerie (section 5.3).
- L'éthanol (section 5.3).

3.2 Bioréacteurs

3.2.1 Boues activées

Les antécédents du procédé de boues activées remontent aux années 1880 lorsque Smith a étudié l'accélération de l'oxydation de la matière organique des eaux usées en aérant un bioréacteur. Le procédé a été appelé « boues activées » par Ardern et Lockett parce qu'il implique la production d'une biomasse activée constituée de micro-organismes capables de stabiliser la matière organique des eaux usées dans les conditions aérobies [150].

Par définition, le procédé basique du traitement par boues activées comprend les trois composants suivants:

- Un réacteur dans lequel les micro-organismes responsables du traitement sont gardés en suspension et aérés,
- Un clarificateur assurant la séparation liquide / solides,

- Un système de recirculation des solides ainsi séparés dans le réacteur.

Un dispositif important du procédé à boues activées est la formation de flocs bactériens capables de sédimenter lors de la clarification. Dans la plupart des cas, le procédé à boues activées est utilisé en complément des procédés physico-chimiques qui assurent le traitement primaire de l'eau usée, ainsi que le traitement tertiaire (désinfection, filtration etc.).

Le réacteur à boues activées utilisé dans la section 5.1 avait un volume de 3 l et est représenté sur la Figure 5.2.

3.2.2 SBR

Le procédé SBR (sequencing batch reactor) s'est popularisé à la fin des années 70, avec l'essor des méthodes de programmation et d'automatisation. A la différence des boues activées, le réacteur SBR fonctionne de façon discontinue selon le principe illustré par la Figure 3.1.

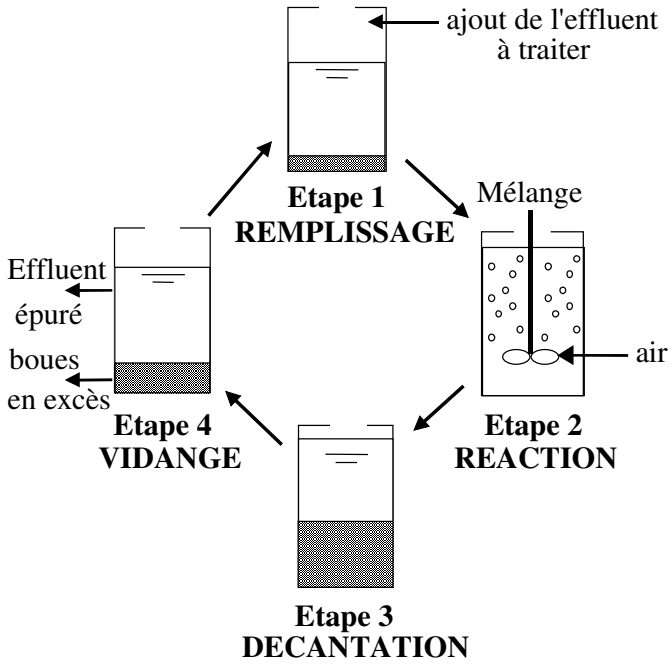

Figure 3.1 Schéma de principe du réacteur SBR aérobie.

Les 4 phases représentées sur la Figure 3.1 se résument comme suit :

- Phase de remplissage : on ajoute l'effluent à traiter dans un réacteur contenant des boues.

- Phase de réaction : on aère le réacteur jusqu'à ce que la pollution soit consommée.
- Phase de décantation : on stoppe l'aération et les boues décantent alors.
- Phase de vidange : on retire le même volume d'effluent traité que l'on avait introduit, et le cycle peut recommencer.

Le procédé SBR peut aussi être employé sans aération : anaerobic SBR (ASBR). La technologie SBR permet ainsi de réaliser le traitement de la pollution et la décantation dans un même volume et donc de réduire les coûts d'investissement. Au lieu d'une séparation spatiale des différentes étapes, elle est temporelle.

Les réacteurs SBR aérobies utilisés dans les sections 4.1 et 4.2 avaient un volume de 5 l et 10 l respectivement et sont représentés sur la Figure 4.1et la Figure 4.8. Les réacteurs SBR anaérobies utilisés dans la section 5.3 avaient un volume de 10 l et sont représentés sur la Figure 5.12.

3.2.3 UASB

La parenté du réacteur UASB (upflow anaerobic sludge blanket) est généralement attribuée à Lettinga dans les années 1970. L'UASB est de loin le système le plus largement répandu pour le traitement anaérobie des eaux usées. Le dispositif le plus caractéristique du réacteur UASB est le séparateur de phase placé en haut du réacteur, qui le divise en une partie inférieure, la zone de digestion, et une partie supérieure, la zone de séparation solides/ liquide / gaz (Figure 3.2). Les eaux résiduaires sont introduites aussi uniformément que possible par le fond du réacteur, traversent le lit de boue et entrent dans la zone de décantation par l'ouverture entre les déflecteurs. Là, en raison d'une vitesse de flux ascendant décroissante, les boues peuvent floculer et/ou précipiter, avant de revenir dans la zone de digestion. Ainsi, la présence de la zone de digestion permet au système de maintenir une biomasse importante dans le réacteur, alors qu'un effluent essentiellement exempt de solides en suspension est évacué par le haut du réacteur. Le séparateur de phases assure également l'évacuation du biogaz par le sommet du réacteur.

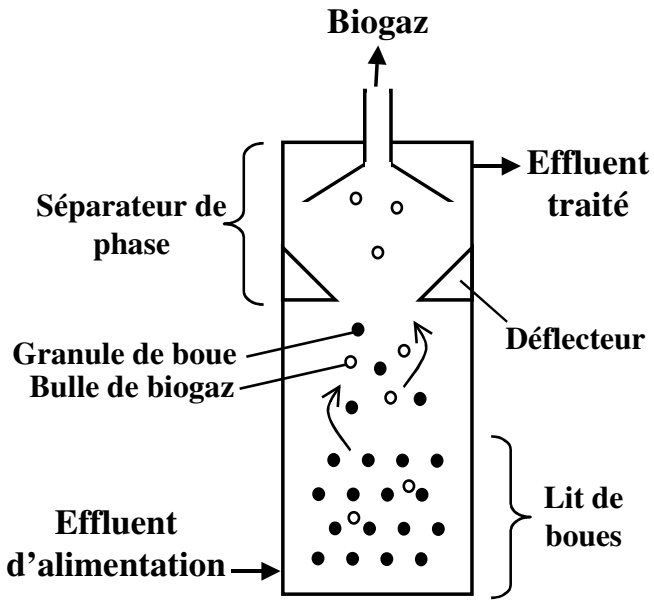

Figure 3.2 schéma de principe du réacteur UASB.

Le réacteur UASB utilisé dans la section 5.1 avait un volume de 5 l et est représenté sur la Figure 5.2.

3.2.4 Réacteur à lit mobile

Le réacteur à lit mobile est un procédé anaérobie à biomasse fixée sur un support mis régulièrement en suspension au moyen d'une pompe à immersion. Les avantages du lit mobile incluent la capacité à accumuler une concentration élevée de biomasse, à supporter des charges organiques relativement élevées, une bonne résistance aux chocs en raison du mélange régulier et un espace requis minimal. Les inconvénients incluent la puissance de pompage exigée pour déplacer le support, le coût du support et une durée de démarrage très longue.

Le réacteur à lit mobile utilisé dans la section 5.2 avait un volume de 5 l et est représenté sur la Figure 5.6.

3.3 Dynamique et caractérisation des populations microbiennes

Les méthodes suivantes ont été utilisées dans les sections 4.1, 5.3 et 6. Les caractéristiques techniques assurant la reproductibilité des expériences sont spécifiées dans la partie matériels et méthodes propre à chaque article.

3.3.1 Suivi de la dynamique de colonisation

Trois étapes successives, récapitulées sur le côté gauche de la Figure 3.3, sont nécessaires pour réussir à observer cette dynamique :

- Extraction et purification des ADN totaux à partir de boues

Il s'agit dans un premier temps de procéder à la lyse des cellules, puis d'éliminer les impuretés et l'ARN et enfin de précipiter et purifier l'ADN total.

- Amplification par PCR de la région V3 de l'ADNr 16S

La PCR (polymerase chain reaction) est une méthode qui permet d'amplifier *in vitro* un fragment d'ADN cible à partir d'un ADN matrice grâce à des amorces spécifiques, une polymérase et des nucléotides. A cette fin, les fragments d'ADN passent par différentes phases assurant leur dénaturation (séparation thermique des deux brins d'ADN), l'hybridation des amorces sur les matrices simples brins au niveau de leurs séquences complémentaires et enfin leur élongation grâce aux nucléotides fournis en excès sous l'action de la polymérase. Le fragment d'ADN amplifié (d'environ 200 pb.) correspond à la région variable V3 de l'ADN_r 16S. Les amorces universelles bactériennes utilisées sont situées dans des régions conservées encadrant la région V3 et sont marquées à l'aide d'un fluorochrome assurant leur détection ultérieure lors de l'analyse SSCP (single strand conformation polymorphism).

- Analyse SSCP

La SSCP est une technique mise au point pour la détection de mutations. Elle est basée sur la structure secondaire qu'adopte l'ADN en conditions non dénaturantes. Ainsi des fragments d'ADN de même taille mais de séquence nucléotidique différant même d'une seule base adopteront une conformation unique qui permettra de les séparer en électrophorèse sur un gel d'acrylamide grâce à la différence de mobilité de la molécule. Appliquée à des écosystèmes complexes et couplée à une PCR pour amplifier le matériel

et le marquer, elle donne immédiatement une vue d'ensemble de la structure et de la diversité d'une communauté microbienne : on obtient un profil de pics où chaque pic correspond à une espèce bactérienne et où la hauteur des pics est représentative de la proportion de cette espèce dans l'écosystème (Figure 3.3).

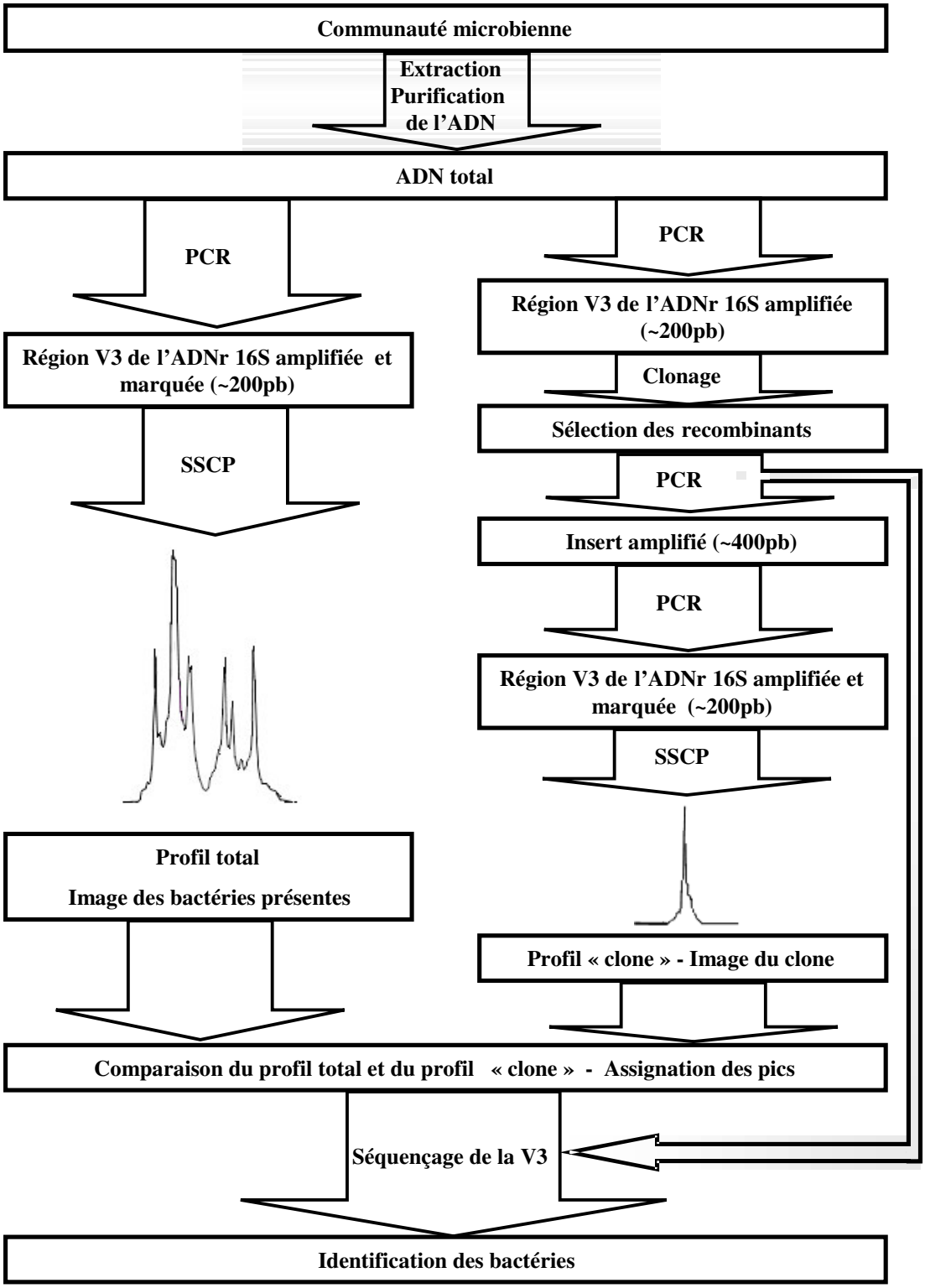

Figure 3.3 Les différentes étapes de l'identification microbienne.

3.3.2 Caractérisation des populations microbiennes

Afin d'identifier chaque pic (ou les pics majoritaires) du profil SSCP sélectionné, un clonage est effectué en plusieurs étapes récapitulées sur le côté droit de la Figure 3.3.

- Amplification de la région V3 de l'ADNr 16S par PCR.
- Insertion de l'ADN amplifié dans un plasmide.
- Transformation : pénétration du plasmide dans des cellules compétentes d'*Escherichia coli* soumises à un choc thermique. Les cellules sont étalées sur un milieu de culture permettant la sélection et l'identification des recombinants.
- Vérification et amplification des transformants par une double PCR. Les produits de PCR sont ensuite analysés par SSCP et comparés au profil total. Cette étape d'analyse des clones par SSCP permet un criblage pour ne séquencer que les clones les plus « intéressants », qui co-migrent avec les pics du profil complexe.
- Identification des clones retenus après séquençage des inserts. Les séquences sont comparées à celles d'une base de données afin de déterminer la famille, le genre ou l'espèce la plus proche phylogénétiquement.

3.4 Méthodes analytiques

Les méthodes analytiques utilisées sont, pour l'essentiel, celles recommandées par l'AFNOR, Association Française de Normalisation [19] ou l'APHA, American Public Health Association [9]. Les mesures de COT, DCO, N-NH_4^+ et NTK solubles ont été effectuées après centrifugation (15 000 rpm., 15 mn.) des échantillons dans les sections 4.1 et 5.3 et après filtration sur filtre en fibre de verre (0,45µm) dans la section 4.2.

Il faut savoir que nombre de méthodes analytiques sont rendues difficiles dans les effluents hypersalins en raison de l'interférence des chlorures. C'est notamment le cas de la détermination de la DBO_5, DCO, du COT, des NO_3^- et des NO_2^-. Par conséquent, des méthodes non sensibles aux ions chlorures ont été sélectionnées autant que possible. Par exemple, le dosage du NH_4^+ et du NTK a été sélectionné pour mesurer le traitement de l'azote en raison de la non-sensibilité des méthodes de détermination de ces paramètres aux ions chlorures. Au contraire, la détermination des NO_3^- (et NO_2^-) dans les effluents hypersalins n'a pas pu être réalisée quelle que soit la méthode utilisée (chromatographie échangeuse d'ions et méthode colorimétrique à l'acide chromotropique à cause de

l'interférence des ions chlorures ; méthode spectrométrique dans les ultraviolets à cause de l'interférence de la matière organique des effluents).

DCO : la demande chimique en oxygène est la concentration d'oxygène équivalente à la quantité d'un oxydant (dichromate) consommée par les matières oxydables dans un échantillon d'eau. C'est un indice de la quantité de matières chimiquement oxydables présentes dans l'effluent. La matière est oxydée, en milieu acide (H_2SO_4) et en présence d'un catalyseur (sulfate d'argent), par le dichromate de potassium ($K_2Cr_2O_7$) introduit en excès. Après chauffage, la DCO est déterminée par dosage en retour : l'excès de bichromate de potassium est déterminé (dosage colorimétrique) à l'aide d'une solution de fer ferreux et d'ammonium (sel Mohr). La méthode utilisée est la norme NF T 90-101 de l'AFNOR [1] pour les sections 4.1 et 5.3, et la méthode à reflux ouvert de l'APHA [9] dans les sections 4.2, 5.1 et 5.2. la méthode de la DCO est sensible à l'interférence des chlorures qui contribuent à accroître le résultat. L'utilisation de sulfate de mercure ($HgSO_4$) en maintenant un ratio massique $HgSO_4$/Cl de 10 est cependant connue pour éliminer cette interférence en faisant précipiter les chlorures [193].

DBO_5 : La DBO_5 est la quantité d'oxygène qui est consommée en 5 j à 20°C et à l'obscurité par les matières oxydables dans un échantillon d'eau, notamment pour assurer leur dégradation par voie biologique. La norme utilisée est celle de l'APHA [9]. La détermination de la DBO_5 peut conduire à une sous-estimation de la biodégradabilité réelle des effluents hypersalins. En effet, l'utilisation de boues activées non adaptées au sel va conduire à de faibles valeurs, qui pourront être améliorées en ayant recours à un inoculum adapté contenant des micro-organismes halophiles ou halotolérants.

COT : le carbone organique total est une mesure de la teneur en carbone des matières organiques présentes dans l'eau. la méthode retenue est la norme NF EN 1484 de l'AFNOR [1]. La combustion catalytique à haute température de la matière organique est réalisée par un analyseur de carbone TOC-V (Shimadzu). Le CO_2 produit est détecté par infrarouge. Le carbone total est obtenu après combustion totale à 680°C avec un catalyseur cobalt/platine, le carbone minéral après acidification et dégazage, le COT par différence entre le carbone total et le carbone minéral. Une dilution maximale des échantillons analysés pour le COT a été effectuée en vue de réduire la concentration de chlorures. En effet, lors de l'oxydation catalytique, les chlorures oxydent la cellule infrarouge, diminuant sa longévité.

MES et MVS : la méthode utilisée pour le dosage des matières en suspension est la méthode par centrifugation, suivie d'une filtration sur filtre en fibre de verre (0,45 µm). Les MES sont déterminées par séchage de l'échantillon à 105°C et les matières volatiles en suspension sont déterminées après calcination à l'étuve à 550°C.La méthode utilisée

est la norme NF EN 872 de l'AFNOR [1] pour les sections 4.1 et 5.3, et celle de l'APHA [9] dans les sections 4.2, 5.1 et 5.2. Là aussi, l'utilisation d'effluents hypersalins peut conduire à une surestimation des MES, en raison de la formation de croûtes de sel lors du séchage, pouvant même emprisonner l'eau et empêcher son évaporation complète, ce qui conduit à des erreurs de pesée. Cette interférence peut être réduite en utilisant un volume d'échantillon aussi grand que possible. Le volume des échantillons était de 25 ml pour les expériences relatées dans les sections 4.1 et 5.3 ; et 5 ml dans les sections 4.2, 5.1 et 5.2.

Salinité/TDS : la salinité d'un échantillon liquide correspond à la somme des anions et cations exprimée en mg l^{-1}. La méthode retenue est la détermination des TDS (total dissoved solids) obtenue par séchage de l'échantillon à 550°C [9]. Cette méthode de détermination par pesée est celle généralement sélectionnée par la plupart des études scientifiques.

NH_4^+ : il s'agit de distiller de l'azote ammoniacal à un pH voisin de 7,4 à l'aide d'une solution tampon pour le doser ensuite par titration. La méthode utilisée est la norme NF T 90-015 de l'AFNOR [1] pour la section 4.1 et la méthode titrimétrique de l'APHA [9] dans la section 4.2.

NTK : le dosage de l'azote total par la méthode Kjeldahl permet de doser l'azote organique et ammoniacal, à l'exclusion des nitrites et nitrates. L'azote organique est d'abord minéralisé à chaud en milieu acide, puis distillé après alcalinisation et recueilli dans une solution acide. Il est ensuite dosé par titration. La méthode utilisée est la norme NF EN 25663 de l'AFNOR [1] pour la section 4.1 et la méthode macro-Kjeldahl avec dosage titrimétrique de l'ammonium, préconisée par l'APHA [9] dans la section 4.2.

O_2 : la concentration en oxygène dissous est déterminée par la méthode électrochimique à la sonde. La méthode utilisée est la norme NF EN 25814 de l'AFNOR [1] pour la section 4.1et celle de l'APHA [9] pour la section 4.2. Les sondes utilisées disposaient toutes d'un dispositif de correction de la salinité.

pH : la mesure électrométrique du pH est en relation étroite avec la concentration des ions hydrogène (H^+) présents dans l'effluent. La méthode utilisée est la norme NF T 90-008 de l'AFNOR [1] pour les sections 4.1 et 5.3, et celle de l'APHA [9] dans les sections 4.2, 5.1 et 5.2.

P-PO_4^{3-} : la mesure des phosphates s'est faite par colorimétrie suivant la méthode à l'acide ascorbique de l'APHA [9]. Cette méthode est l'une des rares qui ne souffre pas d'interférence liée à la salinité.

AGV : le dosage des acides gras volatils a été réalisé par distillation selon la méthode préconisée par l'APHA [9] dans les sections 5.1 et 5.2, et par chromatographie en phase gazeuse dans la section 5.3. La chromatographie en phase gazeuse est une méthode de séparation des composés gazeux, ou susceptibles d'être vaporisés, par chauffage sans décomposition. Le chromatographe utilisé (modèle GC8000 équipé d'un passeur d'échantillons AS800, Fisons Instruments) est équipé d'une Colonne ECONOCAP FFAP (Alltech) d'une longueur de 15 m et d'un diamètre de 0,53 mm ainsi que d'un détecteur à ionisation de flamme. La température du four est de 100°C, celle de l'injecteur de 250°C et celle du détecteur de 275°C. Le gaz vecteur est l'azote et le volume d'injection est de 1 µl.

Alcalinité totale : la détermination de l'alcalinité totale a été réalisée selon la méthode préconisée par l'APHA [9].

Composition du biogaz : la composition en méthane des échantillons a été déterminée par chromatographie en phase gazeuse (Shimadzu GC-8A). La séparation des différents gaz se fait sur deux colonnes : Le CO_2 et le N_2O sont séparés sur une colonne Hayesep Q (80-100 mesh, 2 m x 1/8 inch, Touzart & Matignon) ; les autres gaz (O_2, H_2, N_2, CH_4) sont séparés sur un tamis moléculaire 5Å (80-100 mesh, 2 m x 1/8 inch, Touzart & Matignon). Les deux colonnes sont montées en série et séparées par une colonne à vide de 10 m de long qui ralentit le passage des gaz au niveau de la $2^{ème}$ colonne. La détection se fait sur un catharomètre (principe du pont whestone) dont l'intensité est fixée à 90 mA. Le gaz vecteur est l'argon. la température du four est de 35°C, la température de l'injecteur et du détecteur est de 100°C. le volume d'injection est de 1 ml.

Indice de boue/SVI : il se détermine suite à un test de décantation en éprouvette graduée, d'après la formule suivante : IB = (Résultat du test de décantation (ml l^{-1})) / MES (g l^{-1}).

Chapitre 4. Traitement aérobie d'effluents industriels hypersalins

Articles publiés sous la forme :

(4.1) Lefebvre, O., Habouzit, F., Bru, V., Delgenes, J.P., Godon, J.J., Moletta, R., 2004. Treatment of hypersaline industrial wastewater by a microbial consortium in a sequencing batch reactor. Environ. Technol. 25(5), 543-553.

(4.2) Lefebvre, O., Vasudevan, N., Torrijos, M., Thanasekaran, K., Moletta, R., 2005. Halophilic biological treatment of tannery soak liquor in a sequencing batch reactor. Water Res. 39(8), 1471-1480.

4.1 Traitement biologique aérobie d'un effluent hypersalin d'industrie tartrique

Article : Treatment of hypersaline industrial wastewater by a microbial consortium in a sequencing batch reactor

Résumé

Les effluents hypersalins sont générés par de nombreux secteurs industriels. De tels effluents, riches à la fois en matière organique et en sel (> 35 g l^{-1}) sont difficiles à traiter par les procédés d'épuration aérobie conventionnels. Il est nécessaire de recourir aux bactéries halophiles.

Dans cette étude, un réacteur de laboratoire de type sequencing batch reactor (SBR) a été ensemencé avec un inoculum halophile pour traiter un effluent de l'industrie tartrique contenant 120 g l^{-1} de sel. Les micro-organismes se sont révélés aptes à la dépollution carbonée et azotée de l'effluent, à condition que le pH du réacteur soit neutralisé avec de l'acide phosphorique. Les rendements épuratoires de la DCO soluble et du NTK soluble ont atteint 83% et 72%, respectivement. L'analyse de l'ADNr 16S de la communauté microbienne halophile a révélé une grande diversité.

Abstract

Hypersaline effluents are produced by various industrial activities. Such wastewater, rich in both organic matter and salt (> 35 g l^{-1}), is difficult to treat by conventional wastewater treatment processes. It is necessary to use halophilic micro-organims.

In this study, a bench-scale sequencing batch reactor (SBR) was inoculated with halophilic sediments in order to treat a tartaric acid production effluent containing 120 g salt l^{-1}. The micro-organisms were able to treat carbon and nitrogen, provided the pH in the reactor was neutralised with phosphoric acid. CODs and TKNs removal attained 83% and 72% respectively. 16S rDNA identification of the halophilic microbial community showed high diversity.

Introduction

Worldwide, the pollution removal in hypersaline effluents is likely to represent up to 5% of the total global wastewater treatment requirement. Moreover, the developing countries in particular are confronted with this kind of problem since the industrial and urban wastewater in these countries is often discharged into depressions in saline zones. Thus, the removal of xenobiotics in saline ecosystems is a problem that affects the industrial sector in general and the peri-urban environment of many developing countries.

Various industrial sectors are confronted with the problem of hypersaline effluents generated by their production activity. This is particularly the case in industries that use calcium tartrate crystals as raw material sold to the winemaking and agri-food sectors. The calcium tartrate is recovered from alkaline solutions extracted from the tartar-removing process in wine tanks. These solutions are made up of water, a salt compound (tartar, sodium and potassium), residual soda and organic matter from the wine. The precipitation of calcium tartrate is obtained by acidification (HCl) and the addition of a calcium salt ($CaCl_2$). The crystals are dried and washed to eliminate excess chloride, thus generating hypersaline effluents that must be treated to conform to the French and European legislation.

The level of salt in the effluent, close to 120 g l^{-1} (mainly NaCl and KCl), makes the correct operation of a conventional wastewater treatment system impossible [136]. The main dysfunctions caused by salt have been reviewed in 1995 [236]: first of all, conventional micro-organisms are sensitive to abrupt ionic changes and, in addition, they do not tolerate salt concentrations higher than 50 g l^{-1}. Also, high salt levels disturb the microbial metabolism, with a consequent fall in the removal yields of organic matter: the nitrification reaction, *inter alia*, is particularly inhibited. Moreover, salt tends to increase the suspended solids (SS) in effluents, on account of its lysis effect on many organisms (protozoa amongst others [191]) and because of its inhibiting action on flocculation [236]. Lastly, bacterial acclimation to salt is quickly lost if salinity suddenly drops.

In the treatment of hypersaline effluents, the advantages of using halophilic Bacteria have already been reported. The Bacteria best adapted to the effluent considered in this study are moderate halophiles, i.e. in order to ensure their development, the salt concentration must lie between 30 and 150 g l^{-1} [125].

Among the studies undertaken on halophilic Bacteria, most have aimed at defining the Bacteria's taxonomic position. Such information makes it possible to define the types of

carbon-containing substrates, such as sugars or protidic substances [53; 100], to be used for growing the given micro-organisms. A very small percentage of these halophilic strains have been tested on carbon-containing substrates of an aromatic type like those that are likely to be found in the agri-food effluent studied in this paper. A study was carried out on the representative of the aromatic acid monomers in C6-C1, the benzoate [53]. Later, the removal of phenol (representative of non-acid aromatic monomers) was studied in greater depth in halophilic conditions using a micro-organism identified as *Pseudomonas halodurans* [93].

In non-saline conditions, the Bacteria are known for their varied metabolism, but little information exists with regard to their potential for aerobically degrading polluting molecules under saline conditions. An unidentified bacterial strain, isolated in 1978 from the Great Salt Lake (Utah, USA), was able to degrade a non-aromatic non-polar pollutant, hexadecane, under saline conditions of less than 200 g NaCl l^{-1} [230]. More recently, some recalcitrant compounds such as organophosphoric pesticides have been successfully degraded by a micro-organism identified as belonging to the *Alteromonas* genus [51]. Similarly, the database "Biodegradative Strain Database", available on the Web, refers to the removal of the 2.4-D (2.4-dichlorophenoxyacetate) by a species of the *Halomonas* genus.

The team that obtained significant results on the decomposition of phenol in a saline medium [93] described an aerobic moderate halophile taken out of the Great Salt Lake. This species, identified as belonging to the *Halomonas* genus, was able to degrade 0.1 g phenol l^{-1} as its only source of carbon and energy. This study was carried out using a synthetic saline wastewater similar to the effluents resulting from the oil industry in which phenolic compounds are commonly found. This bacterium degraded phenol in a medium containing NaCl concentrations between 10 and 140 g l^{-1}. Such maximum salt concentrations are similar to those found in some agricultural and food industries or in saline depressions which receive agricultural and urban wastewater in south Mediterranean countries. Under optimal growth conditions (between 30 and 50 g NaCl l^{-1}), the removal of 0.1 g phenol l^{-1} was complete at the end of 13 h for the only strain ever studied. For higher salt concentrations, the complete mineralisation of phenol was slower, reaching 100 h for 140 g NaCl l^{-1} [93].

In 1994, a sequencing batch biofilm reactor (SBBR) was inoculated with moderate halophiles, recovered from the Great Salt Lake, in order to treat a synthetic effluent containing 150 g salt l^{-1} [235]. The removal yields measured on phenol exceeded 99%. The experiment was renewed in 1995 [236], using a SBR with free culture, this time reaching an average yield of 99.5%. In Japan in 2000 [120], a hypersaline agri-food

effluent (150 g NaCl l^{-1}) was purified by halo-tolerant Bacteria. COD removal of about 90% was attained in an aerobic SBR. On the other hand, when the cultivation mode was changed to continuous culture, the COD removal was from 60 to 70%. Other potential applications do exist, e.g. recycling a micro-algae culture medium after a biological treatment [192].

All in all, in the whole corpus of scientific work published on the metabolism of halophilic micro-organisms, there is little reference to the removal by biological agents of hypersaline agri-food effluents and especially to the breaking down of aromatic compounds in saline conditions. However, according to those studies, it seems possible to treat hypersaline effluents using microbiological techniques, despite the considerable obstacles created by salt. Little research has gone beyond the pilot stage and most has used synthetic effluents.

In this paper, we treated a complex industrial hypersaline effluent in a bench-scale aerobic SBR using microbiological techniques. Furthermore, whereas most studies have addressed the removal of carbon only, we also tried to eliminate nitrogen. Lastly, the identification of the micro-organisms involved in the treatment was carried out by 16S rDNA identification.

Materials and methods

Influent

The influent used in this study resulted from the activity of a tartaric acid production plant. The analytical parameters of the influent appear in Table 4.1.

Table 4.1 Characteristics of the tartaric industry effluent.

PH	7.8
Salt (g l^{-1})	120
SS (mg l^{-1})	1760
VSS (mg l^{-1})	460
CODt (mg l^{-1})	4340
CODs (mg l^{-1})	3770
TOCt (mg l^{-1})	1750
TOCs (mg l^{-1})	1580
TKNt (mg l^{-1})	190
TKNs (mg l^{-1})	150

Bench-scale reactor

The bench-scale SBR (Figure 4.1) had a volume of 5 l. The tubes inserted into the top of the reactor ensured the filling and the withdrawal of the effluent. Peristaltic pumps provided a fill flow and a draw flow of 42 and 39 ml min^{-1} respectively. An air compressor ensured bubble aeration through a pumice diffuser, ensuring a dissolved oxygen rate, measured with an electrochemical probe, of 5 mg l^{-1} in the absence of any microbial activity, in the condition of hypersaline effluent treatment.

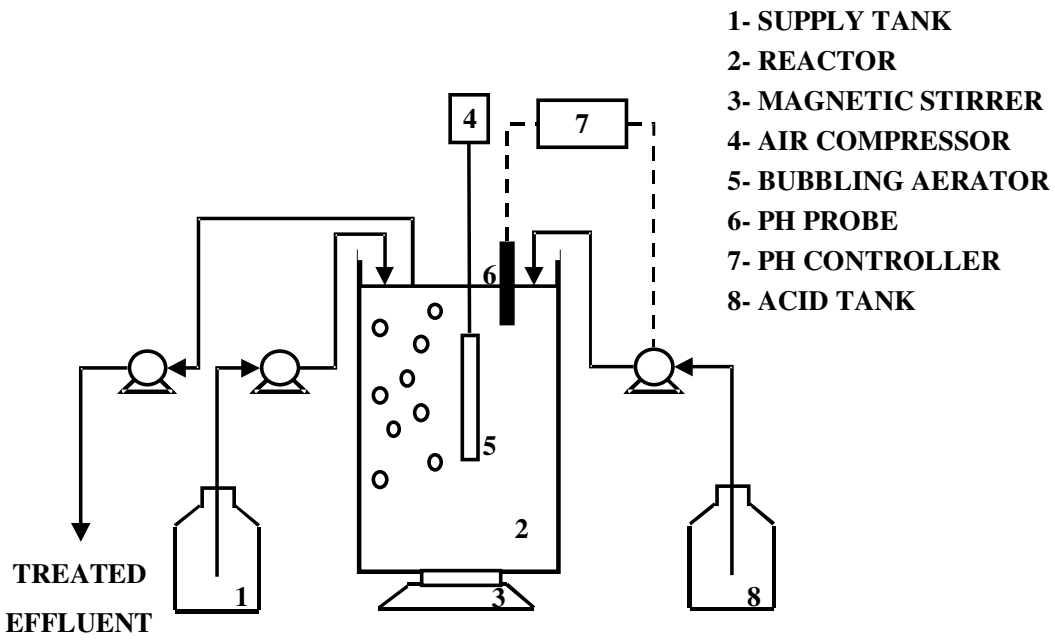

Figure 4.1 Bench scale SBR system operating with a tartaric industry effluent.

After inoculation, the SBR functioned on 24 h cycles, with alternation of the anoxic and aerobic phases (6 h and 17 h, respectively), followed by a sequence of settling, withdrawal and filling (1 h in all) for more than 100 days. The purpose of alternation between anoxic and aerobic phases was to permit the biological treatment of nitrogen [88], in addition to carbon. The hydraulic retention time was 10 days and the sludge retention time 50 days, at the end of the experiment. The volumetric loading rate was 0.2 g COD l^{-1} d^{-1} until cycle 17, afterwards it was doubled to 0.4 g COD l^{-1} d^{-1}, by doubling the flow rate. Thus, the reactor was operated under the conditions of weak load, which ensured better degrading yields and less sludge production. Lastly, the preliminary tests (not presented here) had noted alkalization of the medium, a phenomenon which accompanied a slowing down in the degrading of the organic matter. In order to counter this pH increase in the reactor, pH regulation was set up using hydrochloric acid initially, then phosphoric acid at a later stage. The reactor temperature was ambient (20 to 23 °C).

Inoculum

The reactor, initially filled with fresh water, was first sown with sludge from urban wastewater, treated for carbon, nitrogen and phosphorus. Then the salt content in the reactor was increased very gradually up to that of the influent. When salinity reached a sufficient level (approximately 70 g l^{-1}), sediments recovered from the bottom of the evaporation basins of the industry involved were then added.

Analytical techniques

The analytical methods used were essentially those recommended by AFNOR, the French Standards Authority [1]: measurements of COD, TOC, TKN, N-NH_4^+, SS, pH and dissolved O_2 were taken in accordance with, respectively, standards NF T 90-101, NF EN 1484, NF EN 25663, NF T 90-015, NF EN 872, NF T 90-008 and NF EN 25814. Mercuric sulfate was used to eliminate the interference of chlorides when dosing COD. It has been reported [193] that this interference can be eliminated as long as a 10:1 weight ratio of mercuric sulfate to chloride is maintained.

Volatile suspended solids (VSS) and salt concentration were obtained following measurements of SS after calcination in a drying oven at 525 °C ± 25 °C for 2 to 3 h. The attempts at measuring nitrates (NO_3^-) appeared unfruitful, regardless of the method used [9] (in the case of ionic chromatography and chromotropic acid method, because of chloride interference; with the spectrometric method using ultraviolet rays, because of interference from organic matter).

Extraction and purification of total genomic DNA.

Four millilitre samples were collected from the middle of the digester on days 6, 33, 87 and 121 and centrifuged at 6000 rpm for 10 min. Pellets were resuspended in 4 ml of 4 M guanidine thiocyanate – 0.1 M Tris pH 7.5 and 600 µl of N-lauroyl sarcosine 10%. 250 µl of treated samples were transferred in 2 ml tubes and stored frozen at – 20° C. Extraction and purification of total genomic DNA was carried out, using a protocol based on mechanical cell disruption by heat treatment (70 °C for 1h) in the presence of zirconium beads [81]. Nucleic acids are recovered after several washes with polyvinylpolypirolidone to remove PCR inhibitors before alcohol precipitation. Concentration and size of DNA (around 50 kb) were estimated by electrophoresis on a 0.7% agarose gel and viewed by ethidium bromide with ultraviolet emission.

Amplification, cloning and sequencing of the V3 region of 16S rDNA

Highly variable V3 regions of microbial 16S rDNA genes were amplified by PCR using bacterial primers (W31 – W49) (see Table 4.2). Samples were treated according to the protocol PCR-cloning – Amplification of 16S rDNA previously described [54]. For each reaction the solution, along with enzyme *Tampon Red Taq* ® 5 U/µl in the amount of 5 µl, dNTP 2,5 mM in the amount of 4 µl and 2 - 2 µl of bacterial primers (w31 100 ng/µl – w49 100 ng/µl), was added to 1 µl of DNA diluted in water. PCR conditions were as follows: an initial denaturation step at 94°C for 2 min, followed by 30 cycles of a three-

stage program with 1 min at 94°C, 1 min at 61°C and 1 min at 72°C, the final elongation step running for 10 min at 72°C. PCR products were purified with a QIAamp kit (Quiagen). Purified product sizes (~200pb) and concentrations were checked by electrophoresis on a 2% agarose gel containing ethidium bromide with ultraviolet emission. Correct PCR products were cloned and transformed into *Escherichia coli* using the PCR® 4-TOPO® vector kit according to supplier instructions (Invitrogen).

Table 4.2 Sequences and target positions of primers.

Primer	Sequence	Position in *E.coli* [31]	Target
W31	TTACCGCGCTGCTGGCAC	R[b] 515-533	16S rDNA universal
W49	ACGGTCCAGACTCCTACGGG	F 329-348	16S rDNA *Bacteria*
W104[a]	6-FAM-TTACCGCGCTGCTGGCAC	R 515-533	16S rDNA universal
W91	HEX-ACGGTCCAGACTCCTACGGG	F 329-348	16S rDNA *Bacteria*
T07	TAATACGACTCACTATAGGG	-	plasmid
P13	GACCATGATTACGCCAA	-	plasmid

[a] The primer w104 is marked at 5' end with fluorescent phosphoramidite - TET (Applied Biosystems).

[b] F and R correspond to forward and reverse primer.

E. coli with inserts of proper size were screened by PCR on colonies with plasmid targeted primers T7 and P13. Afterwards, purified PCR products were sequenced with a dye-terminator cycle sequencing reaction kit with AmpliTaq DNA polymerase FS kit buffer (Applied Biosystems) and the T7 primer. Sequence reaction products were analysed on an ABI model 373A genotyper apparatus (Applied Biosystems).

SSCP analysis

Single Strand Conformation Polymorphism (SSCP) analyses were performed for overall detection of microbial populations and for a study of their dynamics. SSCP analysis makes it possible to separate DNA fragments of a similar size according to their configuration (secondary structure). Targeting the 16S rDNA V3 region, which permits the phylogenetic discrimination of microbial species, enables the reactor microbial community to be monitored by one profile of peaks, where each peak corresponds to a

different sequence of 16S rDNA V3 region i.e. to one bacterium. The height of the peaks corresponds to the quantity of 16S rDNA sequence after PCR amplification.

The amplification of the V3 region of 16S rDNA PCR-SSCP was carried out with specific primers w91 – w104 (see Table 4.2) from total DNA. An initial denaturation step at 94°C for 2 min, was followed by 30 cycles of a three-stage program with 30 sec at 94°C, 30 sec at 61°C and 30 sec at 72°C, and a final elongation for 10 min at 72°C. DNA polymerase was *Pfu* turbo (Stratagene). PCR-SSCP products were purified using the kit QIAquick and were estimated by gel-electrophoresis.

For electrophoresis, PCR-SSCP products were diluted in water before mixing with 18.75 µl formamide (Genescan-Applied Biosystems) and 0.25 µl internal standard (ROX, Genescan-Applied Biosystems) [54]. The mixture was denatured by heating at 95^{o}C for 5 min and cooled in watery ice for 10 min. Single strands of DNA molecules made stable secondary conformations which were separated by capillary electrophoresis. SSCP analyses were performed with the automatic sequencer abi310 (Applied Biosystems). DNA fragment detection was done with the fluorescent W34 primer. The results obtained were analysed by GeneScan® 3.1 (Applied Biosystems).

To identify SSCP peaks of interest, 16S rDNA V3 from reactor samples were amplified and cloned into *E. coli* as described above. Cloned inserts were amplified by PCR using the plasmid targeted primers T7 and P13. PCR-SSCP on the resulting DNA fragment produced, after SSCP analysis, single peaks which were compared with total microbial community profiles for peak assignation. Sequencing of interesting cloned V3 regions was carried out and, finally, the identification of micro-organisms corresponding to peaks was successfully established.

Sequence analysis

16S rDNA V3 sequences (of about 200 bp) were identified by comparison with sequences available in databases using the BLAST program. The nucleotide sequence data reported in this work will appear in the GenBank nucleotide database under accession numbers AY188696 to AY188715.

Results and discussion

Evolution of reactor performance during the experiment

The concentrations in soluble COD (CODs) and soluble TKN (TKNs) and corresponding removal yields are shown in Figure 4.2 and Figure 4.3.

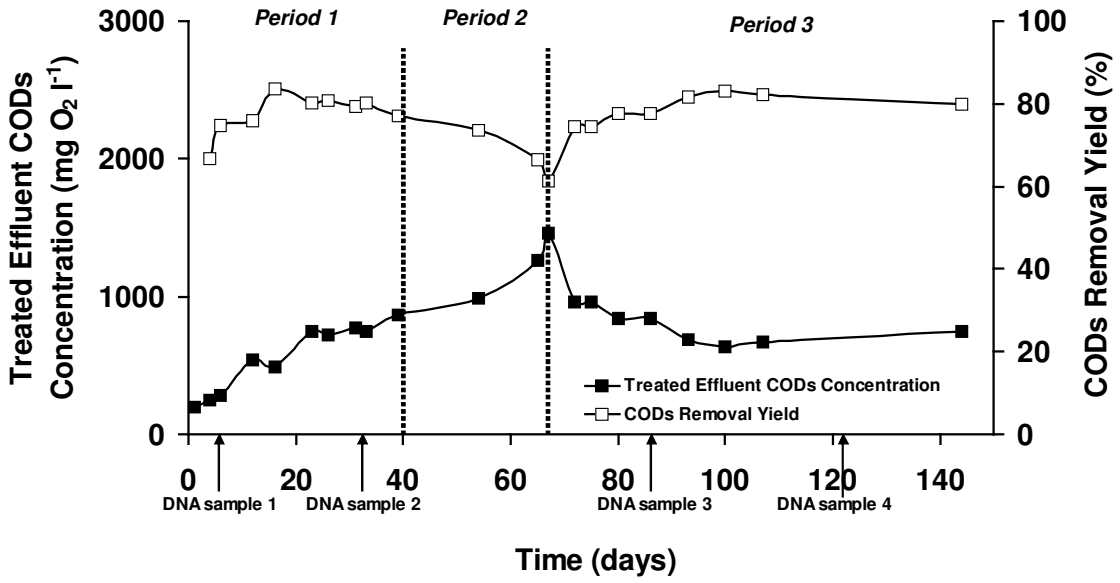

Figure 4.2 Evolution of treated effluent CODs concentration and CODs removal yield obtained during the operation of an aerobic SBR operating with a tartaric industry effluent.
(Days when DNA samples were collected from the middle of the digester are indicated by an arrow)

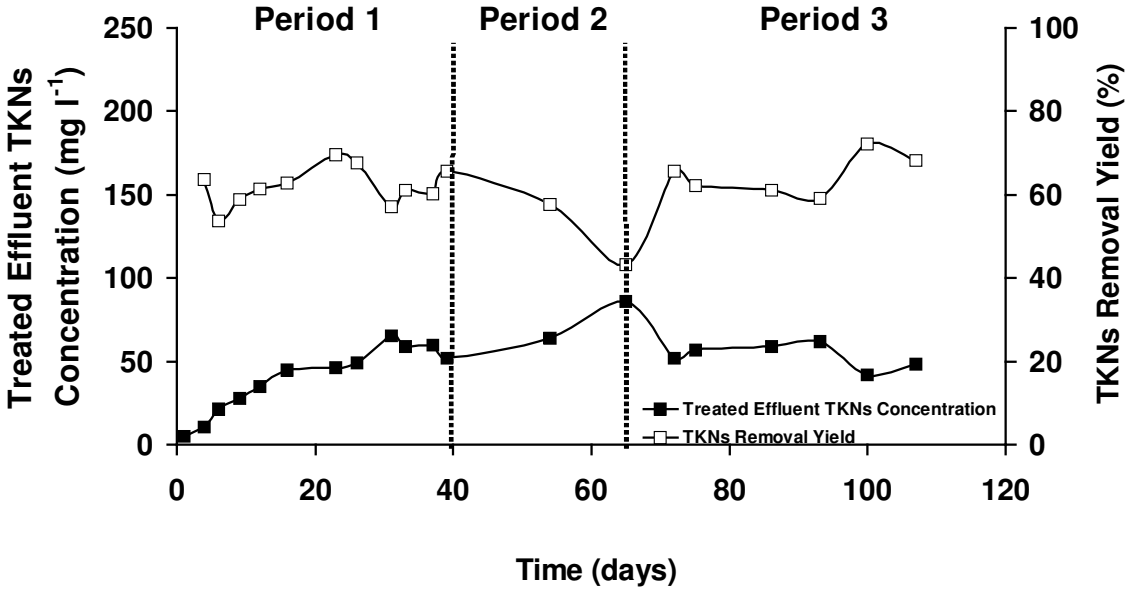

Figure 4.3 Evolution of treated effluent TKNs concentration and TKNs removal yield obtained during the operation of an aerobic SBR operating with a tartaric industry effluent.

The experiment can be divided into three distinct periods:

- Period 1 was a starting period (approximately 40 cycles), during which salinity was increased gradually to reach that of the real effluent (120 g l^{-1}). Other operations were carried out during this phase: halophilic Bacteria adjunction at cycle 17, doubling of the load at cycle 34 by doubling the flow rate. During this period, the reactor was not working under steady state conditions and its performance in removing CODs and TKNs decreased slowly, as the salt concentration increased, and in spite of the halophilic Bacteria adjunction. Four millilitre samples were collected on days 6 and 33 for DNA extraction.

- Period 2 was a period of deterioration of the reactor performance (30 cycles approximately), during which salinity was stabilized near 120 g l^{-1} . This period also corresponded to a phase of microbial adaptation or selection due to the harsh conditions. The removal efficiency fell to 61% and 43% for the CODs and TKNs, respectively. At the same time, the bacterial biomass decreased considerably, from 5.1 g MVS l^{-1} at cycle 26 (the maximum value obtained during the experiment, in the days which followed the inoculation of halophilic sediment) to 1.9 g l^{-1} at cycle 65. As previously considered, the pH in the reactor increased up to 9, probably because of the consumption of residual organic acids, originating in the wine, contained in the

influent. The ecosystem was destabilized by the extreme conditions of the medium (hypersalinity and alkaline pH) and this disturbance generated a considerable reduction in the removal yields, as well as in the biomass growth rate. Furthermore, a neutralisation of pH thanks to hydrochloric acid, carried out as of cycle 60, did not enhance the performance of the system.

- Period 3 was a period of enhancement of the process, followed by a stabilization phase. At cycle 67, phosphoric acid was substituted for hydrochloric acid. Within the next five days, the performance of the process was clearly enhanced. Figure 4.2 and Figure 4.3 show that this period was characterised by a rapid improvement of the removal yields, followed by a stabilization of the system. Analysis of the biomass indicated a growth recovery up to 3.5 g l^{-1} at cycle 93, which is conventional for a low loaded plant treating an industrial effluent without VSS in the influent [151]. Thereafter the biomass remained constant, thus no sludge wastage was required. Four millilitre samples were collected on days 87 and 121 for DNA extraction.

Monitoring the performance of the SBR over time showed it is possible to treat highly saline effluents provided the pH is neutralised with phosphoric acid. The maximum removal yields reached 83% and 72% for the CODs and TKNs, respectively. These yields were obtained during period 3. As previously indicated [120], the inhibition due to alkaline pH added to the inhibition due to salt prevented the optimal operation of the process and it was necessary to remove one of the two inhibitions in order to reach better removal yields. In addition, the positive impact of phosphoric acid indicated a phosphorus deficiency in the influent. The phosphorus requirement could be estimated to 20 mg l^{-1}, using a conventional COD/P ratio of 200/1. After biomass acclimation, phosphorus was brought using phosphoric acid.

The major problem encountered when treating the hypersaline effluent in the halophilic SBR was SS which consisted of dispersed particles that failed to form flocs and settle prior to draw. SS averaged 1800 mg l^{-1} in the treated effluent in this study, one third of this value consisting of VSS, as reported in Figure 4.4. According to this VSS loss, the sludge age ranged from 40 to 50 days during period 3.

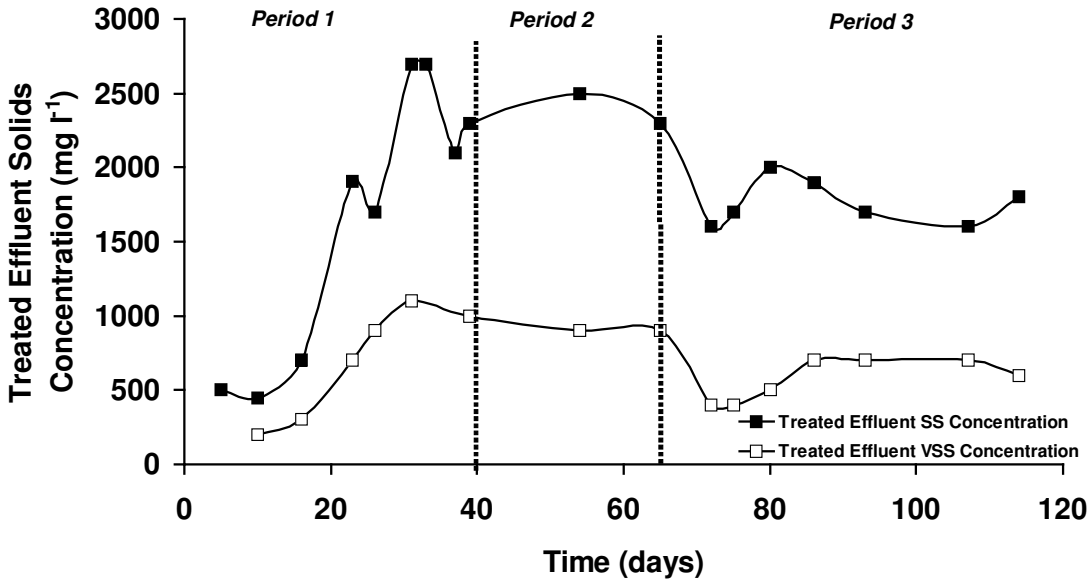

Figure 4.4 Evolution of treated effluent SS concentration and treated effluent VSS concentration obtained during the operation of an aerobic SBR operating with a tartaric industry effluent.

The high turbidity in the effluent explains why CODt and TKNt removal yields were much lower than those of the soluble fraction, optimal yields reaching respectively 68 and 46%, during period 3 (data not showed). These flocculation problems in hypersaline wastewater have already been reported [236]. Several factors may contribute to effluent turbidity: first of all, the lack of filamentous organisms, which were rarely observed during the study period, may contribute to this phenomenon. Filaments do indeed contribute to the mechanical integrity and the structure of the flocs [217]. In their absence, flocs are essentially made of individual particles which are easily disrupted. In addition, the protozoa, which play a role in the elimination of the micro-organisms by grazing them, were rarely observed during the study period. This may also have resulted in increasing effluent turbidity. Finally, the density of salt water is higher than that of fresh water and thus reduces the settling velocity of dispersed particles.

Study of a single operation cycle of the SBR

This study was carried out during cycle 100, during the third period previously described, at a time when the reactor was stabilized. Figure 4.5 shows the removal of soluble TOC (TOCs) during this cycle in parallel with the dissolved oxygen concentration. It appears that carbon removal took place exclusively during the aerobic phase. This removal was very fast (4 h) and took place in two stages, according to the dissolved

oxygen curve: the first stage lasted one hour and corresponded to the removal of easily biodegradable compounds. Then a second three-hour stage was probably linked to the removal of more recalcitrant molecules contained in the influent. It appears from Figure 4.5 that the length of the cycles could be halved since, after 12 hours, there was no further removal of TOCs. The purpose of the anoxic period was to allow nitrification/denitrification to take place in the reactor, but no such phenomenon could be observed during the operation cycle of the SBR (data not showed).

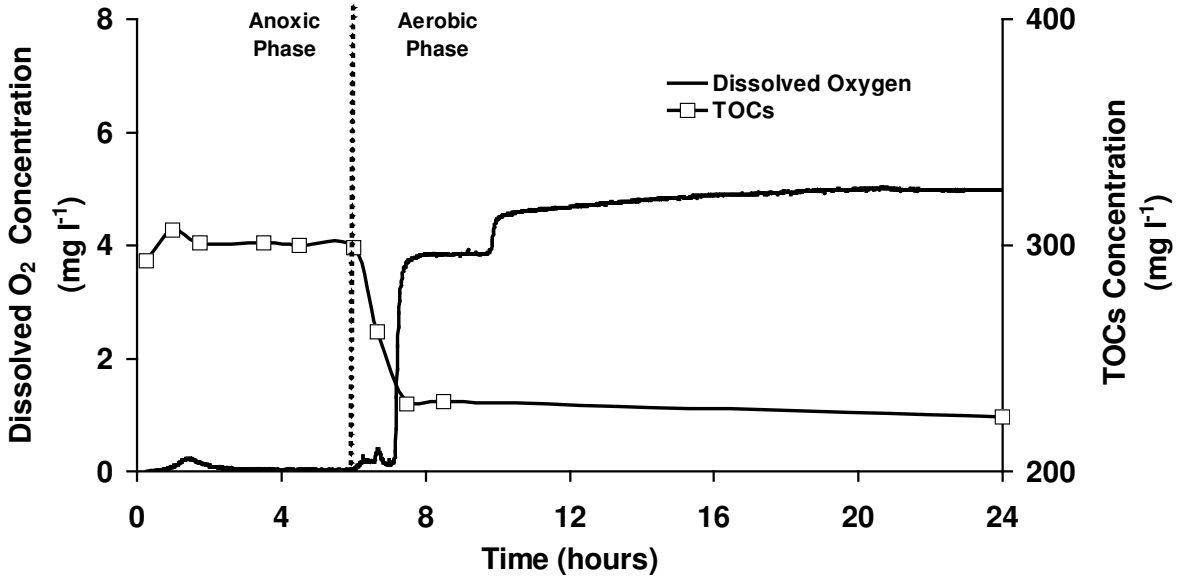

Figure 4.5 Variation in dissolved O_2 concentration and TOCs concentration during day 100 of the operation of an aerobic SBR operating with a tartaric industry effluent.

Dynamics of bacterial communities during the experiment

Profiles of bacterial communities (Figure 4.6) were obtained by PCR-SSCP analysis and their evolution were studied during the experiment. All profiles' areas were large and peaks were not isolated, which indicated high biodiversity. Profile 1 (obtained on day 6) was very different from profiles 2, 3 and 4 (obtained on day 33, 87 and 121): only four peaks (A, B, D and E) on profile 1 were still present later. This first profile probably corresponded to the urban wastewater treatment sludge inoculated on day 1. Then, after halophilic sediments were inoculated on day 17, the profiles changed quickly.

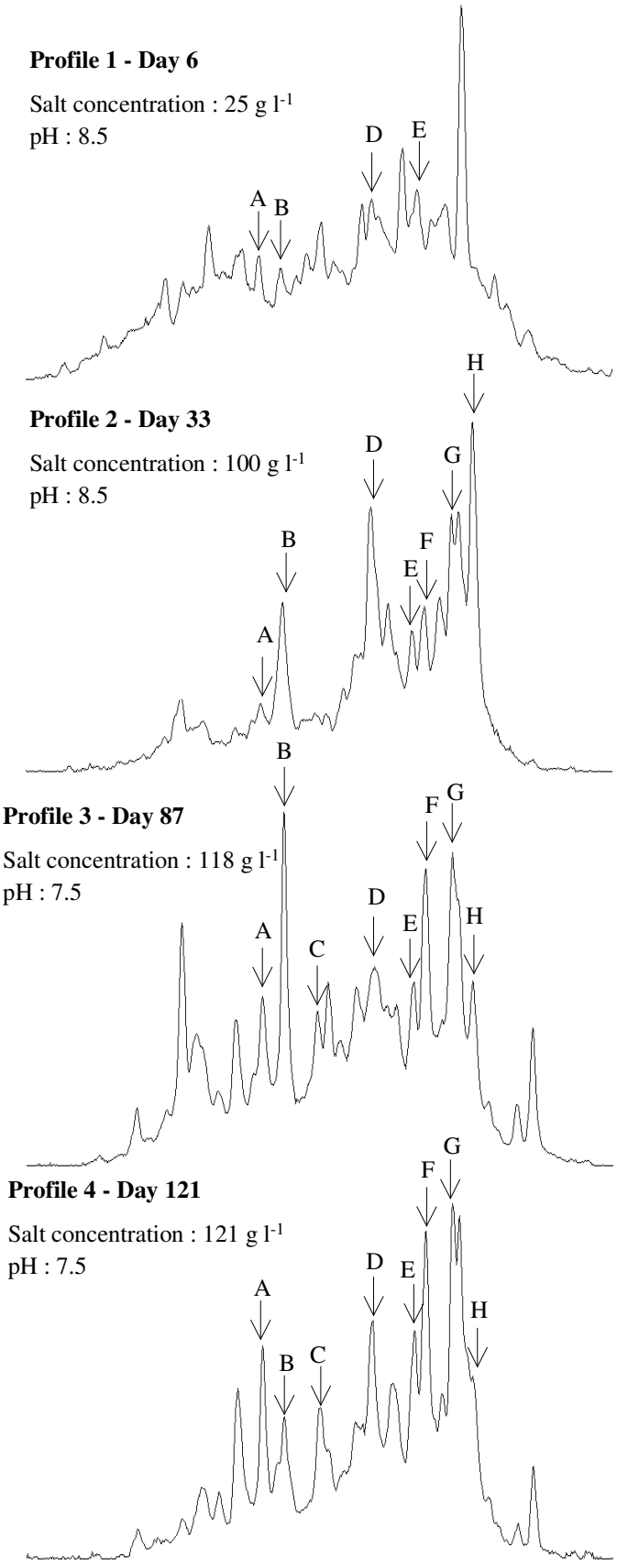

Figure 4.6 Dynamics of SSCP patterns of bacterial 16S rDNA region amplification products of activated sludge operating with tartaric industry effluent.

The area of profile 2 was lesser than the area of profile 1. This indicated a decrease in diversity due to the harsh conditions (high salt concentration and high pH). This period corresponded to microbial adaptation and/or selection. After the pH was neutralized and time passed, the area increased on profile 3, which meant that diversity increased. Finally, profiles 3 and 4 were similar (see peaks A to H. Only the height of the peaks, corresponding to proportions, changed).

Thus, the dynamics of the bacterial communities were an accurate reflection of the evolution during the experiment. The addition of a halophilic biomass completely changed the bacterial community (see the difference between profile 1 and 2). But on profile 2, diversity was less than on profile 1. After acclimation was completed, diversity increased again (see profile 3).

Basically, the diversity (number of dominant 16Sr DNA) of the salt-tolerant ecosystem (profile 4) appeared to be similar to the diversity of the non-salt tolerant ecosystem (profile 1).

Microbial community identification on SSCP patterns on day 121

A 16S rDNA V3 region clone library was established from a bacterial DNA sample and called VM. PCR-SSCP analyses were conducted on 49 bacterial clones for identification. The results of 16S rDNA sequencing analysis are shown in Table 4.3.

Table 4.3 Phylogenetic affiliation of the 16S rDNA sequences of activated sludge operating with tartaric industry effluent.

OTU name	N°. of clones	Divergence within OTU	Division	Closest 16S rDNA	Accession number	Source	Id.
VM40	9	1.2%	γ-Proteo	*Halomonas* sp. BYS-1	AY062217	Unknown	100
VM02	1	0%	γ-Proteo	Uncultured bacterium	AF361651	Deep-sea carbonate crusts	99%
				Halomonas sp. YIM-kkny11	AY121436		98%
VM31	1	0%	γ-Proteo	Uncultured bacterium	AF361672	Deep-sea carbonate crusts	100%
				Halomonas sp. SYM P12	AB085660		99%
VM03	7	1.2%	γ-Proteo	*Marinobacter* sp. DS40M8	AF199440	Subseafloor habitats	100%
VM14	2	0.6%	γ-Proteo	Unidentified eubacterium SCB45	U64022	Marine bacterioplankton	100%
				Marinobacter sp. NK-1	AB026946		98%
VM32	7	0%	γ-Proteo	*Marinobacterium georgiense*	AB021408	Unknown	100%
VM64	1	0%	α-Proteo	*Blastochloris sulfoviridis*	AY117149		98%
VM26	2	0%	α-Proteo	*Sinorhizobium morelense*	AY024335		100%
VM30	2	0.6%	Bacteroidetes	*Marinilabilia salmonicolor*	M62422	Unknown	98%
VM56	1	0%	Bacteroidetes	*Marinilabilia salmonicolor*	M62422	Unknown	96%
VM55	2	0%	Bacteroidetes	Unidentified bacterium	AJ409009	Unknown	93%
				Bacteroides sp.	AB064818		93%

OTU name	N°. of clones	Divergence within OTU	Division	Closest 16S rDNA	Accession number	Source	Id.
VM33	4	1.9%	Bacteroidetes	*Psychroflexus torquis*	AF001365	Antarctic sea ice	94%
VM23	1	0%	Bacteroidetes	Uncultured CFB group bacterium	AF440843	Crude oil-contaminated coastal marsh	92%
				Cytophaga sp.	AB015525		85%
VM50	1	0%	Bacteroidetes	*Flavobacterium salegens*	M92279	Hypersaline Antarctic lake	100%
VM11	3	1.2%	Firmicutes	Unidentified Hailaer soda lake bacterium F24	AF275702	Hypersaline soda lake of Inner Mongolia	92%
				Alkalibacterium olivoapovliticus	AF143512		90%
VM25	1	0%	Firmicutes	*Tetragenococcus halophilus*	AB041349	Fish sauce	98%
VM05	1	0%	Firmicutes	*Haloanaerobium congolense*	U76632	African oil field	98%
VM60	1	0%	Bacteroidetes	Uncultured cytophagales bacterium clone LA7-B21N	AF513957	Hypersaline lake and brackish pond of remote Hawaiian island	93%
VM06	1	0%	Bacteroidetes	Uncultured delta proteobacterium MT23	AF211273	black mud from marine coastal environments	93%
				Cytophaga sp	AB015264		95%
VM51	1	0%	BRC	Uncultured soil bacterium PBS-III-16	AJ390451	Bulk soil and rice roots of flooded rice microcosms	89%

According to 16S rDNA sequencing, the microbial clones belonged to five divisions of *Bacteria*: *Gamma-Proteobacteria* (27 clones), *Alpha-Proteobacteria* (3 clones), *Cytophagales* (13 clones), Gram positive low GC (5 clones), and BRC1 (1 clone). More than half of the clones belong to the division of *Gamma Proteobacteria*. The *Cytophagales* are very well represented, too. Lastly, one clone (VM51) belongs to lineage BRC1 which has been considered as a novel candidate division [55]. Up to the present, this division has included 16S rDNA genes from bulk soil and rice roots of flooded rice environments. VM51 has a sequence similarity of 89% with these 16S rDNA genes from anoxic soil.

Many of the 16S rDNA are close to 16S rDNA of identified marine Bacteria while others are common in oil fields, which are often associated with the presence of saline water. Among them, many *Halomonas sp.* are known for their capacity for biopurification (see introduction) and many *Marinobacter sp.* are known for their capacity in the biological removal of oil hydrocarbons discharged into the marine environment. It can be concluded that these particular Bacteria took part in the removal of pollution in the effluent studied here.

Considering the marine habitat, it has already been reported [70] that most Bacteria identified by molecular methods belong to the divisions of *Proteobacteria* (67%) and *Cytophagales* (25%).Within the framework of this study, 30 clones out of 49 (61%) belong to the division of *Proteobacteria* and 13 clones out of 49 (27%) belong to the division of *Cytophagales*. Thus, the majority of the halophilic 16Sr DNA identified in this study belongs to the same division as those of a marine ecosystem.

The microbial clones clustered within 20 Bacterial OTUs were distributed as follows in the five divisions: *Gamma Proteobacteria*, 6 OTUs – *Cytophagales*, 8 OTUs – Gram + low GC, 3 OTUs – *Alpha Proteobacteria*, 2 OTUs – BRC1, 1 OTU.

For only a few OTUs do the closest 16S rDNA correspond to uncultured or unidentified Bacteria, which may seem surprising. Except for VM51, which belongs to a new division (BRC1), other OTUs belong to previously known divisions. One of the reasons explaining this fact may be the particular attraction of extreme mediums for researchers whose work has thus led them to a good understanding of divisions which include extremophilic Bacteria.

16 OTUs out of 20 correspond to halophilic genes, which indicates that no phenomenon of adaptation occurred but, rather, a selection of halophilic Bacteria. This result was foreseeable given the remarks of Woolard and Irvine already described [236]: non-halophilic Bacteria do not tolerate salt concentrations higher than 50 g l^{-1}.

Final identification tests using primers of *Archaea* gave negative results (data not shown), which means that Bacteria alone were active in the hypersaline reactor.

Conclusions

The data presented in this paper show that halophilic Bacteria can be used to treat hypersaline wastewater. Bacteria taken from the sediments in the evaporation basins of an agri-food plant generating hypersaline effluents were maintained for more than 100 cycles in a SBR. Whereas most of the papers already published have focused on the removal of carbon compounds in synthetic effluents, the Bacteria studied here were able to treat the carbon and nitrogen pollution of a complex effluent produced by the same industry from where they originated. The removal yields reached 83, 88 and 72% for the CODs, TOCs and TKNs, respectively. But high levels of SS remained in the treated effluent.

The results of this experiment have, however, proved the feasibility of treating hypersaline industrial wastewater, providing the parameters of the reactor are fully controlled. The techniques of molecular microbiology have shown the phylogenetic diversity of the halophilic species that developed in the SBR operated for this study. The diversity of a salt-tolerant ecosystem can be similar to the diversity of a non salt-tolerant one.

4.2 Traitement biologique aérobie d'un effluent hypersalin de tannerie

Article : Halophilic biological treatment of tannery soak liquor in a sequencing batch reactor

Résumé

Les effluents hypersalins sont générés par de nombreux secteurs industriels. De tels effluents, riches à la fois en matière organique et en sel (> 35 g l^{-1}) sont difficiles à traiter par les procédés d'épuration aérobie conventionnels. Parmi les industries générant des effluents hypersalins, les tanneries occupent une place importante en Inde. Dans cette étude, un effluent de tannerie résultant du lavage des peaux a été traité dans un réacteur SBR. La caractérisation de l'effluent a montré qu'il était potentiellement biodégradable (bien que difficilement) et très variable selon la nature et l'origine des peaux. La salinité de l'effluent a varié entre 21 et 57 g l^{-1} et la DCO entre 1,5 et 3,6 g l^{-1}. Le réacteur SBR a été inoculé avec des boues halophiles et les performances du procédé ont été évaluées dans différentes conditions opératoires en terme de TSH, cva et concentration en sel. Le réacteur s'est montré plus particulièrement sensible aux changements de salinité. En dépit des variations des caractéristiques de l'effluent d'alimentation, les performances épuratoires du réacteur ont été bonnes, après acclimatation des boues. Ainsi, des rendements épuratoires de 95, 93, 96 et 92% sur DCO, PO_4^{3-}, NTK et MES, respectivement, ont été obtenus avec un TSH de 5 jours, une cva de 0.6 kg DCO m^{-3} j^{-1} et 34 g l^{-1} de NaCl. Les organismes responsables de la dépollution azotée ont été les plus sensibles aux variations de ces paramètres.

Abstract

Hypersaline wastewater (i.e. wastewater containing more than 35 g l^{-1} Total Dissolved Solids (TDS)) is generated by various industrial activities. This wastewater, rich in both organic matter and TDS, is difficult to treat using conventional biological wastewater treatment processes. Among the industries generating hypersaline effluents, tanneries are prominent in India. In this study, tannery wastewater from soak pit was treated in a lab scale SBR for the removal of organic matter. The characterisation of the soak liquor showed that this effluent is biodegradable, though not easily, and highly variable, depending on the origin and the nature of the hides. TDS was in the range of 21 to 57 g l^{-1} and COD was in the range of 1.5 to 3.6 g l^{-1}. This soak liquor was biologically treated

in an aerobic sequencing batch reactor seeded with halophilic Bacteria, and the performance of the system was evaluated under different operating conditions with changes in hydraulic retention time, organic loading rate and salt concentration. The changes in salinity appeared to affect the removal of organic matter more than the changes in hydraulic retention time or organic loading rate. Despite the variations in the characteristics of the soak liquor, the reactor achieved proper removal of organic matter, once the acclimation of the microorganisms was achieved. Optimum removal efficiencies of 95, 93, 96 and 92% on COD, PO_4^{3-}, TKN and SS, respectively, could be reached with 5 days Hydraulic Retention Time (HRT), an Organic Loading Rate (OLR) of 0.6 kg COD m^{-3} d^{-1} and 34 g NaCl l^{-1}. The organisms responsible for nitrogen removal appeared to be the most sensitive to the modifications of these parameters.

Introduction

Hypersaline effluents are generated by various industrial activities. This wastewater, rich in both organic matter and TDS, is difficult to treat using conventional biological wastewater treatment processes [136]. Use of halophilic Bacteria is required [125]. The interest in treating that kind of wastewater is growing at a fast rate.

Among the industries generating hypersaline effluents, tanneries are prominent in India. Tanning is one of the oldest professions in India, with 2000 units spread mostly across Tamil Nadu, West Bengal, Uttar Pradesh, Andhra Pradesh, Karnataka, Rajasthan and Punjab. Leather tanning is almost wholly a wet process from which a large volume of liquid waste is continuously generated. Due to the variety of chemicals added at different stages of processing of hides and skins, the wastewater has complex characteristics. The tanning process and the effluents generated have already been reported in the literature [210; 212; 233] and an overview is presented in Figure 4.7.

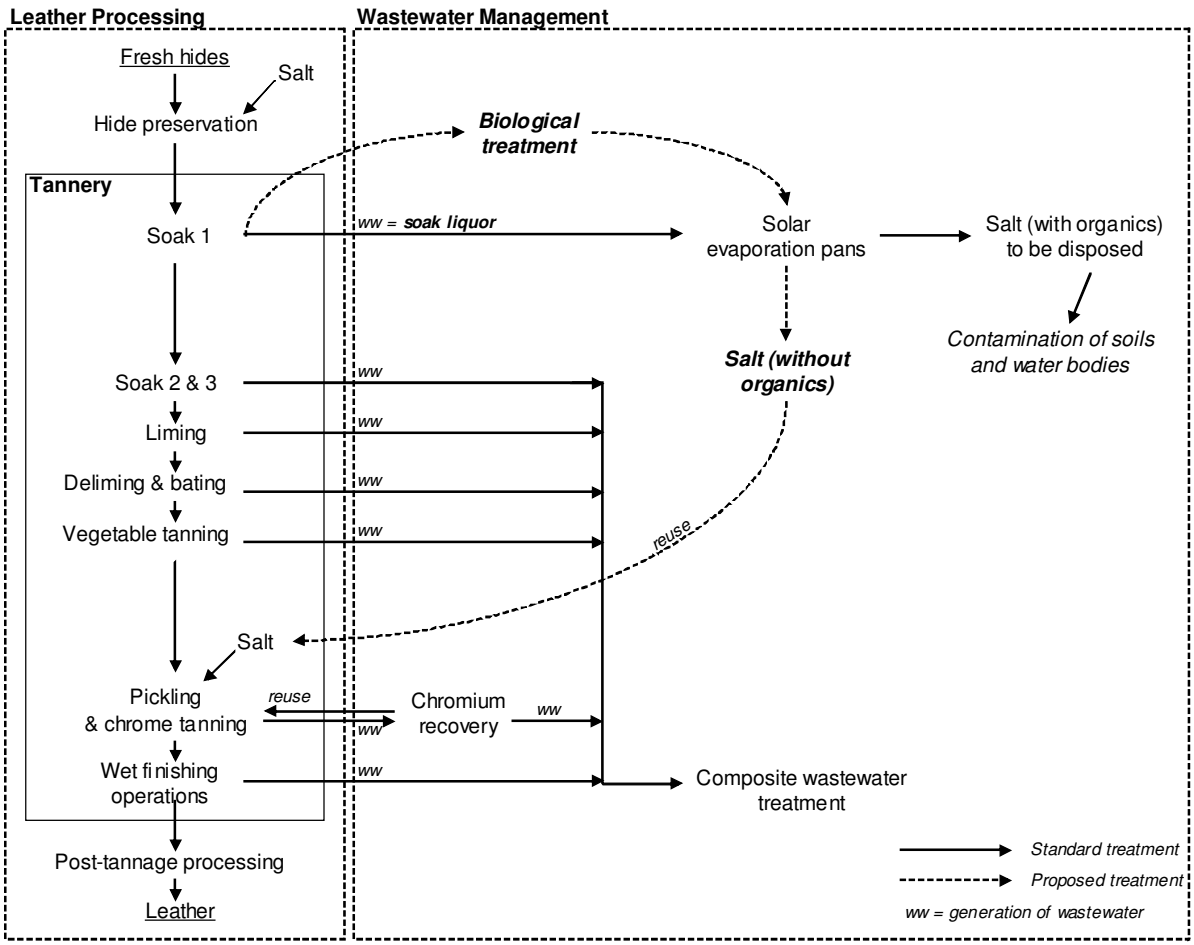

Figure 4.7 Simplified leather production chain and management of the effluents associated.

In this study, tannery wastewater was collected after the soaking of hides and skins. Salt (sodium chloride (NaCl)) is used to preserve the fresh skins from decomposition immediately after they are stripped in the slaughterhouse, and the excess of salt has to be removed in the tannery before further processing. This is done by soaking, using a lot of water, which generates the first source of effluent. This soak liquor is characterised by high organic load, high suspended solids (sand, lime, hair, flesh, dung, etc.) and high salinity. Because of that high salt content, this wastewater is generally segregated and sent to Solar Evaporation Pans (SEPs), as indicated in Figure 4.7. The presence of high concentrations of dissolved organic matter and Suspended Solids (SS) retards the rate of evaporation in SEPs. Thus tanneries require large areas to dispose the soak liquor and the salt obtained cannot be reused because of its high organic content. This salt is then discharged on open land and contributes to soil and water pollution. Treatment of this soak liquor, before sending it to SEPs, in order to remove the excess of organic matter, would accelerate the rate of evaporation, reduce the odour of the effluent and improve the purity of the salt obtained. This salt could then be reused in the tannery itself, during

the pickling stage (treatment of the skins with an aqueous solution of acids and salt, which allows the skins to be further handled without getting swelled). As the amount of salt used for the preservation of the skins exceeds the amount used for pickling, a part of the recovered salt should get another destination (e.g. neighbouring tanneries). Furthermore, improved methods of preservation of hides and skins have been developed that use less amount of salt in admixture with certain biocides and thus restrict the growth of Bacteria to considerable extent. In this case, biological treatment of tannery soak liquor may become more complicated, but, as the amount of salt is reduced, the segregation of the soak liquor may not be required anymore. In some cases, the biocide alone can also be used as a preservation agent.

Degradation of synthetic substrates using halophiles has already often been studied. Panswad and Anan [168] obtained 71% Chemical Oxygen Demand (COD) removal efficiency using an anaerobic/anoxic/aerobic process and a synthetic wastewater containing 3% salt, provided the seeding material was acclimated to high salinity conditions. Dinçer and Kargi [58] treated a synthetic effluent with increasing salt concentrations (0-10%), using an aerobic biological disc system, and could get more than 80% COD removal efficiency, as long as the salt concentration remained below 50 g l^{-1}. The Sequencing Batch Reactor (SBR) is known to be a robust system that stands harsh conditions [92]. It is not surprising that this process has often been used in order to treat saline wastewater. Woolard and Irvine [235] inoculated a sequencing batch biofilm reactor (SBBR) with moderate halophiles, recovered from the Great Salt Lake, in order to treat a synthetic effluent containing 150 g salt l^{-1}. The removal efficiency measured on phenol exceeded 99%. They renewed the experiment [236] with a SBR with free culture that reached an average removal efficiency of 99.5%. Uygur and Kargi [219] used SBR to treat a saline synthetic effluent and noticed that COD removal efficiency decreased from 90 to 32% when salinity increased from 0 to 6%.

Fewer experiments have been conducted on the degradation of complex wastewater in halophily, and, once again, SBR has often been used for that purpose. Kubo *et al.* [120] removed 90% of the COD contained in pickled plum production plant effluent using aerobic SBR. Moon *et al.* [152] treated a 1% salt containing seafood wastewater by SBR, reaching 87.9% COD removal efficiency. Lefebvre *et al.* (see section 4.1) treated an effluent related to the tartaric industry with a SBR and obtained up to 83% COD removal efficiency, provided the pH was neutralised.

In this study, a tannery wastewater (soak liquor) was treated, using an aerobic SBR and halophilic micro-organisms, in order to remove organic matter (carbon, nitrogen, and phosphorus) and suspended solids from the effluent.

Materials And Methods

Influent

The tannery influent (soak liquor) was collected from soak pits in a tannery around Chennai (India). Eleven different samples of soak liquor were collected and used as an influent for the bioreactor during the experimental period.

Bioreactor

An aerobic SBR was operated during 300 days to remove the organic matter from the soak liquor. The lab-scale SBR, illustrated in Figure 4.8, had a volume of 10 l. Tubes were inserted into the top of the reactor to ensure the filling and withdrawal of the effluent using peristaltic pumps. An air compressor delivering airflow of 1.2 l min^{-1} supplied aeration. The ambient temperature of the wastewater in the reactor was close to 30°C. Each cycle lasted for 24 hours: the reaction took place in 22 hours, the settling in 1 hour 30 minutes and the withdrawal and filling of the treated effluent and influent in 30 minutes.

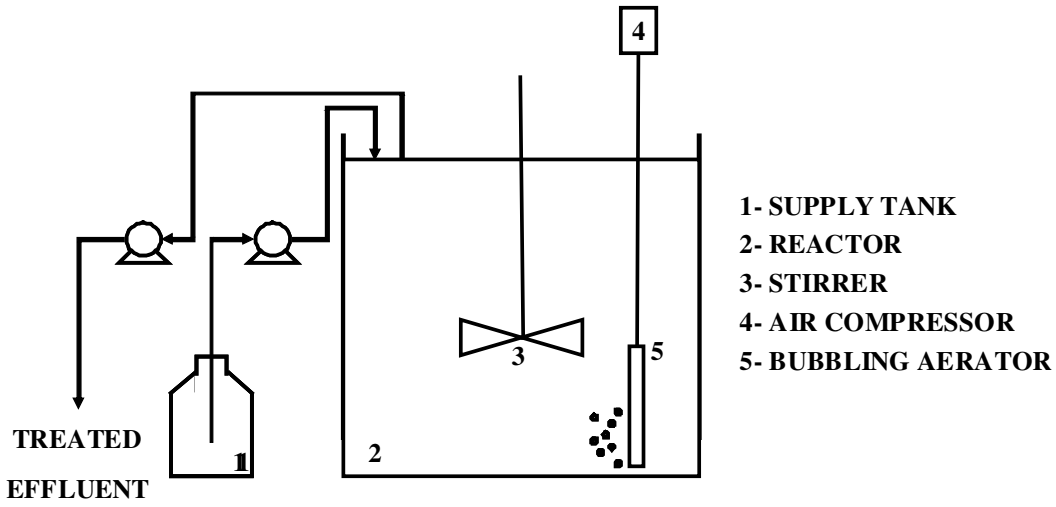

Figure 4.8 Lab-scale aerobic SBR operating with tannery soak liquor.

The influent was delivered to the bioreactor at a flowrate of 2 l d^{-1} until day 100, then at a rate of 3 l d^{-1} between day 100 and 270, finally 4 l d^{-1} until the end of the experiment. Consequently, the hydraulic retention time (HRT) was 5 days until cycle 100, then was reduced to 3.3 days and finally to 2.5 days at the end of the experiment.

The sludge was allowed to accumulate in the reactor. However, in certain periods (between day 189 and 214, and between day 242 and 277), sludge withdrawal was done

by cancelling the settling phase, thus maintaining the sludge mixed in the reactor all over the cycle. Consequently, sludge was withdrawn along with the effluent. In these periods, the sludge was then allowed to settle during 1 hour 30 minutes in a beaker before collecting the supernatant, which was considered as the treated effluent when measuring all analytic parameters (e.g. total COD).

Inoculum

The reactor profited from a quadruple inoculation, which accelerated the installation of a halophilic microbial population and also increased the biodiversity in the reactor. The biological sludge obtained from the secondary basin of the Nesapakkam sewage treatment plant (Chennai, India) was added to the reactor on day 1. Biological sludge from the secondary basin of the common effluent treatment plant of Pallavaram (Chennai, India), treating the composite effluents of 128 tanneries, was also added on day 1. Later, a liquid sample presumably rich in halophilic Bacteria was added to the reactor on day 30. This was collected from a textile effluent treatment plant in the area of Tirupur (India). Finally, sediments collected at the bottom of a salt collection basin in Kelambakkam (Chennai, India) were added on day 30.

Analysis

COD, BOD_5, TKN, $N\text{-}NH_3$, $P\text{-}PO_4^{3-}$, TDS, SS, MLVSS and pH were analysed following APHA's Standard Methods for the Examination of Water and Wastewater [9]. Quality control was ensured using standards as well as duplicates. COD was determined by the open reflux method. Mercuric sulphate was used to eliminate the interference of chlorides when dosing COD. Sawyer and McCarty [193] reported that this interference could be eliminated as long as a 10/1 weight ratio of mercuric sulphate to chloride is maintained. Soluble COD (CODs) was obtained after filtration using 0.45 µm glassfiber filters. Determination of ammonia was done, using the titrimetric method, and phosphates were determined with the ascorbic acid method.

Results and Discussion

Characterisation of the soak liquor

The influent wastewater (soak liquor) was characterised with common parameters (pH, TDS, COD, TKN, etc.). The mean and standard deviations were calculated using 11 different influents during the experimental period. Main results are plotted in Table 4.4. The soak liquor is characterised by substantial organic matter content and high SS content, resulting in an average total COD concentration of 2,200 mg l^{-1} and a SS concentration of 5,300 mg l^{-1}. Very high salinity was reflected by an average TDS concentration of 37,000 mg l^{-1}. TKN, N-NH_3 and PO_4^{3-} averaged 273, 153 and 21 mg l^{-1}, respectively. Finally, pH averaged 7.7. Table 4.4 shows great variability in the quality of the influent, reflected by high standard deviation values. Great variability was observed with respect to the influent, depending on the type of hides and skins and the region from which they came, at the time of the sampling.

Table 4.4 Characterisation of 11 influents coming from the same tannery.

	Influent 1	Influent 2	Influent 3	Influent 4	Influent 5	Influent 6	Influent 7	Influent 8	Influent 9	Influent 10	Influent 11	**Mean**	*Standard Deviation*
pH	-	7.7	7.8	7.9	7.7	7.8	7.7	7.5	7.5	7.6	-	**7.7**	*0.2*
TDS	57300	35100	25800	27800	33000	51700	25300	30200	38600	45900	34000	**36800**	*8600*
SS	10300	3900	7000	6400	7700	3700	2500	3600	7600	5300	4000	**5300**	*2400*
VSS	2600	700	900	1900	1800	1700	500	700	2000	500	800	**1300**	*700*
Total COD	2000	2800	2600	3000	3600	2200	1600	1500	1900	1700	2600	**2200**	*700*
Soluble COD	600	900	600	500	1400	1100	900	800	400	900	900	**800**	*300*
Total TKN	-	300	200	310	470	350	140	140	-	-	-	**270**	*120*
Soluble TKN	-	170	160	190	230	250	90	80	-	-	-	**170**	*70*
Total NH_3	-	90	160	190	280	230	60	70	-	-	-	**150**	*90*
Soluble NH_3	-	50	130	120	190	170	40	40	-	-	-	**100**	*60*
Total PO_4^{3-}	4	15	19	21	28	23	25	41	31	18	8	**21**	*10*
Soluble PO_4^{3-}	5	13	2	6	5	11	8	11	7	6	4	**7**	*3*

Some useful relationships between parameters were calculated. According to it, soluble COD averaged 37% (± 14%) of total COD, which indicates that very little amount of COD is soluble. Most of it must be included in the dungs that come along with the hides and skins. The BOD_5/COD ratio was 0.3, which was very low in comparison to domestic wastewater (i.e. 0.5). Therefore, the biodegradability of the influent was found to be low, according to the criteria of Ahn *et al.* [3]. However, BOD_5 is a controversial parameter, when it is applied to tannery wastewater, since it contains many inhibitors of BOD_5 [13]. The VSS/SS ratio averaged 0.2 ± 0.1, therefore was found to be very low, due to the numerous fibres and inorganic particulate (sand, dust) escaping the soak pit. The VSS/(total COD- soluble COD) ratio averaged 1.2 ± 0.7, thus indicated that every kg of VSS contributed to 1.2 kg of particulate COD. Finally, the COD/N/P ratio averaged 200/22/2 and showed that the soak liquor contained high amounts of nitrogen but lesser amounts of phosphorus. However, no phosphorus deficiency could be identified and this ratio is close to that of domestic wastewater (i.e. 200/22/3.5).

It can then be concluded that the influent used in this experiment was highly variable and suffered from low biodegradability and high inorganic solids content (both soluble and suspended). This showed that high salt content would not be the only obstacle for the good operation of a bioreactor. Yet, the pH and COD/N/P ratio in the soak liquor enabled the bioreactor to be operated without any pH regulation and without adding nutrients such as nitrogen or phosphorus, which would have increased the running costs.

Evolution of HRT, OLR and TDS concentration

High variability in the organic content (reflected by COD concentration) and salinity (reflected by TDS concentration) of the soak liquor might make the proper operation of a biological treatment plant uneasy, causing important disturbance in the equilibrium of the microbial community. Yet, looking forward to applying the process at an industrial scale, decision was taken not to artificially change the influent characteristics in order to make it more homogenous. This choice resulted in frequent changes in the environmental conditions in the bioreactor. In addition, HRT was progressively decreased from 5 to 2.5 days during the experiment, as shown in Figure 4.9, by increasing the influent flowrate. Consequently, OLR ranged from 0.4 to 1.1 kg COD m^{-3} d^{-1}. TDS ranged from 21,200 to 94,300 mg l^{-1}.

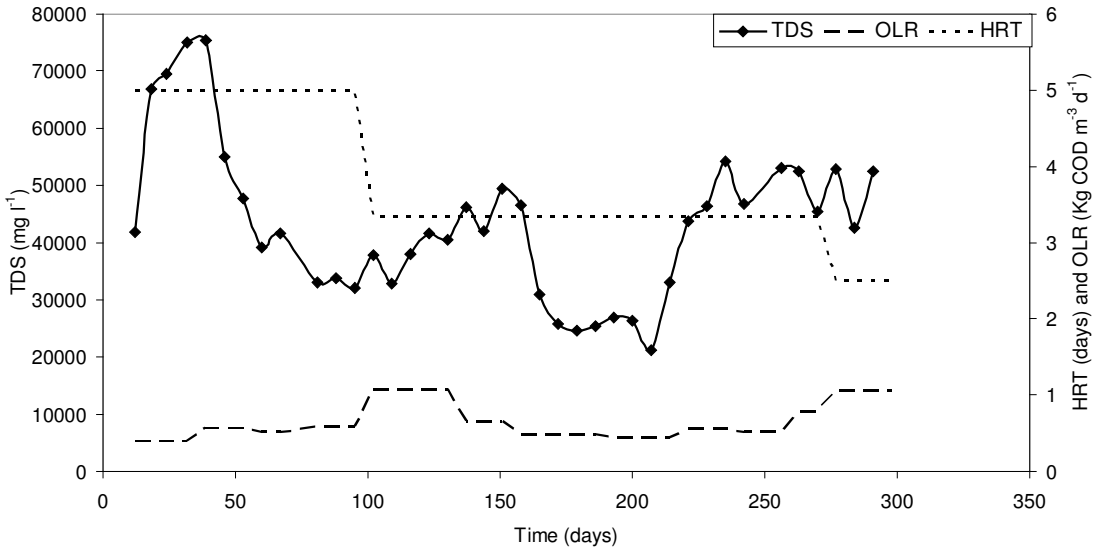

Figure 4.9 Evolution of HRT, OLR and TDS concentration in the SBR operating with tannery soak liquor during the experiment.

COD removal

COD removal by the SBR is reported in Figure 4.10, where the influent wastewater and the treated effluent concentrations are indicated. The influent concentrations are obtained from Table 4.4, for each influent sample used during the experiment. The starting phase, during which salinity was increased gradually to reach that of the real effluent, lasted for about 30 days. At that time, COD concentration was very high in the treated effluent (960 mg l^{-1}), and leading to removal efficiencies as low as 52%. After 30 days, the removal of COD quickly increased up to day 81. At that time, HRT was 5 days, OLR was 0.6 kg COD m^{-3} d^{-1} and TDS concentration was 34 g l^{-1}. Under these environmental conditions, the COD concentration in the treated effluent attained 140 mg l^{-1} (95% COD removal efficiency). This performance then stabilised over 20 days.

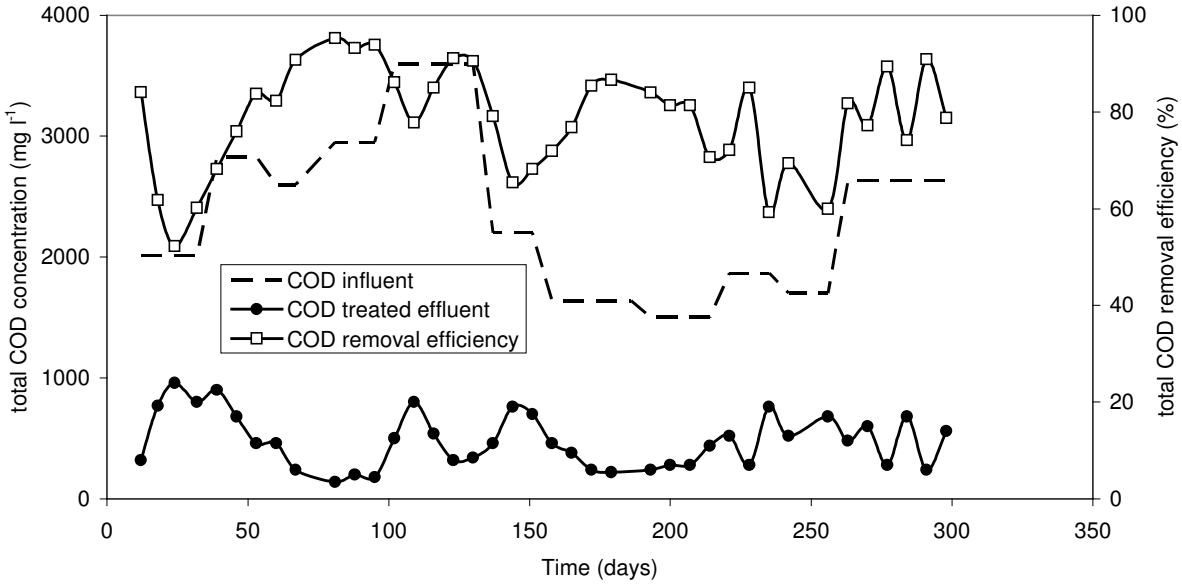

Figure 4.10 Evolution of the total COD concentration in the influent and treated effluent and evolution of the COD removal yield during the operation of an aerobic SBR on tannery soak liquor.

On day 100, the flowrate of the feed was increased from 2 to 3 l d^{-1}. Consequently, HRT decreased to 3.3 days and OLR increased to 1.1 kg COD m^{-3} d^{-1}. Consequently, the COD removal reduced in the first ten days, COD removal efficiency falling down to 78%. Then COD removal increased again, once the acclimation to the new HRT and OLR was done. On day 123, the COD concentration in the treated effluent attained 320 mg l^{-1} (91% COD removal efficiency). This performance then stabilised over 14 days.

On day 137, OLR decreased to 0.7 kg COD m^{-3} d^{-1}, due to a lesser COD concentrated influent wastewater (influent No. 6). It is worthy of note that, at that time (day 137), TDS concentration reached one of its highest value, i.e. 46000 mg l^{-1}, due to the high salinity of influent No 6. This resulted in a new decrease of the COD removal. Then COD removal increased again, as a lesser saline influent wastewater (influent No. 7) was used. On day 179, good performance on COD removal could be attained again. At that time, OLR was 0.5 kg COD m^{-3} d^{-1} and TDS concentration was 25.7 g l^{-1}, and this resulted in a treated effluent containing as less as 220 mg l^{-1} COD (87% COD removal efficiency). This COD removal efficiency remained over 80% during the next 35 days, all environmental parameters remaining more or less constant over that period of time.

Finally, on day 214, TDS concentration started to increase again, due to a more saline influent wastewater (influent No. 9) and remained high until the end of the experiment (influents No. 10 and 11). This TDS raise resulted in a new disturbance that reduced the

efficiency of COD removal. No stabilisation could be observed any more and it is worthy to note that a last modification done on day 277, HRT being reduced from 3.3 days to 2.5 days and OLR being increased from 0.8 kg COD m^{-3} d^{-1} to 1.1 kg COD m^{-3} d^{-1}, did not affect the performance of the system. Therefore, in this last period of time, the COD concentration in the treated effluent varied from 240 to 680 mg l^{-1} (74 to 91% COD removal efficiency).

Specific impact of TDS concentration on COD removal

Monitoring the performance of the SBR in removing COD over 300 days, it appeared clearly that this performance was depending upon the environmental conditions in the reactor and overall upon the TDS concentration. The specific effect of TDS concentration on COD removal was then further analysed. Figure 4.11 shows that the COD concentration in the treated effluent increased and the COD removal efficiency decreased when the TDS concentration increased in the reactor. Up to 50 g TDS l^{-1}, the COD concentration in the treated effluent averaged 390 mg l^{-1} (82% COD removal efficiency). At TDS concentrations higher than 50 g l^{-1}, the average COD concentration was 810 mg l^{-1} (63% COD removal efficiency). It is worthy of note that, on the contrary, no correlation was found between the COD concentration in the influent and in the treated effluent (data not showed). Consequently, these results show the strong inhibitor effect of the highest TDS levels on the performance of the bioreactor. The microbial consortium was unable to adapt efficiently to the highest TDS levels. Yet, the increase in biomass was not affected by TDS concentration (data not showed). As biomass was maintained, only its ability to remove COD was reduced under the highest salinity conditions. This explains why, when TDS concentration decreased again, depending on the influent used during the experiment, recovery was fast. Dinçer and Kargi [58], who noticed a rapid deterioration of a biological disc system in removing COD as soon as salinity reached 50 g l^{-1}, have already indicated this limit value of 50 g salt l^{-1}.

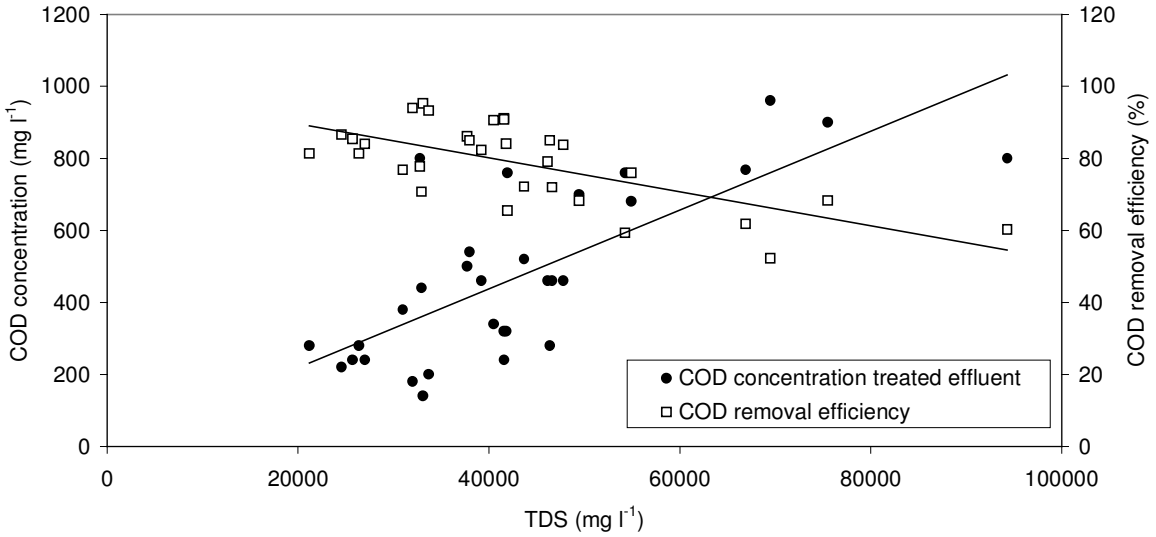

Figure 4.11 Evolution of COD concentration in the treated effluent and COD removal yield with TDS concentration during the operation of an aerobic SBR on tannery soak liquor.

It has already been shown in the literature that good performance could be obtained even with more than 50 g salt l^{-1}. Woolard and Irvine [236] could remove 99.5% of the phenol contained in a 15% salt containing wastewater in SBR. Lefebvre *et al.* (see section 4.1) could remove 83% of the soluble COD of a 12% salt containing wastewater. But it seems that, in our experimental conditions, under fluctuating salinity conditions, the microbial consortium was unable to adapt adequately to the strongest TDS concentrations.

Study of a SBR cycle

A study of a SBR cycle was carried out during day 250. The removal of soluble COD during this cycle in parallel with the dissolved oxygen concentration is shown in Figure 4.12. In five minutes, soluble COD reduced from 350 to 200 mg l^{-1}, which indicates that 40% of the soluble COD disappeared immediately from the liquid phase and was probably adsorbed on to the biomass. After two hours thirty minutes, soluble COD reduced to 120 mg l^{-1}, as the readily biodegradable COD was consumed. At the end of the cycle, soluble COD reduced to 80 mg l^{-1}, after the slowly biodegradable COD was removed.

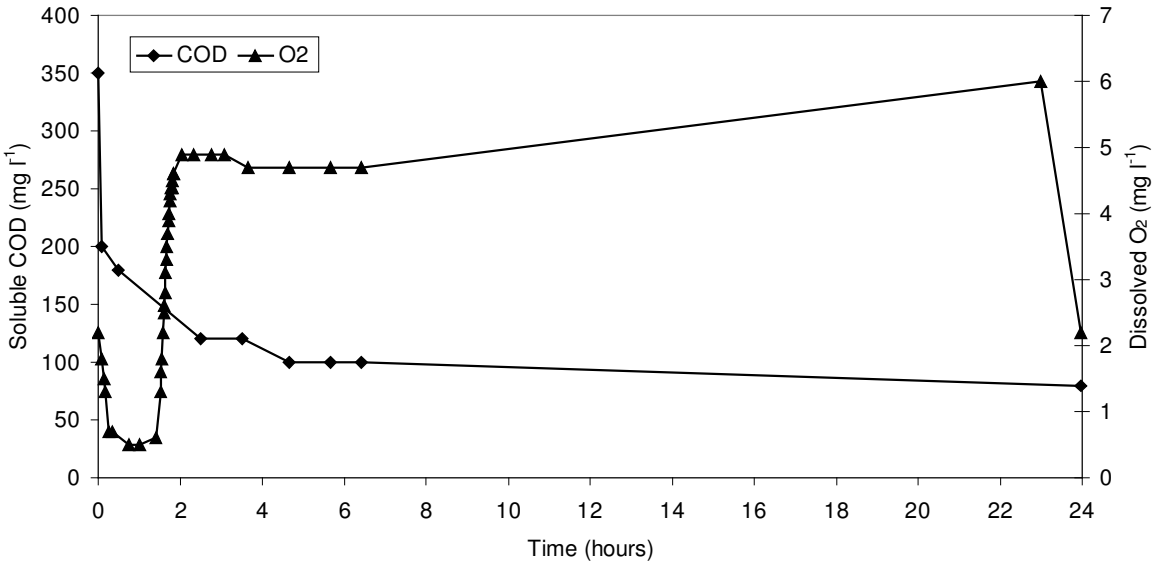

Figure 4.12 Evolution of soluble COD and dissolved O_2 during one cycle of operation of an aerobic SBR operating with tannery soak liquor.

During the first two hours, dissolved oxygen concentration was around 0.7 mg l^{-1}, due to the consumption of the readily biodegradable soluble COD. Then, dissolved oxygen concentration quickly increased up to 5 mg l^{-1} within 30 minutes and progressively reached 6 mg l^{-1} after 23 hours. This last increase was mainly due to the endogenous respiration and to the consumption of slowly biodegradable soluble COD, this last phenomenon being less than the previous one. The last hour of the cycle was dedicated to settling: no more oxygen was added to the reactor and the dissolved oxygen quickly decreased.

Phosphorus removal

After a starting phase of about 30 days, the performance of the SBR in removing PO_4^{3-} increased (data not shown). Every change in the HRT, OLR or TDS affected the removal of PO_4^{3-} in the same way as it affected the removal of COD. Consequently, the PO_4^{3-} concentration in the treated effluent ranged from 0.5 to 10 mg l^{-1}, resulting in a PO_4^{3-} removal efficiency ranging from 50 to 95%. The amount of PO_4^{3-} removed averaged 1.0 ± 0.2 mg for 100 mg of COD removed.

Nitrogen removal

Different observations can be done concerning the removal of TKN and the results are plotted in Figure 4.13. After a starting phase of about 30 days, the performance in

removing TKN quickly improved up to day 100. At that time, 11 mg l^{-1} of TKN only remained in the treated effluent (96% TKN removal efficiency). After day 100, the amount of TKN in the input suddenly rose because of the increase in the influent flowrate and salinity increased too. This resulted in a sudden deterioration of the performances of the SBR in removing TKN. During that period, running from day 100 to day 137, the amount of TKN remaining in the treated effluent averaged 195 mg l^{-1} (54% TKN removal efficiency). Then, even when the quantity of TKN got reduced again, after day 137, and when salinity decreased, the performance of the SBR in removing TKN could not improve. During the last period, running from day 150 to day 200, the TKN concentration in the treated effluent averaged 76 mg l^{-1} (46% TKN removal efficiency). Similar results and conclusions could be drawn with NH_4^+ (data not shown), this parameter being approximately 50 to 60% of TKN. The amount of TKN removed averaged 11.0 ± 0.2mg for 100 mg of COD removed before the first shock (day 100) but only 5.1 ± 1.2 mg for 100 mg of COD removed at the end of the experiment. The amount of TKN removed at the end of the experiment (i.e. 5.1 ± 1.2 mg TKN for 100 mg of COD removed) is a typical value for assimilation of nitrogen. It can then be assumed that, before the first shock (day 100), nitrification took place but, after the shock, only assimilation occurred.

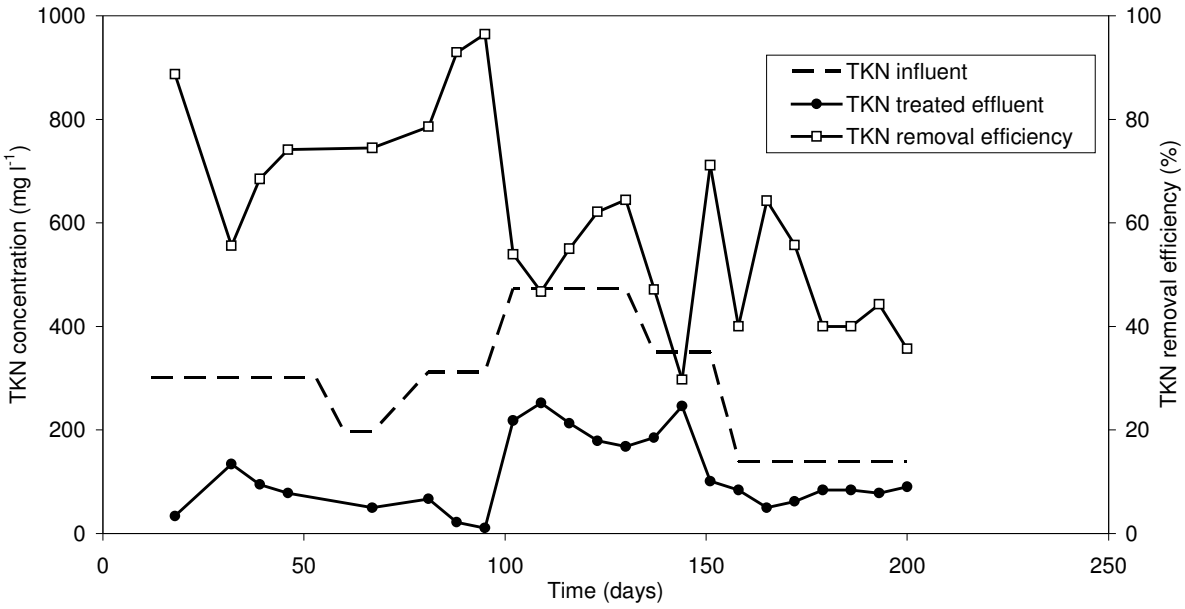

Figure 4.13 Evolution of TKN concentration in the influent and treated effluent and evolution of TKN removal yield during the operation of an aerobic SBR on tannery soak liquor.

These results show that the SBR was able to achieve proper removal of TKN under weak load conditions (i.e. Ammonia Loading Rate (ALR) = 40 mg $N\text{-}NH_3$ l^{-1} d^{-1}) and 32 g TDS l^{-1}. Then, when the TDS concentration and the load increased after day 100, the

removal of TKN decreased. Finally, even when the salt and load were reduced again, the recovery was low, which shows that the bacterial community had suffered from the change in salt and load. Furthermore, because the excess COD was consumed (see Figure 4.10) and not the excess TKN, the COD/N balance was changed, leading to a different behaviour of the bacterial community. It can be assumed that, under weak load conditions, some nitrification took place, but, due to the change in the salt and load, nitrifiers then disappeared.

The inhibition of the organisms responsible for nitrogen removal (mainly nitrifiers) under highly saline conditions has already been studied: Dahl *et al.* [47] found that nitrification could take place under operational conditions up to 20 g Cl l^{-1}, with a maximum nitrification rate of 2 mg N g VSS^{-1} h^{-1}. In addition, they found that a rapid increase of chloride concentration inhibited the nitrifiers. Vredengbregt *et al.* [227] showed that nitrification was possible up to at least 34 g Cl l^{-1} in a fluid bed reactor, provided Ammonia Loading Rate (ALR) was maintained at 15 mg NH_3 l^{-1}_{bed} h^{-1}. Panswad and Anan [168] found that nitrification activity was reduced from 85 to 70% when salinity rose from 5 to 30 g l^{-1}, using salt acclimated seeds, and that the impact was more noticed at the low salt doses of 0-5 g l^{-1} than at the higher runs. Campos *et al.* [34] showed the mixed inhibition effect of salt and ammonia on nitrification: ammonia accumulation started at an ALR of 3 g l^{-1} d^{-1} and a total saline concentration of 525 mM (13.7 g NaCl l^{-1}, 19.9 g $NaNO_3$ l^{-1} and 8.3 g Na_2SO_4 l^{-1}). In our conditions, 96% TKN removal efficiency could be attained under ALR of 40 mg N-NH_4^+ l^{-1} d^{-1} and a TDS concentration of 34 g NaCl l^{-1}. Under these highly saline conditions, a higher ALR of 84 mg N-NH_4^+ l^{-1} d^{-1} was responsible for a loss in the TKN removal efficiency and no complete recovery could be attained when ALR was reduced again.

SS removal

Evolution of solids in the inlet and outlet of the SBR is shown in Figure 4.14. The first 25 days were characterised by high SS amount in the output. This was due to the starting of the experiment, when increasing salt concentration resulted in the low settling of the micro-organisms and their withdrawal with the output. Then, the performance of the SBR in removing SS increased. On day 67, the SS concentration in the outlet reached 580 mg l^{-1} (92% SS removal efficiency), then stabilised over 30 days. Then, with every sudden change in OLR and TDS concentration (see Figure 4.9), the performance of SBR in removing SS fluctuated. A major perturbation occurred after day 137, when the TDS concentration suddenly raised, though OLR suddenly decreased from 1.1 kg COD m^{-3} d^{-1} to 0.7 kg COD m^{-3} d^{-1}. This resulted in the withdrawal of substantial amounts of SS, as shown in Figure 4.14. This resulted in higher amounts of COD (see Figure 4.10) and TKN

(see Figure 4.13) in the treated effluent in the days following day 137. Basically, SS concentration averaged 910 mg l^{-1} in the last 100 days of experiment (79% removal efficiency), which still indicates high turbidity. Approximately 20% of the SS found in the treated effluent were VSS.

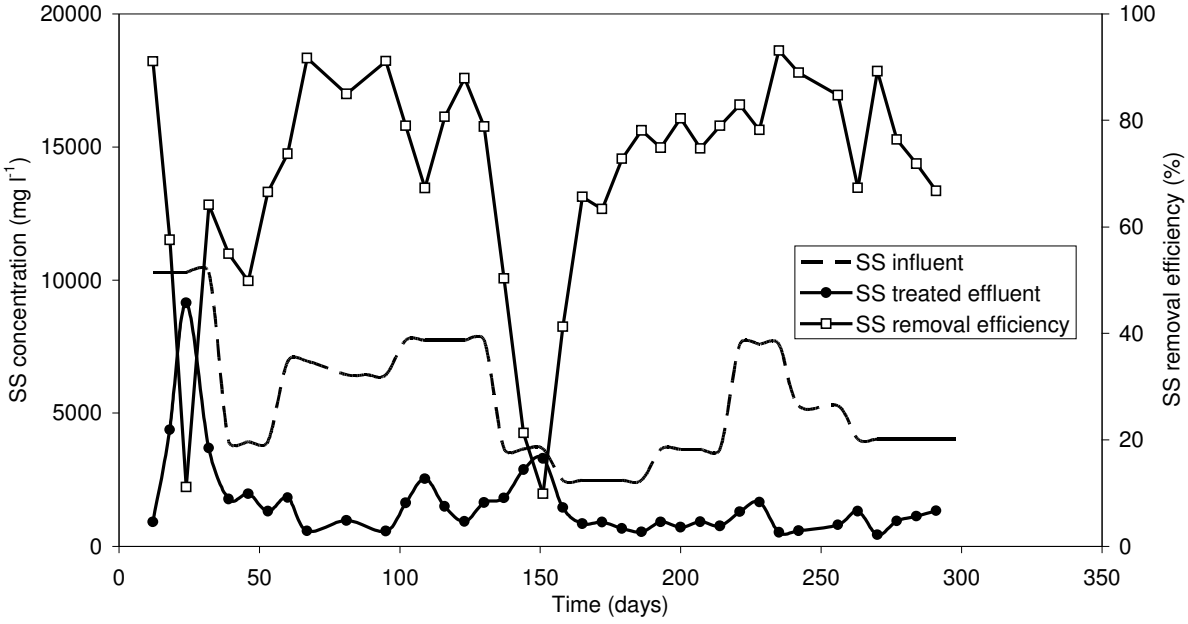

Figure 4.14 Evolution of SS concentration in the influent and treated effluent and evolution of SS removal yield during the operation of an aerobic SBR on tannery soak liquor.

High turbidity is very common in hypersaline effluents, as noticed in the literature. Lefebvre *et al*. (see section 4.1) successfully treated a 120 g l^{-1} salt containing tartaric industry effluent but the SS still averaged 1,800 mg l^{-1} after treatment. Woolard and Irvine [236] gave several reasons to explain this fact, related to the low mechanical integrity of the flocs, as well as to the high density of salt water.

Biomass production

Biomass concentration, measured as MLVSS, was followed and the results are plotted on Figure 4.15. After 50 days dedicated to the starting of the experiment, including the increase in salt concentration, the accumulation of MLVSS averaged 110 mg l^{-1} d^{-1} up to day 189. At that time, MLVSS concentration averaged 18 g l^{-1}. Sludge withdrawal was then initiated by cancelling the settling step, thus removing MLVSS during the withdrawal of the treated effluent. Sludge withdrawal was stopped on day 214, when MLVSS concentration was only 2 g l^{-1} then initiated again from day 242 to day 277. After acclimation of the sludge, the Sludge Volume Index (SVI) decreased and stabilised

around 25 ml g $MLVSS^{-1}$ (see Figure 4.15), which indicated a very good settleability of the sludge. This good property made it possible to retain high sludge concentration (up to 21 g l^{-1}). These results are in accordance with the literature, which shows that high salinity is not an obstacle to biomass growth. Panswad and Anan [168] did not notice an impact of high salinity (30 g l^{-1}) on MLSS in a bioreactor, provided the seeds are acclimated. Kubo *et al*. [120] could also get proper cell growth up to 15% salinity. Furthermore, Panswad and Anan [168] did not notice any significant impact of high salt concentrations and saline shocks on sludge settleability. Campos *et al.* [34] also showed that high salt concentration do not have long term effects on the physical properties of the sludge. They were able to maintain 20 g VSS l^{-1} in an activated sludge reactor, thanks to a SVI value of 11 ml g VSS^{-1}. Finally, Uygur and Kargi [219] found that SVI increased with increasing salt content, but the SVI value (97 ml g^{-1}) obtained at 6% salt in a SBR indicated good settling properties even at high salt content.

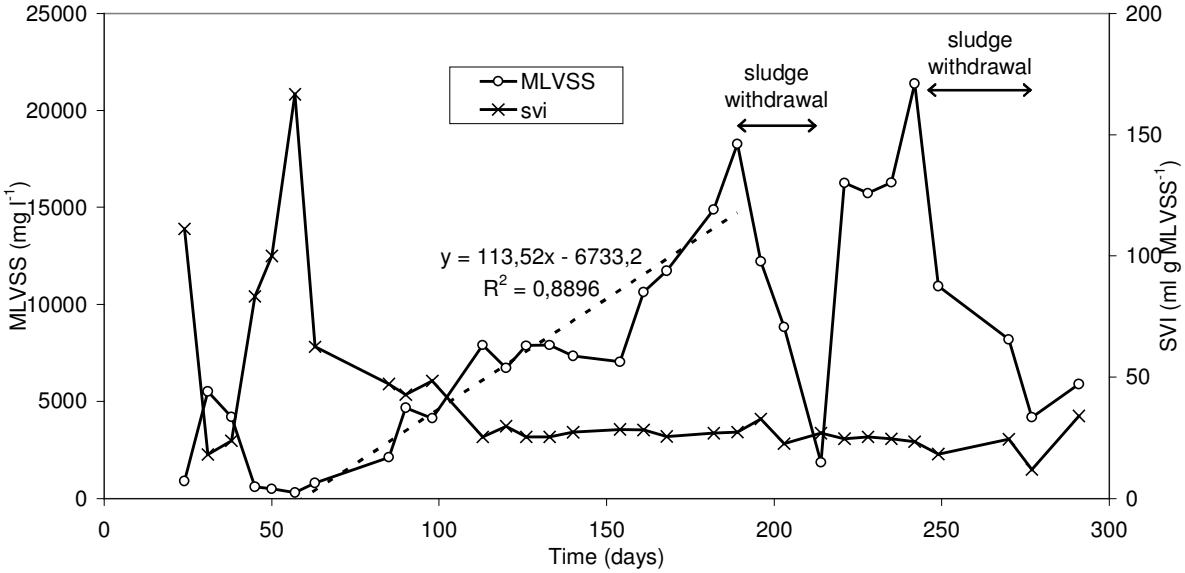

Figure 4.15 Evolution of SVI and biomass concentration during the operation of an aerobic SBR on tannery soak liquor.

Comparison with a SBR treating domestic wastewater

The performance of the SBR operated in this study was finally compared to the performance of a SBR treating domestic wastewater. Ng *et al.* [159] operated an aerobic SBR on domestic sewage with an OLR of 0.9 kg COD m^{-3} d^{-1} and 0.4 days HRT. The characteristics of the influent used in that study were 331 mg total COD l^{-1}, 119 mg SS l^{-1}, 42 mg TKN l^{-1} and 5.7 mg PO_4^{3-} l^{-1}. The effluent concentrations after treatment of that influent and the corresponding removal efficiencies are shown in Table 4.5, along with

the results obtained for the treatment of tannery soak liquor at two different OLRs. It can be seen from Table 4.5 that the removal efficiency of all parameters can be higher for the treatment of tannery soak liquor than for the treatment of sewage. Of course, since the influent concentrations were higher in the case of tannery soak liquor than in the case of domestic wastewater, the treated effluent concentrations remained higher. It can then be concluded that, in the case of a stable halophilic system, the performance of a highly saline system can be similar to the performance of a non saline one.

Table 4.5 Effluent quality and corresponding removal efficiency for SBR operating with domestic wastewater and tannery soak liquor at two different OLRs.

	Tannery Soak Liquor		**Tannery Soak Liquor**		**Domestic Wastewater**	
OLR	0.6 kg COD m^{-3} d^{-1}		1.1 kg COD m^{-3} d^{-1}		0.9 kg COD m^{-3} d^{-1}	
HRT	5 d		3.3 d		0.4 d	
	Effluent Concentration (mg l^{-1})	Removal Efficiency (%)	Effluent Concentration (mg l^{-1})	Removal Efficiency (%)	Effluent Concentration (mg l^{-1})	Removal Efficiency (%)
COD	140	95	320	91	26	81
TKN	11	96	195	54	12	83
PO_4^{3-}	1	93	2.3	92	5.8	0
SS	580	92	3280	82	15	87

Conclusion

This study proved the feasibility of treating a hypersaline tannery soak liquor using halophilic Bacteria in order to remove the organic matter from the effluent. A SBR was operated during 300 days on tannery soak liquor and the evolution of the reactor in removing different parameters (COD, TKN, PO_4^{3-}, and SS) was monitored. The main conclusions are as follows:

(1) The characteristics of the tannery soak liquor showed low biodegradability, high inorganic suspended solids, high nitrogen content but fairly low phosphorus content. In addition, this effluent appeared to be highly variable depending on the origin and the nature of the hides.

(2) The salt concentration appeared to be the main factor that affected the reactor's performance. The COD, PO_4^{3-}, TKN and SS removal efficiencies decreased when salinity

increased. 50 g NaCl l^{-1} appeared to be a limit value for the proper operation of the system.

(3) Optimum removal efficiencies were attained under a low OLR of 0.6 kg COD m^{-3} d^{-1}, 5 days HRT and 34 g NaCl l^{-1}. COD, PO_4^{3-}, TKN and SS removal efficiencies attained 95, 93, 96 and 92%, respectively.

(4) The organisms responsible for nitrogen removal appeared to be the most sensitive to the environmental changes that occurred in the reactor. Other organisms presented adequate recovery capabilities after a salt or load shock.

(5) Biomass growth was not affected by the high salt concentrations, neither was the settleability of the sludge.

(6) The SBR technology appeared to be an adequate solution for the treatment of tannery soak liquor, removing organic matter, as well as the odour of the liquor. After biological treatment, this liquor may be sent to evaporation pans and the improved quality of the salt obtained could allow it to be reused.

(7) The performance of a highly saline system can be similar to the performance of a non saline one.

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Chapitre 5. Traitement anaérobie d'effluents hypersalins

Articles publiés sous la forme :

(5.1) Lefebvre, O., Vasudevan, N., Torrijos, M., Thanasekaran, K., Moletta, R., 2006. Anaerobic digestion of tannery soak liquor with an aerobic post-treatment. Water Res. 40(7), 1492-1500.

(5.2) Lefebvre, O., Vasudevan, N., Torrijos, M., Thanasekaran, K., Moletta, R. Biological treatment of hypersaline tannery soak liquor using anaerobic moving bed. Article inédit.

(5.3) Lefebvre, O., Quentin, S., Torrijos, M., Godon, J.J., Delgenès, J.P., Moletta, R. Impact of increasing NaCl concentrations on the efficiency and microbiology of anaerobic digestion. Soumis.

5.1 Traitement anaérobie d'un effluent hypersalin de tannerie en réacteur UASB

Article : Anaerobic digestion of tannery soak liquor with an aerobic post-treatment

Résumé

L'industrie du cuir est un pan important de l'économie indienne, en raison de son énorme potentiel d'emploi, de croissance et d'exportation. Par ailleurs, l'impact environnemental des tanneries est conséquent. Cette étude se consacre à l'effluent de lavage des peaux, caractérisé par une charge organique importante et une forte salinité. Pour ces raisons, cet effluent devrait être traité à part avant de le mélanger à l'effluent composite, constitué de toutes les sources réunies d'effluents générés par la tannerie. Le traitement anaérobie de cet effluent a donc été étudié en réacteur UASB. Le rendement épuratoire de la DCO a atteint 78% avec une cva de 0.5 kg DCO m^{-3} j^{-1}, un TSH de 5 j et une salinité de 71 g l^{-1}. La combinaison de l'UASB et d'un post-traitement aérobie a permis d'améliorer les performances du traitement, avec un rendement épuratoire de 96% de la DCO. Cependant, ces performances n'ont pu être obtenues qu'à une très faible cva, ce qui limite la viabilité économique de ce procédé.

Abstract

The leather industry occupies a place of prominence in the Indian economy due to its massive potential for employment, growth and exports. The potential environmental impact of tanning is significant. This study focuses on tannery soak liquor, generated by the soaking of hides and skins, which is characterised by high organic load and high salinity. For these reasons, the soak liquor should be segregated and pre-treated separately before being mixed with the composite wastewater, made of all other streams mixed together. The anaerobic digestion of tannery soak liquor was studied using a UASB. COD removal reached 78% at an OLR of 0.5 kg COD m^{-3} d^{-1}, a HRT of 5 days and a TDS concentration of 71 g l^{-1}. The combination of the UASB with an aerobic post-treatment enhanced the performance of the overall wastewater treatment process and the COD removal efficiency of the combined anaerobic/aerobic treatment system reached 96%. However, for effective operation, the system had to be operated at very low OLRs, which affects the economic viability of such a process.

Introduction

Hypersaline effluents are generated by various industrial activities. Such wastewater, rich in both organic matter and total dissolved solids (TDS), is difficult to treat using conventional biological wastewater treatment processes [136]. The use of halophilic Bacteria is required [125]. The number of studies dealing with the biological treatment of hypersaline wastewater is increasing rapidly.

Among the industries generating hypersaline effluents, tanneries are prominent in India. Tanning is one of the oldest professions in India, with 2,000 industrial units spread mostly across Tamil Nadu, West Bengal, Uttar Pradesh, Andhra Pradesh, Karnataka, Rajasthan and Punjab. Leather tanning is almost wholly a wet process from which a large volume of liquid waste is continuously generated. Due to the variety of chemicals added at different phases of processing of hides and skins, the wastewater has complex characteristics. The tanning process and the effluents generated have already been reported in the literature [210; 212; 233] and an overview is presented in the upper part of Figure 5.1, entitled "Successive steps of leather processing". Salt (NaCl) is used to preserve the fresh skins from decomposition immediately after they are stripped in the slaughterhouse, and the excess of salt has to be removed in the tannery before further processing. This is done by soaking, using a lot of water, which generates the first source of effluent. This soak liquor is characterised by high organic load, a high level of suspended solids (sand, lime, hair, flesh, dung, etc.) and high salinity. The soak liquor is usually mixed with the other streams generated by leather processing to form the composite wastewater, which is screened, equalized, physically and chemically treated, and then biologically treated, as indicated in the lower part of Figure 5.1, entitled "Wastewater management".

There would be several advantages in segregating the soak liquor and treating it anaerobically, before mixing it with the composite wastewater, as proposed in Figure 5.1. Firstly, sulphates are known to inhibit anaerobic treatment - as sulphates are reduced into sulphide by sulphate-reducing Bacteria, which retards the anaerobic treatment of carbon - and the composite wastewater contains between 1,000 and 3,500 mg sulphates l^{-1}, whereas the soak liquor does not contain sulphates. Thus, anaerobic digestion of the soak liquor is likely to be more efficient if segregated. Secondly, biochemical oxygen demand (BOD_5) is likely to be higher in the soak liquor than in the composite wastewater, which makes the soak liquor particularly suitable for anaerobic digestion. Thirdly, application of anaerobic digestion to the segregated soak liquor could be used as a pretreatment before aerobic treatment (such as lagooning or with activated sludge). The anaerobically treated soak liquor could be mixed with the composite wastewater

after physico-chemical treatments. Thus, the cost of the treatment would be reduced because fewer chemicals would be used during primary treatments, less oxygen would be required for aeration and less sludge would be generated by aerobic treatment.

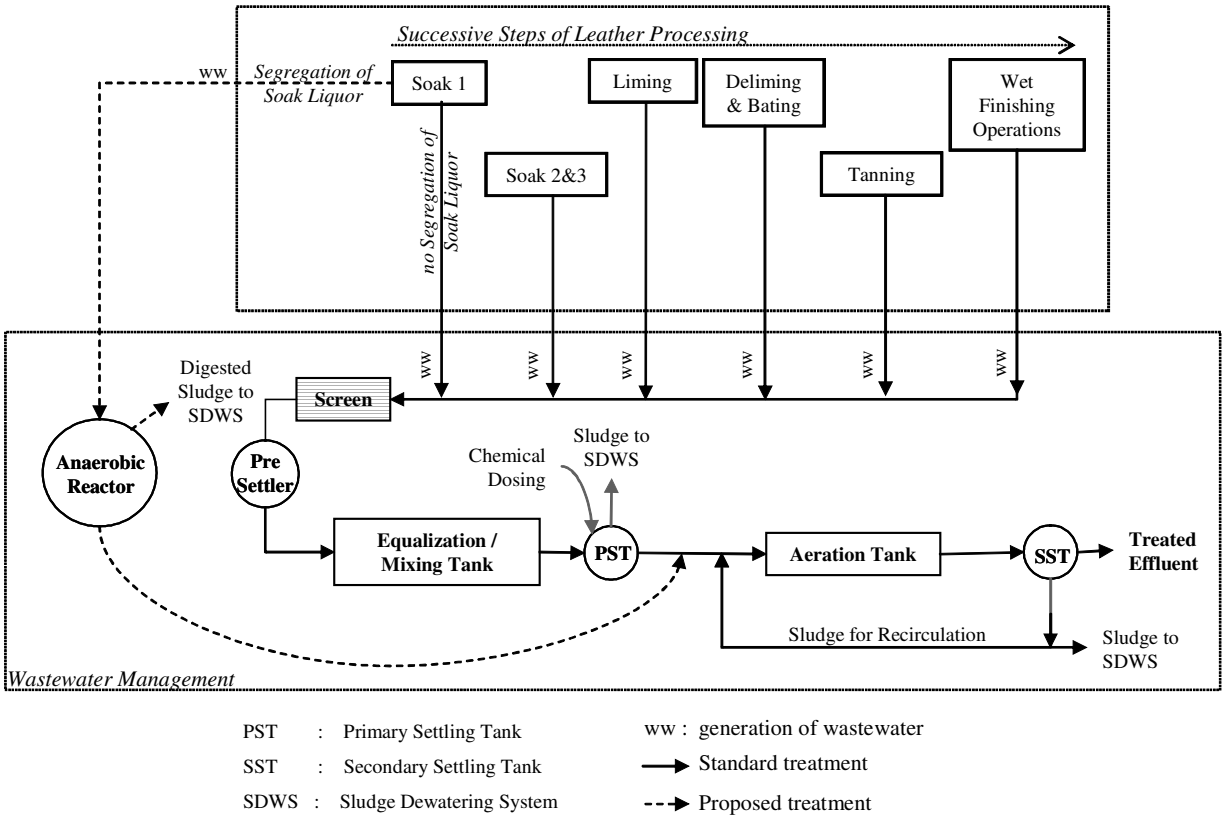

Figure 5.1 Simplified overview of tanning process and tannery effluents management.

High salt content is considered to have an inhibitory effect on anaerobic digestion, mainly due to cations. A sodium content exceeding 10 g l^{-1} has already been reported as inhibiting methanogenesis [84; 121; 182]. However, Omil *et al.* [163] could not show any clear toxic effect of a seafood-processing effluent, with salinity similar to seawater, on an anaerobic contact system pilot-plant. It was therefore proven that the adaptation of an active methanogenic biomass to the salinity of the effluent was possible. They concluded that the efficiency of such a process depended on an adequate adaptation strategy of the biomass to salinity. Feijoo *et al.* [68] specified that sodium toxicity on sludge depended on several factors such as the antagonism effect, due to other ions at adequate concentrations, the nature and the adaptation of the sludge and the methanogenic substrate used.

The scope of this work was to study the anaerobic digestion of hypersaline tannery soak liquor using an upflow anaerobic sludge blanket (UASB) reactor. A UASB was selected because it has already been used successfully for the biological treatment of

tannery composite wastewater [27; 233]. The efficiency of an extensive aerobic post-treatment process was also considered. In order to reduce the cost of such a post-treatment as much as possible, an extensive process was selected, i.e. activated sludge reactor without recirculation of the sludge, to simulate an aerobic lagoon. Finally, the performance of such a treatment was compared to that of an aerobic sequencing batch reactor (SBR) treating similar wastewater (see section 4.2).

Materials and methods

Influent

The tannery influent (soak liquor) was collected from soak pits in a tannery around Chennai (India). 9 different samples of soak liquor were collected and used as an influent for the UASB reactor during the experimental period. The average characteristics of the nine samples are reported in Table 5.1. The high standard deviation values reflect the high variability of tannery soak liquor. The characterisation of tannery soak liquor has already been detailed in a previous study (see section 4.2).

Table 5.1 Characteristics of 9 influents coming from the same tannery.

	pH	TDS ($g\ l^{-1}$)	SS ($mg\ l^{-1}$)	VSS ($mg\ l^{-1}$)	COD ($mg\ l^{-1}$)
Influent No.1	7.5	30.2	3,600	700	1,500
Influent No.2	7.5	38.6	7,600	2,000	1,900
Influent No.3	7.6	45.9	5,300	500	1,700
Influent No.4	-	34.0	4,000	800	2,600
Influent No.5	7.8	63.2	4,200	2,300	3,700
Influent No.6	7.8	32.9	5,100	1,600	2,600
Influent No.7	7.0	50.5	10,700	1,500	4,400
Influent No.8	7.7	58.2	5,100	1,400	2,200
Influent No.9	7.6	72.3	9,600	2,400	2,300
Mean	**7.5**	**47.3**	**6,100**	**1,500**	**2,500**
Std. Dev.	*0.3*	*14.8*	*2,600*	*700*	*900*

Bioreactors

Upflow Anaerobic Sludge Blanket (UASB)

A 5 l lab-scale UASB, fed with soak liquor, was operated for more than 300 days. Influent was continuously provided to the UASB using a peristaltic pump. Temperature was ambient (close to 30°C), which made the treatment mesophilic. The device most characteristic of a UASB is the phase separator. This device is placed at the top of the reactor and divides it into a lower part, the digestion zone, and an upper part, the settling zone. The wastewater is introduced as uniformly as possible over the reactor bottom, passes through the sludge bed and enters into the settling zone via the aperture in the phase separator. Thus, the presence of a settler on top of the digestion zone enables the system to maintain a large sludge mass in the UASB reactor, while an effluent free of suspended solids is discharged. The biogas bubbles are released into the gas phase at the liquid-gas interface. The influent flowrate equalled 1 l d^{-1} initially, then increased to 1.5 l d^{-1} on day 110, decreased to 0.8 l d^{-1} on day 213 and finally increased again to 1 l d^{-1} on day 244.

Aerobic post-treatment

A 3 l lab-scale activated sludge reactor was connected to the outlet of the UASB. Thus, the influent used for this reactor was already pre-treated anaerobically. An illustration of the lab-scale combined anaerobic/aerobic treatment process is provided in Figure 5.2. Aeration was supplied through an air compressor. The temperature of the wastewater in the reactor was close to 30°C. A clarifier was connected to the outlet of the reactor to ensure the clarification of the effluent before discharge. The particularity of the reactor used in this study was the absence of recirculation of the sludge. This made it a hybrid system close to the lagoon in the sense that, operationally, the hydraulic and solids retention times were the same and equalled 3 days in this study. Consequently, the biomass maintained in the reactor was less than that commonly found in activated sludge reactors (i.e. 3-4 g MLSS l^{-1}) and was much closer to that found in lagoons (i.e. 100-400 mg MLSS l^{-1}) [150].

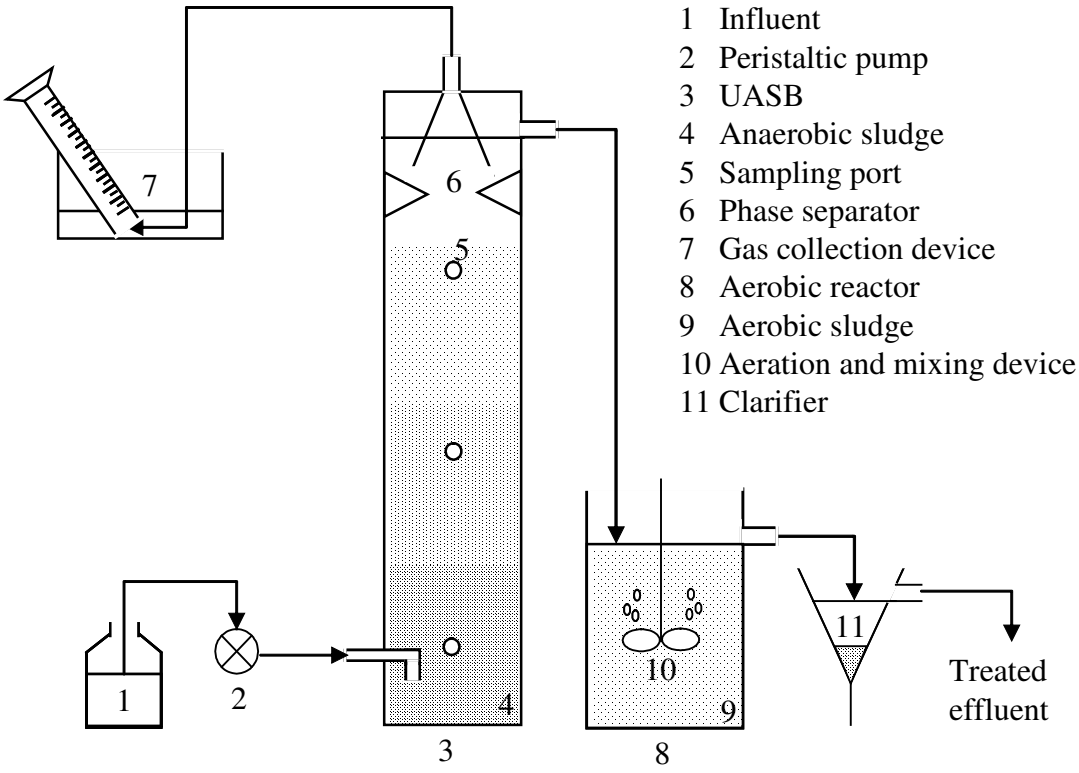

Figure 5.2 Lab-scale combined anaerobic/aerobic treatment process operating with tannery soak liquor.

Inoculum

The UASB reactor profited from the inoculation of granular sludge collected from a pilot-scale UASB treating composite tannery effluent. The MLSS and MLVSS concentrations of this inoculum were 19 and 5 g l^{-1}, respectively. The addition of this inoculum was likely to reduce the start-up time [138] and to introduce an anaerobic population used to treating tannery effluents components. In addition to this inoculum, a permanent addition of Bacteria occurred, as the tannery soak liquor that was used as an influent contained some amounts of Bacteria and even sometimes protozoans. The aerobic reactor was inoculated with aerobic sludge collected from a common effluent treatment plant purifying domestic wastewater from the city of Chennai.

Analysis

COD, TDS, suspended solids (SS), volatile suspended solids (VSS), total alkalinity (TA), volatile fatty acids (VFA) and pH (the abbreviations of these parameters and of other terms is presented at the beginning of this paper) were analysed following APHA's Standard Methods for the Examination of Water and Wastewater [9]. Quality control was ensured using standards as well as duplicates. COD was determined by the

open reflux method. Mercuric sulphate was used to eliminate the interference of chlorides when dosing COD. Sawyer and McCarty [193] reported that this interference could be eliminated as long as a 10/1 weight ratio of mercuric sulphate to chloride is maintained. Determination of VFA used the distillation method, in which the results are measured as mg volatile acids as acetic acid l^{-1}.

Results and discussion

Performance of the UASB

The UASB reactor was fed with tannery soak liquor for more than 300 days. Due to the influent's high variability, which has been described previously, the operational conditions of the bioreactor were subject to frequent and rapid changes, as shown in Figure 5.3, which reports the respective evolution of the TDS concentration, OLR and HRT applied to the reactor.

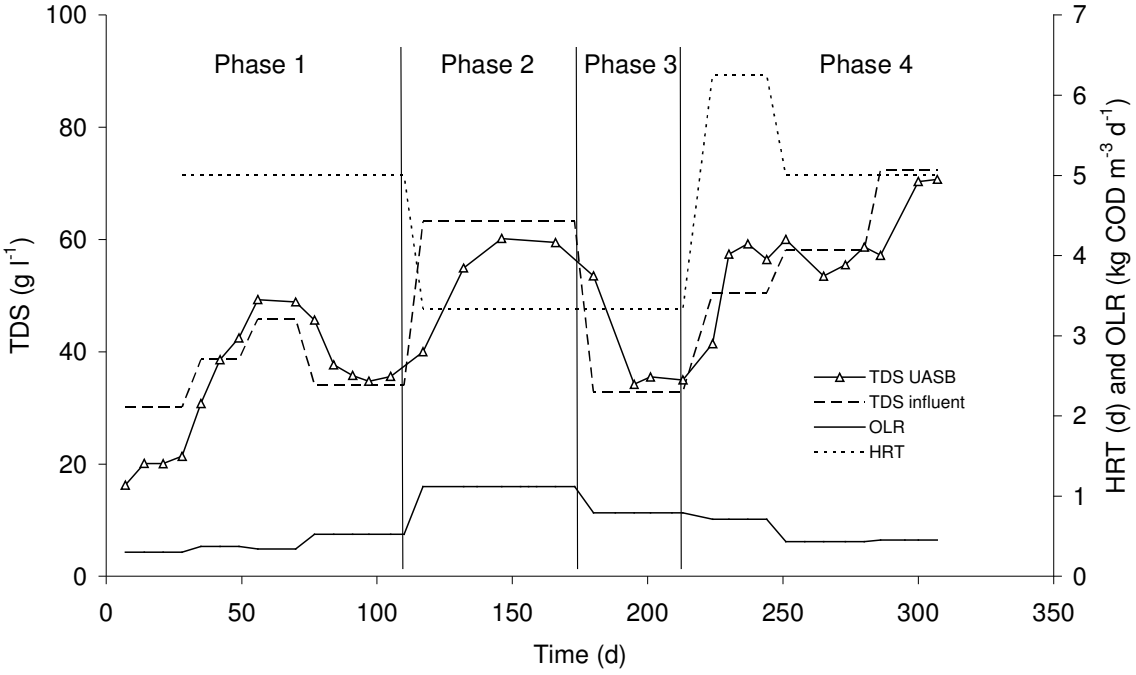

Figure 5.3 Evolution of the environmental parameters applied to the UASB reactor treating tannery soak liquor.

The analysis of Figure 5.3 has permitted the division of the experiment into 4 distinct phases: Phase 1 (days 1-110) corresponds to the starting phase of the reactor and the conditions of its operation at a low OLR, constant HRT and a TDS concentration always

less than 50 g l^{-1}. Phase 2 (days 110-173) corresponds to a rapid hardening of the environmental conditions, with a reduction of HRT and an increase of OLR, along with increasing TDS concentrations. Thus, the operation of a UASB under extreme conditions, in the context of an industrial complex influent (soak liquor) subject to abrupt variations, was analysed during phase 2. Phase 3 (days 173-213) was characterised by a return to the conditions of phase 1, thanks to a sudden decrease of OLR and TDS concentrations. It was therefore possible to test the recovery capabilities of the process after the shock that occurred during phase 2. Finally, phase 4 (days 213-307) was characterised by an increase of salinity in stages, while OLR was maintained at low values and HRT at high values. The impact of increasing TDS concentrations on the performance of the process could therefore be studied. In addition, MLVSS concentration, measured in the lower zone of the reactor, increased linearly from 1.4 g l^{-1} on day 97, at the end of phase 1, to 12.2 g l^{-1}, on day 307, at the end of phase 4.

Phase 1(days 1-110): Operation of the UASB under low OLR

During phase 1, the UASB was operated at a low OLR, ranging from 0.3 kg COD m^{-3} d^{-1} to 0.5 kg COD m^{-3} d^{-1}, and a HRT of 5 days, as shown in Figure 5.3. It also appears from the same figure that the TDS concentration in the UASB increased constantly during the first 70 days of operation until it reached that of influent No.3 (i.e. 46 g TDS l^{-1}). Then, between days 70 and 110, the TDS concentration decreased until it reached the TDS concentration of influent No.4 (i.e. 34 g TDS l^{-1}). On day 91, under an OLR of 0.5 kg COD m^{-3} d^{-1}, HRT of 5 days and 36 g TDS l^{-1}, the COD in the treated effluent reached 600 mg l^{-1}, with a corresponding COD removal efficiency of 77%, as can be seen in Figure 5.4, where the COD values in the influent and in the treated effluent are reported, along with the corresponding COD removal efficiencies. This performance then stabilised until the end of phase 1, showing that the reactor functioned under steady state conditions.

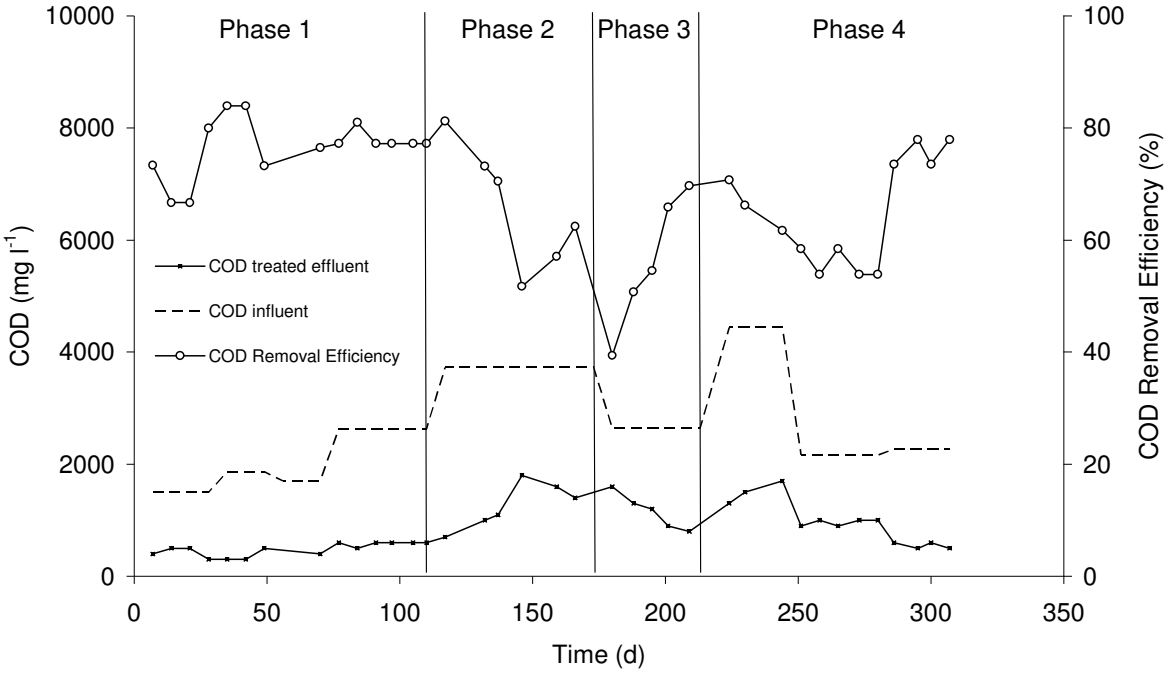

Figure 5.4 COD removal efficiency of UASB reactor treating tannery soak liquor.

Total alkalinity (TA), VFA and pH are efficient indicators of the stability of the reactor and of the correct balance between acidification and methanogenesis. Methanogenesis, in particular, is known to become unstable when the alkalinity ratio (VFA:TA) is above 0.3 [153]. Furthermore, optimal methanogenesis is known to take place at a neutral pH. An inhibition of methanogenesis generally results in an increase of VFA concentration and a sudden drop in alkalinity and pH. At the end of phase 1, the alkalinity ratio was 0.25. The pH was also monitored and averaged 8.0, which corresponds to the pH of the influent but is slightly alkaline and may inhibit methanogenesis. Yet, the amount of biogas produced during phase 1 averaged 750 ml d^{-1}, thus 470 ml g^{-1} of COD removed. This value appeared to be low, which indicated a gas leakage.

The concentration of VFA (measured as acetic acid) was also determined and represented in Figure 5.5. It can be seen that, at the end of phase 1, the concentration of VFA was maintained below 300 mg acetic acid l^{-1}. This shows that the process of hydrolysis and acidification of the organic matter took place in proper conditions.

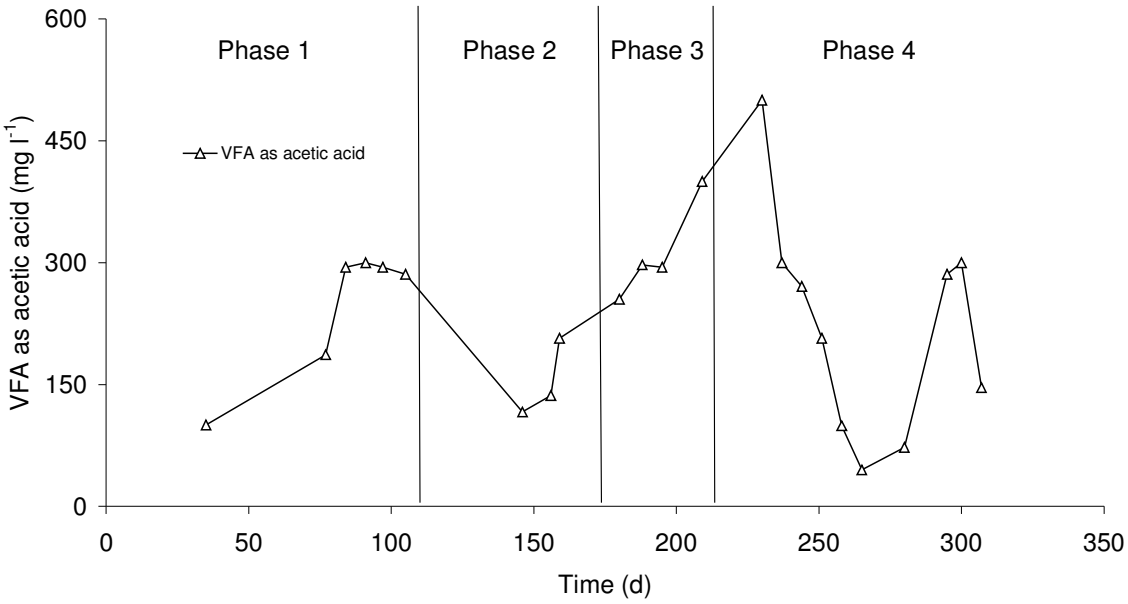

Figure 5.5 Evolution of VFA in the UASB reactor treating tannery soak liquor.

Phase 2 (day 110-173): Operation of the UASB under extreme conditions

On day 110, the flowrate increased from 1 to 1.5 l d^{-1} and, consequently, HRT decreased to 3.3 days and OLR increased to 1.1 kg COD m^{-3} d^{-1} (see Figure 5.3). At the same time, TDS increased at a fast rate due to the higher salinity of influent No.5 (63 g TDS l^{-1}). This resulted immediately in a shock and consequent destabilisation of the system, with COD values in the treated effluent reaching 1,800 mg l^{-1} on day 146, and corresponding COD removal efficiency falling to 52%. Then, it seems that an acclimation process started, as the COD removal efficiency increased once again. Figure 5.5 clearly indicates that this increase of COD in the treated effluent during phase 2 was not due to an increase of VFA. Therefore, it seems that the hydrolysis and acidification processes had been overloaded.

Such overloading of the process at a low OLR could be explained by the high salinity that inhibited the microbial metabolism. In addition, the high influent SS concentration might also have disturbed the operation of the UASB reactor. Indeed, Lettinga and Hulshoff Pol (1991) have already reported that, beyond a certain influent SS concentration and depending on the characteristics of the SS, an anaerobic treatment system like the UASB becomes less feasible. The alkalinity ratio averaged 0.09±0.03 and pH averaged 8.4±0.1 during phase 2.

Phase 3 (days 173-213): Recovery of the UASB after an environmental shock

In an industrial scenario, a bioreactor will frequently be exposed to environmental shocks lasting for several days. Therefore it should be able to withstand variable conditions. In the leather industry especially, the characteristics of the soak liquor are likely to change weekly or monthly, depending on every batch of hides and skins that is supplied to the tannery. The recovery capability of the UASB after exposition to variable load and salt conditions was therefore studied during phase 3. In comparison to influent No.5, influent No.6, which was used throughout this phase, was characterized by a lower organic matter content (2,640 mg COD l^{-1}) and a lower TDS concentration (33 g TDS l^{-1}), as shown in Table 5.1. Consequently, OLR was lower than in the previous phase and the TDS concentration in the UASB reactor started to decrease after day 173 (see Figure 5.3). As a result, the environmental conditions became more favourable to anaerobic digestion and the COD concentration in the treated effluent started to decrease, as can be seen in Figure 5.4. As a consequence, the COD removal efficiency started to increase quickly and reached 70% on day 209. At this time, the TDS concentration was 35 g l^{-1} and OLR was 0.8 kg COD m^{-3} d^{-1}, levels similar to the same parameters at the end of phase 1 prior to the shock. VFA concentration increased regularly during phase 3 (see Figure 5.5) and reached 400 mg l^{-1} at the end of phase 3, which shows that acidification could take place properly again and that methanogenesis became the limiting factor once more, as it had already been the case at the end of phase 1. It can thus be concluded that the bioreactor was capable of good and fast recovery after a shock, as soon as the environmental conditions improved. This proves that the biomass could survive during the shock; only its ability to degrade COD was affected in extreme environmental conditions. The recovery phenomenon was accompanied by a decrease in pH that averaged 7.7±0.2 at the end of phase 3. The alkalinity ratio averaged 0.15±0.04 at the same time. This result is in agreement with the experiment undertaken by Gangagni Rao *et al.* [72], who exposed an anaerobic fixed-film reactor treating saline (i.e. 20 g TDS l^{-1}) pharmaceutical wastewater to organic shock loads and showed that, after a temporary drop, COD removal efficiency could be restored within a week, as soon as the OLR was reduced again.

Phase 4 (days 213-307): Adaptation of the UASB under increasing salt concentration

Influent No.7 was characterised by a very high COD concentration (4,440 mg l^{-1}), as shown in Table 5.1. In order to avoid renewed overloading of anaerobic digestion, a decision was taken to reduce the flowrate on day 213. Thus, HRT was increased again and OLR was maintained around 0.5 kg COD m^{-3} d^{-1}. Influent Nos.7, 8 and 9 were also extremely saline and contained as much as 50, 58 and 72 g TDS l^{-1}, respectively. As a

consequence, the TDS concentration in the UASB increased in stages during phase 4. During a first period, the performance of the UASB was altered. The COD concentration in the treated effluent increased from 800 to 1,700 mg l^{-1} between day 209 and 244, thus the corresponding COD removal efficiency decreased from 70 to 58%. Then, a second period at the end of the experiment was characterised by an enhancement of the reactor performance again, which was due to an influent with lower COD concentration and, probably, a higher biodegradability. Consequently, on day 307, the COD concentration in the treated effluent attained 500 mg l^{-1} and the COD removal yield reached 78%, at an OLR of 0.5 kg COD m^{-3} d^{-1}, a HRT of 5 days and 71 g TDS l^{-1}. These performances are similar to the performances obtained at the end of phase 1, under similar environmental conditions, apart from salinity which was much higher at the end of phase 4. This shows that sludge can still be active at a salinity level over 70 g l^{-1}. Figure 5.5 shows that the increase in the TDS concentration first resulted in a decrease in the VFA concentration, then VFA concentration started to increase again. This is a sign that the first consequence of increasing salinity was an inhibition of acidification, which was followed by a recovery after adaptation occurred. It can be concluded, then, that very high salinity is not an obstacle to anaerobic digestion, provided that the process is progressively adapted to high salt concentrations and OLR is maintained at a low level. At the end of phase 4, the alkalinity ratio averaged 0.24±0.07 and pH averaged 7.7±0.1.

Solids characterisation and management

The evolution of solids in the inlet and outlet of the UASB was also studied (data not shown) and showed very little SS removal. During the experiment, SS removal efficiency seldom exceeded zero. High turbidity problems are very common in hypersaline effluent treatment systems. Woolard and Irvine (1995), in particular, explained that systems treating hypersaline effluents invariably have high concentrations of effluent SS, due to the high density of salt water and to the salt-induced cell lysis phenomenon. Moreover, the nature of the influent itself, with a high inorganic SS concentration, may be responsible for the high SS concentration in the treated effluent. It has indeed already been shown that, in tannery wastewater containing high amount of salt and SS, the settling rate of the raw solids is reduced (Song et al., 2000). Consequently, as most of SS contained in tannery soak liquor are unlikely to be retained or degraded inside the anaerobic reactor, they will probably remain in the treated effluent. Therefore, in order to remove the excess of SS from tannery soak liquor, the use of physico-chemical pre-treatment should be considered, before applying anaerobic digestion. Mishra et al. (2004), for instance, used as a flocculant fenugreek mucilage, a polysaccharide extracted from a leguminous plant grown in India, to remove nearly 85% of SS from tannery effluent within 1h, at a flocculant dose of 0.08 mg l^{-1} and neutral pH. Yet, the effect of

such a pre-treatment on the structure and composition of the sludge and, consequently, its impact on the performance of subsequent anaerobic treatment has to be tested .

At the end of the experiment, MLSS and MLVSS were measured in the upper, middle and lower zones of the reactor. Results are compiled in Table 5.2. High amounts of MLSS and MLVSS were measured, especially at the bottom of the reactor. The MLSS/MLVSS ratio showed that 73 to 87% of the solids that were found in the UASB reactor were inorganic and were probably introduced in the reactor with the influent that contained between 3,600 and 10,700 mg l^{-1} of SS. The higher concentration of MLSS and MLVSS in the lower zone of the reactor can be explained by the low upward velocity (between 14 and 21 cm d^{-1}), which favoured solids sedimentation and accumulation at the bottom of the reactor. Guerrero et al. (1997) noted that MLSS and MLVSS concentrations were higher at the bottom of an upflow anaerobic filter. They explained that this phenomenon could be due to the higher abundance of hydrolytic-fermentative bacteria, which have higher cellular yields, in the zone closest to the inlet of the reactor.

Table 5.2 Mixed liquor solids concentration at different zones of UASB reactor treating tannery soak liquor.

	Upper Zone	Middle Zone	Lower Zone
MLSS (g l^{-1})	9.3	17.2	44.9
MLVSS (g l^{-1})	2.1	2.3	12.2
MLVSS/MLSS	0.23	0.13	0.27

It has been shown that a sludge blanket developped at the bottom of the UASB reactor, but the observation of the biomass revealed the absence of the granular sludge typically found in UASB. This shows that, although the reactor was operated as a UASB and was inoculated with granules, the maintenance of stable granular sludge was made impossible in this reactor due to high salinity. Consequently, the sludge blanket that accumulated at the bottom of the reactor did not consist of granules. Actually, it has already been mentioned that, in thermophilic cultures of *Methanosarcina thermophila*, high sodium concentrations inhibited the microbial production of extracellular polysaccharides, therefore disrupting the granules (Sowers and Gunsalus, 1988). Granule disaggregation could also be due to the change of influent between the pilot plant where the sludge originated from (i.e. tannery composite wastewater) and the lab-scale reactor where they were inoculated.

Aerobic post-treatment

An aerobic post-treatment was set up after the UASB reactor on day 260. Because of no sludge recirculation, the activated sludge biomass was quickly reduced from 3.5 g MLVSS l^{-1} (initial value after seeding on the starting day of operation) to 0.4 g l^{-1}. Then, biomass slightly increased again and stabilised around 1.5 g l^{-1} (data not shown). This biomass value is less than the values usually reported in the literature for activated sludge processes and is closer to these commonly found in lagoons [150]. The operating conditions of this activated sludge process, in the absence of sludge recirculation, made it close to an aerobic lagoon, as the hydraulic and solids retention times were the same, equalling 3 days. Nevertheless, the COD in the treated effluent, after aerobic treatment, attained 100 mg l^{-1} after one month of operation. Thus, the combined anaerobic/aerobic process made it possible to remove 96% of the initial COD contained in the influent, under a total OLR of 0.3 kg COD m^{-3} d^{-1} (0.5 kg COD m^{-3} d^{-1} for UASB and 0.2 kg COD m^{-3} d^{-1} for the post-treatment), a total HRT of 8 days (5 days for UASB and 3 days for the post-treatment) and a TDS concentration of 71 g l^{-1}. Therefore, it appears that the aerobic reactor formed an efficient post-treatment that attained very low COD values in the final treated effluent, at the cost of an increase of HRT and a decrease of OLR.

Comparison with an aerobic sequencing batch reactor treating tannery soak liquor

The performance of the UASB reactor operated in this study was compared to the performance of an aerobic SBR fed with the same influent in a previous study (see section 4.2). The optimal stable performance of SBR, UASB and combined anaerobic/aerobic treatment are compiled in Table 5.3.

Table 5.3 Performance of biological reactors operating with tannery soak liquor

	COD influent (mg l^{-1})	OLR (kg COD m^{-3} d^{-1})	HRT (d)	TDS (g l^{-1})	COD treated effluent (mg l^{-1})	COD removal (%)
Aerobic SBR (low OLR)	2,950	0.6	5.0	35	140	95
Aerobic SBR (medium OLR)	3,600	1.1	3.3	40	320	91
UASB (phase 1)	2,630	0.5	5.0	36	600	77
UASB (phase 4)	2,270	0.5	5.0	71	500	78
UASB + aerobic post-treatment	2,270	0.3	8.0	71	100	96

It appears from Table 5.3 that, whereas the aerobic SBR was able to remove 95% of the COD of the soak liquor at a low OLR of 0.6 kg COD m^{-3} d^{-1}, a HRT of 5 days and 35 g TDS l^{-1}, the UASB was able to remove only 77% of the COD of the soak liquor under similar conditions in phase 1 (i.e. OLR of 0.5 kg COD m^{-3} d^{-1}, HRT of 5 days and 37 g TDS l^{-1}). Thus, the aerobic SBR could remove more organic matter than the UASB, which shows the superiority of aerobic treatment over anaerobic treatment at a similarly low OLR. Furthermore, the aerobic SBR could withstand a higher OLR than the UASB and remove up to 91% of the COD at a moderate OLR of 1.1 kg COD m^{-3} d^{-1}, a HRT of 3.3 days and 40 g TDS l^{-1}. At a similar OLR, the UASB was overloaded and no stable performance could be achieved.

The combination of anaerobic digestion with an aerobic post-treatment was able to achieve a COD removal efficiency of 96% at a total OLR of 0.3 kg COD m^{-3} d^{-1}, a total HRT of 8 days and 71 g TDS l^{-1}. Thus, very little COD remained in the final treated effluent (i.e. 100 mg COD l^{-1}) and the quality of this effluent was similar to that obtained after aerobic treatment in the SBR at a low OLR (i.e. 140 mg COD l^{-1}), but OLR was higher and HRT lower in the case of aerobic treatment used alone, which makes it more competitive.

Conclusion

The aim of this study was to check the advantage of segregating tannery soak liquor from the composite tannery wastewater, in order to treat it separately by anaerobic digestion. The anaerobically pre-treated soak liquor could then be mixed with the composite wastewater to undergo aerobic treatment. The anaerobic digestion of the tannery soak liquor was studied using a UASB reactor over more than 300 days. The conclusions are as follows:

(1) The optimal performance of the UASB reactor treating tannery soak liquor, obtained during the first phase (start-up period), was 77% of COD removal efficiency at an OLR of 0.5 kg COD m^{-3} d^{-1}, a HRT of 5 days and a TDS concentration of 36 g l^{-1}.

(2) Higher OLR and TDS concentrations resulted in a destabilisation of the system.

(3) The UASB showed good recovery capabilities, therefore proving itself able to withstand environmental shocks that always occur in industrial applications.

(4) The UASB showed good adaptability to increasing salt concentrations and achieved 78% COD removal efficiency at 71 g TDS l^{-1}, provided HRT is constant and OLR is maintained at a low level.

(5) No stable granular sludge could be maintained in the reactor. The operation of the reactor under more stable conditions may favour the formation and maintenance of such granules, yet, this hypothesis has to be tested.

(6) The COD removal efficiency of a combined anaerobic/aerobic treatment system reached 96% after one month of operation at an OLR of 0.5 kg COD m^{-3} d^{-1}, HRT of 8 days and TDS concentration of 71 g l^{-1}.

(7) The comparison of anaerobic and aerobic treatment of tannery soak liquor showed the superiority of aerobic treatment, which can withstand a higher OLR and thus remove more COD from the influent.

Acknowledgements

This study was conducted at the Centre for Environmental Studies, Anna University, Chennai (India), thanks to cooperation between Anna University and the French National Institute for Agricultural Research (Institut National de la Recherche Agronomique, INRA), within the framework of the Indo-French Cell for Bioprocesses on Environment

(IFCBE). The authors would like to thank Mr K.V. Emmanuel for his wise recommendations and Ms. J. D'Silva for technical assistance during the study.

5.2 Traitement anaérobie d'un effluent hypersalin de tannerie en réacteur à lit mobile

Article : Biological treatment of hypersaline tannery soak liquor using an anaerobic moving bed

Résumé

Dans un contexte de pénurie d'eau et d'intrusion de sel dans les nappes phréatiques, la gestion des effluents salins est de première importance en Inde. Cette étude se consacre à un effluent de tannerie généré par le rinçage des peaux. Il a déjà été montré que le traitement biologique aérobie de cet effluent est plus efficace que son traitement anaérobie en UASB. Cette étude est donc une tentative d'accroître les performances du traitement anaérobie de cet effluent en utilisant un procédé à biomasse fixée : le lit mobile anaérobie. Cependant, il est apparu que la fixation de la biomasse sur le support utilisé n'était pas effective. En conséquence, les performances du lit mobile anaérobie ont été similaires à celles obtenues avec un réacteur UASB, atteignant un rendement épuratoire de 74 ± 7 % de la DCO, avec une cva de 0.4 kg DCO m^{-3} j^{-1}, un TSH de 6 j et une salinité comprise entre 58 et 72 g l^{-1}. Les raisons pour lesquelles la biomasse ne s'est pas fixée au support font l'objet d'une discussion.

Abstract

In a context of water scarcity and the intrusion of salt into freshwater, the management of saline effluents is of priority concern in India. This study deals with tannery soak liquor, a highly saline effluent generated by the soaking of hides and skins. It has already been shown that the aerobic biological treatment of the soak liquor is more efficient than anaerobic treatment with an upflow anaerobic sludge blanket (UASB). This study constitutes, therefore, an attempt to increase the efficiency of anaerobic digestion of tannery soak liquor using an attached-growth system: the anaerobic moving bed (AMB). However, it appeared that biomass attachment to the packing material did not occur properly. Consequently, the performance of the AMB was similar to that of a UASB, removing 74 ± 7 % of the influent COD, with an OLR of 0.4 kg COD m^{-3} d^{-1}, a HRT of 6 d

and a TDS concentration comprised between 58 and 72 g l^{-1}. The reasons as to why biomass did not to attach to the support medium are discussed in this paper.

Introduction

Hypersaline effluents are generated by various industrial activities. This wastewater, rich in both organic matter and total dissolved solids (TDS), is difficult to treat using conventional biological wastewater treatment processes [136]. The use of halophilic bacteria is required [125]. The number of studies dealing with the biological treatment of hypersaline wastewater is increasing rapidly.

At the present time, the leather industry represents a major sector of the Indian industrial base. The sustainable model of development for this industry is based on the promotion of cleaner technologies and effluent treatment processes in order to enable the industry to enhance its value-added performance. The tanning process and the effluents generated have already been reported in the literature [210; 212; 233]. The tanning process is almost wholly a wet process that generates huge amounts of wastewater. Hence, the treatment of a tannery's wastewater is always required in India and is carried out on a composite effluent made up of all the streams generated by the tannery mixed together. Yet some streams have highly specific characteristics and should be segregated and treated separately.

This paper focuses on the soak liquor, generated by the soaking of hides and skins, which is characterised by high organic load, high amounts of suspended solids and high salinity. Therefore, whenever it is possible, it should be segregated and evaporated. In case of lack of space for evaporation, it should be treated separately before being mixed with the composite wastewater. In both cases, there is an advantage in treating the soak liquor through biological methods as a way of reducing the amount of organic matter in the effluent. For this reason, the aerobic treatment of the soak liquor has already been studied as a pretreatment prior to evaporation in solar evaporation pans using a sequencing batch reactor (SBR) (see section 4.2), and anaerobic digestion has been assessed as a pretreatment step before mixing the soak liquor with the composite wastewater, using an upflow anaerobic sludge blanket (UASB) (see section 5.1). It appeared from a comparison of the anaerobic and aerobic treatment of tannery soak liquor that the aerobic treatment was more efficient, withstanding a higher organic loading rate (OLR), thus removing more COD from the influent.

In this paper, the anaerobic digestion of the soak liquor using an anaerobic moving bed (AMB) was considered as an alternative to UASB. The assumption underlying this work was that the use of an attached-growth process is likely to improve the performance of the anaerobic digestion of the soak liquor. The advantages of AMB include the ability to concentrate biomass, to withstand relatively high organic loading rates and to handle shock loads. Disadvantages include the pumping power required to move the packing material, the cost of reactor packing and the duration of startup [150].

Materials and methods

Influent

The tannery influent (soak liquor) was collected from soak pits in a tannery near Chennai (India). 4 different samples of soak liquor were collected and used as an influent for the AMB during the experimental period. The average characteristics of the 4 samples are reported in Table 5.4. The high standard deviation values reflect the high variability of tannery soak liquor. The characterisation of tannery soak liquor has already been detailed in a previous study (see section 4.2).

Table 5.4 Characteristics of 4 influents coming from the same tannery.

	pH	TDS ($g\ l^{-1}$)	SS ($mg\ l^{-1}$)	VSS ($mg\ l^{-1}$)	COD ($mg\ l^{-1}$)
Influent No.1	7.8	32.9	5,100	1,600	2,600
Influent No.2	7.0	50.5	10,700	1,500	4,400
Influent No.3	7.7	58.2	5,100	1,400	2,200
Influent No.4	7.6	72.3	9,600	2,400	2,300
Mean	**7.5**	**53.5**	**7,600**	**1,700**	**2,900**
Std. Dev.	*0.3*	*16.4*	*3,000*	*500*	*1,100*

Anaerobic moving bed (AMB)

The lab-scale AMB, schematised in Figure 5.6, had a volume of 5 l. The filling of the reactor was done via the bottom, 4 to 5 times a day (depending on the desired hydraulic retention time (HRT)), using a peristaltic pump delivering 200 ml of influent for every

batch. The treated effluent was withdrawn from the top of the reactor. Thus, reactor operation was semi-continuous.

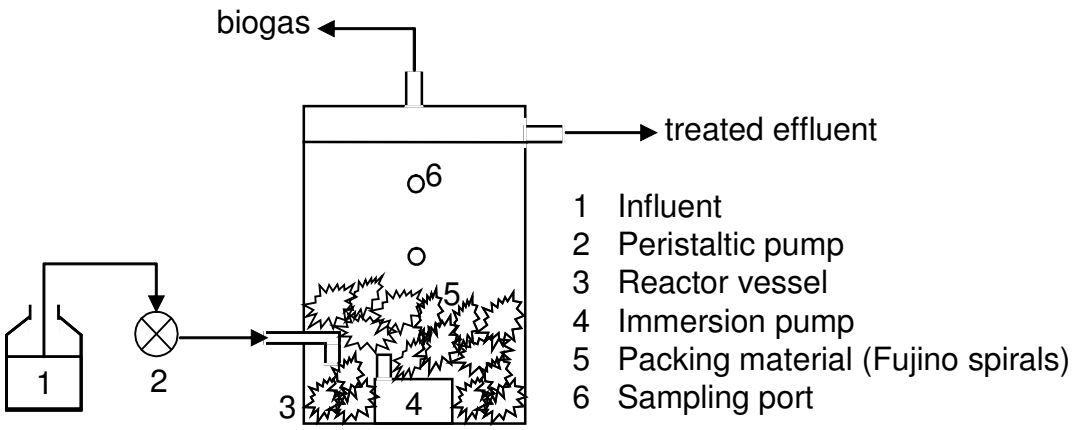

Figure 5.6 Lab-scale anaerobic moving bed.

The packing material used was made of PVC spirals (Fujino Spirals, see Figure 5.7) whose characteristics are indicated in Table 5.5. 50% of the reactor's volume was packed with the spirals and an immersion pump was placed at the bottom of the reactor and operated every hour in order to mix the media. The ambient temperature of the wastewater in the reactor was close to 30°C.

Figure 5.7 Detail of a Fujino spiral.

Table 5.5 Characteristics of Fujino spirals used to pack AMB.

Size (mm)	Surface area ($m^2\ m^{-3}$)	Void ratio (%)	Packing factor ($m^2\ m^{-3}$)	Weight ($g\ l^{-1}$)
55	350	92	272	110

Inoculum

The AMB profited from the inoculation of anaerobic sludge collected from a pilot-scale digestor treating composite tannery effluent. The addition of this inoculum was thought

likely to introduce an anaerobic population used to tannery effluent components, thus reducing the start-up time [138]. In addition, anaerobic sludge collected from a lab-scale UASB treating tannery soak liquor was also added. Finally, a permanent addition of Bacteria occurred because the tannery soak liquor that was used as an influent contained a certain amount of Bacteria and, on occasion, even protozoans.

Analysis

COD, TDS, suspended solids (SS), volatile suspended solids (VSS), total alkalinity (TA), volatile fatty acids (VFA) and pH were analysed following APHA's Standard Methods for the Examination of Water and Wastewater [9]. Quality control was ensured using standards as well as duplicates. COD was determined by the open reflux method. Mercuric sulphate was used to eliminate the interference of chlorides when dosing COD: Sawyer and McCarty have reported that this interference could be eliminated as long as a 10:1 weight ratio of mercuric sulphate to chloride is maintained [193]. Determination of VFA was done using the distillation method, in which the results are expressed as mg l^{-1} of acetic acid.

Results

Conditions of operation of the AMB treating tannery soak liquor

The operation of the AMB treating tannery soak liquor lasted over 120 days. 4 different samples of soak liquor collected from the same tannery were used as an influent during the experiment. The characteristics of the samples showed high variability, especially of organic matter (COD) and salinity (TDS), as specified in Table 5.4. The environmental conditions prevailing in the reactor therefore varied as a function of time, as shown in Figure 5.8. TDS concentration increased in stages throughout the experiment, from 26 g l^{-1} on day 6 up to 72 g l^{-1} on day 124, due to the increase of the influent TDS, whereas OLR was maintained constant at a low value of 0.44 ± 0.10 kg COD m^{-3} d^{-1} by varying the hydraulic retention time (HRT).

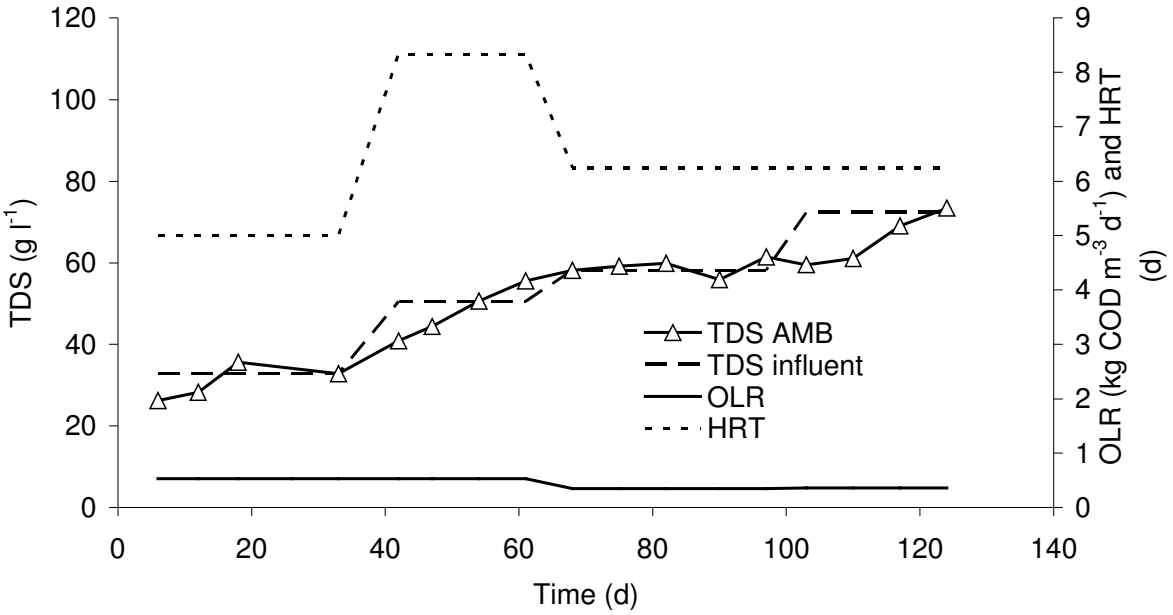

Figure 5.8 Evolution of the environmental parameters applied to the AMB treating tannery soak liquor.

Physico-chemical characteristics of the AMB treating tannery soak liquor

The evolution of total alkalinity (TA), VFA and pH during the experimental period is indicated in Figure 5.9. Total alkalinity showed a tendency to decrease throughout the experiment, passing from 1,680 mg $CaCO_3$ l^{-1} on day 6 to 790 mg $CaCO_3$ l^{-1} on day 124. Meanwhile, the VFA concentration fluctuated between 100 and 500 mg acetic acid l^{-1}. pH remained constant over the experimental period, averaging 7.8 ± 0.2.

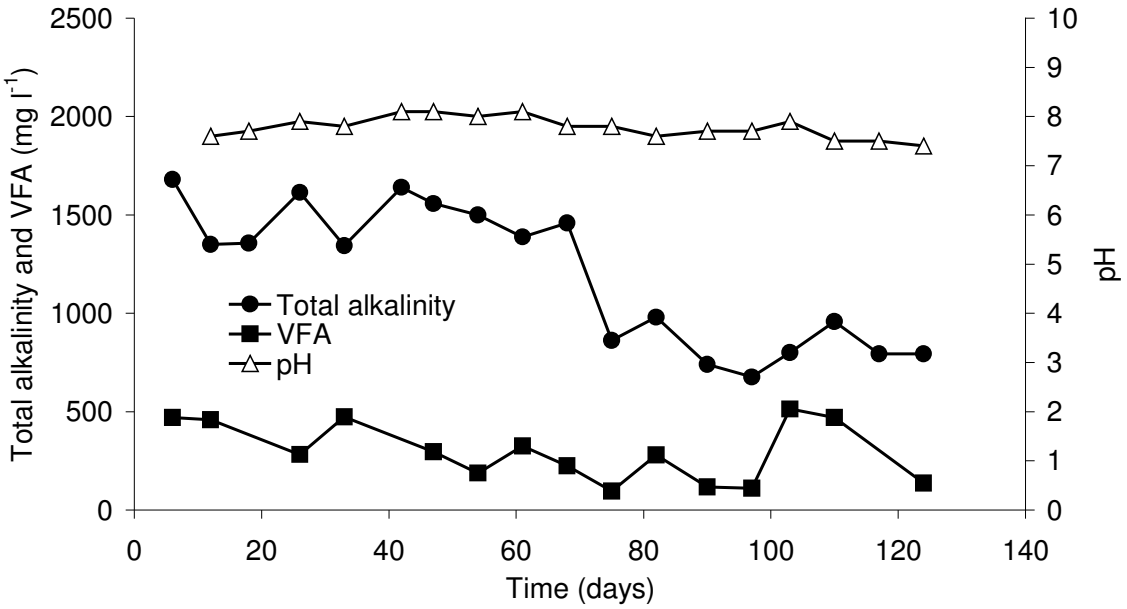

Figure 5.9 Evolution of total alkalinity, VFA and pH in the AMB treating tannery soak liquor.

COD removal

The performance of the AMB over the experimental period, in terms of COD removal, is shown in Figure 5.10. It can be seen from this figure that the performance of the reactor clearly depended on the influent COD. As long as influent No.1 was used, the treated effluent COD slowly decreased, reaching 900 mg l^{-1} (with a corresponding COD removal efficiency of 66%) on day 26, thus indicating an improvement of the treatment efficiency with time, then finally a stabilisation. The shift to influent No.2, which contained more COD, resulted in an increase in the treated effluent COD, in spite of an increase of HRT (see Figure 5.8) aimed at keeping OLR constant. The treated effluent COD attained 1,700 mg l^{-1} (with a corresponding COD removal efficiency of 62%) on day 47, and then stabilised. Finally, the shift to influent No.3, which contained less COD, permitted a decrease once again of the treated effluent COD, which reached 600 mg l^{-1} (with a corresponding COD removal efficiency of 74%) on day 82, and then stabilised until the end of the experiment. Influent No. 4 appeared to be very similar to influent No.3 and did not disturb the performance of the AMB. It appeared, therefore, that the treated effluent COD varied as a function of influent COD. However, the COD removal efficiency remained in the range of 60-75% throughout the experiment.

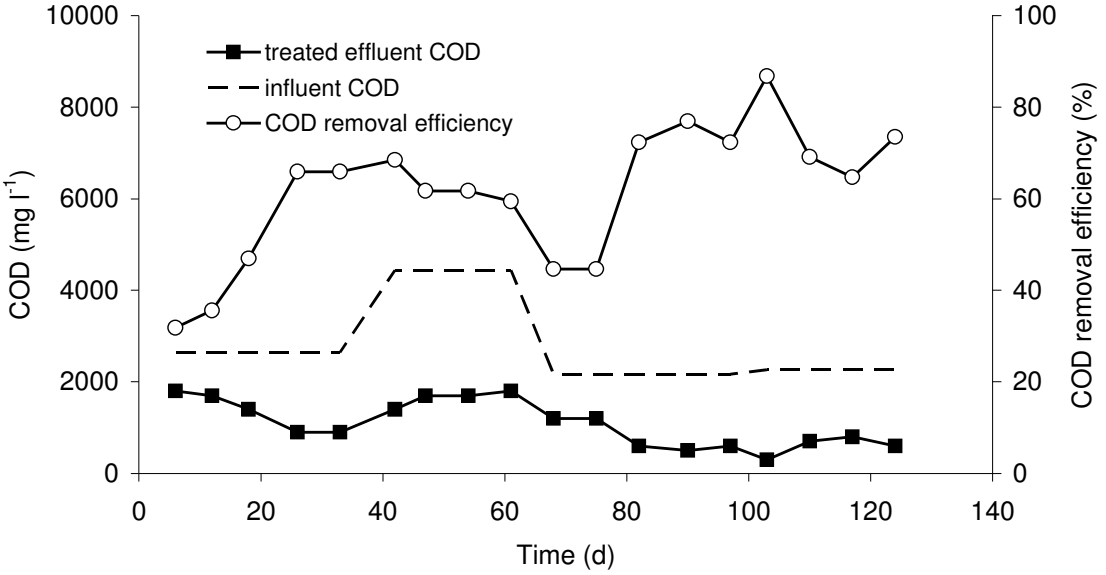

Figure 5.10 COD removal efficiency of the AMB treating tannery soak liquor.

Influents No.1 to 4 were characterised by increasing TDS concentrations, as mentioned above. This resulted in increasingly saline conditions in the AMB (see Figure 5.8). Figure 5.11 shows the evolution of the COD removal rate in the AMB treating tannery soak liquor with an increasing TDS concentration. It can be seen from this figure that the COD removal rate was always between 0.15 kg COD m^{-3} d^{-1} and 0.36 kg COD m^{-3} d^{-1}, whatever the TDS concentration. Therefore, there is no clear indication of any disturbance created by increasing salinity in the AMB.

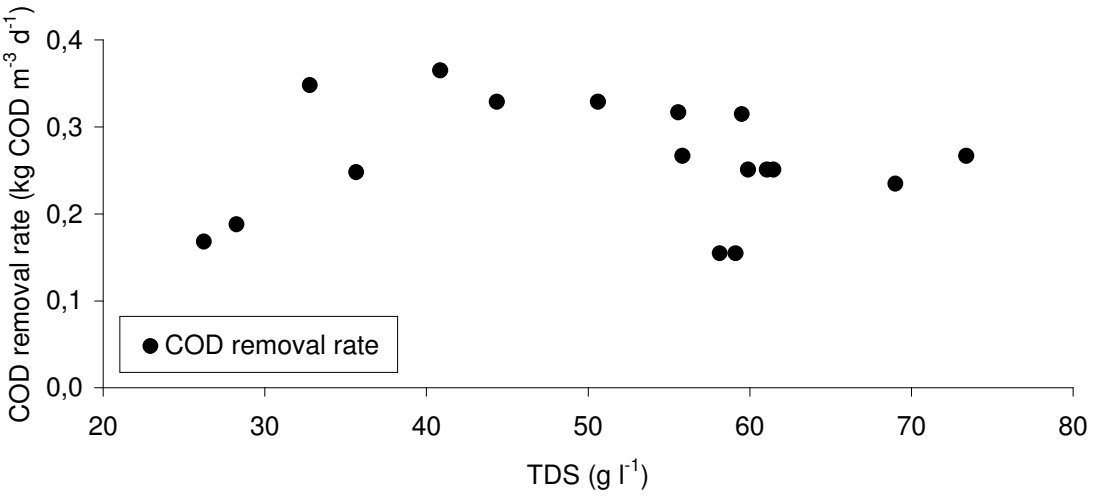

Figure 5.11 Evolution of COD removal rate in the AMB treating tannery soak liquor with increasing TDS concentration.

Biomass production and attachment

Initial and final concentrations of mixed-liquor volatile suspended solids (MLVSS), measured on days 1 and 124 in the AMB, attained 4 and 8 g l^{-1}, respectively. No sludge withdrawal was done during the experiment. Thus, the MLVSS accumulation rate averaged 30 mg l^{-1} d^{-1} throughout the experimental period. At the end of operation, spirals were removed from the bioreactor and no biofilm could be observed on the surface of the spirals. At the beginning of the experiment, the average dry weight of one Fujino spiral, determined by weighing a sample of 30 spirals, was 3.09 g, and the average dry weight of one spiral at the end of the experiment, determined on a sample of 6 spirals, was 3.12 g. This shows that no significant increase of the spirals' dry weight occurred during the experiment. It can therefore be concluded that the biomass was unable to attach itself to the medium and remained suspended throughout the experiment.

Comparison with an anaerobic suspended-growth bioreactor treating tannery soak liquor

The performance of the AMB operated in this study was compared to the performance in a previous study of an upflow anaerobic sludge blanket (UASB) fed with the same influent (see section 5.1). The optimal stable performance of each process is detailed in Table 5.6. It can be seen from this table that the performance of both systems, when operated under similar environmental conditions, is similar. Thus, the use of an attached-growth process was not able to improve the anaerobic treatment of tannery soak liquor.

Table 5.6 Performance of two anaerobic bioreactors operating with tannery soak liquor.

	COD influent (mg l^{-1})	OLR (kg COD m^{-3} d^{-1})	HRT (d)	TDS (g l^{-1})	COD treated effluent (mg l^{-1})	COD removal (%)
UASB	2,270	0.5	5	71	500	78
AMB	2,270	0.4	6	72	600	74

Discussion

In a context of water scarcity and the intrusion of salt into freshwater in India, the management of the soak liquor, which is characterised by high organic load, high suspended solids (sand, lime, hair, flesh, dung, etc.) and high salinity, is of priority concern. In warm countries such as India, the anaerobic digestion of waste and wastewater is likely to be highly suitable: it requires no heating, generates less sludge than aerobic treatment and produces valuable biogas. However, the anaerobic digestion of tannery soak liquor in a UASB appeared to be efficient only at low loading rates, which makes it less attractive than aerobic treatment (see section 5.1). Consequently, this study represents an attempt to increase the efficiency of anaerobic digestion by using an attached-growth process.

Total alkalinity (TA), VFA and pH are efficient indicators of the stability of a reactor and of the correct balance between acidification and methanogenesis. Methanogenesis, in particular, is known to become unstable when the alkalinity ratio (VFA:TA) is above 0.3 [153]. Furthermore, optimal methanogenesis is known to take place at a neutral pH. Inhibition of methanogenesis generally results in an increase in the VFA concentration and a sudden drop in alkalinity and pH. With the total alkalinity ratio averaging 0.3 ± 0.1 throughout the experimental period, the physico-chemical conditions applied to the reactor were likely to permit optimal acidogenesis and methanogenesis. Indeed, the critical value of 0.3 mentioned by Mosquera-Corral *et al.* was only occasionally exceeded around days 33, 103 and 110. pH appeared to be slightly alkaline (i.e. 7.8) but constant. Under these environmental conditions and at a constantly low OLR of 0.44 ± 0.10 kg COD m^{-3} d^{-1}, maintained by varying the HRT, the COD removal efficiency of the AMB was generally comprised between 60 and 75% and was not affected by increasing TDS concentrations.

MLSS and MLVSS accumulated regularly throughout the experiment. In fact, it has already been shown in the literature that high salinity is not an obstacle to biomass growth. Panswad and Anan did not notice any important impact of high NaCl concentration (30 g l^{-1}) on MLSS in an anaerobic/anoxic/anaerobic process treating synthetic wastewater, provided that the inoculum was acclimated [168]. Furthermore, they did not notice any significant impact of high salt concentrations and saline shocks on sludge settleability. However, in our study, the accumulation of MLSS and MLVSS was mainly a consequence of the influent's high SS content that accumulated in the reactor. It also appeared that the biomass was unable to attach itself properly to the medium that was used as a packing material. Little work has yet been done regarding the conditions of biomass attachment to a medium in saline conditions under anaerobic conditions. Mendez

et al. did operate a mesophilic anaerobic filter on fish-canning wastewater (13 g Cl^- l^{-1}) at an OLR of 24 kg COD m^{-3} d^{-1}, reaching an immobilised biomass concentration of 72 g VSS l^{-1}, even leading to clogging problems [149]. Subsequently, the continuous treatment of fish meal processing wastewater containing 15 g TDS l^{-1} was carried out in a mesophilic upflow anaerobic filter, leading to similar results [86]. The biomass immobilised on PVC rings of 14 mm inner diameter attained 55 g VSS l^{-1}, whereas the suspended biomass did not exceed 2 g l^{-1}. The author's conclusion was that the biomass which developed in the reactor was mainly immobilised, either by attachment to the support or by entrapping. More recently, Rovirosa *et al.* treated saline wastewater (i.e. 15 g salt l^{-1}) using a laboratory down-flow anaerobic fixed-bed reactor packed with ceramic rings of 3 mm internal diameter [188]. They observed the formation of a biofilm retaining faecal coliforms, thanks to colloidal suspended solids present in the interstitial spaces or on the surface of the biofilm. Finally, Gangagni Rao *et al.* treated pharmaceutical wastewater, containing 20 g TDS l^{-1}, using a fixed-film reactor packed with PVC pall rings of 35 mm diameter [72]. At an optimal OLR of 10 kg COD m^{-3} d^{-1}, the COD removal efficiency reached 60 to 70%. Furthermore, the scanning electron microscope (SEM) image of the anaerobic biofilm formed on the medium showed a well-defined porous network along with the elongated attachment of filamentous bacteria supporting the overall structure of the biofilm. It can thus be concluded from these studies that a good performance of anaerobic digestion of saline effluents is possible using attached-growth processes and that biomass attachment to a medium is possible in saline conditions. However, in our study this attachment did not occur, which can be explained by a combination of different phenomena. Firstly, the medium used in this experiment (i.e. Fujino spirals) and its nature may not have been suitable for the fixing of the anaerobic micro-organisms. Secondly, the operating conditions, implying a long HRT in the range of 5 to 8 days and a low upflow velocity, may have discouraged biomass fixing by maintaining always a high suspended solids concentration within the reactor. In fact, on account of the operating conditions, the upflow velocity, which was between 0.1 and 0.2 cm h^{-1} according to the desired HRT, is likely to have been insufficient to wash out the suspended biomass and a higher upflow velocity in the initial period would have helped create turbulent conditions, which might have favoured the selection of bacteria that readily attach to the media. Thirdly, as mentioned above, biofilm formation is the result of complex interactions between different groups of Bacteria, including, *inter alia*, filamentous Bacteria that provide the basis of the biofilm structure over which other Bacteria can attach themselves, so creating a complex network. These conditions, requiring high biodiversity, may well be less likely to occur in the case of a saline environment with unadapted bacteria. Even though the biodiversity of a saline environment can be similar to that of a non-saline one, it has already been

shown that the consequence of increasing salinity is initially a reduction of the microbial diversity (see section 4.1). In addition, filaments, which seem to contribute to the integrity and the structure of the biofilm [72], were never observed in the course of our study. Finally, the startup duration of AMB is known to require as much as 3 to 6 months [150]. This period exceeds the total duration of our experiment and it is worthy of note that in most of the studies that have been cited above, the adaptation of anaerobic sludge to highly saline concentrations had generally required a prolonged startup period, itself longer than the total duration of our study. Nevertheless, our study lasted a total of 4 months, which should have been sufficient to reveal at least the beginning of biomass adaptation and fixing on the media.

The reasons listed above may explain the absence of attached biomass on the medium used in our work. At all events, in our study the AMB functioned mainly as a suspended-growth bioreactor. Consequently, it is not surprising that no improvement in the performance of the system was observed in this study in comparison to a UASB reactor. In this study, the AMB's optimal performance was obtained at the end of the experiment, while using influents No. 3 and 4, with an OLR of 0.4 kg COD m^{-3} d^{-1}, a HRT of 6 d and a TDS concentration comprised between 58 and 72 g l^{-1}. Under these environmental conditions, the treated effluent COD averaged 600 ± 150 mg l^{-1}, with a corresponding COD removal efficiency of 74 ± 7 %. Due to such limited efficiency, the use of an aerobic post-treatment would thus be required to improve the process. Under similar conditions (i.e. OLR of 0.5 kg COD m^{-3} d^{-1}, HRT of 5 d and TDS concentration of 71 g l^{-1}), the optimal COD removal efficiency of a lab-scale UASB reactor treating the same tannery soak liquor was 78%, which appears to be very similar (see Table 5.6). In comparison, an aerobic SBR was able to remove 95% of the COD of the same soak liquor at an OLR of 0.6 kg COD m^{-3} d^{-1}, a HRT of 5 d and a TDS concentration of 35 g l^{-1} (see section 4.2). It appears, then, that the aerobic treatment of tannery soak liquor is more viable from an economic point of view than the anaerobic digestion of the same effluent.

Acknowledgements

This study was conducted at the Centre for Environmental Studies, Anna University, Chennai (India), thanks to cooperation between Anna University and the French National Institute for Agricultural Research (Institut National de la Recherche Agronomique, INRA), within the framework of the Indo-French Cell for Bioprocesses on Environment (IFCBE). The authors would like to thank Ms. J. D'Silva for technical assistance during the study.

5.3 Effet de l'augmentation de la salinité sur les performances du traitement anaérobie et sur l'écologie microbienne des boues

Article : Impact of increasing NaCl concentrations on the efficiency and microbiology of anaerobic digestion

Résumé

Le traitement biologique anaérobie des effluents salins au moyen de consortia halophiles et halotolérants est d'un intérêt majeur. La digestion anaérobie des effluents salins est connue pour être inhibée par les fortes salinités, cependant, il semble qu'une stratégie adéquate d'adaptation des boues anaérobies à des salinités croissantes rende possible la dépollution des effluents salins. Dans cette étude, des boues anaérobies non salines ont été inoculées dans deux digesteurs fonctionnant avec un substrat différent (vinasse de distillerie et éthanol), puis soumises à des concentrations croissantes de NaCl. Les performances des digesteurs ont été très variables en fonction du substrat, le réacteur fonctionnant sur éthanol pouvant supporter des concentrations plus élevées en NaCl. La caractérisation de la microflore des boues et son adaptation au sel ont également été analysées par des techniques moléculaires. Ces résultats ont ensuite été corrélés à l'évolution des performances des deux digesteurs anaérobies. L'analyse des communautés microbiennes a révélé une grande diversité qu'il est possible de maintenir en dépit de l'augmentation de salinité.

Abstract

The anaerobic treatment of saline effluents using halophilic and halotolerant microbial consortia is of major interest. Anaerobic digestion is known to be inhibited at high salt content ; however, it seems that a suitable strategy for the adaptation of an anaerobic sludge to increasing salinity makes possible the depollution of saline wastewater. In this study, a non-saline anaerobic sludge was inoculated in two digesters operating with a different substrate (distillery vinasse and ethanol) and then subjected to increasing NaCl concentrations. The performance of the digesters appeared to be highly dependent on the nature of the substrate, the reactor fed with ethanol withstanding higher NaCl concentrations. The characterisation of the microflora and its adaptation to increasing NaCl conditions were also investigated using molecular tools based on the analysis of genomic 16S rDNA. These results were then correlated to the evolution of the

performance of the two digesters. The microbial communities revealed a high diversity that could be maintained in both reactors despite the increase in NaCl concentrations.

Introduction

The biodepollution of saline and hypersaline wastewater could represent as much as 5% of worldwide effluent treatment requirements. Anaerobic digestion is known to be inhibited by high salinities, mainly due to the cations. It has already been reported that a sodium concentration exceeding 10 g l^{-1} strongly inhibits methanogenesis [84; 121; 182]. Furthermore, Feijoo *et al.* noticed a strong impact from the type of methanogenic substrate used [68]. In spite of the obstacle created by high salt concentrations on anaerobic digestion, a certain number of processes have been used successfully for the anaerobic treatment of saline wastewater. Some of them used a halophilic inoculum [11; 153; 224], whereas others required the adaptation of a non-halophilic inoculum to increasing salt concentrations [23; 72; 74; 86; 87; 163; 188]. From the point of view of microbial ecology, the adaptation of a non-saline sludge to high salinities implies the acclimation of halo-tolerant microorganisms to high salt content. Some studies suggest that this acclimation is possible, depending on the nature and the progressive adaptation of the sludge to high salinity [68; 163]. However, little is known regarding the evolution and the diversity of a microbial ecosystem treating highly saline wastewater, even though it has already been shown that the diversity of a salt-tolerant ecosystem treating hypersaline industrial wastewater could be similar to that of a non salt-tolerant one (see section 4.1).

In this study, a non-saline anaerobic sludge was inoculated in two digesters, each operating with a different substrate (distillery vinasse and ethanol) and then submitted to NaCl concentrations increasing in stages. The characterisation of the microflora and its adaptation to increasing NaCl conditions were investigated using molecular tools based on the analysis of genomic 16S rDNA. These results were then correlated to the evolution of the performance of two digesters operating with two different substrates. The present study aims therefore at a better understanding of the levels of NaCl that limit the anaerobic digestion of different substrates and of the adaptability of a non-salt-tolerant microbial ecosystem to increasing NaCl concentrations. Thus, the halo-tolerance of non-saline sludge is considered from the point of view of microbial ecology.

Materials and methods

Bioreactors

This study was conducted using two double-walled anaerobic sequencing batch reactors (ASBR) of 5 l each and maintained at 37°C by a thermostatically regulated water bath (see Figure 5.12). Peristaltic pumps were used for substrate filling and withdrawal and mixing was ensured using magnetic stirrers. The pH and biogas production were measured online. Data acquisition and treatment were performed using the "Modular SPC" software developed by the LBE in Narbonne.

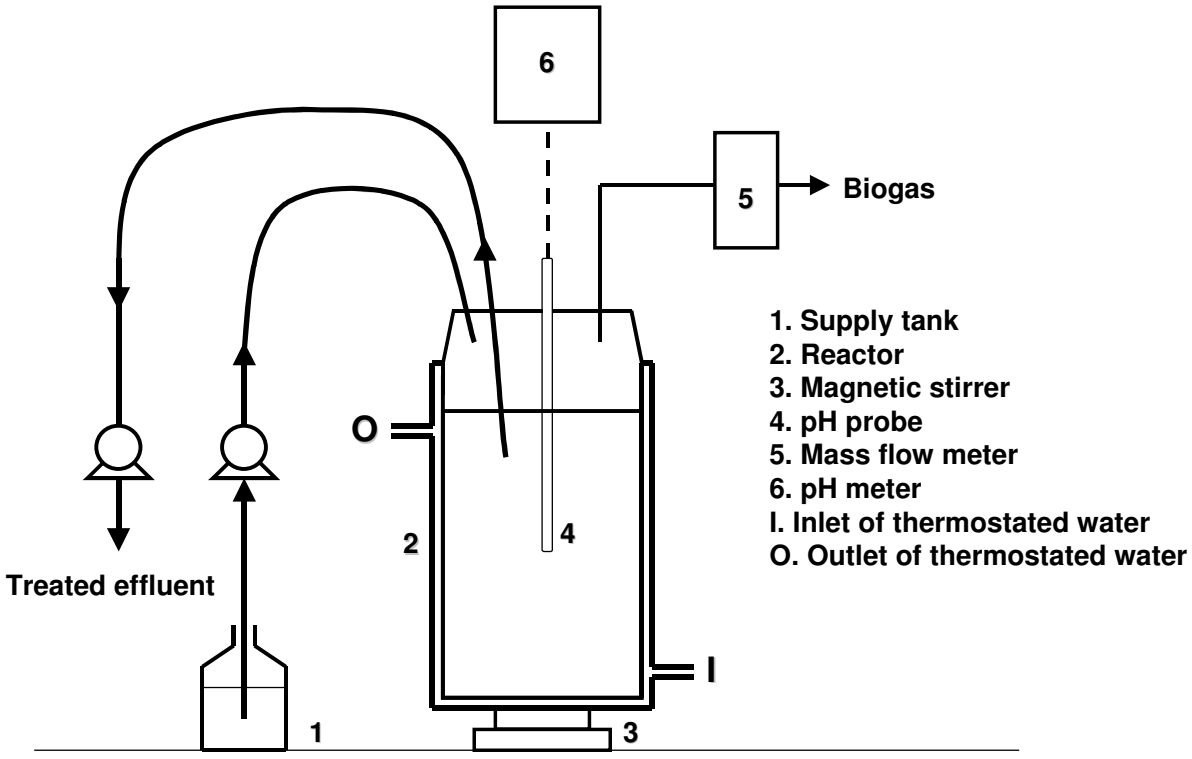

Figure 5.12 Experimental design of ASBR operating with ethanol and distillery vinasse.

Both ASBR were inoculated with anaerobic sludge originating from an upflow anaerobic sludge blanket (UASB) reactor treating sugar wastewater and so not adapted to saline conditions. Initial volatile suspended solids (VSS) concentrations were, respectively, 13.5 and 16 g l^{-1} for the reactors, operating with distillery vinasse and ethanol. The concentrations then decreased due to the start-up of the experiment and stabilised around 8.2 and 7.6 g l^{-1}, respectively.

Substrates

Two different substrates were used to feed the two anaerobic sequencing batch reactors (ASBR), i.e. distillery vinasse, used as a complex substrate, and ethanol, used as a simple substrate. The distillery vinasse originated from a distillery at Narbonne (France). Being the residue of wine distillation, it was composed mainly of proteins and carbohydrates, as already described [226]. This wastewater was characterised by a high organic concentration, with the soluble chemical oxygen demand (COD) varying between 28 and 35 g l^{-1}, and a COD:N:P ratio of 200:3:1. However, in regard to the nitrogen and phosphorus requirements for anaerobic digestion, no deficiency was recorded [91]. In addition, the effluent was characterised by a mean suspended solids (SS) concentration of 1.2 g l^{-1}, 75% of which were volatile, thus showing that the SS were constituted mainly of organic matter. Finally, the pH averaged 3.5, due to the high organic acids content. It was therefore neutralised by NaOH before being introduced into the reactor, in order to maintain reactor pH close to 7 at the end of the filling period. This resulted in an initial Na^+ concentration of 3 gl^{-1} in the reactor operated with distillery vinasse, after it reached the steady-state conditions. This initial addition of Na^+ will not be mentioned any further in this paper since the study focuses on the effect of added NaCl. Yet, it should be kept in mind that the addition of 1, 5 and 10 g l^{-1} of NaCl (i.e. 0.4, 2 and 4 g l^{-1} of Na^+) in the reactor operating with distillery vinasse resulted in a Na^+ concentration of 3.4, 5 and 7 g l^{-1}, respectively. This effluent was stored at 4°C throughout the experiment.

Ethanol with a purity level of 95% was used as a simple substrate for the second reactor because it is known to be highly biodegradable in anaerobic conditions [189]. The oxidation of 1 mol of ethanol requiring 3 mol of O_2, the COD associated with this substrate equaled 2.1 g O_2 g^{-1} of ethanol. Therefore, ethanol presented a COD concentration of 1,600 g l^{-1}.

In order to avoid any nutriment deficiency, both reactors initially received a mineral complement (Fe^{++}, Ni^{++}, Mg^{++}, Ca^{++}, Ba^{++}, Co^{++}, SO_4^{--}), as prescribed by Henze and Harremoës [91]. In addition, the ethanol-fed reactor was supplemented in nitrogen and phosphorus, again following Henze and Harremoës [91].

Analysis

COD, SS, VSS were analysed following the analytical methods recommended by AFNOR, the French Standards Authority [1]. Soluble COD analysis were preceded by a centrifugation step (15,000 rpm, 15 min). COD was determined by the closed reflux method, mercuric sulphate being used to eliminate the interference of chlorides when

dosing COD, in accordance with Sawyer and McCarty who reported that this interference could be eliminated as long as a 10:1 weight ratio of mercuric sulphate to chloride is maintained [193]. Determination of volatile fatty acids (VFA) was done using a gas chromatograph fitted with a flame ionisation detector (Chrompac CP 9000) and coupled with an integrator (Shimadzu CR 3A). The percentage of methane in the biogas was determined with a gas chromatograph (Shimadzu GC-8A), with argon as the carrier gas, equipped with a thermal conductivity detector and connected to an integrator (Shimadzu CR 3A).

Extraction and purification of total genomic DNA

For each level of NaCl, sludge samples were withdrawn from both reactors, after stabilisation of the performance, and frozen for molecular analysis of their microbial communities. 10 ml of mixed liquor were collected from the middle of the reactors and centrifuged at 7,000 rpm and 4°C for 10 min. Cell pellets were suspended in 2 ml of 4 M guanidine thiocyanate 0.1 M Tris-HCl pH 7.5 and 0.6 ml of 10% *N*-lauroyl sarcosine and immediately stored at -20°C. Total DNA was extracted and purified from 10 ml of sample using the QiaAmp DNA stool mini kit, according to the manufacturer's instructions (Qiagen, Hilden, Germany).

Amplification, cloning and sequencing of 16S rDNA

After extraction, bacterial 16S rDNA genes were amplified by PCR using the bacterial forward primer W18 (5'-GAGTTTGATCMTGGCTCAG-3') and the universal reverse primer W31 (5'-TTACCGCGGCTGCTGGCAC-3'), previously described by Snell-Castro et al. (2005), in positions 9-27 and 482-500 in *Escherichia Coli*, respectively [31]. 16S rDNA genes from the Archaea domain were targeted using the archaeal forward primer W17 (5'-ATTCYGGTTGATCCYGSCRG-3') and the universal reverse primer W02 (5'-GNTACCTTGTTACGACTT-3'), *E. coli* positions 3-22 and 1509-1492, respectively. Eukaryotic forward primer W99 (5'-CGGTAATTCCAGCTCC-3') [62], *E. coli* positions 528 to 544, and W02 were used for the partial amplification of 18S rRNA eukaryotic genes. PCR reactions were as follows: each PCR reaction tube contained 2 µl of each primer at a concentration of 100 ng μl^{-1}, along with 5 µl of 1X *Taq* reaction buffer (Perkin Elmer, Forster City, CA, USA), 4 µl of dNTP 2,5 mM, 1 µl of *Taq* DNA polymerase (Perkin Elmer) and water for a final volume of 49 µl. This PCR mix was then added to 1 µl of DNA diluted 5 times in water. PCR conditions were as follows: an initial denaturation step at 94°C for 2 min, followed by 25 cycles of a three-stage program: 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, the final elongation step running for 10 min at 72°C. PCR products were purified with Qiagen microcolumns, according to the manufacturer's instructions

(Qiagen). Purified PCR products were cloned and transformed into TOP10 *E. coli* competent cells using the PCR 4-TOPO vector kit, according to supplier instructions (Invitrogen, Groningen, The Netherlands). Recombinant cells were selected using kanamycine resistance and *ccd* gene killer inactivation. *E. coli* with inserts of proper size were screened by PCR on colonies with plasmid-targeted primers T7 (TAATACGACTCACTATAGGG) and P13 (GACCATGATTACGCCAA). PCR reactions were as follows: each PCR reaction tube contained 0.8 µl of each primer at a concentration of 100 ng μl^{-1}, along with 2 µl of 1X *Taq* reaction buffer (Perkin Elmer), 1.6 µl of dNTP 2,5 mM, 0.5 µl of *Taq* DNA polymerase (Perkin Elmer) and water for a final volume of 20 µl. This PCR mix was then inoculated with recombinant cells sampled from recombinant colonies, using sterile toothpicks. PCR conditions were as follows: an initial denaturation step at 94°C for 10 min, followed by 25 cycles of a three-stage program: 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, the final elongation step running for 10 min at 72°C. Afterwards, the PCR products were sent for sequencing.

Sequence analysis

16S rDNA sequences (of about 500 bp) were identified by comparison with sequences available in databases using the BLAST program. The nucleotide sequence data reported in this work will appear in the GenBank nucleotide database under accession numbers AM117917 to AM117928.

SSCP analysis

Single Strand Conformation Polymorphism (SSCP) analyses were performed for the overall detection of microbial populations and for a study of their dynamics. SSCP analysis makes it possible to separate DNA fragments of a similar size according to their configuration (secondary structure). Targeting the 16S rDNA V3 region, which permits the phylogenetic discrimination of microbial species, enables the reactor microbial community to be monitored by one profile of peaks, each peak corresponding to a distinct sequence of the 16S rDNA V3 region i.e. to one bacterium. The height of the peaks corresponds to the quantity of 16S rDNA sequence after PCR amplification.

The amplification of the V3 region of bacterial 16S rDNA PCR-SSCP was carried out with the bacterial primer pair W49 (-5'-ACGGTCCAGACTCCTACGGG-3'-) and the 6FAM-labeled primer W104 (-5'- TTACCGCGGCTGCTGGCAC-3'-) from total DNA [81]. The archaeal V3 16S rDNA region was amplified using a nested PCR assay combining a first PCR amplification with primers W02 and W17, followed by the PCR-SSCP using primers W104 and W116 (-5'-TCCAGGCCCTACGGGG-3') [81]. The Eukaryota primers used for

18S V7 rDNA amplification were W16 (-5'-CTTAATTTGACTCAACACGG-3'-) and the 6FAM-labeled primer W131 (-5'-GGGCATCACAGACCTGTT-3') [80]. An initial denaturation step at 94°C for 2 min, was followed by 25 to 30 cycles of a three-stage program: 30 sec at 94°C ; 30 sec at 61°C with Bacteria primers, 51°C with Archeae primers, or 55°C with Eukaryota primers ; and 30 sec at 72°C, followed by a final elongation for 10 min at 72°C. DNA polymerase was *Pfu* turbo (Stratagene). PCR-SSCP products were purified using the QIAquick kit and were estimated by gel-electrophoresis.

For electrophoresis, PCR-SSCP products were diluted in water before mixing with 18.75 µl formamide (Genescan-Applied Biosystems) and 0.25 µl internal standard (ROX, Genescan-Applied Biosystems) [18]. The mixture was denatured by heating at 95°C for 5 min and cooled in watery ice for 10 min. Single strands of DNA molecules formed stable secondary conformations that were separated by capillary electrophoresis. SSCP analyses were performed with the automatic sequencer abi310 (Applied Biosystems). DNA fragment detection was done by fluorescence. The results obtained were analysed by GeneScan® 3.1 (Applied Biosystems).

To identify SSCP peaks of interest, 16S rDNA V3 from reactor samples were amplified and cloned into *E. coli*, as described above. Cloned inserts were amplified by PCR using the plasmid targeted primers T7 and P13. PCR-SSCP on the resulting DNA fragment produced, after SSCP analysis, single peaks that were compared with total microbial community profiles for peak assignation. Sequencing of interesting cloned V3 regions was carried out and, finally, the identification of micro-organisms corresponding to peaks was successfully established as described above.

Results

Working conditions of the reactors and experimental strategy

The ASBR reactors were operated with cycles including the following four discrete steps : rapid filling, reaction, settling and withdrawal. The operating conditions applied to both reactors ensured a S_o:X_o ratio ranging from 0.2 to 0.3 g of COD g^{-1} of VSS for the reactor fed with distillery vinasse and from 0.3 to 0.4 g of COD g^{-1} of VSS for the reactor fed with ethanol. The rate of biogas production, measured online, was maximal at the start of the cycle, just after the feed period, and then decreased with time, reaching very low levels at the end of the reaction stage, indicating very low metabolic activity. The reaction stage was interrupted when the biogas production rate dropped below the minimum limit of 0.5 ml min^{-1}, a level indicating that the added organic matter had been

eliminated. At this point, the concentration of VFA was nil and soluble COD concentration at its lowest.

Operating in batches enabled the operation of both reactors during several cycles for each level of NaCl concentration. Then, the comparison of these cycles on the basis of reactor performance (in terms of biogas production rate, reaction time, COD removal, loading rate and methanogenic activity) permitted the determination of the steady-state conditions from which standard cycles were determined for each level of NaCl concentration.

Detailed study of standard treatment cycles without addition of NaCl

Figure 5.13 and Figure 5.14 show the standard cycles, obtained without addition of NaCl, for distillery vinasse and ethanol respectively. In the conditions prevailing in this study, the biogas production rate obtained with distillery vinasse displayed a specific profile (Figure 5.13). Indeed, a treatment cycle could be seen as composed of two phases. The first phase, corresponding to the first 1 h following filling, was characterised by a high biogas production rate, slowly decreasing from 30 to 25 ml min^{-1}, and a decreasing pH related to an increasing concentration of VFA. The concentration of VFA reached a maximum at the end of this phase, attaining 1.7 g of acetate l^{-1} and 1.0 g of propionate l^{-1} after 1 h (data not shown). At the start of the second phase, after 1 h there was a drop in the rate of biogas production followed by a period lasting for several hours characterised by a rate of biogas production that decreased slowly. The pH was at its lowest at the end of the first phase, increasing regularly thereafter throughout the second phase, as the concentration of VFA slowly decreased to nil at the end of the cycle.

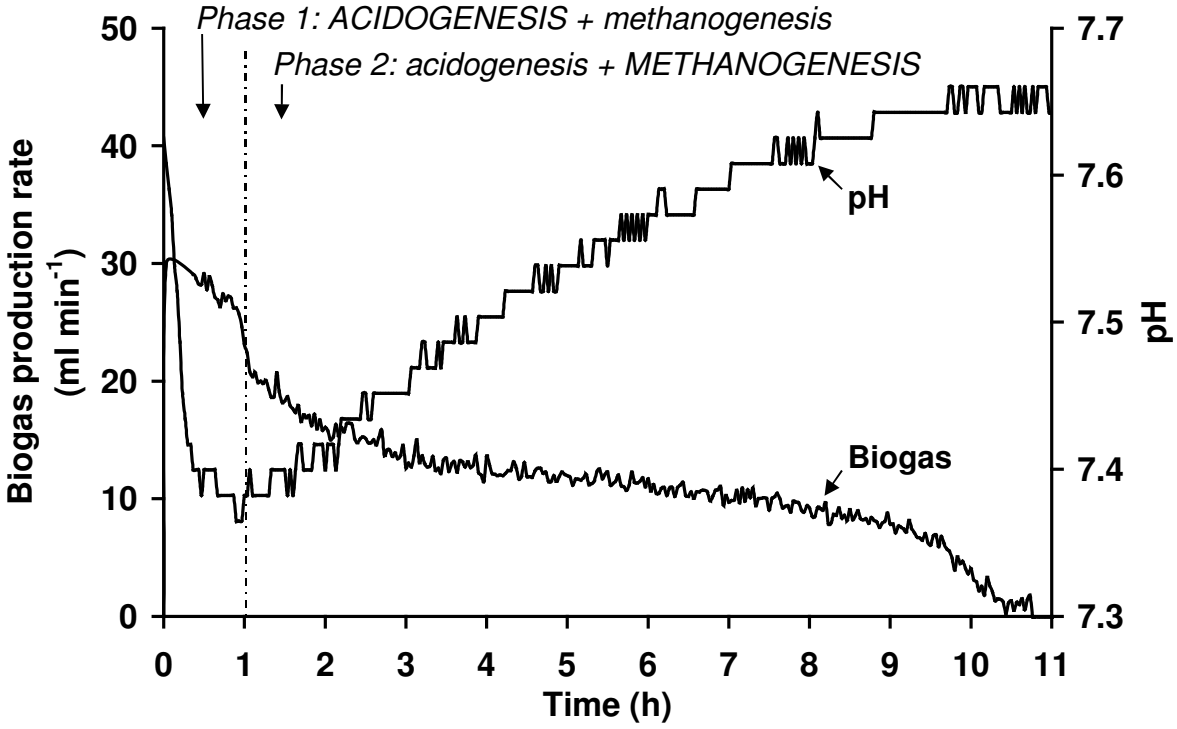

Figure 5.13 Biogas production rate and pH during a standard cycle without addition of NaCl. ASBR operating with winery wastewater.

To better understand these observations, an analysis of the standard cycle obtained in non-saline conditions with ethanol used as the sole carbon substrate is extremely useful. When ethanol alone was added to the reactor, the cycle obtained (Figure 5.14) also showed 2 phases. During the first phase, the biogas production rate was at its maximum (around 42 ml min^{-1}) and remained fairly constant for 2 h. This phase ended with a sharp drop in this rate some 2 h after the beginning of the reaction time. The second phase was then characterised by a lower and fairly constant biogas production rate (around 23 ml min^{-1}) lasting for another 2 h. As for distillery vinasse, the concentration of VFA reached a maximum at the end of phase 1 (after 2 h), attaining 2.3 g of acetate l^{-1}, prior to a slow and constant decrease (data not shown). The concentration of acetate was almost nil at the end of the cycle and, in contrast to distillery vinasse, almost no propionate was produced during the cycle.

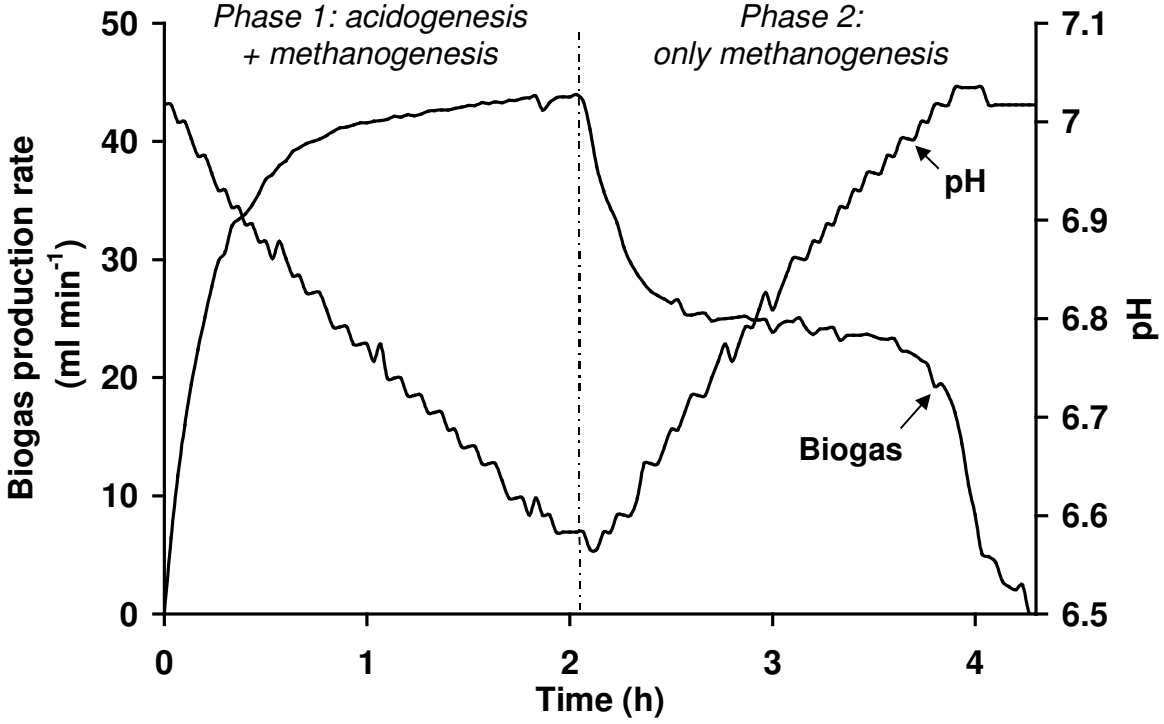

Figure 5.14 Biogas production rate and pH during a standard cycle without addition of NaCl. ASBR operating with ethanol.

A comparison between the results obtained with distillery vinasse and with ethanol suggests that the gas output during the first phase of a cycle using distillery vinasse or ethanol reflects the cumulative production of gas deriving from the acidification of the organic matter and from the methanisation of the VFA that ensue. An accumulation of VFA appears during this first phase, showing that they are produced faster than they can be eliminated. The acidogenesis reaction finished around 2 h after the beginning of the cycle in the case of ethanol (see Figure 5.14), with a resulting sudden drop in the rate of gas production. From this point on, gas production becomes a function only of the degradation of VFA by methanogenesis [189]. The rates of biogas output, along with the clear break observed two hours after the beginning of the cycle, suggest that the rates of the two reactions remain fairly constant for ethanol. In contrast, for distillery vinasse the slope observed during the second phase suggests that some acidogenesis continued but at a slower rate than methanogenesis (see Figure 5.13), thus allowing the elimination of the accumulated VFA and an increase of pH.

Effect of increasing NaCl concentrations on the reactors' performance

Determination of standard cycles

As explained before, the determination of standard cycles was possible for each level of NaCl concentration, on the basis of the reactors' performance in steady state conditions. An illustration of the standard cycles obtained with ethanol as a substrate at 0, 20, 40 and 60 g l^{-1} is shown in Figure 5.15. It can be seen in this figure that the main consequences of increasing NaCl concentrations were a reduction in the biogas production rate and an increase in the length of the cycles. Similar results were obtained with distillery vinasse as a substrate (data not shown).

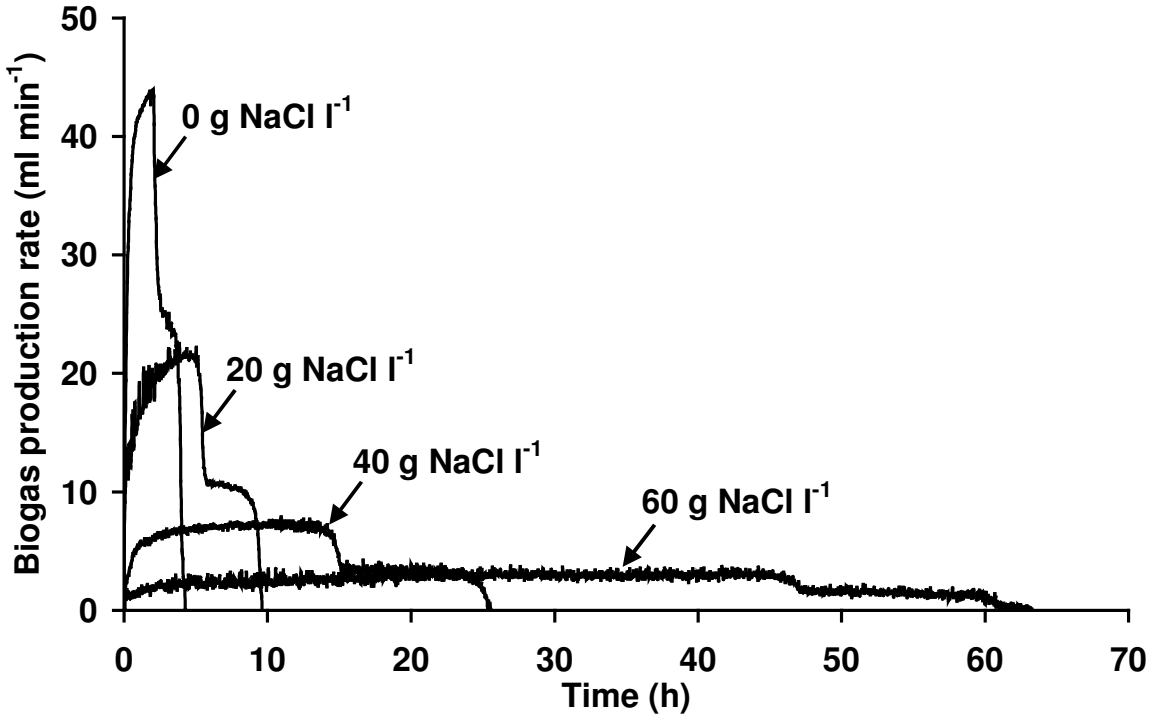

Figure 5.15 Evolution of biogas production rate with increasing NaCl concentrations. Reactor operating with ethanol.

Effect of NaCl on the length of the cycles

From the analysis of standard cycles obtained at each level of NaCl, it was possible to evaluate for each reactor the average length of the cycles at each NaCl concentration and the results are indicated in Figure 5.16. It can be clearly seen from this figure that the consequence of increasing NaCl concentrations was an elongation of cycles for both reactors. The reactor operating with distillery vinasse was already affected at a NaCl

concentration of 1 g l^{-1}, whereas the reactor operating with ethanol was better able to withstand increasing NaCl concentrations. In this reactor, a major effect of NaCl on the length of the cycles only started at a concentration of 40 g l^{-1}. For the reactor operating with distillery vinasse, the length of the cycles increased from 10.7 to 106 h (i.e. an increase of 890%) when NaCl rose from 0 to 10 g l^{-1} whereas, for the reactor fed with ethanol, the length of the cycles increased from 4.5 to 66 h (i.e. an increase of. 1,370%) when NaCl increased from 0 to 60 g l^{-1}.

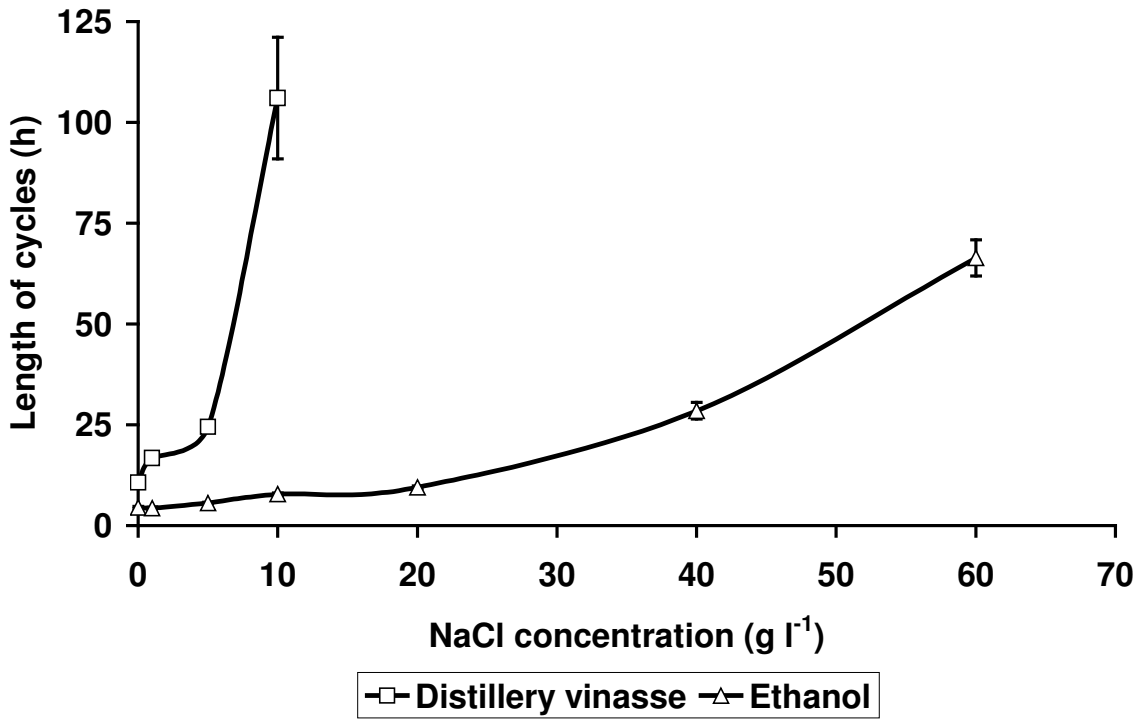

Figure 5.16 Evolution of the length of the cycles with increasing NaCl concentrations, using distillery vinasse and ethanol as substrates.

Effect of NaCl on COD removal

Treated effluent was withdrawn at the end of each cycle, when biogas production was close to zero and COD concentration at its lowest. Thus, the COD measured at that time corresponded to the non- or very slowly-biodegradable fraction of COD. Neither for the reactor operating with distillery vinasse, nor for the reactor fed with ethanol was the COD removal efficiency significantly affected by increasing salt concentrations. The soluble COD removal efficiencies of the reactors averaged 93.5% and 99.9% throughout the experiment, for distillery vinasse and ethanol respectively (data not shown). This result shows that there was no significant decrease of COD biodegradability with increasing NaCl concentrations. Only the reaction time increased.

Effect of NaCl on specific loading rate

The increase of reaction time with increasing NaCl concentrations had severe consequences on the specific loading rate, as shown in Figure 5.17. As for the length of the cycles, a NaCl concentration of 1 g l^{-1} was enough to reduce by 22% the specific loading rate of the reactor fed with vinasse. With 10 g l^{-1} of NaCl, the reduction attained 88%. In contrast, the reactor operating with ethanol could withstand higher NaCl concentrations. A concentration of 5 g l^{-1} induced a reduction of the specific loading rate by 8%, the sharpest drop (about a 73% reduction) occurring at a NaCl concentration of 40 g l^{-1}. Finally, this reduction attained 89% when NaCl concentration reached 60 g l^{-1}, with ethanol as a substrate.

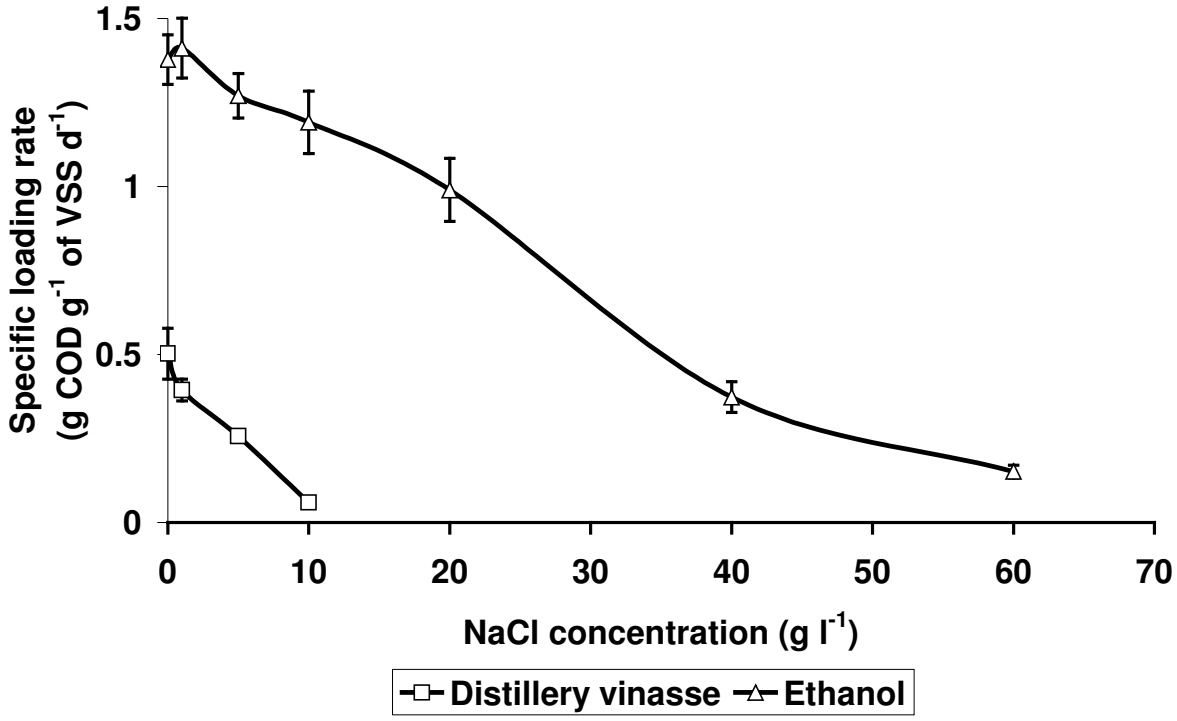

Figure 5.17 Evolution of specific loading rate with increasing NaCl concentrations, using distillery vinasse and ethanol as substrates.

Effect of NaCl on organic loading rate

In industrial applications of wastewater treatment, the organic loading rate is an essential parameter affecting the design of a treatment plant. Increased salt concentrations prolonged the cycles observed for both reactors. Such elongation had drastic consequences on the OLR that could be applied to the two reactors in order to maintain COD removal efficiencies at a maximum. Regarding the reactor operating with distillery wastewater, the addition of 1 g of NaCl l^{-1} was sufficient to reduce the OLR from

4.9 to 3.2 kg COD m^{-3} d^{-1} (i.e. a 33% reduction), and OLR attained 0.5 kg COD m^{-3} d^{-1} (i.e. a 90% reduction) when NaCl concentration reached 10 g l^{-1}. In contrast, the reactor fed with ethanol could withstand higher salinities and its OLR was only significantly affected by a NaCl concentration of 10 g l^{-1} and higher. Consequently, OLR decreased from 17.0 to 9.8 kg COD m^{-3} d^{-1} (i.e. a 42% reduction) when NaCl concentration reached 10 g l^{-1}, and attained 1.2 kg COD m^{-3} d^{-1} (i.e. a 93% reduction) for a NaCl concentration of 60 g l^{-1}.

Effect of NaCl on methanogenic activity

The evolution of specific methanogenic activity was followed for each reactor at each NaCl concentration. COD removal being more or less constant for both reactors, the evolution of methanogenic activity followed the same pattern as for the specific loading rate (data not shown). Consequently, with vinasse as a substrate, specific methanogenic activity decreased from 193 ml CH_4 g^{-1} of VSS d^{-1} at 0 g NaCl l^{-1} to 140 ml CH_4 g^{-1} of VSS d^{-1} (i.e. a 27% reduction) and 24 ml CH_4 g^{-1} of VSS d^{-1} (i.e. a 88% reduction), when NaCl concentrations reached 1 and 10 g l^{-1}, respectively. With ethanol as a substrate, the reactor's specific methanogenic activity only started to be affected at a NaCl concentration of 20 g l^{-1}, thus decreasing from 494 ml CH_4 g^{-1} of VSS d^{-1} at 0 g NaCl l^{-1} to 396 ml CH_4 g^{-1} of VSS d^{-1} (i.e. a 20% reduction) at 20 g NaCl l^{-1}, then to 61 ml CH_4 g^{-1} of VSS d^{-1} (i.e. 88% of inhibition) at 60 g NaCl l^{-1}.

Effect of NaCl on acidogenesis and methanogenesis rates

With ethanol as a substrate, it has been shown that the first phase observed in the standard cycle at 0 g NaCl l^{-1} corresponded to the cumulative production of biogas by acidogenesis and methanogenesis, whereas the second phase corresponded only to methanogenesis [189]. For this reactor, it was therefore possible to distinguish the biogas production rate induced by acidogenesis (i.e. 19 ml min^{-1}) from that induced by methanogenesis (i.e. 23 ml min^{-1}), thanks to fairly constant values during each phase. The establishment of standard cycles for this reactor throughout the experiment enabled this distinction to be made for each level of NaCl. The effect of NaCl on the specific production of biogas by acidogenesis and methanogenesis is thus shown in Figure 5.18. From this figure it can be seen that, on the one hand, methanogenesis started to be inhibited by NaCl at a concentration of 5 g l^{-1}, with a reduction of 7%. This inhibition then reached 93% at a NaCl concentration of 60 g l^{-1}. On the other hand, the specific biogas rate produced by acidogenesis first improved at low salt concentrations (0-10 g NaCl l^{-1}). Reaction time remaining constant over the same period, two hypothesis can explain this fact: either an enhancement of acidogenic bacterial activity or a rise in the concentration

of acidogenic biomass. Mixed liquor VSS actually decreased from 12.4 to 8.2 g l^{-1} at the same time, due to initial wash out (see the Materials and Methods section). It is therefore unlikely that the second hypothesis be confirmed. However, since the acidogenes:methanogenes ratio remains unknown, both hypothesis can explain the phenomenon. Thereafter, at NaCl concentrations of 20 g l^{-1} and higher, a significant reduction in acidogenesis was observed, reaching 11 and 85% at NaCl concentrations of 20 and 60 g l^{-1}, respectively.

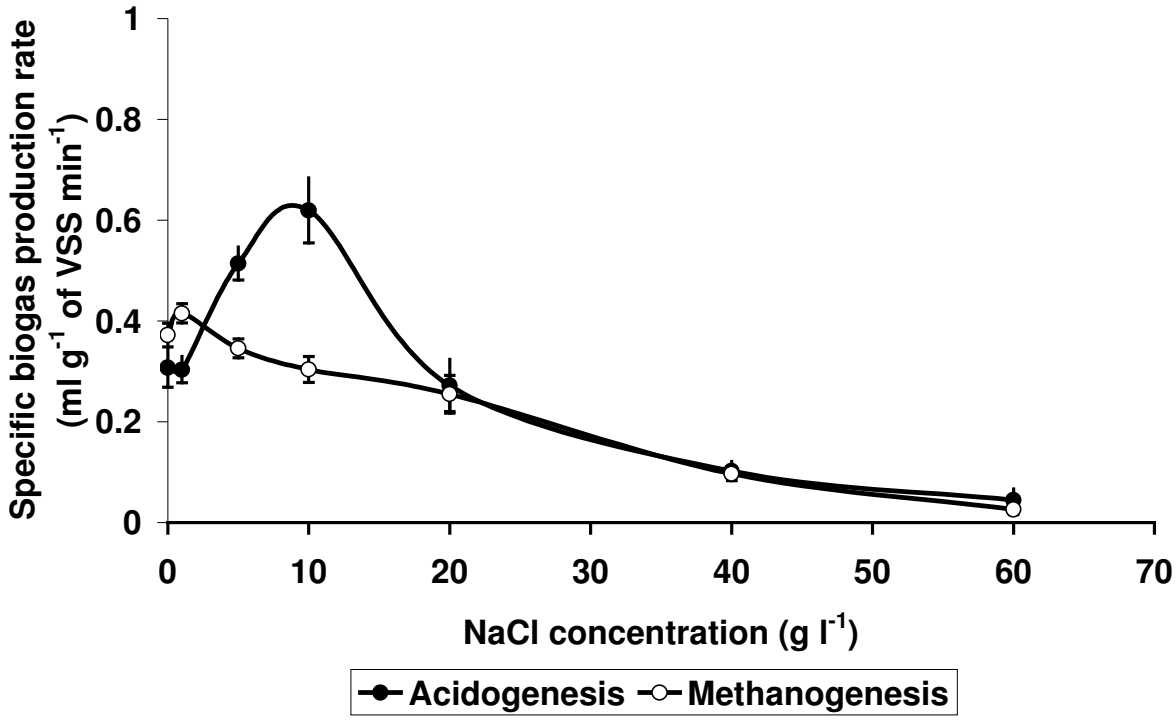

Figure 5.18 Evolution of specific biogas production rate due to acidogenesis and methanogenesis with increasing NaCl concentrations, using ethanol as a substrate.

Evolution of microbial communities with increasing NaCl concentrations

The evolution of microbial communities with increasing NaCl concentrations was monitored by PCR-SSCP. 4 profiles related to the domain of Bacteria were compared for the ASBR operating with distillery vinasse (Figure 5.19). At 0 g l^{-1} of NaCl, the bacterial SSCP profile showed the "mountain-shaped" profile characteristic of very diverse microbial communities usually observed in anaerobic sludge [242]. About 24 distinguishable peaks could be discerned. The profiles obtained at 1, 5 and 10 g NaCl l^{-1} revealed similar diversity, with peaks appearing and others disappearing at each level of NaCl. At the beginning of the experiment, peak 18 appeared to be dominant ; however, with increasing NaCl concentrations, the size of the peak diminished. In contrast, certain sub-dominant peaks (i.e. peaks 5, 13 and 19) progressively became dominant with

increasing NaCl concentrations. Other species (peaks 11 and 14) appeared only within a particular range of NaCl concentration. Lastly, certain peaks (20' and 23') appeared at 10g l^{-1}. Finally, the most important change occurred after the shift from 5 to 10 g l^{-1} of NaCl but the diversity of the profile obtained at 10 g l^{-1} of NaCl was still similar to that of the profile obtained at 0 g l^{-1}.

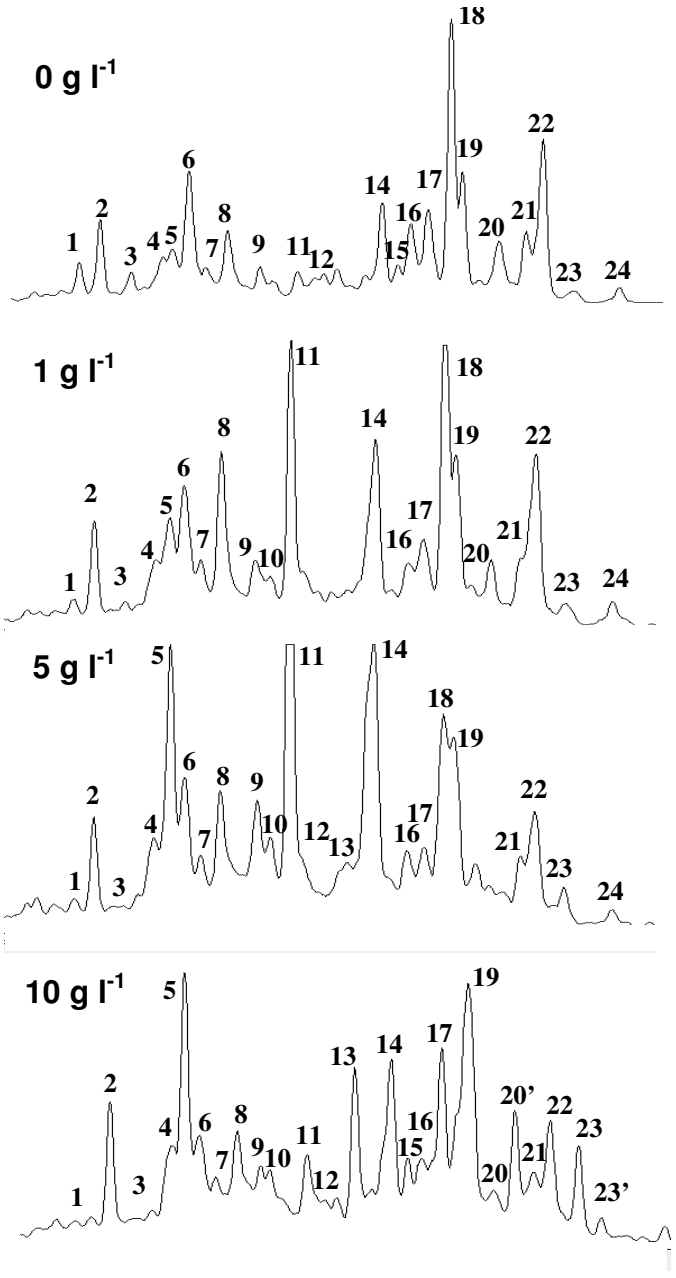

Figure 5.19 Evolution of microbial communities with increasing NaCl concentrations. Reactor operating with distillery vinasse.

7 profiles related to the domain of Bacteria were aligned for the ASBR operating with ethanol, out of which 2 are represented in Figure 5.20. At 0 g l^{-1} of NaCl, the bacterial SSCP profile also showed the "mountain-shaped" profile with about 17 distinguishable peaks, which is less than that of the profile obtained with the distillery vinasse. It also showed less diversity. Afterwards, the profiles obtained at 1, 5, 10, 20, 40 and 60 g NaCl l^{-1} revealed similar diversity, with peaks 2, 9, 15, 17 growing with increasing NaCl concentrations and, on the other hand, the size of peak 12 getting smaller. Peak 13 was dominant throughout the experiment. Finally, the bacterial profiles obtained with ethanol as a substrate appeared to be only slightly modified with increasing NaCl concentrations compared to the profiles obtained with the distillery vinasse.

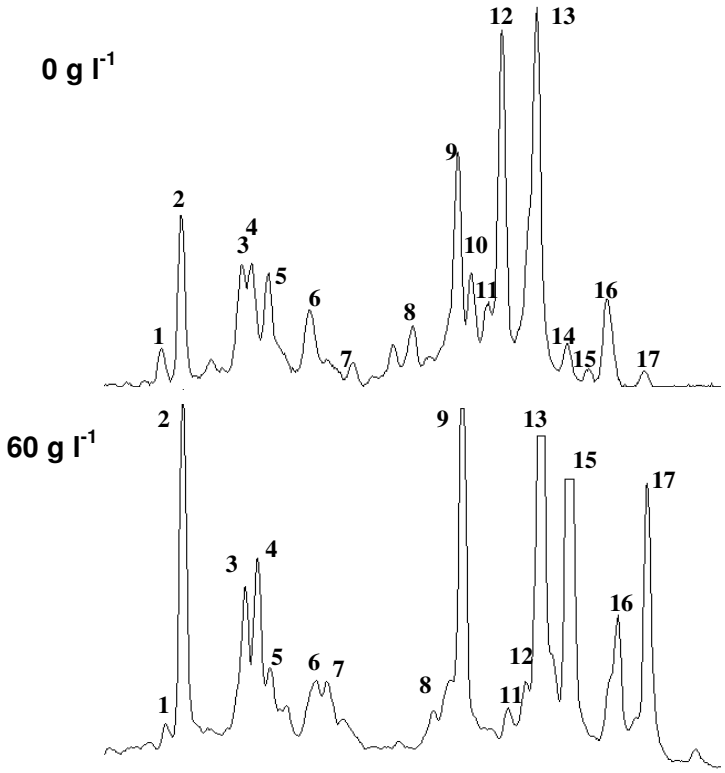

Figure 5.20 Evolution of microbial communities with increasing NaCl concentrations. Reactor operating with ethanol.

The SSCP profiles related to the domain of Archaea were very similar for both reactors, showing little diversity and only 2 dominant peaks remaining throughout the experiment (data not shown). Eukaryota 16S rDNA amplification was also attempted using eukaryotic specific primers and various PCR conditions. However, the amplification failed, which indicates that Eukaryota, if present, were not major components of of the autochtonous microbial community.

PCR-SSCP peak identification

Because the anaerobic sludge isolated from the ASBR operating with ethanol displayed a smaller number of dominant peaks, dominant peak identification focused on it. It has in fact already been shown that peak identification is extremely difficult and time-consuming when the number of dominant peaks exceeds 20. Therefore, 50 bacterial clones were isolated from this reactor at 0 g l^{-1} of NaCl (clones MV1 to 50) and 50 at 60 g l^{-1} (clones MW1 to 50). All were analysed by SSCP and produced a discrete prominent peak that was aligned with the corresponding sludge profile. Only peaks of clones MV1, MV8, MV12, MV13, MV43, MW1, MW13, MW14, MW34, MW41 and MW48 clearly co-migrated with detectable peaks from the corresponding sludge profiles.

In addition, from the same reactor sludge fed with ethanol, 10 archaeal clones were isolated at 0 g l^{-1} of NaCl (clones FL1 to 10) and analysed by SSCP. After alignment with the corresponding profile, 7 clones co-migrated with the first dominant peak (FL1, 2, 4, 5, 7, 8, 9, 10), 1 with the second dominant peak (FL3) and 1 did not co-migrate with any peak (FL6), thus corresponding to an underlying archaeal species.

Finally, clones MV1, MV8, MV12, MV32, MV43, MW13, MW14, MW41, MW48, FL3, FL4 and FL6 were sequenced and the results are indicated in Table 5.7. The 2 dominant archaeal peaks corresponding to clones FL03 and FL04 belong to the Euryarchaeota phylum. FL03 was 100% similar to *Methanosaeteta sp*., isolated from a chlorinated ethene-dechlorinating culture. FL04 showed 99 % similarity with *Methanobacterium beijingense*, isolated from an anaerobic digester. Finally, FL06 showed 99% similarity with the environmental clone LL25A1, isolated from an anoxic soil.

Regarding Bacteria, all the identified peaks except 3 showed more than 95% similarity with environmental clones belonging to the Deltaproteobacteria, Nitrospira and Synergistes phyla. The remaining 3 presented between 90 and 95% similarity with environmental sequences belonging to the Chloroflexi and Spirochaetes phyla. All the Bacteria identified showed clear anaerobic characteristics and most of them are known for their biodegradation capabilities.

Table 5.7 Phylogenetic affiliation of 16S rDNA sequences. Reactor operating with ethanol.

Name	Peak No	Phylum	% ID	Accession No.	Closest microorganism or environmental clone	Isolated from
FL03	-	Euryarchaeota	100	AY780568	Methanosaeta sp. Clone KB-1	Chlorinated ethene-dechlorinating culture
FL04	-	Euryarchaeota	99	AY350742	Methanobacterium beijingense strain 8-2	Anaerobic bioreactor
FL06	-	Euryarchaeota	99	AJ745133	Clone LL25A1	Anoxic soil
MV01	13	Deltaproteobacteria	96	AJ009485	Clone SJA-111	Anaerobic microbial consortium
MV08	1	Nitrospira	99	AB195896	Environmental clone	Anaerobic bioreactor
MV12	9	Deltaproteobacteria	99	AJ306771	Clone SHA-42	1,2-Dichloropropan dechlorinating culture
MV32	5	Chloroflexi	95	AY426440	clone B15	Anaerobic bioreactor
MV43	12	Deltaproteobacteria	99	AY426467	clone E27	Anaerobic bioreactor
MW13	4	Synergistes	100	AY654336	Clone synarJD05	Anaerobic bioreactor
MW14	14	Spirochaetes	90	-	-	-
MW41	16	Firmicutes	98	AY426453	clone B9	Anaerobic bioreactor
MW48	7	Chloroflexi	91	-	-	-

Discussion

In non-saline conditions, the two reactors presented differences due to the different nature of the substrates used. In terms of specific methanogenic activity, specific loading rate and organic loading rate, the performance of the reactor fed with ethanol was 2 to 3 times better than that obtained with distillery vinasse. The soluble COD removal efficiency also differed and reached 99.9% for ethanol which thus appeared to be entirely biodegradable under the initial conditions of the experiment. This removal efficiency was slightly lower (i.e. 93.5±0.5%) for distillery vinasse, which implies the existence of a non-

biodegradable part of the COD of this complex substrate under the experimental conditions. These results are in accordance with Ruiz *et al.* who have already run an ASBR with distillery vinasse and ethanol under similar operating conditions [189].

The impact of NaCl on process performance was then studied. Both reactors underwent a saline stress, increasing in stages. The NaCl inhibition was then quantified using as a criterion the specific loading rate, which was proportional to the specific methanogenic activity, COD removal remaining constant over the experimental period for both reactors. In the reactor operating with distillery vinasse, the addition of 1 g l^{-1} of NaCl was sufficient to induce a reduction of 22% of the specific loading rate. However, the strongest inhibition was observed while increasing NaCl concentration from 5 to 10 g l^{-1}, which caused a reduction of 88% of the specific loading rate. Regarding the reactor operating with ethanol, it is worthy of note that low NaCl concentrations (< 5 g l^{-1})did not inhibit the process. A concentration of 5 g l^{-1} of NaCl then induced a slight reduction of 8% of the specific loading rate and the most important disturbance occurred while increasing NaCl from 20 to 40 g l^{-1} and was characterised by a reduction of 73% of the specific loading rate. The clear conclusion is that the reactor operating with distillery vinasse appeared to be inhibited at a lower salt concentration than the reactor operating with ethanol. A similar inhibition level (i.e. 88% of the specific loading rate) was observed for the two reactors at final NaCl concentrations of 10 g l^{-1} with distillery vinasse and 60 g l^{-1} with ethanol. In the reactor operating with ethanol as sole source of carbon and energy, distinguishing acidogenesis from methanogenesis was possible. It could be seen clearly that methanogenesis started to be affected at a lower NaCl concentration (5 g l^{-1}) than acidogenesis (20 g l^{-1}).

The impact of NaCl thus was different according to the nature of the substrate: NaCl inhibition was observed at lower NaCl concentrations when using a complex substrate. This observation makes it possible to explain the difficulty encountered in the anaerobic biological treatment of complex saline effluents which has frequently been noted in the literature. For instance, the limitation of the load applied to saline complex effluents was observed by Rovirosa *et al.* (2004), when treating complex piggery effluents [188]. At a salt concentration of 15 g l^{-1} (comparable to the maximum concentration tested for the reactor fed with distillery vinasse in our study) and using a down-flow anaerobic fixed bed reactor, a satisfactory soluble COD removal efficiency of 90 % could be reached only with a very low OLR of 0,5 kg m^{-3} d^{-1}. The anaerobic treatment of a fishmeal effluent (10 g Cl^{-} l^{-1}) [86] and of a saline fish farm effluent (10 g Na^{+} l^{-1}) [74] was also limited in both cases to a low OLR (< 3 kg m^{-3} d^{-1}) and a low specific loading rate (< 0.1 kg m^{-3} d^{-1}). These results are very similar to those obtained in this study. It seems, therefore, that the anaerobic treatment of complex saline effluents is only possible on condition there be

a very low loading rate, which thus limits the applicability of this type of treatment. The difficulty of treating complex effluents under saline conditions is likely to be related to the high number of intermediate stages required for their degradation: when the number of stages increases, the probability also increases that one of these stages may be inhibited by salt.

The choice of sludge appears to be a major factor in the effectiveness of biological treatment processes for saline wastewater, which raises the question of the adaptation of the micro-organisms involved in the purification of such wastewater. Molecular biology analyses (SSCP) made it possible to highlight changes in the reactors' microflora in response to saline stress with a view to correlating them with the performance of the reactors. Bacterial SSCP profiles obtained at 0 g l^{-1} of NaCl showed a higher diversity for the reactor operating with distillery vinasse than for the reactor operating with ethanol. This could be a consequence of the nature of the substrates: the distillery vinasse (complex substrate) might indeed require the intervention of a higher number of micro-organisms for its degradation, whereas the degradation of ethanol (simple substrate) may well resuire a lower number of Bacteria.

For the reactor operating with distillery vinasse, the increase in NaCl concentration caused notable changes, with the appearance and the disappearance of dominant peaks on Bacteria SSCP profiles clearly showing no effect of salt on a certain part of the microbial population and, on the contrary, a negative effect on other species. At each level of salinity, moderate differences appeared but the most important were visible between 5 and 10 g l^{-1} of NaCl. It seems therefore that, in the case of this reactor, a salt-induced selection of halotolerant Bacteria occurred. However, this selection took place to the detriment of reactor performance. The profiles of the reactor operating with ethanol were very similar for each level of NaCl, which could be explained by an initial selection phenomenon among ethanol-degrading Bacteria. Thereafter, these Bacteria survived despite increasing salinity, thus revealing great halotolerant capacity. Even at high salt concentrations the performance of these Bacteria continued and they were severely affected only at NaCl concentrations higher than 20 g l^{-1}. Thus, it can be concluded that, according to the nature of the substrate, the same sludge inoculated into two different reactors diverged very quickly and thereafter reacted to increasing salinity in a different way. In the case of a complex substrate (distillery vinasse), the variety of the carbonaceous substrates permitted the salt-induced selection of halotolerant Bacteria whereas in the case of a simple substrate (ethanol), it was the nature of this substrate, rather than salinity, which was responsible for the selection of the micro-organisms.

Lastly, it is worthy of note that the two species of methanogenic Archaea identified in both reactors and close to *Methanosaeteta sp.* and *Methanobacterium beijingense* were found in all the profiles whatever the salt concentration and thus appear to be halotolerant, a fact not previously recorded. As a conclusion, it can be said that the process performances stated in this study were made possible by the adaptation of halotolerant micro-organisms rather than by the operation of halophilic organisms. Thus, increasing NaCl concentrations did not have a huge impact on the biomass composition but only on the micro-organisms' biodegradation rates.

Chapitre 6. Biodiversité des procédés de traitement biologique des effluents hypersalins à travers l'exemple des tanneries

Article : Microbial diversity in hypersaline wastewater: the example of tanneries

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Résumé

Au contraire des procédés conventionnels de traitement des effluents et des environnements salins oligotrophes, la biodiversité des effluents et des boues d'épuration hypersalins est méconnue. Dans cette étude, les communautés microbiennes d'un effluent de tannerie hypersalin, ainsi que d'un réacteur SBR et d'un UASB, tous deux alimentés par cet effluent, ont été explorées grâce au marqueur moléculaire que constitue l'ADNr 16S. De plus, la biodiversité des boues activées d'une station d'épuration traitant des effluents similaires a aussi été explorée. L'analyse comparative de 377 séquences bactériennes a révélé la grande diversité de ce type d'environnement hypersalin, avec 193 phylotypes observés (≥ 97% de similarité), réunis dans 16 des 52 divisions du domaine des Bacteria, La plupart de ces phylotypes sont proches de séquences halophiles et/ou impliquées dans la dégradation de polluants. La diversité de ce type d'environnement a été comparée à d'autres environnements sélectionnés sur leurs critères environnementaux (salinité, taux d'oxygène et charge organique), en utilisant des analyses statistiques.

Abstract

In contrast to conventional wastewater treatment plants and saline oligotrophic environments, little is known regarding the microbial diversity of hypersaline wastewater. In this study, the microbial communities of a hypersaline effluent, tannery soak liquor, of a sequencing batch reactor and an upflow anaerobic sludge blanket, both operating with this effluent, were investigated using 16S rDNA phylogenetic markers. In addition, the diversity of a common effluent treatment plant treating similar wastewater was also considered. The comparative analysis of 377 bacterial sequences revealed the high diversity of this type of hypersaline environment, clustering within 193 phylotypes (≥ 97% similarity) and covering 16 of the 52 divisions of the Bacteria domain, i.e. Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Chlorobi, Planctomycetes, Spirochaetes, Synergistes, Chloroflexi, Thermotogae, Verrucomicrobia, OP3, OP11 and TM7. Most of the phylotypes were related to halophilic and pollutant-degrading bacteria. Using statistical analysis, the diversity of this type of environment was compared to that of other environmental samples selected on the basis of their salinity, oxygen content and organic load.

Introduction

The interest in halophilic micro-organisms is largely motivated by the industrial application of these microbes, notably in the production of enzymes [106] and exopolysaccharides [176], but also for their use in depollution systems [170]. Regarding this last application, pollution removal in hypersaline wastewater is likely to represent up to 5% of the global wastewater treatment requirement. Although the number of studies dealing with the biological treatment of hypersaline wastewater is increasing rapidly, little is known regarding the diversity of these halophilic communities.

It has already been shown that high salinity permits the growth of a complex and diverse halophilic microflora in several types of oligotrophic water, such as seawater [222], salterns [7; 8; 19; 147; 184] and soda lakes [59; 96; 132; 206; 229]. Furthermore, sludge diversity in conventional biological wastewater treatment processes, both anaerobic and aerobic, has also been characterised and appears to be very broad, though different from that in oligotrophic fresh water [24; 80; 81; 203]. However, only scanty knowledge is available regarding the microbial diversity of biological wastewater treatment systems operating with a high salt content. Yet it has already been shown that the diversity of a salt-tolerant ecosystem treating hypersaline industrial wastewater could be similar to the diversity of a non salt-tolerant one (see section 4.1).

In this study, we propose to describe the bacterial diversity associated with a highly saline wastewater, i.e. tannery effluent, and different treatment plants used to clean this pollution. Leather tanning is almost wholly a wet process from which a large volume of highly saline liquid waste is continuously generated. The tanning process and the effluents generated have already been reported in the literature [210; 212; 233]. This study aims firstly at describing the microbial communities involved in the aerobic and anaerobic treatment of hypersaline wastewater ; and, secondly, at comparing the diversity potential of such wastewater to that of biological sludge involved in the aerobic and anaerobic treatment of non-saline wastewater, taking into account saline oligotrophic diversity.

Materials and methods

Sampling

4 different samples were collected for this experiment: first, tannery soak liquor (TSL) was collected from soak pits in a tannery located in a tannery cluster situated in the area

of Chennai, India. Second, activated sludge was collected from a common effluent treatment plant (CETP) in the same area, treating the composite effluents of 128 tanneries and minor amounts of domestic wastewater. The third and fourth samples collected were biological sludge from two laboratory-scale bioreactors run with TSL: one aerobic sequencing batch reactor (SBR) and one upflow anaerobic sludge blanket reactor (UASB). The salt (NaCl) content of these samples was 72.3, 6.9, 52.5 and 45.6 g l^{-1} for the TSL, CETP, SBR and UASB, respectively.

Total extraction, amplification, cloning and sequencing of genomic 16S rDNA

Total genomic DNA was extracted and purified from 10 ml of sample using a QiaAmp DNA stool mini kit, in accordance with the manufacturer's instructions (Qiagen, Hilden, Germany).

After extraction, bacterial 16S rDNA genes were amplified by PCR using the bacterial forward primer W18 (5'-GAGTTTGATCMTGGCTCAG-3') and the universal reverse primer W31 (5'-TTACCGCGGCTGCTGGCAC-3'), previously described by Snell-Castro *et al*. [204], in positions 9-27 and 482-500 in *Escherichia coli*, respectively [31]. 16S rDNA genes from the Archaea domain were targeted using the archaeal forward primer W17 (5'-ATTCYGGTTGATCCYGSCRG-3') and the universal reverse primer W02 (5'-GNTACCTTGTTACGACTT-3'), *E. coli* positions 3-22 and 1509-1492, respectively. Eukaryotic forward primer W99 (5'-CGGTAATTCCAGCTCC-3') [62], *E. coli* positions 528 to 544, and W02 were used for the partial amplification of 18S rRNA eukaryotic genes. PCR reactions were as follows: each PCR reaction tube contained 2 µl of each primer at a concentration of 100 ng μl^{-1}, along with 5 µl of 1X *Taq* reaction buffer (Perkin Elmer, Forster City, CA, USA), 4 µl of dNTP 2,5 mM, 1 µl of *Taq* DNA polymerase (Perkin Elmer) and water, for a final volume of 49 µl. This PCR mix was then added to 1 µl of DNA diluted 5 times in water. PCR conditions were as follows: an initial denaturation step at 94°C for 2 min, followed by 25 cycles of a three-stage program with 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, the final elongation step running for 10 min at 72°C. PCR products were purified with Qiagen microcolumns, in accordance with the manufacturer's instructions (Qiagen). Purified PCR products were cloned and transformed into TOP10 *E. coli* competent cells using the PCR 4-TOPO vector kit, according to supplier instructions (Invitrogen, Groningen, the Netherlands). Recombinant cells were selected using kanamycine resistance and *ccd* gene killer inactivation. Afterwards, 96 individual clones were inoculated onto a plate containing YT medium / 5% glycerol / ampicilline and sent for sequencing after 24h incubation at 37°C.

Sequence analysis

16S rDNA sequences (of about 500 bp) were identified by comparison with sequences available in databases using the BLAST program. To confirm phylum position, all the sequences were fitted into an alignment of about 12,000 full and partial 16S and 18S rRNA sequences using the automated tools of the ARB software package [135]. Partial sequence data were incorporated into trees by neighbour-joining [190]. Potential DNA chimeric structure was searched for by performing fractional treeing on the 5' and 3' ends of the sequenced DNA fragments and by analysis of the suspected sequences by the CHECK CHIMERA program (RDP-II) [43].

Nomenclature

The first letter of each clone name (e.g. MN100) corresponds to the set of primers used for its amplification; the second letter corresponds to an independent PCR amplification event ; and the numeral corresponds to the clone number of the respective library. Each phylotype was usually named according to the first clone identified displaying the representative sequence. An acronym was also chosen in order to facilitate the identification of our samples, i.e. TERSS, standing for Tannery Effluent-Related Saline Sludge. Thus, our samples will usually be referred to as TERSS-TSL, TERSS-SBR, TERSS-UASB and TERSS-CETP. The nucleotide sequence data reported in this work will appear in the GenBank nucleotide database under accession numbers AM157453 to AM157645.

Statistical analysis and sequence population diversity

Coverage was determined using a non-parametric estimator of the proportion of phylotypes in a library of infinite size that would be represented in a smaller library. Good's coverage estimator (C_{Good}) was calculated using the following formula: $C_{Good} = 1 - (n_1/N)$, where n_1 is the number of phylotypes that appeared only once in the clone library and N is the total number of clones analysed [82]. Rarefaction curves and the Chao's abundance-based coverage estimator of species richness (S_{ACE}) [39; 40] were produced by using the EstimateS software package for species richness calculation [44]. Finally, a dendrogram was built up and a principal component analysis (PCA) was performed using the XLSTAT software package. The dendrogram was built up using the aggregation criteria of Ward, based on Euclidean distances, and the PCA was performed using the correlation coefficient of Pearson.

Results

Origin of the tannery wastewater sample

In the leather industry, salt (sodium chloride, NaCl) is used to preserve the fresh skins from decomposition immediately after they are stripped in the slaughterhouse and subsequently the excess of salt has to be removed in the tannery before further processing. This is done by soaking in large quantities of water, which generates the first source of effluent. This tannery soak liquor (sample called TERSS-TSL) is characterised by its high organic load, large amount of suspended solids (sand, lime, hair, flesh, dung, etc.) and high salinity. A similar TSL sample had already been treated at bench-scale: aerobically in a sequencing batch reactor (sample called TERSS-SBR), and anaerobically in an upflow anaerobic sludge blanket (sample called TERSS-UASB) (see sections 4.2 and 5.1). In addition, the microbial diversity of activated sludge from an industrial-scale common effluent treatment plant, shared by 128 tanneries, treating tannery composite wastewater (i.e. the effluent made up of all the streams generated by a tannery, mixed together), was also characterised (sample called TERSS-CETP).

The microbial diversity of these four samples taken from a tannery environment was investigated using 16S rDNA analysis.

Global analysis of the 16S rDNA clone library

Archaeal 16S and eukaryotic 18S rDNA amplification was attempted using archaeal and eukaryotic specific primers and various PCR conditions. However, the amplification failed, which indicates that Archaea and Eukaryota, if present, were not major components of the autochthonous microbial community. 18 phyla among the 52 described by Rappé and Giovannoni were identified, as can be seen in Table 6.1 [178]. With regard to bacterial lineage, the microbial ecosystem observed by 16S rDNA gene comparative sequence analysis appeared to be diverse. The 377 bacterial sequences analysed clustered within 193 phylotypes, on the basis of at least 97% sequence similarity. The phylum most represented was that of Gamma Proteobacteria, which contained 28% of all the sequences analysed and 20% of the phylotypes (see Table 6.1). Other main phyla were Bacteroidetes (20% of the sequences and 21% of the phylotypes), Firmicutes (19% of the sequences and 17% of the phylotypes) and Alpha Proteobacteria (10% of the sequences and 15% of the phylotypes). The other sequences were distributed within 14 phyla representing 23% of the sequences and 25% of the phylotypes.

Table 6.1 Phylogenetic diversity of 16S rRNA sequences from tannery effluent-related samples.

Sequence percentages are indicated in bold characters and phylotype percentages are in parentheses.

Phyla	TERSS-TSL	TERSS-SBR	TERSS-UASB	TERSS-CETP	Total
α-Proteo	**1** (3)	**6** (9)	**1** (2)	**32** (38)	**10** (15)
β-Proteo	**0** (0)	**1** (2)	**2** (2)	**3** (5)	**2** (3)
γ-Proteo	**30** (49)	**53** (41)	**7** (6)	**3** (5)	**28** (20)
δ-Proteo	**0** (0)	**3** (7)	**9** (5)	**3** (5)	**4** (5)
ε-Proteo	**0** (0)	**1** (2)	**0** (0)	**4** (3)	**1** (2)
Bacteroidetes	**12** (11)	**24** (16)	**32** (34)	**16** (15)	**20** (21)
Firmicutes	**55** (34)	**4** (9)	**31** (31)	**0** (0)	**19** (17)
Actinobacteria	**2** (3)	**4** (9)	**3** (2)	**2** (2)	**3** (3)
Chlorobi	**0** (0)	**0** (0)	**0** (0)	**8** (3)	**2** (1)
Planctomycetes	**0** (0)	**2** (2)	**0** (0)	**6** (7)	**2** (3)
Spirochaetes	**0** (0)	**0** (0)	**3** (5)	**1** (2)	**1** (2)
Chloroflexi	**0** (0)	**0** (0)	**1** (2)	**3** (5)	**1** (2)
Thermotogae	**0** (0)	**1** (2)	**2** (2)	**1** (2)	**1** (1)
Verrucomicrobia	**0** (0)	**0** (0)	**1** (2)	**1** (2)	**1** (1)
Synergistes	**0** (0)	**0** (0)	**4** (5)	**0** (0)	**1** (2)
OP3	**0** (0)	**0** (0)	**1** (2)	**0** (0)	**1** (1)
OP11	**0** (0)	**0** (0)	**2** (3)	**15** (7)	**4** (3)
TM7	**0** (0)	**0** (0)	**0** (0)	**1** (2)	**1** (1)

Most of the sequences recovered from the saline samples were not closely related to sequences present in public databases. From the 193 bacterial phylotypes observed, only 54 (28%) presented more than 97% similarity with previously-identified sequences. 79 (41%) showed similarity values in the range of 97-90%; the 60 others (31%) had similarity values between 90 - 80%. The sequences presenting more than 97% similarity are reported in Table 6.2. Only 100 phylotypes (52%) were identified as already cultured

species. These species were widely spread throughout the domain of Bacteria. The most abundant phylotype identified through this study was related to *Halomonas alimentaria* (AF211860) (MT100, 4% of the bacterial sequences), isolated from a traditional Korean fermented seafood. The phylogenetic diversity of each sample, based on phylum and phylotype levels, varied widely from one sample to another (see Table 6.1 and Table 6.2), justifying a separate analysis of each microbial ecosystem.

Table 6.2 Phylogenetic affiliation of 16S rRNA gene phylotypes from tannery soak liquor and treatment plants associated.

Name and origin	No. of clones	Phylum	Closest microorganism or environmental clone (accession No.)	% Id.	Isolated from
TERSS-UASB					
MN094	1	γ-Proteo	*Marinobacter aquaeolei* (AY669171)	100	tropical marine sediment
MN106	6	δ-Proteo	*Desulfomicrobium baculatum* (AJ277895)	98	ND[2]
MN027	1	Bacteroidetes	Clone TSAa22 (AB186816)	99	dechlorinating community
MT054[1]	1	Bacteroidetes	Clone LD54 (AY816826)	99	pig manure storage pit
MN011	2	Firmicutes	*Lactococcus lactis* (AF323179)	99	ND
MN023	1	Firmicutes	*Streptococcus gallolyticus* (AF323911)	99	shea cake digester
MN105	2	Synergistes	Clone GZKB134 (AJ853626)	100	leachate of a landfill
Others[3]	81 (57 phylotypes)			<97	
Total	95 (64 phylotypes)				
TERSS-TSL					
MS113	1	α-Proteo	*Paracoccus aminovorans* (AY256516)	99	ND
MS001	1	γ-Proteo	*Salinivibrio costicola* (AY505534)	98	salt plains
MS023	1	γ-Proteo	*Vibrio parahaemolyticus* (AF388389)	99	ND
MS050	1	γ-Proteo	*Halomonas* sp. (AB167059)	99	ND
MS054[1]	2	γ-Proteo	*Halomonas alimentaria* (AF211860)	97	fermented seafood

Name and origin	No. of clones	Phylum	Closest microorganism or environmental clone (accession No.)	% Id.	Isolated from
MS056	5	γ-Proteo	*Vibrio campbelli* (AY544985)	99	seafood
MS057	1	γ-Proteo	*Idiomarina* sp. (AB167036)	99	ND
MS078	6	γ-Proteo	*Idiomarina baltica* (AJ440215)	98	seawater
MS103	1	γ-Proteo	*Salicola marensis* (DQ019935)	100	saltern
MS120[1]	5	γ-Proteo	*Marinobacter* sp. (AY136115)	99	seawater
MT100[1]	7	γ-Proteo	*Halomonas alimentaria* (AF211860)	98	fermented seafood
MS018	1	Actinobacteria	*Corynebacterium* sp. (AF227828)	97	ND
MS027	2	Firmicutes	*Alkalibacillus salilacus* (AY671976)	99	ND
MS087	4	Firmicutes	*Halobacteroides acetoethylicus* (U32594)	97	ND
MS117	2	Firmicutes	Clone No. 20 (AF395430)	97	ND
Others[3]	51 (20 phylotypes)			<97	
Total	91 (35 phylotypes)				
TERSS-SBR					
MT095	1	α-Proteo	Clone S063 (AJ416665)	98	saline mud volcano
MT097	2	α-Proteo	Clone JP7.1 (AY007679)	97	surface picoplankton
MT025	1	β-Proteo	*Thauera* sp. (AF110005)	97	activated sludge
MT040	1	γ-Proteo	*Shewanella alga* (AF005250)	99	ND
MT053	1	γ-Proteo	*Marinobacter maritimus* (AJ704395)	97	seawater
MT085	1	γ-Proteo	Clone PI (AY580826)	97	coastal bacterioplankton
MT100[1]	9	γ-Proteo	*Halomonas alimentaria* (AF211860)	98	fermented seafood
MT106	4	γ-Proteo	*Marinobacter* sp. (AB089803)	100	coastal seawater
MS054[1]	2	γ-Proteo	*Halomonas alimentaria* (AF211860)	97	fermented seafood
MS120[1]	1	γ-Proteo	*Marinobacter* sp. (AY136115)	99	seawater
MT031	1	δ-Proteo	*Bacteriovorax marinus* (AF084855)	100	salt-water

Name and origin	No. of clones	Phylum	Closest microorganism or environmental clone (accession No.)	% Id.	Isolated from
MT021	1	ε-Proteo	Clone ML615J-7 (AF458288)	99	hypersaline lake
MT046[1]	1	Actinobacteria	*Microbacterium* sp. (AY974047)	98	Antarctic seawater
MT004	1	Bacteroidetes	Clone 2F2 (AJ628010)	99	microbial mat
MT018	9	Bacteroidetes	Clone 2A10 (AJ628003)	99	microbial mat
MT044	2	Bacteroidetes	Clone K413 (AY362005)	99	deep-sea
MT054[1]	2	Bacteroidetes	Clone LD54 (AY816826)	99	pig manure storage pit
MT038	1	Thermotogae	*Geotoga subterranea* (L10659)	98	ND
Others[3]	54 (26 phylotypes)			<97	
Total	95 (44 phylotypes)				
TERSS-CETP					
MU033	1	α-Proteo	*Agrobacterium* sp. (AF482682)	98	ND
MU038	1	α-Proteo	*Caulobacter crescentus* (AE006011)	98	ND
MU042	2	α-Proteo	*Paracoccus yeei* (AY014172)	97	ND
MU059	1	α-Proteo	*Porphyrobacter* sp. (AB033325)	100	ND
MU061	1	α-Proteo	*Paracoccus kawasakiensis* (AB041770)	99	ND
MU073	4	α-Proteo	Clone mle1-13 (AF280850)	98	bioreactor
MU077	1	α-Proteo	*Sphingomonas suberifaciens* (D13737)	97	ND
MU098	1	α-Proteo	*Phenylobacterium falsum* (AJ717391)	97	alkaline groundwater
MU101	1	α-Proteo	*Erythrobacter* sp. (AY646157)	97	seawater
MU102	1	α-Proteo	*Rhodobacter* sp. (AB017798)	98	ND
MU104	1	α-Proteo	*Rhizobium* sp. (U50168)	97	rhizosphere
MU109	2	α-Proteo	*Parvibaculum lavamentivorans* (AY387398)	99	ND
MU045	1	β-Proteo	*Propionibacter pelophilus* (AF016690)	98	estuarine mud
MU050	1	β-Proteo	*Hydrogenophaga intermedia* (AF019037)	97	ND

Name and origin	No. of clones	Phylum	Closest microorganism or environmental clone (accession No.)	% Id.	Isolated from
MU080	1	β-Proteo	*Azoarcus* sp. (AY098637)	99	ND
MU070	1	ε-Proteo	*Sulfurospirillum* sp. (AJ535704)	99	ND
MT046[1]	2	Actinobacteria	*Microbacterium* sp. (AY974047)	98	Antarctic seawater
MU072	1	Bacteroidetes	*Bacteroides* sp. (AY082449)	99	mine drainage system
MU025	6	Chlorobi	Clone E5-46-SC1-138 (AF459066)	100	ultradeep gold mine
Others[3]	66 (42 phylotypes)			<97	
Total	96 (61 phylotypes)				

[1] this phylotype appears twice in this table under the same name, the sequences clustered within this phylotype being shared by two distinct TERSS-samples.

[2] ND: not determined

[3] this category gathers all the phylotypes presenting less than 97% similarity with the closest sequence available in the databases

Comparative analysis of the TERSS-16S rDNA clone libraries

It can be seen in Table 6.2 that the percentage of phylotypes presenting more than 97% similarity with previously-identified phylotypes was only 11, 31, 41 and 43% (15, 31, 43 and 44% of the sequences) regarding TERSS-UASB, TERSS-CETP, TERSS-SBR and TERSS-TSL, respectively. 38, 46, 41 and 40% of the phylotypes (37, 50, 42 and 44% of the sequences) showed similarity values in the range of 97-90%; the remaining 52, 23, 18 and 17% of the phylotypes (48, 19, 15 and 12% of the sequences) had similarity values between 90-80%. Regarding TERSS-UASB, only 6% of the phylotypes (11% of the sequences) were identified as already cultured species on the basis of at least 97% similarity. This proportion of sequences related to already cultured species was higher in the other samples attaining 23, 28 and 40% of the phylotypes (23, 21 and 42% of the sequences) in TERSS-SBR, TERSS-CETP and TERSS-TSL, respectively. Another criterion which was considered was the halotolerant or halophilic characteristics of our sequences: on the basis of at least 90% similarity, 16, 25, 55 and 71% of the phylotypes (18, 35, 63 and 82% of the sequences) of TERSS-UASB, TERSS-CETP, TERSS-SBR and TERSS-TSL, respectively, were related to cultured species showing halophilic properties or to environmental clones isolated from saline environments. The phyla most represented in

TERSS-TSL, TERSS-UASB and TERSS-SBR were the Gamma Proteobacteria, Bacteroidetes and Firmicutes, whereas TERSS-CETP was dominated by Alpha Proteobacteria.

Coverage and similarity of libraries based on phylotype diversity

A deeper understanding of the microbial diversity was attained using various diversity indexes. The Good's coverage values obtained indicated that TERSS- CETP and the TERSS-UASB were more diverse than TERSS-SBR and TERSS-TSL, with respectively C_{Good}= 52, 54, 72 and 82%. The rarefaction curves obtained at a similar level of effort (Figure 6.1) showed weakly curvilinear plots for the curves obtained for TERSS-CETP and TERSS-UASB samples, indicating that the phylotype richness in TERSS-CETP and TERSS-UASB environments was largely underestimated. The estimated number of phylotypes, determined using the Chao's abundance-based coverage estimator of species richness (S_{ACE}), was 50, 108, 144 and 231 phylotypes for TERSS-TSL, TERSS-SBR, TERSS-UASB and TERS-CETP, respectively. Finally, the similarities of the phylotypes in these libraries are represented in Figure 6.2, in which it can be seen that the TERSS-SBR and TERSS-TSL 16 rDNA sequences showed around 30 % similarity, whereas the other samples were very different. The TERSS-TSL and TERSS-CETP samples differed most, having no phylotype in common.

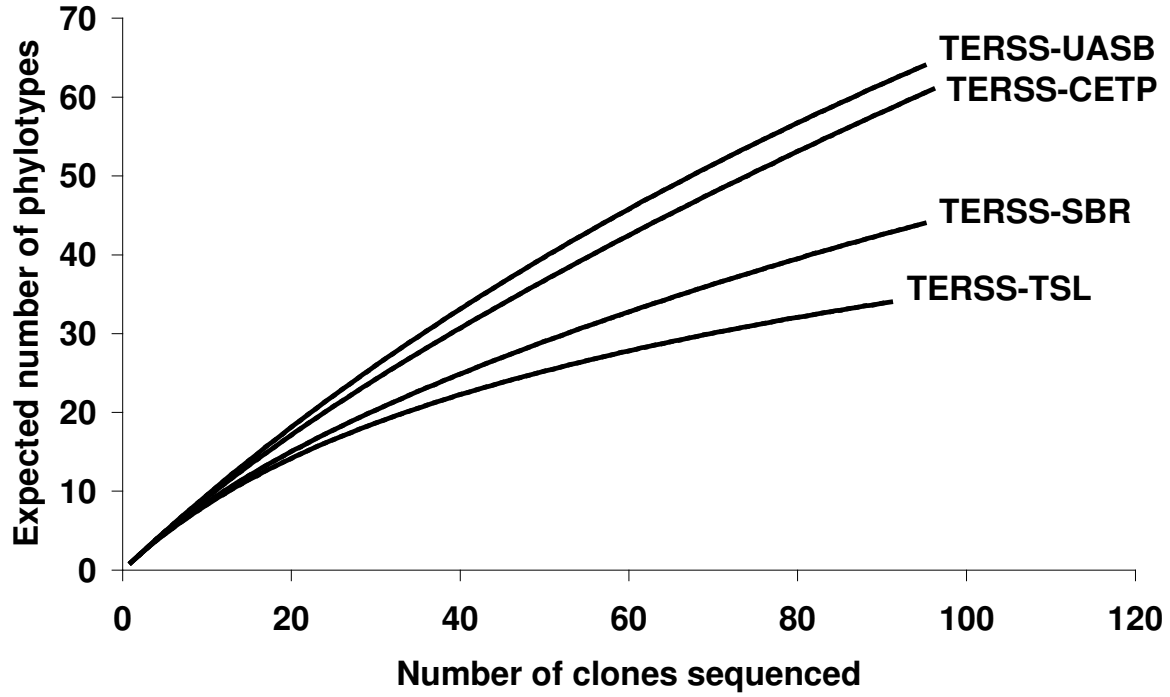

Figure 6.1 Rarefaction curves generated for 16S rDNA clone libraries from tannery effluent-related samples.

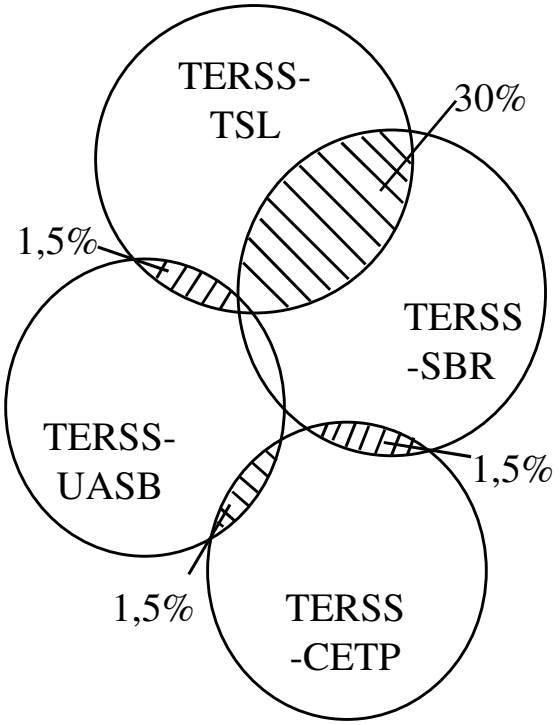

Figure 6.2 Schematic representation of percentage similarities between 4 different samples related to tannery environment.

Effect of salt, oxygen and organic load on phylum diversity

The similarity indexes previously calculated showed that, on the basis of phylotype diversity, all the samples were very different, apart from TERSS-SBR and TERSS-TSL. Given this result, phylum level analysis seemed more suitable for a comparison of the 4 saline wastewater samples analysed here to other published environmental samples, using statistical tools. TERSS-SBR, TERSS-CETP and TERSS-TSL showed aerobic characteristics and can be compared to saline oligotrophic water as regards their high salt content features; and to non-saline wastewater treatment sludge as regards their high organic load. In contrast, TERSS-UASB displayed anaerobic characteristics and thus could be compared to non-saline anaerobic digestors. Therefore, the influence of environmental parameters on the diversity of our samples was studied using statistical analysis (dendrogram and PCA). To this end, our samples were clustered hierarchically into a dendrogram based on the phylum distribution among the samples, in order to present an analytic overview of the diversity. Additional reference data were used to build up the dendrogram, including descriptions of the microbial diversity of saline oligotrophic environments (seawater, salterns, soda lake) and, also, of conventional aerobic and anaerobic sludge from conventional biological treatment processes. Thus, a reference database was built up using 1,412 sequences from seawater [222], 197 from

salterns [18; 154] 212 from soda lakes [99], 1,988 from anaerobic sludge [79; 81; 195] and 256 from aerobic activated sludge [24; 203].

All the samples clearly clustered in the dendrogram within three majors groups: the anaerobic sludge samples gathered in a first group, the aerobic sludge samples in a second group and the third group was composed of all the samples isolated from saline oligotrophic environments (data not shown). Our samples clustered in the third group, apart from TERSS-CETP that was clustered with the aerobic sludge. Among the three major parameters analysed (i.e. presence of oxygen, salt and organic load), salt thus appeared to distinguish clearly TERSS-SBR, TERSS-TSL and TERSS-UASB, whereas oxygen and organic load distinguished TERSS-CETP. A PCA analysis was then performed to further describe the relationship between our samples and the main types of reference ecosystems, as mentioned above. In this analysis, the environmental reference samples were pooled according to their environment and the dendogram results. It was therefore possible to explain 52% of the information with 2 axes, as shown in Figure 6.3: axis 1 appears to be related to the amount of oxygen in the environment, separating samples into the aerobic on the left, the anaerobic on the right. In addition, all the samples appear to be aligned on axis 2 according to their salt concentration. According to Figure 6.3, TERSS-SBR and TERSS-TSL clearly show saline properties. TERSS-UASB stands alone, in an intermediate position between the anaerobic sludge and saline samples, thus displaying characteristics from both. TERSS-CETP also stands alone, in an intermediate position between the conventional aerobic sludge and saline samples.

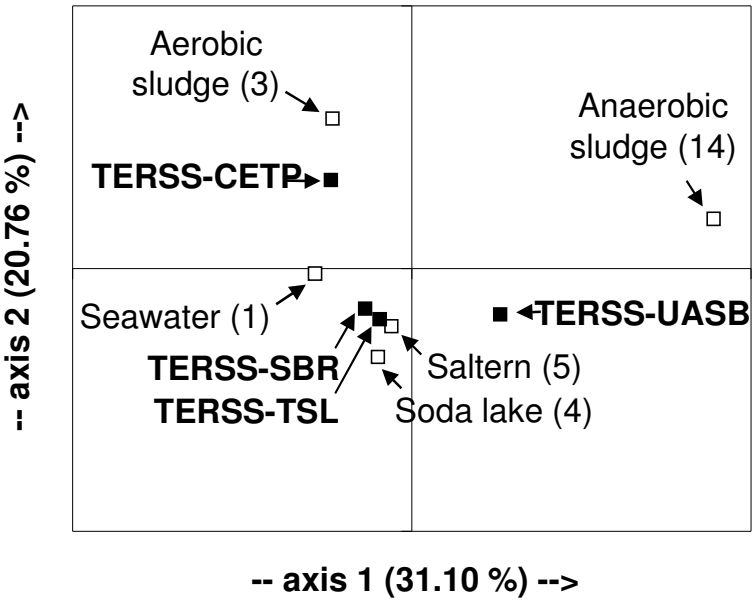

Figure 6.3 Principal component analysis carried out on 4 samples related to tannery wastewater (■) and on other reference samples characterising a specific type of environment (□).

The numerals in parentheses indicate the number of reference samples from **Erreur! Source du renvoi introuvable.** used to characterise the specific environments in the current figure.

Discussion

Prokaryotic biodiversity of tannery effluent-related sludge communities

The tannery effluent-related sludge communities consisted mainly of organisms from the domain of Bacteria. In contrast, Archaea and Eukaryota could not actually be amplified. Though Archaea are seldom amplified in aerobic treatment processes, their absence seems surprising at first sight in TERSS-UASB, as methane production was observed in this digester and had to be induced by methanogenic Archaea. However, at the salinity level of TERSS-UASB (i.e. 46 g NaCl l^{-1}) halophilic Archaea amplification is known to be difficult [184].

The sequences obtained from the tannery soak liquor (TSL) and the treatment systems applied to it appeared to be distant from the identified sequences from the database: only 28% of the phylotypes showed more than 97% similarity with previously-

identified sequences and 31% showed less than 90% similarity. This phenomenon can be explained by the originality of the saline environment screened in this study. Coming from effluent-related samples, it was very likely that the microflora of these ecosystems would be different from the other saline environments that had already been described. In addition, only slightly more than half of the phylotypes were related to already cultured species, which confirms the uncultured majority of halophiles that had already been reported [131].

Environmental characteristics of tannery effluent-related sludge communities

The dendrogram and the PCA analysis emphasise the halophilic characteristics of TERSS-TSL, TERSS-SBR and TERSS-UASB samples, confirmed by the abundance in these samples of the Gamma Proteobacteria and Bacteroidetes that are characteristic of saline environments [18; 154; 222]. A shift in phylum distribution could be observed between TERSS-TSL and the 2 reactors fed with this influent: TERSS-TSL was dominated by Firmicutes whose proportion was reduced and replaced by Gamma Proteobacteria in TERSS-SBR and Bacteroidetes in TERSS-UASB. Regarding TERSS-SBR phylum distribution, it could be stated that the contribution of Proteobacteria and Bacteroidetes (i.e. 64 and 24%, respectively) was similar to that for another aerobic SBR treating hypersaline wastewater generated by tartaric acid production (i.e. 61% and 27% for Proteobacteria and Bacteroidetes, respectively) (see section 4.1).

Regarding TERSS-UASB, the PCA analysis also emphasised its anaerobic characteristics, confirmed by the large number of Firmicutes and Delta Proteobacteria commonly observed in anaerobic digestors [79]. The population of TERSS-UASB was therefore widely influenced by the double constraint of salt and anaerobiosis, which may explain why sequences from this sample prove to be the most distant from identified sequences in the database: the communities colonising this type of environment, being at the same time anaerobic and hypersaline, have seldom been described before. Finally, it is worth noting the presence of Synergistes and Thermotogae, neither of which, to our knowledge, have previously been isolated from saline environments.

Finally, TERSS-CETP, having much lower salinity, was more related to conventional aerobic sludge communities than to saline environments, as can be seen from the dendrogram and the PCA analysis. However, even at this salt content, relatively low in comparison to the other samples, some phylotypes were related to halophilic strains such as *Propionibacter pelophilus* (AF016690), isolated from estuarine mud and *Erythrobacter* sp. (AY646157), found in seawater (see Table 2). It can be concluded, therefore, that the selection of halophiles starts at even a low level of salt content, which supports the

TERSS-CETP's position in the PCA analysis, intermediate between saline environments and conventional aerobic sludge.

Diversity potential of hypersaline wastewater treatment processes

The analysis of the 16S rDNA from whole saline and hypersaline communities has revealed the variable but potentially high diversity of such saline environments [131]. In our study, the saline and hypersaline microbial ecosystems observed by 16S rRNA gene cloning were diverse for the Bacteria lineage. This diversity was estimated by the drawing of rarefaction curves, as well as by the calculation of S_{ACE}. The weakly curvilinear plots for the rarefaction curves obtained for TERSS-CETP and TERSS-UASB emphasised the need for a higher effort of sampling regarding these samples. On the basis of rarefaction curves, the diversity of TERSS-TSL appeared to be higher than that found in some salterns and soda lakes [18; 99] but lower than that found in seawater and in some other salterns [154; 222]. Such a diversity may surprise at first sight, knowing that salt is used in the tannery process in order to inhibit microbial growth and to preserve the skins from biodegradation. It would seem, therefore, that microbial diversity is not affected by such treatment. The diversity was even higher in TERSS-SBR, where the estimated number of phylotypes reached around 100, which is slightly lower than that found in two non-saline aerobic SBR [24], and in TERSS-UASB, where the estimated number of phylotypes was estimated to around 140. The diversity of TERSS-UASB therefore appeared to be similar to that found in a non-saline anaerobic digestor [81]. Finally, the last sample analysed in this study consisted of activated sludge collected in a CETP treating the effluents of 128 tanneries. The diversity of TERSS-CETP was higher than that found in the other activated sludge sample (i.e. TERSS-SBR), though comparable to that found in conventional aerobic sludge communities [24].

In non-saline wastewater treatment, the efficiency of the process is associated with a high diversity of the microbial community in aerobic [24; 203], as well as in anaerobic [81], conditions. In the saline wastewater treatment processes studied in this paper, it has been shown that the efficiency of the process in terms of COD removal was similar to that of the non-saline counterparts (see section 4.2). The diversity found in the hypersaline wastewater (TERSS-TSL) and in the 3 saline processes (TERSS-SBR, TERSS-UASB and TERSS-CETP) analysed in this paper was also in the same range as that of the non-saline counterpart. Therefore, halophilic sludge should be considered as possessing the same resources of diversity as conventional activated sludge. At this level, the two cannot be distinguished. Consequently, it can be concluded that such halophilic sludge biodiversity enables the biological treatment of hypersaline wastewater to be carried out

with an efficiency similar to that observed in the treatment of fresh wastewater, both in aerobic and anaerobic conditions.

Acknowledgements

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Chapitre 7. Résumé et discussion

7.1 Originalité des effluents traités

Il a été montré dans l'étude bibliographique (section 2.3) que le traitement biologique des effluents salins est possible, tant en aérobiose qu'en anaérobiose. Cependant la plupart des recherches menées jusqu'à présent en réacteurs de dépollution se sont limitées à des substrats synthétiques. Les rares exemples de substrats industriels testés sont issus de l'industrie agro-alimentaire (effluents de pêcheries, effluents générés par la transformation des produits de la mer et des légumes saumurés) ou pharmaceutique. Cette thèse accroît la compréhension du traitement des effluents industriels hypersalins en l'appliquant à des types d'effluents originaux. Dans un premier temps, la biodégradabilité aérobie d'un effluent complexe généré par une industrie tartrique a été explorée (cf. section 2.2.2). Cet effluent est caractérisé par une salinité de 120 g l^{-1} (principalement sous forme de NaCl et KCl) et une DCO de 4 g l^{-1}, constituée en grande partie de composés phénoliques aromatiques issus du nettoyage des cuves de vinification. Par ailleurs, son taux de MES, proche de 2 g l^{-1}, lui confère une grande turbidité. Le traitement aérobie du phénol en conditions salines a fait l'objet d'études par Woolard et Irvine [235; 236], mais il n'y a pas d'exemple de traitement aérobie appliqué à un substrat complexe comportant des composés phénoliques aromatiques.

Dans un second temps, c'est la biodégradabilité aérobie et anaérobie d'un effluent spécifique de tannerie hypersalin qui a été considéré : l'effluent de rinçage des peaux (cf. section 2.2.4), le plus concentré en sel (entre 25 et 70 g NaCl l^{-1}, soit jusqu'à 2 fois la salinité de la mer). Cet effluent est également caractérisé par une forte teneur en matière organique (> 2 g DCO l^{-1}), et en MES inorganiques (> 5 g l^{-1}). De plus, il est très variable en fonction de la nature et de l'origine des peaux. Le traitement de cet effluent est d'une importance capitale en Inde, notamment de par l'importance économique du secteur du cuir et de par la toxicité de cet effluent, accentuée par les pénuries d'eau.

7.2 Performances des procédés de traitement appliqués aux effluents hypersalins

7.2.1 Procédés aérobies

Les performances des procédés SBR aérobies appliqués à l'effluent d'industrie tartrique (section 4.1) et à l'effluent de tannerie (section 4.2) décrits plus haut sont résumées dans le Tableau 7.1.

Tableau 7.1 Traitement biologique aérobie des effluents industriels hypersalins considérés dans cette thèse.

Substrat	Inoculum halophile	Salinité ($g\ l^{-1}$)	Procédé	V (l)	DCO alim ($g\ l^{-1}$)	TSH (h)	cva (kg DCO $m^{-3}\ j^{-1}$)	MVS ($g\ l^{-1}$)	cma (kg DCO kg^{-1} de MVS j^{-1})	Rdt DCO (%)
Effluent de tannerie	Oui	35	SBR	10	3	120	0,6	2	0,30	95
Effluent de tannerie	Oui	40	SBR	10	3,6	79	1,1	7,2	0,15	91
Effluent d'industrie tartrique	Oui	120	SBR	5	4,3	240	0,4	3,5	0,12	83

La biodégradabilité aérobie de ces effluents industriels est confirmée par les rendements épuratoires obtenus sur la DCO. La biodégradabilité de l'effluent de tannerie apparaît supérieure à celle de l'effluent d'industrie tartrique, ce qui peut s'expliquer par la nature aromatique des composés du vin mélangés à cet effluent suite au nettoyage des cuves de vinification. Les charges appliquées ont été comparées à celles décrites dans la littérature concernant le traitement biologique aérobie d'effluents salins (cf. Tableau 2.7). Concernant l'effluent de tannerie, les charges appliquées, tant volumiques que massiques, sont inférieures à celles applicables sur effluents synthétiques (à base de mélasses, principalement), mais sont très comparables à celles appliquées sur effluents complexes agro-alimentaires (effluents générés par la production de légumes saumurés et par la transformation de produits de la mer), comme en témoigne le Tableau 2.7. La nature complexe des effluents industriels les rend en effet plus lentement biodégradables que les effluents synthétiques. La cma optimale s'est avérée de l'ordre de 0,3 kg DCO kg^{-1} de MVS j^{-1} et la cva optimale de l'ordre de 1 kg DCO m^{-3} j^{-1}. Ces valeurs correspondent à la gamme de charges généralement appliquées lors du traitement aérobie d'effluents industriels. Les performances de boues activées salines peuvent donc être similaires à celles de boues activées non salines. Cependant une dégradation des performances a été constatée au-delà de 50 g l^{-1} de sel.

Concernant l'effluent d'industrie tartrique, le traitement aérobie en SBR ne s'est avéré effectif qu'à une charge appliquée inférieure à celle de l'effluent de tannerie et de l'ordre de 0,12 kg DCO kg^{-1} de MVS j^{-1} pour la cma et de 0,4 kg DCO m^{-3} j^{-1} pour la cva. Ces valeurs correspondent à un fonctionnement à très faible charge ou aération prolongée. Les performances du traitement aérobie de cet effluent sont par contre très similaires à celles obtenues sur phénol par Woolard et Irvine en SBR (cf. Tableau 2.7) [235; 236]. Cette limitation de charge appliquée observée pourrait alors s'expliquer par la nature aromatique des composés du vin qui se retrouvent dans l'effluent d'acide tartrique et le rendent plus lentement biodégradable.

Les possibilités de traitement aérobie de l'azote ont aussi été mises en évidence dans les deux effluents atteignant des rendements épuratoires du NTK jusqu'à 96 et 72% pour l'effluent de tannerie et celui d'industrie tartrique, respectivement. Là encore, la différence de nature des deux effluents permet d'expliquer cette différence, l'effluent d'industrie tartrique étant moins biodégradable que l'effluent de tannerie. Les bactéries responsables du traitement de l'azote ont cependant été plus sensibles aux chocs de salinité que les bactéries responsables de la dépollution carbonée, ces dernières présentant de bonnes capacités de récupération après un choc. L'épuration de 93% des phosphates de l'effluent de tannerie a aussi démontré les possibilités de traitement du phosphore des effluents industriels hypersalins.

7.2.2 Procédés anaérobies

Les performances des procédés anaérobies appliqués à l'effluent de tannerie décrit plus haut (UASB, section 5.1 et lit mobile, section 5.2) ainsi que celles obtenus avec le procédé ASBR sur vinasse de distillerie et éthanol en conditions salines (section 5.3) sont résumées dans le Tableau 7.2.

Tableau 7.2 Traitement biologique anaérobie des effluents salins considérés dans cette thèse.

Substrat	Inoculum halophile	Salinité ($g\ l^{-1}$)	Procédé	V (l)	DCO alim ($g\ l^{-1}$)	TSH (h)	cva (kg DCO $m^{-3}\ j^{-1}$)	MVS ($g\ l^{-1}$)	cma (kg DCO kg^{-1} de MVS j^{-1})	Rdt DCO (%)
Effluent de tannerie	Oui	71	UASB	5	2.3	120	0,5	5,5	0,09	78
Effluent de tannerie	Oui	72	AMB	5	2.3	144	0,4	8	0,05	72
Vinasse de distillerie	Non	0	ASBR	5	33	160	4,9	9,8	0,50	94
Vinasse de distillerie	Non	10	ASBR	5	33	1600	0,5	8,2	0,06	93
Ethanol	Non	0	ASBR	5	1600	2260	17	12,4	1,37	99,9
Ethanol	Non	20	ASBR	5	1600	4750	8,1	8,2	0,99	99,9
Ethanol	Non	60	ASBR	5	1600	33200	1,2	7,6	0,15	99,9

La biodégradabilité anaérobie de l'effluent de tannerie considéré dans cette thèse a d'abord été testée en réacteur UASB. Le rendement épuratoire obtenu proche de 80% à une salinité supérieure à 70 g l^{-1} correspond au rendement maximal recherché lors de la construction de digesteurs anaérobies industriels et est donc suffisante, même si elle est inférieure à la biodégradabilité aérobie. Cependant, outre une instabilité des performances, ce rendement épuratoire n'a pu être atteint qu'au prix d'une faible cva de l'ordre de 0,5 kg DCO m^{-3} j^{-1}. Une cva plus élevée de l'ordre de 1,1 kg DCO m^{-3} j^{-1} a provoqué une déstabilisation du système mais de bonnes capacités de récupération ont été observées après un retour à une valeur plus basses. Malgré cette limitation de la cva, la cma correspondante de 0,1 kg DCO kg^{-1} de MVS j^{-1} est similaire à celle obtenue avec la plupart des autres effluents industriels testés dans la littérature, notamment issus de l'industrie de la pêche (cf. Tableau 2.8). En fait, il semble que des problèmes de fonctionnement de l'UASB traitant cet effluent, probablement causés par la salinité élevée de l'effluent, se soient traduits par des pertes de biomasse. Par conséquent, la faible biomasse accumulée dans l'UASB traitant l'effluent de tannerie, conjuguée à l'absence de granules, a limité l'applicabilité de ce procédé à cet effluent. C'est pourquoi un digesteur anaérobie à biomasse fixée, le lit mobile, a été par la suite employé pour tenter d'améliorer ces performances. Mais comme il l'a été indiqué auparavant, la fixation de la biomasse sur le support employé dans cette expérience a été très faible, probablement en raison de la faible vitesse ascensionnelle dans le réacteur (de l'ordre de 0,1 à 0,2 cm h^{-1}) qui a empêché le lessivage des bactéries libres et en raison de l'absence de bactéries filamenteuses nécessaires à la structure du biofilm. Par conséquent, aucune amélioration de la biodégradabilité anaérobie et des cinétiques de dégradation n'a été observée dans le lit mobile et les performances de ce réacteur sont très proches de celles obtenues en UASB.

Au regard des performances limitées de la digestion anaérobie de l'effluent de tannerie en comparaison avec le traitement aérobie, une étude plus poussée de la compréhension des phénomènes d'inhibition de la digestion anaérobie par le sel (NaCl) a été menée. Cette étude a montré que la nature du (ou des) substrat(s) carboné(s) utilisé(s) induit une tolérance du procédé de traitement plus ou moins grande au sel. Dans le cas où le substrat utilisé est l'éthanol (substrat simple), une cva de 8,1 kg DCO m^{-3} j^{-1} et une cma de 1 kg DCO kg^{-1} de MVS j^{-1} peuvent être appliquées même à 20 g NaCl l^{-1}. Ces performances sont supérieures à la plupart des substrats complexes (effluents de pêcherie) étudiés à ce jour. Même à 60 g NaCl l^{-1} (2 fois la salinité de l'eau de mer), les performances obtenues restent similaires à celles obtenues dans la littérature sur substrats complexes à des salinités inférieures. Par contre, lorsque le substrat utilisé est un substrat complexe (vinasse de distillerie), les performances du réacteur anaérobie se

dégradent très vite et rejoignent celles obtenues avec l'effluent de tannerie. L'utilisation de l'éthanol comme unique source de carbone a rendu possible la ségrégation des phases d'acidogenèse et de méthanogenèse impliquées dans la dégradation anaérobie de cet effluent, et la quantification de ces phases en terme de production spécifique de méthane. Cette étude a montré que la réaction de méthanogenèse est inhibée à une plus faible concentration en sel que la réaction d'acidogenèse. Ainsi, le risque majeur auquel on peut s'attendre lors de la digestion anaérobie d'effluents salins est une inhibition prioritaire de la méthanogenèse, se traduisant par une accumulation d'acides gras volatils et une réduction du pH. C'est ce phénomène qui a été observé notamment par Isik lors du traitement d'un effluent synthétique simulant un effluent textile à une salinité supérieure à 30 g l^{-1} de NaCl [102]. Dans notre cas, lors du traitement d'un effluent de tannerie hypersalin en réacteur UASB (section 5.1), c'est cependant le phénomène inverse qui a été observé avec une diminution de la concentration en AGV, en réponse à un choc de salinité. Ce phénomène traduit une inhibition simultanée de l'acidogenèse et de la méthanogenèse. Une tentative d'explication avancée peut être l'hypersalinité de ce réacteur (> 60 g l^{-1} de NaCl, i.e. suffisamment élevée pour inhiber à la fois l'acidogenèse et la méthanogenèse), ainsi que la forte teneur en MES inorganiques de cet effluent, qui peut inhiber sa dégradabilité et sa capacité à supporter les chocs lors du traitement en UASB, ainsi que l'ont indiqué Lettinga et Hulshoff Pol [128]. Ainsi des phénomènes différents sont attendus en fonction de la nature de l'effluent salin à traiter, d'où le soin nécessaire à la caractérisation spécifique de chaque nouvel effluent à étudier.

7.2.3 Les principales limites observées : la turbidité des effluents traités et la sensibilité aux chocs

Quel que soit le type d'effluent industriel traité (industrie tartrique ou tannerie) et quel que soit le procédé employé (aérobie ou anaérobie), le principal problème observé lors du traitement biologique des effluents hypersalins concerne la turbidité de l'effluent traité, et ce malgré les bons rendements épuratoires observés (jusqu'à 92% des MES de l'effluent de tannerie épurées en réacteur SBR aérobie). Ainsi dans tous les cas, la nécessité d'un traitement tertiaire visant à éliminer les MES puis les sels de l'effluent traité s'impose, ainsi que le suggère la section 2.3.8.

Egalement, les procédés biologiques se sont révélés sensibles aux variations brutales des caractéristiques de l'effluent de tannerie en terme de salinité et de DCO. Le recours à un bassin d'homogénéisation visant à lisser les rejets apparaît alors comme une

nécessité pour le traitement biologique des effluents industriels hypersalins (cf. section 2.3.8).

7.3 Ecologie microbienne des boues activées en conditions salines

7.3.1 Caractérisation et diversité d'écosystèmes halophiles

Le traitement des effluents fortement concentrés en sel implique l'activité de micro-organismes tolérant ou requérant de fortes salinités et que l'on peut par conséquent qualifier d'halotolérants ou halophiles. Les inventaires microbiens effectués sur les différentes boues d'épuration utilisées dans cette étude (sections 4.1, 5.3 et 6) sont les premiers inventaires de boues salines à hypersalines. Parmi les différents critères environnementaux considérés (présence/absence d'oxygène, salinité, matière organique), il a été montré que c'est la salinité qui influe le plus sur ces communautés en les enrichissant en Gamma Proteobacteria, notamment (cf. section 6). Ainsi la caractéristique halophile de ces boues les rend très similaires aux populations bactériennes que l'on peut retrouver par ailleurs dans les marais salants ou les lacs alcalins.

Par ailleurs la diversité des écosystèmes de boues salines a montré une biodiversité similaire à celle des boues non salines. Cette biodiversité a été mise en évidence en utilisant divers indices (section 6) mais aussi en utilisant les profils SSCP (sections 4.1 et 5.3) qui donnent une image globale de la diversité microbienne. Le constat peut être fait que les performances et la stabilité d'un procédé biologique de traitement des effluents repose sur une importante biodiversité des boues et ce, aussi bien dans les conditions aérobies [24; 203], qu'anaérobies [81]. On peut alors admettre que les ressources bactériennes permettant le traitement des effluents hypersalins sont présentes dans les boues salines et assurent l'efficacité des procédés de traitement biologique aux fortes concentrations en sel.

7.3.2 Adaptation – étude des phénomènes d'halotolérance

Les phénomènes d'adaptation de boues anaérobies aux conditions de salinité croissante ont été observées avec deux substrats différents (cf. section 5.3) : éthanol,

utilisé comme substrat simple, entièrement biodégradable en anaérobiose, et effluent de vinasse de distillerie, comme substrat complexe, aussi connu pour être largement biodégradable en anaérobiose [189]. L'originalité scientifique de cette étude, du point de vue microbien, réside dans l'évaluation de l'halotolérance d'une boue non saline initialement extraite d'un réacteur UASB utilisé pour le traitement d'effluents sucrés. L'observation des profils SSCP obtenus à différents paliers de salinité (0 ; 1 ; 5 ; 10 ; 20 ; 40 et 60 g NaCl l^{-1}) a permis d'observer l'évolution de la microflore des boues aux conditions croissantes de salinité et n'a pas révélé de perte de biodiversité avec l'augmentation de salinité, quel que soit le substrat. Le potentiel d'halotolérance d'une boue activée est donc important. En l'absence supposée d'halophiles, une diversité microbienne se maintient et peut dans certains cas (cas de l'éthanol) maintenir une activité convenable même à des salinités élevées. Et, lorsque l'activité des boues se dégrade avec l'augmentation de la salinité, il semble que ce ne soit pas lié à une perte de diversité, mais plutôt à une inhibition du métabolisme microbien par le sel. On peut aisément imaginer que certaines bactéries peuvent même demeurer inactives, sous des formes de résistance, dans l'attente de conditions plus favorables.

Conclusion et perspectives

Les principales conclusions de la thèse peuvent se résumer comme suit : le traitement biologique aérobie des effluents industriels hypersalins a conduit à des performances supérieures au traitement anaérobie. Le traitement biologique aérobie des effluents hypersalins apparaît donc envisageable au vu des charges appliquées et rendements épuratoires obtenus. Le traitement biologique anaérobie semble moins avantageux de ce point de vue. Par ailleurs le procédé SBR est apparu adéquat pour le traitement de ces effluents. La sensibilité aux chocs (charge organique et salinité) et la forte turbidité observée dans les effluents traités constituent les principales limites de l'épuration biologique des effluents hypersalins, ce qui souligne l'importance des prétraitements (homogénéisation dans un but de lissage des rejets) et des post-traitements (élimination des MES, puis du sel).

Les perspectives à donner à ce travail sont principalement du ressort du transfert technologique avec la mise en place de réacteurs pilotes. Suite à des contacts établis avec l'UNIDO (Organisation des Nations Unies pour le Développement Industriel), il est possible que le traitement UASB des effluents de rinçage de tannerie soit ainsi testé à l'échelle pilote en Inde à Vaniyambadi (Tamil Nadu) où une station de traitement pilote édifiée sous l'égide de l'UNIDO dispose déjà d'une installation UASB. Ce pilote traite présentement des boues d'effluents de tannerie mais les responsables de la station souhaitent essayer le traitement des effluents de rinçage. De même, l'entreprise Faure S.A. est intéressée par la mise en œuvre d'un réacteur pilote aérobie pour traiter son effluent tartrique. A cette fin, des critères de dimensionnement d'un procédé aérobie appliqué à cet effluent sont donnés en Annexe III. D'autres perspectives concernent la mise en place de nouvelles stratégies de traitement visant notamment à améliorer la digestion anaérobie des effluents hypersalins. L'utilisation de bioréacteurs à membrane pourrait notamment régler les problèmes de perte de biomasse et de forte turbidité en sortie des procédés de traitement de ces effluents. Leur coût élevé est cependant une limite majeure. Enfin, les différences importantes de performance constatées en fonction de la nature de l'effluent à traiter soulignent l'intérêt à tester les possibilités de traitement biologique d'autres effluents hypersalins. Le traitement biologique des effluents textiles et pharmaceutiques a notamment été peu étudié en raison du caractère à priori peu biodégradable de ces effluents.

Du point de vue de l'écologie microbienne, il a été montré que les ressources microbiennes des boues sont suffisantes pour assurer le traitement biologique des effluents hypersalins. L'utilisation d'un inoculum halophile permet généralement l'obtention d'une biodiversité importante dans les boues, accompagnée de bonnes performances des procédés biologiques hypersalins. Ce phénomène a notamment été observé dans cette thèse lors du traitement biologique d'un effluent d'industrie tartrique ou d'un effluent de tannerie. En comparaison, le recours à un inoculum non halophile engendre une limitation des performances des boues, même si une bonne activité peut être maintenue à des salinités importantes, selon la nature du substrat utilisé. Par conséquent, il existe un potentiel halotolérant dans les boues non salines. Il serait intéressant d'étudier l'activité et le potentiel de résistance d'une boue halophile en eau douce. On peut s'attendre là aussi à trouver dans une boue activée hypersaline un mélange d'organismes strictement halophiles et d'autres halotolérants, permettant de maintenir une activité de la boue dans des conditions variées de salinité.

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Glossaire

Des mots en français

ADN(r)	acide désoxyribonucléique (ribosomique)
AGV	acide gras volatil
ARN(r)	acide ribonucléique (ribosomique)
Aw	activité de l'eau
$Ca(OH)_2$	hydroxyde de calcium, chaux
$CaCl_2$	chlorure de calcium
CFC	chlorofluorocarbone
CH_4	méthane
CO_2	dioxyde de carbone
DBO_5	demande biochimique en oxygène à 5 jours (g O_2 l^{-1})
DCO	demande chimique en oxygène (g O_2 l^{-1})
$FeCl_3$	chlorure ferrique
H_2	hydrogène gazeux
H_2O	eau
HCl	acide chlorhydrique
IB	indice de boue
KCl	chlorure de potassium
MES	matières en suspension
N	azote
N_2	azote gazeux
NaCl	chlorure de sodium
NH_4^+	ammonium
NO_2^-	nitrite
NO_3^-	nitrate
NTK	azote total kjehdal
O_2	oxygène gazeux
O_3	ozone
P	phosphore
PO_4^{3-}	phosphate
PVC	chlorure de polyvinyle
SO_4^{2-}	sulfate
TSH	temps de séjour hydraulique
UTN	unités de turbidité néphélométrique
UV	Ultraviolet

Des mots en anglais

(U)AF	(upflow) anaerobic filter
ALR	ammonia loading rate
AFFR	anaerobic fixed film reactor
ASBR	anaerobic sequencing batch reactor
BOD_5	biochemical oxygen demand at 5 days (g O_2 l^{-1})
COD	chemical oxygen demand (g O_2 l^{-1})
CETP	common effluent treatment plants
CSTR	completely stirred tank reactor
DFAFBR	down-flow anaerobic fixed bed reactor
(r)DNA	(ribosomal) desoxyribonucleic acid
HRT	hydraulic retention time
(A)MB	(anaerobic) moving bed
OLR	organic loading rate
OTU	operational taxonomic unit
PCR	polymerase chain reaction
(r)RNA	(ribosomal) ribonucleic acid
SEP	solar evaporation pan
SSCP	single strand conformation polymorphism
SVI	sludge volume index
SLR	specific loading rate
(ML)SS	(mixed liquor) suspended solids
TDS	total dissolved solids
TKN	total kjehdal nitrogen
TOC	total organic carbon
UASB	upflow anaerobic sludge blanket
USBF	upflow sludge bed-filter
VFA	volatile fatty acids
(ML)VSS	(mixed liquor) volatile suspended solids

Annexes

Annexe I

Article : Treatment of industrial saline wastewater : a literature review

Résumé

De nombreux secteurs industriels sont susceptibles de générer des effluents hypersalins : on peut notamment citer les industries chimique, pharmaceutique, agro-alimentaire, pétrolière, textile et du cuir. Le rejet de ces effluents sans traitement préalable peut avoir un impact dramatique sur la vie aquatique, la potabilité de l'eau et l'agriculture. Les conséquences sont pires encore dans le cas de régions déjà affectées par les pénuries d'eau, où la compensation de la pollution saline est impossible. Par conséquent, la législation est de plus en plus stricte et le traitement des effluents salins est désormais imposé dans de nombreux pays. Les effluents salins sont généralement traités par voie physico-chimique, parce que le traitement biologique est fortement inhibé par le sel (NaCl, notamment). Cependant, le coût du traitement physico-chimique étant particulièrement élevé, l'intérêt pour des méthodes de traitement alternatives va en s'accroissant, la plupart d'entre eux comprenant une étape biologique aérobie ou anaérobie. Les points principaux étudiés dans cette revue sont : (1) les principaux secteurs industriels générant des effluents salins, (2) l'état de l'art des procédés de traitement, avec un intérêt particulier pour le traitement biologique de la pollution carbonée, azotée et phosphorée, (3) les problèmes de turbidité fréquemment rencontrés avec les effluents salins, (4) l'importance de combiner plusieurs méthodes successives de traitement (biologiques et physico-chimiques) des effluents hypersalins du point de vue de la chaîne de traitement globale.

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Abstract

Many industrial sectors are likely to generate highly saline wastewater: these include the chemical, pharmaceutical, agro-food, petroleum, textile and leather industries. The discharge of such wastewater without prior treatment is known to adversely affect the aquatic life, water potability and agriculture. The consequences are even worse in the case of regions already affected by water scarcity, where the compensation of inorganic salt pollutants is impossible. Thus, legislation is becoming more stringent and the treatment of saline wastewater is nowadays compulsory in many countries. Saline effluents are conventionally treated through physico-chemical means, as biological treatment is strongly inhibited by salts (mainly NaCl). However, the cost of physico-chemical treatments being particularly high, alternative treatment systems are nowadays increasingly the focus of research. Most of such systems involve anaerobic or aerobic biological treatment. The main points reviewed in this paper are: (1) the major industrial sectors responsible for saline wastewater pollution, (2) the state of the art of the treatment processes, emphasising biological treatment of carbonaceous, nitrogenous and phosphorous pollutions, (3) the turbidity problems inherent in saline effluents, (4) the suitability of combined physico-chemical / biological treatment of saline industrial effluents with regard to the global treatment chain.

Introduction

The economic importance of salt (NaCl) is huge in regard to its consumption which exceeds 30 million tons per year in the European Union. The main end markets for salt are the chemical industry (mainly the chloralkali sector), road de-icing and agro-food industries. Other non-negligible uses of salt are found in the petroleum, textile and leather industries as well as for softening hard water. All these sectors generate very large amounts of saline wastewater, rich in both total dissolved solids (TDS) and organic matter. When this wastewater is discharged into the environment without prior treatment, it can cause severe damage by contamination of soil, surface and groundwater.

Concerning the environmental dimension of the problem of salinification, the European Union Directive 2000/60/EC establishing a framework for Community action in the field of water policy requires measures to prevent adverse impact from saline pollution [67]. For inland freshwater, salinity is one of the parameters which must be considered in relation to any body of water, and member States are required to set standards in order to ensure the viability of the ecosystem and the maintenance of a biological community deviating only slightly from that normally associated with the ecotype when conditions

are undisturbed. Thus, under Article 11 of the Directive, member States have to establish measures to ensure that those standards are observed. On the question of control for point sources of saline contamination, Directive 96/61/EC, concerning the integrated pollution prevention and control of industrial activities, requires member States to introduce an integrated permit system for various industrial installations, when their production capacity exceeds certain thresholds [66]. The permits have to contain emission limit values for all relevant pollutants based on best available techniques. The permits must require stricter measures whenever this is necessary to comply with environmental quality standards. According to Directive 2000/60/EC, Annexe V establishes the parameter of conductivity as the standard for measurement of salinity [67]. However, this parameter is seldom used by scientists and the determination of the concentrations of cations or anions (mainly Na^+ and Cl^-) or total dissolved solids (TDS) is usually prefered, which explains why saline wastewater is commonly referred to as high TDS wastewater.

Faced with tightening regulations, the interest in saline effluent treatment processes is increasing rapidly. The rise in knowledge about the treatment of these effluents is depicted graphically in Figure 1, based on a query on the "web of science".

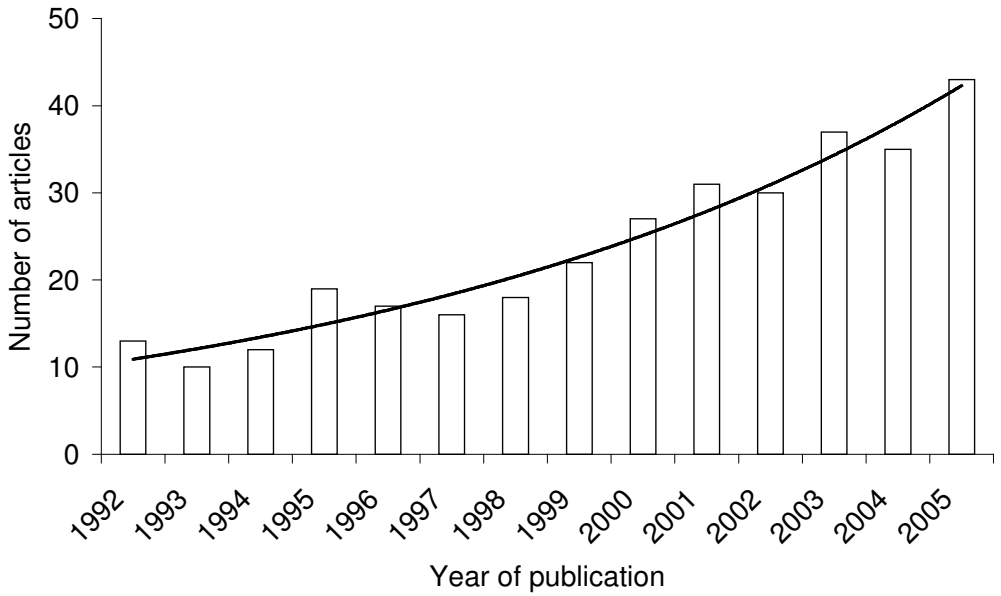

Figure 1 Number of articles dealing with hypersaline wastewater treatment published yearly in the last 13 years.

Saline effluents are usually treated through physico-chemical means, as conventional biological treatment is known to be strongly inhibited by salt (mainly NaCl). However, physico-chemical techniques are energy-consuming and their start-up and running costs

are high. Nowadays, alternative treatment systems are studied, most of them involving anaerobic or aerobic biological treatment. Thus, the purpose of this review is to: (1) list the major industrial sectors generating saline effluents, (2) summarise the latest advances in saline wastewater treatment processes, emphasising biological treatment, (3) focus on combined aerobic/anaerobic treatment processes for carbon, nitrogen and phosphorus removal, (4) focus on the specific problem of turbidity encountered in most of the cases, (5) discuss the relevance of combined treatments for saline industrial effluents, within the context of the global wastewater treatment chain.

Industries generating saline effluents

Food-processing industry

- Salt utilisation in the food-processing industry

The salt destined for human consumption (NaCl) has a double role: for nutrition and for food conservation. The agro-food sectors requiring the highest amounts of salt are meat canning, pickled vegetables, dairy products and the fishing industries. The salt-content of food is highly variable, ranging from of a few percent for some ready-made food, bread and cheese, up to more than 40% in the case of highly concentrated food like packet soups. Salt is known to reduce the water activity and therefore constitutes a microbiological agent of stability [134]. This role of inhibitor-retarder is used to advantage in the fish and meat canning industries. In addition, depending upon salt concentration, salted water permits the development of only certain micro-organisms to the detriment of others, which are either killed or inhibited. Thus, salt acts as a selective antimicrobial or bacteriostatic agent. This role of regulation and orientation in the development of micro-organisms is crucial in, for example, the cheese-making process.

- Generation of saline effluents by the food-processing industry

In the food industry, saline effluents are mainly generated by the use of brine solutions and dry salt (NaCl) for obtaining the finished product. We will focus in this part on two sectors of the agro-food industry that generate high amounts of saline effluents, i.e. the pickled vegetable and the fishing industries. In the pickled vegetables industry, the main source of saline pollution is related to the use of brine for canning and pickling. Consequently, the wash water is polluted by the brine losses and rejections. Olive oil mills, in particular, reject great quantities of solid waste (skins and stones), as well as saline wastewater [225]. In addition, olive oil effluents are characterised by their black-brownish colour due to the presence of sugars, organic acids, tannins, polyphenols,

polyalcohols, pectins and lipids [166; 215], inducing an organic load from 0.1 to 0.3 kg COD m^{-3} [115].

In the fishing industry, the sources of pollution are initially related to the unloading of fish accompanied by seawater. This effluent generates from 13 to 39 kg COD per ton of unloaded fish [12]. The fisheries then generate wastewater rich in proteinic nitrogen, organic matter and salts [6; 146]. Such wastewater also contains considerable quantities of grease (0.25 kg m^{-3}, on average) [145] and sulphates that can limit its anaerobic biodegradability [218].

Leather industry

- Tanning process

The tanning process, which turns raw hides and skins into finished leather products, is a lengthy process that involves several steps, many of which requiring the addition of salt [210; 212; 233]. Before reaching the tannery, raw hides and skins must first be preserved from biodegradation using different methods of preservation. These include salting, chilling, freezing and the use of biocides. After reaching the tannery, the salted skins are then soaked in clean water to dampen them and to eliminate salt, blood and dirt. To remove the hair, the skins are then immersed for one to nine days in a solution of water and lime containing a small quantity of sodium sulphide. Another major source of saline pollution is the pickling step, using weak acid and salt solutions to bring the pelt to the weakly acid state required for most tanning processes. The next step, the actual tanning, converts the protein of the raw hide or skin into a stable material which will not putrefy and is suitable for a wide variety of purposes. Every type of skin can undergo several types of tanning. Process selection depends on the expected use of the leather. There are two principal processes: the first is mineral tanning, using chromium salts *inter alia* ; and the second, vegetable tanning, uses plant substances. Chromium tanning is often carried out in one day whereas vegetable tanning requires several weeks or months. The latter makes it possible to obtain firmer leather, more elastic and resistant to water.

- Pollution impact

The potential environmental impact of tanning is significant. The tanning process is almost wholly a wet process that generates very large amounts of wastewater. Certain streams are hypersaline, such as the pickling and the chromium tanning effluents or the soak liquor generated by the soaking of hides and skins that can contain as much as 80 g l^{-1} of TDS. When they are mixed with the composite wastewater, made up of all the other

effluents mixed together, these highly saline streams can reduce the efficiency of the global treatment. Thus, such streams should be segregated and undergo a specific treatment [127]. In addition, significant volumes of solid wastes are generated by tanneries, including trimmings, degraded hides and hair from the beamhouse processes. The solid wastes can represent up to 70% of the wet weight of the original hides [107]. Large quantities of sludge are also generated. Decaying organic material produces strong odours. Hydrogen sulphide is released during dehairing and ammonia is released in deliming.

Chemical and pharmaceutical industries

Salt is indispensable in the manufacture of chemicals such as hydrochloric acid, sodium hydroxide (caustic soda), sodium bicarbonate (baking soda), chlorine and many other chemicals. Chlorine-based chemicals, *inter alia*, are particularly in demand for pulp and paper bleaching. Other activities requiring salt include production of polyvinyl chloride (PVC) and brine electrolysis in which caustic soda is a co-product.

The manufacture of chemical and pharmaceutical products generates solid waste, gas emissions and highly saline wastewater. The difficulty of treating this wastewater results in the main from its variability, not only from one factory to the other but also within the same factory, depending on the products synthesised. Chemical and pharmaceutical effluents are generally rich in soluble matter (NaCl and sulphates, in particular), suspended solids and toxic substances that are refractory to biological treatment, in particular aromatic compounds [72]. In addition, pharmaceutical effluents are often characterised by high COD and a high COD:BOD_5 ratio [177].

Textile industry

In the textile industry, salts (mainly NaCl and Na_2SO_4) are used in the dyeing process, in order to improve the fixing of the dyes on fibres. The dye fixation yield actually depends on the salt concentration as well as on the desired colour, the darkest shades requiring more salt [194].

All the various operations involved in textile processing have consequent environmental impact. After pretreatments (scouring, washing, mercerizing), the raw material is dyed, which generates an effluent characterised by an alkaline pH, mainly due to the addition of soda and soap, a strong salt concentration required for dye fixation, and a high quantity of COD resulting from the dyeing auxiliaries. In addition, the effluent is particularly rich in residual organic aromatic compounds (mainly phenols) that reduce its biodegradability and the colour of the effluent is not only aesthetically unacceptable but

also seriously damages the water ecosystems by delaying the penetration of UV in water, thus blocking the photosynthetic activity of algae [194].

Petroleum industry

Crude oil is a complex mixture that contains mainly aliphatic, alicyclic and aromatic hydrocarbons [181]. The refining process requires de-emulsifiers and the waste water (called production water) resulting from the decantation of the oil-water emulsion presents a broad range of salinity, from fresh water up to three times the salinity of seawater and beyond [56].

State-of-the-art treatment applied to saline effluents

Clean technologies - Reduction at the source

Simple installations aimed at cleaner production and sustainable development can help the industries to limit the generation of saline pollution. The guiding principle for cleaner production that should always be taken into account in industries generating saline wastewater is the elimination or reduction of the quantity of salt used in the process. Other principles include segregation and recycling.

- Segregation of brine solutions through the installation of a brine equalisation pit.

The use of a brine equalisation pit makes it possible to optimise the retention time of the rejected brine, to improve the decantation of solid salt, to avoid solid salt departure towards the sewer, to smooth salt discharges and to eliminate shock impact on an effluent treatment plant through controlled discharge.

- Recycling after the elimination of suspended solids and grease.

Recycling is another basic principle of cleaner production that should be generalised to all industries generating saline pollution. In the fish industry, Roeckel *et al*. have already proposed the introduction of a new step in the fish reduction process that involves recirculation of the pumping water used to unload the fish, screening of coarse organic matter, flocculation of soluble proteins in the recirculate and its separation by centrifugation, and the incorporation of both coarse and flocculated material into the reduction process. They showed that this stage would make it possible to reduce the COD of the effluent by 91.6% and improve the productivity of the industry by 7% [185]. In fish-processing installations, another case study showed that it is possible to reduce the water consumption by 31.8% through the segregation of the effluents according to their

flowrate and their organic load. This process made it possible to recycle the organic matter from the effluents containing the highest COD, which increased the quantity of processed fish by 5.5 t d^{-1} [183].

Physico-chemical treatment of hypersaline effluents

Hypersaline effluents are often recalcitrant to biological treatment ; Consequently, physico-chemical treatment is generally required to remove the organic matter as well as the TDS from such effluents. The main technologies that have been investigated are evaporation, coagulation-flocculation, membrane techniques and advanced oxidation processes.

- Thermal techniques

Solar evaporation is a low-cost technique commonly applied to concentrate the TDS and organic content of saline effluents, thereby reducing the volume of effluents. In the leather industry, the hypersaline soak liquor generated by the soaking of hides and skins is sometimes segregated from the other streams because of its high salt content and sent to solar evaporation pans. However, the reuse of the solid salt thus obtained is made impossible due to its high degree of impurity. The evaporation rate can be improved using vacuum evaporators or hot air.

- Coagulation-flocculation

Coagulation-flocculation is often used as a pretreatment of hypersaline effluents to remove their colloidal COD along with their TDS (sulphates, *inter alia*). Some examples of coagulation-flocculation applied to saline wastewater are indicated in Table 1.

Table 1 Coagulation-flocculation applied to saline wastewater.

Substrate	TDS (g l^{-1})	Coagulant	pH	Contact time (min)	Dose (g g^{-1} of comp. remov.)	Compound removed	Remov yield (%)	Ref
Pharmaceutical wastewater	29	$Ca(OH)_2$	8	30	4.8	COD	46	[177]
Pharmaceutical wastewater	29	$Ca(OH)_2$	8	30	6.7	SO_4^{2-}	32	[177]
Fish-processing effluent	35	$FeCl_3$	4.3	-	0.4	Proteins	40-50	[145]

It appears from this table that saline pharmaceutical effluents, for instance, can be treated by coagulation for the removal of COD and sulphates [177]. Regarding a fish-processing effluent, flocculation with ferric chloride was able to eliminate the proteins [145]. Finally, Ellouze *et al*. studied colloid coagulation in cuttlefish-transformation effluents, using aluminium sulphate (165.5 mg l^{-1}) followed by a flocculation step using MgO (750 mg l^{-1}) and poly-dimethyl ammonium chloride (35 mg l^{-1}). The coagulation-flocculation of this effluent made it possible to reduce the turbidity and the COD of the effluent by 7 NTU and 90%, respectively [64].

- Membrane techniques

Membrane techniques consist in the transfer of selected molecules under the effect of a concentration or pressure gradient, or an electrical field. Ultrafiltration can be used for the removal of suspended solids and colloidal COD in saline effluents, as already noted by Afonso and Bórquez who used ultrafiltration to concentrate and recycle the proteins contained in seafood-processing wastewater [2]. In the same study, a reduction of abundant suspended solids clogging membranes was obtained by a preliminary microfiltration step. Multiple studies have been devoted to the application of physico-chemical processes to olive oil effluents. Such effluents are generally treated by incineration, concentration by evaporation or ultrafiltration/reverse osmosis [225]. The combination of centrifugation and ultrafiltration steps also achieved the elimination of 90% of the COD as well as the separation of grease in saline olive oil processing effluents [215].

In addition, reverse osmosis can achieve the removal of TDS and is the most commonly-used process in desalination. Again in relation to olive oil processing effluents, reverse osmosis ensured the elimination of 99.4% of the TDS and 98.2% of the COD, as well as complete elimination of the colour and the BOD_5, with a transmembrane pressure at 55.2 bar and a flux of 52.5 l m^{-2} h^{-1} [211].

- Ozonation

Ozone, sometimes combined with UV, can reduce the COD:BOD ratio in saline effluents. This process can achieve the complete oxidation of organic compounds, producing CO_2 and H_2O, even though its operation at such a level of treatment is generally not required. The process is thus especially recommended for the treatment of effluents containing organic compounds very resistant to biodegradation. It is for this reason that ozonation has already been used for the treatment of saline textile effluents, improving their colour and reducing their COD concentration [157].

Aerobic treatment of saline effluents

- Effect of salt on aerobic treatment

High percentages of salt are known to compromise the correct operation of conventional aerobic wastewater treatment processes [136]. Woolard and Irvine reviewed the principal dysfunctions caused by salt [236]: the conventional micro-organisms are sensitive to abrupt changes in ion concentrations and cannot withstand salt concentrations higher than 50 g l^{-1}. In addition, high salinity disturbs microbial metabolism, which reduces the removal of organic matter. Lastly, salt acclimatation is quickly lost if salinity suddenly drops.

- Application of aerobic treatment to saline wastewater

It has been observed that high salinity can strongly inhibit the aerobic biological treatment of wastewater. However, in the 1940s, the follow-up of an activated sludge effluent treatment plant functioning with seawater showed that the efficiency of aerobic treatment was similar to that of a plant operating with fresh water [173]. The interest in the aerobic halophilic degradation of synthetic substrates has been increasing rapidly since the middle of the 1990s, as can be seen in Table 2.

Table 2 Aerobic treatment of high TDS wastewater.
(F:M ratio : food :microorganisms ratio ; HRT : hydraulic retention time ; MLVSS : mixed liquor volatile suspended solids ; OLR : organic loading rate ; SB(B)R : sequencing batch (biofilm) reactor ; SFPW : seafood-processing wastewater ; TDS : total dissolved solids ; V : volume)

Substrate	Halophilic inoculum	TDS ($g\ l^{-1}$)	Process	V (l)	COD in ($g\ l^{-1}$)	HRT (h)	OLR (kg COD $m^{-3}\ d^{-1}$)	MLVSS ($g\ l^{-1}$)	F:M ratio (kg COD kg^{-1} of MLVSS d^{-1})	COD remov (%)	Ref
Synthetic (molasse)	No	20	Fed-batch reactor	15	5	16	7.5	1.1	6.82	80	[111]
Synthetic (molasse)	No	50	Fed-batch reactor	15	5	13	9.3	1	9.23	59	[111]
Synthetic (molasse)	Yes	50	Rotating biodiscs	10	5	4	30	29	1.03	85	[58]
Synthetic (molasse)	Yes	100	Rotating biodiscs	10	5	4	30	28	1.07	60	[58]
Synthetic (phenol)	Yes	150	SBBR	1	0.29	48	0.15	3	0.05	99	[235]
Synthetic (phenol)	Yes	150	SBR	1	0.25	24	0.25	1	0.25	99.5	[236]
Synthetic (glucose. Acetate)	No	60	SBR	5	1.2	6	4.8	-	-	32	[219]
Synthetic (≈SFPW)	Yes	32	Membrane bioreactor	8	5	36	3.4	11.2	0.30	85	[49]
Synthetic (≈SFPW)	Yes	32	Membrane bioreactor	3.6	1.2	13.7	2.1	11	0.19	91	[49]
Synthetic (≈SFPW)	Yes	10	SBR	10	0.55	20	0.7	4.1	0.17	87.9	[152]
Pickling wastewater	Yes	30-60	Activated sludge	Labo	4.6	35	3.2	4.9	0.64	96	[112]

Substrate	Halophilic inoculum	TDS ($g\ l^{-1}$)	Process	V (l)	COD in ($g\ l^{-1}$)	HRT (h)	OLR (kg COD $m^{-3}\ d^{-1}$)	MLVSS ($g\ l^{-1}$)	F:M ratio (kg COD kg^{-1} of MLVSS d^{-1})	COD remov (%)	Ref
Pickling wastewater	Yes	150	Activated sludge	5,000	120	168	17	-	-	60-70	[120]
SFPW	Yes	74	Fixed-bed	1.5	2.7	72	1	8	0.11	60	[76]
SFPW	Yes	20	Activated sludge	5	2.7	72	0.9	2.8	0.32	88	[116]
Tannery wastewater	Yes	35	SBR	10	3	120	0.6	2	0.30	95	[127]
Tannery wastewater	Yes	40	SBR	10	3.6	79	1.1	7.2	0.15	91	[127]
Tartaric industry effluent	Yes	120	SBR	5	4.3	240	0.4	3.5	0.12	83	[126]

It can be seen from this table that the salinity of the effluents treated aerobically ranges from 10 to 150 g l^{-1}. In addition, most of the studies were based on synthetic wastewater which generally underwent treatment at a higher organic loading rate (OLR) and F:M ratio than industrial wastewater.

The contribution of Kargi and his team has been considerable, mainly using synthetic substrates (molasses). In 1996, Kargi and Dincer were initially interested in the effect of salt concentration on the aerobic biological treatment of a synthetic saline effluent using a fedbatch biological reactor [109-111]. The synthetic effluent was made up of diluted molasses, urea, KH_2PO_4 and NaCl up to a concentration of 50 g l^{-1} and characterised by a COD:N:P ratio of 100:10:1. The treatment process used activated sludge. Kargi and Dincer observed that the effluent COD removal efficiency fell from 85 to 59% when salinity increased from 0 to 5% [111]. Thereafter, Dinçer and Kargi tested innovative treatment processes in halophilic conditions including, for instance, a process with aerobic rotating discs whose number and surface area varied [58]. This reactor was used to purify a synthetic effluent under conditions of increasing salinity (0-10%) and made it possible to exceed 80% of COD removal efficiency as long as the salt concentration remained lower than 50 g l^{-1}.

It also appears from Table 2 that halophilic inocula have often been used to improve the performance of the aerobic treatment processes. For instance, the addition of an euryhaline *Halobacter* strain enabled Kargi and Dinçer to significantly improve the performance of activated sludge [110]. According to the same principle, *Halobacter halobium* was added thereafter to an activated sludge culture within an aerobic biofilter in which the cells were immobilised on ceramics particles [113]. The continuous process was then operated at various salt concentrations. The addition of *Halobacter halobium* in the activated sludge led to improved performance of the reactor, in particular at the strongest salt concentrations. Finally, Kargi *et al*. were able to successfully treat an effluent generated by the pickling industry using activated sludge enriched in *Halobacter halobium,* exceeding 95% of COD removal [112]. The same technique (inoculation of the halotolerant bacteria *Staphylococcus* sp*.* and *Bacillus cereus*) applied to another agro-industrial hypersaline effluent (15% of NaCl) generated by the production of plum pickles achieved COD removal efficiency of 90% in a sequencing batch reactor [120]. Finally, the treatment of seafood wastewater revealed the difficulties with the aerobic treatment of saline industrial wastewater in comparison to synthetic wastewater. Using a conventional activated sludge reactor [116] or a continuous fixed bed [76], the OLR (around 1 kg COD m^{-3} d^{-1}) and SLR (< 0.3 Kg COD kg^{-1} of VSS d^{-1}) were lower than that applied to synthetic wastewater in spite of the utilisation of a specific microflora adapted to the effluent.

The sequencing batch reactor (SBR) is known to be particularly robust and to withstand extreme conditions [92]. Consequently, it is not surprising that this process has often been employed to treat hypersaline wastewater. Woolard and Irvine were among the first to inoculate a sequencing batch biofilm reactor (SBBR) with moderately halophilic bacteria isolated from the Great Salt Lake, USA, in order to treat a synthetic effluent containing 150 g l^{-1} of salt. The removal efficiency measured on phenol exceeded 99% [235]. They renewed the experiment in 1995, with a free culture SBR, this time reaching a removal efficiency of 99.5% [236]. More recently, Uygur and Kargi also used a SBR to cleanse a synthetic saline effluent [219]. They observed a drop of COD removal efficiency from 90 to 32%, when salinity increased from 0 to 6%. Another study used a bench-scale SBR inoculated with halophilic sediments in order to treat an effluent from the tartar industry containing 120 g salt l^{-1}. The micro-organisms were able to treat carbon and nitrogen, provided the pH in the reactor was neutralised with phosphoric acid. Soluble COD and TKN removal attained 83% and 72% respectively [126]. Finally, the aerobic treatment in a SBR of a saline tannery effluent (34 g NaCl l^{-1}) achieved 95, 93, 96 and 92% removal of COD, PO_4^{3-}, TKN and SS, respectively, with a HRT of 5 days and an OLR of 0.6 kg COD m^{-3} d^{-1} [127].

There are other potential applications of hypersaline wastewater treatment. One instance is the recycling of a culture medium for microalgae (*Dunaliella salina*) producing beta-carotene, after biological treatment [192]. In this experiment, this effluent rich in organic matter (glycerol) contained between 17 and 25% salt but the removal of glycerol proved possible provided that the sludge was supplemented with nitrogen, phosphorus, potassium and magnesium.

Anaerobic digestion of saline effluents

- Effect of salt on anaerobic digestion

High salinities are known to have an inhibiting effect on anaerobic digestion, mainly on account of the cations. It has been known for quite some time that a sodium concentration exceeding 10 g l^{-1} strongly inhibits methanogenesis [84; 121; 182]. However, Omil *et al*. could not show any clear toxic effect of a fish-processing effluent on an anaerobic pilot plant close to the anaerobic contact system [163]. They showed that the adaptation of a methanogenic biomass active at the salinity level of the effluent was possible and they concluded that the efficiency of such a process depended on a suitable strategy for adapting the biomass to strong salinities. Furthermore, Feijoo *et al*. stated that the toxicity of sodium in sludge depended on several factors, such as the antagonistic effects of other ions at adequate concentrations, the nature and the

progressive adaptation of sludge to high salinity, and the type of methanogenic substrate used [68].

- Application of anaerobic treatment to saline wastewater

The capability of halophilic organisms to biodegrade organic compounds by anaerobic digestion is well known. For instance, the anaerobic degradation of cellulose in a hypersaline lake and in a hypersaline lagoon has already been studied. Results showed that, in the hypersaline lake, the degradation of cellulose occured at a maximum rate with a NaCl concentration of 5%, while the microbial flora of the hypersaline lagoon degraded cellulose most quickly at a salt concentration of 15% [200]. However, anaerobic treatment assays of industrial saline effluents are rare ; most of them are detailed in Table 3.

Table 3 Anaerobic treatment of high TDS wastewater.
(AF : anaerobic filter ; AFFR : anaerobic fixed film reactor ; CSTR : completely stirred tank reactor ; DFAFBR : down-flow anaerobic fixed bed reactor ; F:M ratio : food :microorganisms ratio ; HRT : hydraulic retention time ; MLVSS : mixed liquor volatile suspended solids ; OLR : organic loading rate ; SFPW : seafood-processing wastewater ; TDS : total dissolved solids ; UAF : upflow anaerobic filter ; UASB : upflow anaerobic sludge blanket ; USBF : upflow sludge bed-filter ; V : volume)

Substrate	Halophilic inoculum	TDS ($g\ l^{-1}$)	Process	V (l)	COD in ($g\ l^{-1}$)	HRT (h)	OLR (kg COD $m^{-3}\ d^{-1}$)	MLVSS ($g\ l^{-1}$)	F:M ratio (kg COD kg^{-1} of MLVSS d^{-1})	COD remov (%)	Ref
Inuline effluent	No	10	UASB	1,100,000	7.9	6-8	23-32	18-31	0.7-1.8	65-80	[87]
Piggery manure	No	15	DFAFBR	1.4	1.9	96	0.5	-	-	90	[188]
Pharmaceutical wastewater	No	20	AFFR	9.4	25	60	10	35.7	0.28	67	[72]
Fish-farm wastewater	No	35	CSTR	15	70.1	660	2.5	25	0.10	55	[74]
Fishery effluent	Yes	40	CSTR	1.5	6	72	2	-	-	50	[12]
SFPW	No	15	UAF	1.1	34	288	2.8	57.1	0.05	83	[86]
SFPW	No	7.7-26.3	UASB	1	1.7	3	13.6	27.2	0.50	77	[23]
SFPW	No	13.6-33.7	Anaerobic contact system	15 000	10-60	180-240	1-8	-	-	70-90	[163]
SFPW	Yes	-	Hybrid USBF	2.3	1-1.5	18	1.5-2	7.4	0.2-0.3	70-90	[153]
SFPW	Yes	30	Anaerobic filter	2.5	5.5	9.2	14.3	-	-	70	[224]

It appears from this table that the anaerobic treatment has been tested on a certain number of saline effluents (mainly seafood-processing effluents) at TDS concentrations ranging from 10 to 40 g l^{-1}, a range narrower than that in aerobic treatment.

The anaerobic digestion of seafood-processing wastewater has been widely studied for the past 10 years: a Chilean team focused in particular on the anaerobic digestion of fishery effluents, mainly those generated at the time of fish unloading. After recycling and primary treatment in order to eliminate proteins and grease, Aspé *et al*. showed that the effluent, containing 4-6 kg COD m^{-3}, 1.85 kg SO_4^{2-} m^{-3} et 16.2 kg Cl^- m^{-3}, could be treated anaerobically using a marine inoculum which induced specific methanogenic activity at 37°C of 0.065 kg COD-CH_4 kg^{-1} of VSS d^{-1} [12]. Aspé *et al*. also modelled the ammonia-induced inhibition phenomenon of anaerobic digestion and concluded that methanogenesis was the most inhibited stage [11]. Later on, the treatment of seafood-processing wastewater was studied, using different processes, such as an anaerobic filter [86; 153; 224], a UASB [23] and an anaerobic contact system [163]. The COD removal efficiencies obtained with this type of wastewater generally remained between 70 and 90%, with an OLR ranging from 1 to 15 kg COD m^{-3} d^{-1} and a SLR lower than 0.5 kg COD kg^{-1} of VSS d^{-1}.

Regarding the applications of anaerobic digestion to other saline effluents, the biological treatment assays have been rare. Rovirosa *et al*. were interested in the anaerobic digestion of a piggery effluent diluted in a saline synthetic water (15 g l^{-1} of salt), using a lab-scale down-flow anaerobic fixed-bed reactor (DFAFBR). The COD removal efficiency exceeded 90% for a hydraulic retention time (HRT) of 96h and 68% for a HRT of 12h [188]. Gangagni Rao *et al*. treated a saline pharmaceutical effluent using an anaerobic fixed-bed reactor. In steady-state conditions and with an OLR of 10 kg COD m^{-3} d^{-1}, removal efficiency stabilised between 60 and 70% of the COD and 80 and 90% of the BOD_5 [72].

The anaerobic treatment of textile effluents has also been studied but its efficacy appeared to be generally limited to a decolourisation of the effluent, accompanied by a very modest reduction of its organic load, a result related to the complex aromatic nature of the dyes which gives them a toxic and barely biodegradable profile. Isik and Sponza treated a synthetic effluent simulating a textile effluent (i.e. containing salts, dyes, etc.) in a UASB reactor. Decolourisation of the effluent attained more than 90%, even when HRT was reduced from 100 to 6h. However, the COD removal efficiency fell from 80 to 29% in the same period [103]. The influence of salt concentration was also tested in a UASB reactor on the same type of effluent, leading to similar results, good decolourisation of the effluent (nearly 100%) but strong inhibition of the methanogenesis

when salinity increased. Thus, while increasing NaCl concentration from 0 to 128 g l^{-1} and with a constant HRT and OLR, the COD removal efficicency fell from 80 to 18% and the strongest fall was observed for salinities higher than 30 g l^{-1}. In addition, methanogenesis destabilisation was also characterised by an increase of the VFA concentration and a reduction in pH [102]. On the basis of the maximum methane production rate, Manu and Chaudhari fixed at 400 mg l^{-1} the maximum limit of the dye concentration that could be tolerated before methanogenesis inhibition [144].

Finally, follow-up monitoring was done at a factory producing fructose and inuline whose processing effluents were salted due to the presence of a demineralisation unit [87]. The treatment took place in a reactor of 1,100 m^3 made up of two successive upflow anaerobic sludge blankets (UASB), one functioning at a high, the other at a low, loading rate. The influent COD averaged 8 g l^{-1} and the Cl^- concentration averaged 4 g l^{-1}. Using a F:M ratio of 1 kg COD kg VSS^{-1} d^{-1} and an organic loading rate of 30 kg DCO m^{-3} d^{-1}, reactor operation appeared stable over a three month period. The removal efficiency remained higher than 70% for COD and over 90% for VFA. It remains one of the rare industrial-scale applications of anaerobic digestion to a saline effluent.

Combined anaerobic/aerobic treatment of saline wastewater

- Application of combined anaerobic/aerobic treatment of saline wastewater for the removal of carbonaceous pollution

Because both anaerobic and aerobic treatment of saline effluents have given only moderate performance, the combination of these two modes of treatment has obviously been considered, with an aim to improve the performance of the overall treatment process. Panswad and Anan thus obtained a COD removal efficiency close to 71% by applying an anaerobic/anoxic/aerobic process to a synthetic effluent containing 3% of salt, provided that the inoculum was first acclimatised to high salinity [168]. In the textile industry, it has already been shown that dye cleavage is possible in anaerobiosis. Afterwards, the products obtained are sometimes biodegradable in aerobic conditions, which argues in favour of the application of combined anaerobic/aerobic processes in order to cleave/degrade the residual dyes in textile effluents. Thus, the most conclusive experiment to date used a continuous anaerobic/aerobic process which led to a reduction of 84% of the COD, 86% of the colour and 52.2% of the aromatic amines, with a HRT of 2.9 d and an OLR of 1.17 kg COD m^{-3} d^{-1}. The aromatic amines produced in anaerobiosis were indeed further degraded in aerobiosis [208; 209]. Toxicity tests on daphnids confirmed the reduction of the toxicity of effluent treated in this way [209].

- Application of combined anaerobic/aerobic treatment of saline wastewater for the removal of nitrogen

The combination of aerobic and anaerobic processes also makes possible the treatment of nitrogen in saline effluents.

Denitrification

The capacity of halophiles to use oxyanions as final electron acceptors is a well-known phenomenon. In the middle of the 1980s, extremely halophilic bacteria had already been isolated from various sites and cultivated anaerobically in the presence of nitrate [95]. Many of these isolates were able to produce nitrite, nitrous oxide and nitrogen gas. These results confirmed the existence of extremely halophilic denitrifying bacteria and their presence within a large variety of hypersaline environments. Mancinelli and Hochstein, in particular, showed the capacity of *Halobacterium vallismortis*, *Halobacterium mediterranei* and *Halobacterium marismortui* to grow anaerobically in the presence of nitrate only, and to denitrify it [143]. The same property was then observed with many other moderately halophilic anaerobic bacteria [223]. Thereafter, Shieh and Liu showed the existence of halophilic bacteria capable at the same time of denitrification and fermentation [197]. According to their work, an unidentified gram-negative, facultatively anaerobic strain was able to grow anaerobically and to denitrify nitrates, nitrites or nitrous oxides or, alternatively, to carry out the fermentation of glucose or mannose. Lastly, the determination of the kinetics of nitrate reduction by *Halomonas campisalis* at pH 9 and 12.5% of NaCl showed higher denitrification rates in the case of acetate-enriched cultures [171]. However, the application of these properties to the treatment of nitrogen encountered difficulties: Dincer and Kargi showed that salt concentrations higher than 2% were responsible for significant reductions in the nitrification and denitrification performance of biological reactors and that denitrification was affected by salt more than nitrification [57]. Dincer and Kargi thus contradict Panswad and Anan, who showed that denitrifying bacteria could tolerate high salinities better than nitrifying bacteria, the latter being more tolerant than the heterotrophic bacteria responsible for the carbonaceous depollution [168]. Panswad and Anan were thus able to acclimatise a denitrifying community to saline conditions and maintain a nitrate reduction rate of 2 mg $N\text{-}NO_3^-$ g SS^{-1} h^{-1} at 30 g NaCl l^{-1}.

Nitrification

The salt-induced inhibition of nitrifying bacteria is a well-known phenomenon [136]. But these results have only recently been refined by multiple studies. Chen and Wong showed that the progressive adaptation of a continuous nitrifying activated sludge culture

to chlorides gave better results than those of another culture operated at fixed chloride concentrations [41]. However, beyond 18.2 g Cl l^{-1}, nitrification became unstable. Below 10 g Cl l^{-1}, the dominant ammonia-oxidising bacteria were *Nitrosomonas europaea*, *Nitrosomonas eutropha*, *Nitrosomonas halophila*, and *Nitrosococcus mobilis*, while the dominant nitrite-oxidising bacteria belonged to the *Nitrobacter* genus. At 18.2 g Cl l^{-1}, only *Nitrosococcus mobilis* survived while all the nitrite-oxidising bacteria disappeared. Chen and Wong concluded that the critical concentration permitting the maintenance of a nitrifying population was 18.2 g Cl l^{-1}. Thus, they support the conclusions of Dahl *et al*. who showed that nitrification could take place under operational conditions up to 20 g Cl l^{-1}, with a maximum nitrification rate of 2 mg N g VSS^{-1} h^{-1} [47]. In addition, Dahl *et al*. showed that a rapid increase in the chloride concentration had an inhibiting action on the nitrifying bacteria. Thereafter, Vredengbregt *et al*. showed that in a fluidised bed, nitrification was possible up to 34 g Cl l^{-1} at least, provided that the ammonia load was maintained at 15 mg NH_3 l^{-1} h^{-1} [227]. In 1999, Panswad and Anan observed only a moderate reduction in the nitrifying activity from 4 to 3 mg N-NH_3 oxidised g^{-1} of SS h^{-1} after NaCl concentration increased from 5 to 30 g l^{-1}, provided that the sludge was acclimated to high NaCl concentrations [168]. In addition, the recovery capacities of nitrifying bacteria following a shock of 70 g l^{-1} of NaCl proved to be excellent. Lastly, Campos *et al*. showed a combined salt- and ammonia-induced inhibition of nitrification: the accumulation of ammonia started at an ammonia loading rate of 3 g N-NH_3 l^{-1} d^{-1} and a salt concentration of 525 mM (13.7 g NaCl l^{-1}, 19.9 g $NaNO_3$ l^{-1} and 8.3 g Na_2SO_4 l^{-1}) [34].

- Application of combined anaerobic/aerobic treatment of saline wastewater for the removal of phosphorus

The combination of aerobic and anaerobic processes also makes possible the treatment of phosphorus in saline effluents. Uygur and Kargi operated a SBR with alternating anaerobic, oxic, anoxic and oxic phases at various salt concentrations (0-6% of NaCl) in order to determine the NaCl-induced inhibition on the removal of nitrogen and phosphorus in a synthetic effluent [219]. They observed that the removal efficiency of COD, N-NH_4 and P-PO_4^{3-} decreased when the NaCl concentration increased. The COD removal efficiency fell from 96 to 32% when salinity increased from 0 to 6% and, simultaneously, the removal of N-NH_4 and P-PO_4^{3-} decreased from 96 to 39% and from 84 to 22%, respectively. Another consequence was an increase of the sludge volume index (SVI). The addition of a *Halobacter* strain then made it possible to improve considerably the performance of the process, particularly at salinities higher than 2% [114]. At a salinity of 5%, the COD, N-NH_4 and P-PO_4^{3-} removal efficiencies thus reached

73%, 51% and 31%, respectively, compared to 47%, 36% and 21% without the addition of *Halobacter*.

High turbidity in saline wastewater

Problems of decantation in saline environments have frequently been reported in the literature and multiple reasons have been given for this phenomenon. First of all, the density of salt water is higher than that of freshwater, thus creating greater resistance to decantation. Hypersalinity is also known to reduce the quantity of the filamentous bacteria that play a part in the mechanical integrity and structure of the flocs [217]. Finally, the lack of protozoans can also influence effluent turbidity [236]. Protozoans, indeed, reduce the turbidity of the effluents by grazing the micro-organisms, but it has been shown that their resistance to salinity shocks is limited and they do not normally survive more than 24 hours after a NaCl shock higher than 40 g l^{-1} [191]. This explains why protozoan scarcity is expected in saline environments. However, the observations of Pillai and Rajagopalan showed that under stable conditions seawater is not an obstacle to the development of ciliates and that the performance of an effluent treatment plant operating with seawater was dependent on the number of these protozoans [173]. Holubar *et al.* then demonstrated the influence of protozoans on the turbidity of saline effluents [97]. They adapted protozoans to a saline effluent contaminated by hydrocarbons. In a continuous culture, using a mixed inoculum of protozoans originating from activated sludge, only one species of ciliate protozoa, namely *Uronema nigricans*, could grow. The turbidity of the effluent proved to be dependent on the quantity of *Uronema nigricans*. Moreover, the COD removal efficiency reached 45.8%, against 35.4% in the absence of protozoans.

In spite of the negative effects of salt on effluent turbidity, it seems that it is not an obstacle to microbial growth. Panswad and Anan did not observe any effect of high salinity (30 g NaCl l^{-1}) on the suspended solids concentration in a bioreactor, provided that sludge was previously acclimated [168]. Kubo *et al*. also obtained a suitable biomass growth at a salinity of 15% [120]. In addition, Panswad and Anan did not observe any effect of high salinity on sludge settling [168]. The studies of Campos *et al*. confirmed these results and showed that high salinity does not have a long-term effect on the sludge's physical properties [34]. They were able to maintain a concentration of 20 g VSS l^{-1} in an activated sludge reactor thanks to a SVI of 11 ml g VSS^{-1}. Later, Uygur and Kargi found that SVI increased with salinity, but the SVI value (97 ml g^{-1}) that was achieved at a salt content of 6% in a SBR showed the good settling properties of the sludge, even at high salinities [219]. Lastly, Raj and Anjaneyulu also showed that high salinity and good sludge decantability were compatible [177]. In their study, proper biomass growth was

obtained with a pharmaceutical effluent containing 16 g l^{-1} of salt treated by activated sludge, especially at the highest OLR and HRT values. In addition, the SVI values exceeded 100 only in the case of OLR higher than 1 kg COD m^{-3} d^{-1}.

In order to solve the problems of sedimentation in saline effluents, the use of membrane bioreactors might be considered. They are seldom used in saline wastewater treatment even though they have been used successfully for the treatment of cruiser wastewater [118] and also to prevent the wash-out of a pure culture of a halotolerant sulfate-reducing bacterium, *Desulfobacter halotolerans*, in the treatment of saline sulfate-rich wastewater [220]. Finally, Dan *et al*. compared the performance of a yeast membrane bioreactor (YMBR) to that of a bacterial membrane bioreactor (BMBR) in the treatment of an effluent containing 5 g COD l^{-1} and 32 g NaCl l^{-1} [49]. Through a gradual increase of OLR from 3.4 to 16.3 kg COD m^{-3} d^{-1}, the COD removal efficiency of the YMBR dropped from 85 to 60% and the COD removal efficiency of the YMBR dropped from 91 to 76%, when OLR increased from 2.1 to 7.9 kg COD m^{-3} d^{-1}. The maximum COD removal reached 0.93 g DCO g^{-1} MES d^{-1} for the YMBR with a F:M ratio of 1.5 ; and 0.32 g DCO g^{-1} MES d^{-1} for the BMBR with a F:M ratio of 0.4. Dan *et al*. concluded that the BMBR could be recommended for small F:M ratios, while the YMBR became more competitive when this ratio increased. In addition, they noticed that the silting up of membranes was more limited in the case of YMBR.

Global hypersaline wastewater treatment chain

It appears from the points treated previously that several physico-chemical and biological techniques can be applied to the treatment of saline wastewater. All these techniques present advantages and disadvantages. According to the results obtained with various treatment systems, it appears clearly that the optimal treatment system for saline wastewater involves a combination of several techniques. Figure 2 indicates the generic sequence of the different operations that can be indicated for the treatment of industrial saline wastewater.

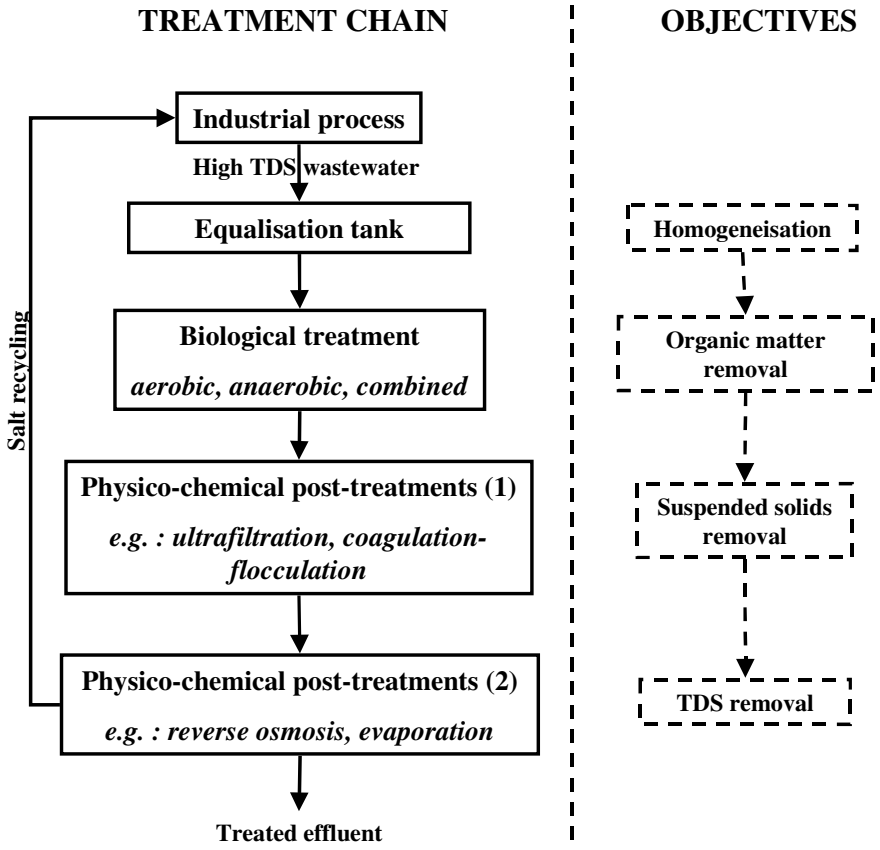

Figure 2 Simplified generic sequence of the global treatment chain of hypersaline wastewater

Firstly, industries generating saline effluents should establish cleaner technology in order to reduce the amount of salt used in the process. The construction of an equalisation tank also appears to be of extreme importance in order to avoid organic and TDS shocks to biological treatment systems. Following pretreatments, biological treatment could achieve proper carbon, nitrogen and phosphorus removal. However, since bad settling and high turbidity are common in saline effluents, this step should be followed by physico-chemical treatment with an aim to eliminate the suspended solids in the effluent. The final post-treatment should consist mainly in the removal of TDS, which can be achieved efficiently by reverse osmosis. Finally, the concentrate residue separated by reverse osmosis should be evaporated and then, if its purity is adequate, recycled at the head of the process, in accordance with cleaner production principles.

Conclusion

In this review it has been shown that the treatment of saline wastewater is feasible, though the obstacles created by high TDS concentrations are considerable. The use of reverse osmosis is particularly efficient in the removal of TDS, yet the high amounts of

suspended solids and organic matter in effluents reduce the life time and the efficiency of the membranes involved. Consequently, the optimal treatment of high TDS wastewater should involve a biological treatment prior to TDS removal. Biological treatment is inhibited by high TDS concentrations. However, it has proved feasible to use halophilic micro-organisms capable of withstanding high salinities and at the same time of degrading the pollutants that are contained in wastewater. The selection of halophilic micro-organisms involves an adaptation of the sludge to high salt concentrations. Furthermore, the effluent organic loading rate and TDS concentration should be equalised as far as possible, as these micro-organisms are sensitive to environmental shocks, especially in anaerobiosis. Nervertheless, after proper adaptation, many halophilic strains have proved capable of removing efficiently the organic matter from saline effluents, including nitrogen and phosphorus. The use of these micro-organisms is therefore recommended in the treatment of saline effluents, prior to suspended solids and TDS removal by physico-chemical treatment.

Annexe II

Critères de dimensionnement d'un procédé biologique aérobie du traitement de l'effluent d'industrie tartrique généré par l'entreprise Faure S.A.

Les critères de dimensionnement utilisés ici sont préconisés dans l'ouvrage « Gestion des problèmes environnementaux dans les industries agro-alimentaires » [151].

Calcul du volume du réacteur

Le calcul du volume du réacteur dépend avant tout de la charge appliquée. Nous avons testé le fonctionnement du réacteur dans cette étude dans les conditions de faible charge : cva = 0,4 kg DCO m^{-3} j^{-1}. Nous recommandons de rester dans ces conditions de fonctionnement qui permettent de produire moins de boues et d'accéder à de meilleurs rendements d'épuration.

Le volume d'un bassin aérobie se calcule à l'aide de la formule suivante :

$V = (Q \cdot l_0) / cva$

avec :

cva = charge volumique appliquée (kg DCO m^{-3} j^{-1})

Q = débit journalier à traiter (m^3 j^{-1})

l_0 = concentration en DCO en entrée (kg DCO m^{-3})

V = volume du réacteur (m^3)

Dans le cas de FAURE S.A., les valeurs à appliquer sont :

cva = 0,4 kg DCO m^{-3} j^{-1}

Q = 18,9 m^3 j^{-1} (prévisions pour 2025)

l_0 = 4 kg DCO m^{-3}

Alors le volume du bassin d'aération sera d'environ 190 m^3.

Calcul des besoins en oxygène

Dans le cadre de notre étude, l'oxygène était fourni en excès, ainsi que nous l'avons déjà montré dans la discussion. Il n'est pas économiquement envisageable d'agir de façon similaire au niveau industriel. La quantité théorique d'oxygène à fournir est estimée à partir de l'équation empirique suivante :

Besoins en O_2 (kg O_2 j^{-1}) = $a' . Q . (l_0 - l_s) + b' . x . V$

avec :

l_s = concentration en DCO en sortie

a' = coefficient de consommation d'O_2 à des fins énergétiques (kg O_2 kg DBO_5^{-1})

b' = coefficient de consommation d'O_2 pour l'auto-oxydation (kg O_2 kg MVS^{-1})

x = concentration en micro-organismes (kg MVS m^{-3})

Dans le cas de FAURE S.A., les valeurs de ces paramètres sont les suivants :

l_s = 0,6 kg DCO m^{-3}

a' = 0,66 (conditions de faible charge appliquée)

b' = 0,07 (conditions de faible charge appliquée)

x = 3,5 kg MVS m^{-3}

Donc, Besoins en O2 ≅ 90 kg O_2 j^{-1}, en boues activées et avec une concentration en sel de 120 g l^{-1}. En considérant la production quotidienne de DCO prévue pour 2025 (≅ 75 kg DBO_5 j^{-1}), les besoins en oxygène avoisineront 1,2 kg O_2 kg DBO_5^{-1}.

Calcul de la production de boues en excès

Dans les conditions de l'étude, on n'a pas eu besoin de purger de boues en excès, parce que la temps de séjour des boues particulièrement long favorisait la réaction d'auto-oxydation par rapport à la production de biomasse et aussi parce que la purge des boues se faisait naturellement par le rejet. En conséquence la concentration en micro-organismes en fin d'expérience avoisinait 3,5 g MVS l^{-1}, ce qui est une valeur

conventionnelle dans le traitement aérobie des eaux. Au stade industriel, il faudra envisager de valoriser les boues en excès.

En première approximation, si les autres paramètres sont optimisés selon les critères décrits ci-dessus, on a coutume de considérer qu'un kg de DCO éliminée par voie biologique aérobie conduit à la formation de 0,5 kg de boues en matières sèches. Par ailleurs on considère que les MES apportées quotidiennement au système sont récupérées sous forme de boues et ne sont pas dégradées, dans le cas d'un effluent ne contenant que peu de MES dans l'entrée. La valeur obtenue est une estimation haute de la quantité de boues sèches produites. Cette production de boues en excès peut alors être mise en équation :

B (en kg de MES j^{-1}) = $0,5 . (l_0 - l_s) . Q + m . Q$

avec : m = concentration en MES en entrée (kg de MES m^{-3})

Dans le cas de FAURE S.A., m = 1,8 kg MES m^{-3}

Dans le cadre de notre étude, la production maximale de boues serait alors de 66 kg MES j^{-1}, soit 1 kg MES kg^{-1} de DCO éliminée. Ces boues en excès gagneront alors à être valorisées par épandage agricole après compostage préalable, si leur composition le permet (ni métaux lourds, ni organismes pathogènes).

APPLICATION DES MICRO-ORGANISMES HALOPHILES AU TRAITEMENT DES EFFLUENTS INDUSTRIELS HYPERSALINS

Résumé. De nombreux secteurs industriels sont susceptibles de générer des effluents hypersalins : on peut notamment citer les industries chimique, pharmaceutique, agro-alimentaire, pétrolière, textile et du cuir. Le rejet de ces effluents sans traitement préalable peut avoir un impact dramatique sur la vie aquatique, la potabilité de l'eau et l'agriculture. Les conséquences sont pires encore dans le cas de régions déjà affectées par les pénuries d'eau, où la compensation de la pollution saline est impossible. Par conséquent, la législation est de plus en plus stricte et le traitement des effluents salins est désormais imposé dans de nombreux pays. Les effluents salins sont généralement traités par voie physico-chimique mais, leur coût étant particulièrement élevé, l'intérêt pour des méthodes de traitement alternatives va en s'accroissant, la plupart d'entre eux comprenant une étape biologique aérobie ou anaérobie. Cependant, les procédés de traitement biologique conventionnels sont fortement inhibés par le sel. Le recours à des micro-organismes tolérant les fortes concentrations en sel (halotolérants), voire les requérant (halophiles) est nécessaire. Ces micro-organismes de l'extrême sont présents dans les océans mais aussi dans des environnements hypersalins, tels que les marais salants ou les lacs alcalins. Leur survie et leur diversité dans les boues d'épuration hypersalines ainsi que leur capacité à dégrader la pollution organique carbonée, azotée et phosphorée des effluents hypersalins (avec l'accent sur les effluents de tannerie) sont l'objet de cette thèse.

APPLICATION OF HALOPHILIC MICRO-ORGANISMS TO THE TREATMENT OF HYPERSALINE INDUSTRIAL WASTEWATER

Abstract. Many industrial sectors are likely to generate highly saline wastewater: these include the chemical, pharmaceutical, agro-food, petroleum, textile and leather industries. The discharge of such wastewater without prior treatment is known to adversely affect the aquatic life, water potability and agriculture. The consequences are even worse in the case of regions already affected by water scarcity, where the compensation of inorganic salt pollutants is impossible. Thus, legislation is becoming more stringent and the treatment of saline wastewater is nowadays compulsory in many countries. Saline effluents are conventionally treated through physico-chemical means, as biological treatment is strongly inhibited by salts (mainly NaCl). However, the cost of physico-chemical treatments being particularly high, alternative treatment systems are nowadays increasingly the focus of research. Most of such systems involve anaerobic or aerobic biological treatment made possible by use of salt-tolerant micro-organisms. These halophilic micro-organisms are retrieved in the oceans but also in other hypersaline environments such as salterns and soda lakes. This thesis studies their survival and diversity in hypersaline sludge, as well as their application to carbonaceous, nitrogenous and phosphorous depollution of hypersaline industrial wastewater (focusing on tannery wastewater).

Mots-clés : effluents hypersalins, traitement biologique, SBR, UASB, lit mobile, industrie du cuir, industrie tartrique, bactéries halophiles et halotolérantes, biodiversité, ARNr 16S, SSCP

Keywords : hypersaline wastewater, biological treatment, SBR, UASB, moving bed, leather industry, tartaric acid industry, halophilic and halotolerant bacteria, biodiversity, 16S rRNA, SSCP

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