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1 Safe recycling of sewage sludge on agricultural land - BIOWASTE

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13 Abstract

14 More than 50,000 wastewater treatment plants are operating in the European Union, producing more than 7.9
15 millions tons of dry solids per year. The amount of sewage sludge will continue to increase as the Urban
16 Wastewater Treatment Directives continues to be implemented in the different member countries. It is now
17 undeniable that various toxic organic compounds, such as surfactants, hydrocarbons and residues derived from
18 plastics are found in sewage sludge. The BIOWASTE project, under the EU 5th framework programme, offers
19 an integrated study of xenobiotics throughout sludge recycling, using a combination of complementary
20 approaches such as biotechnology, eco-toxicology, plant toxicology, analytical chemistry, microbiology,
21 mathematical modelling, life cycle costing and life cycle analysis. This paper presents an overview of the results
22 as well as their implication on the current EU regulatory work in progress concerning sewage sludge application.
23 Particularly, two major findings are here detailed: the isolation, characterization and use of anaerobic xenobiotic-
24 degrading microorganisms, and the modelling of the fate and impact of xenobiotics on anaerobic digestion.

25

26 **Keywords:** agricultural reuse, anaerobic degraders , sludge, xenobiotic, modelling

27 Introduction

28 Disposal and handling of sewage sludge is a growing problem in Europe due to the increasing quantity of sludge
29 produced (8.3 million Tonnes in 2005 (Magoarou, 2000)). Agricultural disposal remains one of the most popular
30 options for disposal in the EU (4.5 million Tonnes in 2005 (Magoarou, 2000)), and has long-term environmental
31 and economic benefits, which make it competitive with other technologies such as incineration, wet oxidation,
32 and landfill. It therefore needs to be validated as a long-term, safe, and environmentally friendly option.
33 Sewage sludge contains small fractions of toxic organic chemicals, which may cause problems if safe use of

34 sewage sludge on agricultural land is to be implemented (Langenkamp and Part, 2001). The European Union has
35 identified a number of compounds in the Legislation addressing water pollution (Directive 2455/2001/EC), listed
36 in Annex X. These include halogenated organic compounds, metals, and a several other compound classes. The
37 use of halogenated organics in Europe is decreasing, and they are readily removed by reductive dechlorination.
38 The other compounds are of more concern, and include phthalic acid esters (PAEs), nonyphenols and
39 nonylphenol ethoxylates (NPs, NPEs), polycyclic aromatic hydrocarbons (PAHs), and linear alkylbenzene
40 sulfonates (LAS). These all readily adsorb onto solids, and are present in activated, and primary sludge (Figure
41 1). Most of these compounds are degraded under aerobic conditions (Spormann and Widdel, 2000), but as most
42 sludge treatment is anaerobic, they may persist through anaerobic sludge treatment. All these compounds have
43 environmental impacts, and are relatively surface active, which means they will follow the general path as shown
44 in Figure 1, and are therefore a concern in the sludge. Anaerobic degradability is limited for all compounds, and
45 assessing the impact of their presence on the process itself is also a concern.

46

47 The BIOWASTE project takes an integrated approach for xenobiotic management. This means complete cycle
48 management from source (in this case, the treatment plant), to sink (the farm, and its impact on plant life).

49 First of all, analytical techniques were developed, validated and used to determine the environmental burden of
50 the target contaminants in European sewage sludge, soil and sewage sludge amended soils. A part of the analysis
51 involves a broad survey of treatment systems in Europe for the presence of the model compounds, as well as
52 variation between different laboratories. Results so far have indicated a broad variation of the different
53 compounds between countries. However, it seems that some compounds have very high level (LAS, NPE and
54 PAE) with medium toxicity and persistence and some other low level (PAH) but high toxicity and persistence. In
55 some cases, mild or no treatment is required to reduce below legislated levels (as assessed in countries that have
56 legislation). In other countries where no legislated levels exist, concentrations of xenobiotics in sludge are high.
57 Legislation at a national level would be needed to comply with new EU directives.

58 Bioassays for evaluating the environmental safety of using bioprocessed and non-bioprocessed sewage sludge as
59 organic fertilisers were developed. To evaluate the impact of the xenobiotic compounds in soil, the environment
60 and on product crops, a number of new toxicity tests have been established. One novel test assesses the end-
61 phytotoxicity by evaluating impact on rhizospheric activity in the interface between soil and root. It was found
62 that an impact could only be found in soil adhering strongly to the roots, and that this should be used as a
63 standard. Alternative tests are being used to assess mutagenic tests, seed viability, and accumulation of

64 xenobiotics in food plants. These tests are being used to assess impacts in a large scale factorial test using
65 lysimeters. Novel testing methods have been submitted to standards authorities for approval as standardised
66 tests.

67 Life cycle assessment work seeks to determine the global and local environmental impacts of different treatment
68 alternatives. In addition, economic impacts have been examined using life cycle costing methodology. An
69 environmental risk assessment of untreated sewage sludge on a local scenario is also being conducted. The other
70 package deals with micromanagement within the treatment process, and may be used to evaluate individual
71 technologies.

72 Also, different packages within the project address degradation in both aerobic and anaerobic treatment, isolation
73 and enhancement of microbes degrading the model compounds under aerobic and anaerobic conditions and
74 modelling of the compounds through the treatment plant. Anaerobic digesters and compost reactors were
75 developed to reflect the existing systems in use. Different strategies were investigated to find parameters of
76 significance for detoxification of sewage sludge. These studies included the effect of hydraulic retention time,
77 temperature, and reactor set-up on degradation of the target compound. The aerobic processes were particularly
78 efficient. Nevertheless, the anaerobic processes showed a significant potential of biodegradation. As anaerobic
79 digestion is worldwide used, we mainly focussed on anaerobic degraders and on in situ enhancement of the
80 anaerobic degrading ability.

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83 **Specific Project Components**

84

85 *Microbial Isolation, Characterisation, and Bio-augmentation.* The microbiology of aerobic and anaerobic
86 conversion of these compounds is interesting from a fundamental, as well as practical point of view. Facultative
87 aerobic isolates have been previously achieved on all of the model compounds, but anaerobic isolates degrading
88 the model compounds have not been found.

89 Because of the difficulty involved in achieving isolates, we took a two-prong parallel approach in microbial
90 characterisation. Enrichment procedures were tested and compared according to their potential for enhancing
91 xenobiotic removal efficiency: one series of successive Fed-batch reactors, and one continuous CSTR reactor.
92 For each, one abiotic control - chemically sterilized – representing the abiotic losses, and one population control
93 - a blank without xenobiotic addition – were performed. In order to enrich the microbial ecosystems in

94 xenobiotic-degrading microorganisms, the reactors were continuously or sequentially fed with a specific
95 anaerobic synthetic medium. During enrichment, isolate candidates were taken from the enrichments. During
96 enrichment, with verified removal (99%) of target compound compared to the abiotic control (31%), the
97 microbial community through the whole experiment was examined using SSCP (single strand conformation
98 polymorphism; Delbes et al. 2001). A strong correlation was observed between the PAE degradation kinetics and
99 the growth of new dominant species within the enriched ecosystems : three bacteria (*bact1*, *bact2* and *bact3*)
100 were identified as being potentially selected by the PAE enrichment procedure, with *bact1* as the main dominant
101 species (Figure 2). A limited clone library was then assembled from the final enrichment, and the peaks
102 identified phylogenetically. The dominant Eubacteria species (*bact1*) was clearly identified as being very close
103 to *Soehngenia saccharolytica*, already described as a benzaldehyde-converting bacterium. As well, the dominant
104 Archaeobacteria species was identified as *Methanosaeta concilii*, known for its hydrophobic properties. These
105 results suggest the existence of specific relationships between these microorganisms for PAE degradation. This
106 approach has also been extended for the characterization of microbial communities and/or the identification of
107 bacteria involved in the biodegradation of PAH and NPE in complex environmental samples. The sequence data
108 could also be used to design molecular probes for further *in-situ* monitoring of the abundance of the degrading
109 consortium and describing their spatial configuration. It appears that the dominant specie (*bact1*) was mainly
110 found in flocs in the enriched cultures (Figure 3). This may suggest that the dominant species of the enriched
111 culture grows essentially where hydrophobic compounds such as PAEs are trapped, i.e. into the biofilm matrix.
112 Moreover, this kind of spatial arrangement favoured symbiotic relationships with methanogens.

113 Similar work has been done with the isolates. We have had very encouraging anaerobic isolate results, achieving
114 isolates on all target compounds except LAS. Figure 4 summarizes the ability of PAE isolates to degrade the
115 target compound. As shown in control reactors, the abiotic losses were considered as non significant during the
116 experiment (< 5%). Each isolate exhibited different removal performances from around 50% for isolate A for
117 more than 90% for isolate C.

118

119 Bio-augmentation work has indicated mixed results. Very promising results has been obtained with batch
120 augmentations, with high proportions of target microbe in the augmentation mix and with very efficient results
121 in biodegradation as shown on Figure 5. Therefore, a continuous reactor could not be augmented for PAE
122 degradation, due to sharp decrease of the target population indicating survival and maintenance problems
123 (protozoa grazing for example).

124

125 *Modelling of movement through the treatment plant.* Behaviour of these compounds in the treatment plant is
126 critical to the project, and modelling is an integral part of the project. The target compounds are surface active,
127 with high K_{OW} and K_{OC} coefficients. We have created models to both assess degradation of the compounds in
128 the treatment, as well as evaluate the impact of the compounds on the processes. The base models are the
129 Activated Sludge Model no.1 (Henze et al. 1987), and the Anaerobic Digestion Model No. 1 (Batstone et al.
130 2002), together with specific extensions for adsorption, stripping, and biological conversion of the xenobiotic
131 compounds. Parameters from these models are sourced from experiments, theoretical data (for example
132 adsorption, and stripping), and full-scale data. So far, ADM1 model was successfully fitted to the experimental
133 data and the parameters estimated were lower than the suggested values. Statistical analysis performed in the
134 parameters estimated showed that xenobiotics had no apparent inhibitory effect on the anaerobic digestion
135 process at typical concentrations (Figure 6) but that also, the xenobiotics are not readily degraded in typical non
136 adapted anaerobic systems.

137 The anaerobic degradation of hydrophobic compounds in the sludge was modelled in the case of PAE based on
138 the PAE concentration on the bioavailable sites of the sludge (Figure 7). The sites considered bioavailable are
139 those accessible by the microorganisms. These sites are located on the surface of the biosolids and in the aqueous
140 phase. Since PAE is considered insoluble in water, it was assumed to partition between the surface and the inner
141 part of the biosolids through first order kinetics. Biodegradation of PAE was considered to be a co-metabolism
142 result of the anaerobic consortium degrading sludge and took place on the surface of the biosolids through
143 simplified Monod kinetics. The overall PAE decrease rate was limited by the desorption of the compound from
144 the inner part to the surface of the biosolids.

145

146 We are also using a new thermodynamic modelling approach to assess favourability of potential pathways. This
147 can also be used to assess degradability of a proposed compound. If free energy change of reaction is positive or
148 zero in a range of reactor operating conditions, bioconversion is fundamentally not possible. All compounds
149 examined so far have been degradable, with the exception of some high MW PAHs.

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151

152 **Conclusions**

153 The study has shown so far that xenobiotics in sludge can be an issue at a national level. However, action is also
154 relatively straightforward. Current aerobic composting or aeration as a final conditioning can remove the
155 compounds, and we have shown in the laboratory that anaerobic digestion also has the potential to remove a
156 significant proportion. Introduction of *de-novo* ability into existing reactors is a more difficult issue, as bio-
157 augmentation has not worked, especially in continuous systems. However, the results are supported by new
158 microbial isolates and enrichments, together with molecular tools that assess establishment of the target culture.
159 The project has also resulted in a number of new tools, including easier analytical testing of the compounds,
160 improved mutagenic and toxicity testing, mathematical models, and application of existing tools such as life-
161 cycle assessment to this new application.

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174

175 **Acknowledgements**

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177 CT-2002-01138.

178 Figure Captions:

179 Figure 1: Route of xenobiotic organic compounds from raw wastewater to the farm.

180 Figure 2: Effect of different enrichment procedures on specific PAE removal rate and Bacterial SSCP profile
181 evolution during the CSTR enrichment .

182 Figure 3 : Picture of DBP (PAE) enriched floc showing (left) all bacteria using EUB-338 probe, and (right)
183 organism responding to DBP-EN1 probe developed from SSCP results.

184 Figure 4: Phthalic Acid Ester (PAE) removal measured after 2 days of batch cultivation under anaerobic
185 conditions. The culture medium correspond to a mixture of synthetic medium, 2g/L yeast extract and 30 mg/l
186 DBP. CTA and CTB correspond to control reactors (non inoculated). Isolate X corresponds to PAE-non
187 degrading bacteria growing on Yeast Extract. Isolates A, B, and C corresponds to pure isolate bacteria degrading
188 PAEs.

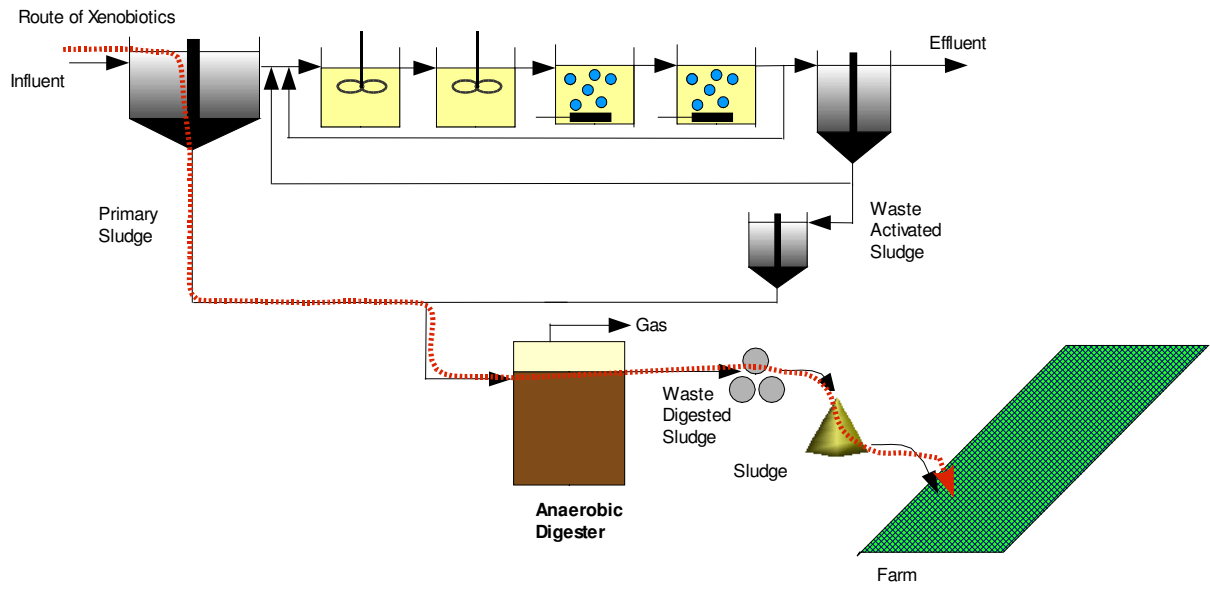
189 Figure 5: PAE removal in anaerobic batch tests for sludge amended or not with PAE-degrading isolates in pure
190 culture or in mixture (5 % v:v added).

191 Figure 6 : Km and Ks values (symbols) for acetate and propionate degradation and 95% confidence intervals
192 (uncorrelated estimates of the error) as estimated from dynamical experiments conducted in three anaerobic
193 bioreactors (two of them were fed with secondary sludge contaminated with xenobiotics at typical
194 concentrations, while the third one was fed with non contaminated sludge; control). The 95% joint confidence
195 areas (lines) of the parameters overlap, meaning that there is no statistically significant difference caused by the
196 presence of xenobiotics in the sludge.

197 Figure 7 : PAE (particularly DEHP) concentration in the anaerobic CSTR fed with secondary sludge
198 contaminated with DEHP and model prediction. The variation of DEHP in the feed is also shown.

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202 Figure 1: Route of xenobiotic organic compounds from raw wastewater to the farm.

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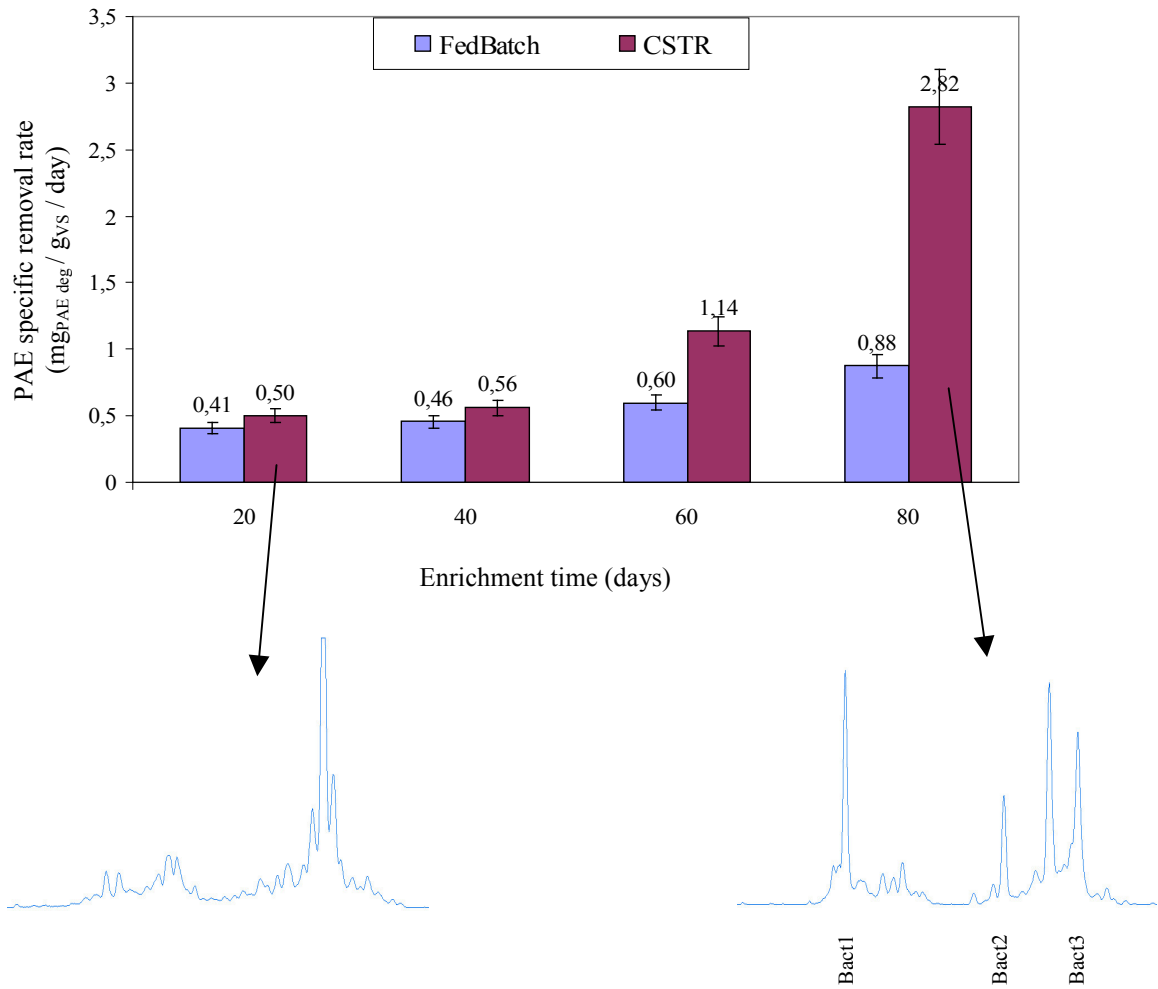
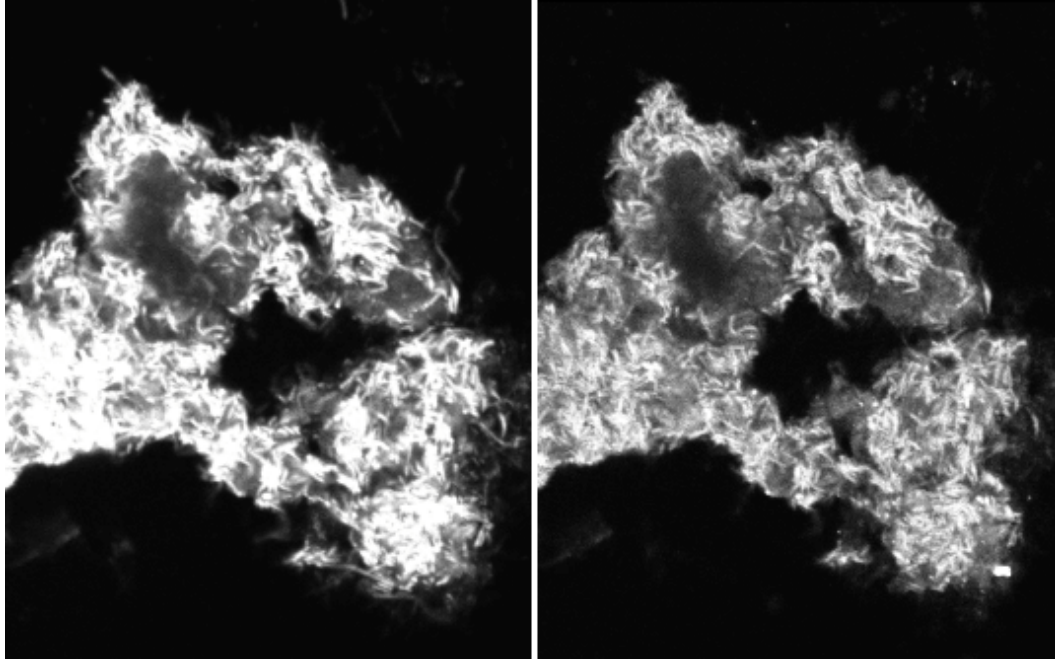


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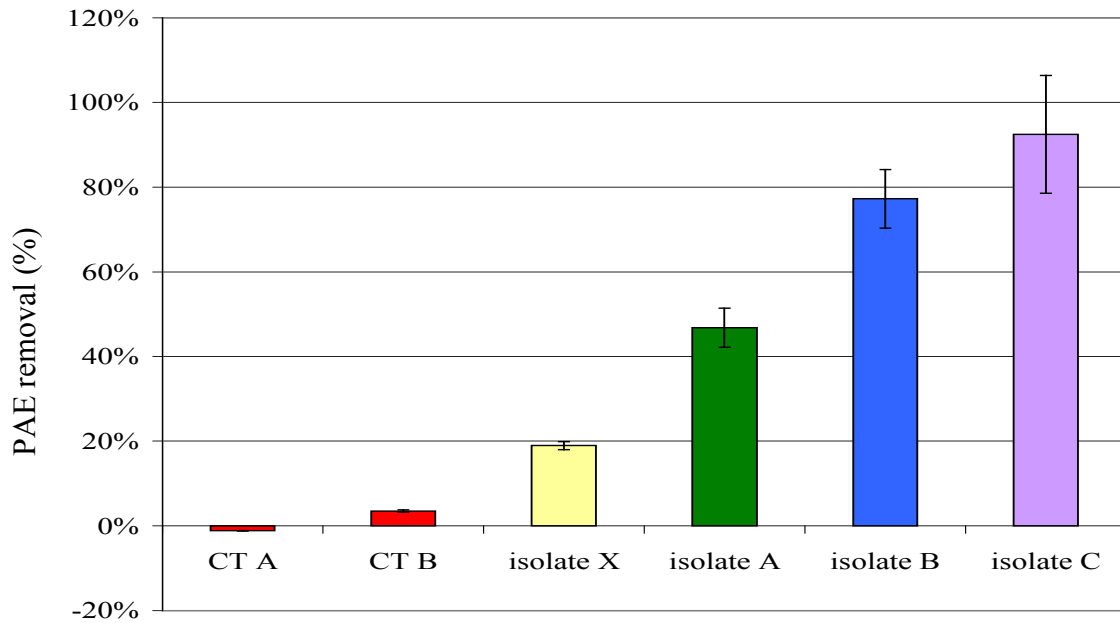
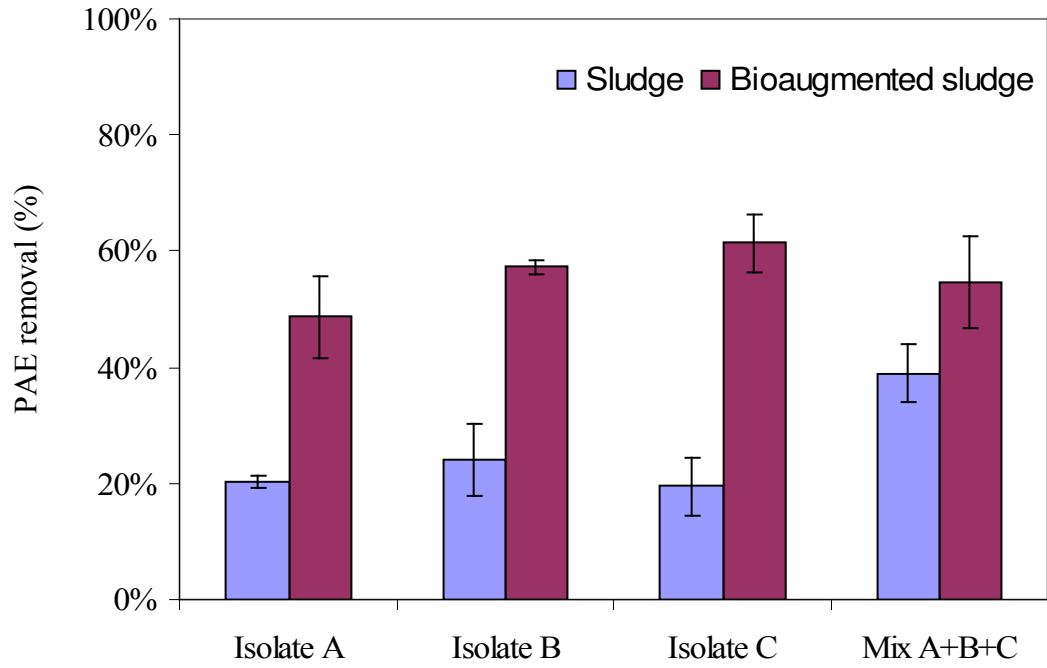


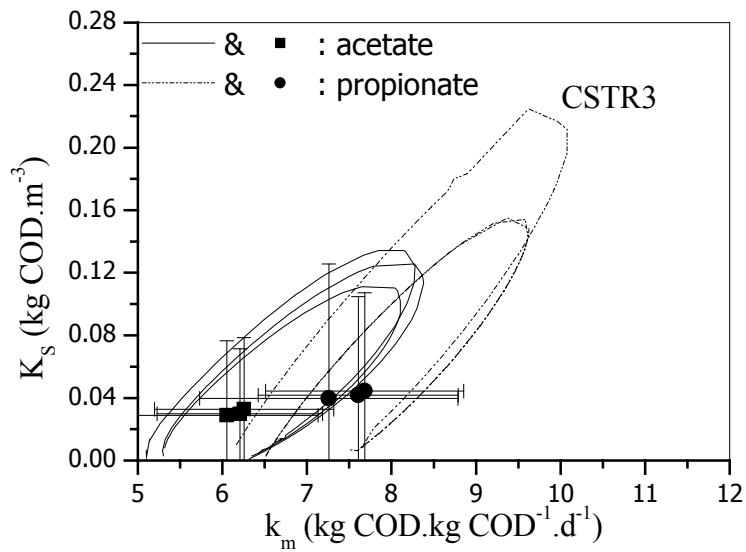
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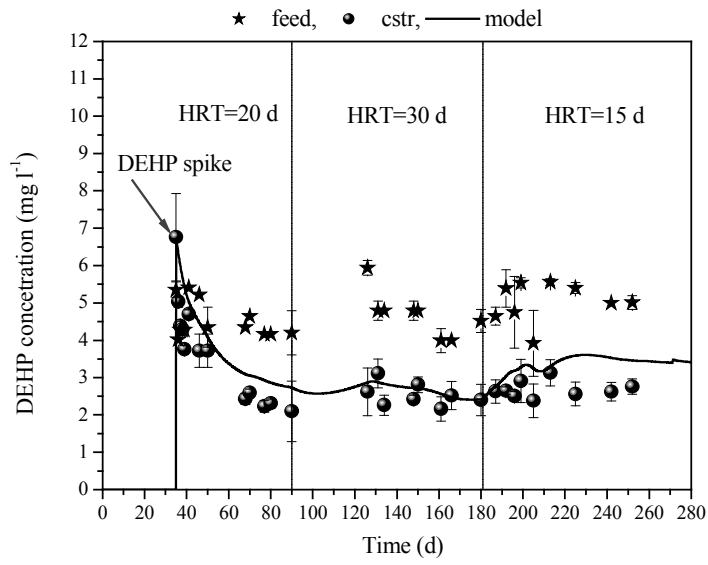
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