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The environmental biorefinery: state of the art on the production of hydrogen and value-added biomolecules in mixed-culture fermentation

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Abstract:

The environmental biorefinery consists in recovering and adding value to waste, possibly through a multi-product approach. A first implementation of such concept is the production of methane and nutrient-rich digestate by anaerobic digestion in biogas plants. However, methane and digestate have only a low added-value and biogas plants still require feed-in tariff policies to be economically viable. The aim of this article is to provide a meta-analysis of current biomass recovery technologies compatible with environmental applications (*i.e.* non-sterile conditions and carried out by microbial mixed culture). A particular focus on those able to produce high value-added fermentation metabolites was made. To achieve this objective, both qualitative (*e.g.* substrates, pretreatments) and quantitative data (*e.g.* yields, productivities, process parameters) were retrieved from 624 manually-checked research articles, excluding review papers, and 134 patents published after 1997. In addition, a straightforward market study was carried out for nine promising biomolecules: H_2, ethanol, acetate, propionate, lactate, 1,3-propanediol, butyrate, caproate and polyhydroxyalkanoates (PHAs). Finally, the feasibility of producing each biomolecules in the context of an environmental biorefinery is discussed in the light of the current process performances and their related bottlenecks.

Keywords: Biohydrogen; Bioeconomy; Biomolecules; Dark fermentation; Microbial electrosynthesis; Microbial consortia; Photofermentation; Pretreatment

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1. Introduction

Anaerobic digestion (AD) is currently going through a strong industrial development in the renewable energy sector all around the world[1,2]. It is a mature biological process involving a complex association of microbial communities (*i.e.* mixed microbial cultures) able to convert into methane a variety of organic substrates including industrial and municipal wastewater, sewage sludge, municipal solid waste and residues and effluents from agricultural activities[3]. To date, the main product considered in AD processes is biogas, consisting of a mixture of methane and carbon dioxide. However, hydrogen gas (H_2) which is an intermediate product of this process (Figure 1) has a higher added value than biogas and is predicted to become a wide energy carrier for transportation and energy storage in a near future[4]. Several process control strategies and microbial selection procedures have been explored over the past 15 years to promote H_2 production during the acidogenic phase of AD in a process called dark fermentation (DF)[5]. During DF, H_2 production is concomitant with the accumulation of soluble metabolites (carboxylic acids and alcohols, Figure 1), which have a higher added-value than biogas and could potentially be extracted prior to their conversion into methane[6]. Despite the numerous scientific studies published on mixed-culture DF and the few dozens on pilot-scale reactors, all showing great perspectives for this technology, there is still no industrial-scale implementation of such environmental biorefinery.

< Figure 1 >

The aim of this article is to provide a meta-analysis of the biomass recovery technologies operated under non-sterile conditions. Mixed-culture DF processes and, more broadly, mixed-culture bioprocesses used for the production of H_2 and value-added fermentation metabolites were considered. To achieve this objective, both qualitative (*e.g.* substrates, pretreatments) and quantitative data (*e.g.* yields, productivities, process parameters) were retrieved from 624 manually-checked research articles, except review articles, and 134 patents published after 1997 (Figure 2). In addition, a straightforward market study was carried out for nine promising biomolecules (including H_2). Finally, the feasibility of producing each biomolecule in the context of environmental biorefinery is discussed in the light of the current process performances and their main bottlenecks.

< Figure 2 >

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2. Methodology

2.1. Database building

The main database used in this study was established from the Scopus (title, abstract and keywords of research articles) and Orbit (full-text patents) databases and includes documents published from 1997 to January 2017. Documents were first selected by automatic queries based on a keyword list (Table 1) according to the following approach:

- Patents containing keywords from the "Hydrogen" AND "Process" lists
- Patents and research articles containing keywords from ("Hydrogen" OR "Biomolecules") AND "Process" AND "Mixed culture" lists
- Patents and research articles containing keywords from "Biomolecules" AND "Combined processes") AND "Mixed culture" lists

< Table 1 >

This strategy led to the identification of 8853 research articles and 1654 patents (not considering non-extended Chinese patents). In a second step, the database was adjusted through the use of a keyword-based and automatic procedure and further manual evaluation to remove documents dealing with:

- methane production only
- ethanol production by yeasts
- microbial fuel cells
- pure cultures of either wild type or genetically modified organisms
- published results in non-peer-reviewed scientific journal
- state-of-the-art reviews or opinion articles

The final database includes 624 research articles and 134 patents (see Supplementary information). This database was then checked for the presence of a pre-established list of 25 representative articles to ensure that no key publication was missing. The database was further manually enlarged with most recent articles (2017) to consider the most up-to-date observations about biomolecule production by mixed-culture fermentation.

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2.2. Calculations

2.2.1. COD mass balance

In some research articles, the Chemical Oxygen Demand (COD) was experimentally measured and was directly retrieved. When this information was not available, the COD equivalents of the substrate (and the products) were assessed from the elemental composition of each compound. More precisely, for a compound $C_wH_xO_yN_z^{n-}$, the COD equivalent corresponds to:

$$COD_{molecule}\ (g_{COD}/g_{molecule}) = 8.\frac{4.w+x-2.y-3z+n}{12.w+x+16.y+14.z} \quad (1)$$

The COD equivalents of the most often encountered molecules are provided in Table 2. In addition, when the macromolecular characterization was provided (i.e. lipid, protein, carbohydrate and moisture content), the following molecular formulas were used: $C_{57}H_{104}O_6$ for lipids, $C_5H_7O_2N$ for proteins and $C_6H_{10}O_5$ for carbohydrates (corresponding to triolein, *Escherichia coli* and cellulose respectively). Additives used in fermentation media such as yeast or beef extract were also considered as pure proteins. According to these hypotheses, the COD equivalent calculation when macromolecular characterization was provided is:

$$COD_{substrate}\ (g_{COD}/g_{substrate}) = 1.19x(g_{carbohydrate}/g_{substrate}) + 1.42x(g_{protein}/g_{substrate}) + 2.90x(g_{lipid}/g_{substrate}) \quad (2)$$

< Table 2 >

2.2.2. Productivities

All productivities reported in this article correspond to average productivities. Depending on the process configuration (*i.e.* continuous or discontinuous), the productivities of the different biomolecules were calculated as follows:

- For batch and fed-batch processes: average productivities were calculated by dividing final concentrations (or total gas production) by the total duration of the fermentation.
- For continuous and semi-continuous processes: in the case average productivities in the stationary phase were not provided, they were assessed by dividing concentrations of the biomolecules during the stationary phase by the hydraulic retention time.

2.2.3. Hierarchical clustering and PLS-DA

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To obtain clusters based on metabolic profiles, all studies were first thoroughly examined to keep the results where more than 60% of initial COD was recovered as products at the end of the process or in the stationary phase, in discontinuous and continuous processes, respectively. The metabolic profiles were then considered as "successful" fermentation where results can be rigorously compared. COD profiles were then analysed by hierarchical clustering using the "pvclust" function of the R package pvclust[7], using the "average" method and the Euclidean distance. The clusters retained were those gathering more than five fermentation profiles and for which the existence was statistically significant (p-values < 0.05). Significance was assessed by bootstrap procedures (10,000 bootstraps). The five significant clusters were then graphically represented using a Partial Least Square Discriminant Analysis (PLS-DA) based on COD profiles. The PLS-DA was carried out using the "plsda" function of the R package mixOmics[8].

3. Bioprocesses for hydrogen production by mixed microbial consortia

To strengthen the results and conclusions of this section, only the research articles for which it was possible to calculate COD mass balances and showing an H_2 yield higher than 0.01 $g_{COD}.g_{COD}^{-1}$ (based on total initial COD) were considered (400 articles). Patents considered in this section were those explicitly claiming H_2 production (100 patents).

3.1. The H_2 market

Dihydrogen (H_2) is a molecule used in industry as chemical reagent, especially for hydrogenation reactions as widely used in petrochemistry or for ammonia and methanol production. Currently, the global hydrogen consumption is about 60,000 kt/yr (~ 700.10^9 Nm^3) and is predicted to reach a near-exponential growth in the coming years. Indeed, the use of H_2 as decarbonated energy carrier, both for energy storage or in the transportation sector, could represent up to 30% of H_2 world's consumption in 2030 and even reach more than 60% of the world consumption in 2050 (estimated to be ~ 480,000 kt/yr, source: Mcphy-energy).

Currently, bio-based dihydrogen is not present on the market. About 96% of the hydrogen currently present on the market is derived from fossil fuels such as natural gas trough steam reforming, an environmental-impacting process, emitting more than 10 kg of fossil CO_2 per kilogram of H_2 produced. Current H_2 production costs by natural gas reforming

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is between 1.0 and 2.0 €/kg but strongly depends on the hydrocarbons market price, and could also be negatively impacted by potential future regulations on CO_2 emissions.

The remaining 4% of worldwide hydrogen is produced by water electrolysis, an electrochemical process in which electric current is used to split water into dioxygen and dihydrogen. The cost of electrochemically-produced H_2 is estimated between 3.5 and 5.0 €/kg. Environmental impacts are directly related to the source of the electricity used. Thus, a reasonable target for the overall production costs of a future biobased H_2 production process could be assessed between 1.5 and 3.5 €/kg_{H2} to be economically competitive with the existing market. It is likely that the environmental impact of biohydrogen production would be favourable when compared to the existing processes[9,10], but it should be determined on a case-by-case basis through Life Cycle Assessments (LCAs).

3.2. Current biological technologies for bioH_2 production

Three technologies have been developed for producing H_2 with microbial consortia: DF, photofermentation and microbial electrolysis (Figure 3). DF corresponds to the anaerobic conversion of organic substrates through fermentation in the absence of light. DF leads to the production of hydrogen and soluble molecules such as short-chain carboxylic acids (acetic, propionic, butyric acids, Figure 1)[5]. DF is by far the most studied technology (75.75% of the scientific articles) but also the most patented (40% of the patents). The main advantage of this technology relies on the possibility to use complex low-cost substrates such as industrial or agricultural effluents or residues (Section 3.3). However, only a maximum of 33% of the substrate COD content can be converted into biohydrogen by DF[5]. A way to recover the energy remaining in the liquid phase is to inject the DF effluents into a digester to produce methane. Following such coupling, almost all of the COD of the feedstock can theoretically be converted into biohydrogen and methane, either separately or as a mixture that can be sold as hythane[11]. The studies reporting a coupling between DF and AD represent 8.75% of the scientific publications of the field, and 34% of the patents (Figure 3). This high patent proportion probably reflects a relative easiness of implementing DF upstream from a pre-existing AD plant, also known as two-step AD.

< Figure 3 >

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Photofermentation is a technology involving photosynthetic organisms that can produce H_2 from organic substrates in presence of light[12]. This additional energy input, which can be artificial or natural (sun), makes thermodynamically favorable H_2-producing reactions that are not possible in DF, thus allowing a more complete conversion of organic substrates into biohydrogen. However, this technology is less flexible than DF regarding the types of substrate. It is usually necessary to convert complex substrates into a mixture of carboxylic acids and alcohols prior to H_2 production by photofermentation. This feature makes possible the coupling between DF and photofermentation, in which DF effluents, rich in carboxylic acids, can be converted into biohydrogen. Overall, research on photofermentation and its coupling with DF accounts for 9.25% of scientific articles but only 5% of patents (Figure 3).

Finally, microbial electrolysis is the latest technology that has been investigated for the production of biohydrogen by mixed cultures. This technology requires specifically designed bioreactors compatible with the presence of electrodes (see Krieg et al. (2018) for more details)[13]. External supply of electrical energy can here be used to make thermodynamically favorable chemical reactions[14]. When used for hydrogen production, microbial electrolysis cells use the presence of electro-active microorganisms on the anodic surface to convert organic substrates into electric current, protons and CO_2. This electric current can then be used on the cathode surface to abiotically convert protons into biohydrogen. When the anodic and cathodic compartments are separated by a membrane, the hydrogen produced at the cathode is nearly pure. Similarly to photofermentation, microbial electrolysis allows a more complete conversion of organic substrates into biohydrogen when compared to DF. Here, mixtures of carboxylic acids, as found in DF effluents, can be used as substrate at the anode. While microbial electrolysis accounts for only 6.25% of the published articles in the field, 20% of the patents are dealing with this technology, suggesting a high industrial interest.

3.3. Substrates and pretreatments

3.3.1. Diversity of the substrates used for H_2 production

One of the main benefits of using mixed-culture fermentation is its flexibility on converting a wide range of substrates[15]. Within the scientific articles, synthetic fermentation

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media are employed in 53% of the studies with the use of simple substrates such as glucose, sucrose, cellulose, mixtures of volatile fatty acids (*e.g.* acetate, propionate, butyrate) or purified glycerol (Figure 4A). The COD concentration of these simple substrates is usually low, with an average value of 12.1 and 3.4 $g_{COD}.L^{-1}$ when sugars and volatile fatty acids are provided as substrates, respectively (Figure 4B). Usually, studies using synthetic fermentation media aim to elucidate the fundamentals of bioH_2 production (*e.g.* effect of pH, effect of microbial population selection procedures) rather than demonstrating an actual feasibility of, for instance, a sugar-based bioH_2 production process. Indeed, using such purified substrates represents a significant cost in biohydrogen production processes, particularly in comparison with food waste or co-products from the industrial sector. In addition, the use of readily edible sugars (mainly beet, sugar cane and maize crops) for commodity chemicals production competes with food production and raises societal debates[16,17].

BioH_2 production from organic residues or agricultural/industrial process co-products concerns 47% of the scientific articles in the field (Figure 4A). Most of these substrates are issued from agriculture and green waste (20.3% of the scientific studies, 43.2% of the complex substrates). They include rice and wheat straws, corn stalks, sugar molasses or fruit production residues. Energy crops (*e.g.* sorghum, sugarcane, cassava) are only employed in 22.9% of the studies using biomass from agriculture and green waste (*i.e.* 10.8% of the studies using complex substrates). On average, biomass from agriculture and green waste are used in H_2 production process with a COD concentration of 19.4 $g_{COD}.L^{-1}$ (Figure 4B). The second most important category of complex substrates gathers industrial effluents which are used in 12% of the studies reporting biohydrogen production (25.5% with complex substrates). These substrates are of various kinds and include, among others, residues from the paper, dairy and oilseed industries, as well as crude glycerol generated from the biodiesel industry. An average COD concentration of 29.7 $g_{COD}.L^{-1}$ is reported in all the identified studies. For the rest of the complex substrates, four categories are distinguished: food waste (8% of the studies, average organic matter concentration of 55.9 $g_{COD}.L^{-1}$), municipal waste such as sewage sludge or the organic fraction of municipal solid waste –OFMSW (3.2 % of the studies, average organic matter concentration of 44.2 $g_{COD}.L^{-1}$), macro/ microalgae (2% of the studies) and dark or ethanol fermentation effluents (1.5% of the studies).

< Figure 4 >

3.3.2. Substrate pretreatments

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Due to their complex structure, some organic compounds cannot be directly and easily converted in biological processes. For instance, lignocellulosic materials, agriculture residues or urban green waste are composed of cellulose and hemicelluloses, but also of lignin, a polymer giving stiffness to plants and protecting them from microbial attack[18–21]. Municipal waste may also contain recalcitrant biomass such as cardboard[22] or bacterial cell walls in sewage sludge[23]. To exploit these biomasses in fermentation processes, pretreatments are applied to make their sugars more soluble and biologically more accessible. These pretreatments are mainly classified into three categories: mechanical (grinding, sonication), physico-chemical (acid/alkaline hydrolysis, heat treatment, steam explosion) or enzymatic (by microorganisms or enzyme cocktails) methods.

About 45% of the studies employing complex substrates reported the use of one or more biomass pretreatment methods. The most common methods are thermal pretreatments (19%), acid or alkaline hydrolysis (18%) and enzymatic pretreatments (8%), whether applied alone or in combination with other pretreatments. Biomasses from agriculture & green waste and municipal waste are the most preferred substrates to be pretreated, representing 67.9% and 61.5% of the studies employing at least one pretreatment method, respectively (Figure 5). It is important to note that these pretreatments may represent significant costs prior to fermentation. As an illustration, the authors of a techno-economical study using corn stalks as substrate recently estimated that the price of raw substrate treatment (90 €/$t_{cornstarch}$) was at least doubled to ~ 180 €/$t_{cornstarch}$ (equivalent to 330 €/$t_{solubleCOD}$) when the pretreatment cost was taken into account[24]. Most industrial effluents and food waste do not require any pretreatment and are directly used as fermentation substrate (Figure 5). For more detailed information about substrate pretreatments, readers may refer to Carrere *et al.* (2016)[23].

< Figure 5 >

3.3.3. Inoculum pretreatments

In biological ecosystems, hydrogen is an energy vector favouring electron transfer between microorganisms. To optimize its production, it is not only recommended to favour microbial species that release their excess of electrons as H_2, but also to prevent the growth of hydrogen-consuming microorganisms such as methanogenic archaea or acetogenic bacteria. The choice and adaptation of an inoculum is one of the most crucial elements when designing a biohydrogen production process. To obtain a suitable inoculum, the most common method (56.1% of the studies, Figure 6A) is to pretreat inocula originating from the environment or

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from parent reactors to remove undesirable microorganisms[21,25]. The most widely used technique is thermal pretreatment (70.4% of the pretreatments, Figure 6B) which consists in applying a thermal shock to the inoculum. As a result, the microorganisms capable of surviving by forming spores are specifically selected, such as *Bacillus* and *Clostridium* species. These genera contain many efficient hydrogen-producing microorganisms[21], as well as acetogenic species. However, thermal pretreatment is efficient to prevent methanogenic archaea growth and is usually sufficient for the start-up of hydrogen-producing processes. Following the same principle, other pretreatment methods aims to the elimination or inhibition of hydrogen-consuming bacteria and the indirect selection of hydrogen-producing bacteria, including acid or alkali treatments (11.7% of the pretreatments), addition of methanogenesis inhibitors such as chloroform and 2-bromoethanesulphonate (6.1%), or aeration (3.6 %) methods.

Studies that do not use pretreatment techniques (43.9% of the studies) employ other population selection pressure through process operating parameters. For example, it is possible to maintain acidic conditions in the fermentation medium to inhibit the methanogenic archaea activity[26]. In continuous processes, it is also possible to wash-out archaea, which have a lower growth rate than hydrogen-producing bacteria, by applying a short hydraulic retention time[5]. Operating parameters have also been widely optimized throughout the studies using pretreatments to prevent the re-emergence of hydrogen-consuming microorganisms during the process. For more detailed information about inoculum pretreatments, readers may refer to Rafieenia *et al.* (2017)[21].

< Figure 6 >

3.4. Production performances

As indicated in Sections 3.2 and 3.3, various technologies and a broad range of substrates can be used for biohydrogen production, thus leading to highly variable performances. Hydrogen yields as a function of the technologies and the initial COD are shown in Figure 7. Considering all the technologies, the total substrate COD concentration was lower than 22.2 $g_{COD}.L^{-1}$ for 75% of the studies. Regarding hydrogen yields, values below 0.16 $g_{COD\_H2}.g_{COD}^{-1}$ were observed in more than 75% of the studies. Yields higher than this value were only reached when substrate with lower COD content were employed (8.4 $g_{COD}.L^{-1}$ on average) and mostly by photofermentation, microbial electrolysis technologies or by coupling them with DF. When initial substrate concentrations were higher than 22.2 $g_{COD}.L^{-1}$,

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the maximum average hydrogen yield was only 0.07 $g_{COD\_H2}.g_{COD}^{-1}$. These observations emphasize that, under the current state of the art, there is a compromise to be found between reaching high hydrogen yields and valorizing substrates at high COD content. In the following sections, the biohydrogen production performances obtained for each technology will be detailed and put into perspective with regard to this specific issue.

< Figure 7 >

3.4.1. Dark fermentation performances

One of the main advantages of DF processes is its flexibility regarding a wide range of substrates. However, hydrogen production performances can greatly vary depending on the nature and complexity of the substrates (Figure 8A). The average yield observed in DF was about 0.108 $g_{COD\_H2}.g_{COD}^{-1}$. This value represents ~ 33% of the maximum theoretical yield, *i.e.* 0.33 $g_{COD\_H2}.g_{COD}^{-1}$, in DF[5]. Best performances were observed when synthetic fermentation media were employed rather than complex substrates (p-value < 0.0001). The average yield reached then 0.124 and 0.089 $g_{COD\_H2}.g_{COD}^{-1}$ with purified sugars and complex substrates, respectively. Regarding complex substrates, the highest average yields were achieved with industrial effluents and biomass from agriculture and green waste (mostly after pretreatment) with average yields of 0.096 and 0.094 $g_{COD\_H2}.g_{COD}^{-1}$, respectively. Interestingly, food and municipal waste are the substrates for which the lowest hydrogen yields were obtained, with average yields of only 0.064 and 0.056 $g_{COD\_H2}.g_{COD}^{-1}$, respectively. Surprisingly, when considering all the data retrieved from the studies dealing with DF for H_2 production from organic biomass, only the intrinsic composition and the structural features of the organic substrates seem to have an influence on the H_2 yields. Although each microbial community had its own optimal parameters, the observed hydrogen yields were not statistically different in all studies, whatever the process parameters (Figure 8B) such as working volume (ranging from 0.01 to 3,300 L) and temperature (15 to 80°C), or the mode of operation of the bioreactor (batch, semi-continuous or continuous).

< Figure 8 >

Beyond the hydrogen yield, the choice of process parameters can strongly influence the composition of the microbial community (Section 3.3) and thus the hydrogen production kinetics or the stability of the process (Figure 9). In particular, the choice of operation mode (batch VS continuous) plays an important role, especially regarding the easiness of process

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implementation and the related performances. The batch reactor is the simplest configuration. In this mode, all the substrate is added at start of reactor operation and no withdrawal of the medium is carried out before the end of fermentation. Because of its simplicity, most of the reactors have been carried out in batch mode (60.7% of the studies, Figure 9A) with hydrogen productions generally ranging from 0.70 to 2.76 $L_{H2}.L_{medium}^{-1}$ (1st and 3rd quartiles, Figure 9B), with a maximum[27] of 12.88 $L_{H2}.L_{medium}^{-1}$. In general, batch processes are not the most efficient from a kinetic point of view, because (i) a lag phase is often observed due to microbial inoculum storage and the time to adapt to the fermentation medium; (ii) batch tests are ended after a time chosen by the operator which is not necessarily optimal; (iii) most studies using batch reactors do not focus on microbial kinetics. Thus, relatively low hydrogen productivities were achieved in batch mode, with values generally ranging between 0.22 and 1.22 $L_{H2}.L_{medium}^{-1}.d^{-1}$ (1st and 3rd quartiles, Figure 9C), the median and maximum[28] values being 0.55 and 6.28 $L_{H2}.L_{medium}^{-1}.d^{-1}$, respectively. To attain higher productivities, solutions were to operate bioreactors in continuous (31.4% of the studies) or semi-continuous (7.6%) mode. In these cases, bioreactors have both inlet and outlet flows for feeding the substrate and withdrawing the products continuously or sequentially, respectively. Hydrogen production kinetics and productivities were optimized with these modes of operation and usually ranged between 1.20 and 7.80 $L_{H2}.L_{medium}^{-1}.d^{-1}$ (1st and 3rd quartiles, Figure 9C), the median and maximum[29] values being 3.34 and 346.8 $L_{H2}.L_{medium}^{-1}.d^{-1}$, respectively.

< Figure 9 >

3.4.2. H_2 production performances of photo-fermentation and microbial electrolysis

To improve hydrogen yields, DF can be coupled with photofermentation or microbial electrolysis (Section 3.2). These two processes are based on a common principle: the conversion of volatile fatty acids and alcohols into H_2 and CO_2 is made thermodynamically favourable by providing an additional source of energy.

In the case of photofermentation, this external energy is provided by either artificial or natural light. However, the energy conversion efficiency of photofermentation, *i.e.* the ratio of the energy recovered as H_2 on the energy provided as light, does not exceed 10% under well-controlled conditions[12]. That makes this technology non-profitable if artificial light is used and if H_2 is the only product recovered. About 27% of the articles focusing on photofermentation concern the study of photofermentation alone while 73% of the articles deal with its coupling with DF. In both cases, the hydrogen yields were not significantly

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different, with average values of 0.279 and 0.246 $g_{COD\_H2}.g_{COD}^{-1}$ respectively (Figure 10). Overall, the average hydrogen yields of 0.255 $g_{COD\_H2}.g_{COD}^{-1}$ obtained by both photofermentation or its coupling with DF were significantly higher than those obtained in DF alone, *i.e.* 0.108 $g_{COD\_H2}.g_{COD}^{-1}$ (p-value < 0.0001). Nevertheless, these results do not necessarily reflect realistic working conditions, particularly regarding the use of small working volumes (< 0.25 L in 75% of the studies) and substrates with low COD concentration (< 11.2 $g_{COD}.L^{-1}$ in 75% of the studies). A pilot-scale photofermentation process dedicated to H_2 production was developed with a pure culture of *Rhodobacter capsulatus*[30], but no large scale mixed-culture process have been carried out so far. Adaptation to higher concentrations of organic matter or higher loading rates as well as the improvement of energy conversion efficiencies remain the main challenges of photofermentation prior to scaling up at larger scale. For more detailed information about photofermentation, readers may refer to Hallenbeck and Liu (2016)[31].

< Figure 10 >

In microbial electrolysis cells, electric energy is provided through an applied voltage between two electrodes. When producing H_2, these cells require voltage between 0.2 and 0.8 V, which is much lower than the values of 1.8 to 3.5 V typically applied in water electrolysis processes[14]. The energy conversion efficiency of microbial electrolysis is defined as the ratio of the energy recovered as H_2 over the electric energy provided to the system. As most of the energy is provided by the oxidation of organic matter at the cathode, efficiency calculated in this way can theoretically by as high as 1094% if based on the higher heating value of H_2 and when acetate is used as substrate[32]. Experimentally, the average energy conversion efficiency is 199 ± 22% in the scientific studies identified in the present article. Regarding the COD conversion efficiencies, an average hydrogen yield of 0.479 $g_{COD\_H2}.g_{COD}^{-1}$ was reported in microbial electrolysis or by coupling it with DF. However, performances are extremely variable (Figure 10). Interestingly, yields higher than 0.950 $g_{COD\_H2}.g_{COD}^{-1}$ were obtained in microbial electrolysis process[33,34]. In addition to the high conversion efficiencies, another advantage of microbial electrolysis cells is the possibility to produce nearly pure H_2 when anodic and cathodic compartments are separated by a membrane.

The high performances of microbial electrolysis cells regarding hydrogen yields and energy efficiencies, as well as the high purity of the biohydrogen recovered, makes this technology particularly attractive as a complement to DF. However, this technology is still

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mostly studied at a small scale (working volume < 0.50 L in 75% of the studies) and with substrates at low COD concentration (< 3.2 $g_{COD}.L^{-1}$ in 75% of the studies) that mostly correspond to synthetic mixtures of volatile fatty acids (73% of the studies). Nevertheless, few pilot-scale reactors (volumes from 100 to 1000 L) operated with wastewaters at low organic loading rates (0.5 to 2.0 $g_{COD}.L^{-1}.d^{-1}$) have been recently implemented and exhibit very promising results[35–38]. Similarly to photofermentation, research efforts are required to develop efficient microbial electrolysis cells at higher organic loading rates and treating real DF effluents, prior to its implementation at industrial scale. For more detailed information about microbial electrolysis, readers may refer to Zhen *et al.* (2017)[39].

< Table 3 >

3.5. Downstream processes for H_2 production

Biohydrogen production during DF and photofermentation is always concomitant with CO_2 production. If the biogas is not diluted, the proportions encountered range generally from 30 to 60% for H_2 and 40 to 70% for CO_2, with possible traces of CH_4 and /or H_2S. Mature technologies for hydrogen separation that are currently used in petrochemical processes are easily applicable to bioH_2 production processes[40,41]. For instance, the Pressure Swing Adsorption (PSA) process can produce H_2 at a purity of 99.999% with a H_2 recovery ranging from 75 to 92% while a purity of 90-99% and a H_2 recovery of 85-95% can be achieved with membrane permeation technologies[42]. Therefore, the biohydrogen separation step is not a technological obstacle, but remains one of the most costly step of the overall process[43–45].

4. Toward the waste-based biorefinery

Optimization of H_2 production has been the main objective of the last 15 years of research concerning DF. However, the hydrogen yields achieved so far (average of 0.108 $g_{COD\_H2}.g_{COD}^{-1}$) and the intrinsic metabolic limitations during DF severely limit the implementation of this process alone in a context of biomass recovery. A first solution, as presented in Section 3.4, is to couple DF with another hydrogen-producing process such as photofermentation or microbial electrolysis. An alternative is to implement a biorefinery approach in which several product streams are considered.

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4.1. Two-stage anaerobic digestion for H_2 and CH_4 production

The environmental biorefinery approach has been first and logically considered through the coupling between DF and AD leading to the production of H_2 and CH_4 (Section 3.2). This two-stage process presents several advantages[11]:

- At equal COD conversion rates, an energy yield up to 10% higher than the one-stage AD can theoretically be achieved, because of the higher energy content of H_2 in comparison with CH_4, *i.e.* heating value of 17.7 $MJ/kg_{COD\text{-}H2}$ versus 12.5 $MJ/kg_{COD\text{-}CH4}$, respectively.
- The two-stage process is more stable than the one-stage AD and operating parameters can be more easily optimized as hydrolysis/acidogenesis and acetogenesis/methanogenesis steps are separated (Figure 1).
- Methane yields can be increased in the two-stage process due to better biomass hydrolysis in the first DF step
- The two-stage process can be successfully carried out at high organic loading rates, increasing subsequently the methane productivity.

In the studies focusing on two-stage AD, 72.5 ± 19.1% of the total COD content of substrates were recovered as H_2 and CH_4, at an average initial total COD concentration of 47.8 ± 38.2 $g_{COD}.L^{-1}$ (based on 34 articles). Average hydrogen and methane yields were 0.055 ± 0.032 and 0.670 ± 0.187 $g_{COD}.g_{COD}^{-1}$, respectively. Most studies used complex substrates (88%) that are representative of the categories presented in Figure 4, thus demonstrating the applicability of such coupling.

In addition, the two-stage AD could also increase, theoretically, the added value of the process. Indeed, the hydrogen market price is ranging between 1.5 and 5.0 €/kg (Section 3.1) while the feed-in tariff of methane is comprised within a range of 0.09 to 0.20 €/kg (through injection into the natural gas network in France). Even considering a low case scenario with an H_2 price of 1.5 €/kg and an identical COD recovery (*i.e.* 72.5%), the added-value of a two-stage process would increase by 23 to 65% (23.9 - 45.0 $€/t_{CODfed}$) the economy of a one-stage AD (14.5 - 36.5 € / t_{CODfed}) process. Such increase is mitigated by the costs related to hydrogen purification and DF reactor operation that should be evaluated on a case by case basis. Consistently, the first technical-economic studies showed that the two-stage process can be financially advantageous compared to single-stage AD for substrates such as food

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waste[46,47]. For more detailed information about two-stage AD, readers may refer to Xia *et al.* (2016)[11].

4.2. Production of fermentation by-products

A wide range of molecules accumulate during AD (Figure 1) and mixed-culture fermentation processes. These compounds represent new opportunities for recycling waste into added-value molecules, such as short chain carboxylic acids and alcohols. Among the scientific articles identified in this study, the most present metabolites at the end of fermentation in batch and fed-batch reactors, or at the steady state in continuous and semi-continuous reactors were acetate (86.7% of the studies), butyrate (79.3%), ethanol (49.9%) and propionate (46.5%) (Figure 11). This result emphasizes that soluble metabolites are mostly produced as a mixture in mixed-culture fermentation. That represents a major challenge regarding the following separation/purification steps. To provide a better overview of the most commonly observed metabolites, a hierarchical clustering was performed based on the fermentation profiles reported in the literature (Figure 12). This clustering took into account the studies with more than 60% of the COD recovered as by-products (57% of the studies) and revealed five "standard" fermentation profiles (Figure 12A).

< Figure 11 >

(1) The first cluster corresponds to the production of H_2 by photofermentation, microbial electrolysis and their coupling, as described in section 3.4.

(2) The second cluster brings together studies focused on two-stage AD as described in the previous section.

(3) Cluster 3 is predominantly composed of studies focusing on DF and includes 129 scientific articles (Figure 12). This cluster is characterized by a fermentation profile dominated by butyrate (0.371 ± 0.148 $g_{COD}.g_{COD}^{-1}$), acetate (0.167 ± 0.100 $g_{COD}.g_{COD}^{-1}$) and H_2 (0.117 ± 0.062 $g_{COD}.g_{COD}^{-1}$). It is mainly observed during the fermentation of sugars (68% of the articles) or of sugar-rich complex substrates such as food waste, dairy or sugar industry wastewaters or hydrolysed lignocellulosic biomasses. The cluster 3 profile, characterized by a predominant production of butyrate and acetate, can be considered as a "typical" profile of DF.

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(4) Similarly, cluster 4 essentially contains studies focusing on DF and includes 20 scientific articles. In these studies, the dominant metabolites were ethanol (0.341 ± 0.079 $g_{COD}.g_{COD}^{-1}$), followed by acetate (0.195 ± 0.120 $g_{COD}.g_{COD}^{-1}$), butyrate (0.111 ± 0.123 $g_{COD}.g_{COD}^{-1}$) and H_2 (0.105 ± 0.066 $g_{COD}.g_{COD}^{-1}$). The substrates mainly corresponded to sugars (55% of the articles) and effluents from the sugar and oleaginous (crude glycerol) industries or hydrolysed lignocellulosic biomasses. The main difference with cluster 3 lies in the mode of inoculum selection: in cluster 4, only 25% of the inocula were pretreated whereas 55.1% of the studies involved at least one inoculum pretreatment in cluster 3. Rather than selecting *Clostridiaceae* species using heat shocks, studies in cluster 4 mainly used aerotolerant inocula (*e.g.* activated sludge) and/or maintained an acidic pH (< 5) during fermentation. These conditions allowed the enrichment of ethanol-producing bacteria such as species from the *Enterobacteriaceae* family.

(5) Finally, cluster 5 corresponds uniquely to studies that used glycerol (pure or crude) as substrate and gathers 9 studies. In this particular case, the main product is 1,3-propanediol (0.593 ± 0.114 $g_{COD}.g_{COD}^{-1}$) which is accompanied by a variable mixture of carboxylic acids, ethanol and H_2.

< Figure 12 >

The results of this hierarchical clustering highlight that fermentation profiles are relatively stable and repeatable despite the great diversity of substrates and fermentation process conditions. In the following sections, production performance and contextual market elements are discussed for each of the main DF metabolites (acetate, butyrate, ethanol) as well as other molecules with high potential (1,3-propanediol, propionate, caproate, lactate, PHA). Other high-valued metabolites such as butanol and succinate will not be discussed as they are not commonly observed in mixed-culture fermentation (see Figure 11).

4.3. Applications and economy of dark fermentation co-products

4.3.1. Acetic acid

Acetic acid is a commodity chemical which has a very wide range of applications, including plastics manufacturing, its use as food additive or solvent. The total world market volume was 13,570 kt/yr in 2015[48] with more than 50% of the market located in Asia (mainly

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China and India). The current market is relatively stable in Europe but growing in Asian countries and its annual growth rate has been estimated at 5% for the period from 2014 to 2020. Its production is mostly oil-based while bio-based acetic acid represented only 10% of the global market in 2015. The market price of acetic acid is comprised between 0.33 and 0.67 €/kg, equivalent to 0.31 to 0.63 €/kg_{COD} (Table 4).

< Table 4 >

A stable production of acetate can be achieved during DF at an average yield of 0.167 ± 0.100 $g_{COD\_acetate}.g_{COD}^{-1}$ when a butyrate-dominated fermentation profile is observed (Cluster 3, Figure 12). However, because substrates with low initial COD content are often used in DF, acetate concentrations in the fermentation medium are often low. A final acetate concentration higher than 2.4 $g.L^{-1}$ was reached in only 25% of the studies in which acetate production was observed (Figure 13). Nonetheless, acetate can be produced at higher concentrations and the best performance was reported with sugarcane bagasse, reaching a final concentration of 35.3 $g.L^{-1}$ (Table 5)[49]. By maximizing the acetate yields, values as high as 0.56 $g_{COD\_acetate}.g_{COD}^{-1}$ in fermentation processes[50] or even 0.90 $g_{COD\_acetate}.g_{COD}^{-1}$ were reached in microbial electrosynthesis processes[51] (Table 6). Finally, high acetate productivity values of 57.0 $g.L^{-1}.d^{-1}$ were achieved even when employing complex substrates (Table 5)[52].

The main limitation of acetate recovery when it is produced by mixed microbial cultures is the extraction/purification step of the molecule. Indeed, acetate is mostly produced together with other molecules having similar chemical characteristics (short chain carboxylic acids). That makes inefficient the extraction techniques traditionally used in fermentation processes. No scientific publication concerning the specific extraction of acetate from a mixture of carboxylic acids in fermentation processes was identified in the considered database. Moreover, no techno-economic study focused on acetate production by mixed-culture fermentation was carried out yet, making difficult to assess precisely the limits and the optimal operating range of potential recovery processes. Nonetheless, few strategies of *in-situ* acetate purification were proposed with, for instance, the case of hyper-thermophilic AD (70°C) carried out at low hydraulic retention time (<3j)[53–55]. Here, the acetate re-consumption pathways were inhibited while all other compounds were converted into CH_4. As only acetate remained in the liquid phase, traditional techniques such as liquid-liquid extraction processes were used to efficiently recover acetate[56]. However, the proof of concept of such process remains to be carried out on real effluents. Another example is the case of microbial

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554 electrosynthesis, in which pure acetate can be produced in the cathodic compartment and then
555 extracted by migration through membranes (electrodialysis). Although the principle is
556 interesting, microbial electrolysis processes still suffer from low productivities that limit their
557 applicability[57]. Instead of extracting acetate, it is also possible to upgrade an acetate-rich
558 fermentation broth into a more easily-extractable compound using a secondary biological or
559 chemical process. For instance, a mixture of carboxylic acids can be esterified[58], converted
560 into lipids by yeasts[59], or used to produce caproic acid or polyhydroxyalkanoates (see Section
561 4.4).

562 **< Figure 13 >**

563 **< Table 5 >**

564 4.3.2. Butyric acid

565 Butyric acid is a precursor of esters used as food additives or in perfume formulation.
566 It can also be directly used as antibacterial agent in the field of animal nutrition[6] and for
567 bioplastic applications, although the former application would require approval of regulatory
568 authorities such as the FDA in USA or EFSA in EU. The butyrate market is currently quite
569 limited, with a current production capacity of about 30 kt/yr. This low capacity can be partly
570 explained by selling prices that are still too high for the commodity market (from 1.67 to 2.09
571 €/kg, Table 4). Nonetheless the butyrate market could reach an annual growth rate as high as
572 12% between 2016 and 2020, depending on its availability (production volume) and the
573 subsequent price decrease[60].

574 Significant production of butyrate was observed in DF at an average yield of 0.371 ±
575 0.148 $g_{COD\_butyrate}.g_{COD}^{-1}$ (Cluster 3, Figure 12). Although butyrate is generally produced at
576 low concentration (Figure 13), final concentrations can reach up to 21.4 $g.L^{-1}$ (Table 5). The
577 conversion yield in DF can be optimized to achieve 0.74 $g_{COD\_butyrate}.g_{COD}^{-1}$ (Table 5)[61].
578 Butyrate production can also be promoted by chain elongation reactions[62] in which a mixture
579 of acetate and ethanol is converted into butyrate (Table 6). Finally, butyrate productivities are
580 high with 73.2 $g.L^{-1}.d^{-1}$ as best value achieved with complex substrates (Table 5)[52].

581 Butyrate is mostly produced together with other carboxylic acids in mixed-culture
582 fermentation which represents a severe limitation to its specific extraction. However, the
583 possible conversion of a mixture of lactate, acetate and ethanol by chain elongation (possibly
584 requiring the addition of ethanol) makes theoretically possible the production of butyrate as

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sole soluble carboxylic acid. Butyrate could then be extracted by liquid-liquid extraction[56] or electrodialysis. However such proof of concept remains to be demonstrated. Chemical routes such as esterification are also possible to facilitate extraction steps while upgrading butyrate into valued chemical such as butanol or butyl-butyrate[58,63].

< Table 6 >

4.3.3. Ethanol

Ethanol is a molecule with a large world market of 76,700 kt/year (2015)[48] . Ethanol is predominantly a bio-based product (93%) and is mainly used as biofuel (80 to 85%), or in the food industry and as solvent. A strong market growth has occurred over the past ten years due to energy transition policies, particularly in Europe, Brazil and United States. The largest producers are the United States (59% of bioethanol) and Brazil (27%)[64], which mainly use maize and sugar cane as raw materials, respectively. The ethanol market price is between 0.30 and 1.50 €/kg depending on the product purity and the raw material (corresponding to 0.14 - 0.72 €/kg_{COD}, Table 4). It is noteworthy that ethanol market price rely heavily on support policies, either through direct subsidies or fuel blend obligation[65].

Under certain conditions, ethanol can be produced by mixed cultures of fermentative bacteria at an average yield of 0.341 ± 0.079 $g_{COD\_ethanol}.g_{COD}^{-1}$ (Cluster 4, Figure 12). Similarly to acetate and butyrate, ethanol is generally not the targeted product and therefore accumulation is limited to low concentrations around a median of 0.5 $g.L^{-1}$ (Figure 13). Nonetheless, several studies aimed at optimizing the ethanol/H_2 production from glycerol. Using crude glycerol directly issued from the biodiesel industry, the highest concentration achieved with mixed cultures reached 26.0 $g.L^{-1}$, with a yield and productivity of 0.59 $g_{COD\_ethanol}.g_{COD}^{-1}$ and 1.6 $g_{\_ethanol}.L^{-1}.d^{-1}$, respectively[66]. This titer must be compared with yeast-based fermentation of sugars which can typically attain titers as high as 150 $g.L^{-1}$ [67]. The highest yield was observed from crude glycerol with a value of and concentration and productivity of 0.91 $g_{COD\_ethanol}.g_{COD}^{-1}$, 8.0 $g_{\_ethanol}.L^{-1}$ and 4.8 $g_{\_ethanol}.L^{-1}.d^{-1}$, respectively[68]. However, the productivity when using a complex substrate remained low with maximum value around 9.5 $g.L^{-1}.d^{-1}$ (Table 5)[52].

The extraction and purification of ethanol from fermentation media is traditionally carried out by distillation followed by dehydration. However, a large amount of energy is required for these two purification steps, depending strongly on the ethanol concentration. In

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particular, the energy required for the distillation step is higher than the heating value of ethanol (28.9 $MJ.kg^{-1}$) for ethanol concentrations lower than ~14 $g.L^{-1}$, thus making its extraction clearly unsustainable in that case [69]. However, within an environmental biorefinery, a part of the energy required for distillation could be provided by recycling heat from the cogeneration biogas plant. Ethanol concentration remains the main parameter to be optimized prior to extraction, with an objective of, typically > 40 $g.L^{-1}$ [69]. Thus, considering the current state of the art, ethanol production by mixed-culture fermentation is far from being economically or even energetically competitive in most cases when compared with corn-based or sugar cane-based ethanol. However, Varrone et al. (2013)[66] estimated that bioethanol production costs using crude glycerol issued from a biodiesel production plant could be as low as 0.27 €/kg considering a mixed-culture fermentation reaching only $26g_{\_ethanol}.L^{-1}$, making the process economically competitive. Thus, exploring mixed-culture ethanol production processes base on non-edible substrates that cannot be fermented by yeasts could reveal interesting niche with the potential of outcompeting current bioethanol production plants, especially regarding environmental and societal impacts[70].

4.4. Other value-added metabolites produced in mixed-culture fermentation

4.4.1. 1,3-propanediol

1,3-propanediol (PDO) is a bio-based molecule entirely produced by biotechnological processes (currently by genetically modified organism cultures) from glucose or glycerol. PDO is mainly used as precursor of polytrimethylene terephthalate (PTT), a polymer used in the textile industry, or also directly used in the food, cosmetic and pharmaceutical sectors. In 2015, its market volume was 128 kt/yr and its selling price was estimated at 1.76 €/kg, equivalent to 1.05 $€/kg_{COD}$[48]. This price could be raised to more than 3 €/kg, equivalent to 1.79 $€/kg_{COD}$, in the coming years[71].

Only few studies are available on PDO production by mixed-culture fermentation but this is a growing research area, representing more than 50% of the articles published after 2015. Among these studies, the substrate used for producing PDO is categorized as pure or crude glycerol. The yields are generally high, with an average of 0.593 ± 0.114 $g_{COD\_PDO}.g_{COD}^{-1}$ (Cluster 5, Figure 12), a value close to the maximum theoretical yield (0.82 $g_{COD\_PDO}.g_{COD}^{-1}$)[72]. Interestingly, the best production performances were achieved in a fed-batch reactor fed with raw glycerol, with a final concentration of 82.7 $g.L^{-1}$, a yield of 0.75 $g_{COD\_PDO}.g_{COD}^{-1}$ and a productivity of 73.7 $g.L^{-1}.d^{-1}$ (Table 5)[73]. These results are comparable

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with the best performances achieved so far during glycerol fermentation by pure culture of unmodified strains[74,75].

Many methods have been developed for 1,3-propanediol extraction and purification from fermentation media[74]. First, a three-step process is based on the high boiling point of 1,3-propanediol (214 ° C) and is composed of (1) a filtration step for biomass separation, (2) an evaporation step to remove compounds more volatile than PDO such as water and organic acids and, (3) a rectification step to produce PDO with a purity higher than 99%[76]. The overall extraction yield of the whole chain can be as high as 90%[76] but this method requires a large amount of energy[74]. An effective alternative is based on a succession of three successive steps, *i.e.* (1) biomass separation by microfiltration and activated carbon; (2) concentration by vacuum distillation and (3) final separation by silica gel chromatography. Following this procedure, a purity of 98% and an extraction yield of 75% were achieved[77]. An interesting improvement of this second downstream pipeline could be the implementation of a simulated moving bed as alternative chromatographic step, as described in a patent by Archer Daniels Midland Co (2001)[78]. Research on low-cost PDO extraction and purification is still active, but the mature existing technologies make possible the scaling up of a mixed-culture process for 1,3-propanediol production.

4.4.2. Propionic acid

Propionic acid can serve as food preservative in the fields of human and animal nutrition and is also a platform molecule that is used for example as flavour precursor[79]. However, similarly to butyrate, the use of waste-based propionic acid for food or feed applications would require approval of regulatory authorities such as FDA or EFSA in USA and UE, respectively. It is mainly produced by petrochemical routes[79] and represents a market of 400 kt/yr (2013)[6,79]. Its market price is ranging between 1.25 and 1.38 €/kg, which is equivalent to 0.83 - 0.91 €/kg_{COD} (Table 4). However, a lower price would be necessary to meet the demand of the feed industry and expand the propionate market.

In DF processes, propionate production is generally avoided as this pathway is anti-correlated with H_2 production[5]. As a result, only very few studies have so far focused on the optimization of propionate production by microbial mixed culture and from complex substrates. The highest propionate final concentration found is 15.8 $g.L^{-1}$ using food waste as substrate[80].The best yields and productivities are 0.31 $g_{COD\_propionate}.g_{COD}^{-1}$ and 22.0 $g.L^{-1}.d^{-1}$, observed in two different studies (Table 5). Better yields up to 0.45 $g_{COD\_propionate}.g_{COD}^{-1}$ were

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attained when using defined fermentation media[81], in particular when refined glycerol is used as substrate (Table 6). For more detailed information about propionate biological production, readers may refer to Es *et al.* (2017)[79].

Similarly to acetate and butyrate, no low-cost process that could specifically extract propionate from mixtures of short-chain carboxylic acids has been developed. Nonetheless, AD can be used to convert into methane all soluble end-products generated by fermentation except propionate, and more particularly under high ammonium concentration (> 2.9 $g.L^{-1}$)[81,82]. In that case, traditional carboxylic acid extraction techniques could be used to produce pure propionic acid, but the proof of concept remains to be demonstrated.

4.4.3. Lactic acid

Lactic acid is an alpha-hydroxy acid widely used in the food industry (bacteriostatic, preservative, flavour enhancer), but also in the pharmaceutical sector and more recently in the polymer industry for polylactic acid manufacturing (PLA, bioplastic). The market value is high, ranging between 0.84 and 1.51 €/kg, equivalent to 0.79 – 1.41 €/kg_{COD} (Table 4). The market volume is 472 kt/yr and is expected to grow in the coming years due to the increasing demand in PLA. The lactic acid is currently 100% bio-sourced[48] as high isomeric purity lactic acid can be produced by simple fermentation[83]. This aspect is particularly important for PLA production, which biodegradability depends on the L-isomer purity of the lactic acid[83]. First life cycle assessments have shown that

Similarly to propionate, lactic acid production is not desired in DF as it does not promote the H_2 production[5]. Nevertheless, very good performances have been achieved regarding lactate production by mixed-culture fermentation from both glucose and food waste (Table 5 and Table 6). In particular, lactate was produced with high selectivity even from complex substrates. Using food waste as the substrate, the highest lactate concentration achieved so far is 64.0 $g.L^{-1}$, with a yield and productivity of 0.63 $g_{COD\_lactate}.g_{COD}^{-1}$ and 12.8 $g.L^{-1}.d^{-1}$, respectively (Table 5)[84]. Productivities as high as 40.0 $g.L^{-1}.d^{-1}$ were reached using food waste as substrate[85].

Similarly to carboxylic acids, lactate extraction is suitable when its selectivity is sufficiently high. Some studies demonstrated the feasibility of lactate extraction after food waste fermentation, using a process combining centrifugation, nanofiltration and electrodialysis steps with an overall recovery rate of 73%[85]. Lactate can also be extracted *in-*

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situ from fermentation medium by adsorption on activated carbon that can be further desorbed with acetone[86]. However, research on the improvement of such extraction processes, as well as on the techno-economic aspects of lactate production are still necessary before considering a scale-up of the process.

4.4.4. Caproic acid

Caproic acid is a medium chain fatty acid (6 carbon atoms) used as antimicrobial agent, animal feed additive, food flavouring and potential biofuel precursor (e.g. decane)[87,88]. The industrial production of this molecule remains low, with a production capacity of only 25 kt/yr. Its current selling price ranges between 1.88 and 2.09 €/kg, equivalent to 0.85-0.95 €/kg_{COD} (Table 4) that is far too high for a commodity product. This price would likely decrease if the caproic acid production is intensified.

Caproate can be produced from a mixture of acetate, butyrate, ethanol and lactate by chain elongation according to the following global reactions (butyrate being a reaction intermediate)[87]:

$$12\ C_2H_6O\ (\text{ethanol}) + 3\ C_2H_3O_2^-\ (\text{acetate}) \rightarrow 5\ C_6H_{11}O_2^-\ (\text{caproate}) + 4\ H_2 + 8\ H_2O + 2\ H^+ \quad (3)$$

$$15\ C_3H_5O_3^-\ (\text{lactate}) + 10\ H^+ \rightarrow 5\ C_6H_{11}O_2^-\ (\text{caproate}) + 15\ CO_2 + 10\ H_2 + 5\ H_2O \quad (4)$$

Caproate production can therefore be a way to recover value from a mixture of these metabolites. Caproate production by mixed cultures is a recent topic, with more than 90% of the identified articles having been published after 2013. In most studies, chain elongation reactions are favoured by ethanol addition (80% of the studies dealing with caproate). However, such external ethanol addition has a high environmental impact, and increase the costs of the process, and therefore should be minimized [89]. Using complex substrates, the best performances achieved so far are a maximum concentration of 11.9 $g.L^{-1}$, a yield of 0.81 $g_{COD\_caproate}.g_{COD}^{-1}$ and a productivity of 26 $g.L^{-1}.d^{-1}$ (data issued from different studies, Table 5)[90,91]. For more detailed information about caproate production, readers may refer to Cavalcante *et al.* (2017)[87].

Caproate extraction is greatly facilitated by the low water solubility of its acid form (~ 11 $g.L^{-1}$). When caproate is concentrated in an acidic compartment by electrodialysis, caproic acid accumulation forms an organic phase on the top of the aqueous phase that can be physically removed[88]. Direct liquid-liquid extraction from the fermentation medium is also

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741 possible[91–93]. For instance, using trioctylphosphine oxide as solvent, caproate recovery yields
742 of 97.3% were achieved[93]. Moreover, long-term caproate production (> 1 year) was
743 demonstrated[93] and at least one pilot-scale reactor was already implemented, incorporating an
744 extraction technology[94]. In summary, caproate production by microbial mixed culture could
745 reach a pre-industrial stage in the coming years, the main constraint being the reduction of the
746 external supply of ethanol.

747 4.4.5. Polyhydroxyalkanoates (PHAs)

748 Polyhydroxyalkanoates (PHAs) are a family of biodegradable polyesters that can be
749 produced by fermentation. Indeed, in presence of an excess of carbon source content, some
750 microorganisms are able to accumulate PHAs within their cell as a way to store carbon and
751 energy[95]. Depending on the substrate available for the PHA-accumulating microorganisms,
752 polymers have different physicochemical characteristics that can be exploited through
753 numerous applications in the fields of packaging and health, *e.g.* surgery[95]. The most common
754 PHAs are poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
755 (PHBV) although a wide variety of PHAs can be produced. In 2015, the market volume of
756 PHAs was only 17 kt/yr and its production was 100% bio-based[48]. Its selling price is currently
757 varying between 2.20 and 5.00 €/kg, equivalent to 1.38 - 3.14 €/kg_{COD} (Table 4) but is not yet
758 economically competitive when compared to equivalent petro-based plastics (~1 €/kg)[96]. As
759 one of the main contributor to the overall process operating costs is the carbon source[97], using
760 waste as substrate appears to be a promising way to reach economic viability[98]. Moreover, it
761 would also improve environmental performances of PHA production, for instance when the
762 process is integrated in a wastewater treatment plant[99].

763 PHAs can be produced from a mixture of carboxylic acids (*i.e.* acetate, propionate,
764 butyrate, valerate, caproate). Similarly to caproate, PHAs production can be used as a way to
765 valorise DF effluents. When microbial mixed cultures are used, PHA production is generally
766 carried out in three steps: (i) an acidogenesis step (*e.g.* DF) to produce carboxylic acids; (ii) a
767 PHA-producing bacteria selection phase using feast/famine cycles and (iii) a PHA
768 accumulation phase. The selection phase (ii) is generally kinetically limiting the whole
769 process and explains the low productivities observed, typically 1 $g_{PHA}.L^{-1}.d^{-1}$ (Table 7)[100]. The
770 best conversion efficiency was 0.41 $g_{COD\_PHA}.g_{COD}^{-1}$ using paper industry effluents[101]. For
771 more detailed information about PHAs production from waste, readers may refer to Valentino
772 *et al.* (2017)[96].

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To date, several PHA extraction methods have been developed. After a biomass recovery phase (*e.g.* centrifugation), two strategies are generally employed: the most commonly used method consists in solubilizing PHAs in a solvent (*e.g.* chlorinated solvent) followed by a precipitation step, for example using ethanol[96]. High purities are achieved with this method but require high operating costs related to solvent recycling. The other method aims to disrupt or digest the cellular biomass using chemical or enzymatic treatment to release the PHAs as particles[96]. This second technique is still under development to obtain better extraction yields and a better stability of the PHA for thermoplastic applications. Pilot-scale reactors for PHAs production using mixed cultures and incorporating extraction/purification processes have recently been implemented[100,102]. The current technologies are however limited by their high production costs as well as their low productivities that does not yet fit with the demand of bioplastic purchasers.

< Table 7 >

5. Conclusions

Residual materials represent a significant source of organic matter[103] that can directly contribute to a circular and environmentally friendly economy. The development of AD is a first step towards residual materials conversion and recycling, particularly in a context of energy transition. However, methane has a limited added-value and requires feed-in tariffs to ensure the economic viability of the biogas plants. Recovery of other biomolecules upstream biogas plants could therefore be a way to improve the economic competitiveness of this sector while maintaining environmental and societal services, *i.e.* waste treatment and recycling. Thus, biogas plants have the potential to become environmental biorefinery in which biogas production would only be a final stage dedicated to the most recalcitrant organic fractions.

Biohydrogen is by far the most studied molecule that can be easily implemented in "second generation" biogas plants when considering DF processes. Moreover, H_2 should have an important place in the future economic landscape as chemical reagent and energy carrier, particularly as substitute for petro-based fuels in the transportation sector. Thus, H_2 production could be a first way to improve the economic viability of the biogas sector as there is both an emerging market demand and a high price for this molecule. By implementing H_2 production in AD plants, the territorial grid offered by the biogas plants would significantly

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reduce the costs related to H_2 transportation. Research on biological H_2 production under non-sterile conditions has been active for more than 15 years, and mainly focused on the study of DF by mixed microbial communities. This process has been widely tested at laboratory scale for a wide range of operating conditions, such as temperature (from 15 to 80 °C), working volume (from 0.01 to 3300 L), operation modes (discontinuous, semi-continuous and continuous) and for a variety of complex substrates that are representative of most of the resources available for AD (Figure 4). However, an average of only 8.9% of the organic load is converted into H_2 when complex substrates are used in DF (9.6% for industrial effluents, 9.4% for green and agricultural waste, 6.4 % for food waste and 5.6% for municipal waste). Therefore, there is still room for improvement to reach the theoretical maximum yield of 33%, especially regarding easily biodegradable sugar rich substrates such as food and municipal waste. Because of these low yields, most economic scenarios comparing the different bioH_2 production technologies conclude that DF is not economically competitive yet with technologies such as biomass gasification or biogas reforming[10]. However, these scenarios do not take into account the environmental benefits offered by DF when compared to more traditional H_2-producing processes, as shown by recent LCA[10], as well as products other than H_2 that can be coproduced and the possible couplings within a biorefinery that could improve economic performances of DF.

Nonetheless, DF acts also as pretreatment of the complex organic matter and the effluents are more easily degradable than the initial materials. Thus, DF effluents can be directly injected into biogas plants to recover soluble COD content as methane. Such coupling, also known as two-stage AD, is a mature technology supported by numerous studies and patents that could be readily implemented in a short term (TRL7, see supplementary information for more information about TRL scale). In addition to this coupling, new complementary routes with better added-value could be implemented in a near future:

- Producing more bioH_2 (TRL5-6): it is possible to inject DF effluents in a microbial electrolysis cell or in a photofermentation reactor. However, the development of both processes is currently limited by high capital expenditures and the low organic load rates they can withstand.
- Extracting biomolecules from DF effluents or bioelectrochemical processes (TRL2-3): acetate, butyrate and ethanol can be stably co-produced with bioH_2 during mixed-culture DF or produced purely by bioelectrochemical processes. Specific and low-cost extraction methods still need to be developed for DF

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effluents whereas bioelectrochemical systems are still limited by low productivities.

- Producing biomolecules from DF effluents (TRL6): additional fermentation processes can be carried out to produce more easily extractable molecules such as caproate or PHAs from DF effluents. Caproate production is undergoing a scale-up phase while PHA production processes still suffers from low productivities.
- Redirecting DF toward the production of metabolites without H_2 production (TRL3-4): because of their anti-correlation with H_2 production, propionate and lactate production by mixed cultures has received little attention. Nevertheless, these metabolites have a high market value and early studies show that they could be produced with good performance upstream of biogas plants. It is also possible to produce 1,3-propanediol in the case where glycerol is used as substrate.

All these complementary or alternative processes are at unequal levels of technological maturity ranging from laboratory-scale pre-studies (lactate, propionate and 1,3-propanediol production by mixed cultures and specific extraction of DF metabolites) to pilot-scale processes (microbial electrolysis, photofermentation, PHAs and caproate production by mixed cultures). Modelling approaches such as life cycle assessment and techno-economic studies would be helpful to further scaling up steps by providing boundaries for economic and environmental viability. In all cases, the higher added-value of fermentative products when compared to methane leaves room for the potential addition of fermenters and extraction systems to biogas plants when these technologies are more mature. Whatever the recovery scenarios considered, AD will act as a final way to recover the last part of non-valorised organic matter.

To conclude, biogas plants transformation into environmental biorefineries responds not only to a concern for better economic viability, but could also represent an interesting source of bio-based platform molecules for the future bioeconomy. By keeping easily fermentable substrates for biomolecules production (*e.g.* agro-food industries effluents, food waste) and recalcitrant waste for AD (*e.g.* manure, sludge), significant volumes of biomolecules could be produced. For instance, if 1% of the French methane production expected in 2030[103] is diverted to produce lactate, a total production of 130 kt/yr could be achieved, which represents nearly a quarter of current world consumption. Thus, the

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870 environmental biorefinery has the potential to become a major supplier of biobased molecules
871 with the lowest environmental impact and contribute as a sustainable way to the future
872 bioeconomy.

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874 **Abbreviations**

875 **AD:** Anaerobic digestion

876 **DF:** Dark fermentation

877 **COD:** Chemical oxygen demand

878 **LCA**: Life cycle assessment

879 **PDO**: 1,3-propanediol

880 **PHA**: Polyhydroxyalkanoate

881 **PLA**: Polylactic acid

882 **PLS-DA**: Partial least square discriminant analysis

883 **TRL:** Technology readiness level

884

885 **Competing interests**

886 The authors declare that they have no competing interests.

887

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Legends

Figure 1. The different phases of anaerobic digestion.

Figure 2. Publication of research articles and patents related to biomolecule production by mixed-culture fermentation between 1997 and January 2017. Information regarding patents after mid 2015 is incomplete due to the 18 month delay between patent filing and publication.

Figure 3. Technologies used for biological hydrogen production. The documents represented correspond to scientific articles displaying a hydrogen yield higher than 0.01 $g_{COD}.g_{COD}^{-1}$ (400 documents) and to the patents explicitly claiming hydrogen production (100 documents).

Figure 4. Substrates used for the biological production of hydrogen (A) and their total COD concentration (B). The total COD concentration corresponds to the initial concentration of the substrate for batch processes and to the concentration of the feed for continuous/semi-continuous processes. The documents represented correspond to scientific articles displaying a hydrogen yield higher than 0.01 $g_{COD}.g_{COD}^{-1}$ (400 documents). N corresponds to the number of scientific articles taken into account for each category. Only the categories with $N \geq 10$ are represented. Red dots represent the average of the distributions.

Figure 5. Substrate pretreatment carried out before biohydrogen production. N corresponds to the number of scientific articles taken into account for each category. Mechanical pretreatments: grinding, sonication; physico-chemical pretreatments: acid/alkaline hydrolysis, heat treatment, steam explosion; enzymatic pretreatments: by microorganisms or enzyme cocktails.

Figure 6. Part of the studies focused on dark fermentation using inoculum pretreatment (A) and the different pretreatment methods employed (B). The documents represented correspond to scientific articles for which the information concerning inoculum pretreatment is available, displaying a hydrogen yield higher than 0.01 $g_{COD}.g_{COD}^{-1}$ and focused on dark fermentation, or on a coupling between dark fermentation and another technology (346 documents).

Figure 7. Hydrogen yield as a function of the total COD concentration of the substrates. The total COD concentration corresponds to the initial concentration of the substrate for batch processes and to the concentration of the feed for continuous/semi-continuous processes. The documents represented correspond to scientific articles displaying a hydrogen yield higher than 0.01 $g_{COD}.g_{COD}^{-1}$ (400 documents).

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Figure 8. Hydrogen yield as a function of the substrates employed (A) and process parameters (B). The documents represented correspond to scientific articles displaying a hydrogen yield higher than 0.01 $g_{COD}.g_{COD}^{-1}$ and focused on dark fermentation (303 documents). N corresponds to the number of scientific articles taken into account for each category. Only the categories with $N \geq 10$ are represented. Red dots represent the average of the distributions.

Figure 9. Frequency of use (A) and performance (B-C) of the different operation modes used for hydrogen production by dark fermentation. The documents represented correspond to scientific articles displaying a hydrogen yield higher than 0.01 $g_{COD}.g_{COD}^{-1}$ and focused on dark fermentation (303 documents). N corresponds to the number of scientific articles taken into account for each category. Hydrogen production and productivities are normalized by the working volumes.

Figure 10. Hydrogen yields achieved by photofermentation and microbial electrolysis. The documents represented correspond to scientific articles displaying a hydrogen yield higher than 0.01 $g_{COD}.g_{COD}^{-1}$. N corresponds to the number of scientific articles taken into account for each category.

Figure 11. Occurrence probability of common metabolites within the database. Scientific articles in which only hydrogen was measured, as well as those dealing with the coupling between fermentation and anaerobic digestion were excluded. Metabolites were considered present when their respective yields were higher than 0.01 $g_{COD}.g_{COD}^{-1}$. The result is standardized on 353 documents.

Figure 12. Standard fermentation profile identification by hierarchical clustering. Average metabolic profiles of the clusters (A) and their representation by discriminant analysis (PLS-DA)(B). Only scientific articles reporting more than 60% of the substrate total COD recovered as products are taken into account (222 documents). N corresponds to the number of scientific articles of each cluster identified by hierarchical clustering. Only the clusters with $N \geq 5$ are represented. Error bars corresponds to standard deviations. Ellipses represent 95% confidence intervals.

Figure 13. Final concentrations of acetate, butyrate and ethanol produced by mixed-culture fermentation processes. N corresponds to the number of scientific articles taken into account for each category.

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1161 **Table 1.** Keywords used for the database building.

Category	Keywords
Process	Fermentative process, Photofermentation, Photoautotrophy, Phototrophy, Fermentation / Light, Photosynthesis, MEC, Microbial electro*, Bioelectrochemistry, Bioelectrolysis, Electrofermentation, Electromicrob*, Dark Fermentation, Obscure fermentation, Anaerobic digestion, Anaerobic condition, Anaerobic process, Acidogenesis, Acetogenesis, Methanogenesis, Solventogenesis, Chain elongation
Combined processes	Association of two processes (see previous line)
Hydrogen	Hydrogen, Dihydrogen, H_2
Biomolecules	List of 130 biomolecules according to Straathof (2014)[104]
Mixed culture	Consortium, Consortia, Co-culture, Microbiome, Microbiota, Microflora, open-culture, Symbiosis, Mixed culture, Community, Population, Dominant

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View Article Online
DOI: 10.1039/C8GC00572A

1164 **Table 2.** Molecular formulas and COD equivalents of commonly encountered biomolecules.

Biomolecule	Molecular formula	COD equivalent (g_{COD}/g)
1,3-propanediol	$C_3H_8O_2$	1.68
2,3-butanediol	$C_4H_{10}O_2$	1.96
Acetate	$C_2H_4O_2$	1.07
Butanol	$C_4H_{10}O$	2.59
Butyrate	$C_4H_8O_2$	1.82
Caproate	$C_6H_{12}O_2$	2.21
Cellulose	$C_6H_{10}O_5$	1.19
Ethanol	C_2H_6O	2.09
Glucose/Fructose	$C_6H_{12}O_6$	1.07
Glutamate	$C_5H_9O_4N$	0.98
Glycerol	$C_3H_8O_3$	1.22
Hydrogen	H_2	8.00
Lactate	$C_3H_6O_3$	1.07
Methane	CH_4	4.00
Methanol	CH_4O	1.50
PHA	$(C_4H_6O_2)_n$	1.67
Propanol	C_3H_8O	2.40
Propionate	$C_3H_6O_2$	1.51
Succinate	$C_4H_6O_4$	0.95
Sucrose/Lactose	$C_{12}H_{22}O_{11}$	1.12
Valerate	$C_5H_{10}O_2$	2.04
Xylose/Arabinose	$C_5H_{10}O_5$	1.07

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1167 **Table 3.** Conversion performances and features of the bioH_2 production technologies.

Technology	TRL	Substrate	Theoretical maximum yield ($g_{COD}.g_{COD}^{-1}$)	Experimental average yield ($g_{COD}.g_{COD}^{-1}$)	Energy conversion efficiency (%)
Dark fermentation	7	Sugars Waste/wastewater	0.333	0.124 (sugars) 0.089 (waste/wastewater)	-
Photofermentation	6	Volatile fatty acids Fermentation effluent	1.000	0.255	<10
Microbial electrolysis	6	Volatile fatty acids Fermentation effluent	1.000	0.479	199

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1170 **Table 4.** Market price and volume of biomolecules of interest.

Molecule	Market price (€/kg)*	Market price (€/kg_{COD})*	Global market volume (kt/yr)	References
PHA	2.20 – 5.00	1,38 – 3.14	17	[96], this study
1,3-propanediol	1.76 – 3.00	1.05 – 1.79	128	[48,71]
Butyric acid	1.67 – 2.09	0.92 – 1.15	30**	This study
Caproic acid	1.88 – 2.09	0.85 – 0.95	25**	This study
Propionic acid	1.25 – 1.38	0.83 – 0.91	400	[6,79], this study
Lactic acid	0.84 – 1.51	0.79 – 1.41	472	[48], this study
Acetic acid	0.33 – 0.67	0.31 – 0.63	13,570	[48], this study
H_2	1.50 – 5.00	0.19 – 0.63	60,000	This study
Ethanol	0.30 – 1.50	0.14 – 0.72	76,700	[48,64], this study
CH_4	0.09 – 0.20***	0.02 – 0.05***	-	This study

1171 * Excluding transport costs

1172 ** Production capacity

1173 *** Feed-in tariff for methane injection into the natural gas network in France

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Table 5. Best performance of soluble metabolite production by mixed cultures from complex substrates.

Metabolite	Substrate	Concentration ($g.L^{-1}$)	Yield* ($g_{COD}.g_{COD}^{-1}$)	Productivity* ($g.L^{-1}.d^{-1}$)	Process configuration	Process or start-up duration (d)**	Ref.
Highest concentrations							
Acetate	Sugar cane bagasse	**35.3**	0.24	1.1	Semi-continuous	90	[49]
Butyrate	Food waste	**21.4**	0.26	2.7	Batch	8	[80]
Caproate	Fermented municipal waste + ethanol	**11.9**	0.46	26.0	Continuous	140	[90]
Ethanol	Raw glycerol	**26.0**	0.59	1.6	Fed-batch	15.8	[66]
Lactate	Food waste	**64.0**	0.63	12.8	Batch	5	[84]
Propionate	Food waste	**15.8**	0.16	2.0	Batch	8	[80]
1,3-propanediol	Raw glycerol	**82.7**	0.75	73.4	Fed-batch	1.1	[73]
Highest yields							
Acetate	Food waste	7.9	**0.56**	1.8	Batch	4.3	[50]
Butyrate	Municipal waste	1.6	**0.74**	0.8	Batch	2.1	[61]
Caproate	Liquid phase of alcoholic fermentation	NA***	**0.81**	2.1	Continuous	350	[91]
Ethanol	Raw glycerol	8.0	**0.91**	4.8	Batch	1.7	[68]
Lactate	Food waste	64.0	**0.63**	12.8	Batch	5	[84]
Propionate	Sago starch wastewater	2.2	**0.31**	1.1	Semi-continuous	NA	[105]
1,3-propanediol	Refined glycerol	82.7	**0.75**	73.4	Fed-batch	1.1	[73]
Highest productivities							
Acetate	Beverage industry wastewater	3.6	0.18	**57.0**	Continuous	155	[52]
Butyrate	Beverage industry wastewater	4.6	0.39	**73.2**	Continuous	155	[52]
Caproate	Fermented municipal waste + ethanol	11.9	0.46	**26.0**	Continuous	140	[90]
Ethanol	Beverage industry wastewater	0.6	0.06	**9.5**	Continuous	155	[52]
Lactate	Food waste	40.0	0.41	**40.0**	Continuous	152	[85]
Propionate	Cheese whey	6.9	0.26	**27.6**	Continuous	40	[106]
1,3-propanediol	Raw glycerol	82.7	0.75	**73.4**	Fed-batch	1.1	[73]

* Yields are normalized on the total COD content of the substrate. Productivities correspond to average productivities (batch, fed-batch and semi-continuous reactors) or productivities during steady states (continuous reactors).

**Process duration stands for total batch/fed-batch duration if applicable. Start-up duration corresponds to the time required to reach the best performing steady state in continuous/semi-continuous processes. Both durations are not necessarily optimal due to experimental design.

*** Continuous extraction

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DOI: 10.1039/C8GC00572A

Table 6. Best performance of soluble metabolite production by mixed cultures from synthetic fermentation media.

Metabolite	Substrate	Concentration ($g.L^{-1}$)	Yield* ($g_{COD}.g_{COD}^{-1}$)	Productivity* ($g.L^{-1}.d^{-1}$)	Process configuration	Process or start-up duration (d)**	Ref.
Highest concentrations							
Acetate	Glucose	**34.4**	0.31	3.1	Fed-batch	11	[53]
Butyrate	Refined glycerol	**13.5**	0.19	0.5	Fed-batch	27	[107]
Caproate	Lactate	**23.4**	0.72	1.5	Fed-batch	16	[108]
Ethanol	Refined glycerol	**11.1**	0.42	3.7	Batch	3	[109]
Lactate	Glucose	**21.5**	0.92	43.0	Continuous	38	[110]
Propionate	Refined glycerol	**22.6**	0.45	0.8	Fed-batch	28	[81]
1,3-propanediol	Refined glycerol	**81.4**	0.56	23.8	Fed-batch	3.4	[111]
Highest yields							
Acetate	CO_2 + electricity	4.7	**0.90**	0.9	Fed-batch	5	[51]
Butyrate	Acetate + ethanol	11.5	**0.80**	20.0	Continuous	NA	[62]
Caproate	Lactate	23.4	**0.72**	1.5	Fed-batch	16	[108]
Ethanol	Xylose	3.1	**0.61**	1.0	Batch	3	[112]
Lactate	Glucose	21.5	**0.92**	43.0	Continuous	38	[110]
Propionate	Refined glycerol	22.6	**0.45**	0.8	Fed-batch	28	[81]
1,3-propanediol	Refined glycerol	1.7	**0.77**	2.0	Batch	0.8	[113]
Highest productivities							
Acetate	Sucrose	2.7	0.14	**127.9**	Continuous	27	[114]
Butyrate	Sucrose	4.9	0.30	**237.1**	Continuous	NA	[29]
Caproate	Acetate + ethanol	8.7	0.51	**52.2**	Continuous	75	[115]
Ethanol	Sucrose	0.5	0.05	**22.5**	Continuous	27	[114]
Lactate	Glucose	19.2	0.90	**115.0**	Continuous	35	[110]
Propionate	Starch	1.0	0.07	**46.1**	Continuous	NA	[116]
1,3-propanediol	Refined glycerol	81.4	0.56	**23.8**	Fed-batch	3.4	[111]

* Yields are normalized on the total COD content of the substrate. Productivities correspond to average productivities (batch, fed-batch and semi-continuous reactors) or productivities during steady states (continuous reactors).

**Process duration stands for total batch/fed-batch duration if applicable. Start-up duration corresponds to the time required to reach the best performing steady state in continuous/semi-continuous processes. Both durations are not necessarily optimal due to experimental design.

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1193 **Table 7.** Best performance of PHA production by mixed cultures.

Maximized variable	Substrate	Accumulation step		Global process		Ref.
		Yield ($g_{COD}.g_{COD}^{-1}$)	Productivity ($g.L^{-1}.d^{-1}$)	Yield ($g_{COD}.g_{COD}^{-1}$)	Productivity ($g.L^{-1}.d^{-1}$)	
Synthetic fermentation media						
Yields & productivities	Acetate + Propionate + Butyrate	**0.84**	**29.3**	**0.49***	**1.2**	117
Complex substrates						
Yields	Paper mill wastewater	**0.75**	NA	**0.41****	NA	101
Productivity (accumulation)	Sugar-cane molasses	0.63	**259.2**	NA	NA	118
Productivity (global)	Snack industry wastewater	NA	12.0	0.30**	**1.0**	100

1194 * Yield calculated for the selection and accumulation steps

1195 ** Yields calculated for the fermentation, selection and accumulation steps