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Review

Biosystems analysis and engineering of microbial consortia for industrial biotechnology

The development of industrial biotechnology for an economical and ecological conversion of renewable materials into chemicals and fuels requires new strategies and concepts for bioprocessing. Biorefinery has been proposed as one of the key concepts with the aim of completely utilizing the substrate(s) and producing multiple products in one process or at one production site. In this article, we argue that microbial consortia can play an essential role to this end. To illustrate this, we first briefly describe some examples of existing industrial bioprocesses involving microbial consortia. New bioprocesses under development which make use of the advantages of microbial consortia are then introduced. Finally, we address some of the key issues and challenges for the analysis and engineering of bioprocesses involving microbial consortia from a perspective of biosystems engineering.

Keywords: Biomass / Chemicals and biofuel / Industrial biotechnology / Microbial consortia / Systems biology

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1 Introduction

Industrial biotechnology refers to the application of scientific and engineering principles to the processing of natural resources by biological means to provide goods such as chemicals, materials and fuels. It is widely regarded as a key technology to provide future alternatives for the diminishing fossil resources (Fig. 1).

Products such as bread, wine, distilled spirits, vinegar, cheese, pickles and other fermented materials have been with us for centuries, being provided by biological means such as fermentation using bacteria and fungi. In such bioprocesses, natural microbial flora or a small amount of the previous fermented material is usually used as inocula. The processes are carried out thus with a mixed culture (also referred to as microbial consortium in this study). This praxis of fermentation industry with mixed cultures was gradually replaced more and more with pure cultures to avoid contamination of the fermentation process and the product with undesired microbes. In fact, the establishment of submerged fermentation processes with pure cultures was the prerequisite for the successful development of antibiotic production in the 1940s and 1950s and was viewed as the land marker of biochemical engineering and modern biotechnology. To date, the multi-billion-dollar market values of bulk biotechnological products such as amino acids, organic acids and antibiotics and high-value products such as vitamins, enzymes and pharmaceutics are almost exclusively generated by pure cultures of micro-organisms or mammalian cells. There are presently only a few exceptions where mixed culture-based bioprocesses are used. These include processes for traditional foods, beverages/alcohols, waste water treatment and biogas production. In some industrial bioprocesses such as fermentations for acetic acid and vitamin C, a mixed culture may be practically used. Because one species in these processes is so dominating for the whole process, they are normally not considered as a mixed culture process. This applies also to many industrial fermentation processes which are not operated under strictly aseptic conditions.

We argue that the emerging of industrial biotechnology for large-scale production of chemicals and fuels and its ultimate goal of effectively utilizing natural resources requires a rethinking of present strategies of bioprocess development. Processes with pure cultures are normally targeted on a single product. The results are often low-product concentration (typically less than 10% of wt.) and limited yield (typically less

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Abbreviations: **ADM1**, Anaerobic Digestion Model 1; **AHL**, acyl homoserine lactone; **LB**, lignocellulosic biomass; **PDO**, 1,3-propanediol; **PHA**, polyhydroxyalkanoate; **PHB**, polyhydroxybutyrate; **QS**, quorum sensing; **UASB**, up flow anaerobic sludge bed

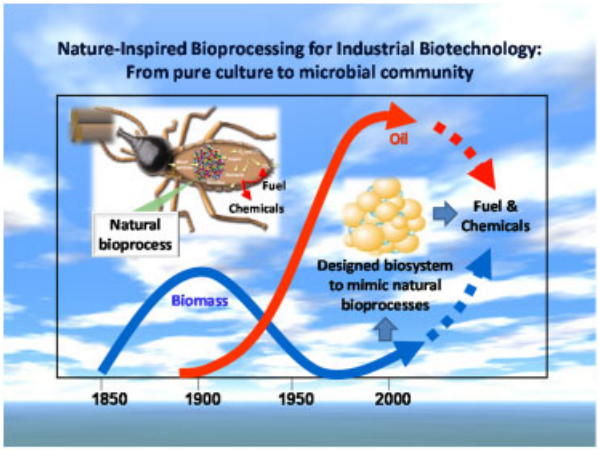

Figure 1. Industrial biotechnology and nature-inspired bioprocessing of biomass: from pure culture to microbial consortia with designed and synthetic biosystems.

than 50% wt. of the substrate). This imposes economical and ecological constraints on the processes due to the high costs of substrate and high energy demand for product purification to remove the large amount of water [1]. If two or more products can be produced in the same process, the utilization of substrate will be more complete and the costs of product recovery could be significantly reduced due to the less amount of water to be removed *per* unit of product. To this end, a mixed culture may be used, in which each organism could use a specific substrate and produce a specific product in the same bioreactor. Mixed cultures can also have several other advantages over pure cultures. A primary advantage is given by synergies of different enzymatic systems and combination of metabolic pathways of different microorganisms that can result in more efficient utilization of substrates and increased product yield. For example, a typical problem of many fermentation processes with pure cultures is the production of byproducts in form of organic acids or alcohols which are toxic to cell growth. In a bioprocess with a mixed culture, the toxic byproduct(s) could be degraded or even converted to another useful product by one of the species, leading to bioprocessing with multiple products and a more efficient use of the substrate(s). This is exactly the goal of the new concept biorefinery that has been proposed as a future direction of industrial biotechnology [2]. Indeed, it might be the only ecologically and economically feasible solution for an effective utilization of complex and abundant biomass materials such as cellulose and hemicelluloses. However, despite great efforts worldwide, biorefinery of renewable materials has demonstrated only the limited success in industrial scale production so far. For the utilization of complex substrates such as lignocellulosic materials, the bioprocesses developed so far are often too complicated and thus associated with high costs and often also low efficiency. In this respect, it is necessary to learn from the nature. In fact, natural efficient "biorefinery processes" of cellulosic and lignocellulosic materials are always associated with microbial consortia. Examples for this can be found in soil, in the hindgut of termites and in ruminant animals (Fig. 1). In this respect, it should be mentioned that about 90–99.8% of the microbes present in natural environmental niches cannot be cultured with currently available technologies, and hence cannot be exploited further for biotechnology with pure culture approach [3, 4]. It may be stated that the biorefinery concept can be best realized with mixed cultures in many cases.

A further advantage of mixed cultures is the possibility of utilizing cheaper secondary products (*e.g.* whey and molasses) or even biomass as substrates for biotechnological production of chemicals. It was recently shown that processes based on the mixed cultures can be established to generate a narrow product spectrum from a mixed substrate [5]. Finally, on an industrial scale, working with mixed cultures or natural consortia under unsterile condition will lower the production costs and thus will open new markets for many biotechnological products. Thus, mixed culture technology could become an attractive addition or alternative to traditional pure culture-based biotechnology for the production of chemicals and bioenergy in industrial biotechnology.

This review article first briefly illustrates some examples for the use of mixed cultures and microbial consortia in established industrial processes. The potential to improve existing processes as well as examples for some novel processes under development are then introduced. It should be mentioned that in this article we alternatively use the terms mixed culture, coculture and microbial consortium. With coculture, we normally refer to cultures with defined species of microorganisms. With mixed culture and microbial consortium, we normally refer to situations where the species are not defined or identified. In the general discussion, however, we also use the terms "mixed culture" or "microbial consortium" to refer to all the cases for simplicity. The emphasis of this article is put on the discussion of major issues and challenges in the analysis and engineering of microbial consortia for industrial biotechnology from a biosystems engineering perspective. In this respect, future research needs and directions are discussed.

2 Examples of industrial bioprocesses involving microbial consortia

In comparison to the many biotechnological processes based on the pure cultures, examples for the application of mixed cultures or microbial consortia in the production of chemicals are relatively few. However, bioprocesses involving microbial consortia were and are still the predominant practice for traditional food and beverage industries. Furthermore, microbial consortia are industrially applied for wastewater treatment, biogas production and biological soil remediation. Bader *et al.* [6] gave recently an excellent overview of relevant processes. We describe in the following briefly a few examples to illustrate the scope and complexity of such processes.

2.1 Traditional food and beverage

Substrates used in producing traditional food and beverage are normally nonsterile and may contain many different types of microorganisms. Thus, there may be activities and growth of

various microorganisms that can ferment the substrate. As a consequence, the natural fermentation is carried out through a sequence of different microbial species. This was done in very early time of the human being, with records dating back to 6000 B.C. in some old civilizations [7]. The number of food products that rely on fermentation in one or more microbial steps for their production is tremendous. In fact, in the dairy field, several new brands of fermented foods are introduced into the market every year [8]. Many of these products belong to the so-called "functional foods" and are made by mixed culture fermentation containing selected bacteria such as *Lactobacillus acidophilus* or *Bifidobacterium* spp. which are claimed to provide several prophylactic and therapeutic benefits [9]. Mixed culture approach was also used to enhance the quality, texture and shelf life of food products. Lozo *et al.* [10] reported that *L. paracasei* subsp. *paracasei* produced bacteriocin on traditionally homemade white-pickled cheese and that this substance inhibited the growth of other pathogenic microbes.

Generally, food fermentations are carried out by mixed cultures which are composed of indigenous microbiota present in the food substrate. This implies that variations in the indigenous biota may affect the composition and activity of the fermenting microorganisms. This has a direct effect on the product quality and the reproducibility of fermentations. To better understand the fermentation process, most of the relevant microbes have been studied, but often separately. Here, population dynamics play a crucial role in the performance of the fermentation. For many years, therefore, studies on mixed culture food fermentations have been focused on analyzing the microbial population and its dynamics using classical and molecular methods. For example, it was shown in the wine industry that mixed culture fermentations using controlled inoculation of *Saccharomyces cerevisiae* starter cultures and non-*Saccharomyces* yeasts represent a feasible way toward improving the complexity and enhancing the particular and specific characteristics of wines. It was reported that the use of selected starter cultures plays an important role in the suppression of wild yeasts that may contribute negatively to the wine [11]. Such studies were supported by the growing demand for new and improved wine-yeast strains that are adapted to different types and styles of wines.

Mixed culture food fermentations are of primary economic importance. The performance of such cultures is not the simple result of "adding up" the individual single-strain functionalities but is largely determined by interactions at the level of substrates, the exchange of metabolites and growth factors or inhibiting compounds. Because of the complexity of most food fermentation, only a limited number of studies are available where quantitative and systems-level approaches are used to study the processes, especially the cellular interactions. Hence, a systems biology approach is highly relevant and desirable for this field. Successes in replacement or addition of more desired probiotic strains in mixed culture fermentation will undoubtedly boost research on dynamics and interactions within these complex microbial populations to support the development of prebiotics and probiotics food.

2.2 Biogas production and granule development in anaerobic bioreactors

Biogas production *via* anaerobic digestion has been industrially used for many years. Recently, biogas production has gained again much attention due to rising energy prices and the need for a better waste treatment. High-rate anaerobic bioreactors are commonly applied for industrial biogas production. The upflow anaerobic sludge bed (UASB) is one of the most common types of bioreactors used. The success of UASB relies on the establishment of a dense sludge bed in the bottom of the reactor, in which most biological processes take place [12]. In these reactors, methanogenic microbial consortia are present as granules, typically in the size of 0.2–5 mm. The granules are characterized by a high-density and high-methanogenic activity [13]. The different types of microorganisms are in close vicinity of each other, representing a paradigm of spatially organized consortia enhancing interspecies electron transfer and thus facilitating high-rate methane formation [14]. The microbial composition of methanogenic granules has been studied by several groups [13, 15, 16]. Generally, the transformation of organic wastes into biogas is considered to occur in four stages (Fig. 2) [17]. During the first stage (hydrolysis phase), biological macromolecules are broken down into oligo- or monomers which are transformed during the second stage (acidogenesis phase) into volatile organic acids, alcohols, aldehydes, ketones, CO_2 and H_2. In the third stage (acetogenesis), the molecules produced in stage 2 are metabolized into acetic acid as well as some CO_2 and H_2. The transforming organisms in the first three stages are primarily bacteria. In the fourth stage (methanogenesis phase), CH_4 is formed *via* decarboxylation of acetate and methanization of CO_2 and H_2 by acetogenotrophic and hydrogenotrophic archaea. Because of the special growth requirement of some of the bacteria within granule consortia, such as a low hydrogen

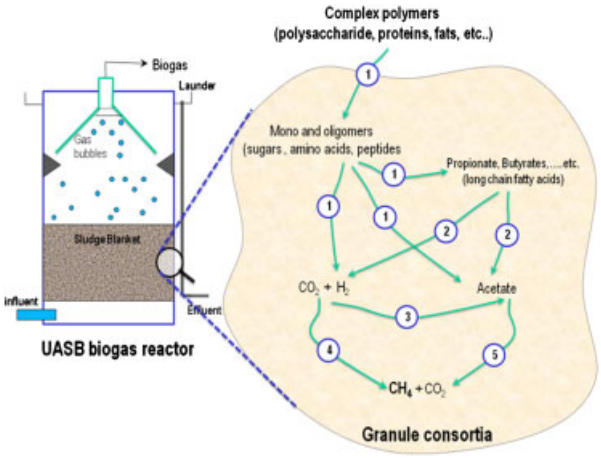

Figure 2. Example of a well-established bioprocess using microbial consortium: the UASB biogas reactor. Also shown are the microbial groups and steps involved in methane production within granules. 1, Fermentative bacteria; 2 obligate hydrogen-producing acetogenic bacteria; 3, hydrogen-oxidizing acetogens; 4, carbon dioxide reducing, H_2-oxidizing methanogens and 5, aceticlastic methanogens.

partial pressure, some of those bacteria are difficult to cultivate using the traditional culturing methodologies. It was proven that spatial organization of the bacteria is critical for thermodynamic reasons and many interspecies syntrophic reactions are only energetically beneficial if hydrogen transfer occurs over distances of a few microns or less [13, 14]. Because of the need for such close proximity, random cell–cell associations would lower the metabolic efficiency. In this respect, signaling mechanisms to organize the syntrophic species can be predicted within the UASB granules [13].

For biogas production, it is rather difficult to describe the whole process by reliable kinetics. Reaction complexity involves the different types of bacteria, the various process parameters (pH, temperature and mixing), and the nature of substrates being hydrolyzed (origin, soluble and insoluble, *etc.*). Therefore, microbial diversity and dynamics of the process are normally not considered, and a lumped organism capable of catalyzing most typical pathways is assumed. Ideally, a synthetic consortium, with well-studied and defined microorganisms is needed to further understand the commensal relationship among microorganisms and to optimize biogas production of anaerobic digesters [18]. Establishing such synthetic biogas sludge will indeed help biogas engineers to introduce new species, and to extend the substrate range to include ready available biomass wastes.

2.3 Bioethanol

Although synthetic ethanol production from the petrochemical ethylene was once the predominant source of industrial ethanol, fuel ethanol is currently produced from sugarcane, corn, wheat and sugar beets by fermentation in a large scale [19]. Industrial ethanol fermentations are normally not performed under sterile conditions, and a variety of Gram-positive and Gram-negative bacteria have been isolated from fuel ethanol fermentations [20]. Generally, the natural nutrient media used permit a growth pattern favoring ethanol fermenting yeast and prevent bacterial overgrowth. Nevertheless, contamination is a serious problem in ethanol fermentation, especially with the fast growing *Lactobacillus* and hence antimicrobial agents are introduced in such fermentation [20].

The industrial bioethanol production from starch generally involves liquefaction with α-amylase, enzymatic saccharification, and finally the fermentation of the sugars to ethanol. Simultaneous saccharification and fermentation with a mixed culture was suggested to reduce the costs of the process. Still, the high feedstock cost poses a major obstacle to large-scale implementation of ethanol as a transportation fuel. Therefore, interest has recently shifted to replacing these traditional feedstocks with the nonfood-based lignocellulosic biomass (LB) feedstocks such as agricultural wastes (*e.g.* corn stover) or energy crops (*e.g.* switchgrass). Many factors, such as lignin content, crystallinity of cellulose and particle size, limit the digestibility of the hemicellulose and cellulose present in the LB [4, 21]. In fact, the carbohydrate composition of the hydrolysate (mainly C6 and C5 sugars) impedes the complete conversion by pure cultures. Genetically modified organisms have been developed. But on an industrial-scale, the production process is still in early stage as the cost will be relatively high with genetically modified organisms. Therefore, the production of ethanol from LB waste was intensively studied and mixed culture approach was thoroughly discussed. Simultaneous saccharification and fermentation of cellulosic material was suggested by Mamma *et al.* [22], and a coculture fermentation with *S. cerevisiae* and *Fusarium oxysporum* was performed. More recently, *Zymomonas mobilis* and *Candida tropicalis* were evaluated for the production of ethanol from enzymatically hydrolysed LB and 97.7% of the theoretical yield of ethanol was obtained [23]. Considerable improvement in this area has been observed using cocultivation of different micro-organisms for ethanol production from cellulose [24, 25]. The same trend was also followed recently by using the cheese whey powder as a cheap substrate for ethanol production by immobilized mixed culture [26].

3 New mixed culture bioprocesses in development

In the following, examples of novel mixed culture processes which are still under development are briefly described.

3.1 Biohydrogen

Hydrogen as the "fuel of the future" has received much attention in the past and renewed interest these days. Presently, hydrogen gas is produced by steam reforming of natural gas and other hydrocarbons requiring high-energy inputs [27]. Fermentation of carbohydrate-rich raw materials for biohydrogen production offers some potential advantages over chemical processes such as mild operation conditions (30–35°C, 1 atm). In anaerobic fermentations, hydrogen is a typical byproduct for regeneration of reducing equivalents. However, regeneration of reducing equivalents can take place by the production of other metabolites as well. If acetate is the only byproduct a maximal theoretical yield of 4 mol H2/mol hexose can be achieved. However, typical yields are <1 mol/mol. Moreover, most bacteria favor to the production of other metabolites (acetate, butyrate and ethanol) because they show little tolerance toward elevated hydrogen partial pressure. In fact, the major challenge for large-scale biohydrogen production is the low fermentation rate and the low hydrogen yield. Hydrogen production from different feedstocks by mixed cultures has been well covered in several recent reviews (*e.g.* [28, 29]). To inactivate most hydrogen scavengers of methonogens or homoacetogens, alkaline, acid and heat pretreatment methods have been investigated [30]. Heat pretreatment turned out to be most promising with respect to hydrogen yield. Methanogens may be suppressed completely in this way but heat-pretreatment is not necessarily effective against homoacetogens [31]. A more recent approach is to use a thermophilic consortium and very efficient hydrogen production could be shown. Moreover, thermophilic bacteria produce fewer byproducts beside hydrogen and the thermodynamic conditions concerning hydrogen partial pressure is more favorable [32]. Heat-treated anaerobic sludge and pure

cultures of *Clostridia* and *Enterobacter* species were used for biohydrogen production by dark fermentation [32]. Recently, Ozmihci and Kargi [33] compared different mixed cultures for biohydrogen production by combined dark and light fermentation and a combination of the anaerobic sludge and the fermentative bacteria *Rhodobacter sphaeroides* yielded the highest hydrogen yield. One has to conclude unfortunately that the hydrogen production *via* fermentation (in pure and mixed cultures) is not sustainable on its own [34]. The substrate energy is converted to the energy carrier hydrogen even under optimized conditions by a relatively low percentage (<40%). The residual energy is retained in the byproducts. This is the reason why a second fermentation stage is suggested for a complete substrate conversion.

3.2 The use of mixed cultures for the production of multiple enzymes

One of the major advantages using mixed cultures is its ability to produce more than one product while simultaneously utilizing either one or mixed substrates. However, few investigations were done in this area. Sun *et al.* [35] used a mixed inoculum of two different strains of *Aspergillus niger* M_2 and M_3 (2:1 w/w) to produce multiple enzymes (proteinase, pectinase and cellulase). They also used the same strains to degrade pectin and tannins. The results showed that the strain M_2 could secrete a high amount of cellulase and pectinase, wheras strain M_3 produced a high amount of proteinase. The two strains benefited from each other and increased the enzyme activities mutually in mixed culture fermentation. Pectin and tannins were degraded by 99 and 66%, respectively.

3.3 Mixed culture process for hydrogen and methane

In 2004, Sapporo Breweries, Shimadzu Corporation and Hiroshima University announced the setup of a pilot plant for hydrogen and methane production. Over several months, a stable hydrogen/methane production was reported. Nishio and Nakashimada [36] investigated this process and showed that this two-stage process is useful for the treatment of sugar-rich wastewater and bread wastes (Fig. 3). Bread waste fermented with thermophilic anaerobic sludge at 55°C was converted to hydrogen and volatile fatty acids (mainly acetate and butyrate) in the first stage and to methane in a second stage; the suspended solids were reduced by 91%. Similarly, another Japanese group established a two-stage process in pilot-scale [37]. A thermophilic microflora produced hydrogen and methane with garbage and waste paper as substrate at 60°C. Despite the unsterile process, *Thermoanaerobacterium* species from the inoculated microflora were dominating in the hydrogenotrophic stage. Hence, the thermophilic process strategy reduced the risk for contamination effectively. In another process, ethanol, hydrogen and methane are produced in a "Maxifuel" concept by mixed culture consortia [38]. After an efficient pretreatment of LB, the C6-sugars are converted at 37°C to ethanol by a pure yeast culture and in a second stage at 70°C C5 sugars are converted by a pure *Thermo-*

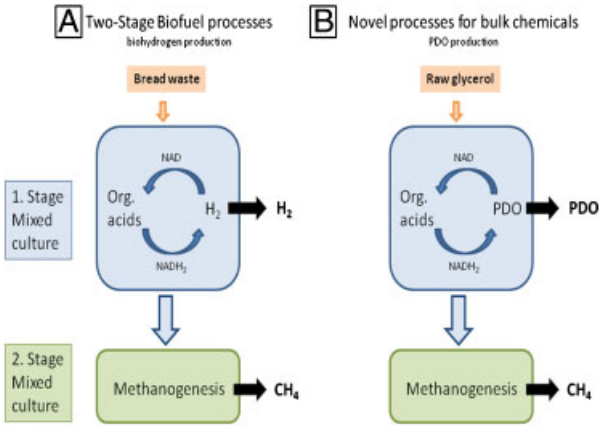

Figure 3. Examples of multiproduct mixed culture fermentation processes.

anaerobacterium culture to ethanol and hydrogen. After evaporation of ethanol, the effluent is digested by mixed consortia to methane. It was reported that the second step may also be carried out with a mixed culture under the same conditions [39].

3.4 Mixed culture for 1,3-propanediol

Glycerol, especially crude glycerol as byproduct directly from biodiesel production plants is an interesting substrate for the industrial biotechnology. Crude glycerol normally contains impurities such as alcohols, salts, heavy metals and water, and may need to be purified for certain fermentation processes with pure culture [40]. Recently, mixed culture processes were developed to convert crude glycerol into 1,3-propanediol (PDO). PDO is a building block of the polymer poyltrimethyleneterephthalate which represents a new generation of polyester with superior properties [41, 42]. Conventionally, microbial production of PDO is carried out by using single microorganism, either natural strains with glycerol as substrate or genetically engineered one with glucose as a substrate [43, 44]. The bioconversion of crude glycerol into PDO is of particular interest as a part of a biorefinery concept either for biodiesel or for bioethanol production. In both cases, glycerol can be obtained as a byproduct with impurities as mentioned above. The microbial conversion of glycerol to PDO is, however, always associated with production of organic acids as byproduct because of the necessity of balancing the reducing power. This results in two major problems. First, organic acids are toxic and limit cell growth and thus productivity of the process. Second, only about half of the substrate (glycerol) is converted into PDO, leading to an incomplete use of the substrate. Fermentation with mixed culture was proposed as an interesting and effective solution to these problems [45]. In this mixed culture process, the toxic byproducts from glycerol bioconversion are converted to methane by methanogenic organisms, simultaneously in the same bioreactor or in a subsequent stage (Fig. 3). This concept was successfully demonstrated in laboratory scale and is being scaled-up in

pilot plant (results not published). Using crude glycerol (80% glycerol) as a carbon source and inocula adapted from a local wastewater treatment plant, PDO can be produced as the main product at concentration as high as 60 g/L in a not yet optimized semi-batch culture. A high yield of 0.6 mol PDO/mol glycerol, which is close to the theoretical maximal yield of anaerobic glycerol conversion, has been achieved (own data not published). The byproducts, mainly acetate and butyrate, are degraded to methane and CO_2. Another major advantage of this mixed culture process is to operate the process in a very simple bioreactor without sterilization, leading to significant reduction in investment and operation costs. Furthermore, unlike with pure culture for anaerobic glycerol conversion, sparging during the fermentation (with N_2 in this case) is not necessary, obviously because of the generation of an anaerobic environment by member(s) of the microbial consortium and synergetic effects concerning generation and consumption of CO_2 and H_2. The avoidance of aeration can not only reduce investment and operation costs, but also simplify the downstream processing by reducing foaming in the filtration and evaporation stages.

Selembo *et al.* [46] tested glycerol and glucose, separately, for their ability to simultaneously produce PDO and hydrogen under unsterile condition in 300 and 500 mL serum bottles. The inoculum was derived from four different origins: tomato soil, wheat soil, compost and sludge and each was pretreated with slightly different heat techniques. Among the inocula, wheat soil was found to be the best for glycerol fermentation, whereas compost was most suitable for glucose fermentation. Both pure and industrial glycerol was used in the experiments. However, no PDO was produced using glucose as substrate. Simultaneous production of PDO and hydrogen was also obtained in continuous fermentations with mixed culture by Temudo *et al.* [47]. The anaerobic mixed culture used as inoculum consisted of a mixture of two types of sludge, obtained from distillery wastewater treatment plant and potato starch processing acidification tank. However, the yield obtained from the experiments was not satisfactory.

3.5 Propionic acid

The production of propionic acid by *Propionibacteria* has been investigated over the last decades. To overcome the slow growth rate and product inhibition, several strategies such as cell retention systems have been proposed [48], but the process is still not economically feasible. Usually, cheese whey or whey permeate derived from dairy industries at high concentrations are used as substrates. However, since lactose, the main sugar found in whey, is not the ideal fermented sugar for *Propionibacteria*, better substrate affinity was realized in a coculture with *L. plantarum* [49]. *L. plantarum* converts lactose to lactate which can be converted more rapidly by *Propionibacteria*. This carbohydrate to propionate fermentation *via* the intermediate lactate was proposed in the 1980s [50]. It is reinvestigated recently to ferment whey to propionate with a coculture of *L. zeae* and *Veillonella criceti* and to use the dried fermentation broth as a conservative agent in bread against mould growth. Recently, it was also shown that sourdough fermented with *L. buchneri* and *L. diolivorans* can be used for bread preservation as well [51]. Thus, a nonsterile process with cheap substrates can be used for propionate production in a mixed culture process.

3.6 Polyhydroxyalkanoate

Another potentially interesting process utilizing microbial consortia is the production of polyhydroxyalkanoate (PHA) as raw material for biodegradable plastics. Microorganisms such as *Ralstonia eutropha*, *Alcaligenes lactus* and *Burkholderia sacchari* are used in their wildform in industrial production processes [52]. The major polymer produced in the microorganisms is polyhydroxybutyrate (PHB, [52]). Apart from PHB, other PHAs synthesized include polyhydroxyvalerate, polyhydroxymethylvalerate and polyhydroxymethylbutyrate, depending on the substrate(s) used. The commercial production of PHB currently employs genetically modified *Escherichia coli* and *Alkaligenes* species [5]. Disadvantages of the pure culture production of PHB include the high costs for the pure substrates utilized and the costs for sterile operation of the final production process. Therefore, the potential use of mixed microbial cultures for the production of PHA and the simultaneous treatment of waste streams was investigated recently [5, 53]. Natural selection of the microbial consortia for PHA accumulation was successfully realized by alternating between periods with substrate (feast phase) and without substrate (famine phase) in an aerobic sequencing batch reactor. Using such natural microbial consortia in an unsterile operation, it was shown that up to 80% of the dry biomass formed in the process can be recovered as PHA [5]. This is only slightly worse than the genetically modified *E. coli*-based process (http://www.metabolix.com), and potentially sufficient for establishing an economically feasible process [5].

4 Major issues and challenges for the analysis and engineering of bioprocesses involving microbial consortia

4.1 The need for novel microbial cultivation methods

More than 90% of the microbes present in natural environment cannot be cultured with currently available technologies [3, 4]. Accessing this "missing" microbial diversity is of significant interest for both basic and applied sciences, and has been recognized as one of the principal challenges for contemporary microbiology. Even in the era of post-genomics with the different molecular and omics tools and methods such as RNA profiling, metabolomics and proteomic, the establishment of pure cultures is still obligatory for detailed physiological characterization to further study the mixed culture. The traditional culturing strategies are generally selective and biased toward growth of specific microorganisms. This is the major reason for the failure to cultivate most microorganisms in pure culture. Hence, research is needed and novel techniques for the isolation of pure culture are expected to show more success in the next few years. In this connection,

it is interesting to mention that the addition of quorum-signaling compounds or helper strains can facilitate growth of otherwise "uncultivable" microorganisms [54]. More recently, Nichols *et al.* [55] reported a microfluidic microsystem approach as a high-throughput screening method for uncultivable organisms.

There is also a need to develop cultivation systems for defined or identified microbial consortia for functional studies. One difficulty in applying functional genomic methods to study microbial consortia lies in the separation of the organisms for extracting intracellular materials. A two-chamber reactor system as shown in Fig. 4 could be used to solve this problem for defined coculture process. This reactor system was originally developed for dialysis high-cell density fermentation [56]. It is being used to study minimal microbial consortia for a simultaneous production of PDO and methane as shown in Fig. 3. The cultivation system allows the study of molecular interactions between two organisms and the intracellular processes in the individual organism. Membranes with different cut-off of molecular weights can be used to selectively study interactions due to different classes of substances (*e.g.* low and high molecular weight). This reactor system can also be used to study microbial communities with more than two organisms by constraining each time one organism in one chamber and the rest in the other one and studying the effects and behavior of the constrained one successively. The overall behavior of this reactor system can be compared with that of mixed culture in order to identify possible effects of direct cellular interaction. Alternatively, effects of concentration gradient can be studied which even better mimic natural environment in many microbial consortia.

4.2 Understanding the type of microbial interactions

There are several types of microbial interactions that may be involved in a mixed culture fermentation process, mutualism, synergism, amensalism, food competition, predation and parasitism. Either individually or in combination they may influence the functioning of the desired microbial process. In fact, detailed studies of the physiology of individual or predominating microorganisms should be performed to establish their requirements with respect to various environmental factors (nutrients, temperature, pH, oxidation–reduction potential, removal of waste products or toxic materials) and then to determine how these factors affect their capabilities. The sum total of this information will indicate the possible interactions between the different microorganisms and will form the basis for conducting experiments either in the laboratory or with the mathematical models. It is, however, the complex web of interactions among the species that defines the structure of communities and how those communities respond to environmental change.

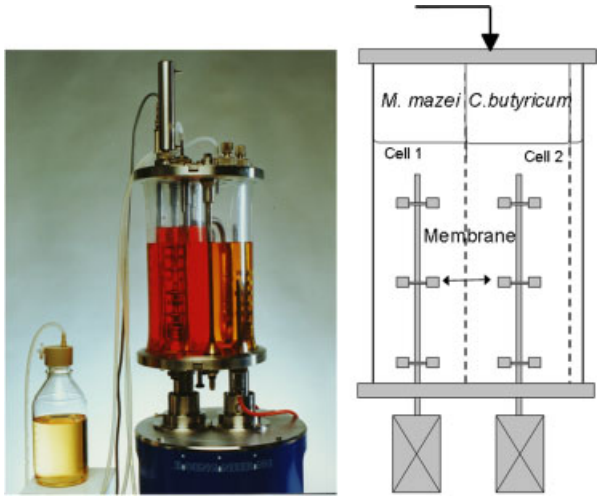

Figure 4. A two-chamber bioreactor system suitable to study interactions of microbial consortia.

One form of interspecies interaction, that is frequently evident in mixed culture industrial operation, is mutualism, in which two or more species provide a net benefit to one another. There are numerous examples of mutualisms of different degrees of coupling in nature. For instance, a mutualistic interaction between archaea and bacteria is suggested to be responsible for the anaerobic oxidation of methane, an important component of global methane cycles [57]. In the dairy industry, the cooperative behavior of *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus* for the manufacture of yoghurt was documented for many years. This behavior resulted in improved quality and stability of the final product compared with monocultures [58]. Mutualisms may be especially important in microbial consortia, where multiple species are involved in degrading organic substrates and thus the huge potential for the utilization of the metabolic pathways of all involved strains in a coculture situation. In fact, "syntrophy" is one form of microbial mutualism that is commonly involved in the degradation of organic substrates by microbial consortia. In syntrophic interactions, the transfer of metabolites between species is essential for growth [59]. An example of such interactions is the widely distributed phenomenon of interspecies hydrogen transfer between methanogens, which use hydrogen to gain energy by reducing carbon dioxide into methane in biogas production process (Fig. 2).

Amensalism is an interspecies interaction in which one organism adversely affects the other organism without being affected itself. It frequently occurs in food fermentations and the major end products by one group of the microorganisms are effective growth inhibitors of spoilage organisms [60]. Another example is the production of antimicrobial compounds such as bacteriocins that are produced by many food-fermenting lactic acid bacteria and play an important role in mixed culture population dynamics.

Microbial competition for limited natural resources within a community is believed to be the selective force that promotes biosynthesis of antimicrobial compounds. Inhibition of growth of different competitors by the produced antimicrobial compounds is common for surviving and thriving in most natural environment. In fact, the biological role of antimicrobials compounds has recently been the subject of some controversy and several investigators have proposed that the true function of these molecules in nature is to act as signal

molecules within and between species [61]. It was proven that growth rate and population dynamics in mixed dairy fermentations are largely determined by the ability to utilize amino acids efficiently [7].

Parasitism is the interaction in which one species benefits at the expense of another. A well-known example of parasitism in the microbial world is represented by bacteriophages. In some cases, phage attack may suddenly inactivate dominant strains in a fermenting culture, leading to failure and product losses in industrial fermentations. Interestingly, the recombination machinery of bacteriophages and their ability to transfer DNA from one bacterial cell to another may accelerate evolutionary processes in bacterial communities and contribute to the diversity in mixed culture fermentation processes. Recently, understanding this type of interaction was the basis for a potential new strategy for treating some bacterial disease [62].

Finally, predation as interaction between microbes is also abundant in many natural environments. However, very few potential applications in industrial biotechnology are presented in the literature. Nevertheless, the sociobiology of bacteria, largely unappreciated and ignored by the microbiology research community two decades ago is now a major research area, catalyzed to a significant degree by studies of communication and cooperative behavior among the predatory Gram-negative Myxobacteria [63].

Despite of intensive research in this field, elaborating the exact type(s) of microbial interaction(s) involved in a consortium remains a major challenge. One recent example is the production of plantaricin by *L. plantarum* NC8 which takes place only after cocultivation with specific Gram-positive strains or even the addition of heat-killed cells from some of the inducing strains [64]. More recently, it was proven that Lactacin B production by *L. acidophilus* was only induced if it sense live target bacteria [65]. These behaviors and others cannot be explained by merely one of the microbial interactions mentioned above. It is a challenge to understand the simultaneous involvement of several different microbial interactions in one and the same industrial process. It is even more challenging but of great importance to control the interactions of microbes. Brenner *et al.* [66] proposed a synthetic biology approach to engineer the interactions of microbes (see below). For this purpose, it is necessary to understand the molecular mechanisms involved in microbial interactions.

4.3 Characterizing the molecular mechanisms involved in microbial interactions

As a means of coordinating responses to changing environmental settings and surrounding microbes in a mixed culture population, bacteria have evolved a number of complex communication systems [67–76]. Bacterial communication, often termed as quorum sensing (QS), has attracted intense research interest in recent years, especially for pathogenic bacteria, where the release of some virulence factors is shown to be controlled by QS. QS is characterized by the secretion and detection of small molecules within a bacterial population, leading to the realization of coordinated behaviors upon establishment of a sufficient quorum. Typically, cell-free supernatants collected from cultures at high density will elicit responsive gene expression when presented to cells at lower density, and this response is often strain specific [67]. The natural QS systems in Gram-negative bacteria often use acyl homoserine lactones (AHLs or autoinducer) as communication signals. Gram-positive bacteria often use small peptides as the QS signals. In all QS systems, signals are produced intracellularly and transported to the extracellular environment. The smaller AHLs diffuse freely across bacterial cell membranes, whereas peptides and large AHLs appear to be actively transported by pumps. During the growth of a bacterial population, the concentration of signal molecules increases and they act on neighboring bacterial cells. Achievement of a critical threshold concentration results in: (i) activation of a sensor/response regulator, responsible for signal transduction, which in turn triggers the expression of multiple genes and (ii) activation of a positive autoinductive feedback loop to amplify QS signal molecule generation (Fig. 5). Peptide signals and also some AHLs are typically sensed by membrane-associated receptors to initiate a phosphorylation cascade that leads to target gene expression. Briefly, the canonical quorum-sensing function is assumed to assure an individual cell of a critical population density before undertaking expression of specialized functions. Nevertheless, under some conditions, the secretion of autoinducers may become too taxing on available resources or may even stimulate unwanted attention from neighbors or host cells. Therefore, contact-dependent signaling cascades offer a means for more direct, and possibly less costly, communication between bacteria [68]. Like QS, contact-dependent signaling is also prone to modulation by cell density, with high cell numbers increasing the likelihood of interbacterial contact and subsequent signaling.

4.4 Recognizing the types of signaling molecules and the keystone species "leader" in a mixed microbial population

AHLs are far from unique as intercellular signals between bacteria. Many studies in the last few years have led to the identification of many additional classes of signaling molecules. Beside qunilone produced by *P. aeruginosa* or butyrolactones produced by *Streptomyces* species which are of low-molecular-weight diffusible substances analogous to AHL system, many other distinct signaling molecules including branched chain fatty acids, amino acids, peptides, oligopeptides and even proteins are now involved. Peptidoglycan fragments of the cell wall of both Gram-positive and Gram-negative were reported to have also a signaling function [69]. Moreover, antimicrobials and toxins were described in some cases to serve as signals molecules and the overdose of a particular signal can be lethal, as with mammalian hormones like insulin. It has been suggested that, although the definition of signaling is too stringent to include most antimicrobials and other secondary metabolites, these molecules might act as cues or chemical manipulators as well as serving other functions, such as altering central metabolic pathways, contributing to nutrient scavenging or participating in developmental

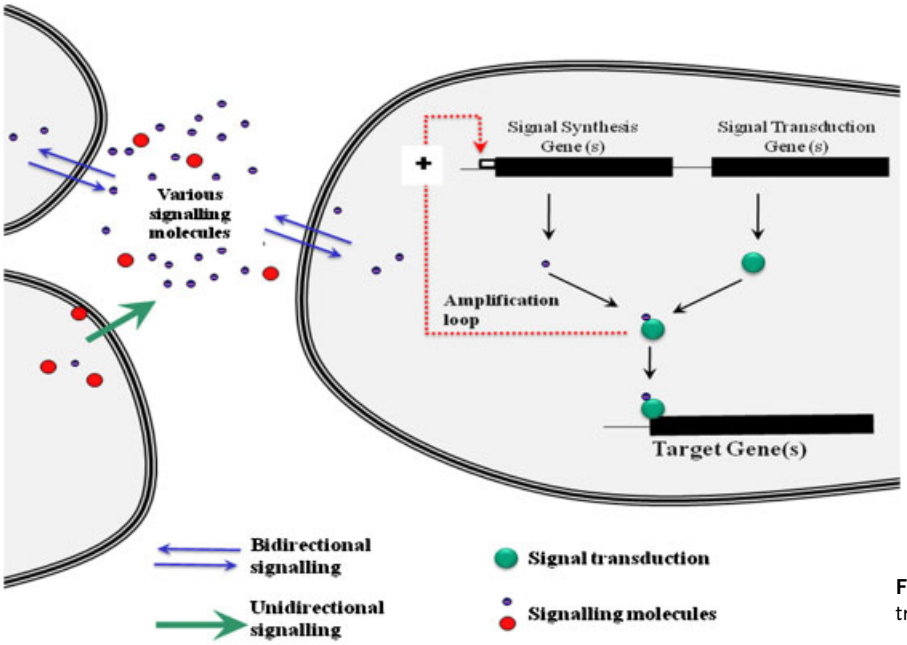

Figure 5. QS signal generation and transduction circuit.

pathways. In agreement with this, it was shown that the redox-active phenazine pyocyanin pigment produced by *P. aeruginosa* influence gene expression in several bacterial species [70]. It should be stressed here that the tremendous diversity of oligopeptides makes them especially suitable when a high degree of discrimination is required, as for instance the building up of a mixed consortium or biofilm formation.

One major challenge of understanding a mixed microbial culture is to identify the "keystone" species within a multi-species consortium. The existence of strain producing anti-microbial metabolites will indeed constrict the mixed microbial living in its vicinity. Moreover, bacteria are often thought of as unable to differentiate into distinct coexisting cell types. This involves the autocrine signaling in which all the cells in the population produced and respond to the same signal. Yet, it was shown that bacteria can develop into morphologically complex multicellular communities composed of different subpopulations of specialized cell types [71], and evidence was presented for paracrine signaling ([72], Fig. 5). It was shown that some more experienced cells produce a signal that induces neighboring cells to adopt a different fate and hence communicate in unidirectional conversations [72, 73]. The new findings strengthen the view that bacterial communities can be thought of as multicellular organisms. One-way signaling occurs also when the *cyano-bacterium Anabaena* grows as filaments and, under nitrogen-limiting conditions, about every tenth cell differentiates into a nitrogen-fixing heterocyst that prevents adjacent cells from becoming heterocysts, but in this case the two cell types rely on each other for survival [72, 73]. This newly discovered phenomenon raised interesting questions about how and why distinct subpopulations arise and coexist in a specific microbial consortium and whether a leading population is then responsible for the consortium's success. In this respect, Goldman and Brown [77] demonstrated that although multispecies interactions can play a part, more often a single "keystone" species solves the target of consortia selection.

4.5 Tools for characterization and quantifications of mixed microbial cultures

An important aspect in working with microbial consortia is the characterization and quantification of species involved and their dynamics. The techniques developed so far can be classified in three groups: molecular biological, biochemical and microbiological [78]. Table 1 summarizes techniques that are frequently used in quantification and qualification of microbial consortia. Among the methods, molecular biological ones are of particular importance since the material (samples) for analysis can be directly extracted from the microorganisms and it does not involve specific culturing. On the contrary, the majority of the biochemically and microbiologically based methods lie on the ability to culture the microorganisms. However, when a defined culture is used, these methods can provide an economical and fast way to characterize and quantify the population dynamics.

New techniques of sequencing allow the sequencing of the genomes of all the organisms in a microbial consortium (metagenomics) in a rather short period of time now. This opens up a new horizon to characterize the population, the roles of individual organisms and their interactions in a microbial consortium as demonstrated for the anammox bacteria [79]. These bacteria are important since they convert nitrite and ammonium directly to nitrogen and are major players in the nitrogen cycle. However, the very slow growth rate (2 wk doubling time) and the unavailability in pure culture hinder their examination by classical methods. Recently, through the use of novel techniques such as

Table 1. Different techniques used for qualification and quantification of microbial consortia (modified from [78]).

Category	Method	Description
Molecular biology method	*PCR based*	
	Denaturing gradient gel electrophoresis (DGGE)	Separates amplified 16S rDNA molecules by %G-C content by denaturing agents
	Amplified ribosomal DNA restriction analysis (ARDRA)	Separates amplified 16S rDNA molecules by restriction patterns
	Terminal-restriction fragment length polymorphism (T-RFLP)	Separates amplified 16S molecules by restriction patterns, labeling by fluorescent dyes
	Hybridization	
	FISH (Fluorescence *in situ* hybridization)	Identifies the presence of desired sequences using fluorescent-labeled probes
	DNA microarrays	Extremely high-throughput multiple probe hybridization
Biochemical method	Metabolic assays	Profiles total metabolites produced by a consortia
	Lipid analyses	Profiles based on the distribution of various membrane lipids
Microbiological methods	Cell counting	Direct cell counting, indirect cell counting and morphological counting
	Flow cytometry and cell sorting	Physically separates microbial assemblages on the basis of measurable properties

community genome sequencing, the metabolism of anammox bacteria from the community genome was partially deciphered [79]. Indeed, large metagenomics sequencing projects that analyze genomic DNA directly from environmental samples are providing much detail on the genetic diversity and potential within selected environments. Present emphasis of metagenomics is in finding new molecules as potential biocatalysts or pharmaceutics. How functional genomic tools (transcriptomics, proteomics, fluxomics and metabolomics) can contribute to the qualitative characterization and quantification of cellular processes in a microbial consortium is a compelling question. There are only a few examples in the literature where DNA microarray and metaproteomics have been used to characterize anaerobic biodegradation and biomethane production [80]. The major obstacle to the utilization of microarray tests for transcriptome analysis of a multiple species population is the cross-hybridization of the partner species' DNA on the spots of the microarray test, which is defined for a specific organism. Protein identification from metaproteomic analysis is also difficult. Furthermore, technical challenges such as extraction and isolation of RNA, proteins and metabolites from complex substrate media in a mixed microbial culture need to be addressed.

4.6 Mathematical modeling and network analysis of mixed culture processes

Mathematic modeling of bioprocesses can be done at macroscopic or molecular levels. The well-known macroscopic model for mixed culture is the Anaerobic Digestion Model 1 (ADM1) [81]. This model considers biochemical (*e.g.* hydrolysis) and physico-chemical (*e.g.* dissociation) processes. It is aimed at direct and simple implementation in full-scale plants and should assist transfer from research to industry [81]. It already proved to be beneficial for full-scale industrial applications [82]. For example, in a UASB system treating recycling paper mill wastewater the industry partner investigated the cost-effectiveness of pH regulation for reducing $CaCO_3$ precipitation. It could be shown that the impact of acid dosing would be less than 10% and is therefore not recommendable. Another industry partner wanted to decide, whether a thermophilic processing would reduce ammonia inhibition. After implementing the system into the ADM1, it was calculated that the changing to thermophilic conditions will have no significant impact on ammonia inhibition or reactor stability. ADM1 seems to be quite suitable for degradation processes. A general problem of this model is that anabolic reactions are predicted worse than catabolic reactions. For instance, ADM1 has fixed stoichiometric parameters for glucose fermentation products which limit the product spectrum and product concentrations that may be predicted. This is the reason why recent research studies implement product formation, *e.g.* hydrogen production into ADM1 [83]. Still it is not possible to use the macroscopic approach to model complex organic acid and alcohol production processes and their regulation.

To this end, mechanistic and dynamic models are highly desirable. Such models could be theoretically very useful for quantitatively understanding interactions between organisms in a microbial consortium. With this respect, impressive progress has been made for pure culture in recent years. However, the models and algorithms for mechanistic and dynamic modeling can be generally only applied to small systems with a few variables. For mixed cultures, they are hardly applied due to the complexity. Furthermore, cellular interactions and population dynamics are generally not considered in models developed for pure cultures. New concepts and algorithms are needed in this respect.

Metabolic flux and control analyses have been developed as very useful tools to study metabolic pathways of pure cultures (for a review, see [84]). Various levels of sophistication in modelling approaches are in use. From a methodological viewpoint, stoichiometry-based metabolic flux analysis is

a mature tool. However, without energy balancing and information about the intracellular processes the flux balances are often underdetermined. To overcome this problem and especially to estimate intracellular fluxes and their distribution around branching points, sophisticated labeling techniques (*e.g.* with ^{13}C labeling) and algorithms have been developed. This method involves the extraction of intracellular metabolites or proteins of cells for isotopomer modelling. Because the metabolites and proteins of individual organisms in a mixed culture population can be hardly distinguished, the isotope-labeling techniques cannot be directly applied to mixed culture for the purpose of metabolic flux analysis.

Rodriguez *et al.* [85] adopted an energy-based metabolic flux analysis approach to predict the product spectrum in an undefined mixed culture. Stolyar *et al.* [57] and Bizukojc *et al.* [44] incorporated an optimization algorithm into metabolic flux analysis of a defined microbial consortium at a relatively large network level. For this purpose, the set of reaction fluxes that can yield the highest amount of energy are calculated. An optimization problem is defined in which the reaction fluxes are the decision variables and the total energy yield is the objective to be maximized while satisfying several constraints (*i.e.* mass conservation and thermodynamic laws). Specifically, Bizukojc *et al.* [44] analyzed the mixed culture composed of a glycerol degrader (*C. butyricum*) and a methanogen (*M. mazei*) as described above (Fig. 6) for the simultaneous PDO and methane production. Simulations were performed for different scenarios. In addition to the effects of exchange of the metabolites acetate, CO2, H2 and formate, the influence of methanol was also investigated, because it is added in excess during biodiesel production and remains in the glycerol water. Usually it is recovered by evaporation but this energy-consuming operation unit could be omitted in the suggested PDO production process. In this case, the methanol can enhance the methane production and the growth of the methanogens. This consequently reduces the concentration of acetate, a compound that inhibits glycerol degrader. The

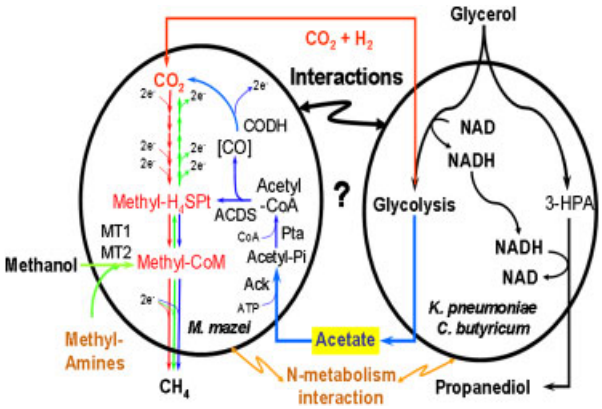

Figure 6. Simplified scheme of methanogenesis pathways in *M. mazei*, glycerol metabolism in propanediol producers (*K. pneumoniae* and *C. butyricum*) and their possible interactions in a coculture. The three methanogenesis pathways (methylotrophic pathway in green arrows; acetoclastic pathway in blue arrows and hydrogenotrophic pathway in red arrows) converge on the reduction of methyl-CoM to methane.

analysis revealed that if *C. butyricum* produced no hydrogen, it would be preferable for acetate scavenging. This is exactly the ideal case for an optimal PDO production [86]. In the case of methanol addition to the defined coculture, a favorable effect on methane production can be proven. Under certain consumption rate of methanol, a somewhat higher PDO yield can be predicted. In addition, acetate and formate utilization can be facilitated, leading to a better methane production. This conceptual study shows the potential of metabolic flux analysis for the optimization of a mixed culture for the industrial utilization of a waste stream from biodiesel production. However, the method applied cannot provide information about selection and regulation of some of the key intracellular pathways in this mixed culture such as the three methanogenesis pathways (methylotrophic pathway; acetoclastic pathway and hydrogenotrophic pathway) in *M. mazei* which converge on the reduction of methyl-CoM to methane (Fig. 6).

Modelling at a network level is an important aspect of systems biology. This starts with the reconstruction of networks at different molecular levels with respect to metabolism, regulation and signal transduction. Impressive progresses have been made in these areas for single organism with data from functional genomics and with the help of computational tools. However, the reconstruction of metabolic and regulatory networks of the individual organisms in such a community is still a not solved problem that deserves further research. Since genome-scale networks are generally very large and complex and a fully kinetic description of the reactions and regulations involved is out of reach, it is a compelling issue and challenge to quantitatively describe and to predict the behavior of cells under various environmental conditions in a microbial consortium at a network level. New approaches and algorithms are also needed to combine the methods of detailed mechanistic modelling with a network-oriented approach to address some of the important issues such as cellular communication in a microbial consortium in a systems biology approach.

4.7 New types of bioreactors and operation strategies

From an engineering perspective, new types of bioreactor and operation strategies are needed for a successful use of mixed cultures or microbial consortia for production purposes. For the example of glycerol bioconversion by a microbial consortium as described above (Figs. 3 and 6), a high yield of 0.6 mol PDO/mol glycerol can be reached which is close to the theoretical yield in pure culture. However, the concentration of the biogas obtained was still not sufficiently high with the microbial consortium in a "one-pot" bioreactor. A two-stage continuous fermentation process seems to be favorable to enhance the biogas production. Two bioreactors with different retention times can be coupled. By this, an optimal growth for the fermentative bacteria and methanogenic microorganisms, respectively, can be optimised. In the first reactor, PDO and organic acids are produced as main and byproducts, respectively. In the second reactor, PDO remains in the reactor, whereas the acids are degraded to biogas. New concept in bioreactor design may also enable running the process effectively in a one-stage process. One example of such a potential

bioreactor design is the fixed-film fermenter UFP from HF Biotec GmbH Berlin, Germany (Fig. 7). In this bioreactor, the retention of biomass (as biofilm) is provided by the fixed film in the upper part. No stirring device is needed. High throughput is possible at low retention time (2–4 days). This reactor provides compartments for different organisms which need different conditions and residence time. Also other types of biofilm bioreactors may be adapted for microbial consortia [87]. For a technical application of such biofilm reactors, extensive engineering investigations and optimizations are needed which range from mass and heat transfer, flow patterns, residence time and microbial heterogeneity in the different zones to reactor configuration and scaleup.

The compartmentation of microbes can also be achieved by immobilization of the microbes in structured carrier materials. It is conceivable to design "onion"-like or multilayer porous structures as "bioparticles" to immobilize different microbes for a defined microbial consortium to carry out a desired bioconversion process as shown in Figs. 2 and 3. Nutrients or signal molecules may be precoated in such porous materials to support cell growth and cellular communication. Even enzymes may be introduced as components of the bioparticles. In a long term, such bioparticles may be developed to mimic natural microbial consortia as found in the gut of termites (Fig. 1). A number of fundamental issues related to material sciences such as manufacturing of the bioparticles with the desired pore structure, particle size and mechanic strength and to chemical engineering such as mass transfer an flow patters should be studied.

4.8 Engineering microbial consortia by synthetic biology approach

Another prospective for engineering microbial consortia is the use of synthetic biology approach. Brenner [66] reviewed recent efforts to engineer communication among different organisms for the development of synthetic microbial consortia. These synthetic microbial consortia can be used to study the behavior (*e.g.* commensalism, amensalism and parasitism) of interacting populations in a minimal microbial consortium or to mimic microbial interactions under controlled conditions. These clearly defined and engineered consortia can be described through mathematical models more easily than natural systems are. They can thus be used to develop and validate models of more complex systems. From an application perspective, Brenner *et al.* [66] argued that synthetic consortia can have interesting potential uses in healthcare such as more efficient drug-delivery devices and gene-delivery vehicles due to two favorable traits: (i) great complexity of functions available and (ii) robustness to changes in the environments. No examples of such potential uses of synthetic consortia for the production chemicals and fuels in the context of industrial biotechnology have been reported so far. In fact, engineering microbial consortia with molecular biological tools for industrial application is just in the infancy and faces several challenges such as overcoming the problem of horizontal gene transfer and maintaining homeostasis [66]. For application in industrial biotechnology, it appears to be more attractive and realistic to select suitable natural microorganisms and combine them in synthetic way for novel functions as illustrated in this article with the examples of hydrogen production and bioconversion of glycerol.

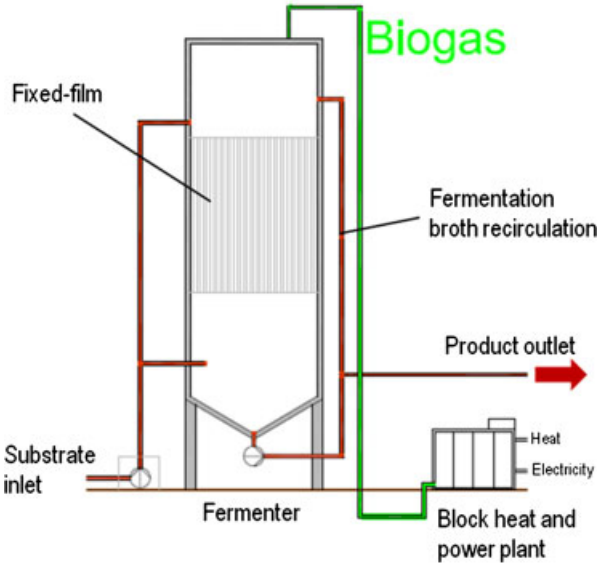

Figure 7. Example of a fixed-film bioreactor for compartmentation of microbes (Picture from HF Biotec, Berlin).

5 Concluding remarks

Microbial consortia play important roles in many traditional industrial bioprocesses such as for food and beverage production, waste material treatment and biogas production. The advantages of bioprocesses involving microbial consortia for the production of chemicals from renewable materials, especially in combination with the production of biofuels in the frame of the concept of biorefinery, have been recently also recognized as illustrated in this study with the examples of production of PDO and H_2. Rapid development in the sequencing of microbial consortia opens up both many opportunities and challenges for industrial biotechnology with microbial consortia. As briefly discussed in this article, new microbial cultivation techniques and bioreactors, new tools for qualitative and quantitative characterization of species and their interactions, new methods to study cellular communication and signaling and new concepts and algorithms for mathematical modeling are desperately needed. In particular, functional genomic studies and systems biology of microbial consortia are just in their infancy and many technical problems and conceptual issues have to be solved. In a short to middle-term perspective, the use of defined or minimal microbial consortia involving a few species seems to be promising. They can well serve as model system(s) for method and technology development [88, 89]. They are particularly useful for engineering of microbial consortia using synthetic biology approach. The synthetic biology adds one dimension more on the possibility of making use of microbial consortia for

industrial uses. In this connection, it appears particularly promising to integrate technologies from material sciences into this exciting area. With the purposeful design of structured materials, we could develop synthetic biotechnological systems to better mimic efficient natural microbial consortia for bioprocessing of complex materials such as lignocelluloses. To achieve this goal, we need to have a systemic and engineering understanding and description of the different phenomena and processes from molecular to process levels in a multiscale and interdisciplinary approach.

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Conflict of interest

The authors have declared no conflict of interest.

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