Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Synthetic co-cultures: novel avenues for bio-based processes

Martijn Diender, Ivette Parera Olm and Diana Z Sousa

In nature, microorganisms live in multi-species communities allowing microbial interactions. These interactions are lost upon establishing a pure culture, increasing the metabolic burden and limiting the metabolic potential of the isolated microbe. In the past years, synthetic microbial co-cultivation, using well-defined consortia of two or more microbes, was increasingly explored for innovative applications in biotechnology. As such, interspecies interactions take place without the complexity of an open mixed culture, minimizing undesired side reactions. Ultimately, synthetic co-cultivation allows to take well-characterized microbes 'off-the-shelf' to create ecosystems with improved process capabilities.
This review highlights some of the recent developments on co-cultivation, focusing on waste-to-chemicals conversions. It also addresses fundamental knowledge on microbial interactions deriving from these studies, which is important to further develop our ability to engineer functional co-cultures for bioproduction.

Address
Laboratory of Microbiology, Wageningen University and Research, Stippeneng 4, 6708 WE Wageningen, The Netherlands

Corresponding author: Sousa, Diana Z (diana.sousa@wur.nl)

Current Opinion in Biotechnology 2021, **67**:72–79

This review comes from a themed issue on **Environmental biotechnology**

Edited by **Robbert Kleerebezem** and **Diana Z Sousa**

For a complete overview see the Issue and the Editorial

Available online 28th January 2021

https://doi.org/10.1016/j.copbio.2021.01.006

Introduction

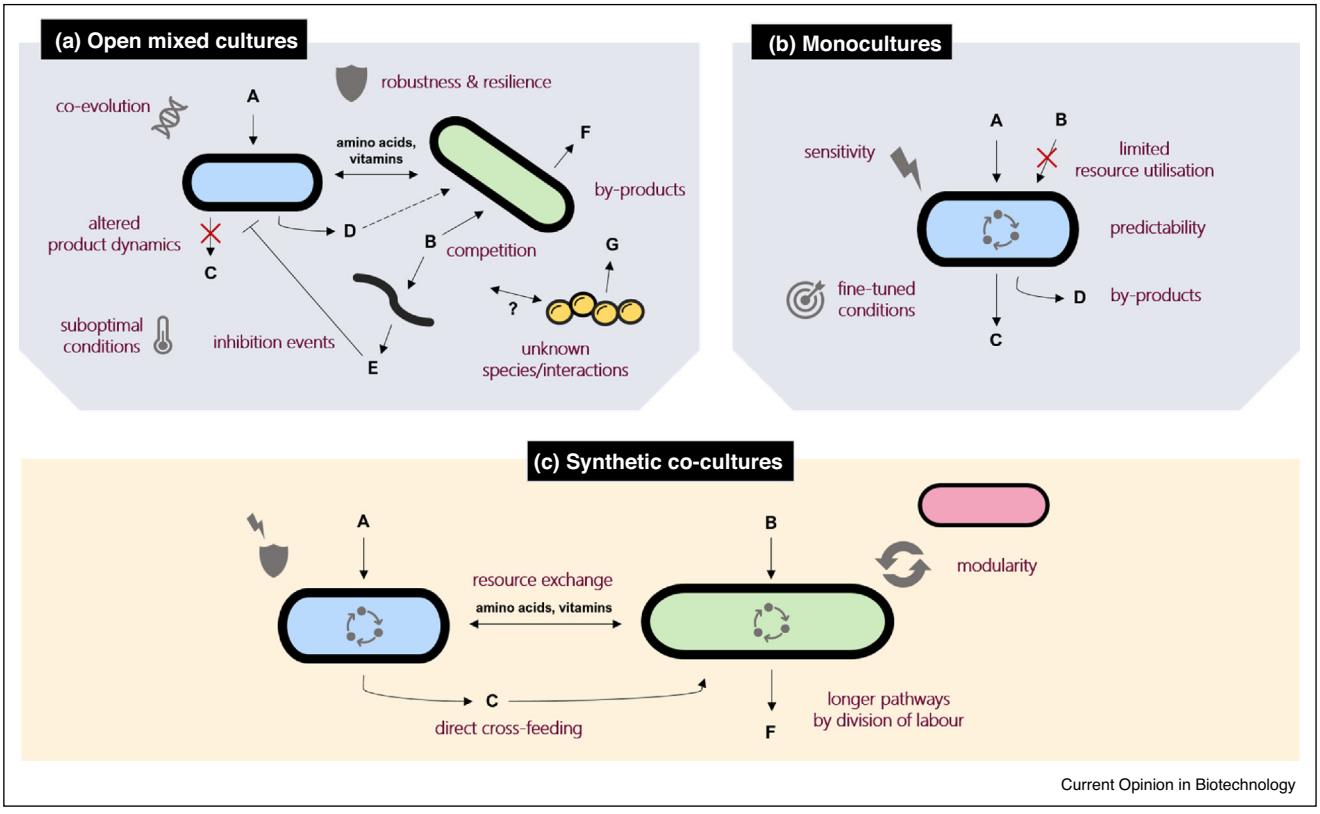
The production of bio-based chemicals will play a vital role in replacing fossil fuels and derivatives, while contributing to closing carbon and nutrient cycles associated with human activities. Microbes are regarded as promising catalysts to produce chemicals from renewable sources. Organic streams (e.g. non-food lignocellulosic biomass or municipal wastes) or CO_2 are currently the preferred sources for biotechnological processes for their availability and overall sustainability. Common examples of such microbial-based processes are the conversion of lignocellulosic biomass to alcohols by solventogenic bacteria, or the conversion of municipal wastes to methane (biogas) by anaerobic mixed communities (anaerobic digestion) [1]. Conversion of CO_2 in biotechnological processes can be performed by cyanobacteria or algae [2]. Additionally, anaerobic acetogenic bacteria are recently receiving increased attention to produce alcohols or organic acids from gases containing CO_2 (and CO) [3]. Anyhow, conversion of these substrates is challenging for microbes: lignocellulosic materials due to their rigidity, and CO_2 for the high demand of reducing power. Microbial catalysts are traditionally used in the form of i) a pure culture of microorganisms (i.e. single microbial strains, natural or genetically engineered) specialized on a specific conversion, or ii) open mixed cultures (mainly undefined, naturally occurring microbial communities). The use of open mixed cultures works well for the conversion of complex substrates to products at the end of the anaerobic food-chain, such as methane [4], or products that can be selected by steering process parameters, such as microbial storage compounds [5]. However, competition for substrate by different metabolic groups (decreasing e.g. product specificity) or utilization of the desired (intermediary) product in the food chain are common problems in open mixed cultures approaches (Figure 1). On the other hand, monoculture production systems offer more control over the production process but lack the robustness and resilience of mixed cultures and are prone to contamination events. In monocultures, genetic engineering is often a promising approach for improving production of specific cell metabolites, although it can also have effects on microbial fitness.

In the past years, a new approach for developing microbial catalysts has emerged that consists of designing and constructing well-defined consortia of microbes [6]. Synthetic co-cultures are composed of two or more microbes that form a system where the different players interact, establishing a reaction network for chemical production (Figure 1). Co-cultures can be cultivated in 'one-pot', functioning differently than when each microorganism is cultivated independently (e.g. in a sequential process). By design, the synthetic co-culture can be formed as such that the culture has the advantages of microbial mixed culture interactions but lacks strains that negatively influence the yield of the process.

Microbial co-cultures for biotech industry

Microbial co-cultures can be used to produce a variety of products ranging from small molecules, such as hydrogen or short chain fatty acids, to more energy intense

Figure 1



Summary of the main traits that characterize **(a)** open mixed cultures, **(b)** monocultures and **(c)** synthetic co-cultures, including possible microbial interactions. Synthetic co-cultures have traits of both mixed and monocultures. They are relatively simple (i.e. limited amount of microbial species) and yet display significant robustness and resilience. Like monocultures, the individual behavior of the species in synthetic consortia can be, to some extent, well-predicted. Metabolic modelling can be applied to describe the microbial interactions involved and to identify opportunities for process optimization. Complex substrates can rarely be completely utilized by a single microbial strain, which makes monocultures unsuitable for several biotechnological applications. Synthetic co-cultures can be purposely designed to optimize resource utilization and to promote direct cross-feeding of intermediates, thereby minimizing the presence of by-products. End-products that require longer pathways can be produced by exploiting this division of labor. Exchange of public goods strengthens the fitness of the consortia as a whole and contributes to co-evolution, just as it occurs in open mixed cultures. The latter, though, are inherently much more complex; multiple microbial interactions take place simultaneously in open mixed cultures, which constantly reshape community composition and product dynamics. In addition, predictability of open mixed cultures is limited due the technical impossibility of characterizing all microbial species and interactions present. Modularity is a unique feature of synthetic co-cultures: partners in the consortia can be replaced, added or removed in order to meet the process needs. Improving robustness and minimizing undesired interactions are targets to realise in synthetic co-cultures to increase their applicability in bio-based processes.

products, such as wax esters or polyhydroxy-butyrate (Table 1). Co-cultures can be designed for converting/producing a specific substrate/product. They present several advantages over mono and mixed cultures (Figure 1) and allow for microbial interactions while being less complex as mixed cultures. One has to keep in mind, however, that co-cultures are likely less robust than mixed cultures, and that some microbial interactions can also negatively influence some aspects of culture performance (e.g. parasitism). In this review we will address different examples of synthetic microbial co-cultures, highlight their advantages and disadvantages and discuss their applicability, mainly focusing on applications to produce chemicals from waste or other renewable sources.

Direct cross-feeding and division of labor for more efficient valorization of waste streams

For biotechnological applications, a system is favored that can convert a cheap, widely available substrate/energy source (e.g. CO_2, complex organic waste, light) into intermediate metabolite(s) that can subsequently be converted to a higher-value product by sequential and/or parallel conversions. An example of such a co-culture is the cultivation of the genetically engineered strain of the cyanobacterium *Synechococcus elongatus* together with several bacterial partners [22,23]. The engineered *S. elongatus* strain excretes ~85% of its photosynthetically fixed carbon in the form of sucrose, allowing growth of sucrose consuming partners for production of, for

Table 1

A selection of microbial co-cultures, their substrates and their target products. Here only the target product is listed, and in most cases side products are formed. N.D. not determined, GMO genetically engineered organism

Substrate class	Co-culture composition	Substrate	Intermediates	Target products	Ref.
Monosaccharides	*C. acetobutylicum* + *C. ljungdahlii*	glucose	H_2, acetate, acetone, acetoin	2,3-butanediol, isopropanol	[7,8••]
	Gluconobacter oxydans + *Ketogulolicigenium vulgare*	D-sorbitol	L-sorbose	2-keto-L-gulonic acid	[9]
	E. coli (GMO) + *S. cerevisiae* (GMO)	xylose	taxadiene, acetate	oxygenated taxanes, sesquiterpenes	[10]
	C. butyricum, A. bayily (GMO)	glucose + oxygen	acetate, butyrate	wax esters + hydrogen	[11]
	E. coli K12 + *A. bayily*	glucose	acetate	biomass	[12]
Polysaccharides	*Trichoderma reesei* + *Lactobacillus pentosus* + *Clostridium tyrobutyricum, Veilonella criceti, Megasphaera elsdenii*	lignocellulose	lactate	C_3–C_6 fatty acids	[13••]
	C. acetobutylicum + *C. celluliticum*	cellulose	pyruvate	butanol	[14]
	C. acetobutylicum + *E. aerogenes*	glucose/ cellobiose	N.D.	hydrogen	[15]
	Shewanella oneidensis + *Klebsiella pneumoniae (GMO)*	glucose/ cellobiose	lactate	electricity	[16]
Small organic molecules	*E. coli* + *E. coli* (GMO)	glycerol	3-dehydroshikimic acid	muconic acid	[17]
	G. sulfurreducens + *C. pasteurianum*	glycerol + acetate	electron transfer	propanediol, butyrate	[18]
	C. kluyveri + *Methanogen 166*	ethanol + acetate	hydrogen	caproate, methane	[19]
	E. coli + *M. formicicum*	glycerol	formate	succinate	[20,21•]
CO_2 and CO	*Synechococcus elongatus (GMO)* + *B. subtillis, E. coli, S. cereviseae, Halomonas boliviensis*	light, CO_2	Sucrose	Polyhydroxybutyrate, α-amylase	[22,23]
	C. autoethanogenum + *C. kluyveri*	CO, H_2, CO_2	Acetate/ethanol	C_4–C_8 fatty-acids and alcohols	[24•,25]
	C. ljungdahlii + *C. kluyveri*	CO, H_2, CO_2	Acetate/ethanol	C_4–C_8 fatty-acids and alcohols	[26]
	M. thermoautotrophicus + *C. hydrogenoformans*	CO, H_2	H_2/CO_2	methane	[27]

example, polyhydroxybutyrate (PHB) or α-amylase. This allows for the controlled and efficient production of a valuable end-product from light and CO_2. Such a result might be difficult to obtain by genetically modifying *Synechococcus* alone, as the physiological/genetic chassis for these specific end-products in this strain might either be unsuitable or not well enough understood for to be effective. Next to direct cross-feeding, this co-culture shows another advantage of synthetic co-cultivation: modularity. By pairing *S. elongatus* with different acceptor strains that are suited to produce specific products from sucrose, there is large potential for the generation of different end-products from light and CO_2.

Direct cross-feeding and modularity are not the sole advantages of synthetic co-cultivation. Scavenging of toxic compounds from the environment by microbes can be used to create process conditions amenable to other microbes. An example of this is the growth of strict anaerobes in co-culture with aerobes, making use of the high energy yields of aerobic metabolism while simultaneously utilizing fermentative aspects of strict anaerobes. This is exemplified by a co-culture of the aerobic fungus *Trichoderma reesei* together with lactic acid bacteria and other different fermentative bacteria, converting lignocellulose to different end products (propionate, butyrate, caproate) [13••]. The authors from that study exploited biofilm formation, adding spatial organization, yet another important element of co-cultivation, to create a redox gradient in the reactor and benefit from both aerobic and anaerobic microbial metabolisms. Modularity of the co-culture was used to switch between production of butyric acid (*Clostridium tyrobutyricum*), propionic acid (*Veilonella criceti*) and longer chain acids (*Megasphaera elsdenii)*. A combination of aerobic and anaerobic microorganisms has been tried out before, also as means of producing 'energy-intense' products. An example of this is the co-culture of *Clostridium butyricum* with a *Actinetobacter bayily* mutant made deficient in glucose utilization, enabling production of wax esters from glucose with enhanced carbon and electron recovery compared to solely the aerobic process [11]. The strict anaerobic *C. butyricum* produced volatile fatty acids (VFAs) and hydrogen from glucose, while *A. bayily* utilized VFAs to produce wax esters and simultaneously deoxidizing the environment (important for *C. butyricum* to operate fermentatively).

Division of labor, by distributing functions over different partners, allows microbes to evolve towards a smaller genome, being energy efficient for the individual strains and therefore promoting the efficiency of the overall culture [28••]. About 98% of the microbes show amino acid auxotrophy (i.e. inability to synthesize the full spectrum of required amino acids for growth), which is seen as a consequence of co-evolution [29•]. Laboratory evolutionary experiments also show that prolonged co-cultivation of amino acid auxotrophs resulted in a metabolic cooperation between the strains where both partners evolved to produce significantly more of the exchanged amino acid compared to the initial strains [30•]. Exchange of metabolites synthesized in different microbes can take place with both small metabolites and macromolecules. An example of this is the co-culture of *Clostridium ljungdahlii* and *Clostridium acetobutylicum* [7]. While the products 2,3-butanediol and isopropanol were not formed from glucose by monocultures, potential exchange of intermediates (e.g. acetone, acetoin) via direct cell–cell contact could have resulted in the increased formation of 2,3-butanediol and isopropanol [7]. Additionally, the same co-culture was shown to perform transfer of macromolecules such as proteins and RNA, causing the strains to potentially influence each other's metabolism directly [8••]. Direct cell–cell contact appears to be required for these molecules to be transferred, suggesting that the cells physically connect. Observations that cell–cell contact in some cases is required for a stable culture was shown before in a co-culture of *C. acetobutylicum* and *Desulfovibrio vulgaris* [31]. *D. vulgaris* can normally not thrive in an environment with glucose as sole substrate, but in conditions where it could physically interact with *C. acetobutylicum* it was shown to do so.

Environmental constrains to steer synthetic microbial co-cultures

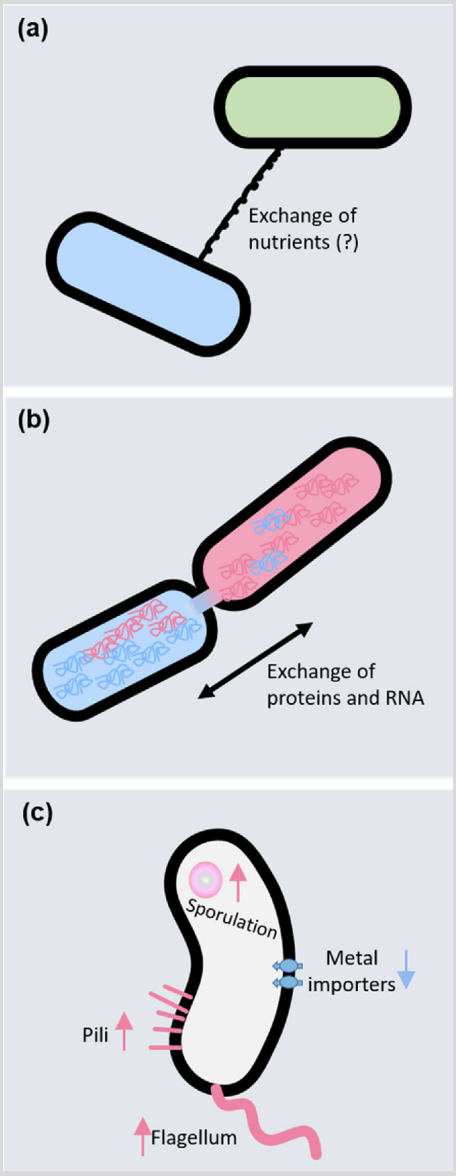
As seen in the previous section, cross-feeding and division of labour are beneficial for microbes and can increase the overall efficiency microbial metabolism. However, metabolically active microbes also influence the concentrations of compounds in their environment and, consequently, the thermodynamics and affinity of reactions of other microbes. A famous example of this is the syntrophic degradation of fatty acids [32]. Oxidation of these acids is made possible by generation of a low hydrogen pressure by the methanogenic partner. A more recent discovery in the field of syntrophy was the interaction between an Asgard archeon and a bacterium for the degradation of amino acids, hypothesized to form a basis for eukaryogenesis [33]. This syntrophic interaction is theorized to be both dependent on the removal of hydrogen/formate and the exchange of secondary metabolites by a bacterium to support anabolism and catabolism of the archaeon.

Like in syntrophy, the removal or production of intermediates by non-obligate syntrophic species can alter the thermodynamics of certain reactions and, in turn, their feasibility. This directly influences the end-product spectrum and growth characteristics of the overall culture. An example of this is the co-culture of CO-utilizing acetogens like *Clostridium autoethanogenum* and *C. ljungdahlii* with the reverse β-oxidizer *Clostridium kluyveri*, converting CO to chain elongated products [25,34]. When growing on CO, *C. autoethanogenum* produces acetate and ethanol, acting as substrates for chain elongation by *C. kluyveri*. It was noticed, however, that the ethanol production in monocultures of *C. autoethanogenum* could not have been sufficient to support the observed chain elongation activity in co-cultures. While direct electron transfer mechanisms cannot be ruled out, a follow-up study suggests that *C. kluyveri* enhances solventogenic metabolism in *C. autoethanogenum* by lowering the ethanol concentration in the environment [24•]. Interestingly, *C. autoethanogenum* shows no changes in gene transcription of its central metabolism, implying that the origin of the ethanol shift is thermodynamically driven. This is supported by findings that claim that the flux trough gas fermentation metabolism of acetogens is controlled by thermodynamics rather than gene expression [34,35]. A similar example of a metabolic shift driven by a non-syntrophic metabolic interaction is the co-cultivation of *Escherichia coli* grown on glycerol together with the formate-oxidizing methanogen *Methanobacterium formicicum* [20]. Natively, *E. coli* produces mainly ethanol and formate as end-product of glycerol fermentation, with succinate and acetate as byproducts. In co-culture with *M. formicicum*, the spectrum shifts to succinate production instead [20]. Additionally, glycerol consumption increased 11-fold in the co-culture and the growth rate of the culture was ~10-fold higher, suggesting that the accumulation of formate in single cultures of *E. coli* is potentially determining its spectrum of end products. Prolonged cultivation of this co-culture showed that the yield of succinate could be further increased to almost twice the initial amounts compared to non-adapted co-cultures [21•]. Additionally, the methane yield in the final cultures increased significantly.

In contrast to the two cases above, where a significant amount of intermediates are transferred between the strains, sometimes only a small push is needed to obtain a large effect in product shift. An example of this is the co-culture of *Clostridium pasteurianum* and *Geobacter sulfurreducens*, where *G. sulfurreducens* donates its electrons from acetate oxidation to *C. pasteurianum* [18]. While during co-cultivation *C. pasteurianum* only obtained 0.6% of its electrons from *G. sulfurreducens*, a significant shift was observed in its product spectrum: from butanol and ethanol to butyrate (+38%) and propanediol (+37%).

The product spectrum shifts discussed above pose an interesting effect of co-cultivation that is challenging or potentially impossible to achieve in monocultures. Like

Box 1 Discovering new interspecies interaction mechanisms



In addition to application in bioprocesses, synthetic co-cultures can aid in the discovery and elucidation of interspecies interaction mechanisms. Syntrophic exchange of metabolites, such as H_2 and formate, in obligate syntrophic co-cultures is well studied [32,42]. However, other types of interaction can occur that are not so well explored or even not known. For example, formation of nanotubes (a) has been documented for several co-cultures, mainly in auxotrophs [43,44]. The study by Pande *et al.* [43] suggests the exchange of amino acids via nanotubes between *A. baylyi* and *E. coli*. However, a recent study [45] shows that *Bacillus subtilis* forms tubular structures on their membrane as a postmortem phenomenon, questioning the role of these structures in species interaction. Another type of interaction gaining interest recently is cell membrane fusion (b). This has been serendipitously discovered while studying co-cultivation, and has shown that this might result in the formation of persistent hybrid cells [7,8••]. Similar observations regarding physical cell–cell contact had been previously reported for co-cultures of *D. vulgaris* and *C. acetobutylicum* [31]. Whether this applies to other species needs to be further assessed. All seems rather incipient in this field, and it is possible that many other mechanisms are important. It often occurs that when two organisms are put together, some yet unexplained interactions take place. This happened too with the co-culture of *C. autoethanogenum* and *C. kluyveri* developed at our laboratory [24•]. Despite that no changes were observed in transcripts of central metabolic pathways of *C. autoethanogenum* in mono-culture versus in co-culture, expression of flagella and pili genes by *C. autoethanogenum* was significantly higher during co-cultivation (c). If these expression patterns are related to interspecies interaction or cell sensing remains to be investigated. The expression of electrically conductive pili (e-pili) has been reported for direct electron transfer in different microbes [46]. The role of flagella in electron transfer is more controversial [47]. Some authors defend that it mainly facilitates interspecies electron transfer by allowing the establishment of biofilms and provision of increased surface area for the anchoring of cytochromes in the biofilm matrix [48]. In archaea, archaella may have a similar role to bacterial e-pili, as it has been recently shown for *Methanospirillum hungatei* [49].

during syntrophy, housing these interactions in the same cell compartment would not allow for the proper intracellular conditions to run two different types of metabolisms simultaneously.

Mutualism, competition or parasitism

Interactions in microbial communities are strongly affected by the environment (e.g. medium composition, temperature, pH), by the microbial diversity in culture, but also by the experimental set-up. An example of this is the co-culture of *Enterobacter aerogenes* and C. *acetobutylicum* producing hydrogen from glucose or cellobiose [15]. The Thauer limit poses that maximally 4 mol of H_2 per mole of sugar can be formed via dark fermentation of sugars to acetate [36]. However, the co-culture of *E. aerogenes* and C. *acetobutylicum* shows a production of 5.6 mol of H_2 per mol sugar consumed (~40% higher than the Thauer limit) [15]. Because of the competitive nature of these two strains for sugars, initiating the co-culture at a 1:2 ratio (*E. aerogenes:C. acetobutylicum*) resulted in *E. aerogenes* overgrowing the culture, removing the benefits of co-cultivation. Tuning the initial condition to a ratio of 1:10000 resulted in an efficient co-culture system, increasing H_2 yield significantly and decreasing byproduct formation. While this co-culture is probably not stable for prolonged cultivation, this example shows that production processes involving bacteria, that otherwise would outcompete each other, can be tuned by changing the initial cultivation conditions.

One might expect that co-cultures that are dependent on each other's activity result in more stable cultures over time. However, some 'dependent' co-cultures are not viable over time when the microbes grow in the same compartment and lose their stability because of a 'winner-takes-all' behavior of one of the microorganisms. This is shown by a tri-culture of *Azotobacter vinelandii*, *Bacillus*

licheniformis and *Paenibacillus curdlanolyticus* depending on each other for nitrogen and substrate availability [37]. In a single compartment, this tri-culture was unstable in both rich (making strains independent) and poor (making strains-dependent) medium conditions, resulting in a single strain dominating at the end of cultivation. However, when applying spatial separation in different chambers connected via channels with the same medium, the tri-cultures were shown to be viable for all three microbes. This shows that a thin line exists between competition and mutualism, and that diversion from one to the other behavior can be prompted by applying the proper cultivation conditions.

Other co-cultures are stable and can be transferred and sustained over multiple cultivations. In several of these cases, both microbial partners benefit from the interaction. Examples are the aerobic/anaerobic systems where the anaerobe profits from oxygen removal, while the aerobe profits from products produced via fermentative metabolism [11,13••]. However, in some co-cultures one of the strains is strictly dependent on the other but not the other way around. For example, in the co-culture of *C. autoethanogenum* and *C. kluyveri* [25], the acetogen has the advantage of having substrate (in this case, CO) and does not need to adapt to *C. kluyveri* to survive. On the contrary, a shift towards more ethanol production would be energetically less favorable for *C. autoethanogenum* and even diminishes its overall ATP yield. This downside for *C. autoethanogenum* is also seen back in the biomass density of *C. autoethanogenum*, estimated to be lower in co-culture compared to monoculture conditions [24•]. As no direct energetic advantage for *C. autoethanogenum* appears to be the case in these co-cultures, *C. kluyveri* can be viewed as a parasite, profiting from ethanol production by *C. autoethanogenum*. On the other hand, one could argue that some of these thermodynamically driven product shifts are not under the control of the microbes themselves but are the result of kinetics and thermodynamic parameters of the microbial metabolism. For now this leaves the question open on why these co-cultures are very stable over multiple transfers.

Conclusions and outlook

In the transition to a circular economy, and aiming at closing carbon and nutrient cycles, it is important to create innovation in the field of biotechnology. Microbial synthetic co-cultures are a promising alternative and are potentially more robust and resilient than monoculture approaches, while being less complex compared to mixed culture approaches. Therefore, co-cultures offer possibilities for efficient conversion of complex substrates towards specific products of interest. In addition to the *in vivo* studies reviewed here, there are increasing efforts to model synthetic co-cultures *in silico* based on genomic and/or kinetic data [38–41]. These approaches can shed light on microbial function and interactions in co-cultures, and eventually aid the optimization of these systems. A microbial co-culture is more than the sum of its parts and, by combining experimental work and *in silico* simulations, understanding of the consortia ecology can be accelerated, resulting in further advancing microbial cultivation technology.

While making use of cross-feeding and division of labor is the most intuitive approach for the design of synthetic communities, some microbial interactions such as competition and excretion of unfavorable secondary metabolites (e.g. antimicrobials/signaling molecules) can possibly result in non-optimal functioning of the co-culture. However, these 'undesired' interactions could also be used to create 'artificial' interdependencies to aid in getting more robust cultures. Negative relations could be exploited — for example, strains that produce toxins to kill an undesired contaminant, or a strain that produces inhibitors of specific metabolic activity by microbes in the co-culture (or that triggers a certain metabolism). Additionally, we can likely still learn substantially from microbial interactions observed in natural obligate syntrophic co-cultures and in synthetic co-cultures (Box 1). In addition to exploring microbial interactions, questions regarding robustness of co-cultures and effects of prolonged co-evolution during application in production processes remain largely unanswered. These topics will need to be addressed by future studies to gain further insight in the applicability of the co-cultures for the biotech industry.

In the future, synthetic microbial communities for biotechnology may include hybrid solutions involving native and genetically engineered microorganisms. The challenge is to explore directions and implement rules for designing and constructing effective synthetic microbial communities that can be applied in biotechnology. These rules may be related with the adequate selection of microorganisms for setting-up synthetic microbiomes or involve genetic engineering of microbial player(s) to provide communities with key social traits (e.g. effective mechanisms for electron transfer, inclusion of quorum sensing pathways, etc.).

Author contributions

The authors contributed equally to all aspects of the article.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors would like to acknowledge the Netherlands Science Foundation (NWO) (Project NWO-GK-07), the NWO domain Applied and Engineering Sciences (AES) (Perspectief Programma P16-10), and the Ministry of Education, Culture and Science, Netherland (Project 024.002.002) for financial support. Any opinions, conclusions or

recommendations expressed in this review are those of the authors and do not necessarily reflect the views of the funding sources.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Yang L, Xu F, Ge X, Li Y: **Challenges and strategies for solid-state anaerobic digestion of lignocellulosic biomass**. *Renew Sustain Energy Rev* 2015, **44**:824-834 http://dx.doi.org/10.1016/j.rser.2015.01.002.

2. Desai SH, Atsumi S: **Photosynthetic approaches to chemical biotechnology**. *Curr Opin Biotechnol* 2013, **24**:1031-1036 http://dx.doi.org/10.1016/j.copbio.2013.03.015.

3. Takors R, Kopf M, Mampel J, Bluemke W, Blombach B, Eikmanns B, Bengelsdorf FR, Weuster-Botz D, Dürre P: **Using gas mixtures of CO, CO_2 and H_2 as microbial substrates: the do's and don'ts of successful technology transfer from laboratory to production scale**. *Microb Biotechnol* 2018, **11**:606-625 http://dx.doi.org/10.1111/1751-7915.13270.

4. Chynoweth DP, Owens JM, Legrand R: **Renewable methane from anaerobic digestion of biomass**. *Renew Energy* 2001, **22**:1-8 http://dx.doi.org/10.1016/S0960-1481(00)00019-7.

5. Korkakaki E, Mulders M, Veeken A, Rozendal R, van Loosdrecht MCM, Kleerebezem R: **PHA production from the organic fraction of municipal solid waste (OFMSW): overcoming the inhibitory matrix**. *Water Res* 2016, **96**:74-83 http://dx.doi.org/10.1016/j.watres.2016.03.033.

6. Tsoi R, Dai Z, You L: **Emerging strategies for engineering microbial communities**. *Biotechnol Adv* 2019, **37**:107372 http://dx.doi.org/10.1016/j.biotechadv.2019.03.011.

7. Charubin K, Papoutsakis ET: **Direct cell-to-cell exchange of matter in a synthetic Clostridium syntrophy enables CO_2 fixation, superior metabolite yields, and an expanded metabolic space**. *Metab Eng* 2019, **52**:9-19 http://dx.doi.org/10.1016/j.ymben.2018.10.006.

8. •• Charubin K, Modla S, Caplan JL, Papoutsakis ET: **Interspecies microbial fusion and large-scale exchange of cytoplasmic proteins and RNA in a syntrophic *Clostridium* coculture**. *mBio* 2020, **11**:e02030-20 http://dx.doi.org/10.1128/mBio.02030-20

The authors build on previous work on a syntrophic synthetic *Clostridium* co-culture displaying direct cell-to-cell interactions. Using advanced microscopy techniques, they show the occurrence of cell-membrane fusion events allowing the exchange of proteins and RNA. This cell fusion eventually leads to hybrid cells that continue to grow, divide and even sporulate as one entity.

9. Wang EX, Ding MZ, Ma Q, Dong XT, Yuan YJ: **Reorganization of a synthetic microbial consortium for one-step vitamin C fermentation**. *Microb Cell Fact* 2016, **15**:21 http://dx.doi.org/10.1186/s12934-016-0418-6.

10. Zhou K, Qiao K, Edgar S, Stephanopoulos G: **Distributing a metabolic pathway among a microbial consortium enhances production of natural products**. *Nat Biotechnol* 2015, **33**:377-383 http://dx.doi.org/10.1038/nbt.3095.

11. Salmela M, Lehtinen T, Efimova E, Santala S, Mangayil R: **Metabolic pairing of aerobic and anaerobic production in a one-pot batch cultivation**. *Biotechnol Biofuels* 2018, **11**:187 http://dx.doi.org/10.1186/s13068-018-1186-9.

12. Santala S, Karp M, Santala V: **Rationally engineered synthetic coculture for improved biomass and product formation**. *PLoS One* 2014, **9**:e1137869.

13. •• Shahab RL, Brethauer S, Davey MP, Smith AG, Vignolini S, Luterbacher JS, Studer MH: **A heterogeneous microbial consortium producing short-chain fatty acids from lignocellulose**. *Science (80-)* 2020, **369**:eabb1214 http://dx.doi.org/10.1126/science.abb1214

An exceptional work describing the establishment of a synthetic consortia growing across an oxygen gradient in a membrane-aerated bioreactor. The oxygen gradient is established naturally upon stepwise inoculation of three microorganisms: an aerobic fungus, a facultative anaerobic lactic-acid bacteria and anaerobic lactate-fermenting strain. The consortium converted lignocellulose in to fatty acids. This study demonstrates the potential of rational engineering of synthetic consortia combined with reactor engineering for the development of novel bioprocesses.

14. Salimi F, Mahadevan R: **Characterizing metabolic interactions in a clostridial co-culture for consolidated bioprocessing**. *BMC Biotechnol* 2013, **13**:95 http://dx.doi.org/10.1186/1472-6750-13-95.

15. Ergal İ, Gräf O, Hasibar B, Steiner M, Vukotić S, Bochmann G, Fuchs W, Rittmann SKMR: **Biohydrogen production beyond the Thauer limit by precision design of artificial microbial consortia**. *Commun Biol* 2020, **3**:443 http://dx.doi.org/10.1038/s42003-020-01159-x.

16. Li F, An X, Wu D, Xu J, Chen Y, Li W, Cao Y, Guo X, Lin X, Li C *et al.*: **Engineering microbial consortia for high-performance cellulosic hydrolyzates-fed microbial fuel cells**. *Front Microbiol* 2019, **10**:409 http://dx.doi.org/10.3389/fmicb.2019.00409.

17. Zhang H, Li Z, Pereira B, Stephanopoulos G: **Engineering *E. coli-E. coli* cocultures for production of muconic acid from glycerol**. *Microb Cell Fact* 2015, **14**:134 http://dx.doi.org/10.1186/s12934-015-0319-0.

18. Moscoviz R, De Fouchécour F, Santa-Catalina G, Bernet N, Trably E: **Cooperative growth of *Geobacter sulfurreducens* and *Clostridium pasteurianum* with subsequent metabolic shift in glycerol fermentation**. *Sci Rep* 2017, **7**:44334 http://dx.doi.org/10.1038/srep44334.

19. Yan S, Dong D: **Improvement of caproic acid production in a *Clostridium kluyveri* H068 and Methanogen 166 co-culture fermentation system**. *AMB Express* 2018, **8**:175 http://dx.doi.org/10.1186/s13568-018-0705-1.

20. Richter K, Gescher J: **Accelerated glycerol fermentation in *Escherichia coli* using methanogenic formate consumption**. *Bioresour Technol* 2014, **162**:389-391 http://dx.doi.org/10.1016/j.biortech.2014.04.011.

21. • Kim NY, Kim SN, Kim O, Bin: **Long-term adaptation of *Escherichia coli* to methanogenic co-culture enhanced succinate production from crude glycerol**. *J Ind Microbiol Biotechnol* 2018, **45**:71-76 http://dx.doi.org/10.1007/s10295-017-1994-0

An example of a metabolic shift in a non-syntrophic microbial interaction. Succinate production from glycerol by a wild-type strain of *E. coli* was increased twofold through successive rounds of co-cultivation with a formate-consuming methanogen.

22. Hays SG, Yan LLW, Silver PA, Ducat DC: **Synthetic photosynthetic consortia define interactions leading to robustness and photoproduction**. *J Biol Eng* 2017, **11**:4 http://dx.doi.org/10.1186/s13036-017-0048-5.

23. Weiss TL, Young EJ, Ducat DC: **A synthetic, light-driven consortium of cyanobacteria and heterotrophic bacteria enables stable polyhydroxybutyrate production**. *Metab Eng* 2017, **44**:236-245 http://dx.doi.org/10.1016/j.ymben.2017.10.009.

24. • Diender M, Parera Olm I, Gelderloos M, Koehorst JJ, Schaap PJ, Stams AJM, Sousa DZ: **Metabolic shift induced by synthetic co-cultivation promotes high yield of chain elongated acids from syngas**. *Sci Rep* 2019, **9**:18081

In this study, it is demonstrated that thermodynamics, in contrast to gene regulation, is the main driver of the microbial interactions in a syngas-fermenting synthetic consortia.

25. Diender M, Stams AJM, Sousa DZ: **Production of medium-chain fatty acids and higher alcohols by a synthetic co-culture grown on carbon monoxide or syngas**. *Biotechnol Biofuels* 2016, **9**:82.

26. Richter H, Molitor B, Diender M, Sousa DZ, Angenent LT: **A narrow pH range supports butanol, hexanol, and octanol production from syngas in a continuous co-culture of *Clostridium ljungdahlii* and *Clostridium kluyveri* with in-line product extraction**. *Front Microbiol* 2016, **7**:1773.

27. Diender M, Uhl PS, Bitter JH, Stams AJM, Sousa DZ: **High rate biomethanation of carbon monoxide-rich gases via a thermophilic synthetic coculture**. *ACS Sustain Chem Eng* 2018, **6**:2169-2176 http://dx.doi.org/10.1021/acssuschemeng.7b03601.

28. •• Noack S, Baumgart M: **Communities of niche-optimized strains: small-genome organism consortia in bioproduction**. *Trends Biotechnol* 2019, **37**:126-139 http://dx.doi.org/10.1016/j.tibtech.2018.07.011

An interesting genome-reduction approach to establish synthetic communities of niche-optimized strains for application in biotechnology. The immediate surrounding environment (i.e. niche) is advocated as key determinant of gene targets for strain optimization.

29. • Zengler K, Zaramela LS: **The social network of microorganisms - how auxotrophies shape complex communities**. *Nat Rev Microbiol* 2018, **16**:383-390 http://dx.doi.org/10.1038/s41579-018-0004-5

An insightful overview of naturally occurring auxotrophic interactions in microbial communities and how they shape network dynamics.

30. • Preussger D, Giri S, Muhsal LK, Oña L, Kost C: **Reciprocal fitness feedbacks promote the evolution of mutualistic cooperation**. *Curr Biol* 2020, **30**:3580-3590.e7 http://dx.doi.org/10.1016/j.cub.2020.06.100

A laboratory evolution experiment to test the metabolic cooperation of auxotrophic *E. coli* strains in co-culture. This study shows that cooperation via cross-feeding of essential metabolites is strongly favored in self-organized multicellular clusters (versus in cells outside clusters) and is driven by positive feedback loops.

31. Benomar S, Ranava D, Cárdenas ML, Trably E, Rafrafi Y, Ducret A, Hamelin J, Lojou E, Steyer JP, Giudici-Orticoni MT: **Nutritional stress induces exchange of cell material and energetic coupling between bacterial species**. *Nat Commun* 2015, **6**:6283 http://dx.doi.org/10.1038/ncomms7283.

32. Morris BEL, Henneberger R, Huber H, Moissl-Eichinger C: **Microbial syntrophy: interaction for the common good**. *FEMS Microbiol Rev* 2013, **37**:384-406 http://dx.doi.org/10.1111/1574-6976.12019.

33. Imachi H, Nobu MK, Nakahara N, Morono Y, Ogawara M, Takaki Y, Takano Y, Uematsu K, Ikuta T, Ito M *et al.*: **Isolation of an archaeon at the prokaryote–eukaryote interface**. *Nature* 2020, **577**:519-525 http://dx.doi.org/10.1038/s41586-019-1916-6.

34. Richter H, Molitor B, Wei H, Chen W, Aristilde L, Angenent LT: **Ethanol production in syngas-fermenting: *Clostridium ljungdahlii* is controlled by thermodynamics rather than by enzyme expression**. *Energy Environ Sci* 2016, **9**:2392-2399.

35. Valgepea K, De Souza Pinto Lemgruber R, Abdalla T, Binos S, Takemori N, Takemori A, Tanaka Y, Tappel R, Köpke M, Simpson SD *et al.*: **H_2 drives metabolic rearrangements in gas-fermenting *Clostridium autoethanogenum***. *Biotechnol Biofuels* 2018, **11**:55.

36. Thauer RK, Jungermann K, Decker K: **Energy conservation in chemotrophic anaerobic bacteria**. *Bacteriol Rev* 1977, **41**:100-180 http://dx.doi.org/10.1128/mmbr.41.1.100-180.1977.

37. Hyun JK, Boedicker JQ, Jang WC, Ismagilov RF: **Defined spatial structure stabilizes a synthetic multispecies bacterial community**. *Proc Natl Acad Sci U S A* 2008, **105**:18188-18193 http://dx.doi.org/10.1073/pnas.0807935105.

38. Liu F, Mao J, Kong W, Hua Q, Feng Y, Bashir R, Lu T: **Interaction variability shapes succession of synthetic microbial ecosystems**. *Nat Commun* 2020, **11**:309 http://dx.doi.org/10.1038/s41467-019-13986-6.

39. Kong W, Meldgin DR, Collins JJ, Lu T: **Designing microbial consortia with defined social interactions**. *Nat Chem Biol* 2018, **14**:821-829 http://dx.doi.org/10.1038/s41589-018-0091-7.

40. Benito-Vaquerizo S, Diender M, Parera Olm I, Martins dos Santos VAP, Schaap PJ, Sousa DZ, Suarez-Diez M: **Modeling a co-culture of *Clostridium autoethanogenum* and *Clostridium kluyveri* to increase syngas conversion to medium-chain fatty-acids**. *Comput Struct Biotechnol J* 2020, **18**:3255-3266 http://dx.doi.org/10.1016/j.csbj.2020.10.003.

41. Li X, Henson MA: **Metabolic modeling of bacterial co-culture systems predicts enhanced carbon monoxide-to-butyrate conversion compared to monoculture systems**. *Biochem Eng J* 2019, **151**:107338 http://dx.doi.org/10.1016/j.bej.2019.107338.

42. Stams AJM, Plugge CM: **Electron transfer in syntrophic communities of anaerobic bacteria and archaea**. *Nat Rev Microbiol* 2009, **7**:568-577 http://dx.doi.org/10.1038/nrmicro2166.

43. Pande S, Shitut S, Freund L, Westermann M, Bertels F, Colesie C, Bischofs IB, Kost C: **Metabolic cross-feeding via intercellular nanotubes among bacteria**. *Nat Commun* 2015, **6**:6238 http://dx.doi.org/10.1038/ncomms7238.

44. Shitut S, Ahsendorf T, Pande S, Egbert M, Kost C: **Nanotube-mediated cross-feeding couples the metabolism of interacting bacterial cells**. *Environ Microbiol* 2019, **21**:1306-1320 http://dx.doi.org/10.1111/1462-2920.14539.

45. Pospíšil J, Víťovská D, Kofroňová O, Muchová K, Šanderová H, Hubálek M, Šiková M, Modrák M, Benada O, Barák I *et al.*: **Bacterial nanotubes as a manifestation of cell death**. *Nat Commun* 2020, **12**:4963.

46. Lovley DR: **Happy together: microbial communities that hook up to swap electrons**. *ISME J* 2017, **11**:327-336 http://dx.doi.org/10.1038/ismej.2016.136.

47. Sure S, Ackland ML, Torriero AAJ, Adholeya A, Kochar M: **Microbial nanowires: an electrifying tale**. *Microbiol (United Kingdom)* 2016, **162**:2017-2028 http://dx.doi.org/10.1099/mic.0.000382.

48. Liu X, Zhuo S, Jing X, Yuan Y, Rensing C, Zhou S: **Flagella act as *Geobacter* biofilm scaffolds to stabilize biofilm and facilitate extracellular electron transfer**. *Biosens Bioelectron* 2019, **146**:111748 http://dx.doi.org/10.1016/j.bios.2019.111748.

49. Walker DJF, Martz E, Holmes DE, Zhou Z, Nonnenmann SS, Lovley DR: **The archaellum of *Methanospirillum hungatei* is electrically conductive**. *mBio* 2019, **10**:e00579-19 http://dx.doi.org/10.1128/mBio.00579-19.