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To cite this article: Gloria Padmaperuma, Rahul Vijay Kapoore, Daniel James Gilmour & Seetharaman Vaidyanathan (2018) Microbial consortia: a critical look at microalgae co-cultures for enhanced biomanufacturing, Critical Reviews in Biotechnology, 38:5, 690-703, DOI: [10.1080/07388551.2017.1390728](https://doi.org/10.1080/07388551.2017.1390728)

To link to this article: <https://doi.org/10.1080/07388551.2017.1390728>



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Published online: 12 Dec 2017.



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Microbial consortia: a critical look at microalgae co-cultures for enhanced biomanufacturing

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ABSTRACT

Monocultures have been the preferred production route in the bio-industry, where contamination has been a major bottleneck. In nature, microorganisms usually exist as part of organized communities and consortia, gaining benefits from co-habitation, keeping invaders at bay. There is increasing interest in the use of co-cultures to tackle contamination issues, and simultaneously increase productivity and product diversity. The feasibility of extending the natural phenomenon of co-habitation to the biomanufacturing industry in the form of co-cultures requires careful and systematic consideration of several aspects. This article will critically examine and review current work on microbial co-cultures, with the intent of examining the concept and proposing a design pipeline that can be developed in a biomanufacturing context.

ARTICLE HISTORY

Received 14 April 2017
Revised 30 August 2017
Accepted 3 October 2017

KEYWORDS

Bioproducts; metabolites; proteins; microbial biotechnology; microbial communication

Introduction

Axenic monocultures are predominantly used in biomanufacturing, due to the ease of monitoring and to meet stringent safety regulations [1]. However, such monocultures are at high risk of contamination that results in capital and product losses during manufacturing [2,3]. Controlled, symbiotic co-cultures possess features that provide solutions to surmount these bottlenecks. Though not universally applicable to all cell systems, co-cultures have shown improvements in yields of biomass, lipids [4] and high-value products [5].

Symbiotic microbial communities have existed from the beginning of time, within benthic mats and fossil remains [6–8]. The first human civilizations used combinations of various microbes, for the production of fermented food and alcoholic beverages [9,10]. Nowadays, industry has harnessed microorganisms as a means of production, due to their innate abilities to synthesize complex compounds and the ease of scale-up. Cells derived from mammals, such as Chinese Hamster Ovary cells [11,12], HeLa cells and mouse cells are workhorses of the biopharmaceutical industry, alongside yeast [13,14] and bacteria [9], which are used predominantly in the food industry, due to their quick turn-around times. The need for sustainable production routes has

seen microorganisms deployed for bioremediation of water and soils and as carbon capture and storage options to minimize greenhouse gas emissions. Microbial communities are increasingly being investigated for the production of valuable accessory pigments [15–17] and in microbial fuel cells for electricity generation [18,19].

Maintaining axenic cultures has proved to be expensive and labor intensive, given the recurrent problem of contamination by bacteria, viruses, protozoa, yeast, fungi and microplasma [20]. Parasites or grazers can out-compete the working cell culture and influence cell health and production outputs. The Fifth Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production by Langer [20] reported that a failure rate of 7%, would amount to US\$1–2 billion in expenses. Across 434 biomanufacturing companies, contamination was the main reason for batch spoilage. Biomanufacturing with the help of defined artificial co-cultures and consortia may hold a key to increase production rates and tackle contamination [21–23].

In recent years, researchers have started to question whether an axenic culture is strictly the best way forward, as in the natural environment, microorganisms thrive alongside other organisms. As thinking processes have evolved, research into harnessing consortia into

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biotechnological applications has increased [21] and thanks to synthetic biology and “omics” analysis, the knowledge pool on microbial communication is expanding.

This review aims to examine critically the utility and characteristics of controlled co-cultures in biomanufacturing. An insight into natural consortia and the characteristics that are relevant and transferrable to the industrial world is presented followed by a case study scenario of the application of this principle in developing processes that employ microalgae.

Microbial consortia

Consortia in nature

Microbial consortia are encountered within various natural habitats, such as mammalian guts [24], foods [25], soils [26–28], water bodies and wastes [29]. A question that arises is, ‘Why do naturally occurring microorganisms prefer to live as part of a community? As with human communities, in which a group of individuals play a role in the advancement of society, so do microorganisms. Microbial associations may be symbiotic [6,30,31], which include mutualism and commensalism [32], parasitic or predator–prey type [33–35].

Compared to a single taxon, microbial assemblages have been proven resilient when faced with adverse

conditions [36] and resist invasion from other species [37]. A consortium can overcome challenges through communication [38–41] and division of labor [22,23,36,37], evolving into a stable assemblage [42,43]. Biofilms are good examples of community assemblages [44–46]. Studies conducted by Brenner et al. [39] elucidate the bi-directional patterns present within complex systems, which shape and govern the mode in which the populations within the matrix grow, evolve and assert their roles [47].

Communication through metabolites [6,48–50] plays a key-role in defining relationships, protection, evolution, selection of partners and division of labor [40], as shown in Figure 1. Primary metabolites shape growth, development and reproduction, as seen in quorum sensing. During quorum sensing, bacterial populations release regulatory metabolites, such as N-acylhomoserine lactones [51–53], as the population density grows [54]. The same applies to interactions in the rhizosphere, where sugars, polysaccharides, amino acids and sterols are chemical cues [55]. Secondary metabolites facilitate external interactions [10,56]: toxins, pigments, antibiotics, alkaloids and carotenoids, are accumulated by cells as responses to abiotic and/or biotic factors [49,57,58], and can be extracted and marketed. A balanced competition within the consortium does not allow other microorganisms to be able to “readily

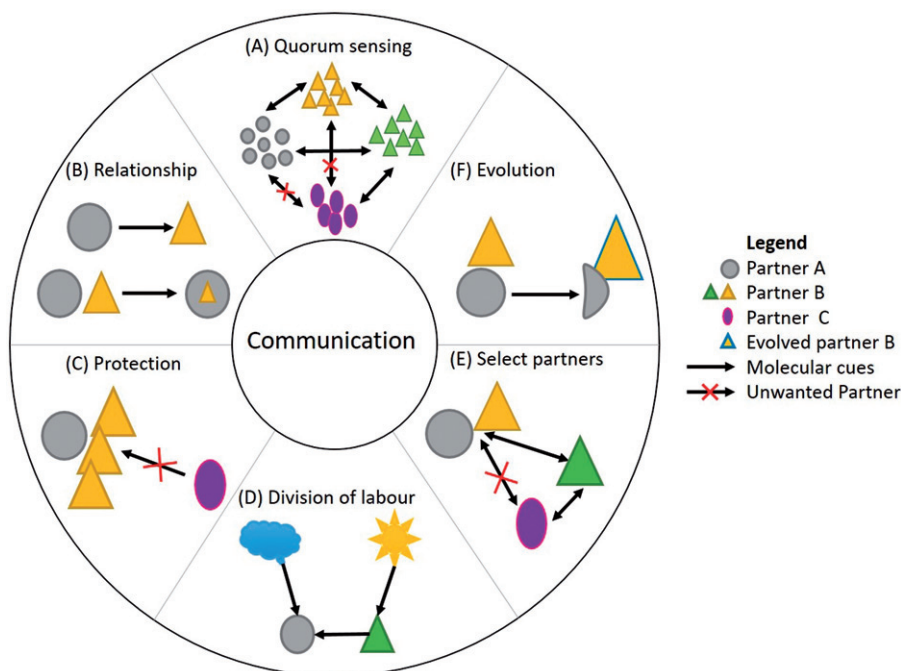


Figure 1. Communication within microbial communities. Metabolite exchanges (arrows) facilitate various modes in which microorganisms (geometrical shapes) exhibit intra- or inter-species interactions. Communication is used for (A) quorum sensing and defining the abundance of each species and (B) type of symbiosis and roles played by partners, such as in (C) protection and (D) nutrient acquisition and division of labor. Further to this, as the community evolves, so does the communication, with the effect of causing changes to the microbial communities that are part of it, for example, by recruiting new partners (E) or by evolving existing members (F).

plunder” nutrients. Division of labor has applications in bioremediation [59,60], with microorganisms working together, for example, to counteract the effect of toxins [61–63]. Thanks to these overarching characteristics, consortia are robust and readily adaptable [64], and better at outcompeting microbial contaminants and predators.

Microbial communities have successfully evolved in nature, from macro- to micro-sphere natural scenarios. This widespread natural occurrence gives reason to believe that synthetic consortia have the potential to drive production and improve industrial biotechnology.

Artificial co-cultures: learning from nature

The argument for moving towards co-cultures stems from the following: (a) current technology such as transcriptomics, metagenomics, metabolomics coupled to computer modeling allow for better understanding of microbial interactions [65,66], (b) contamination issues can be minimized or completely eliminated [22,23,67]; (c) growth profiles of primary producers can be improved [9,68]; (d) the release of new molecules can be triggered [69]; and (e) bioremediation and production can be coupled [70]. From a biotechnological perspective, a good consortium would be scalable, robust, self-sustainable, reproducible, versatile in terms of feedstock and/or production [38,71–73] and profitable [3,74].

When constructing an artificial consortium, factors to consider include: priority effects, community backgrounds and competitiveness for resources. Overyielding or underyielding effects [75] may arise, with overpowering microorganisms monopolizing the nutrients or with competition inhibiting growth of all members [76,77]. Nevertheless, artificial co-cultures have outperformed monocultures, when used for the production of antioxidants, pigments and aromatic compounds, as shown in Table 1.

Co-culture design

A bottom-up pipeline is proposed in Figure 2 to design and set-up co-cultures. This involves starting with the end-product to then shortlisting a handful of suitable primary partners (A). The primary partner will then dictate the nature of the secondary partner (B), usually an aider, ideally with bioproduction capabilities. A two-way “trigger and response” system would be ideal, such as mutualism or a commensal symbiosis [32]. It is important to realize that growth increments do not always translate into more products, as productivity can be additionally dependent on the activity of co-culture

partners. This is true for microalgae, where co-culture of partner A with B may increase biomass of A, but appropriate stress inducers may be needed to increase specific product yields [78,79].

Shortlisting suitable candidates

The secondary partner (B) should possess some of the following characteristics: (a) be nontoxic, (b) be capable of co-habiting [59], (c) match in growth rates, (d) provide nutrients and/or stimulators to enhance A [80], (e) not cause underyielding effects [75] (f) enhance the capability of A to utilize multiple feedstocks [81], (g) remove inhibitory molecules (h) use A’s waste as a feed [82], (i) maintain genetic integrity over prolonged periods of culture, and (j) function as a bioproducer.

Selecting co-culture partners

Co-culture partners are selected according to: (a) communication (metabolite/peptide/protein) profiling and/or (b) from existing natural associations. Screening based on communication profiling involves surveying the literature for secondary partners that release compounds to enhance the primary partner (A). Whilst, the second method consists of selecting partners from a natural symbiotic consortium. Angelis et al. [69] tested combinations between eight Basidiomycetes and four strains of microalgae, to evaluate the best co-culture partners. The candidates were selected according to exopolysaccharide (EPS) production, on the basis that co-culturing fungi with algae would increase overall EPS production. An increased yield with a diverse composition of EPS was recovered, and the co-culture of *Agaricus blazei* (Basidiomycete) and *Chlorella vulgaris* (microalgae) was chosen for further studies [69]. Similarly, *Weissella confusa* 11GU-1 (a yeast) and *Propionibacterium freudenreichi* JS15 (a bacterium) were deemed to be a working co-culture in bread-making, as the molecules released through their association served to be better antifungal, texture-building and anti-stalling agents [83].

Co-culture media

A communal growth medium is required for co-culturing. Microorganisms isolated from symbiotic consortia will thrive in their original media. However, for artificial co-cultures, a new recipe has to be developed and tested. Conventionally, a growth medium of the primary partner, A [4] or a mixed medium of A and B [84] in which both partners can grow are used. In a mutualistic symbiosis, co-culturing in growth medium A, should be

Table 1. Microbial co-cultures in bio-production.

Product	Reported organisms	Mode	Reported product yield/concentration			References
			Monoculture	Co-culture		
Acetate	<i>Weissella confusa</i> 11GU1 <i>P. freudenreichii</i> JS15	Fermentation at 1:1 culture ratios	0.08g/kg	0.09g/kg	0.5g/kg	[83]
Astaxanthin	<i>Haematococcus pluvialis</i> , <i>Phaffia rhodozyma</i> AS2-1557	Gas Exchange: CO ₂ and O ₂ 3g/L of glucose	3.68mg/L	1.09mg/L	12.95mg/L	[5]
Biomass	<i>Haematococcus pluvialis</i> , <i>Phaffia rhodozyma</i> AS2-1557	Gas Exchange: CO ₂ and O ₂ 25g/L of glucose	0.62g/L	5.02g/L	5.70g/L	[5]
	<i>Scenedesmus obliquus</i> , Institute of Hydrobiology	Direct mixing, 3:1 ratio	3.5g/L	n.d.	4.38g/L	[145]
	<i>Candida tropicalis</i> , Institute of Microbiology					
	<i>Isochrysis galbana</i> 8701	Direct mixing, 1:1 ratio	1.17g/L	0.31g/L	1.32g/L	[143]
	<i>Ambrosiozyma cicatricosa</i>					
	<i>Spirulina platensis</i> UTEX 1926 <i>Rhodotorula glutinis</i> 2.541	Direct mixing, 2:1 ratio	0.20g/L	1.7g/L	3.6g/L	[156]
	<i>Chlorella vulgaris</i> TISTR 8261 <i>Trichosporonoides spathulata</i>	Direct mixing	0.75g/L	10.23g/L	12.2g/L	[77]
	<i>Chlorella</i> sp. KKUS2 <i>Toluraspore</i> YU5/2	Direct mixing	1.933g/L	8.333g/L	8.010g/L	[157]
	<i>Chlorella</i> sp. KKUS2 <i>Toluraspore</i> Y30	Direct mixing	1.933g/L	8.267g/L	8.733g/L	[157]
	<i>Rhodotula glutinis</i> DBVPG 3853, <i>Debaryomyces castellii</i> DBVPG 3503	Fed-batch system with co-culture 1:1 ratio	5.3mg/L, batch co-culture	8.2mg/L		[85]
Carotenoids (β-carotene, torulene, torularhodin)						
EPS	<i>Weissella confusa</i> 11GU1 <i>P. freudenreichii</i> JS15	Fermentation at 1:1, with 15% w/w added flour	n.d.	1g/kg	1.52g/kg	[83]
	<i>Agaricus blazei</i> LPB03, <i>Chlorella vulgaris</i> LEB106	Direct mixing, 1:1 ratio	4g/L	0.95g/L	5.17g/L	[69]
2-keto-L-gulonic acid	<i>Gluconobacter oxydans</i> , <i>Ketogulonigenium vulgare</i>	Fermentation with gene manipulation	n.d.	n.d.	76.6g/L (89.7%)	[158]
Propionate	<i>Weissella confusa</i> 11GU1 <i>P. freudenreichii</i> JS15	Fermentation at 1:1 culture ratios	1.15g/kg	0g/kg	0.59g/kg	[83]
Lipids	<i>Chlorella pyrenoidosa</i> FACHB-9 <i>Rhodospiridium toruloides</i> AS2.1389	Wastewater, co-culture 1:1 ratio	3g/L	3.4g/L	4-4.6g/L	[159]
	<i>Spirulina platensis</i> UTEX 1926 <i>Rhodotorula glutinis</i> 2.541	Direct mixing, 2:1 ratio	0.013g/L	0.135g/L	0.467g/L	[156]
	<i>Chlorella vulgaris</i> TISTR 8261 <i>Trichosporonoides spathulata</i> JU4-57	Direct mixing	4.14g/L	n.d.	5.74g/L	[77]
	<i>Chlorella</i> sp. KKUS2 <i>Toluraspore</i> YU5/2	Direct mixing	0.052g/L	1.141g/L	2.424g/L	[157]
	<i>Chlorella</i> sp. KKUS2 <i>Toluraspore</i> Y30	Direct mixing	0.052g/L	0.920g/L	1.564g/L	[157]

Co-cultures employed for specific products are listed, along with the organisms employed, cultivation mode and reported product yields/productivity/concentrations in mono and co-cultures. The monoculture data provided lists the yield/concentration of the primary partner (A) followed by the secondary partner, if both organisms produce the desired product (n.d.: not determined).

sufficient. In commensal symbiosis, a supplement to help partner B may be needed. For example, glucose, yeast extract [4] and/or corn syrup [85] were added to the algal media to assist the yeast strains.

Inoculation: ratio and timing

The inoculum density of each partner will affect the final co-culture outcome. This can be determined by analyzing the growth rate of the organisms in co-culture media. Buzzini [85] demonstrated that when the

inoculation ratio of *Rhodotorula glutinis* (yeast) and *Debaryomyces castellii* (starch accumulating bacteria) was 1:1, it resulted in a 150% increase in β-carotene production (by the yeast). This is not always the case, as seen in the *C. vulgaris* and *R. glutinis* (algae–yeast) co-culture where higher yields of lipids and biomass were achieved compared to monoculture, irrespective of the starting inoculum [76]. The timing, order and growth phase at which the inocula are introduced into the culture vessel will influence the general structure of the co-culture and its performance. This phenomenon has

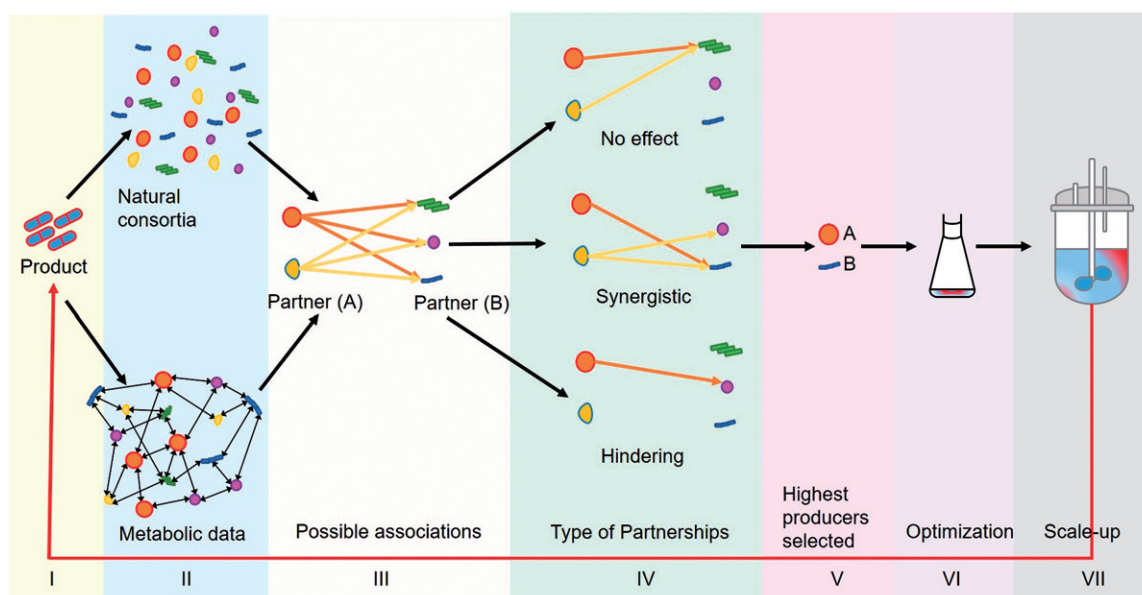


Figure 2. Steps involved in constructing an artificial co-culture. A bottom-up approach is shown. The desired product is defined first (I), the microbial producers are short-listed next. This can be based on metabolite profiling or on natural associations (II). From selected candidates (III) co-cultures need to be investigated to elucidate the type of partnership (IV). The highest yielding co-culture is to be selected (V), optimized (VI) and upscaled (VII).

been termed the priority effect [86,87], and can be an integral factor in bioreactor systems, as shown by Zhang et al. [84]. The co-culturing of *C. vulgaris* and *R. glutinis*, achieved higher levels of biomass and lipids, reaching 17.3 and 70.9%, respectively, when each culture was inoculated in their respective log-phase, at a ratio of 1:1. Similarly, the co-culture of *Dinoroseobacter shibae* (a bacterium) and *Thalassiosira pseudonana* (a diatom), required *T. pseudonana* to be in exponential growth phase before the bacterial inoculation [88].

Reactor design and available technologies for co-culture

Bioreactors (photo, airlift, pulsed, stirred, packed, fixed-bed, fluidized, etc.) that can be run in continuous, semi-batch/fed-batch and batch modes have been devised for the culturing of axenic cultures, where monitoring and nutritional requirements are relatively simpler when compared to co-cultures. The challenges rest in finding suitable methods to maximize the growth of co-cultures.

One non-compartmentalized approaches, such as co-inoculation, pelletization [89], biofilms, and encapsulation [77], allow for close contact of the organisms facilitating metabolite exchange. However, this approach has problems with respect to monitoring population dynamics, third party contamination, and meeting nutritional requirements of the primary partner to ensure it is not outcompeted. In compartmentalized

approaches the physical contact of the interacting organisms is limited [70]. However, it offers the advantage of independent harvesting and easier monitoring of the bioreactor environment. Each culture is treated as a monoculture, whilst exploiting co-culture characteristics. Approaches here include: membrane segregation [88] including dialysis/hydrogel system [90], transwell systems [70,91] and adhesion matrix, bead entrapment [77], agar plate growth [92], growth in microfluidic channels, gaseous separation [93], cell droplets [94], and matrix immobilization [95].

Critical considerations

Setting up a co-culture for a biotechnological application will involve compromising certain species characteristics. Trade-off between optimal conditions and the growth conditions, in the two or more species selected, need to be taken into account. Trade-off may involve a slower growth rate of the organisms, compared to optimal growth levels, but with higher product yields. This has an impact on processing times. However, the higher titers may outweigh the disadvantage. Viabilities of the co-culture can then be pre-determined with an overall system mass balance. Monitoring the population dynamics to prevent competition, over-/under-yielding effects [96], contamination, toxicity, priority effects [43,86] and abiotic factors have to be addressed for system reproducibility and to prevent production failures or diminishing yields

Case study: microalgae co-cultures for biotechnological application

Microalgae can be prokaryotic (cyanobacteria) and eukaryotic photosynthetic microorganisms. They play a major role in the function of both aqueous and non-aqueous ecosystems due to their ability to grow photo-autotrophically, hence converting inorganic to organic matter that may serve as a source of nutrition for other microorganisms [97]. The simplicity of microalgae, in terms of nutrient requirements and manipulation, makes them ideal candidates for biofuel production [98–103], with some strains of *Schizochytrium* sp. reportedly accumulating oil up to 77% dry wt. [104].

The multitude of high-value biomolecules, such as: astaxanthin, β -carotene, omega-3 fatty acids, phycocyanin, EPS, organic acids and allelopathic chemicals [10,105–108], that can be produced by these organisms, makes them organisms of commercial interest in the pharmaceutical and nutraceutical industries. However, their performance is affected by various factors, such as: contamination, pH, temperature, nutrient limitations, and light availability [109–113]. Lipid accumulation [114–118], and accumulation of other bio-active compounds is usually a response to stress caused by nutrient starvation, high light, temperature, pH and salinity [119–123]. Usually, the biomolecules are chemically extracted, however, in the case of algae belonging to the genera *Chlorella* and *Dunaliella*, they are also secreted into the growth medium [124].

Current established industrial productions include: β -carotene using *Dunaliella salina* [125], astaxanthin using *Haematococcus pluvialis* [126], proteins from *Spirulina platensis* [127], fatty acids from *Chlorella* sp. [128] and pigments using *Nostoc* sp. [129]. Other products also include: lutein, xanthophylls, antimicrobials, anticoagulants in addition to carbohydrates (starch and other polysaccharides) [71,130–134]. Table 2 lists examples of high-value products from species of microalgae, which have been commercially successful. The market value for lutein, for example, was estimated to be US \$187 million in 2009 [135] with astaxanthin products being worth about US \$200 M per year [136]. Though some of these compounds can be synthesized artificially, manufacturers are steering towards natural products, due to limitations in biological functions and implications in food safety [137].

Microalgae co-cultures: current status

Microalgae are good candidates for co-culture, and research in this field is yet to harness its full potential.

There is a considerable body of work on consortia and co-cultures in the wastewater treatment and anaerobic digestion, where microalgae are increasingly being investigated as co-culture partners. Here, we focus primarily on microalgae co-cultures that can be used in biomanufacturing. Work with bench scale and small pilot scale trials have been carried out on the interaction between microalgae and other microorganisms. Popularly, bacteria have been the focus of the investigation, as many bacterial species are endogenous in most non-axenic microalgal cultures. The tight-knit relationship that exists between bacteria and algae comes to the fact that many microalgae rely on exogenous sources of cobalamin (vitamin B₁₂), thiamin (vitamin B₁) and/or biotin (vitamin B₇) to grow [138–140]. These compounds are widely synthesized by a vast array of bacterial species [68,139,141] and are available for consumption.

Investigations have shown that co-culture of the bacterium *Mesorhizobium loti* with the green alga *Lobomonas rostrate* [138,139] and the bacterium *Sinorhizobium meliloti* 1021 (*Ensifer meliloti*) with the green alga *Chlamydomonas reinhardtii* [140] are based on vitamin associations. Furthermore, cobalamin producing bacteria, such as: *Mesorhizobium* sp., *Mesorhizobium plurifurium*, *Roseomonas mucosa*, *S. meliloti* Mn04-gfp, *S. meliloti* 1021, *Alcaligenes faecalis*, and *Pseudomonas putida* mt2, have also been shown to live in successful symbiotic associations with the microalgae *C. reinhardtii*, *L. rostrate* and *C. nivalis* [138]. The studies concluded that the consortium established a defined algal morphology development, nutrient acquisition as well as bacterial growth [140].

A further potentially important relationship is between microalgae and yeast, where the microalgae provide O₂ for yeast to assimilate carbon substrates and the yeast release CO₂ to aid algal photosynthesis. Work conducted in the co-culturing of yeast and algae has shown increases in overall biomass with an impact on lipid profiles. The coupling of microalgal species with a symbiotic organism led to an increase in biomass and desired products, and has gained popularity in bioremediation and biodiesel production, as shown in Table 1. When using microalgae assemblages for bioremediation, the waste streams are high in nutrients, which may cause bacterial strains to out-grow the algal strains. This would affect the lipid profile for biodiesel production, as bacterial strains are low lipid producers. Similarly, with no nutrient starvation, lipid synthesis may not occur within the algal strain. Thus, other forms of energy recovery, such as anaerobic digestion and hydrothermal liquefaction are more suitable.

Table 2. A selection of high-value products derived from microalgae species as monocultures.

Bioproduct	Reported species	Reported product yield/concentration	Reference
Astaxanthin	<i>Chlorella zofingiensis</i> ATCC 30412	10.3 mg/L	[160]
	<i>C. zofingiensis</i> , CCAP 211/14	0.1 pg/cell	[161]
	<i>Haematococcus pluvialis</i> LB 16	91.7 pg/cell	[162]
	<i>H. pluvialis</i> 26	40.25–51.06 mg/L	[163]
	<i>H. pluvialis</i> , 34/7	2.7% dry wt	[164]
β-carotene	<i>Dunaliella salina</i> , Sambhar Salt Lake	4.21 pg/cell	[122]
	<i>D. salina</i> - 19.3	7.05–8.26 pg/cell	[165]
	<i>D. salina</i> SAG 42.88	3.99 pg/cell	[123]
	<i>D. salina</i> , CONC-007	72.7 pg/cell	[166]
	<i>D. salina</i> , CCAP 19/18	31.6 pg/cell	[166]
	<i>D. salina</i> , Urmia Lake isolate	8.94–11.4 pg/cell	[167]
	<i>D. salina</i> , KU01	56.25 pg/cell	[168]
	<i>Dunaliella bardawii</i> – KU01	52.91 pg/cell	[168]
	<i>D. salina</i> , CCAP 19/18	70 pg/cell	[169]
	<i>Dunaliella</i> sp., Sambhar Salt Lake	94.26 pg/cell	[122]
Glycerol	<i>Botryococcus braunii</i> , UTEX 572	5.51–21 mg/L/d	[170]
Lipids	<i>Chlorella vulgaris</i> , KCTC AC10032	6.91 mg/L/d	[170]
	<i>Scenedesmus</i> sp., KCTC AG20831	20.65–39 mg/L/d	[170]
	<i>Chlamydomonas acidophila</i>	20 mg/L	[171]
Lutein	<i>Muriellopsis</i> sp., isolate from Empordàmarsh	1.4–0.8 mg/L/d	[135]
	<i>C. zofingiensis</i> CCAP 211/14	4 mg/g dry wt	[161]
	<i>Nostoc muscorum</i>	0.0229% p/v	[134]
Phycobilin	<i>Gloeotrichia natans</i>	0.21 g/L	[129]
Phycocyanin	<i>Galdieria sulphuraria</i> 074G	8–28 mg/g dry wt	[172]
	<i>Spurilina platensis</i>	46% w/w	[71]
	<i>S. pluriformis</i>	9.6% w/w	[71]
	<i>Nostoc</i> sp.	20% dry wt	[129]

The species involved and reported product yields/productivity/concentration are provided in different units as reported in the references.

Factors affecting microalgae co-cultures

As in monocultures, pH, nutrients, N/P ratio, availability of carbon source, light intensity and salinity will affect the growth kinetics of the co-culture. Likewise, the priority effects and history of the community, as discussed in the section “Co-culture media”, will influence the co-culture. A limiting step would be co-culturing an organism with a higher growth rate compared to algae (bacteria/yeast), which may result in the algae population being outcompeted, with light limitation due to shading, and competition, being factors affecting the final product yield [37,139,142].

Studies carried out by Cai et al. [143] investigated the growth and biochemical composition of alga *Isochrysis galbana* and the yeast *Ambrosiozyma cicatricosa* co-cultures for aquaculture food. A co-culture inoculum ratio of 1:1 was employed yielding a higher biomass of 1.32 g/L compared to the maximum obtained from *I. galbana* 8701 (1.17 g/L) and *A. cicatricosa* (0.31 g/L) monocultures, with enhancements in C14 and C18 fatty acid content, 18.85 and 9.03% of the total fatty acids. At the conclusion of the experimental period, the co-culture population was 96.64% algae cells. Zhang et al. [84] demonstrated that inoculating *C. vulgaris* and *R. glutinis* co-culture during logarithmic growth improved biomass and lipid yields of 17.3 and 70.9%, with seeding ratios of 1:1 and 1:2 (yeast:algae).

Shu et al. [144] investigated *Chlorella* sp. and *Saccharomyces cerevisiae*, at the following seeding

ratios, 1:2, 1:1, 2:1, the best ratio was 2:1. (algae:yeast), with higher lipid and biomass produced. In the case of *Scenedesmus obliquus* with *Candida tropicalis* and *S. cerevisiae*, a ratio of 3:1 (algae:yeast) increased the algal biomass yield by 30% [145].

Microalgae co-culture: future potential

In the case of eukaryotic microalgae, the partnership with other organisms, such as bacteria, yeast or cyanobacteria may be beneficial in production outputs. Selecting symbiotic/synergistic/mutualistic organisms for artificial co-cultures, that themselves produce marketable products, allows for a biorefinery mode of production [71,72]. Extrapolating this concept to symbiotic poly-cultures, thus mimicking natural consortia in the laboratory, would fully exploit the system. A possible future multi-production scheme, for an algae photobioreactor is represented in Figure 3.

Co-cultures and consortia: challenges and future possibilities

The literature presented in this review describes the benefits of a co-culture, with the design of co-cultures on trigger-response mechanisms to increase outputs [49,58]. However, slight variations in the culturing system could modify the behavior of the consortium and destabilize the synergistic balance, leading to loss of

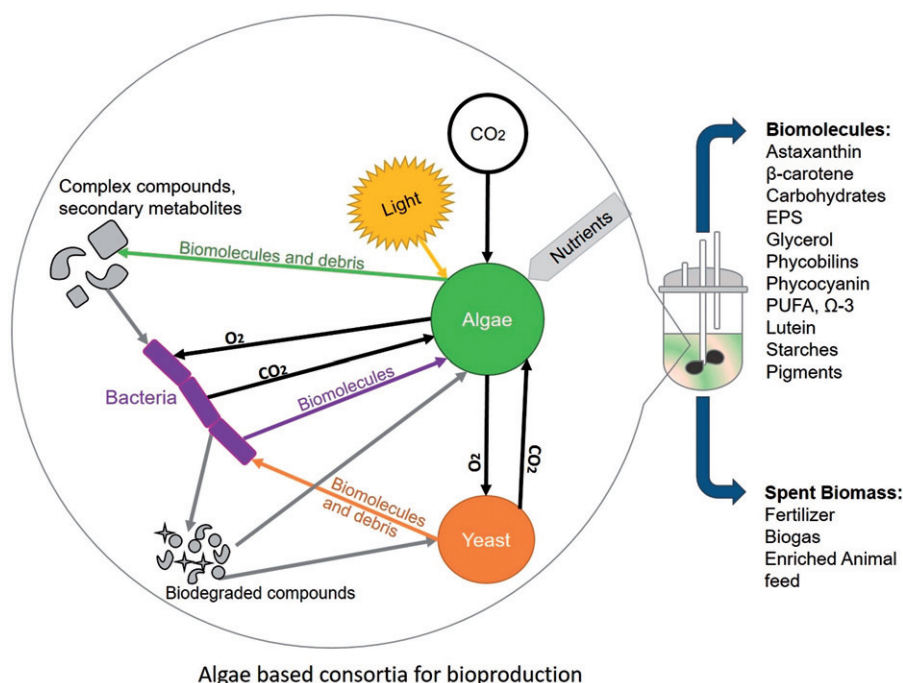


Figure 3. Representation of a microalgae-based consortium for biotechnological applications. A photo-illuminated bioreactor for culturing an artificially created synergistic consortium between algae, yeast and bacteria within a small-scale reactor is represented. The microalgae take up carbon dioxide and produce oxygen (through photosynthesis) that is, consumed by the aerobic bacteria and yeast, which in turn supply carbon dioxide (through respiration) to be consumed by the algae. Cell secretions and degradation will release biomolecules (vitamins, proteins, carbohydrates, nucleic acids and secondary metabolites) into the growth media. The bacteria will break these materials into simpler compounds to be consumed by all members of the consortium.

product. Potential reactor design based on the actual metabolic fluxes, as proposed by Stenuit and Agathos [64], is a tool to be used to monitor and predict culture behavior, and from which to build upon for further optimization.

Understanding the underlying communication and population dynamics is necessary to engineer a successful industrial consortium. Identifying the extracellular chemical cues (metabolites/peptides/proteins) released by species within a co-culture/consortium would provide a canvas from which to develop the consortium production [34,57]. Various methods have been used to track molecular exchanges between microorganisms, outlined by Narihiro and Sekiguchi [146] and Beale et al. [147]. These include extraction using organic solvents, cation exchange [148] combined with chromatography techniques and Mass Spectrometry [149] in combination with intracellular metabolic profiling [150,151]. Challenges exist with respect to trapping and concentrating the molecules of interest [91,147], sample processing, and separation of intra- and extra-cellular metabolites. In addition, the interference from matrix components, such as salts found in growth media of marine algae need to be considered [151,152].

Co-culture database

Natural consortia have evolved over long periods and the associations constructed by the microorganisms themselves have progressed through selection phenomena to produce the extant scenarios. In the biotechnological environment, it would be unworkable to screen all positive associations. A valuable tool would be to have an open access database, detailing successful and failed, co-culture trials, with proper documentation of extracellular compound yields and relevant metadata. This would be beneficial for academic research and facilitate the transition from bench-scale to industrial applications.

Databases have found their role in engineering and more recently in synthetic biology. The compilation of databases, such as the Synthetic Biology Open Language database allows the user to search and find the right combinations to meet the research requirements. The standardization of key aspects that govern biological phenomena has propelled research in synthetic biology. In a similar fashion, databases have been created for the metabolites and metabolic pathways, for pathogens and drugs, as outlined by the Metabolomics Society [153]; these databases are viewed by millions of

users on a daily basis, who consult, update and contribute data. The identification of communication systems would benefit structuring future artificial co-cultures. Some quorum sensing, allelopathic chemical and signaling molecules from various extracellular polymeric subclasses have been identified [154,155]. It is important to preserve the bio-molecular interactions within a database that is easily accessible. Many extracellular substances are of great interest to the industry. A compendium incorporating such information also improves on the understanding and provides a better framework in which co-culturing can be exploited.

A useful co-culture database would provide standardized culturing conditions or at least valuable meta-data. This database should contain information on the microorganisms, relating to their growth dynamics, biomolecules released in axenic and in co-cultures, in addition to bioreactor conditions. The addition of an online simulator, such as HYSIS and UniSim in Chemical Engineering, would facilitate analysis, simulation and design of co-cultures and consortia in biomanufacturing.

Conclusions

Research for the creation of artificial co-cultures in biomanufacturing has its merits. As discussed in this review, benefits include minimization of contamination and enhanced co-production of similar products. Assembling and implementing co-cultures, derived naturally or artificially, is not straightforward. The ability to create very stable lichen-like systems in the laboratory may not be feasible for at least another decade. However, the first steps to take should be in the direction of understanding the trigger-response mechanisms in co-cultures in order to build a versatile engineering framework. With the appropriate tools and systematic approaches, such as the proposed database, the use of co-cultures can be developed and steered towards more complex and dynamic consortia, that can be used in biomanufacturing. In this regard, microalgae-based co-cultures offer promise, given their natural associations, versatility and ability to thrive with dissimilar species. The advantages of using them as the core on which to build the consortia rests on the fact that they are widely available, to produce an array of products with significant importance in the welfare of humans and animals. They offer environmentally sustainable biomanufacturing routes to be developed, given their ability to fix atmospheric carbon dioxide. In future, systematic construction of consortia with appropriate documentation and development should enable co-cultures to be effectively used in biomanufacturing.

Disclosure statement

The authors declare no conflicts of interest.

Funding

GP acknowledges financial support from Engineering and Physical Sciences Research Council DTA PhD studentship. The authors are also grateful to Biotechnology and Biological Sciences Research Council (BB/K020663/1), whose support helped in the construction of this work.

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