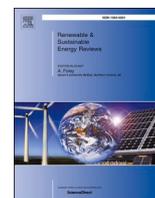


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Algal–bacterial consortia for bioproduct generation and wastewater treatment

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ABSTRACT

Microalgae have been developed as promising candidates for bioenergy production, coupled with answering challenges related to water pollution and global warming. When combined with wastewater, microalgal biomass production could be freed from a strong dependence on freshwater and chemical nutrients, as well as achieve the additional advantage of wastewater reclamation. However, despite those dual benefits, certain limitations on the growing of algae in wastewater remain to be resolved, including the inevitable presence of bacteria in wastewater, which influences biomass productivity and quality in various ways. Pointing at microalgal-bacterial relationships, this study provides an updated review of the application of microalgal-bacterial consortia (MABC) to benefit biomass yield and harvest, and in wastewater remediation, focusing on the main interactions established between the microorganisms integrated within MABC and the factors influencing the behaviours of MABC. The challenges faced by the MABC biotechnology are also discussed, which are primarily rooted in undesirable bacteria that parasitically eat microalgal products and inhibit algal growth through nutrient competition, lysate exudation, or reducing the algal resistance to biotic stress. However, there is a lack of systematic studies on maintaining stable and effective operation of MABCs for wastewater cultivation and high-value bioproduct generation. Knowledge gaps are identified as including systematic information about the responses of MABC to culture conditions and wastewater-borne bacterial communities, and the metabolic mechanisms underpinning the interactions between algae and bacteria in wastewater. Further research focuses and methodologies are proposed in this review, making full use of the advent of omics and computational technology.

1. Introduction

Microalga-based biofuels cater for increasing energy demand while decreasing greenhouse gas emissions, on account of their competitive lipid production and effective CO₂ utilization [1–3]. However, the current cultivation cost is too high to allow commercial application, since nutrient use constitutes half of the cost and energy input of the cultivation process, mainly comprising nitrogen and phosphorus [4]. In the quest for inexpensive algal bioenergy, the combining of wastewater treatment and microalgal cultivation was touted as a viable means of significantly reducing the nutrient cost as well as the freshwater input [5]. In the process of growing algae with wastewater, diverse and rich microbial communities within the wastewater posed an obstacle. Wastewaters usually contain large amounts and high diversities of microorganisms, as indicated by high Chao values and Shannon indices,

where members of the Proteobacteria, Bacteroidetes, Firmicutes, Synergistetes and Actinobacteria phyla generally dominate [6–8]. It is the biotic stress of undesirable communities disturbing the sustainability of wastewater-cultured algae and disrupting algal biomass production that requires the collection of information on wastewater-borne bacteria and their interaction with microalgae.

However, the impacts of bacteria on microalgae are not all adverse. The versatile microbes affect algal growth and biomass accumulation in various ways that cover a wide range of relationships from mutualism and commensalism to parasitism [9–11]. Ecological studies have identified three main types of interactions between microalgae and bacteria through observing natural lichen or constructing artificial co-cultures. The natural interactions involve nutrient substrates, signal chemicals, or gene expression driven by evolution [10,12,13]. Accumulating evidence suggests that these interactions depend on the specific species in microalgal–bacterial consortium (MABC) and have the potential to

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Abbreviations:

AIP	Autoinducing peptides
AOB	Ammonia-oxidizing bacterium
c-di-GMP	bis (3'-5') diguanylic acid
Chl-a	Chlorophyll α
COD	Chemical oxygen demand
DIC	Dissolved inorganic carbon
DO	Dissolved oxygen
EPS	Extracellular polymeric substances
GC-MS	Gas chromatography-mass spectrometry
HRAP	High rate algal pond
HRT	Hydraulic retention time
IAA	Indole-3-acetic acid
MABC	Microalgal-bacterial consortium
MGPB	Microalgal-growth promoting bacteria
NGS	Next generation sequencing
PGPB	Plant-growth promoting bacteria
PPCPs	Pharmaceuticals and personal care products
qPCR	Quantitative polymerase chain reaction
WWTP	Wastewater treatment plant

accelerate the development of microalgal biotechnology in designing co-culture platforms. Nitrogen-fixing bacteria and microalgal/plant-growth promoting bacteria (MGPB/PGPB), along with other symbiotic bacteria, have been applied to promote algal growth, bioproduct accumulation, and nutrient removal at laboratory scale. What is the 'secret ingredient' that these bacteria provide? It has been proved or hypothesized that certain specific bacteria could work as nitrogen suppliers or producers of the hormone indole-3-acetic acid (IAA) [14,15]. Increased biomass harvest efficiency is another benefit from the action of bacteria as biofloculants, reducing the energy demand in centrifugation or facilitating harvest by settling [16].

In addition to species-dependence, both environmental conditions and nutrient availability also have effects, even potentially reversing the relationships between algae and the accompanying bacteria. When co-cultured with *Azospirillum brasilense*, the α -amylase activity in *Chlorella sorokiniana* decreased under nitrogen-replete conditions, but increased under nitrogen-starved conditions, in comparison to mono-cultured algae [17]. The detected concentrations of signal molecules tryptophan and IAA reduced in the co-culture of *C. sorokiniana* and MGPB *A. brasilense* in nitrogen-free or carbon-free media, compared with those in nutrient-rich medium [18].

The aforementioned research was mostly carried out under constant conditions and with uncomplicated combinations of algae and bacteria in a chemically defined medium or pre-treated wastewater, which cannot provide enough knowledge to develop a commercial MABC system fed with real wastewater in continuous operation outdoors, characterized by fluctuating local climates and wastewater characteristics.

Although there are several published studies concerning certain applications of algae with bacteria for biomass production and/or wastewater treatment, only simple and elementary information about MABC in wastewater has been related, due to the complex biotic and abiotic elements in wastewater [12,19–23]. A relatively early review published by Kouzuma and Watanabe briefly summarized the interactions between algae and bacteria in natural and suggested applying algae-bacteria interactions in biotechnology process [12]. Subsequently Ramanan et al. reported contemporary understanding about algae-bacteria interactions that impact on microorganism evolution and habitat ecology, as well as on environmental mitigation and biotechnological potential [10]. Both of them provide an elementary theory about algae-bacteria interactions in natural environments from the aspects of evolution and ecology,

however, they only show simple information about elaborate algae-bacteria interactions in wastewater. Lately Quijano et al. concerned the harvest of algal cells and focused on the factors relating to form algal-bacterial aggregates [22], and Zhang et al. reported the advantages of algal-bacterial consortia on wastewater treatment together with the effects of bioreactor and carrier, but without full consideration about operating environmental factors [23]. A systematic overview is not yet available covering the aspects from creating an MABC to its growth in wastewater, which remains a knowledge gap in this important topic.

The present article constitutes a state-of-the-art review on applying MABC for the dual purposes of bioproduct generation and wastewater recovery, including (i) algal and bacterial sources in conjunction with cultivation mode, (ii) advantages achieved by the MABC over individual algae or bacteria, (iii) factors studied to affect the behaviour of MABC, and (iv) challenges of smoothly and stably operating MABC. The knowledge gaps regarding managing an effective MABC system under variable conditions and wastewater are identified as (i) methodical responses of MABC to culture condition changes and microbial communities inherent in wastewater, (ii) metabolic interactions between organisms involving algae, their associated bacteria, and wastewater-borne bacteria, and (iii) an *in silico* tool integrating a wealth of data about the activities of MABC in varying ambient conditions to guide commercial application of MABC. Hence, by taking advantage of guidance and perspectives from omics and computational technology, we propose a pipeline for establishing an algal-bacterial consortium platform for outputting bioproducts and cleaning wastewater.

2. Interactions between microalgae and bacteria

Algae and bacteria have historically co-existed, and their interactions in the environment and artificial lichen have been revealed by extensive studies as falling into three types, namely nutrient exchange, signal transduction, and gene transfer (Fig. 1) [10,12]. These interactions result in three cooperative or competitive partnerships between algae and bacteria: mutualism, commensalism, and parasitism [9, 10].

2.1. Nutrient interactions

Nutrient interactions commonly occur, affecting algal and bacterial growth rates by either providing nutrients or causing competition for nutrients with each other. The most common materials that are circulated between algae and bacteria are O₂ and CO₂. As shown in Fig. 1, O₂ can be excreted from photosynthetic algae and consumed by heterotrophic bacteria, whereas CO₂ can act as a reagent in photosynthesis within algal cells and be produced through bacterial respiration. Besides that, some specific algal-bacterial partnerships still depend on organic carbon sources, amino acids and vitamins. In coastal environments, a globally distributed diatom, *Pseudo-nitzschia multiseriata* PC9, and its accompanying bacterium, *Sulfitobacter* sp. SA11, exhibited a complex symbiotic exchange of nutrients that improved each other's performance, including diatom-excreted organosulfur molecules and bacterially excreted ammonia [24]. Moreover, the lower growth rate of axenic PC9 and little growth of axenic SA11 in organic carbon-free medium indicated a mutual relationship between these specific strains. The transcriptome proved this relationship by showing increased transcription of the enzymes for biosynthesizing taurine in PC9, together with upregulating the gene expression for taking up and catabolizing taurine. Although nitrate was provided in the co-cultured medium, PC9 preferred SA11-derived ammonia to nitrate. However, the authors did not dig into the reason that the mutual phenomenon only occurred in specific strains, rather than in other closely related strains; i.e. other associated bacteria showed little or even negative effect on PC9 and non-responsive diatoms were also observed when co-cultured with PC9.

Genetically engineered sugar-secreting cyanobacteria have been

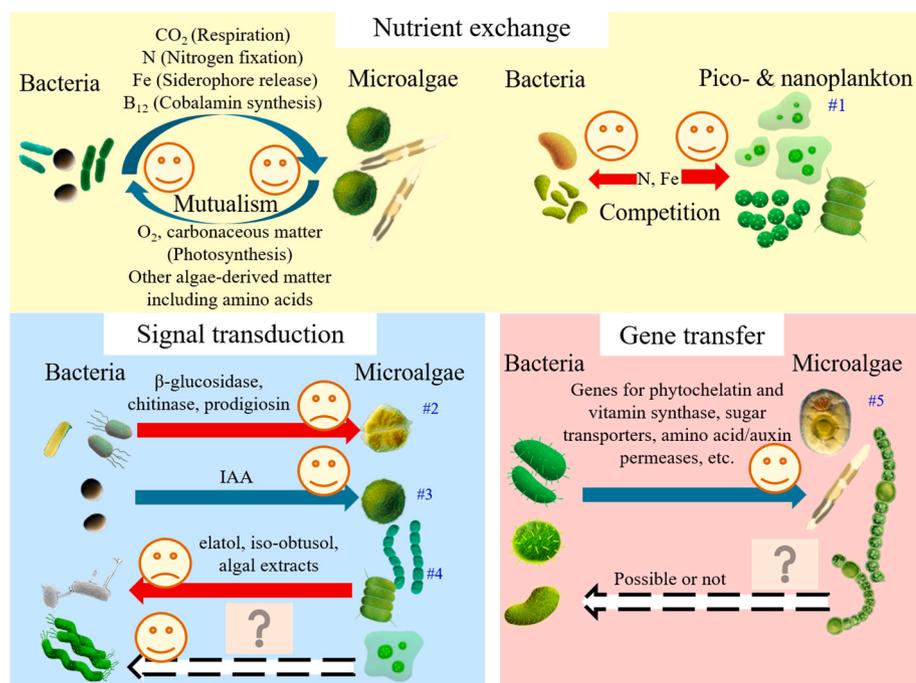


Fig. 1. The interactions between microalgae and bacteria in natural lichen or simply designed artificial co-culture systems. #1, pico- and nanoplankton of size 0.8–25 μm , mostly composed of microalgae. #2, algae and cyanobacteria involved in blooms were the main microorganisms studied. #3, diatoms and green algae were demonstrated to respond to bacterial IAA. #4, cultured green algae and diatoms contributed greatly to this interaction type. #5, eukaryotic diatoms, green algae and eustigmatophytes were found in this group.

explored as sole carbon supplier for the growth of *Escherichia coli* and *Bacillus subtilis* [25,26]. With a simple laboratory two-species system, by modelling the growth dynamics Grant et al. elucidated that the bacterium *Mesorhizobium loti* provided vitamin B₁₂ to the B₁₂-dependent alga *Lobomonas rostrate*, while *M. loti* received photosynthates from the alga [27]. Moreover, this mutualism comprised complex regulation of the synthesis of vitamin B₁₂ in *M. loti*, rather than simply passive lysis, release and reception, wherein the underlying regulatory pathways are still awaiting exploration. *Chlorella vulgaris* was able to release dissolved organic carbon to its associated bacterium and, in return, obtain inorganic carbon and low molecular mass organic carbon from the bacterium to increase cell number and growth rate [28].

The exchanged nutrients are accounted for by the different requirements of algae and bacteria; however, nutrient competition was not unexpected for some limiting elements or compounds in specific environments. On an Fe-fertilized shore, diatoms predominated over heterotrophic bacteria through competing over Fe and secreting limited amounts of organic carbon [29]. By excluding phytoplankton from bacterial cultures and adding a carbon source, Fourquez et al. proved that phototrophs and heterotrophs could compete for Fe through observation of increased bacterial Fe uptake rates, in comparison with the bacteria co-existing with phototrophs [30].

To better understand the nutrient exchange processes between algae and bacteria, besides transcriptome technology, isotope labelling could shed light on the cooperation or competition.

2.2. Signal transduction and gene transfer

In signal transduction, the chemicals that mediate the interactions do not serve as nutrients but rather activate or inhibit gene expression and/or physiological activities, thereby modifying behaviours and growth. The bacteria *Alteromonas* sp. and *Thalassobius aestuarii*, co-existing in the phycosphere of *Alexandrium tamarense*, were able to produce β -glucosidase and chitinase to directly lyse algal cells [31]. Prodigiosin has attracted hot attention due to its broad damage to algal membranes. It is a red pigment synthesized by bacteria usually found in algal bloom areas, including *Serratia*, *Streptomyces*, *Vibrio*, *Hahella*, *Zooshikella*, and *Pseudoalteromonas* [32,33]. Amin et al. found that the bacterium *Sulfobacter* sp. SA11, associated with *Pseudo-nitzschia multiseriis* PC9,

promoted diatom cell division via secretion of IAA which was synthesized by the bacterium using both diatom-secreted and endogenous tryptophan [24]. Here IAA and tryptophan served as signalling molecules to create an exclusive feedback loop between PC9 and SA11.

From the perspective of algal exudation, metabolites against bacterial survival and growth were commonly reported. Secondary metabolites of the red algae *Laurencia* spp., including elatol and isoobtusol, exhibited strong and broad-spectrum antibacterial activities [34]. The crude extracts of *Scenedesmus bajacalifornicus* BBKLP-07 have been tested against *E. coli*, *C. perfringens* and *B. subtilis* bacteria [35]. Usually the interspecies signal transduction did not require direct contact [36]. Although several signal molecules were identified in the environments or cultures containing intermingled photo- and heterotrophs, there remain massive numbers of substances that potentially underlie the regulation process and its pathway of interactions between algae and bacteria, such as basal interspecies recognition and the potential signals awaiting bacterial survival.

Another algal-bacterial interaction has been found as horizontal gene transfer in natural samples. Horizontal transference of a gene between neighbouring microbes is defined as gene transfer and plays a critical role in evolution [37]. Phylogenetic analyses of chloroplast genomes have revealed that two open reading frames were part of an operon-like gene cluster conserved in bacteria, which indicates horizontal gene transfer between the chloroplast genomes of the diatom *Seminavis robusta* and bacteria [38]. Employing transcriptomic sequencing together with bioinformatic analyses and an automated annotation tool, Olsson et al. identified that novel phytochelatin synthase genes in two extremophilic green algae had transferred from bacteria [39]. Yurchenko also reported a eustigmatophyte operon closely related to the one from *Phycorickettsia* and suggested that the gene transfer between bacterium and its host explains the early evolution of eustigmatophyte [40]. According to current knowledge, gene transfer mostly occurs in natural long-term systems for microbial evolution and adaptation to environmental change.

In natural processes, the three kinds of interactions (nutrient interactions, signal transduction and gene transfer) usually work together to form tightly connected networks for acclimatisation and control the physiological response of co-occurring algae and microbes to different environments, and even possibly act as facilitators of speciation [24,41].

In artificial platforms, the diverse interactions have facilitated many aspects, including controlling harmful algal blooms, accumulating pharmaceutical feedstocks, bioenergy production, and wastewater treatment [12,42–45].

3. The application of MABCs in biofuel production

While algae have attracted significant attention for their capacity to produce biofuels and industrial materials, the presence of bacteria could hardly be avoided in the process of algal cultivation, considering the impracticality of achieving sterile environments in commercial cultivation and the inevitable organic substances arising from algal metabolism. The bacterial presence would affect algal biomass yield in various ways — either positive or negative. Positive impacts are achieved when bacteria provide nutrients needed by algae or secrete growth-promoting signals.

Besides biodiesel, co-culturing algae with bacteria was reported to improve the yield of other bioenergy products, such as hydrogen and electricity [43,46,47]. The abovementioned algal–bacterial interactions are the basis of understanding algal–bacterial behaviours in MABCs, in which the positive effects of bacteria on algal growth and bioproduct yield have been explored, as shown in this section together with Fig. 2 and Table 1. Fig. 2 visually and briefly summarizes the positive impacts of bacteria as nutrient supplier and growth-promoting signal producer to algae, followed by increased production of algal biomass and harvest efficiency. Table 1 shows recent application of bacteria to promote algal activities, including bacterial source, promotion on biomass accumulation and cellular metabolism as well as harvest efficiency, culture conditions and analysis methods.

3.1. Bacterial promotion of algal growth as nutrient supplier and growth-promoting signal producer

In terms of algal biotechnology, the main purpose herein is to obtain high-quality algal biomass without adverse impacts by bacteria, based on revealing the effects of associated bacteria on algae.

In this context, Kim et al. isolated and identified *Rhizobium* sp. as the most prevalent bacterium in the phycosphere of green algae, for which this bacterium increased the algal cell count by 70% and received fixed organic carbon in return [14]. Another symbiotic *Rhizobium* sp. was isolated by Fei et al.: it supplied nitrogen to its host, *Chlorella variabilis*, leading to an enhanced growth rate, but stimulated fatty-acid oxidation in algal cells, resulting in low-quality biodiesel [48]. *Azotobacter vinelandii* was also employed as a nitrogen fixer for its co-cultured cyanobacterium, with the aim of reducing the cost of the nitrogen source [49].

In addition to nutrients, studies have also shown that some PGPB can provide signal stimulation of algae by cell communication. To cite an

instance, Pagnussat et al. succeeded in promoting the growth of *Scenedesmus obliquus* C1S and alleviating its oxidative stress under nitrogen deprivation through co-culturing *Azospirillum brasilense* Sp245, a supplier of IAA [15]. Similarly, the symbiotic bacterium *Achromobacter* sp. CBA4603 of *Haematococcus pluvialis* elevated the algal cell density and chlorophyll content and prolonged the exponential stage through the exudation of IAA [50]. In addition to contact co-culture, the growth of *Chlorella vulgaris* OW-01 was able to be efficiently augmented by the volatile compounds of its phycospheric bacteria — including *Hyphomonas* sp., *Rhizobium* sp., and *Sphingomonas* sp. — in a non-contact culture apparatus [51]. The possible growth-promoting agent was verified as indole by the responses of algal growth to the supplementation of synthetic chemicals.

3.2. Bacterial promotion of algal bioproducts

Bacterial–microalgal interactions can induce metabolic changes in cells, which could be a useful strategy to raise the production of lipids and other possible valuable compounds. The siderophore-producing bacterium *Idiomarina loihiensis* RS14 slightly enhanced the lipid and protein contents in *Chlorella variabilis* ATCC 12198 in iron-deficient conditions [52]. Besides growth, the nitrogen-fixing aerobic bacterium *Mesorhizobium sangaii* also trebled the lipid production of *Chlorella vulgaris* under nitrogen deficiency conditions [53]. Without any alteration of growth rate, the bacterium *Vibrio cholerae* enhanced metabolism related to the fatty acids of the microalga *Isochrysis galbana*, with increased production of total lipids and polyunsaturated fatty acids [54]. However, the underlying pathway that regulates metabolic behaviours promoting lipid accumulation has not been discussed much in the literature so far.

When co-culturing with *Escherichia coli*, *Pseudomonas stutzeri*, *Pseudomonas putida* or an unknown bacterial community, *Chlamydomonas reinhardtii* enhanced H₂ production because O₂ that it photosynthesized was in turn consumed through bacterial respiration, thereby mitigating the inhibitory effect of high O₂ concentration on hydrogenase activity [43]. Respiration was considered to be the main reason for increased H₂ production in the co-culture system, so the bacterial respiration rate, the algal/bacterial ratios and the conditions related to algal photosynthesis efficiency were discussed to optimize H₂ generation [55,56]. The synergistic effects on CO₂ and O₂ between algae and bacteria also facilitated electricity generation, algal biomass growth, and lipid production in algally assisted microbial fuel cells [47,57,58].

Both nutrient exchange and signal transduction have been reported to occur in the co-cultivation of algae and bacteria to improve algal growth and biomass production, while as yet very few published reports relate to gene transfer that required a long-term partnership [40]. Moreover, reported exchanges of nutrients and signal molecules were

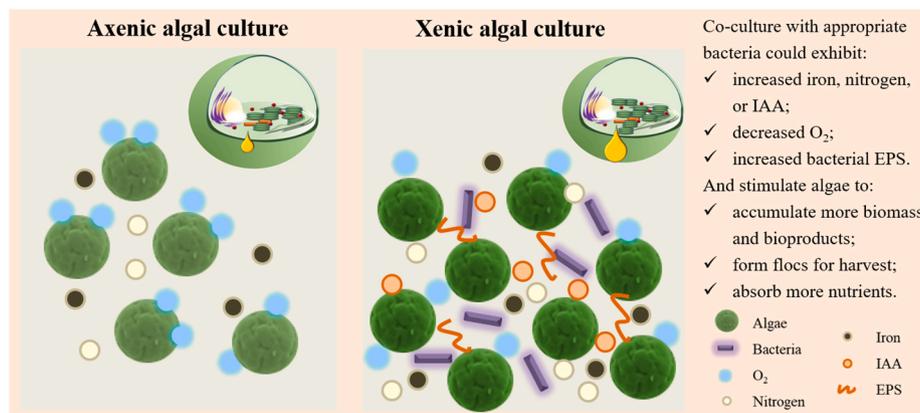


Fig. 2. The symbiotic effects of bacteria on the growth and lipid production in co-cultivated microalgal cells, in conjunction with harvest efficiency. Herein bacteria could work as nutrient supplier, growth-promoting signal producer or flocculant.

Table 1
The application of bacterial improvement to algal processes.

Bacteria / source	Algae	Improved algal response	Culture medium	Main methods used for analysis	Ref
<i>Rhizobium</i> sp. / isolated from phycosphere of <i>Chlorella vulgaris</i>	<i>Chlorella vulgaris</i>	Increased algal growth by 70.3%	BG11 medium	Denaturing gradient gel electrophoresis for bacterial community and qPCR for quantifying <i>Rhizobium</i> sp.	[14]
<i>Hyphomonas</i> sp., <i>Rhizobium</i> sp., and <i>Sphingomonas</i> sp. / isolated from phycosphere	<i>Chlorella vulgaris</i> OW-01	Enhanced algal growth to at least 1.5-fold higher biomass and lipid productivity	Algae in BG11 medium; while bacteria in Lysogeny broth	GC-MS for bacterial volatile compounds	[51]
<i>Rhizobium</i> sp. / isolated from phycosphere	<i>Chlorella variabilis</i>	Increased algal growth by 27% through bacterial-derived nitrogen	Bold's basal medium	Transcriptome analysis for algal metabolic response, including nitrogen and fatty acids	[48]
<i>Azospirillum brasilense</i> / purchased IAA producer	<i>Scenedesmus obliquus</i>	Decreased algal reactive oxygen species levels, increased algal cell densities, and ameliorated algal cell bleaching	Nitrogen-limited BG11 medium	Recombinant strains to confirm the interaction between algae and bacteria	[15]
<i>Achromobacter</i> sp. CBA4603 / isolated from phycosphere	<i>Haematococcus pluvialis</i>	Elevated cell density and prolonged exponential phase	Optimal <i>Haematococcus</i> medium	Auxin quantification and next-generation sequencing (NGS) platform for bacterial community analysis	[50]
<i>Bacillus licheniformis</i> / purchased	<i>Microcystis aeruginosa</i> and <i>Chlorella vulgaris</i>	Promoted dry cell mass and chlorophyll-a production in <i>Chlorella vulgaris</i>	Synthetic wastewater	HPLC for target signal molecules and q-PCR for quantifying the genes related to chlorophyll metabolism and bacterial genes	[83]
All bacteria in activated sludge	<i>Scenedesmus</i> sp. 336 and <i>Chlorella</i> sp. 1602	Increased dry biomass production, possibly due to the dominant existence of IAA-secreting bacteria	Artificial municipal sewage	NGS for bacterial community structure and microbial diversity analysis	[88]
All bacteria naturally associated with the microalgae	<i>Ettlia</i> sp. YC001	Increased algal flocculation efficiency	BG-11 medium	SDS-PAGE analysis of protein bands, and NGS for the bacterial communities	[62]

quite limited to inorganic nutrients (such as CO₂, O₂, and nitrogen) and algal growth promoter (mainly IAA) in most algal cultivation research. The current findings are not sufficient to propel MABC development to the goal of understanding the interactions between these microorganism species and further commercial application of bioenergy. The stability of MABCs should be assessed first of all, which requires as much information as possible about signals for recognizing and regulating the target microorganisms, their behaviour, and the compounds exchanged, together with the factors influencing the MABC's activities.

3.3. Bacteria as flocculants

Besides enhancing the growth rate, certain bacteria were studied to facilitate the aggregation of algal cells, thereby streamlining the algal harvest process and reducing the energy and cost requirements [16,59]. Xenic culture of *Chlorella vulgaris* (in the presence of live microalga-associated bacteria) exhibited effective flocculating activity that was 46 times higher than that in the axenic culture [60]. Li et al. applied the bacterial cells of *Micrococcus* sp. hsn08 to harvest negatively charged microalgal cells with the help of amino acids in the cell walls and positively charged calcium ions in the culture [61]. Even for self-flocculating *Ettlia* sp. possessing high concentrations of extracellular polymeric substances (EPS), the associated bacterial community was able to further increase flocculation efficiency and strengthen the aggregate integrity via additional bridging provided by the long filamentous EPS structure [62]. The mechanisms underlying microalgal bioflocculation by bacteria can probably be attributed to polymeric substances on the surface or secreted out of bacterial cells, along with the contribution of the positively charged ions around cells.

This part shows the advantages of MABC as a platform of biofuel production. Firstly, bacteria as nutrient supplier and growth-promoting signal producer to algae increase production of biomass and lipid. Secondly, bacterial respiration consumes O₂ and then improves H₂ yield of H₂-generated algae. Additionally, bacterial EPS contributes to forming algal-bacterial flocs that facilitate harvest by gravity.

4. MABCs for wastewater treatment

Algal systems, with or without the designed help from bacteria, have

been extensively used in the treatment of wastewater since the 1950s. The reason of choosing algae for wastewater treatment lies in their high growth rate and strong adaptation to different environments, even extreme polar environments. Elaborate mechanisms of algal adaptation include membrane fluidity, enzyme flexibility, cellular compatible solute accumulation, extracellular substances, light acclimation, antioxidant synthesis, and dark adaptation [63]. Further, with the recognition of potential benefits arising from the inevitable presence of bacteria, rapid developments were made in the use of MABC for treating wastewater, and for producing biomass, over the past decade.

Focusing on MABC system in wastewater, the specific operational strategies and the key advantages from recently formed algal-bacterial consortia are reviewed and discussed in this section, as summarized in Table 2.

4.1. Operational strategies to form an MABC

The operation of treating wastewater through the use of MABC usually includes screening appropriate oleaginous algal species tolerant to wastewater environments, choosing bacterial species working with the target alga, and growing the suspended or immobilized consortium in open or closed bioreactors. The following section is a compilation of the information available in the recent literature on this matter.

In an MABC system, algae are supposed to play an important role in three respects: (i) recovering inorganic nutrients (especially nitrogen and phosphorus) from wastewater to achieve pollutant remediation; (ii) producing photosynthetic O₂ and photosynthate to support the metabolism of aerobic bacteria; and, foremost, (iii) accumulating high-value compounds, such as polyunsaturated fatty acids and lipids. Hence the ideally selected algae would play a vital role in an MABC system. Most algal species employed in this biotechnology were isolated from wastewater or polluted natural waters due to their strong adaptability to harsh environments and high generation of bioproducts.

Regarding the other member of the consortium, the function of bacteria is designed to exert influence on the following aspects: (i) degrading organic materials and photosynthate to achieve advanced treatment of wastewater; (ii) producing inorganic carbon and possible algal growth promoting substances (mostly from specific bacteria) to enhance biomass production; and (iii) regulating the metabolism in

Table 2

Application of microalgal–bacterial consortia in nitrogen, phosphorus and carbon removal from different wastewaters.

Microorganisms	Waste stream	System and operating mode	Algal growth	Nitrogen removal efficiency %	Phosphorus removal efficiency %	Carbon removal efficiency %	Ref.
<i>Auxenochlorella protothecoides</i> with wastewater-borne microbial community	Effluent from oxidation tank at a winery plant	200 mL suspension in batch mode	No significant increase	100 ± 0 A: 99 ± 1 B: 16 ± 17	100 ± 0 A: 100 ± 0 B: 1 ± 1	38 ± 6 A: 44 ± 11 B: 36 ± 14	[8]
<i>Chlorella</i> sp. with <i>Acinetobacter</i> sp. isolated from wastewater	Sterilized centrate from WWTP	100 mL suspension in batch mode	1.08 g/L A: 0.80 g/L B:	79.12 A: 73.10 B: 73.10	96.26 A: 83.43 B: 48.75	79.11 A: 63.91 B:	[67]
<i>Chlorella vulgaris</i> with its co-occurring bacterium <i>Rhizobium</i> sp.	Sterile synthetic wastewater based on BG11 medium supplemented with glucose, peptone, meat extract, urea and KH ₂ PO ₄	350 mL suspension in fed batch mode with HRT of 7 days 9 L suspension in continuous mode with HRT of 7, 5 and 3 days	0.63 ± 0.03 g/L A: 0.52 ± 0.02 g/L B: < 0.05 g/L	54.7 A: 46.8 B: 50.7	~96 A: < 58 B: < 69	48.7 A: 18.3 B: 42.2	[66]
<i>Chlorella</i> sp. with <i>Bacillus firmus</i> and <i>Beijerinckia fluminensis</i> isolated from wastewater	Sterilized vinegar fermentation wastewater	200 mL suspension in batch mode	Reduced algal biomass in co-culture with bacteria	86.7 (1% <i>B. firmus</i>) - (10% <i>B. firmus</i>) 74.2 (1% <i>B. fluminensis</i>) 78.7 (10% <i>B. fluminensis</i>) A: 66.7 52.11 ± 7.31 (NH ₃ -N)	68.9 (1% <i>B. firmus</i>) 61.3 (10% <i>B. firmus</i>) 74.8 (1% <i>B. fluminensis</i>) 74.3 (10% <i>B. fluminensis</i>) A: 56.7	69.7 (1% <i>B. firmus</i>) 75.9 (10% <i>B. firmus</i>) 73.4 (1% <i>B. fluminensis</i>) 76.7 (10% <i>B. fluminensis</i>) A: 62.8	[68]
A stable algal community containing various green algae and cyanobacterial species collected from a raceway pond that was initially inoculated with <i>Chlorella vulgaris</i>	Synthetic wastewater mimicking acid mine drainage	Biofilm in continuous mode with HRT of 3 days	–	–	98.96 ± 0.69	95.53 ± 3.37	[70]
<i>Euglena gracilis</i> with <i>Emticicia</i> sp. EG3 isolated from the algal phycosphere in municipal wastewater effluent	Autoclave-sterilized and non-sterilized wastewater effluent	1 L suspension in batch mode	Increased algal biomass production by a factor of 3 in the co-culture system	–	–	–	[80]
<i>Chlorella pyrenoidosa</i> with ammonia-oxidizing bacterium (AOB) <i>Kluyvera</i> sp. isolated from activated sludge	Autoclaved and raw municipal wastewater	Suspension in batch mode	~0.25 g/L (sterile) A: ~0.41 g/L (sterile) ~0.35 g/L (raw) A: ~0.3 g/L (raw)	91 (NH ₃ -N)	–	–	[81]
<i>Chlorella vulgaris</i> with <i>Bacillus licheniformis</i>	Sterilized synthetic wastewater	Suspension in batch mode	0.9 g/L A: 0.5 g/L	88.95 A: 35	80.28 A: < 60	86.55 A: < 60	[83]
<i>Chlorella vulgaris</i> with <i>Exiguobacterium</i> sp. and <i>Bacillus licheniformis</i>	Piggery wastewater after filtration, decolouration, and autoclave treatment	Suspension in batch mode	2.7 mg/L ^a A: 1.3 mg/L	~80 A: 70	~82 A: 60	~85 A: 34	[84]
Mixed algae (dominated by <i>Leptolyngbya</i> sp.) with aerobic granular sludge	Synthetic domestic wastewater	Aggregates with diameters of 0.61 ± 0.12 mm in batch mode	2 g/L	44.3	65.4	84.6	[72]
<i>Scenedesmus</i> sp. 336 and <i>Chlorella</i> sp. 1602 with activated sludge	Artificial municipal sewage	100 mL suspension in batch mode	Increased algal biomass production by 30% in the co-culture system	100% (NO ₃ -N on the first day) A: 100% (NO ₃ -N on the fifth day)	100% (no statistically significant difference between algae and co-culture)	94.42 (no statistically significant difference between algae and co-culture)	[88]

A: axenic algae, B: individual bacteria without algae, -: not mentioned; ^a: chlorophyll content.

algae to improve assimilation of inorganic pollutants. The bacteria could be screened or isolated from particular species reported as potential PGPB, or from wastewater communities, or from among those originally co-existing with the corresponding microalga.

Kumsiri et al. screened over 190 actinomycetes for their ability to grow in digestate effluent and produce IAA, and selected *Nocardia bhagyanarayanae* I-27 for co-culturing with *Tetrademus obliquus* AARL G022, which then exhibited elevated chlorophyll *a*, biomass and lipid contents [64]. Wastewater-borne bacteria are good candidates to work with algae to recover nutrients from wastewater. Ferro et al. paired *Chlorella vulgaris* and its co-occurring bacterium, *Rhizobium* sp., isolated from municipal wastewater for fed-batch cultivation in sterile synthetic

wastewater [65,66]. Liu et al. selected a wastewater-borne bacterium, *Acinetobacter* sp., that could co-exist with *Chlorella* sp. in WWTP centrate, to determine the effects of the bacterium on nutrient removal [67]. The bacterium *Beijerinckia fluminensis* was isolated from wastewater arising from vinegar production to achieve effective growth and nutrient recovery with *Chlorella* sp. cultivated in sterilized wastewater [68].

In addition to the abovementioned simple artificial microalgal–bacterial consortia, complex communities isolated from mature wastewater treatment processes could also be used as inocula to investigate the interactions and purification of wastewater [69,70]. Zhou et al. constructed a revolving algal biofilm reactor to treat synthetic wastewater mimicking acid mine drainage, wherein the biofilm was

grown with a stable consortium collected from a four-year-old raceway pond [70]. To treat ammonium-rich wastewater with microalgae isolated from a wastewater treatment plant (WWTP), Arun et al. enriched the system with ammonia-oxidizing bacteria from the fermenter of the WWTP as well as methanol-utilizing denitrifiers obtained from activated sludge from a sewage treatment plant [71]. Algal granules have been successfully employed as a newly typical formation for wastewater treatment that is established through introducing algae into activated sludge or aerobic granules [72–74].

Most of the MABC systems have treated wastewater in homogeneous suspension, although immobilization was also occasionally studied as a possible means to carry the algae and bacteria: for instance, the biofilm attached on a belt employed by Zhou et al. [70], alginate algal–bacterial beads used in the study by Hu et al. [75], and a membrane bioreactor inoculated with symbiotic sludge bacteria and mixed algae [76]. Algal granules are considered to be non-homogeneous cultures due to their large size (the particle diameters are millimetre-sized) [77]. The immobilization systems were primarily designed for wastewater reclamation rather than algal biomass production for biofuel, so little information about biomass yield and compositions was provided. Despite effective wastewater purification and easy harvest of biomass, the immobilization might not be suitable for biofuel applications on account of the low growth rate (negligible daily increment) and lipid production (10% of dry biomass) [72,78].

Obviously, because of the complexity of bacterial communities in wastewater, the research into and application of algal–bacterial consortia are still in the early stages and have mainly focused on suspended cultivation of simple consortia containing one algal species and one bacterial species in closed bioreactors with sterilized wastewater (Table 1).

It is important to note that there is a lack of systematic studies on the growth conditions and wastewater-borne bacterial communities that effect the formation of stable and sustainable consortia during real wastewater treatment, which certainly constitutes an important research direction.

4.2. Key advantages of MABCs for wastewater treatment

Among the potential advantages of MABCs for wastewater treatment, three of them are worth highlighting: (i) the excellent nutrient removal performance by the synergistic effect of co-occurring microalgae and bacteria; (ii) the algal growth and potential high-value bioproduct accumulation facilitated by specific nutrients and signal molecules provided by associated bacteria; (iii) the facilitation of biomass harvest by gravity sedimentation without the addition of synthetic flocculants. Of these three aspects, the latter two advantages can be obtained with a regular medium, as in the previous section, which also occur in wastewater cultivation.

4.2.1. Wastewater bioremediation and high-value biomass production

Table 2 lists the promising carbon, nitrogen and phosphorus removal efficiencies for MABCs in wastewater, which were mainly followed by enhanced growth of the consortium and the complementary interaction between algae and bacteria. In comparisons of algal growth in raw and autoclaved WWTP centrate, synergistic effects of centrate-borne bacteria on algal growth during the exponential phase and nutrient removal performance were verified [78]. Acting alone, the bacterial community was able to reduce the COD loads by 36–60% over a 5-day period but had limited removal rates for nitrogen, phosphate, and sulfate in effluent from an oxidation tank at a winery plant. In contrast, the lone addition of algae in sterile wastewater increased the COD while effectively removing inorganic substances, including nitrogen, phosphate and sulfate. To take advantage of algal–bacterial complementarity in wastewater treatment, culturing algae together with bacteria inherent in wastewater significantly reduced the contents of both COD and nutrients [8]. Holmes et al. developed a simplified process consisting of a model

bacterium (*Escherichia coli*) and a model alga (*Auxenochlorella protothecoides*) for removing succinate, finding a 18–66% higher removal rate of COD in the presence of algae than that in the axenic bacterial system, due to bacterial oxygen demand offset by algal photosynthetic aeration [79].

The microalgal-growth-promoting bacterium *Emticia* sp. EG3 was isolated from an *Euglena gracilis*–municipal wastewater effluent culture and it tripled the algal biomass and lipid productivities, compared with EG3-free control [80]. An ammonia-oxidizing bacterium (AOB), *Kluyvera* sp., helped remediate ammonia in autoclaved municipal wastewater, enhanced the growth of *Chlorella pyrenoidosa*, and further promoted the lipid production in the algal stationary phase by competing with algae for nitrogen [81]. In return, O₂ provided from microalgal photosynthesis supported the rapid development and activity of AOB and then achieved high overall removal of ammonia in WWTP centrate [82]. In complicated microalgal–bacterial biofilms, the dominant Proteobacteria also contributed a lot to the oxidation of ammonia, which was able to alleviate the toxicity of ammonia to algae and promote algal growth [70,71].

Ji et al. showed upregulation of a chlorophyll-related gene in *Chlorella vulgaris*, when it was co-cultured with *Bacillus licheniformis*, through quorum sensing, which was proved by increased concentrations of signal receptor autoinducing peptides (AIP) and messenger bis (3′–5′) diguanylic acid (c-di-GMP), eventually followed by the increase of dry biomass and nutrient removals [83]. In addition to basic exchange of oxygen and carbon dioxide, *Exiguobacterium* in MABC activated the enzymes associated with nitrogen metabolism in the algal photosynthesis pathway and led to increases in chlorophyll content, net photosynthetic activity, and wastewater purification [84]. Although the bacterium *B. fluminensis* borne in vinegar production wastewater was reported to inhibit the growth of algae, it still effectively enhanced the accumulations of chlorophyll a & b, and carotenoids, and promoted nutrient recovery [68].

Besides common pollutants, MABC were reported to effectively and economically remove pharmaceuticals and personal care products (PPCPs) that exert strong pressure on wastewater treatment plants, since widespread presence of PPCPs in natural waters poses high environmental risks to all organisms [85].

In these systems, a synergistic effect occurred and was believed to favour biomass production, nutrient recovery and pollutant removal by both algae and bacteria. Microalgae assimilate the nutrients — especially nitrogen and phosphorus and occasionally organic pollutants — and supply oxygen to bacteria. Bacteria can remove the input organic substances inherent in wastewater and photosynthate from algae via heterotrophic growth (producing carbon dioxide). Moreover, the inorganic carbon, along with possible signal materials, from the bacterial activities boosts algal growth for further treatment of wastewater. However, regarding the mechanisms underlying the interactions within MABC, additional work is needed to investigate: (i) the separate contribution of individual algae and bacteria to wastewater reclamation; and (ii) the conditions for optimal and continuous operation of MABC systems.

4.2.2. Biomass harvest efficiency

Bacteria are known to favour the flocculation of algae, increasing the floc size and aiding biomass gravity sedimentation. Like flocculation occurring in an ordinary medium, the MABCs in wastewater also facilitated the biomass harvest without addition of synthetic flocculants, which originated from charge attraction and adsorption bridging. The flocculating efficiency of *Chlorella vulgaris* SAG 211–19 reached up to 92% with the help of bacterial EPS in seafood wastewater effluent, while only less than 9% of flocculating efficiency was obtained by individual algal culture [86]. Owing to flocculation of algae and bacteria, most high rate algal ponds (HRAPs) employed gravity harvest to collect MABC biomass in algal settling cones where only 3–6 h were needed to settle higher than 80% of algal biomass. Centrifugation has been utilized

to collect unicellular algal biomass and occupied 20–30% of operation cost in algal cultivation process [22], which might be eliminated in MABC system.

4.2.3. Cooperation between microalgae and bacteria in wastewater

For the cooperation of microalgae and bacteria in wastewater, among the three types of interaction between phototrophic and heterotrophic, activities about nutrient exchange and signal transduction have been usually observed for microalgae and bacteria in wastewater.

O₂ provided by algal photosynthesis facilitates bacterial respiration, nitrification, organic matter degradation, and so on [79,82]. In some cases, algal extracellular polysaccharides or amino acids feed bacteria as well [14]. In turn, CO₂ from bacteria shows as carbon source for algal growth and nitrogen-fixing bacteria also transfer nitrate or ammonium to algae [81,87]. Signals from bacteria have been found to algal growth and metabolism in wastewater and mostly are identified as IAA, AIP and c-di-GMP [83,88]. Cooperation through O₂ and CO₂ commonly occurred in MABC in wastewater, moreover, this cooperation between physically close organisms might decrease the cost of algae and bacteria to get necessities, in comparison with getting them from aeration [89]. However, utilization of algal EPS and nutrient supply from bacteria exhibit exclusiveness at a certain degree, for example, nitrogen supplier mostly belong to *Rhizobium* sp. and *Azotobacter* sp. Research about signal transduction between algae and bacteria in wastewater is still relatively less than that in nature and more signal mechanisms are waiting for being found.

A comparative metabolomic analysis of *Chlorella sorokiniana* cultivated in an axenic culture and in a consortium with *Pseudomonas* H4 in artificial wastewater revealed algal cell responses to the synergistic growth with the bacteria and possible metabolic regulations of microalgae by the bacterium effecting nutrient degradation [90]. Over one hundred detectable differential metabolites were identified as biomarkers responding to the bacterium, 73% of which increased, while only 27% decreased. The differential metabolites revealed: (i) bacterium H4 enhanced algal assimilation of nitrogen and its synthesis into structural proteins, seen by observing significantly lower levels of amino acid biomarkers and slightly higher nitrogen content in the consortium than those in the axenic algae group; (ii) H4 was able to upregulate several pathways related to lipid accumulation, as proved by increases in relevant intermediate metabolites in the consortium; (iii) the algal carbohydrate metabolism rarely responded to the existence of the bacterium; and (iv) proteins in algae associated with the tricarboxylic acid cycle were decreased by the presence of H4, which reduced the COD removal due to downregulated acetate assimilation.

Besides above two types, a unique interaction has played an important role to improve MABC behaviour in wastewater that is detoxication. Several specific bacteria, such as AOB, with high efficiencies on oxidating ammonia and organic materials alleviates ammonia toxicity to microalgae [81]. The co-culture of *Scenedesmus* sp. 336, *Chlorella* sp. 1602 and activated sludge effectively removed harmful reactive oxygen species under oxidative stress in sterile synthetic wastewater, compared to axenic algal culture [88].

4.3. Vital factors for constructing a successful MABC in wastewater

In this section, the specific operational strategies that influence the performance of MABCs are reviewed and discussed. It is important to note that there is a lack of systematic studies on the experimental conditions that are suited to operating a stable and effective MABC for wastewater cultivation and high-value bioproduct generation, which certainly constitutes an important research direction to support the construction of a feasible and practicable algal–bacterial system. Some critical factors for MABC construction are summarized from available literature in the following sub-sections.

4.3.1. Aeration

The aeration rate would affect the intensity of shear forces, gas balance, and the diffusion of the substrate, thereby leading to different properties of a microalgal–bacterial consortium. The gas balance between O₂ and CO₂ could further play a vital role in algal metabolism, because both O₂ and CO₂ need the catalytic site provided by ribulose-1,5-bisphosphate carboxylase/oxygenase to be integrated into the Calvin cycle [91]. That is to say, the algal efficiency of CO₂ fixation can be impaired in the presence of copious O₂ under high aeration rate [74]. Considering the much higher growth rate of bacteria (0.5 h⁻¹) than algae (0.2 d⁻¹), insufficient oxygen production usually occurs in MABCs and makes aeration with CO₂ that usually adopted by algae culture not necessary. On the other hand, additional aeration blew CO₂ away that was followed by inorganic carbon deficiency, which resulted in competition for the carbon source between autotrophic bacteria and algae, worsened algal growth conditions, and adverse effects on the nutrient assimilation process [92]. Compared to 20 mL/min, aeration of 100 mL/min mixed O₂ and N₂ decreased DIC concentration in solution by 67%, increased nitrogen and phosphorus concentrations in effluent by 70% and 50% respectively, decreased Chl-a concentration by 70% [92]. Hence, the optimal aeration should depend on air and a low rate not causing large amount of CO₂ escape, meanwhile, well-mixing with agitation can also promote O₂ and CO₂ availability for bacteria and algae, respectively. Moreover, from biomass yield and energy saving, aeration rate could change with growth-state-based schemes to match the demands of algae and bacteria in the whole cultivation period, as Han et al. have done for *Chlorella* sp. in BG11 medium [93].

4.3.2. Light irradiation

As one of the indispensable constituents of MABCs, algae are photosynthetic organisms and their metabolic activities strongly depend on light irradiation. Previous studies demonstrated the remarkable impacts of illumination — including parameters such as light intensity, photoperiod and wavelength — on algal growth, lipid production and nutrient utilization [3,94–96]. Based on that, the light irradiation would further affect the behaviour of MABCs. For the system containing *Chlorella vulgaris* and its co-occurring bacterium, *Rhizobium* sp., in synthetic municipal wastewater, Ferro et al. concluded that illumination led to better performance of fatty acid production and nutrient recovery, and eliminated the competition for nutrients between algae and bacteria, in comparison to the response in the dark condition [97]. In algal–bacterial granules, functional bacteria, Comamonadaceae and Nitrosomonadaceae, were able to be enriched under high light intensity of 225 μmol/m²/s and promoted the NH₄⁺-N removal capability. Moreover, strong illumination shifted the dominated genus to filamentous *Stigeoclonium*, which comprised 68% of the granules formed with 225 μmol/m²/s light intensity and triggered the high N-removal performance and lipid accumulation of the algal–bacterial granules [98]. Although increased light irradiation enhanced the nitrogen removal and lipid production of algal–bacterial granules, the mechanisms underpinning the changes in community composition and triggering bioactivity in microbes were not deeply discussed in the above study. Moreover, light limitation often occurred, on account of cell shading by bacteria and/or algae, the light period, and dark-coloured wastewater [99]. Additionally, algal–bacterial granules might go through severe photoinhibition and even chloroplast degradation under provided surface light intensity of 3000 lux, due to their large aggregates (>200 μm diameter) [74,77,99]. Hence, the behaviour of MABCs should be further and more deeply researched for practical application, taking the natural day/night rhythm and seasonal light fluctuation into consideration. Regarding the formation of algal–bacteria granule in wastewater being the main factor inducing photoinhibition, the relationship between light availability and granule sizes should be accurately explored for achieving a size-dependent regulation of light conditions.

4.3.3. Ratios of algae and bacteria

Bacteria could positively, negatively or neutrally affect algae in various ways, according to the reported findings on natural consortia or artificial co-cultures; hence, the proportions of algae and bacteria might be set differently to obtain mutualism or commensalism, and to avoid parasitism. The growth-promoting effect of *Emticia* sp. on *Euglena gracilis* increased as the initial bacterial density increased [80]. Ji et al. set different inoculation ratios of *Chlorella vulgaris* and *Bacillus licheniformis* in synthetic wastewater and found that greater bacterial addition resulted in higher chlorophyll content and nutrient removal efficiency [83]. However, the most pronounced biomass production and wastewater treatment occurred at the lowest ratio of 1:3 (algae–bacteria) set in that study. By investigating bacterial addition at concentrations of 1% and 10%, Huo et al. also verified that a higher concentration of bacteria showed a faster removal rate for nitrogen and phosphate, and higher pigment concentrations than at the lower concentration [68]. About algal-bacterial flocs formation for harvest, Nguyen et al. [86] found that only initial microalgae concentration higher than 20 mg/L could occur flocculation.

However, these research studies leave open three questions: (i) whether the nutrient removal efficiency and algal growth could be further promoted by continuously increasing the dose of bacteria; (ii) how the synergistic effects would be influenced by the algae–bacterium ratios; and (iii) in what ways the MABC respond to culture conditions that facilitate adjusting the ratios of algae and bacteria to adapt to environments and achieve the dominant position of target bacteria when addressing a specific goal.

4.3.4. Pretreatment of wastewater

When using wastewater to grow algae, pretreatment of wastewater is commonly employed to boost the algal growth rate. These measures include removing solid particles, decreasing the prevalence of unwanted organisms, and adjusting nutrient levels, which are achieved through filtration, centrifugation, and sterilization. The pretreatment could change the status of wastewater-borne bacteria and subsequent inoculation of algae — and possibly exogenous bacteria — yet only a few reports have studied the performance of MABC in wastewater that had been pretreated in different ways. To improve the algal–bacterial bioactivity in industrial wastewater, Hu et al. pretreated the dark and rich-bacterial wastewater with a strong oxidizing agent, NaClO, which was effective at low doses. However, due to residual free chlorine in the wastewater from a beef-packaging plant, pretreatment with a high level of NaClO inhibited the growth of an MABC and reduced its performance in wastewater reclamation [75]. Hence, the pretreatment method should also be optimized to help construct a stable and healthy MABC in wastewater. Considering the economic and environmental issues, filtration or settling might be a winner though they cannot effectively avoid unwanted organisms and is only suitable for MABC with strong resistance to diversity environments.

4.3.5. Other culture parameters awaiting consideration

As algal and bacterial growth also depend upon temperature, pH, and nutrient availability, these parameters should be considered for their possible influence on the sustainability and productivity of algal–bacterial consortia in wastewater.

Sudden temperature rises on the change of seasons triggered the growth of bacteria associated with microalgal culture, which made the medium ammonium-limited and worsened microalgal performance [100]. Competition for organic carbon sources occurred between *Chlorella vulgaris* and heterotrophic bacteria when temperature was increased to 30 °C [101]. The co-occurring bacterial community of *Nannochloropsis salina* CCMP1776 in secondary treated wastewater responded to changes in temperature, light, and macronutrient availability [102]. Nutrient limitations, along with high algal growth rate, enhanced bacterial diversity. Nutrient resupply increased the abundance across numerous genera of the taxonomically rich Proteobacteria

phylum, while the Bacteroidetes genera showed a complex response to changes in both nutrient status and other growth parameters, including substrate type. Trait et al. also provided evidence that nutrient concentrations affected bacterial community composition and their relationship with associated microalgae [103]. The dominant bacterial species associated with *Chlorella* sp. was *Pseudomonas* sp. in BG11 medium containing luxury nutrients, while the relative abundance of *Brevundimonas* spp. increased when less nitrogen and phosphorus were provided. In low nutrient conditions, the promotion of algal growth and chlorophyll content by a *Hydrogenophaga* strain increased.

Salinity was also found to disturb the formation, settleability and stability of algal–bacterial systems [77]. Salinity of 4% caused disintegration of the aerobic granules, reflected in decreased granule diameter, and negatively influenced granular stability by decreasing extracellular polysaccharide contents, especially the alginate-like exopolysaccharide. The nitrification process of converting ammonia to nitrite was almost completely inhibited at the salinity of 4%, while increased salinity encouraged bacteria to secrete more IAA, which improved algal growth and lipid production as well as phosphorus removal.

When it comes to the behaviours of MABC, not only species-specific interactions but also the environmental conditions should be fully understood in order to operate a stable system. Since the MABC system involves multiple strains, the interaction models (physical contact, physical barrier, concentration gradient) could play a vital role in adjusting co-culture behaviour as well [104,105]. For co-cultures, real contact is easier to implement and enables high diffusion rates of substance exchange, although that would hinder the harmony of MABC if the constituent organisms required different conditions, competed over space or nutrients, or imposed survival stresses on another strain. With the latter in mind, physical distance (droplets, solid systems), physical barriers (microfluidic systems, dialysis membranes) and concentration gradients might help to construct a stable system to prevent direct detrimental effects on other strains. For example, the polyester membrane separating *Nostoc* sp. and *Aspergillus niger* boosted their biomass production from primary effluent, in comparison with the mixed co-culture [105]. Actually, well-mixed culture with direct-contacted cells were the main model when applying MABC to treat wastewater, while other interaction models have been neglected so far and were rarely considered. Considering the complex bacterial community and following from the complicated algal–bacterial relationships in wastewater, it is necessary to assess the effects of the interaction model on MABC in wastewater, together with the abovementioned ambient conditions.

4.4. Economic and environmental impacts of applying MABCs in wastewater

In addition to technically operating an MABC system with wastewater, the potential impacts of MABC in wastewater on economy and environment should also be fully assessed. Considering integrating MABC wastewater treatment plant (WWTP) by replacing biological process (such as active sludge system, A²/O, MBR), more land will be needed due to lower wastewater treatment efficiency by achieved MABCs. However, this portion of investment likely depends on location, it is not included by economic analysis. Moreover, limited research has been done about applying MABCs with wastewater in closed large-scale photobioreactor due to high construction and operation cost, so open HRAPs have been considered as suitable technology for large-scale application of MABCs. In the view of algal biomass production, the economic and environmental impacts of MABC systems are usually compared with algal cultivation in chemically defined medium.

During the operation of wastewater treatment plant (WWTP), the provision of O₂ through mechanical aeration is required by heterotrophic bacteria in active sludge and other biological methods [106], which consumes much energy that can be partially dismissed since co-existed algae photosynthesized O₂ (e.g. activated sludge system consumes

power around 0.02 kWh/m³ of water vs. power around 0.02 kWh/m³ of water for HRAP) [107]. Meanwhile, cost for algal cultivation can also be reduced by 90%, 78% and 98% on water, nitrogen and phosphorus, respectively, since they are provided by wastewater [108]. Medium strength domestic wastewater (40 mg/L of nitrogen and 8 mg/L of phosphorus) generates 0.6 g of microalgae per liter, which means that a total yield of 77.6 × 10⁶ kg microalgae can be reached from all domestic wastewater collected in USA without any chemical input [109].

The waste of chemical elements through common biological methods used in WWTP could be effectively eliminated since some elements (such as C, N and P) have been transferred into algal biomass for downstream utilization. Absorption of CO₂ by algae contributes to achieving carbon neutral and decrease the global warming issues, while approximately 50% of the influent carbon in activated sludge system ends up in the atmosphere [110,111]. Meanwhile, owing to CO₂ absorbed into algal biomass, the production of algae-based biogas or biodiesel production leads higher than 6 times less of greenhouse gas emissions than the production of conventional diesel [108].

The dual benefits for wastewater and algal biomass make MABC as an integrated economic and environmental friendly system.

This section reviewed the operational strategies regarding the source of algae and bacteria, wastewater type and operation mode, finding that co-culture bacteria in MABC mainly originate from phycosphere or wastewater. The key advantages of MABC over axenic culture are exhibited on increased removal rate of nutrients (especially COD), amplified biomass and pigment production, and easy-implemented harvest method of gravity sedimentation due to algal-bacterial flocculation. The mechanisms underlying above advantages include nutrient exchange, signal transduction and bacterial detoxication for algae. Vital factors affecting MABC operation are aeration, light irradiation, algal to bacterial ratio and wastewater characteristics. Applying MABCs treating wastewater and producing biomass exhibits dual economic and environmental benefits through reducing energy consumption for intensive aeration in conventional wastewater treatment, eliminating chemical investment for algal growth along with absorbing CO₂ by algal photosynthesis.

5. Challenges of applying MABCs in wastewater treatment

Although the presence of certain bacteria facilitated algal cultivation and wastewater bioremediation by providing nutrients and growth-promoting signals, the sustainability and stability of the consortium are still challenged by non-target bacterial blooms. Regarding the work that isolated bacteria from the phycosphere or from wastewater, only a minority of bacteria — even as little as one in one hundred — were identified as benefitting algal growth [44,64,68,80]. The bloom of other unwanted bacteria could occur with a great probability. This is termed “biological contamination”: it is deleterious for algal cultivation, has frequently led to culture crashes, and has hindered the commercial development of algal biomass production — especially in those applications using wastewater as the medium, for the purpose of reducing cost [19,112,113].

5.1. Parasitic consumption of microalgal products

For the bacteria that are parasitic on algae, they feed on algal intracellular compounds by lysing algal cells under the action of lipase, glucosidases, aminopeptidase, alkaline phosphatase, cellulases, and other enzymes [31,114,115]. The consumption of algal products in these processes has been raised as a concern, owing to the consequent decrease in biomass yield and quality [12,116].

5.2. Direct inhibition of microalgal growth

The biotic stress engendered by bacteria, manifest as nutrient competition and growth-inhibition, commonly hindered the

development of algal biofuels, especially in oligotrophic environments and large-scale cultivation.

In wastewater, the possible nutrients that algae and bacteria can compete for include nitrogen, phosphorus and small organic molecules, such as organic acids, monosaccharides, amino acids, etc. Mayo demonstrated the competition for glucose between *Chlorella vulgaris* and heterotrophic bacteria in synthetic wastewater containing low concentration of glucose [101]. As bacteria possessed more effective uptake of phosphate than microalgae, algal bioactivities were obviously inhibited when phosphorus deficiency occurred [100,117]. In wastewater, ammonium can be utilized by algae as the first choice of nitrogen source [118], whilst AOB were usually present in anaerobic membrane bioreactor effluents and competed with microalgae for ammonia leading to impaired microalgal growth [119,120]. However, Mantovani et al. found that microalgae could outcompete AOB for limited phosphorus in centrate from WWTP when optimal environmental conditions were provided for microalgal growth [82].

The allelopathic effects in wastewater have not been fully investigated, however a few research studies verified that certain bacteria in natural environments could exude lytic substances to lyse algae [31]. Combining high-throughput sequencing and gene databases, the expression of terpenoids and polyketides, as algicidal secondary metabolites, was found to be increased and coincided with the death or damage of large *Chlorella sorokiniana* cells in synthetic wastewater, which could be considered as a defensive mechanism on the part of the bacteria [98]. From a poorly performing outdoor 200 L industrial bioreactor in which *Nannochloropsis salina* was growing, Fulbright et al. isolated an alga-inhibiting bacterium, *Bacillus pumilus*, that secreted inhibitory molecules against algal growth rather than competing for growth-limiting nutrients in regular medium [121,122]. This inhibition was reinforced at a high cell ratio of bacteria to algae, and was not altered by pH fluctuations, although the specific molecules inhibiting the algae were not determined in that work [122].

In addition to above methods, bacteria could degrade algal microenvironments by driving the pH away from the optimal value desired by algae, which is usually neutral or alkaline. Certain plant-growth-promoting bacteria and nitrifying bacteria would release some acidic substances (e.g. poly-γ-glutamic acid, volatile fatty acids) that acidify the culture system [123,124]. In the co-culture of *Chlorella vulgaris* and *Bacillus licheniformis*, the pH value quickly dropped from its initial neutral level to less than 4, which severely damaged algal cells and sharply decreased the chlorophyll *a* (Chl *a*) content, whilst the pH of the single algal culture increased and was maintained around 8 during the whole growth period [125]. The pH regulation to 7 achieved large increases in Chl *a* content and nitrogen removal in the combined algal-bacterial system, which confirmed the adverse changes in algal microenvironments caused by bacteria [125].

5.3. Indirect inhibition of microalgal growth

Direct inhibition of algal activity would be followed by susceptibility of MABCs to other pathogens, such as fungi, protozoa, or zooplankton. By analysing algal extracts, several antifungal compounds were found, such as phenolic and carotenoid substances, and the production of these bioactive compounds could be influenced by culture conditions (temperature, light irradiance, and growth stage) [126,127]. That is to say, algae possess certain resistance to fungi, but the implementation of this ability was limited, according to the ambient conditions and algal status. Even though it has rarely been studied, the co-existence of detrimental bacteria has a high possibility to influence the algal protective system and cause susceptibility to other pathogens by direct inhibition of algal growth.

5.4. Insufficient information about MABCs in large-scale cases

Besides above bacterial inhibitions to algae, variations of these

effects play important roles in putting wastewater treated and biomass produced by MABC into pilot-scale application. Still today the delicate behaviour that occur in MABC during upscaling process are unclear. Although many researchers have studied pilot-scale wastewater treatment by MABC, few of them focus on anticipation of bacteria, as well as how algal-bacterial interactions affect the algal biomass production and wastewater treatment efficiency [128,129]. When upping small-scale HRAP to full-scale system, Sutherland et al. [129] find that deeper HRAPs of 400 mm may be more suitable than 200 mm to offer more carbon from bacterial respiration to release the hold of carbon limit to algal photosynthesis, however, no details about bacteria is presented.

In fact, it is still a challenge to upscale the application of MABC in wastewater and guarantee desired yield of biomass due to biological and engineering factors, which needs the help from process control and mathematical model. To develop an efficient tool for constructing and optimizing HRAPs to commercially yield microalgal production, Solimeno et al. implemented activated sludge models (ASM) in COMSOL Multiphysics™ platform to develop a mechanistic model BIO_ALGAE describing the relative proportion of algae and bacteria in an MABC system [130]. With the help of the BIO_ALGAE model, Solimeno and García successfully investigated the influence of hydraulic retention time (HRT) and seasonal variations of temperature and irradiance on the individual growth of algae and bacteria, and then increased algal biomass production by 30% along with ammonia removal rate to 92% through controlling HRT in an outdoor HRAP [131].

The data used by Solimeno et al. [130,131] was obtained from samples that were taken once a week in the same HRAP. Considering that ample parameters differ among wastewaters and play vital roles in MABC activities, reconstruction of models should be done according to the characteristics of organisms in MABC, wastewater, and local climates before employing models to guide process control. For more accurate control, data used for simulations might be obtained in higher frequency, even in real-time situation to improve the precision of models, which makes a claim on faster analysis and more advanced algorithms.

The challenges faced by applying MABC in wastewater comprise the potential adverse impacts of bacteria to algae and insufficient knowledge of MABC behaviour in large scale. The adverse effects of bacteria on algal biotechnology include: (i) lowering algal biomass quality by the consumption of high-value algal bioproducts; (ii) directly inhibiting algal growth through nutrient competition, or an allelopathic effect; and (iii) indirectly increasing the susceptibility of microalgal culture to other pathogens. Creating a mathematical model to control a specific HRAP has been successfully developed, while more common methods are still needed to globally upscale MABC for wastewater treatment and biomass production where advanced algorithms and process control cannot be left out.

6. Concluding remarks and future perspectives

Based on the recently achieved progress, MABCs have been examined as a promising technology for the dual purpose of economical alga-based biofuel applications along with wastewater reclamation. Current developments of this biotechnology are significant; however, there remain challenges along the way. In particular, control strategies to maintain long-term operation of a consortium, despite changes in conditions and biological contamination, still need to be comprehensively investigated. More research work and data analysis should be devoted to the widespread application and scale-up of MABCs, as per the pipeline shown in Fig. 3.

6.1. Selection and breeding of strains

Both microalgal and bacterial strains need to be selected or bred for specific wastewater to enhance the sustainability and efficiency of growing algal-bacterial consortia, which can critically improve the

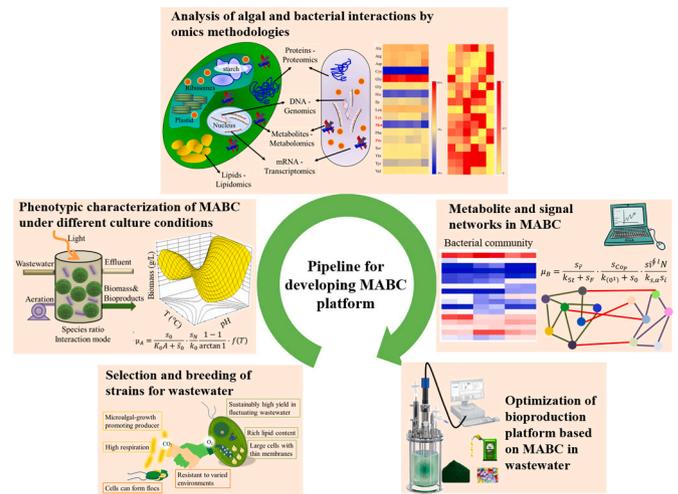


Fig. 3. Prospective pipeline for developing an algal-bacterial consortium platform for bioproduct generation based on understanding the interactions inside the system.

processes of biomass production and nutrient removal. The ideal algal species for MABCs should include certain features: competitive lipid productivity, resilience to harsh environments, strong self-regulation to fluctuation of environmental conditions, and low sensitivity to growth-inhibiting molecules from bacteria or viruses. The abovementioned characteristics cater for sustainable algal growth and lipid production from wastewater. Besides growth and lipid yield, some other characteristics would endow extra benefits on an MABC system coupling wastewater treatment and biofuel generation. For instance, algae that readily form flocs in cooperation with bacteria and have thin cell walls could simplify and economize the biomass harvest and oil extraction processes.

Associated bacteria in the MABC system should be screened to improve the above algal properties and maintain their dominance even when subject to alterations of the environment. Isolating an algal species, along with its beneficial bacteria, from target wastewater is encouraged to alleviate the inhibition from adverse factors encountered in wastewater and to promote the overall performance of high-quality biomass production.

The ideal algal species could likely be isolated from local samples and some harsh or even extreme environments, like local waters, wastewater, seawater, and brackish water. Isolation process might protect local ecology from bio-invasion and obtain strains with high harmony with local climates. A prior acclimatisation stage before commencing the real cultivation might help a lot for the consortium to achieve stability, preeminent growth, and outstanding nutrient removal in wastewater [132].

6.2. Phenotypic characterization of MABCs under different culture conditions

The behaviour of an MABC could be either promoted or impaired by the inoculum, aeration, nutrient availability and other cultivation conditions. Most of the current studies were conducted under indoor batch conditions (Table 2); moreover, only a few concerned the impact on consortia of certain individual factors. There is little systematic information about the responses of microalgae and bacteria to complex changes to conditions or to microbial communities inherent in real wastewater when algae were cultured for biofuel production in conjunction with nutrient recovery. For future application in alga-based biofuel production from wastewater, the high-value bioproduct yields and pollutant removal efficiencies of MABC systems operated long-term need to be evaluated under outdoor continuous operation, with focuses

on local climates and wastewater characteristics, including but not limited to temperature, daylight, and nutrient bioavailability, and fluctuations therein. In addition to that, one of the other upcoming challenges is to understand the response of the wastewater-borne microbial community to algal status and condition changes. A number of algicidal bacteria might be dormant at the beginning of cultivation but have the potential to recover rapidly as environmental conditions change, especially those that cause deterioration of algal cultures [102]. Upon collecting this information, *in silico* experiments should be employed to formulate rigorous mathematical modelling to investigate the microalgal–bacterial behaviours under various physicochemical, biological, environmental and/or operating conditions [133,134].

To monitor organism and chemical variation in MABC system, technologies and database probably need to be created, including waterproof visual tools for collecting individual organism morphology information, a database for blasting organism characteristics and *in situ* detector for nutrients and algaecides. Supported by elaborate tools and the understanding of the relationships of the MABC with complex biotic and abiotic processes, the establishment and management of stable and productive microalgal–bacterial systems could be achieved by exploiting growth-promoting bacteria and suppressing the presence or activity of detrimental bacteria.

6.3. Mechanisms underlying the interactions between strains in MABCs

Although the discussion in section 3.1 and 3.2 showed that a few research studies have been executed to elucidate possible algal–bacterial relationships at the molecular level, as yet there is little detail about their interaction mechanisms and mutual benefits when the consortia were cultured in wastewater for biofuel production. The interactions between microalgae and bacteria could occur in multifarious ways, from metabolic shifts to gene expressions. Furthermore, the complexity of wastewater would be intensified by changes in nutrient availability and bacterial abundances over various timescales and batches. Hence, the research into MABCs in wastewater is still in its infancy, lagging well behind research into axenic microalgal or bacterial cultures in chemically defined media.

Comprehensive understanding of the structure and functionality of interspecific relationships in nature or designed simple co-cultivation could be reached through state-of-the-art metaproteomics, metatranscriptomics, metametabolomics and computational modelling [24,36,90,135–137]. The term ‘omics’ involves the characteristics and quantification of large datasets of entire biological processes in organisms with the aim of understanding cell activities on the molecular and gene levels, encompassing genomics, transcriptomics, proteomics, lipidomics and metabolomics [138]. The emergence of omics methodologies, along with computational simulation and prediction, would also shed light to unravel the metabolic fluxes, quorum sensing, and gene expression between microalgae and bacteria in wastewater cultivation.

To cite an example, high-throughput sequencing (next generation sequencing, NGS) technology has already been applied in preliminary investigation of the response of bacterial communities in the phycosphere to algal growth stages [50,62]. The identification of bacterial communities during algal growth revealed the reaction of individual bacteria to algal growth and flocculation, and a shift in the relative abundance of bacteria when algae went through different growth stages. However, this technique still faces some limitations to obtain absolute microbial abundances, which requires combination with other technology, such as flow cytometry, real-time qPCR, or microbial biomass carbon measurement, to calculate the absolute abundance from relative abundances [139,140]. The accuracy arising from such combination is challenged due to variations among results obtained under different experimental conditions [141]. Another challenge in applying NGS to analyse MABC microorganisms in wastewater is to remove the barriers arising from differences between the NGS results from 18S rRNA sequences of eukaryotic algae and 16S rRNA sequences of prokaryotic

bacteria [142]. Although some efforts have been made to improve these shortcomings [141,143], they should still be taken into consideration before being employed for MABC analysis, due to the added complexity.

Comprehensive gene and metabolite information hinder the analysis and understanding of interactions occurring in MABC. To face big data, artificial intelligence techniques, such as machine learning and deep neural network, can also be employed to find the key interreacted pathways between algae and bacteria and the routes to improve the performance of MABC. By means of omics analysis and computational techniques, trading of metabolites or signalling molecules with ecological regulation of the microorganisms in MABCs can be better elucidated, together with the response to condition changes and culture status. Thus, a platform might be achieved that lays a foundation to accurately regulate the behaviour of target MABCs in wastewater for fast accumulation of target bioproducts, easy flocculation or settling of biomass, and effective reclamation of wastewater.

Integrating ideal species and optimal culture conditions, commercialization of MABC should be on the way to fruition, thereby driving sustainable generation of bioproducts and leading to truly ‘green’ wastewater treatment.

Author statement

Liquan Jiang: Conceptualization, Writing – original draft, Investigation. Yizhen Li: Investigation. Haiyan Pei: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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