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Potential interactive effect on biomass and bio-polymeric substances of microalgal-bacterial aerobic granular sludge as a valuable resource for sustainable development

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Abstract

The algal/bacterial biomass and extracellular polymeric substances (EPSs) existing in microalgal-bacterial aerobic granular sludge (MB-AGS) offer a promising bioresource. The current review-based paper presents a systematic overview of the compositions and interactions (gene transfer, signal transduction, and nutrient exchange) of microalgal and bacteria consortia, the role of cooperative or competitive partnerships of MB-AGS in the treatment of wastewater and recovery of resource, and the environmental/operational factors affecting their interactions and EPS production. Moreover, a brief notes is given on the opportunities and major challenges of utilizing the microalgal-bacterial biomass and EPS for phosphorus and polysaccharides chemical recovery, renewable energy (i.e. biodiesel, hydrogen, electricity) production. Overall, this compact review will pave the way for developing MB-AGS future biotechnology.

Key words: Microalgal-Bacterial aerobic granular sludge, Extracellular Polymer Substances, interactions, bio-products, biofuels

1. Introduction

Wastewater treatment facilities are striving to remove contaminants from wastewater and recover resources (particularly, highly value-added products) through the created sludge. More enticingly, generated sludge is able to be utilized in the form of feedstock for the recovery of numerous valuable resources or products, such as bioplastics, biogas, alginic acid, phosphorus (P), cellulose, etc. (Chen et al., 2022b).

The Aerobic Granular Sludge (AGS), a stable self-immobilized ecological structure of granular sludge, is now a promising wastewater treatment technique over the last 20 years. In comparison to traditional activated sludge processes, AGS technology enjoys a number of advantages including self-immobilized biofilm, high biomass retention, superior settling qualities, and the capacity to concurrently remove nutrients and Chemical Oxygen Demand (COD) (Amorim et al., 2014; Mery-Araya et al., 2019). A recently recognized potential technique is algal-bacterial AGS, which is a combination of bacterial AGS and microalgae, and it features the merits of microalgae and bacteria together.

On the basis of bacterial AGS, a microalgal-bacterial symbiosis system called microalgal-bacterial aerobic granular sludge (MB-AGS) has been produced. In contrast to bacterial AGS, MB-AGS not only preserves the bacterial AGS benefits but also exhibits superior pollutant removal capacities (nitrogen (N), organics, and phosphorus (P)) via the production of additional products with added value (Liu et al., 2022b). Moreover, researchers found the MB-AGS to be compact in structure with granular stability when dealing with different levels of organic compound, at even low COD/N ratios such as COD/N = 1 with lower greenhouse gas (GHG) emission (Zhao et al., 2018). This new microalgal-bacterial system represents a more economically viable and sustainable platform that taking the advantages of the interactions between microalgal and bacterial organisms (Zhang et al., 2020). However, biofilm development involves many complex influencing parameters; the initiation of a biofilm reactor can be tediously challenging and constraining the production of biofilm biomass. Thus, it is crucial to comprehend how bacteria and microalgae interact.

The present study reviewed the bacteria-and-algae interaction in the EPS and biomass production of MB-AGS technology with the aim of providing recent advances, specifically: This paper updated the recent findings about the multilayer biofilm structure, the symbiotic relationship, and the key interactions in the MB-AGS system. This paper also reviewed the research initiatives on the interactive effects of microalgae-bacteria consortia on extracellular biopolymers, biomass, and valuable products recovered from MB-AGS. Furthermore, the influencing factors of MB-AGS design and operation for wastewater treatment and the possibility and effective approaches of recovering resources from MB-AGS were discussed.

2. Composition and interactions of microalgal-bacterial aerobic granular sludge

2.1. Brief review the composition of microalgal-bacterial granules

The MB-AGS consists of several layers/zones in the symbiotic system; it can generally contain four different functioning bacteria and microalgae working under anaerobic/anoxic/aerobic conditions and zones (as depicted in Fig. 1a). In the outer layers of granules, both bacteria and microalgae can proliferate. Fungi and ordinary heterotrophic organisms (OHO) can aerobically break down organic matters (Qixin et al., 2022). The aerobic condition could be manipulated by supplying air via aeration and/or created by utilizing the oxygen produced by microalgae (Zhang et al., 2020). Meanwhile, nitrification takes place in the obligate aerobic zone (outer layer) for the aerobic bioconversion of ammonia to nitrite by

Ammonium Oxidizing Bacteria (AOB) and then nitrite to nitrate by Nitrite Oxidizing Bacteria (NOB) (Chen et al., 2022c). The produced nitrite/nitrate will be consumed by denitrifiers (Van Kessel et al., 2015); also, it is possible for ammonia into N_2 through the anaerobic ammonia oxidation (anammox) process (Chen et al., 2022c).

The above-mentioned nitrite/nitrate reducers and anammox bacteria are generally alive in the inner layers of granular sludge. Moreover under anaerobic condition, Polyphosphate-Accumulating Organisms (PAOs; i.e. the functioning bacteria to remove phosphate) can take up Volatile Fatty Acids (VFAs) from the bulk liquid and internally save them to produce poly-hydroxyalkanoates (PHAs) with poly-phosphate hydrolysis and P-release, and then take-up more phosphate and stored as poly-P for excess P removal during the aerobic/anoxic conditions (Chen et al., 2020). Being a carbon and energy source, the PHA is used by the PAOs for energy production and cell growth in the aerobic zone when oxygen or nitrate in anoxic conditions acts as an external electron acceptor. Due to such special feature, the PAO bacterial group tends to grow between anaerobic and anoxic/aerobic zone, so that to complete the enhanced biological phosphorus removal (EBPR) under alternative conditions which can be realize that the integration of micro-aerobic condition/zone created by microalgae has a potential to maintain EBPR process (Valverde-Pérez et al., 2016).

Besides the microorganisms (bacteria and microalgae), the EPS could be the key content of the MB-AGS (as depicted in Fig.1b). Generally, both eukaryotic (algae, fungi) and prokaryotic (Bacteria, Archaea) microorganisms produce EPS (Wingender et al., 1999) in both floc and granular type of micro consortia, representing an intricate compound of humic substances, nucleic acids, polysaccharides (PS), proteins (PN), lipids, and the leaked intercellular polymers in a wide spectrum (Felz et al., 2016; Siddharth et al., 2021; Wingender et al., 1999).

The granular sludge's EPS is stratifiable as slime, which can be classified as loosely-bound EPS (LB-EPS) and tightly-bound EPS (TB-EPS) as drawn out via centrifugation and mild heating approach as reported in the research paper for bacterial-AGS (Li & Yang, 2007). In microalgal-bacteria AGS system, EPS can contribute to the stability and integrity of mature microalgal-bacterial consortia. Microalgae can produce more EPS through metabolic processes, whereas bacteria are inclined to secrete more EPS to shield themselves from the effects of algae growth, light, and free nitrous acid (Abbew et al., 2022). Functionally, the EPS could protect the cells from the hostile outside environment, provide energy and carbon in the "hunger" condition, and preserve the sustainable stability of granular sludge (Lin et al., 2010). What's more, the EPS content in MB-AGS enjoys much greater stability in a saline condition (up to 4%) than that of bacterial-AGS (Meng et al., 2020).

Furthermore, EPS is classified as gel-forming or non-gel-forming types, depending on the capacity to create hydrogels. The gel-forming EPS is present in large quantities (100–300 mg/g VS sludge) and is, unlike a non-gel-forming EPS, regarded as essential components that give matrix structure to biofilm and support the structural integrity, hence called structural EPS. The main constituent of the structural EPS is Alginate-Like Exopolysaccharides (ALE), which resemble the alginate produced by macro-algae like seaweed comprising nucleic acids, uronic acids, neutral saccharides, lipids proteins, phenolic compounds, and so on (Chen et al., 2022a). They can be extracted from both floccular and granular sludges and represents a major factor in granulation following increase in the ALE concentration along with granulation (Zahra et al., 2022). Of note, a typical method of ALE extraction merely extracts structural EPS from biomass instead of all the existing EPS that exhibit hydrogel properties, which are thought to ensure structural stability and mechanical strength. It is recommended that this extracted substance can be considered a structural EPS given its complicated composition compared to commercial alginate (Chen et al., 2022a) and as a valuable product for further commercialized application (Feng et al., 2021).

Although study on algal-bacterial AGS remains in its primary lab-scale steps, the existing information of EPS recovery and economic feasibility is still incomplete. It is well recognized that the abilities of bacterial strains to auto-aggregate play a significant part in the granulation process. The results point to the possibility of accelerating not only the formation but also sustainable functioning of an algal-bacterial AGS by introducing a few suitable microalgal organisms into the bacterial AGS. This, in turn, offers hope for a swift conversion of the bacterial AGS into a microalgal-bacterial AGS. The ability of the *Chlorella sp.* strain throughout a 5-day cultivation period shows a clear increasing trend under a 12h/24h light/dark cycle. In the test dark condition (0 h/24 h), the entire EPS content of the *Chlorella sp.* strain showed a weakly positive correlation (r^2=0.42) with auto-aggregation capability, whereas a substantial correlation between them (r^2=0.95) was found under light/dark cultivation (12 h/12 h) (Nuramkhaan et al., 2019).

Moreover, the ALE content in Algal-AGS enjoys greater stability in a saline condition than the AGS; it drops rapidly following increase in salinity from 1% to 4%, causing AGS to disintegrate at 4% salinity (Meng et al., 2020). As a stress response mechanism, microbes typically secrete EPS into their surrounding environments, where Reactive Oxygen Species (ROS) are scavenged and hydrated biofilm or a granular matrix is produced by these microbes acting as antioxidants. EPS contributes to the stability and integrity of mature microalgal-bacterial consortia in microalgal-bacterial consortium systems. Microalgae can produce more

EPS through metabolic processes, whereas bacteria are inclined to secrete more EPS to shield themselves from the effects of algae growth, light, and free nitrous acid (Abbew et al., 2022).

2.2. Microalgae-bacteria interaction

The interaction between the bacterial and micro-algae consortia can be of different types: (a) nutrient exchange, (b) signal transduction, and (c) gene transfer (Jiang et al., 2021). These interactions produce either competitive or cooperative collaboration between microalgae and bacteria through such patterns of interaction as commensalism, parasitism, and mutualism in order to establish and maintain the microalgal-bacterial symbiosis (Zhang et al., 2020). Microalgae can ingest CO_2 and other inorganic byproducts that bacteria release in consortia as a result of their metabolic processes. Microalgae also release oxygen, with the estimated oxygen production rate of 1.83 mg-O_2/L-h in a steady state (Praveen et al., 2015), where bacteria consume to grow which obviates the requirement for aeration in a bioreactor (Fig. 2).

Findings indicate that during nutrient exchange interactions, bacteria release substances such as vitamin (B12) and hormones (e.g. indole acetic acid, gibberellins, cytokinin), ethylene, siderophores, and so on, contributing to microalgal growth and biomass formation (Bhatia et al., 2022). Furthermore, according to a research piece, having been co-cultured with green algae, *Rhizobium sp.*, i.e., the most common and governing *C. vulgaris*-derived bacterium, could increase the number of algal cells by about 72%. It was demonstrated that bacteria would help the algae to grow. Meanwhile, steady growth of the mentioned *Rhizobium* co-cultured with green algae is followed by a significant rise in cell density (determined by increase in copy numbers of *rirA* and *rhtA* genes as *Rhizobium* specific). Algae and *Rhizobium* both experienced average growth rate increases of 11% and 110%, respectively, demonstrating mutualistic interactions (Kim et al., 2014).

The molecules facilitating the interactions in signal transduction cannot act as nutrients; instead, they inhibit or activate gene expression and/or physiological processes, thus changing the growth and behavior (Zhang et al., 2020). While the information is deliverable within and between cells, their main role is to make interactions with cells via sticking to certain receptors on the surface and communicating cellular messages (Qixin et al., 2022). Typically, quorum Sensing (QS) and allelopathic effect are the ways for the transmittance of signal between algae and bacteria. QS involves communication in biofilms or other biological niches through the production of such signaling molecules as autoinducers and acyl-homoserine lactones (AHLs). These signal compounds support population-dependent coordinated gene expression in bacteria. Communication between cells of bacteria and algae (cell-to-cell) is also possible. Microalgae may detect bacterial signals and react favorably or unfavorably. In addition, it has been demonstrated that the microalgae discharge quorum quenching chemicals such as AHL inactivating enzymes, causing bacteria to exhibit different behaviors (Bhatia et al., 2022).

Another signaling method in the microalgal-bacterial system is called "allelopathy," which describes how certain specific chemicals are digested and produced by microbes or plants. These chemicals then ruin any chance for bacteria and algae to survive in the microenvironment. Allelopathic effects include restraining bacteria to algae, algae to algae, and algae to bacteria and these effects illustrate the significance of regulating the physiological functions of the algal-bacterial system (Qixin et al., 2022).

Horizontal Gene Transfer (HGT) has emerged in a long co-evolutionary process in the microalgal lineages, and it involves a gene being transferred between two nearby microbes and is important for evolution. Whereas the majority of gene transfer cases occur in bacteria is largely by chance and as a result of the large metabolic diversity that bacteria possess. The integration between bacterial genes and the microalgal genomes of engineered microalgal-bacterial consortia helps build up adaptive resistance against the pressures from a specific environment (Jiang et al., 2021; Zhang et al., 2020). For instance, for algae to reside in toxic environments limited in iron and nitrogen, they obtain many genes from bacteria through a prolonged course of co-evolution. These genes include those for ferritin uptake, ornithine-urea cycle enzymes, and metal detoxification. Algae can acquire some basic abilities through gene transfer, making it possible for them to coexist and grow steadily in an inter-algal ecosystem (Qixin et al., 2022).

2.3. Interactions in extracellular polymeric substances production

Cell-to-cell interactions between bacteria and algae positively contribute to the community and affect various physiological processes, including uptake of nutrients, production of virulence factors, formation of biofilms, and stability of ecological niches (Bhatia et al., 2022). For ensuring maximum population rise and survival in various environments, a majority of bacteria utilize QS to synchronize population behaviors, e.g., virulence factor secretion, exoenzyme generation, and biofilm formation (Tan et al., 2015). QS represents a cell-to-cell communication approach based on which bacteria release certain molecules, sense them, and exert regulatory control on gene expression to deal with population density. Quorum Quenching (QQ) obstructs QS system and prevents or constrains gene expression affecting bacterial behaviors (Huang et al., 2016). The bacterial community has identified a large number of signal molecules that are broadly categorized as follows: (a) Gram-negative bacteria producing AHLs (most studied); (b) Gram-positive bacteria typically use modified oligopeptides or autoinducing peptides; and (c) Gram-negative/-positive bacteria make perfect use of autoinducer-2 for interspecies communication (Huang et al., 2016). The AHLs-mediated QS is among the best communication systems for bacteria and is found in about ten percent of the proteobacteria belonging to different ecological niches (Tan et al., 2015).

The microbial attachment potential and granular stability are directly impacted by the amount of AHLs, according to an analysis of algal bacterial granular sludge. As QS molecules are controlled by population densities, more QS molecule activities are observed in algal bacterial granules. Moreover, it was discovered that inhibition of AHLs reduced the protein content and attachment potential of microalgal-bacterial granular sludge, in which case granules may lose their structural strength and balance. The microbial population associated with AGS differed from the microalgal-bacterial granular sludge according to high-throughput sequencing analysis; in particular, microalgal-bacterial granulation increased the number of EPS producers and AHLs, including genera *Acinetobacter*, *Chryseobacterium*, and *Flavobacterium* (Zhang et al., 2018a). According to the add-back studies, the C6- and C8-HSL in particular helped regulate EPS synthesis. AHLs-mediated QS may play a significant ecological part in EPS production and algal bacterial granulation, according to the statistical analysis that found a strong correlation for the AHLs content with the production of extracellular polysaccharides, the 50th percentile of the particle size distribution, and the formation of microbial communities (Zhang et al., 2019).

3. Key factors associated with EPS and biomass production

The microalgae-bacteria interaction is susceptible to several factors such as salinity, shear force, illumination intensity, and so forth which can have a direct influence on the EPS, biomass production, and granule stability, which are completely explained in the following sections and indicated thoroughly in the Table 1.

3.1 Environmental Factors

3.1.1. Ratio of carbon to nitrogen

Reducing the wastewater COD/N ratio (from 8, 4, 2, 1) will decrease the stability of the MB-AGS granules and the EPS contents. But it is not the same case in polysaccharides content, in which: the granules' polysaccharides content steadily increased from their initial 20 mg/g-VSS to 50 mg/g-VSS, and it was consistently high as the influent COD/N ratio declined (Zhao et al., 2018). Moreover, the introduction of a co-substrate (glucose and sodium acetate) was studied in another research which triggered a self-protective response, resulting in the secretion of EPS with elevated protein levels and glucose. This led to an increase in biomass production, surpassing the effects of sodium acetate. Intracellular degradation was the mode of action for sulfonamides, instead of EPS-mediated degradation (Wang et al., 2022c).

3.1.2. Salinity

The mature MB-AGS protects its internal microorganisms and maintains its stable structure in case of salinity changes in influent wastewater owing to its high and stable EPS content. Dong et al.'s research demonstrated that the whole EPS content increased considerably from the initial 120 mg/g-VSS to 210 mg/g-VSS with 1% salinity and further to 230 mg/g-VSS with 3% salinity - this finding indicates that excessive EPSs are excreted as a result of MB-AGS acting to protect themselves in response to an increase in osmotic pressure brought on by an increase in influent salinity (Dong et al., 2021).

According to a study on activated sludge, the increased salinity caused increase in contents of PS and PN in LB-EPS and TB-EPS. The ratio of PN/PS in the case of LB-EPS was reduced from 4.8 to 0.9 while in the case of TB-EPS, from 2.9 to 1.4, following a rise in salinity from 0.5% to 6% (Nguyen et al., 2021). The same holds for MB-AGS, showing a sharp decline in PN/PS ratio along with the salinity increased from 0 to 1% and this indicates that greater salinity might encourage more PS excretion from the MB-AGS to deal with the significant osmotic pressure in salinity stress conditions (Dong et al., 2021).

Of note, high NaCl concentration could reduce the EPS production following the rise in NaCl concentration from 75 g L^{-1} to 250 g L^{-1}, EPS productivity fell from 370 down to 320 mg EPS per g cell dry weight (Cui et al., 2017). A good symbiotic interaction between the bacteria and microalgae (*Nitzschia*) could not only achieve rapid microalgae growth and twice as much lipid production, but also reduce the negative effect on EPS production (Meng et al., 2020). As reported in this study, there were 17.3 and 28.5 mg/g-VSS ALE contents in the granules from AGS and MB-AGS, respectively. The blocks of ALE biopolymer are based on mannuronic acid (M) and guluronic acid (G), which are categorizable into 3 different types of homopolymeric blocks, i.e., GG, MG, and MM. GG (supplying gel-forming capacity) and MM contents in the AGS were respectively reduced by 86.5% and 88.9%. On the contrary, the GG and MM contents (endowing the chains with flexibility) in the microalgal-AGS were merely reduced by 21% and 40%, respectively, in a saline condition (Meng et al., 2020).

3.1.3. Heavy metals

Backed by its wide range of genes and high metabolic capacity, the multi-species system performs more effective ecological functions in comparison to the single-species system. Due to its significant friendliness to the environment compared to single-species systems, toxicity testing with emphasis on multi-species systems, like microalgal-bacterial systems, might gain high popularity in the future in terms of pollutant risk assessment (You et al., 2021b). Cells can overcome cytotoxicity through avoidance, tolerance, and detoxification mechanisms. Heavy metals may penetrate the cells; however, due to the tolerance and neutralization skills, they cannot have toxicity effects. Internalized metals can be converted into a less toxic form through metallothioneins and phytochelatins as metal-binding proteins as well as other organic compounds e.g. citrate, phosphates, and exo-polysaccharides (Bhatia et al., 2022; Kaplan & biotechnology, 2013).

Microalgal-bacterial aggregates are formed when EPS acts as a glue to bind algal and bacterial cells. Organisms in microalgal and/or bacterial aggregates frequently exhibit more effective tolerance against contaminants than individuals due to the abundance of EPS within the aggregates. Furthermore, microalgal EPS act as nutrients to support bacterial growth, and bacterial EPS mitigate the negative impact of metals on algae (You et al., 2021b). The performance and growth of the microalgal-bacterial consortia can be impacted by heavy metals, which are important toxic compounds.

The consortium controls the detoxification and evolution of both non-essential and essential metal ions as well as the corresponding biotechnological applications through a number of mechanisms, including the sequential processes of diffusion, adsorption, volatilization, hydrolysis/photolysis, and biotransformation (Fig. 3) (Kim, 2011). These versatile removal mechanisms against the toxic substance(s) are attributed to the diverse communities in the MB-AGS system.

3.2 Operational factors

3.2.1 Illumination

Owing to the non-mediated impact of light intensity and photoperiod on photosynthesis and photo-degradation, they might either promote or restrict growth and physiological activities in microalgal-bacterial consortia. A minimum light intensity of ≥90 μmol m^{-2} s^{-1} suffices to yield a sufficient amount of O_2 from micro-algae to create an aerobic/anoxic zone area (0.3–0.6 mg-O_2/L) in granules (Meng et al., 2019). With sufficient illumination, like under the summertime sunlight condition (12/12 h of light/dark cycle) in a 6 h-cycle comprising mainly 120 min of anaerobic phase, 90 min of oxic phase and 144 min of anoxic phase, the microbial granulation could be achieved in two short periods: In the first three days, the induced water-born microalgae and filamentous bacteria interacted primarily on the granules surface with an excess of polysaccarides, protein and humic acid substances. The maturation time (about 4-7days) included the shift and growth of algae that penetrated the inner compartment of the granules (He et al., 2018). The microalgal-bacterial granules exposed to sunlight irradiation generated 34.7% more proteins and 13.1% polysaccharides. In addition, they displayed high structural stability compared to a zero sunlight irradiation condition. The microalgal-bacterial granules had about 70 mg g-SS^{-1} lipid content, being approximately 2.1 times greater than the granules associated with the bacterial-AGS reactor (Huang et al., 2020). Stronger light illumination in the reactors could lead to the extended removal of nitrogen and phosphorus, possibly as a consequence of the microalgae improvement (e.g. *Navicula* and *Stigeoclonium*) and AOB (e.g. *Comamonadaceae* and *Nitrosomonadaceae*). However, *Nitrospiraceae* were found to be adversely affected by Illuminance ≥180 μmol m^{-2} s^{-1} (Meng et al., 2019).

Moreover, there is a fascinating study (Cockell et al., 2011) - demonstrating how algae, bacteria, and cyanobacteria could withstand prolonged ultraviolet (UV) exposure in an outer space, and illustrating that the environment might serve as a selection pressure on the compositions of microbial communities. In this study, the UV light-exposed rock samples with a wavelength greater than 200 nm (simulation of the supposed worst UV exposure on the early

Earth) show that land masses were prone to epilithic colonization at that time; yet, the UV exposure of anoxic planets serves as definite selection pressure on the organisms residing on the surface.

3.2.2 Oxygen

Oxygen produced by microalgae increased bacterial activity, leading to the secretion of more EPSs with higher polysaccharide ratios. Given the varying rates of metabolic and microalgae and bacteria quantities, gas exchange (O_2 and CO_2) between microalgae and bacteria must reach a mutually matched status in order to obviate the need for the external aeration process for MB-AGS (Sun et al., 2022b; Tang et al., 2021). The potential for operating an MB-AGS system without an external oxygen supply is very high. In closed conditions with no external oxygen and only oxygen supplied by microalgal photosynthesis (the produced oxygen was circulated into the reactor by a gas/liquid separation), the EPS content decreased from 321.79 ± 20.92 mg/g-VSS to 184.82 ± 9.30 mg/g-VSS. Given the oxygenase activities of ribulose bisphosphate carboxylase/oxygenase, a high level of O_2 that may have amassed within the algal cells might reduce the net photosynthetic carbon fixation. This low O_2 environment is likely to constrain bacterial growth, while it facilitates algal growth. While the PS content of the EPS fractions did not significantly decrease, the PN content was reduced as well, especially for the loosely bounded PN, which nearly decreased by half from 31.04 ± 0.58 to 14.40 ± 2.04 mg/g-VSS (Wang et al., 2020). Excessive dissolved oxygen would lead to microalgae photorespiration by slowing down their growth since the ribulose bisphosphate carboxylase/oxygenase (Rubisco) is initiated in order to keep oxygen fixed rather than CO_2 when O_2 concentration is significant. This issue leads to reduced microalgae development rate and malonaldehyde accumulation in micro-algal cells, being harmful to cell metabolism (Giordano et al., 2005; Sun et al., 2022a).

3.2.3 Shear force

The shear force in the bioreactor hosting microalgae and bacteria has an important role in granule formation, and structure which could be induced by external aeration and/or hydraulic recirculation/mixing (Jiang et al., 2022). Microalgae could be immobilized more effectively on the granules as a result of increased shear force. The causes of this matter are as follows: (1) higher shear force increased the abundance of smaller granules, providing more space for microalgae to grow in the reactor under similar suspended solids; (2) the shear force positively affected the bacteria's surface hydrophobicity and EPS secretion (Liu et al., 2017).

To adapt the changes to operational conditions, the aerobic granules may adopt EPS regulating mechanism, particularly through increased secretion of fluorescence protein. Each of the parent reactors received three distinct hydrodynamic shear force levels produced from changing time (90, 120, and 150 min) and the aeration intensity (1.5, 0.9, and 0.6 L/min), indicating that the best system performance and granular characteristics were favored by moderate aeration intensity coupling aeration time (0.9 L/min and 90 min). In addition, as a response mechanism, a larger number of EPSs (particularly PN) were generated following the highest hydrophobicity (He et al., 2019). However, the higher shear force typically makes the biofilms thinner, denser, and stronger. The adhesive strength of the biofilm could increase as a result of higher shear force, turning it highly resistant against 'mechanical' as well as combined 'mechanical and chemical' treatments. Further to the above, the basal layer of each biofilm was remarkably resistant to mechanical and chemical removal (Chang et al., 2020).

3.2.4 Solid retention time

The effects of SRTs on the performance of aeration-free microalgal-bacterial nitrification system as well as microbial activities and their population were investigated, which indicated a comparable accumulation of nitrite at different SRTs, including ten, twenty, and forty days. However, due to the significant abundance and activity of NOB, the process abruptly failed on the 20th and 40th days (SRT) in the case of a durable operation. Also, the syntrophic interactions between nitrifying bacteria and phototrophic microbes could result in stable and effective functioning of partial nitrification in the algal-bacterial system at a shorter SRT of 10 days (cyanobacteria and eukaryotic algae). SRT significantly affected the bacterial populations compared to the eukaryotic ones. At a 10-day SRT, the network analysis pointed to the increased possibility of a competition occurring between bacterial and eukaryotic taxa as well as considerable network complexity, which improved the complexity and stability of the network and consequently, enhanced the functional strength and balance of the mentioned system. Overall, a shorter SRT produces highly stable partial nitrification through reducing NOB activities, enhancing the structure of bacterial community, and controlling interactions of microbes so that an equilibrium-based microalgal-bacterial consortium ecosystem can be created (Zhi et al., 2023).

3.2.5 Acyl-homoserine lactone content

As previously stated, cell-to-cell communication is so significant in the community that it affects different physiological-related processes, including biofilm formation, virulence factors generation, nutrient uptake, and stability of ecological niches (Bhatia et al., 2022). Microalgal-bacterial granular sludge produced hydrophobic proteins more favorably as a result of the improvement of AHL-based QS, e.g. by adding QS molecules, which helped the granular sludge develop well. AHL inhibition reportedly decreased the attachment possibility and protein content of algal-bacterial granular sludge, thus threatening granules' structural stability. Due to the prevalence of the genera Acinetobacter, Chryseobacterium, and Flavobacterium, algal-bacterial granulation promoted the number of AHLs and EPS producers (Zhang et al., 2018a). A rather new study reported that AHLs featuring a side chain of $\leq C_{10}$ were frequently found in MB-AGS, and those that were produced in low light conditions had higher concentrations of C_6- and $3OC_8$-HSL, which helped promote the growth of biofilms. According to the add-back research findings, AHLs helped regulate EPS synthesis (Zhang et al., 2019).

4 Chemicals recovery from microalgal-bacterial aerobic granular sludge

In the pharmaceutical, cosmetic, and food industries, one may manufacture goods whose microalgae, or their components, endow them with specific features besides the high potential of utilizing micro-algal biomass as a source of carbohydrates or fatty acids for energy generation. This is possible given the knowledge about the microalgae-bacteria interaction. Despite algae receiving much attention for their ability to produce biomaterials, bacteria are almost inevitably present during the cultivation of algae due to the impossibility of creating environmentally clean and uninfected place for commercial farming and the inevitability of organic materials produced by algal metabolism. The existence of bacteria could affect the algal biomass yield positively or negatively. When bacteria supply algae with the nutrients they require or release signals that encourage growth, positive effects are obtained (Jiang et al., 2021).

4.1 Polysaccharides and phosphorus production

In order to support a sustainable circular economy, the perception regarding wastewater must be changed from being solely an environmental concern to also including the recovery of value-added products. Due to the steady rise in production and manufacturing costs and the scarcity of resources (some of which are limited), focus has shifted to the transformation of wastewater treatment plants into useful sources (Hamza et al., 2021). Granular sludge differs from flocculated sludge in that it contains a significant amount of extracellular biomaterials, which can be used to replace industrially produced synthetics like alginate and sulfated polysaccharides (Tan et al., 2021). When considering the hydrogel-forming capacity of ALE, the majority of prior studies have concentrated on the transformation of P into EPS, but the P and ALE existing side by side offers more intriguing potentials in practice, such as bio-fertilizer and soil aggregates enhancer (Chen et al., 2022b).

EPS can assist in creating an anaerobic microenvironment by preventing contact between anoxic organisms and oxygen, making it a valuable tool in artificial consortia maintenance. Incorporating EPS-producing members and interkingdom species into artificial consortia may further contribute to their resilience, resistance, and tolerance to inhibitory elements, as EPS can create a protective barrier and prevent essential growth factors from diffusing outward (Zhang et al., 2018c). A recent study on granulation in non-phototrophic and phototrophic cultures found that the EPS content of non-phototrophic cultures was 27.7 mg/g-VSS PN and 7.3 mg/g-VSS PS, while the EPS content of phototrophic cultures was 36.5 mg/g-VSS PN and 8.8 mg/g-VSS PS. The phototrophic cultures produced more EPS, particularly aromatic and tryptophan-like compounds, suggesting that proteins played a significant role in the development and stability of algal-bacterial granules (Zhang et al., 2018b). Additionally, Wang et al., 2022b investigated the primary constituents of activated sludge and algae-bacteria consortia for treating coking wastewater and found that they shared many of the same primary elements, indicating that the composition of EPS was unaffected by the growth of algae.

Recently, researchers investigated the use of CO_2 to enhance the microalgal-bacterial granular sludge process. Results showed that CO_2 increased the production of extracellular polysaccharides and aromatic protein, favoring granular stability of the microalgal-bacterial granular sludge. Without the addition of CO_2, the physical structure of the MBGS tended to be loose and disintegrated, with a sludge volume index at 5 min (SVI_5) of above 150 mL g^{-1} and an average pore size of 35 nm after 60 days of operation. Conversely, CO_2 was able to preserve the compact and integrated structure of microalgal-bacterial granular sludge, with an SVI_5 lower than 50 mL g^{-1} and an average pore size range from 10 to 13 nm (Ji & Liu, 2022). Moreover, the growing concerns regarding the increase of CO_2 in the atmosphere have led to the shift from conventional wastewater treatment processes to algae-bacteria symbiosis. A recent study indicated that the specific carbon removal rate of CO_2 by MB-AGS is around 2.0 mg C/(g VSS h), suggesting that CO_2 could be employed to treat residential wastewater using MB-AGS to achieve a carbon-negative objective (Ji & Liu, 2022).

The decline in EPS-Protein content from the growing period of 0-28 days was indicative of age-related changes in the strength and adhesiveness of the biofilm in native microalgae-bacteria consortia. The synthetic wastewater's blue-green filament began to gradually separate from the flask at day 15, and by the end of the experiment, it was floating freely in the culture media. Conversely, in real food processing wastewater, most of the blue-green filaments (*Leptolyngbya sp.* and *Phormidium sp.*) fell off between days 7 and 14, leaving the diatoms (*Nitzchia palea*) and a few green unicellular cells (*Chlorella sp.*) to settle in the sediments at the bottom of the flasks. Research indicates that diatom-associated bacteria play a crucial role in diatom aggregation, which aids in the development of local microalgae-bacteria consortia. Diatoms also release mucilage for gliding, with proteins and polysaccharides being the main components, contributing to the formation of biofilms (Amadu et al., 2023).

Of note, microbial EPS components can have both positive and negative effects. While some EPS components act as growth hormones and sources of vitamin B12 in probiotic symbiosis, others, such as alkaloids and functional enzymes, can be toxic and inhibit microalgal growth. Quinolone derivatives and β-glucanases are among the most significant biotoxic EPS components responsible for microalgal death, each exhibiting unique characteristics. Quinolone derivatives can impede DNA and protein production as well as microalgal respiration, while β-glucanases damage the cell wall. In a similar vein, certain biotoxic EPS substances released by microalgae can also pose a risk to bacteria (Chen et al., 2022c).

Another recoverable chemical is Sulfated Polysaccharide (SP) that generally refers to any type of polysaccharide with sulfate groups producible from marine algae and some different kinds of animal tissues. Recent research identified SPs in waste-activated sludge produced by the treatment of saline wastewater and predicted the possibility of economical resource recovery in the case of coastal cities with direct seawater flushing and/or seawater intrusion. Considered valuable material, SPs run the possibility of being removed from the sludge and applied to various fields in practice (Liu et al., 2022a; Xue et al., 2019).

Unlike the traditional EBPR approach, the MB-AGS allows phosphorus harvesting through both bacterial up-taking and microalgal assimilation to ensure P removal and recovery. Thus, one may reasonably assume that the MB-AGS is able to provide an efficient P harvesting method from (Ji et al., 2020). Meanwhile, the phosphate harvest process allows for the realization of almost all potential mutualism relationships. This finding that PAOs releasing phosphate in aerobic conditions could be disrupted as a result of direct microalgae assimilation leading to phosphate removal is a notable benefit for microalgae-bacteria consortia (Zhang et al., 2021).

To ensure better phosphorus removal and recovery, a self-sustaining synergetic MB-AGS was investigated that would result in 86% removal of the influent phosphorus in 6 hours, which included a 2-hour dark phase and a 4-hour light phase. A small amount of phosphorus release was seen at the dark phase and then, considerable phosphorus uptake as well as accumulation of poly-phosphorus in micro-algal cells were seen at the light phase with the main phosphorus-accumulating organisms being the microalgal species of the Pantanalinema genus (Ji et al., 2020). This new microbial consortium could be applied to phosphorus recovery out of industrial wastewater. In one previous study, researchers looked into how photosynthetic microorganisms grew on wastewater and found that co-culturing of *C. vulgaris* and *R. sphaeroides* in a mixture of starch and piggery wastewater improved phosphorus recovery (up to 96%) (You et al., 2021a).

4.2 Challenges of extracellular polymeric substances extraction

The significance of perceiving EPS regulation and its composition in terms of its features and dimensions has helped plants dealing with wastewater treatment to properly manage waste sludge and treat wastewater. Moreover, a deeper understanding of the above significance promotes the production of viable EPS as valuable biomaterials, creating a technologically viable substitute for sludge management (Feng et al., 2021). Notably, there are two major difficulties in recovering important polysaccharide compounds, the first involves a thorough scrutiny of the association or any link between the polysaccharide characteristics and the effectiveness of biological treatment. Due to the complexity of real wastewater in treatment plants, the inhibitory effects of toxic compounds have always been a challenge, which can decrease the performance, EPS production, and biomass retention. How to manipulate the microalgae-bacteria interactions and avoid the inhibitory effects should be study more, despite some key factors has already been studied (as summarized in Section 3). Second, it is essential to optimize extraction, separation, and purification approaches to obtain highly pure polysaccharides because wastewater and sludge are complex and produce polysaccharides of low purity (Liu et al., 2022a).

Based on specific research objectives, there is a need to alter the optimization protocol for EPS recovery (Feng et al., 2021). It is difficult to identify ALE when it is further separated from EPS. Different outcomes can be obtained depending on if the granular structure is dismantled (fully solubilized) throughout the ALE extraction procedure. Given that only the same category of ALE is more comparable, it is critical to determine if the extracted ALE is of sodium form, acid form, or acid-soluble EPS depending on the termination step in the process of extraction. The strict supervision of consistent recycling operations appears to be one important factor that is key to effective extracellular biopolymer recovery (Chen et al., 2022a).

Notably, the chemical compounds extracted from EPS could provide numerous binding sites (including hydrophilic and hydrophobic groups), which could be further modified into functional materials. In this respect, investigation of the extraction of exopolymers that form hydrogels as well as an understanding of their physicochemical characteristics and their interactions are useful in revealing the roles that EPSs play in the formation of granules as well as their possible use cases as sizing and coating agents (Feng et al., 2021).

5. Energy production from microalgal-bacterial aerobic granular sludge

5.1 Traditional biofuel production

Symbiotic microalgae-bacteria consortia are viable enough to increase the generation of micro-algal biomass and enhance it by means of useful energetic and chemical substances. Future microalgae-based biomass and biofuel generation will benefit from the proper management of such bacterial and microalgae biological interactions (Yao et al., 2019). Through the coordinated relation between algae and bacteria in the form of micro-algal fuel cells, photosynthetic algae can absorb solar energy and convert it into electricity without the need for any external input of organic materials. Biodiesel can be produced by converting the triacylglycerol and other lipids produced by algae into fatty acid and methyl esters (Yong et al., 2021). Moreover, biodiesel can be produced by digesting biomass in fermenters to produce natural gas (Chew et al., 2017).

Compared to biogas, liquid biofuels like biodiesel are generally considered highly profitable because of the higher oil-to-natural-gas price ratio (Bohutskyi et al., 2018). The highest biodiesel production of MB-AGS is 66.21 ± 1.08 mg/g SS, being much greater than the AGS (35.44 ± 0.92 mg/g SS). Even though methyl palmitate was predominant in the biodiesel produced from the two granules, poly-unsaturated fatty acid in the MB-AGS appeared at a greater percentage (21.86%) than AGS (1.2%) because of *Scenedesmus* addition (Liu et al., 2018).

Moreover, the use of only green microalga *Scenedesmus acutus* for treatment of pre-treated wastewater indicated 79.9 mg/L biomass productivity and 280 mg/L lipid accumulation, rendering 249.4 mg/L of biodiesel (de Alva et al., 2013). Although the algal-bacterial granule yields more biodiesel, the outcome indicates that the biodiesels generated via algal-bacterial granules might not remain stable over a prolonged storage. However, linoleic acid (C18:2) and linolenic acid (C18:3) represent vital poly-unsaturated fatty acids for human body. Therefore, microalgal-bacterial granules are assumed raw materials of the mentioned fatty acids (Meng et al., 2019). In another research, six photobioreactors (625, 393, 272, 193, 178, and 167 mg/L Soluble COD (SCOD)) were operated with diluted anaerobic digestion effluent. Relevant findings indicate that algal cell growth is significantly inhibited by the considerable concentration of SCOD = 625 mg/L. The 272 mg/L SCOD obtained the highest lipid productivity (59.13 mg/L.d), and nutrients (nitrogen and phosphorus) were removed via a photobioreactor under the SCOD of 167 mg/L (Xie et al., 2018).

The average production rates of biodiesel between 9435–76,705 tons from Scenedesmus and 5521–113,011 tons from Chlorella were measured based on the assumption of the theoretical urban wastewater volume treated annually in Campania. This figure only accounts for 0.32 to 6.62% of the present diesel consumption of the region (Catone et al., 2021). However, prior research indicates that algal biomass featuring a 42% lipid content and a US$ 217.22/ton production cost may compete with oil, costing per barrel US$ 60.00 (Xie et al., 2018).

5.2 Green-fuel (hydrogen) production

Microalgae and cyanobacteria encapsulate the metabolic, genetic, and enzymatic properties required for the production of bioH_2 gas. Given that only water (H_2O) is produced as a byproduct of the combustion of hydrogen, this energy source is renewable and unsoiled. Because of its significant conversion efficiency ranging from 122 to 142 kJ/g, being 2.75 times greater than that of combustible hydrocarbons, H_2 is regarded as a fuel of the future (Catone et al., 2021; Rashid et al., 2013b). Naturally, green microalgae generates biohydrogen and it is categorized into (a) direct photolysis and (b) indirect photolysis (Goswami et al., 2021). To be specific, a process where biohydrogen in generated via dividing water into electrons and protons by sunlight is called direct photolysis, while indirect photolysis occurs when Sulphur-free media are utilized where photosynthesis yields carbohydrate compounds and decreases the amount of oxygen in the cell. Hydrogenase sensitivity to oxygen hampers the production of H_2 in direct photolysis and this problem is overcome via indirect bio-photolysis (Catone et al., 2021).

Low light results in O_2 transformation, which is both high enough to allow acetic acid consumption and low enough to maintain hydrogenase activity. Microalgae ability to produce oxygen has an inhibitory impact on hydrogenase expression and activity that are associated with hydrogen generation. At low light levels, Chlamydomonas can produce hydrogen and go into anoxia. Here, the effect of co-cultivating bacteria with Chlamydomonas on hydrogen production at low light intensities shows that it can increase H_2 photo production by up to 56% (Fakhimi & Tavakoli, 2019). Another study demonstrated that when Chlamydomonas-bacteria co-cultures were grown on sugars and the bacteria produced acetic acid, the Algal H_2 photo production would be possible. The function of acetic acid in this alga is not just dependent on its capacity to encourage O_2 uptake, it may also be related to H_2 production through metabolic assimilation. A promising technique for producing synergetic H_2 is co-culturing Chlamydomonas with bacteria. Among the key disadvantages of the dark-fermentation for hydrogen generation is the accumulation of acetic acid. However, this disadvantage can turn into an advantage upon co-cultivating H_2-producing bacteria with Chlamydomonas (Fakhimi et al., 2019). In addition, dark and photo fermentation processes help produce it (Goswami et al., 2021). In one study, autotrophic microalgae cultivation, dark fermentation, and photo fermentation were combined using the four carbon sources examined to create a high yield biohydrogen generation system. Sucrose was the most efficient carbon source for the sequential dark and light fermentation processes, which continued a stable operation for almost 80 days and gave a H_2 generation rate of 670 ml/h/l and maximum H_2 yield of 12 mol H_2/mol sucrose (Lo et al., 2010).

The production of hydrogen through biofilm formation is dependent on the amount of EPS secreted by bacteria. The yield of hydrogen is directly proportional to the amount of EPS produced. As the biofilm is formed, the carbon source for EPS synthesis becomes limited, leading to the conversion of more energy into hydrogen energy (Wen et al., 2020).

5.3 Direct electricity generation via microbial fuel cell

The microbial fuel cell (MFC) is a novel renewable-energy technology that incorporates photosynthetic organisms to achieve direct electricity generation. MFC employs microorganisms' anaerobic respiration to directly turn organic waste (fuel) into useful electricity (Gajda et al., 2015). The microalgae–microbial fuel cells containing photosynthetic microorganisms can convert solar energy into electricity via the metabolic reactions of photosynthetic microorganisms. In addition to bioelectricity generation, microalgae–microbial fuel cells are able to sequestrate CO_2 from air and eliminate nitrogen pollutants from waters (Lee et al., 2015). Microalgae have frequently been utilized in MFCs as a substrate for bacteria or to minimize oxygen in the cathode. However, a considerable amount of electricity can also be produced in the anode, where cytochromes facilitate indirect transfer of electrons produced in the photosystem II of the algae, creating what is known as a photosynthetic algal microbial fuel cell (Shukla et al., 2018).

Genetic engineering strategies including gene knock-in and gene knock-out in conjunction with proteomics, transcriptomics, and metabolomics in stress conditions have been highlighted in order to increase the potential and viability of microalgal-assisted MFCs for electricity production and micro-algal biomass generation. Genetic engineering targets important metabolic pathways of microorganisms through up- or down-regulation, genes overexpression, and silencing of the important gene in order to modify the needed metabolite content without affecting the physiological characteristics of microorganisms. Through the mentioned techniques for genetic modulation, it is possible to increase the electro-activity of electro-genic microorganisms, which will ultimately improve electricity generation. These changes include QS system gene modifications, genetic engineering or overexpression of e-shuttle synthesis genes, knockout of native global regulators, and deletion or mutation of genes for enhancing biofilm formation on electrodes (Sharma et al., 2022).

MFCs utilize microalgal biomass and activated sludge as substrates to generate power. In the anode, activated sludge serves as an inoculum and a source of nutrients. Dry algal biomass at different concentrations (1–5 g/L) is studied and an attempt is made to determine if under 1000 Ω electric resistance, 5000 mg COD/L will produce maximum voltage (about 1 V) and power density (about 2 W/m^2) of all concentrations that were evaluated (Rashid et al., 2013a). In another study, to produce oxygen in situ, sediment MFCs were built by adding algae (Chlorella vulgaris) to the cathode. The rate of power generation of sediment microbial fuel cells with an algae-assisted cathode was 21mW/m, which was extended up to 38mW/m with a cathode coated with carbon nanotube, being 2.4 fold greater than the sediment MFCs with a bare cathode. Based on the results of dissolved oxygen and cyclic voltammetry analysis, it was found that C. vulgaris in the cathode could elevate the amount of the dissolved oxygen and the rate of oxygen reduction (Wang et al., 2014). In a different study, algal cathode microbial fuel cells were fed landfill leachate at various concentrations (5–40%) for energy generation as well as COD and nutrient removal. With 5% leachate, the highest cell voltage was achieved at 300 ± 11 mV; however, as the leachate percentage increased, the voltage of the cell decreased (Nguyen et al., 2017).

Nitrite/nitrate and oxygen are used by the membrane photosynthetic microbial fuel cell as cathodic electron acceptors, which, in turn, help treating organic carbon/nitrogen and reducing CO_2 in the cathode compartment. Our study reports an unconventional cathodic process developed based on in situ nitritation through microalgal photosynthesis during the day to ensure shortcut removal of nitrogen from ammonium-rich wastewater. Final NH_4^+ and NO_2^- concentrations in the effluent of 0.10 mg NH_4^+/L and 0.02 mg NO_2^-/L were attained, which led to 99 ± 0.5% nitrogen removal efficiency and 56 ± 0.1 mA electricity production (Arun et al., 2022).

The organisms are embedded in EPS, which is known as the "house of the biofilm cells." As microorganisms mature, the density of the biofilm gradually increases due to a higher concentration of EPS. However, the electrochemical performance of the biofilm depends not only on its density, but also on the spatial distribution and metabolic activity of live and dead cells within the biofilm. Dead cells tend to accumulate in the inner layer of the biofilm, which can impede electron transfer and increase diffusion resistance, resulting in a decrease in electrochemical activity. Therefore, it is suggested that the presence of active, electron-transferring live organisms in the outer layer of the biofilm plays a pivotal role in the generation of high current density in the system. The formation of a biofilm on the anode is crucial to the effectiveness of microbial fuel cells, and optimizing biofilm structure and composition could lead to improved performance and efficiency. EPS is crucial for producing hydrogen, and the yield of EPS determines the amount of hydrogen released. With the passage of time, a high-density biofilm may contain dead cells in the inner layer, which cause a decrease in performance. Angelaalincy et al., 2018 have shown that the temporal and spatial locations of live and dead cells within a biofilm contribute to the electrochemical performance of the system. Similarly, Xiao et al., 2017 investigated the role of EPS in the electron transfer process of *Shewanella oneidensis* MR-1 cells by removing the EPS layer from the cells through heating at 38°C for 30 minutes. They found that this treatment did not damage the viability of the cells but led to a 35 ±

15-nm EPS layer being removed. The electrochemical performance of MR-1 cells in microbial fuel cells can be influenced by the presence of EPS. When a control group of cells was incubated at 30°C for 30 minutes, their EPS layer was preserved. In contrast, MR-1 cells depleted of EPS displayed maximum current increments 40-90% higher than the control group with EPS retained. This highlights that MR-1 cells lacking EPS can transport electrons to extracellular electron acceptors more efficiently than those with EPS present. The reason for this may be that EPS acts as an insulating layer that impedes the flow of electrons, thereby reducing the effectiveness of electron transfer. These findings underscore the crucial role of EPS in microbial fuel cells, and suggest that altering EPS content has the potential to enhance the electrochemical performance of these systems.

6. Research needs and future directions

The development of industrial EPS and biomass production using algal-bacterial consortia as a valuable commercialization source requires a thorough understanding of the consortium properties that can help in manipulating the process for more productive and valuable outcomes. Based on various objectives, the standard for biopolymer extraction should be established. It must take into account whether EPS extraction for industrial applications can be done according to the conventional standards. Cell lysis is less significant than the sizeable yield amounts with an ideal structure. Certain granular sludge EPS components are not able to be successfully extracted based on the conventional extraction protocol. Any novel EPS extraction techniques should consider efficacy, cost-effectiveness, user-friendliness, and minimal chemical additives. Furthermore, the current EPS recovery process is hampered by the purification techniques for the extracted EPS. The purification method should also be optimized to recover high-quality biopolymer, depending on the intended use of the extracted product (Feng et al., 2021). Improving the purity of functional extracellular biopolymers is essential for obtaining high yields of high-quality biomaterials with desirable properties. The properties of these biopolymers, such as gel-forming, film-forming, mechanical, amphiphilic, adsorption, viscoelastic, aggregation, and self-extinguishing, can be optimized by adjusting the operating parameters and incubation conditions of the biosystem (Chen et al., 2022a).

The treatment of wastewater is a crucial process for removing harmful pollutants from water, but what happens to those pesky pollutants after they are removed? They are transferred to the sludge, which is a by-product of the treatment process. The sludge is like a mixed bag of goodies, containing both organic and inorganic substances, including heavy metals, pathogens, micro-plastics, and other unsavory pollutants. To ensure that biomaterials derived from this sludge are safe for use, a comprehensive risk assessment is needed to identify and quantify any potential risks associated with residual pollutants (Tan et al., 2021).

In recent years, the effective reduction of GHG emissions has become a critical international issue, spanning across scientific, environmental, economic, and political sectors. Among the GHGs, CO_2 is one of the most prominent pollutants being emitted into the atmosphere (Kao et al., 2014). Flue gases have been recognized as a promising source of CO_2 for microalgae, owing to their high concentrations of the gas, typically ranging between 5% and 15% v/v (Vale et al., 2020). However, the impact of flue gas on algal-bacterial consortia in terms of bio-polymer production, and the negative impact of other compounds such as sulphur oxides (SOx) and nitrogen oxides (NOx) on the process, has not been fully elucidated and requires further investigation.

7. Conclusions

Recent studies on the interactive algae-bacteria consortia effect on biomass and bio-polymeric substances of MB-AGS were reviewed. The characteristics, influencing factors, and challenges associated with cooperative or competitive partnerships between algae and bacteria through interaction patterns on biomass, valuable source recovery, and EPS were discussed comprehensively. It is still necessary to conduct a thorough investigation into the application of control strategies to keep a consortium operating over a long term despite environmental changes and biological contamination.

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Figures and Table

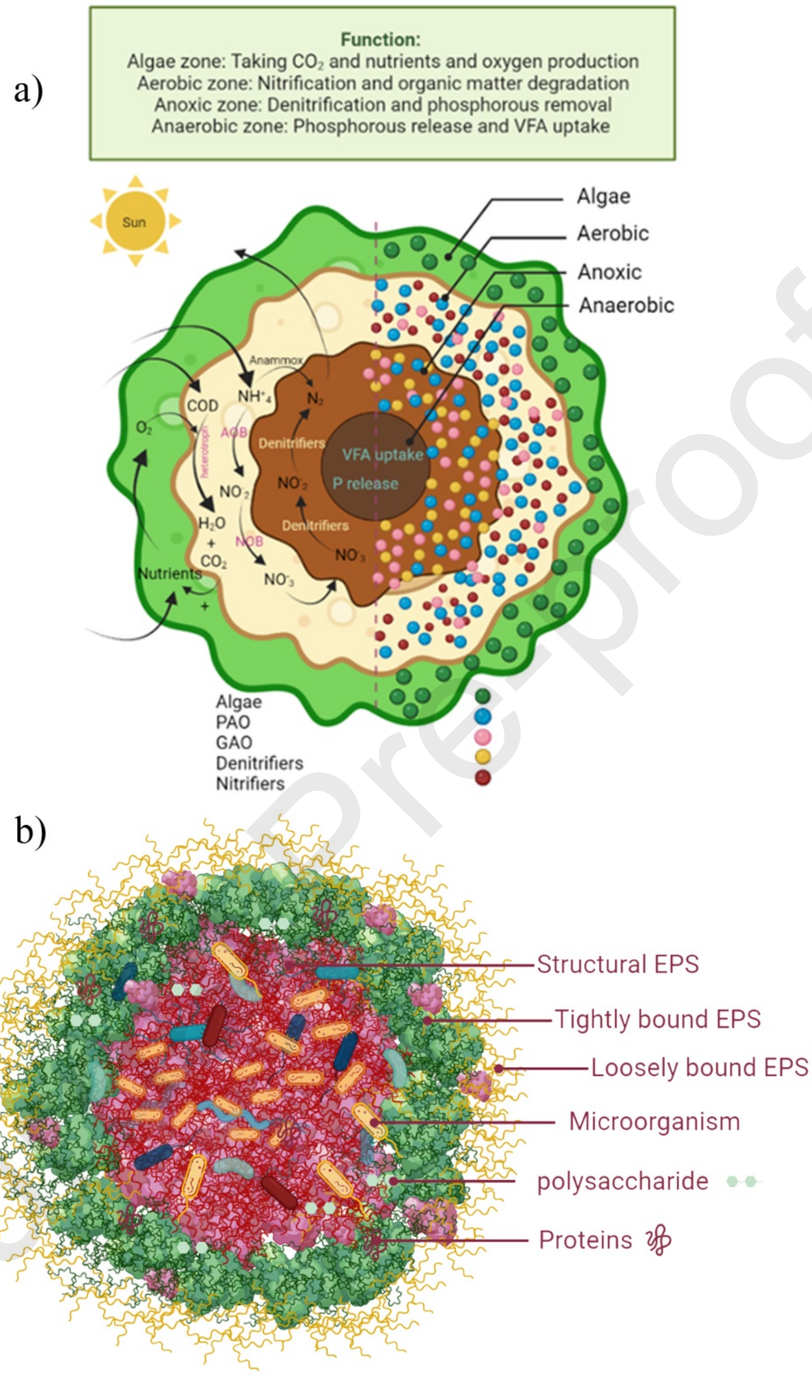
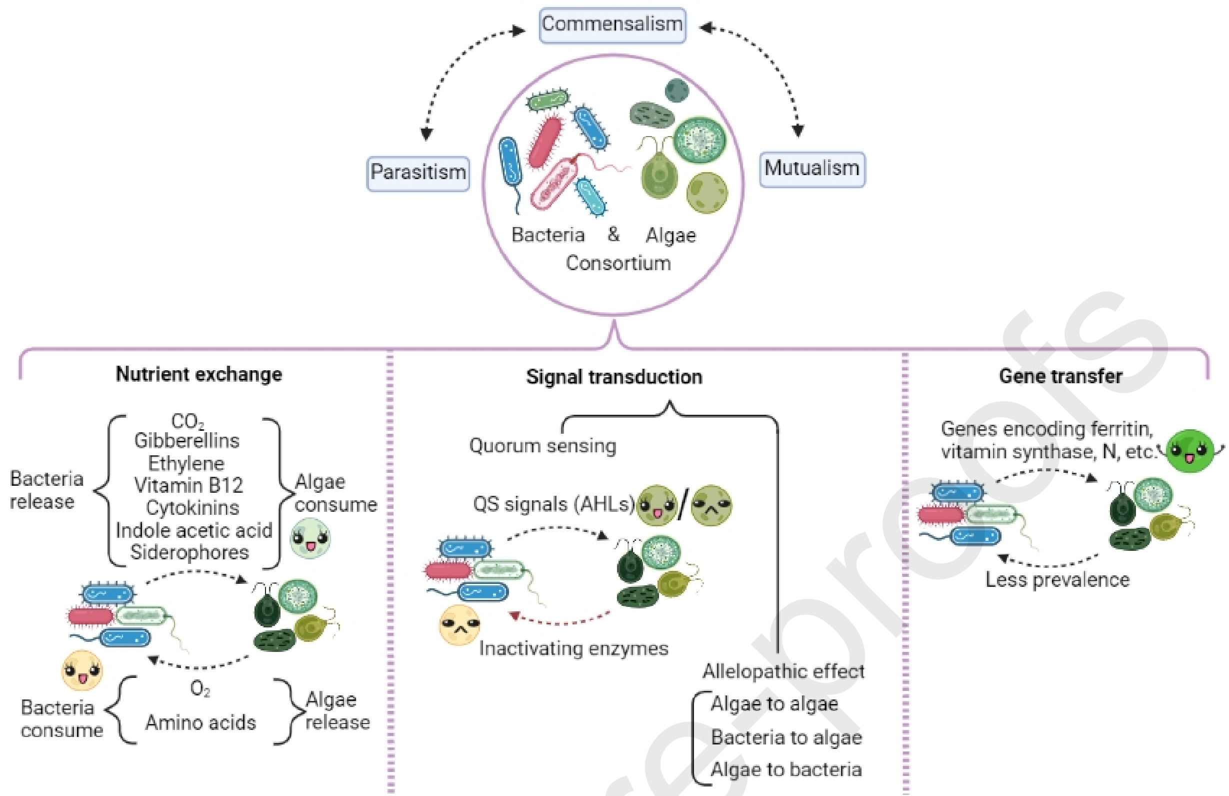


Fig. 1. The composition of microalgal-bacterial granules (a) and extracellular polymeric substances (b).



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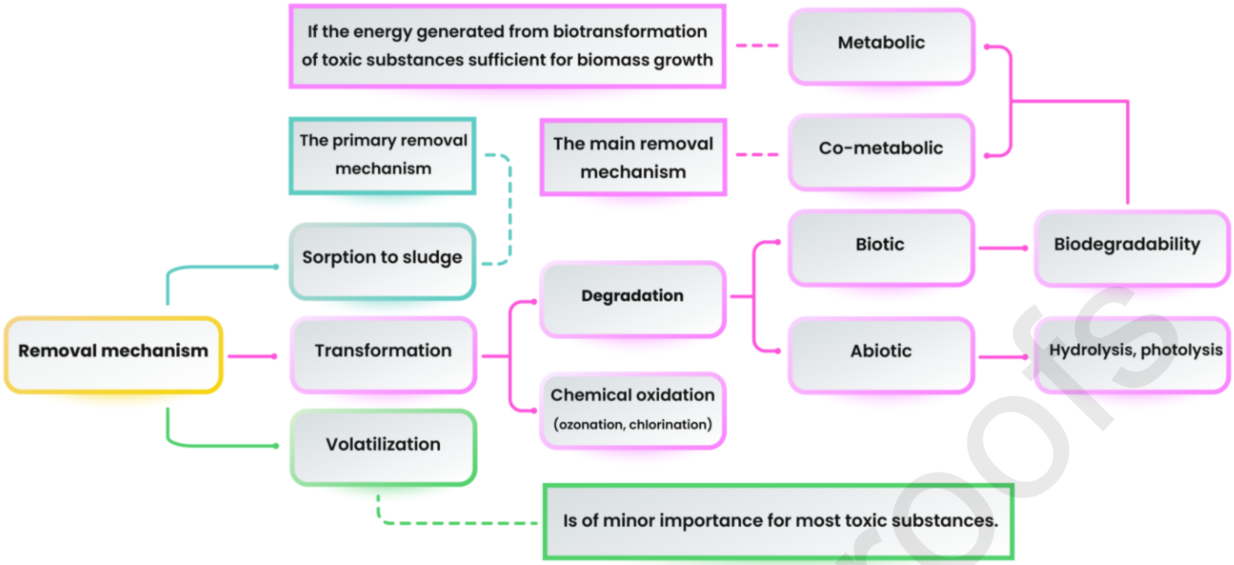
1010 **Fig. 2. The microalgal-bacterial symbiosis interactions**



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1013 **Fig. 3. Removal mechanism of heavy metals by algae-bacteria consortia**

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Table 1. Influencing factors on EPS and biomass production

Factors	Reactor	Feed stock	Characteristic of feed stock	Condition	Productivity	Removal efficiency	Reference
Carbon to nitrogen ration	Algal-Bacterial AGS	Synthetic wastewater	Influent COD: 400 to 50 mg/L, 10 mg PO_4^{3-}-P/L, 50 mg NH_4^+-N/L, 5 mg Fe^{2+}/L, 10 mg Ca^{2+}/L, 5 mg Mg^{2+}/L, and 1 ml of trace element per liter	Decreasing the COD to nitrogen ratio (8, 4, 2, 1); Light cycle 12 h/12 h; light illuminance 7200 lux on surface of water; Room temperature (25 ± 2 °C); 2 min feeding, 60 min no aeration, 173 min aerobic, 2 min settling, 3 min decanting	PS content steadily increased from their initial 18.0 to 51.9 mg/g-MLVSS; the MLSS decreased from 5.30 to 3.21 g/L; the EPS content decreased from ration of 4 to 2 to about 114.5 from 196.2 mg/g-MLVSS.	The dissolved organic carbon (DOC) removal decreased from 96% to 82.5 %. Low COD/N ratio (4-1) impact the nitritation and denitrification.	(Zhao et al., 2018)
Salinity	Algal-Bacterial AGS	Synthetic wastewater	300 mg COD/L, 5 mg PO_4^{3-}-P/L, 30 mg NH_4^+-N/L, 5 mg Mg^{2+}/L, 10 mg Ca^{2+}/L, 5 mg Fe^{2+}/L, and 1 ml of trace element per liter	Salinity shock (1–3%)	EPS content enhanced from initial 119.7 to 213.8 mg/g-VSS (1% salinity) and 233.5mg/g-VSS (3% salinity)	Decrease in removal of DOC (from 97% to 94%); Ammonia (100 to 23%), TN (66 to 16%) and TP (70 to 38 %)	(Dong et al., 2021)
	Algal-Bacterial AGS	Synthetic saline wastewater	COD: 600 mg/L, ammonia: 50 mg/L, phosphate: 10 mg /L.	Increase of salinity smoothly (1–4% salinity)	ALE content decrease about 10.7% and reach the 28.5 mg/g-VSS	91–92% of DOC removal efficiencies	(Meng et al., 2020)

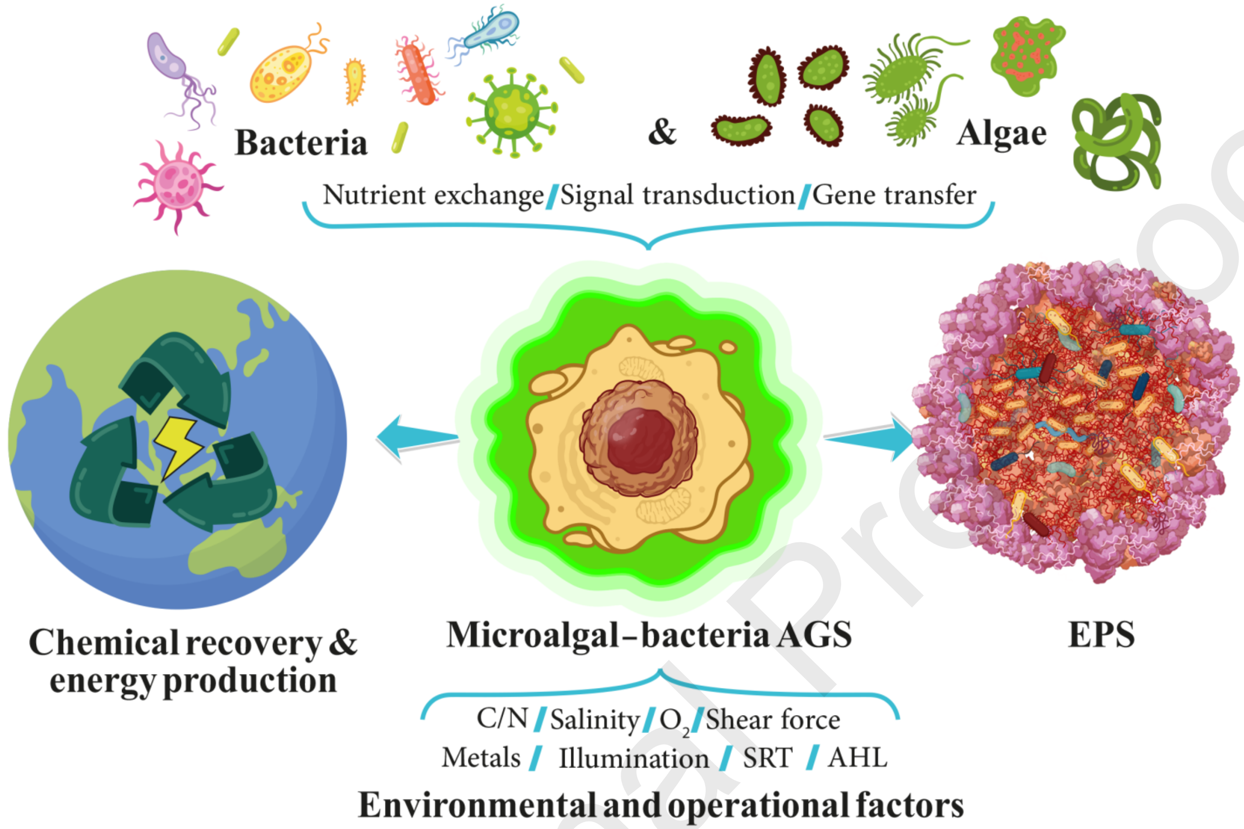
Heavy metals	Algal-Bacterial AGS	Synthetic wastewater	K_2HPO_4: 3.5 g/L, KH_2PO_4: 1.5 g/L, NH_4Cl: 1 g/L, $MgSO_4$: 0.3 g/L, and NaCl: 0.5 g/L, CH_3COONa: 0.82 g/L	5 mg/L of Cr(VI); 5 g-total solid /L of granules using rotary shaker; rpm: 125; temperature: 25 ± 2 ◦C. pHs of biosorption experiments were 6.	5.2 % increase of LB-EPS and soluble microbial products enhanced by 140% exposed to Cr(VI) for 16 h.	-	(Yang et al., 2021)
Illumination	Bacterial-Algal Coupling System	Mariculture wastewater	714 0.0 ± 35.7 mg COD/L, Salinity of 2.2 ± 0.1%, 35.2 ± 1.8 g VSS/L, pH: 7.32 , 3.7 ± 0.2 mg TP/L, 47.6 ± 2.4 mg NH_4^+-N/L.	Light intensity: 5000 Lux; light(12h)/dark(12h) cycle; temperature: 25 ◦C; mixing through shaking	Biomass productivity: 0.465 g/L.d	COD removal: 98.1%; Ammonia removal: 70.7%; Phosphorus removal: 90.0%	(Gao et al., 2021)
	Algal-bacterial AGS	Synthetic wastewater	300 mg COD /L, 30 mg N/L, 5 mg P/L, 5 mg Mg/ L, 10 mg Ca/L, 100 mg/L $NaHCO_3$, 0.25 mg Fe/L, and 1 mL trace elements per Liter.	Light intensity on surface water: 12.3 klux; 5 min filling, 115 min no mixing, 231 min aerobic, 3 min settling, 5 min decanting; temperature: 20 ± 2℃, air flowrate: 5.6 L/ min, HRT: 12h; VER: 50%; SRT:32 days	MLSS increase from the 4.44 to 6.29 g/L	Less than 0.05 mg-P/L in effluent; more than 0.99 % organics and ammonia removal efficiencies.	(Wang et al., 2022a)
Oxygen	Algal-Bacterial AGS	Synthetic wastewater	500 mg COD/L, 50 mg NH_4^+-N/L, 10 mg P/L, 5 mg Mg^{2+}/L, 23 mg Ca^{2+}/L, 0.25 mg Fe^{2+}/L, and 1 mL trace elements per Liter.	No external oxygen and only oxygen supplied by algal photosynthesis, the produced oxygen was circulated into the reactor by a gas/liquid separation (Closed system)	EPS content decreased from 321.79 to 184.82 mg/g-MLVSS	~100% COD removal, and the reduction of ammonia removal efficiency.	(Wang et al., 2020)
Shear force	AGS	Synthetic wastewater	200 mg COD/L, 50 mg NH_4^+-N /L, 4 mg TP/L, 10 mg Ca^{2+}/L, 10 mg Mg^{2+}/L, and trace solutions.	Different hydrodynamic shear forces by applying airflow of 1.5, 0.9, and 0.6	More EPS (particularly PN) were produced	Stable carbon and nutrients removal regardless of the	(He et al., 2019)

				L/min at time of 90, 120, and 150 min. optimum: 0.9 L/min and 90 min; water temperature: 19 ± 2℃; VER: 50%		applied conditions (aeration rate and time)	
Solid retention time (SRT)	Algal-bacterial consortia	Synthetic wastewater	Ammonia: 50–70 mg /L, nitrite: 0–1.50 mg /L, Nitrate: 0–0.50 mg /L, and COD: 20–71 mg/L.	SRTs (5 d, 10 d, and 20 d). 5 min of feeding, 23.5 h of reaction, 20 min settling and 5 min discharging, Light intensity of 30 μmol photons·m^{-2}·s^{-1}., HRT: 1.2 d; VER: 83%; aeration-free	Higher SRTs increased the biomass and removal of nitrogen by denitrification, but play an inhibitory role on nitrite accumulation.	Short SRT (10 d) could result in a partial nitrification.	(Zhi et al., 2023)
AHL content	Algal-Bacterial AGS	Synthetic wastewater	1.2 g/L COD; 0.472 g/L of $(NH_4)_2SO_4$; 0.040 g/L of KH_2PO_4; 0.03 g/L of $FeSO_4·7H_2O$; 0.025 g/L of $MgSO_4·7H_2O$; 0.03 g/L of $CaCl_2$	Light/ dark cycle of 6 h/18 h (4000 ± 300 lx); 25℃; SRT: 20 d.	Increase of AHL-based QS help the production of hydrophobic protein.	-	(Zhang et al., 2018a)

Credit Author Statement

Moein Besharati Fard: Conceptualization, methodology, investigation, formal analysis, visualization, writing – original draft preparation. **Di Wu**: Conceptualization, writing-reviewing and editing, funding acquisition, resources.

Graphical Abstract



Highlights

- Interaction of Microalgae and bacteria on EPS discussed thoroughly
- Environmental and operational factors influencing the EPS and biomass production
- Patterns of mutualism, commensalism, and parasitism in MB-AGS discussed
- EPS bio-product as a valuable source pave the way to sustainable development

- Green-blue energy could be produced by converting/utilizing MB system