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Multifaceted roles of microalgae in the application of wastewater biotreatment: A review☆

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ABSTRACT

Microalgae have become imperative for biological wastewater treatment. Its capability in biological purification of wastewaters from different origins while utilizing wastewater as the substrate for growth has manifest great potentials as a sustainable and economical wastewater treatment method. The wastewater grown microalgae have also been remarked in research to be a significant source of value-added bioproducts and biomaterial. This paper highlights the multifaceted roles of microalgae in wastewater treatment from the extent of microalgal bioremediation function to environmental amelioration with the involvement of microalgal biomass productivity and carbon dioxide fixation. Besides, the uptake mechanism of microalgae in wastewater treatment was discussed in detail with illustrations for a comprehensive understanding of the removal process of undesirable substances. The performance of different microalgae species in the uptake of various substances was studied and summarized in this review. The correlation of microalgal treatment efficacy with various algal strain types and the bioreactors harnessed for cultivation systems was also discussed. Studies on the alternatives to conventional wastewater treatment processes and the integration of microalgae with accordant wastewater treatment methods are presented. Current research on the biological and technical approaches for the modification of algae-based wastewater system and the maximization of biomass production is also reviewed and discussed. The last portion of the review is dedicated to the assertion of challenges and future perspectives on the development of microalgae-based wastewater treatment technology. This review serves as a useful and informative reference for readers regarding the multifaceted roles of microalgae in the application of wastewater biotreatment with detailed discussion on the uptake mechanism.

1. Introduction

Due to world's growing population, intensive agricultural and rapid industrialization, increased urban wastewater production has shown to be one of the most critical environmental problems envisaged by humanity. Scarcity of clean water supply in many developing countries might be due to failure in conducting appropriate treatment of wastewater or discharging effluent below an environmentally safe level to nearby waterbodies. In particular, water quality in overpopulated nations like India, Kenya, Ethiopia and Nigeria has reached a worrying state (Onuoha, 2012). Organic and inorganic impurities and various contaminants ranging from micropollutants to heavy metals and excessive nutrient loads are discharged into nearby waterbodies by virtue of industrial, agricultural and domestic activities (Rathod, 2015). An array of pollutants like industrial waste, pathogens, heavy metals and pesticides are present in wastewater from different origins and their impacts on human health as displayed in Figure S1 (Yu et al.,

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Wastewaters contain substantial amounts of organic and inorganic nutrients, which cause ecosystem imbalances with their high biological and chemical oxygen demand (BOD & COD). The presence of excessive nutrients like nitrogen (N) and phosphorus (P) will cause eutrophication of waterbodies and thereby disrupting the health of water systems. The investigated concentrations of COD, nitrogen, and phosphorus found in a variety of wastewaters are deduced in Table S1. This phenomenon leads to environmental concerns which comprise solid waste and by-product generation, undesirable product emissions to air, excessive growth of undesirable microbes that endanger aquatic life form and worsening quality of water consumption that contributes to widespread health-related problems in areas nearby the discharge range (Amenorfenyo et al., 2019).

Wastewater treatment shall be operated at primary, secondary or tertiary stages implementing physical, biological, or chemical procedures. The primary treatment eliminates the easily settled materials which result in operational problems in subsequent treatment stages. In contrast, the secondary treatment involves physical or biological processes that degrade the organic material present in wastewater by consuming the dissolved organic matter and oxidizes the major nutrients to nitrate and orthophosphate. As a result, secondary effluent is rich in inorganic nitrogen and phosphorus which will give rise to eutrophication and long-term environmental issues due to the intractable organic compounds and heavy metals that are being released (Rambabu et al., 2020c; Rathod, 2015). On the other hand, the tertiary treatment which is a progressing treatment process that reduces nitrates, phosphates and organic matter is essential to produce clean and harmless effluent that will be discharged into waterbodies (Molazadeh et al., 2019). Tertiary treatment involves denitrification where nitrate is reduced to nitrite in the first process and then nitrite will be reduced to nitrogen gas which will escape into the atmosphere (Farazaki and Gikas, 2019). The primary factors that are accountable for the defecting state of wastewater treatment facilities in some countries might be owing to the design weaknesses of the water treatment process, lack of expertise and insufficient funds. Therefore, the evolution of well-structured wastewater treatment technologies and practicable economic approaches is hence becoming more significant.

Conventional wastewater treatment system mainly focuses on the eradication of solid suspension and reduction of BOD by activated sludge (Bolognesi et al., 2019). Therefore, the capability of conventional water treatment methods in the elimination of micropollutants, inorganic nutrients are still undesirable. With the implementation of the Environmental Quality Act's (EQA) effluent standards, the removal of nutrients specifically dissolved nitrogen and phosphorus, are among the essential requirements. The absolute value of total nitrogen and phosphorus discharged to rivers or stream is 20 mg/L for the approval of the wastewater treatment system. The biodegradation process with a limited capacity of conventional wastewater treatment technologies involving disintegration of organic and inorganic constituents will likely be ineffectual when the significant amount of other components like heavy metals, xenobiotics and nutrient loads are present in water (Rambabu et al., 2020a; Wollmann et al., 2019). This phenomenon will lead to lethal environmental issue that affects the ecosystem, which is oxygen depletion and a greater degree of effluents toxicity to aquatic life (Umamaheswari and Shanthakumar, 2016). In addition, the untreated nutrients in wastewater effluent will also abate the functionality of the disinfection stage, causing increment in chlorine demand which is deleterious to the aquatic ecosystem and human health (Falakh and Setiani, 2018). Consequently, there is a huge demand for the treatment process that can remove these nutrients before the effluents are discharged (Mohamad et al., 2017). Recent wastewater treatment techniques include capacitive deionization (Rambabu et al., 2020b) and membrane separation (Velu et al., 2018).

Algae-based wastewater treatment technologies offer a compelling solution due to their effective fixation of inorganic compounds, including carbon dioxide and heavy metals (Chen et al., 2018; Koppel et al., 2018, 2019; Li et al., 2020; Suganya et al., 2016). Microalgae show a great capacity for the uptake of inorganic nutrient as they require nitrogen and phosphorus for proteins synthesis and heavy metals as micronutrients for growth (Chen et al., 2018 ; B.-L. Liu et al., 2020). In this regard, usage of algae as bioremediation agents for wastewater can effectively draw nitrogen and phosphorus out of wastewater, maintain the dissolved oxygen content and assist in reducing pathogens and faecal bacteria present in wastewater (Das et al., 2019). Observations obtained from the research have derived that wastewater that has been in contact with microalgae resulted in an immense decrease in the level of heavy metals, nitrates and phosphate (Rathod, 2015). Microalgae treatment is also a more efficient approach to wastewater treatment as it is capable of treating wastewater in a single step in contrast to conventional wastewater treatment that require multiple processes to fix the carbon, nitrogen and phosphorus ratios (C:N:P). It is also a sustainable option from an environmental point of view as it has the capacity of converting carbon dioxide into chemical substance and fuel products without causing pollution, aiding the reduction of greenhouse gas emissions. To compensate its production cost, the microalgal biomass harvested from wastewater treatment can also be converted into valuable bio-based products, such as health supplements, biohydrogen, bioalcohols and biohydrocarbons (Chandini, 2016; Klinthong et al., 2015; Koyande et al., 2019; Perez-Garcia and Bashan, 2015; Show et al., 2017).

Microalgae have been widely applied in wastewater treatment, the microalgae species that are commonly employed in experiments of sewage treatment are eukaryotic and prokaryotic blue-green algae (Chalivendra, 2014). The attraction in microalgal cultures stems from the fact that conventional treatment poses drawbacks like high operational costs, inevitable secondary pollution from chemical processes, considerable space demand for operation, unfulfilled utilization of natural resources and great potential for carbon dioxide leakage over time for the storage method in practice. In addition, concentrations of a non-renewable resource like phosphorus and another essential nutrient like nitrogen present in wastewater are sufficient to facilitate the generation of growth substrates of microalgae cells, biomass yields and carbon neutrality (Delrue et al., 2016). Therefore, the demand for freshwater and industrial nutrients that are usually added to achieve conventional biological purification can be materially diminished, thus reducing the operational cost and environmental impact of the whole treatment process.

This review aims to exemplify the potential of microalgae in wastewater treatment and provide available results from previous studies and research conducted to evaluate the overall performance of microalgae in treating wastewater. In addition, Section 2 discussed the mechanism of wastewater treatment by microalgae with representative illustrations. The sustainable value of microalgae in the prospective of environment, economic and society is also deduced in Section 3 by discussing the biomass production of microalgae and its impact of environmental remediation with its carbon dioxide sequestration feature. Lastly, the future outlook and challenges in the application of microalgae for wastewater treatment is discussed in Section 4.

2. Uptake mechanism of microalgae

Wastewater treatment with the microalgae-based system is effective in removing inorganic compounds such as nitrate, phosphate, heavy metals, inorganic carbon, toxic substances (organic and inorganic), BOD, COD and other impurities dissolved in wastewater through their uptake mechanism. The brief idea of the microalgae uptake mechanism involving bacterial oxidation in wastewater is illustrated in Fig. 1.

Microalgae are microscopic organisms made up of eukaryotic cells that are impelled by the same photosynthetic process as higher plants. Microalgae cells comprise cell wall, plasma membrane, cytoplasm, nucleus and organelles. Microalgae also have plastids that contain chlorophyll which is responsible for manufacturing food by carrying out photosynthesis. Dissimilar to higher plants, the absence of a vascular system for nutrient transport in microalgae, cut down the requirement of a vascular system for nutrient transport, as every cell is photoautotrophic with direct absorption of nutrients.

Microalgae assimilate photons in the form of energy in its chloroplast cell and extract CO_2 from exhaust gases generated by combustion process or bacterial respiration along with nutrients from wastewater to synthesize their biomass and concurrently producing oxygen. The whole process of microalgae in uptaking and utilizing nutrients is illustrated in Fig. 2. Thereby, the microalgae biomass is obtained for further processes, and O_2 is released into the atmosphere. The conversion of CO_2 and water into organic compounds does not require extra energy addition which also prevents secondary pollution. The released oxygen from microalgae is enough to attain the desired aerobic requirement of bacteria to metabolize the residual organic substances in treated wastewater. Additionally, microalgae also require a light and dark regime for productive photosynthesis, where the former is utilized for a photochemical phase in the production of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), while the latter condition is for the biochemical phase to synthesize essential molecules for growth.

2.1. Nutrients removal

The uptake and consumption of nitrates and phosphates by microalgae cells for growth can significantly reduce the nitrogen and phosphorus content in wastewater and enhance the wastewater discharge quality (Emparan et al., 2019). An assessment of nutrients uptake by four microalgae species, namely *S. dimorphus, S. quadricauda, C. sorokiniana, and C. vulgaris ESP-6* was conducted. Each microalgae was mixed with diluted anaerobically digested wastewater in membrane photobioreactor (MPBR) and normal photobioreactor (NPBR). The concentrations of ammonia nitrogen and phosphate remained in MPBR and NPBR with different microalgal strains observed until day 9, which confirmed that microalgae cultivated with MPBR are able to remove more nutrients than microalgae with NPBR.

Furthermore, the ability of different microalgae strains (*Chlamydomonas sp., Chlorella sp.,* and *Oocystis sp.)* on the removal of nitrogen and phosphate was studied by assessing NO_3^--N and PO_4^{3-}-P loss (Rasoul-Amini et al., 2014). Three types of which the latter is of one strain, *Chlamydomonas sp.* (YG04 & YG05), *Chlorella sp.* (YG01 & YG02) and *Oocystis sp.* (YG03), were isolated from a paddy-field soil sample and were selected for the experiment. Figure S2 shows the removal percentages of phosphate and nitrate recorded every 4 days throughout the experiment, respectively. The results indicated that all microalgal strains attained their highest removal efficacies for PO_4^{3}—P on the 14th day, which is the last day of assessment. This proves that longer cultivation period will increase the amount of nutrients uptake by microalgae.

A series of experiments was conducted using *Chlorella vulgaris* to indicate the effect of temperature on the microalgal removal efficacies of nutrients, BOD and COD (Azeez, 2010). Figure S3 demonstrates the removal percentage of total N and P by microalgae and the growth rate of microalgae for 2 days. Results have shown that both algal growth and nutrients uptake started decreasing rapidly after the temperature reaches 30 °C. This indicates that 30 °C is the critical temperature in the experiment.

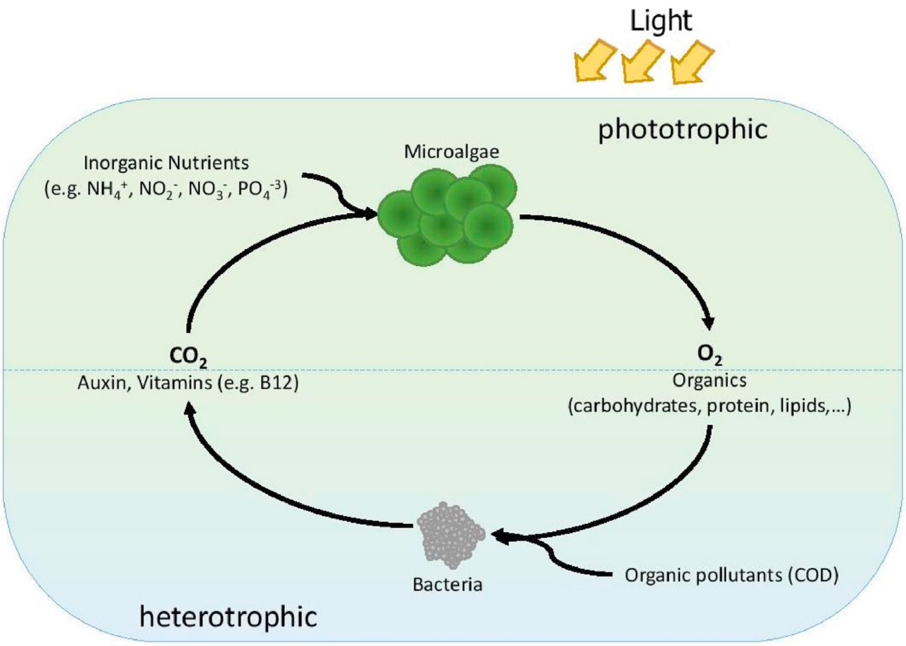

Fig. 1. Wastewater treatment involving algal-bacterial activity (Emparan et al., 2019).

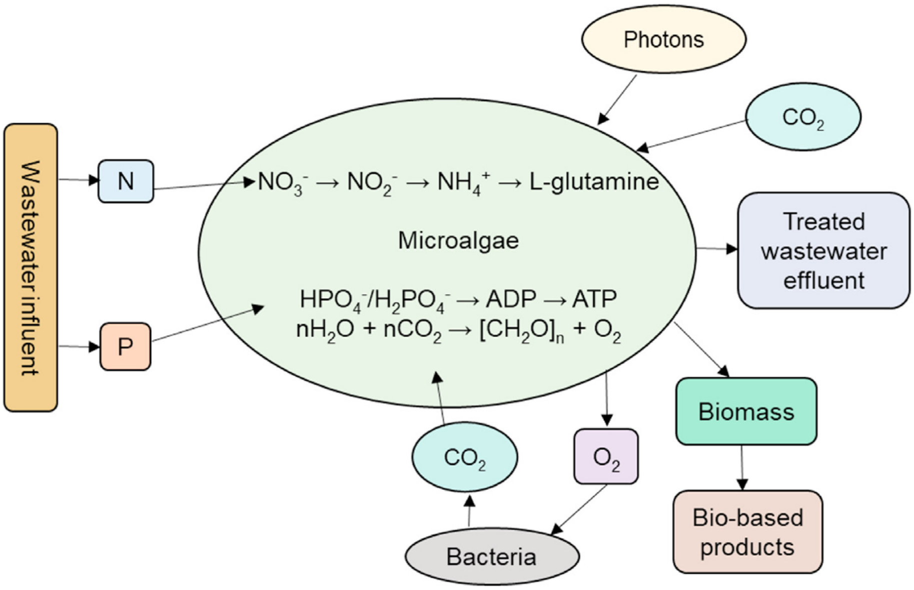

Fig. 2. Uptake mechanism of nutrients and interactions with bacteria in intracellular of microalgae.

2.1.1. *Phosphorus*

Inorganic phosphorus that can naturally be found in lipids, nucleic acids, and proteins present in wastewater plays an important role in microalgae energy metabolism and growth. Translocation of inorganic phosphates takes place across the plasma membrane of microalgae cells. In the course of algae metabolism, inorganic phosphorus in the forms of monohydrogen and dihydrogen phosphate (HPO_{42-} & $H_2PO_4^-$) are integrated into organic compounds which is adenosine diphosphate (ADP) in this case through phosphorylation. The process of phosphorylation requires energy to produce its final product, ATP. Energy can be sourced from oxidation of respiratory substrates, electron transport system of mitochondria found in eukaryotic microalgae, and light utilized in the process of photosynthesis (Emparan et al., 2019).

For photosynthesis, the light-dependent reaction involves both photochemical and redox reaction steps. Overall equation (Eq. (1)) for light dependent phosphorylation which involves ADP, phosphate (P), and NADP is as follows (Razzak et al., 2013):

$$2H_2O + 2NADP^+ + 3ADP + 3P + Photons(light) \rightarrow 2NADPH + 2H^+ + 3ATP + O_2 \qquad \text{Eq. (1)}$$

From the equation, light energy is used to synthesize energy storage molecules (ATP and NADPH). The chemical equation of the photosynthesis process delineates the generation of ATP that is derived from ADP takes place in the presence of energy input.

2.1.2. *Nitrogen*

Organic nitrogen can access wastewater through sewage effluent from land where animal manure is stored or applied. Organic nitrogen is the key element in biological substances like enzymes, peptides, proteins, chlorophylls and energy transfer molecules such as ADP and ATP. Organic nitrogen is derived from inorganic sources encompassing nitrite (NO_2^-), nitrate (NO_3^-), nitric acid (HNO_3), ammonia (NH_3), ammonium (NH_4^+), and nitrogen gas (N_2). The presence of nitrogen in wastewater is usually in the form of NH_4^+, NO_2^- and NO_3^-.

The conversion of inorganic nitrogen into organic forms can be carried out by eukaryotic microalgae via assimilation. Briefly, the transformation mechanism that take place across the microalgae plasma membrane is the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and to ammonium (NH_4^+) subsequently, which is then integrated into amino acids (the organic form of nitrogen). The reduction process of nitrogen is illustrated in Fig. 3. The primary step of the assimilation of nitrate involves nitrate reductase (NR) which is the reduced form of nicotinamide adenine dinucleotide (NADH), $C_{21}H_{27}N_7O_{14}P_2$, present within the microalgae to transfer two electrons in the reaction of converting nitrate to nitrite. Subsequently, ferrodoxin (Fd) from microalgae along with nitrite reductase which is NADPH, $C_{21}H_{29}N_7O_{17}P_3$ produced from the photosynthesis reaction that involves ADP, phosphate and NADP (Equation (1)) transfer six electrons in the reaction of reducing NO_2^- to NH_4^+. From this action, all the inorganic forms of nitrogen will be reduced to NH_4^+ within the intracellular fluid of microalgae. Finally, glutamic acids (Glu), $C_5H_9NO_4$ which are neuroactive amino acids found in microalgae and adenosine triphosphate (ATP) released from phosphorylation (process of assimilation of phosphates into organic compounds) incorporate ammonium into amino acids (glutamine) within the intracellular fluid of microalgae (Emparan et al., 2019).

2.2. *BOD and COD reduction*

Microbial in wastewater consumed dissolved oxygen released by microalgae to degrade organic material to carbon dioxide and water during algae-bacterial interaction. This process is a demonstration that microalgae provides substantial amount of molecular oxygen as oxidizing agent for bacterial oxidation hence reduces BOD and COD in wastewater. The extent of microalgae in treating BOD and COD level in wastewater by implementing algal inoculation was reported in Table S2. Results proposed that the microalgal inoculation with textile wastewater had significantly brought down the values of BOD and COD in comparison to the control treatment. Most microalgae displayed relatively high removal efficiency of BOD and COD (>80%), with the exception of *Anabaena flos aquae* (Elsadany, 2018).

Another study was conducted on batch reactor experiments of COD removal by *Chlorella vulgaris*, *Selenastrum gracile*, and *Scenedesmus quadricauda* (Lee et al., 2016). The COD concentrations of

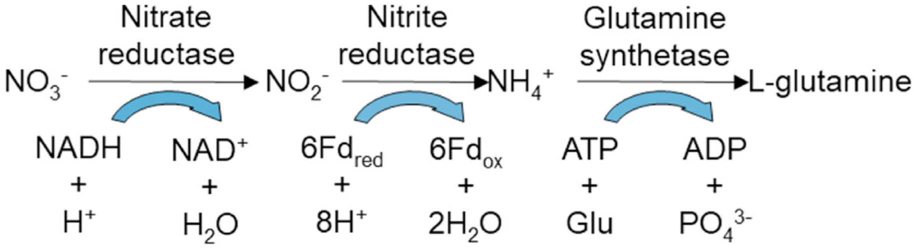

Fig. 3. Schematic of the conversion of inorganic nitrogen to its organic form via assimilation (Chalivendra, 2014).

wastewater remained after treated with *C. vulgaris* AG10032 and *S. gracile* UTEX 325, which have been decreasing until day 2 and 4, respectively, where they started to increase gradually and rebound to reach beyond the initial concentration after day 8. Using *Galdieria sulphuraria*, a 700-L pilot scale wastewater treatment system has shown consistent removal rates of BOD in 5 days retention time (Henkanatte-Gedera et al., 2017) and another test system with enclosed bioreactor reported BOD removal rate of 16.4 ± 3.3 mg/L-d in primary effluent after 2 days of retention time (Tchinda et al., 2019).

The effect of temperature was also studied on the microalgal removal (*C. vulgaris*) efficacies of nutrients, BOD and COD (Azeez, 2010). The COD and BOD removal percentages are shown in Table S3. Results have shown that COD and BOD removal percentages were increasing continuously and peaks at 30 °C. Therefore, the critical temperature is approximately 30 °C where any further increment in temperature will lead to reduction of algal growth rate and decreased oxygen demand removal efficiencies. Hence, less molecular oxygen will be released resulting in the increase of BOD and COD levels.

2.3. *Heavy metals removal*

Microalgae strains have demonstrated high efficiency in the elimination of heavy metals as well. Heavy metal uptake by microalgae encompasses passive biosorption by dead biomass and active biosorption by living microalgae cells. In the process of passive biosorption, metal ions in the cationic form are physically adsorbed over the microalgal cell surface that contains functional groups like hydroxyl (-OH), carboxyl (-COOH), amino (-NH_2), and sulfhydryl (-SH). During active biosorption, the metal ions are translocated across the cell membrane into the cytoplasm (Chalivendra, 2014). Intracellular polyphosphate bodies of microalgae can also supply a storage influx and seclude metals such as Cd, Co, Hg, Ni, Cu, Ti, Pb, Mg, Zn. Once metal amasses and accumulates inside the cell, the metal ions are antecedently situated within certain organelles and astricted to metal binding ligands such as phytochelatins and metallothioneins which is depicted in Fig. 4. Extracellular metal binding can be performed by physical adsorption, chemisorption, complexation, chelation, and reduction (Salam, 2019). It was reported that cyanobacterial microalgae produce extracellular polymeric substances (EPS) which form as a cover on the algal cell surface and able to turn into released polysaccharides (RPS) to circulate in the surroundings. EPS and RPS contain ionizable functional groups such as carboxyl, phosphoric, amine, and hydroxyl groups which aid in the extracellular sorption of metal ions (Pereira et al., 2011).

Results in Table S4 indicate the reduction percentages and the unconsumed concentration of different types of heavy metals remained in industrial wastewater effluent after being incubated for four weeks with various microalgae strains under constant illumination utilizing white cool light bulb exposure (3000 Lux) at 25 °C (Elsadany, 2018). The outcome of this incubation has certified that majority of tested microalgae strains have very high capacity in removing heavy metals suspended in the wastewater. For copper, each of the strain tested showed good removal effectiveness, recorded relative high reduction percentages which surpass 80% as a whole. *Nostoc ellipsosporum* particularly excels in removing all of the heavy metals utilized, attaining reduction percentages of >95% for all metal ions. In general, the removal efficiency of Arsenic was generally low by all of the algal strains in exception of *N. ellipsosporum* and *Chlorella vulgaris*. For instance, *Anabaena variabilis* managed to deliver reduction percentages of 97.57 (Cr), 94 (Pb), 97.94 (Fe), 91.77 (Cu), and 93.46 (Mo) but obtained a 66% reduction of As. Besides, *Chlorella vulgaris* also showed great removal property of heavy metals as it recorded the second highest removal rates after *N. ellipsosporum*.

A series of batch equilibrium experiment to indicate the biosorption capacity of seven microalgal strains towards cadmium (Cd) (Hashim and Chu, 2004). Figure S4 shows that the brown algal species possess the greatest adsorption capacity for Cd, followed by green algae and red algae. In another study, six algas species were studied towards chromium (Cr) binding capacities (Murphy et al., 2008). Results indicated that brown algae demonstrated great binding capacity towards Cr(III) and Cr(IV). Red and green algae performed worst towards Cr(III) and Cr(IV) binding capacity, respectively. On different oxidation state, the microalgae exhibited slightly different binding capacity.

2.4. *Pathogens removal*

The pathogen removal mechanism of microalgae in wastewater include competition of nutrients, the elevation of pH and dissolved oxygen level, adhesion and sedimentation of pathogens, and algal toxins which is illustrated in Fig. 5 (Dar et al., 2019). In microalgae cultivation, microalgae assimilate nutrients and carbon sources which are the main energy sources of bacterial cells. The competition of nutrients between microalgae and bacteria will result in starvation of bacterial cells and ultimately lead to bacterial cells die off.

pH value usually increases during cultivation of microalgae because of the CO_2 assimilation in photosynthesis. Absorption of nitrogen by microalgae also increases the pH value of the medium, as every nitrate ion reduces to ammonia produces one OH^- ion. This phenomenon will lead to pathogen elimination. Attribution of the limited transfer of carbon dioxide from the atmosphere and the process of microbial oxygenation, microalgae will also further increase pH levels that could result in pathogens die off. Fluctuations in pH are also recognized to adversely affect the survival of *E. coli* and will therefore give rise to a remarkable elimination of faecal coliforms such as *Escherichia coli, Enterococci, and Clostridium perfringens* in waterbodies (Ansa et al., 2011). Oxygenation carried out by bacterial respiration in treatment ponds that attributes to algal growth has been identified to give rise to faecal bacteria

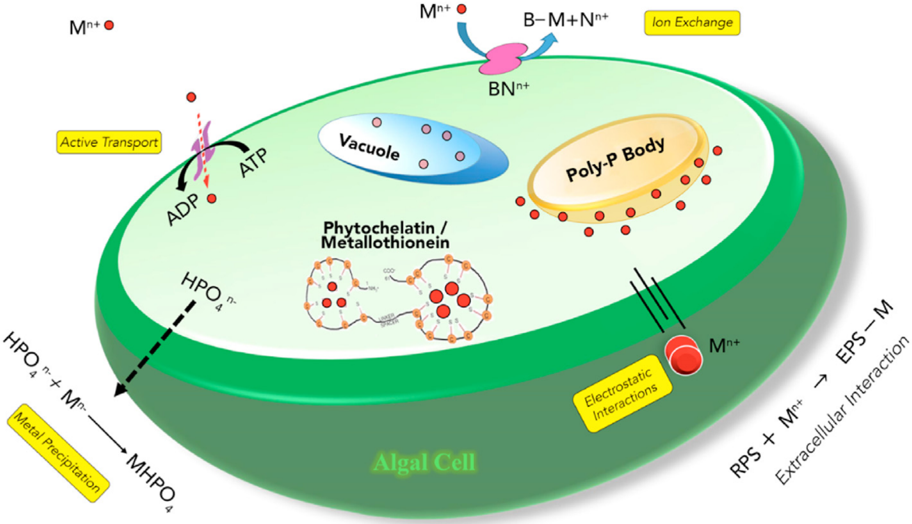

Fig. 4. Metal binding sites of a microalgal cell (Salam, 2019).

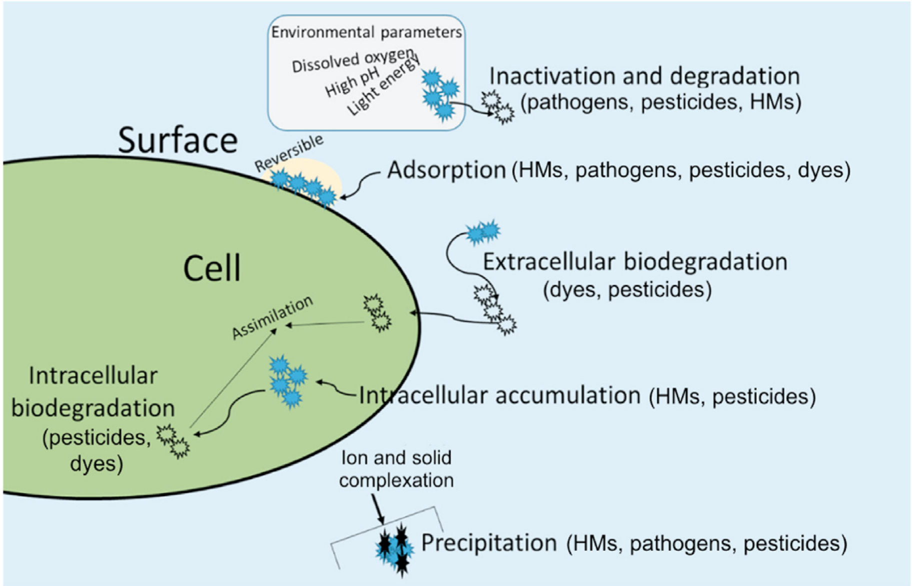

Fig. 5. Removal mechanism involving pathogens, pesticides, dyes and heavy metals by microalgae (Markou et al., 2018).

annihilation owing to the presence of toxic formations of oxygen. The photosynthesis activity by microalgae is also sufficient to elevate the oxygen concentrations of waterbodies to levels that is deleterious to faecal bacteria. Oxygen concentration at above 0.5 mg/L has been deduced to have faecal bacteria removal denotations (Ansa et al., 2011). Adhesion of faecal bacteria to microalgae in wastewater is imperative as it ensures bacterial cells are in close proximity when the elevation of pH and dissolved oxygen performed by microalgae occurs. For adhesion to happen, pathogens will first attach to the solid matter that will sink as sediment and deposit on the surface of microalgal cells. Subsequently, the available polysaccharides expressed by bacterial cells will form positively charged amino groups. The positively charged polymers will then neutralize the negatively charged microalgal surface, which will form a bridge between the particles, resulting in adhesion of bacterial cells to microalgae (Dar et al., 2019). Furthermore, a toxin named microcystin-LR generated by algal strain, *Synechocystis sp.* and toxins of long-chain fatty acids produced by *C. vulgaris* under high pH conditions have been discovered to be harmful to pathogen and faecal bacteria (Mohamed, 2008). Green algae can remove faecal coliforms by secreting and elevating levels of chlorophyll-a (Ansa et al., 2012).

Another pathogen, *Salmonella enterica* was found to be eliminated by a microalgae species, *Scenedesmus sp*. in experiment (Mezzari et al., 2017). *Scenedesmus sp.* (30% v/v, 70 mg/L dry weight) inoculated with *Salmonella enterica* (105 CFU/mL) were incubated under mixotrophic condition utilizing red light emission diode at 630 nm and at room temperature under continuous mixing. *Salmonella enterica* was eliminated in the existence of microalgae under 48 h of treatment. Algal species like *Rhizoclonium implexum* also has been observed to be competent in the elimination of coliform bacteria in conjunction with total suspended and dissolved solids, COD, BOD, nitrogen, and phosphorus (Dar et al., 2019). *Galdieria sulphuraria* is capable of reducing coliform bacteria *Enterococcus faecalis* and *Escherichia coli* to very low levels (<2 copies/mL) resulting in 3.8 and 5.4 log reductions in primary effluent within 3 days of retention time (Tchinda et al., 2019).

The effect of pH and dissolved oxygen (DO) levels elevated by microalgae on the removal and inactivation of *E. coli, total coliforms, Enterococci* and *C. perfringens* were assessed (L. Liu et al., 2020). Figure S5 shows that increment in pH, DO levels in *Mougeotia sp.* and *Hydrodicty sp.* jars are 3.9, 3 and 3, 1.3, respectively. The residual concentrations of four indicator organisms were tested under five pH conditions over 9 days period and different DO levels over 13 days period. The initial concentrations of *E. coli, total coliforms, Enterococci* and *C. perfringens* in secondary effluent were 3.16, 4.75, 3.44 and 3.17 log CFU/100 mL, respectively. Experiment with pH as a controlling factor demonstrated that all pathogens attained their maximum removal percentages under the highest pH value, which is pH 10.5 except for *Enterococci*. The optimum pH value for *Enterococci* is pH 4 where it reached its fastest removal rate. *Enterococci* was seen to be eradicated completely on day 3 of the experiment under pH 4. Moreover, experiment with DO levels as a controlling factor indicated that high DO levels caused a rapid reduction in *E. coli* and *Enterococci* and a gradual decrease in *C. perfringens*. Whereas in the case for total coliforms, despite both high and low DO levels achieved the same removal percentage at the end of the experiment, low DO levels were more ideal for its removal. Low DO level has shown a consistent decrease of total coliforms throughout the experiment while high DO levels caused its concentration to rebound beyond its initial concentration on day 9 and then decreased rapidly on day 11 until it reached the maximum removal rate which was rather inconsistent.

2.5. Pesticides removal

Microalgae are capable of assimilating a wide range of organic pollutants, including pesticides, as an energy source for their growth in wastewater through biosorption and biodegradation. Biosorption comprises mechanisms of absorption, adsorption, surface complexation, ion exchange, and precipitation., which takes place in the cell wall of both living and dead cells. Biodegradation occurs when microalgae generate enzymes that disintegrate the bonds in the pesticide molecules. In a study conducted by, *Chlorella vulgaris* was exposed to four common fungicides; propamocarb, mandipropamid, cyprodinil and metalaxyl in two experiments: short-term involving biosorption (60 min) and long-term involving biodegradation (4 days). The pesticide solution was added to sterile distilled water to obtain an initial concentration of 2.0 μg/L. Table S5 shows the remaining concentrations of pesticides after being in contact with *Chlorella vulgaris* in the experiment. Cyprodinil pesticide was removed effectively, as shown by lowest remaining pesticide for both short and long term, followed by mandipropamid.

Another short term and long term experiments analysing the removal percentage of pesticides by microalgae was conducted (Hussein et al., 2016). The microalgae utilized in the experiment is *Chlorella vulgaris* and the pesticides are molinate, simazine, isoproturon, atrazine, propanil, carbofuran, dimethoate, pendimethalin, metoalcholar, and pyproxin. In short term experiment, the samples containing respective pesticide mixed with sterile Milli Q water (initial concentration of 2 μg/L) and *Chlorella vulgaris* (0.6 g dry weight per litre) were stirred at a speed of 380 rpm for 1 h at room temperature. In long term experiment, the samples of pesticide mixed with sterile BG11 (initial concentration of 2 μg/L) and growing *Chlorella vulgaris* (inoculum of 10% (v/v)) were kept for 5 days. Table S6 displays the removal percentages of respective pesticide after being in contact with microalgae. Results have demonstrated that microalgae achieved better removal of pesticides in long term experiment in comparison to short term experiment.

2.6. Dyes removal

Microalgae have been utilized in colour and vinyl sulfone dye removal from textile wastewater due to their high surface area and binding affinity (Andrade and Andrade, 2018; Chu and Phang, 2019). In the removal mechanism of dyes, cell wall of microalgae involves biosorption, electrostatic attraction, complexation and bioconversion. Dye ions adhere and accumulate to the algal biopolymers surface and subsequently diffuse onto the solid phase of biopolymer. The extracellular polymers which comprise functional groups will assist the biosorption of dye molecules onto the polymer surface (Kumar et al., 2014). Biomass of a microalgae species, *Spirogyra* has been proven to be an efficient biosorbent for reactive dye removal. Biomass of *Caulerpa lentillifera* and *Caulerpa scalpelliformis* are capable in removing basic dyes through biosorption. Furthermore, *C. vulgaris* has also been prevalently utilized as a biosorbent for the removal of reactive dyes such as Remazol Black B (Aksu and Tezer, 2005).

For bioconversion, microalgae disintegrate the dyes to simpler compounds. *Chlorella vulgaris* can reduce 63–69% of the colour from mono-azo dye by converting it to aniline. demonstrated the colour removal efficacy of *Chlorella vulgaris* by adding them to different concentrations of textile wastewater (Supranol Red 3BW) for a culture period of 10 days. Table S7 displays the colour removal percentage of dyes by *C. vulgaris* when added in medium containing different concentrations of textile wastewater. A low reduction in colour (1.40%) of the Supranol Red 3BW textile wastewater in the medium without *C. vulgaris* was recorded. This indicates that the colour removal is resulted from the active growth of microalgae. Moreover, five microalgal strains namely *A. flos aquae, N. elepsosporum, N. linkia, A. variabilis*, and *C. vulgaris* were assessed for their performance to abolish the red colouring originated from textile industrial effluent (Elsadany, 2018). The experiment disclosed that all tested microalgae strains were capable of abolishing the red dye of the treated textile wastewater effluent with assorted reduction percentages. N. elepsosporum recorded the complete dye removal, followed by for *C. vulgaris* (96.16%), *A. variabilis* (88.71%), *N. linkia* (79.03%) and *A. flos aquae* (50.81%). The complexity of the textile wastewater with entangled compositions comprising other chemicals like heavy metals does not seem to affect the efficacy of colour removal by microalgae.

2.7. Carbon dioxide fixation

Carbon dioxide that can be found from microbial respiration in wastewater can cause pH imbalance if presented in significant amounts. Certain microalgae strains are effective in pH neutralisation of wastewater since they have great tolerance to the loaded amounts of carbon dioxide. Microalgae are capable to uptake carbon in the form of CO_2 biologically from the atmosphere as well as from the bacterial oxygenation in wastewater. In general, carbon fixation produces final carbohydrate products, $[CH_2O]_n$. The overall reaction can be divided into two channels. They are light-dependent reaction and dark or light-independent reaction.

In light dependent reaction like oxygenic photosynthesis, water is the electron donor, and oxygen is released after hydrolysis. Equation (2) for photosynthesis involving water and carbon dioxide can be simplified as follows:

$$H_2O + CO_2 + Photons\ (light) \rightarrow [CH_2O]_n + O_2 + H_2O \qquad \text{Eq. (2)}$$

In the light-independent reaction or dark reaction, ATP and

NADPH formed from photosynthesis process (Eq. (1)) are required to act as electron donors. The overall equation (Eq. (3)) for the light-independent reaction is as follows:

$$CO_2 + 4H^+ + 4e^- \xrightarrow{2NADPH, 3ATP,\ Enzymes} [CH_2O]_n + H_2O \qquad \text{Eq. (3)}$$

The carbon skeletons that are produced by light dependent and light independent reactions are then used in a variety of ensuing processes, forming other organic compounds. For example, a form of carbohydrate which is cellulose can be used as the precursor for lipids and amino acids biosynthesis, which explains the designation of CO_2 fixation process. This reaction also releases oxygen which is then consumed by the bacteria to reduce the high organic loads in the wastewater system by microbial metabolism, aiding the bioremediation of wastewater (Satpati and Pal, 2020).

2.8. Biomass production

The biomass produced by microalgae in wastewater treatment can be extracted and converted into a variety of fuel bioproducts and value-added chemicals via biorefinery processes (Chew et al., 2017). For instance, derivation of biodiesel and biochar through pyrolysis, biodiesel through transesterification, bio-oil through thermochemical conversion, biomethane through anaerobic digestion and so on (Klinthong et al., 2015; Yu et al., 2018). Biomass is also imperative for heavy metals removal in wastewater as it performs passive biosorption process in heavy metals uptake mechanism. Besides, biochar is a great adsorbent of dyes and toxic chemicals such as antimicrobials and surfactants present in wastewater treatment. Biochar generated from sludge also reduces carbon footprint of wastewater treatment plant and improve soil properties. Slow pyrolysis is an anaerobic process that converts biomass into charcoal residue also known as biochar at elevated temperatures (360–800 °C) and under atmospheric pressure. Through slow pyrolysis, the product distribution of biochar converted from biomass is 35 wt% solid, 30 wt% liquid, and 35 wt% gas (Mohan et al., 2014).

The uptake mechanism and biosynthesis process to form biomass by photosynthetic microalgae with uptake of ammonium, phosphate, nitrate found in wastewater is depicted as Eq. (4) (Anbalagan and Nehrenheimph, 2016):

$$\begin{aligned} &CO_2 + H_2O + NH_4 + NO_3 + PO_4 + Photons(light) \\ &\quad + Algae \rightarrow C_{106}H_{263}O_{110}N_{16}P + C_6H_{12}O_6 + O_2 \end{aligned} \qquad \text{Eq. (4)}$$

Heterotrophic microalgae consume organic carbon, soluble carbonates dissociated from carbon acids either by direct intake or by converting CO_2 to carbonates through carbonic anhydrase activity. The carbonic anhydrase process can be depicted as Eq. (5):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3{}^- + H^+ \leftrightarrow CO_3{}^{2-} + 2H^+ \qquad \text{Eq. (5)}$$

Therefore, heterotrophic microalgae that use organic carbon substrates ($[CH_2O]_n$) provided as sole carbon source can omit light energy and replace the synthetic process to form organic carbon by assimilating CO_2 in the photosynthetic metabolism. The depiction of biomass productivity by heterotrophic microalgae that utilizes carbonic acids as its substrates and energy needs can be delineated by the following chemical equation:

$$\begin{aligned} &16NH_4{}^+ + 92CO_2 + 92H_2O + 14HCO_3{}^- \\ &\quad + HPO_4{}^{2-} \rightarrow C_{106}H_{263}O_{110}N_{16}P + 106O_2 \end{aligned} \qquad \text{Eq. (6)}$$

In the equation above, algal biomass is represented by the chemical formula $C_{106}H_{263}O_{110}N_{16}P$. In addition to nutrient availability, algal biomass production also requires light energy. A stoichiometric formula for the most common constituents in a microalgal cell is $C_{106}H_{181}O_{45}N_{16}P$, and these elements should appear in the medium for optimal growth of microalgae and successful elimination of the inorganic compounds. Therefore, the equation above deduces the role of algae in wastewater treatment with the simultaneous production of biomass (Randrianarison and Ashraf, 2017).

The removal rates of pollutants in different origins of wastewater by microalgae are displayed in Table 1.

3. Limitations and future perspectives

3.1. Limitations associated with microalgae-based wastewater treatment

Although microalgae-based wastewater treatment is oriented towards efficient removal of nitrogen and phosphorus, not all emerging pollutants and heavy metals can be eradicated effectively. Selection of different algal strains and the inhibition factors from the environment and wastewater itself need to be considered before integrating microalgae with wastewater treatment since they have a huge impact on the growth and treatment efficiency of microalgae.

Significant amount of solids suspensions and high turbidity of industrial wastewater may affect the radiation of light through the wastewater, hence affecting the photosynthesis process of microalgae and interfering its growth. Therefore, an additional wastewater method that possess high solids removal efficiency such as sedimentation, adsorption, coagulation and so on can be employed in order to ensure great photosynthesis efficiency (Amenorfenyo et al., 2019).

Despite the cultivation technique of microalgae in wastewater is simple and effective, in terms of economic aspect, it is still not a convincing alternative wastewater treatment technique. The contributing factors to the less profitable property of this treatment method are high downstream processing cost, small scale of production, and only selected microalgae species and cultivation modes can produce high quality biomass that can be converted into useful bioproducts (Chia et al., 2018). Moreover, enclosed photobioreactors that require artificial light source and chemical agents for sterilization increase overall production cost (Umamaheswari and Shanthakumar, 2016). The top three total equipment cost are as follows: photobioreactors, freeze-dryer and storage and decanter (Acién et al., 2012).

Selection of microalgae species for wastewater treatment is critical. As a result of the different physical and chemical composition of wastewater from different sources, the selected microalgae species should be able to cope with the variations in environmental factors (Alkhamis and Qin, 2013; Azov, 1982; Cheirsilp and Torpee, 2012; Chew et al., 2018; Gour et al., 2020; Takabe et al., 2016; van Elsas et al., 2011). On top of that, the species should have the ability to share metabolites to accommodate stress and sustain any attack of unwanted species and nutrient limitations (Amenorfenyo et al., 2019). For heterotrophic and mixotrophic microalgae, species of microalgae that is facultative in using organic carbons as sole substrates and cut off any light source for its cultivation is also limited.

In industrial wastewater, as a result of low concentrations of N and P containing compounds besides the high levels of toxins, lower algal growth rates are obtained. Hence, the potential for large scale treatment of industrial wastewater is much less comparatively, because of the high level of heavy metal ions which is

Table 1
Removal rates of pollutants in different origins of wastewater and biomass productivity by microalgae.

Microalgae species	Wastewater type	Treatment efficiency (%)	Biomass productivity (g/L-d)	Ref
Algal- bacterial symbiosis (*Chlorella* + *Nitzchia*)	Settled domestic sewage	N: 92 P: 74 COD: 87 BOD: 97	N/A	Wang et al. (2010)
Auxenochlorella protothecoides	Concentrated municipal wastewater	TN: 9.8 TP: 13.5 TOC: 16	0.193	Renuka et al. (2015)
Chlamydomonas mexicana	Piggery wastewater (filter sterilized)	TN: 3.12 TP: 1.4 TOC:1.45	0.028	Renuka et al. (2015)
Chlamydomonas polypyrenoideum	Dairy industry wastewater	TDS: 89.8 TSS: 91 Cl^-: 78 NO_3^-: 62 Fl^-: 66.6 PO_4^{3-}: 69 NH_4^+: 63 COD: 80	2.2 g/L	Umamaheswari and Shanthakumar (2016)
Chlamydomonas sp.	Industrial wastewater	NH_4^+:100 PO_4^{3-}: 33	1.34 g/L	Umamaheswari and Shanthakumar (2016)
	Industrial wastewater	NO_3–N: 10 NH_4–N: 10 PO_4–P: 3.3	0.134	Renuka et al. (2015)
Chlorella pyrenoidosa	Soybean processing wastewater	TN: 88.8 TP: 70.3 COD: 77.8 NH_4–N: 89.1	0.64	Renuka et al. (2015)
	Piggery wastewater	N: 75.7–82.5 P: 62.5–74.7 NH_4–N: >90	0.04	Umamaheswari and Shanthakumar (2016)
	Settled domestic sewage	N: 93.9 P: 80	N/A	Wang et al. (2010)
	Domestic sewage and industrial wastewaters	N: 60-70 P:50-60 BOD: 80-88 COD: 70-82	N/A	Wang et al. (2010)
Chlorella sorokiniana and aerobic bacteria	Industrial wastewater	N: > 95 P: 80.7 COD: 84.8	N/A	Bhatt et al. (2014)
Chlorella sp.	Municipal wastewater centrate	TN: 6.3 TP: 5.7	0.07	Wang et al. (2010)
	Digested dairy manure wastewater	NH_4–N: 100 TN: 75.7–82.5 TP: 62.5–74.7	N/A	Li et al. (2019)
Chlorella vulgaris	Tertiary wastewater by Forward osmosis membrane photobioreactor (OMPBR)	TN: 86-99 TP:100	5 g/L	Yu et al. (2017)
	Textile wastewater	TN: 44.4–45.1 TP: 33.1–33.3 COD: 38.3–62.3	0.73 g/L	Bhatt et al. (2014)
	Brewery wastewater	TN:87.27 TP:79.75	2.28 g/L	Umamaheswari and Shanthakumar (2016)
	Citric acid effluent	TN: 94.4 TP:90.6 BOD: 95.7 COD: 94.9	0.765 g/L	Umamaheswari and Shanthakumar (2016)
	Diluted pig manure (suspended solids content to 0.2%)	TN: 54-98 TP: 42-89 BOD: 98	N/A	Satpal and Khambete (2016)
	Domestic wastewater	NO_3–N: 1.52 NO_2–N: 3.26 NH_4–N: 2.17 PO_4–P: 2.6	N/A	Renuka et al. (2015)
	Tertiary municipal wastewater	TN: 25 TP: 24	0.04	Renuka et al. (2015)
Cyanobacteria	Secondarily treated domestic effluent + settled swine wastewater	N: 95 P: 62	N/A	Satpal and Khambete (2016)
Galdieria sulphuraria	Primary effluent	NH_3–N: 6.26 P: 1.41 BOD: 16.4 (mg/L-d)	N/A	Tchinda et al. (2019)
Gloeocapsa gelatinosa	Chemical (based products) wastewater collected from the Periyor		N/A	Emparan et al. (2019)

(continued on next page)

Table 1 (*continued*)

Microalgae species	Wastewater type	Treatment efficiency (%)	Biomass productivity (g/L-d)	Ref
		NO3-:80.9 NO_2^-: 100 PO_4^{3-}: 75		
Nannochloris oculata	Aquaculture wastewater -recirculation aquaculture system (RAS)	TN: 78.40 NO_2^-:84.38 PO_4^{3-}: 14.7	N/A	Emparan et al. (2019)
Oedogonium sp.	Digested piggery wastewater	NH_4—N: 13.7 TP: 13.2	N/A	Renuka et al. (2015)
Pithopora sp.	Thermal wastewater collected from the power station	BOD:88.23 COD:87.75 NO_3^-:23.07 PO_4^{3-}:89.37	N/A	Emparan et al. (2019)
Scenedesmus acutus	Municipal effluent from activated sludge plant	COD: 77.3 NO_3^-: 71.1 NH_4^+: 93.6 PO_4^{3-}: 66.2	N/A	Emparan et al. (2019)
Scenedesmus obliquus	Piggery effluent	TN: 58 TP: 24 TN: 60 NO_3^-: 84 NH_4^+: 57 TP: 83	N/A	Emparan et al. (2019)
	Brewery effluent	TN: 1.48 TC: 4.37	0.1	Renuka et al. (2015)
	Aquaculture wastewater with Polyvinylidene fluoride (PVDF) hollow-fiber microfiltration MPBR	N: 86.1 P: 82.7	0.0426	Yu et al. (2017)
Scenedesmus quadricauda	Domestic wastewater from sewage wastewater treatment plant	BOD:89.21 COD:70.97 NO_3^-:70.32 PO_4^{3-}:81.34	N/A	Emparan et al. (2019)
Scenedesmus sp.	Modified effluent of a wastewater treatment plant by photo-membrane bioreactor	N: 46 P: 100	N/A	Bhatt et al. (2014)
	Effluent from pre- treated sewage by Outdoor flat-plate bioreactor	N: 5.84 P: 0.85 (mg/L-d)	0.0523	Yu et al. (2017)
Spirulina sp.	Dairy wastewater collected from the factory	COD: 77 NO3$^-$: 80 PO_4^{3-}: 72	N/A	Emparan et al. (2019)
Synechocystis salina	Chemical (based products) wastewater collected from the Periyor	NO3$^-$:82.5 NO_2^-:96.23 PO_4^{3-}:64.52	N/A	Emparan et al. (2019)
Synedra affinis	Sewage wastewater collected from the drain opens into river, Yamuna	NO3$^-$: 100 NO_2^-: 100 PO_4^{3-}:100	N/A	Emparan et al. (2019)

inefficient for algal culture concurrent with CO_2 fixation (Cheah et al., 2015; Molazadeh et al., 2019).

3.2. Future perspectives

Various studies have been conducted to pave for industrial scale application of microalgae by establishing the associated processes and technologies. However, the transformation from pilot to industrial scale operations often subject microalgae to unfavorable conditions, greatly reducing the bioproduct yields (Matamoros et al., 2015). Therefore, integration of robust microalgae cells and bioprocess engineering methods to ensure economic and environmental feasibility still requires more studies and research to be done. Novel biotechnological approaches regarding genome modification of microalgal cells to instil them with different properties are swiftly increasing. However, the full potential of genetic engineering of some microalgal species, particularly diploid diatoms, only can be thoroughly perceived if the conventional cultivation methods become firmly entrenched, thereby enabling practical mutations to be integrated to achieve greater performance (Zeng et al., 2011). Current biotechnological approaches to modify pollutants removal properties and cultivation of microalgae (illustrated in Fig. 6) and suggestions of synergistic treatment methods to be integrated with microalgae for elevated performance of microalgal wastewater treatment in future perspective are discussed in this section.

4. Concluding remarks

Current and available literature studies have exhibited and certified the bioremediation properties of microalgae in wastewater. Microalgae have been proven to be efficient in nutrient and heavy metals removal from different types of wastewaters and appear to be a promising candidate for carbon capture technology. The potential of microalgae to eliminate emerging contaminants is relatively high. Each microalgae species, however, has more specialized property and ability to eradicate various types of contaminants. More research has shown that study the novel species of microalgae that have yet to be discovered will attach great importance to searching for more efficient and stable strains to degrade contaminants. Studies on the emerging pollutants in our environment and various properties of microalgal strains in remediating the environment must be conducted more. More research is required to investigate the industrial scale of microalgae and the enhancement of bioproducts quality in terms to enhance the feasibility and compatibility of microalgae cultivated in full scale. Experiments to assess the removal efficacy of a wide variety of heavy metals ions by different microalgal strains in combination or

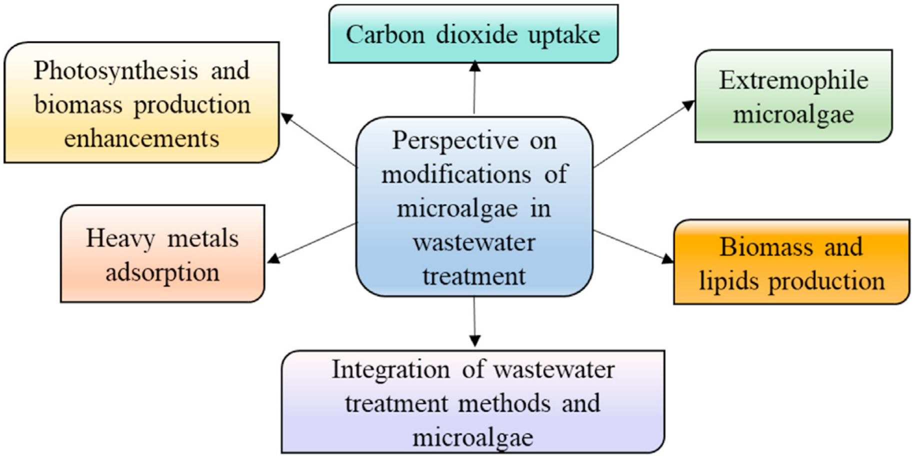

Fig. 6. Perspectives on microalgae modification in wastewater treatment.

1. **Enhancement of photosynthetic efficiency**: Two approaches can be implemented, which are light-harvesting complex refinement, and light utilization improvement, by amending the light constitution and diminishing non-photochemical satiate (Durnford, 2003). Increased light intensity resulted in growth enhancement for *Chlorella reinhardtii* (30%) and *Chlorella vulgaris* (44.5%) (Zeng et al., 2011). An intracellular spectral reconstitution of light in *P. tricornutum* demonstrated a 50% increment in the efficacy of photosynthesis and biomass productivity (Fu et al., 2019).
2. **Amelioration of carbon uptake**: Carbon dioxide is important for the reproduction, growth and production of secondary metabolites for microalgae cells. Carbon uptake has been ameliorated utilizing gene shuffling through polymerase chain reaction, which has simultaneously enhanced the carboxylation reaction. The Calvin cycle has been revised in another stage to increase the requirement of carbon, therefore approaching the equilibrium of Rubisco towards carbon fixation (Vazquez-Villegas et al., 2018). CO_2 can be sequestrated by Rubisco to generate two molecules of 3-phosphoglycerate inside the chloroplast. Nonetheless, oxygen can compete with CO_2 for Calvin Cycle by rubisco. Two new green microalgal species which are *Chlorella sp. UK001* and *Chlorococcum littorale* demonstrated high CO_2 uptake rates exceeding 1 g/L CO_2 per day. Moreover, *C. littorale* also exhibits a relative high tolerance to great CO_2 concentrations which scores up to 40% (v/v) (Zeng et al., 2011).
3. **Utilization of extremophilic microalgae**: Temperature effect possesses the major challenge to microalgae growth (Ras et al., 2013). The extremophilic microorganisms (Table S8) have been proven to possess better adaptability to unfavorable photochemical conditions and are highly adaptive in a wide range of environmental factors. For example, thermophilic *Chlorogleopsis sp.* unveiled great light adaptability, with successful growth under strong and weak light intensity (246.1 and 36.9 mmol m^2/s), as compared to the conventional microalgae strains, *Chlorella* and *Scenedesmus sp.* (200 mmol m^2/s) (Fu et al., 2019). *Galdieria sulphuraria* is able to grow in highly acidic environments, down to pH 1.8 (Gross and Schnarrenberger, 1995) and also demonstrated thermophilic growth nature by sustaining up to 56 °C. The metabolic versatility and production of value-enhancing phycocyanin of *G. sulphuraria* have made it a promising candidate for remediating high COD-loaded, acidic or high-temperature wastewater.
4. **Inducible expression of phytochelations**: Algae have shown great potential for heavy metal recovery, including inducible expression of heavy metal binding phytochelatins (PCs) as well as their linear copolymer, alginate which is formed by a-l-guluronate (G) and a-d-mannuronate (M), which can be found in 10–40% of the dry weight of brown algae. *Laminaria digitate* exhibited better biosorption performance of heavy metal compared to commercial alginate beads and other biosorbents (Papageorgiou et al., 2006). Adsorption capacity of metal ions by alginate-calcium bead decreased in the order of $Pb^{2+} > Cu^{2+} > Cd^{2+}$, in compliant with the hard-soft-acid-base (HSAB) theory (Cheng et al., 2019). The bonding strength of the metal affects the impacts of fluctuations in pH and also the way of bonding (Papageorgiou et al., 2006).
5. **Production of biomass**: Microalgal biomass contributes greatly to the adsorption of the heavy metals in wastewater as it involves in passive biosorption of heavy metals. Besides, increase in the scale of microalgae production for wastewater treatment plant required to be coherent with a simultaneous reduction in production costs. Therefore, biomass production is a crucial parameter for the derivation of useful bioproducts which can be recycled into raw materials utilized in water treatment and increasing its potential as feedstock for second-generation biofuels and biocha that can compete with fossil fuels. Hence, biomass is imperative to maintain the financial viability of microalgae-based wastewater treatment. According to a study, an approximate amount of 1.6–2 g of CO_2 is captured for each gram of microalgal biomass produced (Zeng et al., 2011). Lipid production was also widely studied in various wastewater sources, such as palm oil mill effluent and seafood wastewaters (Cheah et al., 2018a, 2018b, 2020; Lee et al., 2020; Nguyen et al., 2019). These studies can create a better alternative to clean the wastewater from these sources.
6. **Encapsulation of microalgae**: It can be encapsulated that microalgae as bioremediation agent is capable to eliminate pollutants in wastewaters up to certain removal degree. Nevertheless, the technology is limited in terms of solids, odour, colour removal and pH balances. Pollutants like dissolved solids, excess nitrogen, phosphorus, heavy metals, bacteria, and protozoa will inhibit the microalgae growth in wastewater. Therefore, the development of microalgae technology requires incorporation with methods for effective removal of contaminants in wastewater. Numerous methods have been reviewed for their removal efficacies of pollutants aforementioned. However, these methods have disadvantages such as high reagent requirement, uncertainty in heavy metals removal, possible production of toxic biomass and so on. Therefore, adsorption processes that encompass simple operation and effective removal rate will be reviewed (Emparan et al., 2019). The comparison of performance by each treatment is demonstrated in Table S9.

individually should be widely conducted for the future development of algal technology. Studies on the integration of existing treatment systems and microalgal treatment should be conducted and reported more to provide more opportunities for the implementation of algal treatment in wastewater plants.

Author statement

Wai Siong Chai: Conceptualization, Writing- Original draft preparation. **Wee Gee Tan**: Data curation, Writing- Original draft preparation. **Heli Siti Halimatul Munawaroh**: Visualization, Investigation. **Vijai Kumar Gupta**: Writing – Review & Editing, Validation. **Shih-Hsin Ho**: Project administration, Supervision. **Pau Loke Show**: Writing – Review & Editing, 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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Appendix A. Supplementary data

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