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THE ROLES OF MICROALGAE AND BACTERIA IN WASTEWATER TREATMENT

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SUMMARY

Nature-based wastewater treatment employing microalgae and bacteria has gained serious attention due to its combination with valuable biomass production. In wastewater, microalgae serves as the primary source of dissolved oxygen (DO) production for bacterial organic matter degradation. In addition, microalgae can effectively remove nutrients, pathogens as well as various heavy metals. Bacteria, on the other hand, has been widely applied in biological wastewater treatment for stabilization of organic matter, nitrification, denitrification, and under some specific conditions, enhanced biological phosphorus removal. When cultured together, microalgae and bacteria can cooperate effectively for wastewater treatment as well as form big flocs which can be harvested easily via sedimentation. However, some natural antagonistic interactions between them should be expected. Various environmental and operational factors showed significant influences on microalgae and bacteria in wastewater. They can impact system performance individually or in combination with others. Therefore, those factors should be carefully monitored for improving performance of the system.

Keywords: bacteria, microalgae, nature-based wastewater treatment, nutrient removal, organic matter degradation.

INTRODUCTION

During the last decades, nature-based wastewater treatment using microalgae and bacteria has been receiving serious attentions. The core of this technology lies at the symbiotic cooperation between microalgae and bacteria in wastewater. Thanks to this cooperation, organic matter as well as inorganic nitrogen and phosphorus can be removed effectively (Muñoz, Guieysse, 2006). As the technology bases on natural processes of microalgae and bacteria with requiring only sun light as the main energy source, it can save a lot of energy as well as simplify the process of operation and maintenance. In addition, algal bacterial biomass produced during the treatment process can also be used for several purposes, including biogas, biofuels, fertilizers or biopolymers production. Therefore, the algal bacterial wastewater treatment system has great potential for dual purposes system including wastewater treatment and biomass production (Park *et al.*, 2011).

Obviously, harmonious interaction between microalgae and bacteria in wastewater is the key for a successful application of the system in real case. It was suggested that co-culturing microalgae and bacteria could enhance their strength against environmental instability, permanence for partners, sharing of metabolites and nutrient restriction, and control against invasive species (Mehta *et al.,* 2015). However, to date, there have been very few studies evaluating the role of bacteria and microalgae in wastewater treatment, which plays an important role in enhancing the efficiency of the system as well as in up-scaling process. Therefore, assessing the role of microalgae and bacteria in wastewater needs to be further studied.

In this review, insight knowledge about the roles of microalgae and bacteria in wastewater treatment in separation as well as together were provided. Moreover, various factors influencing the wastewater treatment process employing microalgae and bacteria were also be discussed.

THE ROLES OF MICROALGAE IN WASTEWATER TREATMENT

Major microalgal processes in wastewater

In prokaryotic microalgae (cyanobacteria or blue-green algae), photosynthesis is performed by photosynthetic membranes parallel and close to the cell surface (Richmond, 2008). Eukaryotic microalgae, on the other hand, has special organelles, the chloroplasts, containing photosynthetic pigments for harvesting and conversion of light energy (Staehelin, 1986). Microalgae can be categorised according to their light harvesting photosynthetic pigments such as red, golden, brown or green microalgae.

In the absence of light, microalgae relies on endogenous respiration to provide energy for its living activities. During the process, part of the carbohydrates generated during photosynthesis is oxidized to carbon dioxide and chemical energy. Therefore, microalgae consumes oxygen in water at night causing fluctuation pattern in dissolved oxygen profile, especially in places experiencing eutrophication (Howarth *et al.,* 2011).

Moreover, it was found that various microalgae species can live in different irradiance-limited environments such as beneath polar ice (Palmisano *et al.,* 1985) or under the shade of a dense periphyton population (Johnson *et al.* 1997) where the light intensity was as low as 0.6 $\mu E.m^{-2}.s^{-1}$ (Palmisano *et al.,* 1985). Under such condition, these microalgae species could utilize organic materials for their growth (heterotrophic growth) via active transport of the molecules through their cytoplasmic membrane (Morales-Sánchez *et al.,* 2015). The active transport was found to be light-dependent which microalgae only consumes organic substrate at insufficient irradiance level for photosynthesis (Tuchman *et al.,* 2006).

The applications of microalgae in wastewater treatment

The role of microalgae in wastewater treatment was early recognized in conventional waste stabilization ponds as the main source of dissolved oxygen (DO) production (Oswald, Gotaas, 1957). The use of microalgae in wastewater treatment is promising due to its natural occurrence in water beside bacteria as well as fast growth and the ability of tolerance in various environmental conditions. Thanks to microalgal photosynthetic aeration, both chemical demand and biological demand of oxygen (COD and BOD, respectively) for organic matter removal is fulfilled (Travieso *et al.* 2008). High level of DO is frequently the case in wastewater treatment system employing microalgae, the DO value could be as high as 29 mg/L (or 350% saturation) during sufficient light condition (Zhai *et al.,* 2017). The high DO concentration in the system is much higher than normal DO level maintained in aeration tank from 0.5 to 2.5 mg/L (Aboobakar *et al.* 2013). It was also suggested that optimal DO concentration for effectively removal of COD by activated sludge ranged from 0.36 to 0.54 mg/L (Pittoors *et al.,* 2014).

Moreover, during its growth and reproduction, microalgae consumes large amount of inorganic nitrogen and phosphorus for proteins and other cellular materials, attributing from 40 to more than 60% of microalgae dry weight (Muñoz, Guieysse, 2006). Moreover, high pH level due to microalgal photosynthesis also moves the NH_3/NH_4^+ equilibrium in wastewater toward NH_3 formation which ammonia fraction could increase from 0.4% at pH 7 to 80% at pH 10 (water temperature of 20°C) (Li *et al.*, 2012) and thus increasing nitrogen removal via ammonia gas stripping (Abdel-Raouf *et al.*, 2012). It was earlier reported that NH_3 stripping could be one of the most important mechanisms for nitrogen removal in open treatment systems like high rate algal pond (HRAP) contributing up to 47% of total nitrogen removed (Garcia *et al.*, 2000).

High efficiency in removal of ammonium nitrogen and ortho-phosphate was usually obtained via microalgal uptake with nearly complete removal in some cases (Table 1).

Table 1. Wastewater nutrients removal efficiencies of some microalgae

Algal Species	Wastewater types	Nitrogen removal (%)	Phosphorus removal (%)	References
Spirulina platensis	Synthetic urban wastewater	81.5 – 92.6	80.5 – 94.1	(Zhai *et al.,* 2017)
Desmodesmus sp.CHX1	Livestock waste water	78.5 – 88.3	91.7 – 95.1	(Luo *et al.,* 2018)
Chlorella vulgaris	Factory wastewater	90.2	85.5	(Travieso *et al.,* 2008)
Chlorella vulgaris	Textile wastewater	44.4 – 45.1	33.1 – 33.3	(Lim *et al.,* 2010)
Chlorella vulgaris	Municipal wastewaters	35 – 93	65 – 100	(He *et al.,* 2013)
C. sorokiniana	Mixture of municipal and piggery wastewater	100	40 – 60	(Leite *et al.,* 2019)
Chlorella vulgaris, Scenedesmus obliquus and *Consortium C*	Urban wastewater	84 – 98	92 – 100	(Gouveia *et al.,* 2016)

Besides, microalgal consumption of inorganic carbon results in alteration of the carbonate equilibrium, frequently raising pH level to a value above 10 (Zhai *et al.,* 2017). This phenomena was found to have negative effect on pathogen in water. Moreover, it was indicated that intracellular substances excreted by microalgae inhibit pathogens in wastewater (Najdenski *et al.*, 2013). High pathogen removal efficiencies were reported in wastewater treatment systems employing microalgae which was as high as 99.8% of total coliform removed (Abdel-Raouf *et al.*, 2012; Komolafe *et al.*, 2014; Slompo *et al.*, 2020).

Another application of microalgae in wastewater treatment was heavy metal removal. Microalgae are efficient absorbers of heavy metals, mostly via bioaccumulation in their cells. In addition, the increase in pH associated with microalgae growth can enhance heavy metal precipitation (Suresh Kumar *et al.*, 2015). Research showed that popular microalgae species found in wastewater such as *Scenedesmus spp.* and *C. vulgaris* are effective in removing various heavy metals including Fe, Zn, Mn, Cr, Cd and Ni with the removal efficiency ranging from 52.3 – 100% in both batch and continuous systems (Hammouda *et al.* 1995). In addition, high tolerance of microalgae with high concentration of heavy metals was reported which 40 – 80% of Fe and Mn at concentrations of 10 – 20mg/L were successfully removed by two microalgae *Desmodesmus* sp. MAS1 and *Heterochlorella* sp. MAS3 (Abinandan *et al.,* 2019). Other species such as *Chlorella vulgaris* and *Spirulina platensis* also showed effective removal of 40% Cd at 10 mg/L within 7 days (Sandau *et al.*, 1996).

It was indicated that heterotrophic growth microalgae could process a wide range of organic carbon sources such as pyruvate, acetate, lactate, ethanol, saturated fatty acids, glycolate, glycerol, C6 sugars (e.g. glucose and fructose), C5 monosaccharides (e.g. xylose and arabinose), disaccharides (e.g. lactose, sucrose and cellobiose) and amino acids (Tuchman *et al.*, 2006; Morales-Sánchez *et al.*, 2015; Turon *et al.*, 2015). Moreover, microalgae under heterotrophic growth showed higher growth rate in comparison to autotrophic growth leading to its extensive application for valuable algal cellular chemicals production (Gim *et al.*, 2014; Morales-Sánchez *et al.*, 2017). It was reported that heterotrophic growth of *Chlorella protothecoides* with cassava starch hydrolysate as the main carbon source could reach 15.8 g/L of biomass with high lipid accumulation up to 4.19 g/L (Wei *et al.*, 2009). As a consequence, the application of heterotrophic microalgae for coupled purposes of wastewater treatment and biomass production has received serious attraction (Venkata Mohan *et al.,* 2015). Wastewater from brewery industry was used for heterotrophic microalgae *Aurantiochytrium* sp. KRS101 production yielding biomass production up to 6.69 g/L/d with biomass concentration as high as 31.8 g/L (Ryu *et al.,* 2013). High microalgal lipid production in domestic wastewater was also reported for three species *Scenedesmus* sp. ZTY2, *Scenedesmus* sp. ZTY3 and *Chlorella* sp. ZTY4 with the lipid contents reaching 69.1%, 55.3% and 79.2%, respectively. After 11 days of cultivation, these microalgae could remove 63.4%, 52.9% and 64.4% dissolved organic carbon (DOC) yielding 1.65, 1.98 and 2.31 g biomass/g DOC, respectively (Zhang *et al.,* 2013). Shen et al. (2017) also suggested the heterotrophic microalgae *Botryococcus* sp. NJD-1 for raw domestic wastewater treatment (up to 64.5%, 89.8% and 67.9% for nitrogen, phosphorus and total organic carbon, respectively) and biofuel production (up to 61.7% lipid content) (Shen *et al.,* 2017).

Recent development in applications of microalgae in wastewater treatment

It was pointed out that genetic engineering could convert obligate photoautotrophic microalgae to grow heterotrophically through the induction of specific transporters (Morales-Sánchez *et al.,* 2015). The introduction of a gene encoding a glucose transporter (*glut1* or *hup1*) to microalga *Phaeodactylum tricornutumcan* was reported to allow its heterotrphic growth on exogenous glucose in the absence of light (Zaslavskaia *et al.,* 2001). Similar genetic engineering methodologies was used in C. *reinhardtii* strains, which the HUP1 (hexose uptake protein) hexose symporter from *Chlorella kessleri* was introduced. The microalgae was then be able to use externally supplied glucose for heterotrophic growth in the dark (Doebbe *et al.* 2007).

Besides, genetic engineering could also improve the growth and photosynthetic activity of microalgae, resulting to enhancement in wastewater treatment and biomass production (Balamurugan *et al.,* 2021). It was reported that the introduction of malic enzyme gene *PtME* from the oleaginous diatom *Phaeodactylum tricornutum* in green microalga *Chlorella pyrenoidosa* was resulted to 3.2-fold increasing in cellular neutral lipid content even at nitrogen deprivation condition (Xue *et al.,* 2016). Two key lipogenic genes including glycerol-3-phosphate acyltransferase (GPAT1) and lysophosphatidic acid acyltransferase (LPAT1) were expressed in oleaginous *Phaeodactylum tricornutum* which also showed significant enhancement in growth and photosynthetic efficiency (Wang *et al.*, 2018). Although genetic engineered microalgae showed great potential, their application in wastewater treatment, especially in open cultivation mode remains largely unknown. Moreover, the ecological consequences as well as complex interactions with the native species once released to the environment should also be investigated (Balamurugan *et al.*, 2021).

THE ROLES OF BACTERIA IN WASTEWATER TREATMENT

In wastewater treatment engineering, depending on the carbon source utilized, bacteria can be classified as heterotrophic or autotrophic ones. Heterotrophic bacteria consume or absorb organic carbon to produce energy and sustain life while autotrophic bacteria can use energy inorganic chemical reactions to produce a carbohydrate, fat or protein substrate. The chemical reactions happening in bacterial cell to produce energy are oxidation-reduction reactions involving the movement of electrons from one molecule (electron donor, oxidized molecule) to another (electron acceptor, reduced molecule) (Tchobanoglous *et al.*, 2002). Those molecules can be either inorganic or organic in nature. In case of aerobic environment, heterotrophic bacteria can use dissolved oxygen in water as electron acceptor while other molecules such as dissolved nitrate or nitrite can be used in the absence of dissolved oxygen (anaerobic environment) (Gerardi, 2003).

Bacterial aerobic oxidation of organic pollutants

Aerobic heterotrophic bacteria use oxygen, which is added mechanically, to break down wastewater contaminants, converting it into energy. Bacteria use this energy to grow and reproduce (Samer, 2015).

Besides oxidizing organic matter to CO_2 and H_2O, bacteria also incorporates inorganic nutrients such as ammonium nitrogen and phosphate to growth (Henze *et al.*, 2008). The process of organic matter oxidation and biomass growth can be at very high rate due to the mechanical intensive addition of oxygen, especially in aeration tanks or lagoons of the wastewater treatment plant. It was indicated that the aeration process could consume from 50 to 90% of the total electricity used by a treatment plant and contribute between 15 and 49% of total costs within the plant (Drewnowski *et al.*, 2019). In a recent study on energy requirements of 17 activated sludge wastewater treatment plants in Greece, Siatou et al. (2020) reported that aeration process consumed 67.2% of the total electricity consumption in a plant accounting for 0.618 kWh/m^3 (Siatou *et al.*, 2020).

Another option is to attach the biomass on carriers in trickling filter or rotating biological contactor (RBC) units. Although RBC and tricking filter systems showed better oxygen-transfer efficiencies comparing to activated sludge system at recommended power consumption, activated sludge system is more economically feasible (Patwardhan, 2003).

Regardless of the system applied, as a consequence of aerobic biological oxidation, organic matter concentration and in a lesser degree, nutrients concentration in treated wastewater are effectively reduced with typical removal efficiency ranging from 96 to more than 99% of COD removal and above 97% of BOD removal (Rani Devi, Dahiya, 2008; Jamal Khan *et al.*, 2011).

Bacterial nitrification in wastewater treatment

Besides organic matter oxidation, biological nitrification is another important process happening in the aeration tank which ammonium ions are oxidized to nitrite ions and then to nitrate ions (Pathak *et al.*, 2020). The process is comprised of two main steps including the conversion of ammonium nitrogen to nitrite nitrogen and from the newly converted nitrite to nitrate nitrogen (Gerardi 2003). Each step was performed by unique autotrophic bacterial groups called ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) with the proportion in activated sludge ranging from 4.1 to 13.2% for AOB and from 2.1 to 5.7% for NOB (You *et al.*, 2003).

As a consequence of nitrification in aeration tank, an increase of nitrate level and at the beginning of the process, some peaks in nitrite concentration are expected. Moreover, for every one nitrate or nitrite ion, from two to three hydrogen ions are released which greatly decreases pH level in water (Gerardi, 2003). Via the process, most of the ammonium can be converted to nitrate with the efficiency normally between 97 and 99.9% (Tchobanoglous *et al.*, 2002).

Bacterial denitrification in wastewater treatment

In the absence of dissolved oxygen or anaerobic condition, various groups of facultative aerobic bacteria in wastewater can turn to use nitrite ions and nitrate ions as electron acceptor to degrade organic matter. As the result, those ions are reduced to free nitrogen which is then escaped to atmosphere as a gas (Gerardi, 2003). In conventional wastewater treatment plant, denitrification process is implemented in a separated reactor without aeration resulting in anoxic condition. The anoxic reactor can be located before or after the aeration tank for a complete nitrification/denitrification process (Tchobanoglous *et al.*, 2002) with the removal efficiency of total nitrogen ranging from 43 to 58% (Collivignarelli, Bertanza, 1999). Recent researches indicated that by maintaining low level of dissolved oxygen (DO) concentration of around 0.5mg/L, the simultaneous nitrification and denitrification (SND) processes could be triggered (Zhao *et al.*, 1999). As a consequence, both organic matter oxidation and nitrogen removal can be achieved with single aeration reactor with total nitrogen removal efficiency of 55 – 98% (Bueno *et al.*, 2018). However, incomplete denitrification process sometime produces nitrous oxide (N_2O) and being released into the atmosphere. This compound is classified as one of the greenhouse gases with 300 times more powerful than CO_2 and hence should be prevented (IPCC, 2018).

Bacterial phosphorus removal in wastewater treatment

Conventional wastewater treatment plant effluent has to keep phosphorus concentration from 0.1 to 2.0 mg/L for preventing eutrophication in natural water bodies (Tchobanoglous *et al.*, 2002). However, the removal of phosphorus via cell uptake by conventional activated sludge can contribute around 15 to 25% of the total phosphorus in many municipal wastewaters which is around 0.015 mg of P incorporated in every mg of total suspended solids (TSS). Therefore, chemical reagents such as alum or iron salts are usually used to precipitate phosphorus in wastewater in addition to biological uptake (Henze *et al.*, 2008).

It was indicated that, under some specific conditions, various bacterial groups in activated sludge could uptake and store a significant larger amount of phosphorus in their cells. The process was then called enhanced biological phosphorus removal (EBPR) and these microorganisms were called phosphorus-accumulating organisms (PAOs). In order to stimulate the growth of PAOs, an anaerobic condition with absence of nitrate must be applied. Then the mixed liquor can experience a subsequent aerobic condition where PAOs can accumulate large quantity of phosphates in their cell under the form of polyphosphate (Seviour *et al.*, 2003). As a result, in the EBPR process, the amount of P incorporated in the sludge mass can increase to around 0.05 to 0.10 mgP/mgTSS (Henze *et al.*, 2008).

THE COOPERATION OF MICROALGAE AND BACTERIA IN WASTEWATER TREATMENT

Synergistic interactions between microalgae and bacteria in wastewater treatment

In most of wastewater treatment systems employing microalgae and bacteria, these microorganisms are suspended in wastewater due to various types of mixing such as paddle wheeling or bubbling aeration. The mixing also allows well distribution of materials and microbes as well as avoids stagnant zone, hence improving the efficiency of the entire system (Grobbelaar, 1994). Under light exposure, microalgal photosynthesis is activated releasing O_2 that supports heterotrophic bacterial degradation of organic pollutants. At the same time, microalgae also benefits on the CO_2 generated from bacterial respiration as well as the inorganic nutrients released from bacterial oxidation process (Schumacher *et al.,* 2003). Moreover, as being primary producer in many aquatic food chain, microalgae also release organic matters from their cells during growth as well as when they die, which are available as substrate for heterotrophic bacteria (Kouzuma, Watanabe, 2015). In addition, studies also reported that certain bacteria can excrete microalgal growth-promoting compounds or vitamins such as thiamine or biotin (Droop, 2007; Ramanan *et al.,* 2016; Higgins *et al.,* 2016).

In addition of autotrophic growth, microalgae could also remove organic substrate, especially in case of high strength wastewater such as piggery or food-processing industrial wastewaters under mixotrophic growth (Wang *et al.,* 2012; Gupta *et al.,* 2016; Nirmalakhandan *et al.,* 2019). Moreover, denitrification was sometimes reported in large scale microalgal bacterial treatment system during the night, when the dissolved oxygen levels were < 2 mg/L (Park and Craggs 2010; Sutherland *et al.* 2020). Overall, thanks to the cooperation between autotrophic microalgae and bacteria, both organic and inorganic pollutants in wastewater can be effectively removed in a single-step treatment (Mohsenpour *et al.,* 2021).

When culturing together, microalgae and bacteria may clump together to form bigger floc and therefore have a faster settling rate which can be harvested by simple sedimentation or filtration. Researches indicated that the flocculation between microalgae and bacteria could result in high gravity settling efficiencies ranging from 30 to 98% (Gutzeit *et al.,* 2005; Medina, Neis, 2007; Van Den Hende *et al.,* 2014). It was also showed that flocculation between microalgae and bacteria provided little impact on treatment efficiency, especially when activated sludge was used as bacterial source. Studies on combination of microalgae and activated sludge for wastewater treatments showed high removal efficiencies of COD (up to 93%), total nitrogen (TN) (up to 73%) and total phosphorus (TP) (up to 82%) (Gutzeit *et al.,* 2005; García *et al.,* 2006; Sutherland *et al.,* 2014; Van Den Hende *et al.,* 2014).

In addition to suspended cultivation, the application of microalgae and bacteria in wastewater treatment in the form of attached growth or biofilm was extensively studied due to its advantages of biomass handling and up-scaling (Moreno Osorio *et al.* 2021). The biofilm carrier could be fixed in tray structure (Babu *et al.,* 2010), rotating contactor (Mukherji, Chavan, 2012) or in the form of membrane which wastewater was continuously fed through the biofilm (Gou *et al.,* 2020). Besides, the biofilm was also cultivated on compact carriers allowing suspended growth in the reactor (Tang *et al.,* 2018). Moreover, various types of carrier were studied such as wood (Babu *et al.,* 2010), polyvinyl chloride (PVC) (Posadas *et al.,* 2014) or recycled plastic bottle (Chaiwong *et al.,* 2018). Han *et al.,* 2020 studied the efficiency of algal bacterial biofilm growth on four commercial biofilm carriers including braided cotton, polypropylene brush, polystyrene foam and carbon fiber sponge. The results showed that braided cotton biofilm carrier providing the best support for biofilm growth (Han *et al.,* 2020). It was evident that the attached growth of microalgae and bacteria still maintains their natural interactions as well as treatment roles in wastewater (Kouzuma and Watanabe 2015). The algal photosynthesis – bacterial oxidation relationship was also confirmed by a study on open algal–bacterial biofilm photobioreactors for domestic wastewater treatment (Posadas *et al.,* 2014). Moreover, study on sequencing batch suspended algal bacterial biofilm reactor reported a significant TN removal (47.65%) via denitrification occurring at the anaerobic inner section of the biofilm besides nutrient assimilation by the biomass (Tang *et al.,* 2018). Oxygen level was also a key factor controlling nitrification rate in algal bacterial biofilm in wastewater stabilization pond (Babu *et al.,* 2010).

Antagonistic interactions between microalgae and bacteria in wastewater treatment

Beside synergistic cooperation between microalgae and bacteria, their antagonistic interactions are also existed (Cole, 1982). As the optimal pH level for bacteria to growth being around neutral value, high pH level due to microalgal photosynthesis could cause bacterial activities to be inhibited (Sutherland *et al.,* 2015). In terms of nutrient consumption, the amount of inorganic nitrogen and phosphorus in wastewater is generally at high value, providing enough nutrients for both microalgae and bacteria to growth. However, low nutrient level due to quick consumption by microorganisms or low strength influent wastewater could cause competition between microalgae and bacteria (Grover, 2000). In addition, under some conditions, microalgae was shown to excrete compounds with bactericidal or fungicidal action (Dellagreca *et al.,* 2010; Najdenski *et al.,* 2013). Similarly, several bacteria species showed capability of producing algicidal chemicals (Natrah *et al.,* 2014).

Study on the interactions between microalgae and bacteria in wastewater treatment

The use of mathematic models to simulate the algal-bacterial processes showed potential as a rapid and cost-effective method to study the interactions between these microorganisms (Solimeno, García, 2017). Besides of simple processes of microalgal-bacterial growth, aerobic and anoxic respiration, anoxic growth on different nutrients and substrates, hydrolysis of particulate matters and various chemical equilibriums allowing to simulate pH level could also be simulated (Reichert *et al.,* 2001; Wágner *et al.,* 2016). In terms of algal processes, the impacts of photoinhibition (Wu, Merchuk, 2002) as well as light penetration (Benson *et al.,* 2007) were added for better simulation. The reliability of kinetic model on simulating complex system of microalgae and bacteria in wastewater is also promising which dissolved oxygen, pH as well as microbial biomass were successfully simulated (Solimeno *et al.,* 2017).

Besides, community analysis with the aid of genomics could also provide insight knowledge into interactions between microalgae and bacteria in wastewater (Perera *et al.,* 2019). Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) together with 16S rDNA sequencing was used to analysing bacterial community in microalgal bacterial consortia treating municipal wastewater under different inoculum ratio (Su *et al.,* 2012). The use of PCR-amplified bacterial 16S rRNA genes as domain-, division-, and subdivision-level probes in fluorescence *in situ* hybridization (FISH) was also useful to assess the proportions of polyphosphate-accumulating organisms (PAOs) in the enhanced biological phosphorus removal (EBPR) from wastewater (Crocetti *et al.,* 2000). Quantitative polymerase chain reaction (qPCR) assays were used with specific rRNA gene markers for studying the change in microalgal bacterial composition under different photoperiods (Lee *et al.,* 2015). The bacterial populations associated with microalgae in different stages of synthetic anaerobic digestates were identified using 16S rDNA phylogeny. Results showed a considerable shift of dominant organisms in the community which can only be revealed via genomic studies (Vasseur *et al.,* 2012).

THE INFLUENCING FACTORS ON MICROALGAL BACTERIAL WASTEWATER TREATMENT PERFORMANCE

pH

At pH 8.5, coliform bacteria and other harmful microorganisms are reduced, with pH 9.5 resulting in the greatest eradication of the wastewater bacterial population (Tchobanoglous *et al.,* 2002). High pH level is not beneficial for microalgae as the optimum pH range for the majority of freshwater microalgae species varying between 7 and 9 (Richmond, 2008). Moreover, elevated pH in wastewater also causes the equilibrium between NH_3 and NH_4^+ changing to the direction of NH_3 production (Garcia *et al.,* 2000) which is toxic for the growth of microorganisms (Collos, Harrison, 2014). It was indicated that, depending on microalgal species and environmental conditions, the NH_3 concentration where growth rate was reduced by 50% (EC_{50}) could range from a low level of 1 - 3 µM to a high value of nearly 2000 µM (Rossi *et al.*, 2020).

Since microalgae use CO_2 as the main carbon source, another application of microalgal bacterial system is to purify flue gas, especially from burning processes. Moreover, elevated CO_2 concentration also decreases the pH via shifting carbonate equilibrium which could minimize negative effects of high pH level. However, one of the most difficult aspects of incorporating CO_2 into a full-scale wastewater system is ensuring that sufficient CO_2 can be provided at a reasonable cost (Beardall, Raven, 2013). The use of CO_2-rich flue gas for maintaining pH level at appropriate values (from 7.5 to 8) has been suggested by various studies (Park, Craggs, 2010; Van Den Hende *et al.*, 2011; Sutherland *et al.*, 2015).

Light intensity

Below the light saturation threshold, the rate of photosynthetic activity is proportional to the strength of the irradiance (Gonçalves *et al.*, 2014), yet intensities over this point showing no relationship with microalgal growth where its photosynthetic apparatus becomes saturated (Richmond, 2008). The optimum level of light intensity may vary depending on the microalgal species and temperature but often stated to be between 200 and 400 $\mu Em^{-2}s^{-1}$ (Muñoz, Guieysse, 2006). Furthermore, exposure to extreme level of light intensity results in photoinhibition effect in microalgae. Moreover, since natural sunlight is the main energy source for such system which is generally designed to enhance light exposure within the photoreactor, the disinfection impacts toward bacteria as well as pathogens under high level of irradiance could also be the case (Richmond, Hu, 2013).

Temperature

Most microalgae can survive at temperatures ranging from 10 to 30°C, with the optimum temperature falling within a narrower range, often between 15 and 25°C (Singh, Singh, 2015). It was suggested that temperature higher than 35°C could reduce maximum growth rate of microalgae (Bouterfas *et al.*, 2002). In addition, low temperature below 10°C was reported to reduce microalgal nutrients removal by 46% and 20% for TN and TP, respectively (Grönlund *et al.*, 2010). Similarly, it was indicated that optimal temperature for nitrification ranges between 28 and 32°C with nitrification rate falling by 50% at 16°C and 80% at 10°C. Extreme values of temperature of higher than 45°C or lower than 5°C would cease nitrification process (Gerardi, 2003). Flocculation efficiency of activated sludge could also decrease at temperature higher tha 45°C (Morgan-Sagastume, Grant Allen, 2005).

Mixing

Vertical mixing influences both the quantity and frequency of light exposure every individual microalga encounters. During the high growth of microalgae and bacteria causing low light attenuation in photoreactor, mixing is critical because it ensures that all cells are at least briefly exposed to saturating light at frequent time scales, allowing for maintaining high productivity (Grobbelaar *et al.*, 1996). Several studies have successfully proven that enhanced vertical mixing increases microalgal photosynthesis and production owing to increased light/dark cycles, known as the ‘‘flashing light effect" (Sforza *et al.*, 2012; Vejrazka *et al.*, 2012).

Increased mixing can also aid in nutrient uptake under limiting conditions, leading to increased growth (Grobbelaar, 1994). Mixing also prevents cell sedimentation on the pond bottom. However, strong mixing resulting to high shear force could damage the formation of biomass flocs and hence decreasing settling efficiency (Hadiyanto *et al.*, 2013). Therefore, the mixing frequency, as well as the mixing velocity, are likely to be important in maintaining desirable large colonies of microalgae and bacteria.

Hydraulic retention time

The hydraulic retention time (HRT) influences the biomass content in the pond by permitting or inhibiting biomass accumulation on longer or shorter HRT, respectively. HRT in wastewater HRAPs generally ranges from 3 to 9 days depending on season and latitude (Sutherland *et al.,* 2015). HRT can be adjusted by changing the pond depth, which changes the light environment in the pond, or by diluting it with harvester effluent. By altering the total nitrogen taken by the microalgae, changing the HRT will change the nutrient load into the HRAP as well as the water quality of the effluent (Garcia *et al.,* 2000). It was suggested that by increasing HRT, the wastewater treatment system employing microalgae and bacteria could maintain effluent quality during harsh conditions such as winter or very high strength wastewater (García *et al.*, 2006; Aguirre *et al.*, 2011).

Nutrient concentration

Carbon dioxide in wastewater treatment system employing microalgae and bacteria mainly comes from bacterial activities, satisfying 25-50 % microalgal demand (Sutherland *et al.*, 2015). Moreover, due to the low C:N ratio in wastewater (7:3) in comparison with the ratio in algal biomass (15:6 C:N), algae usually endure carbon-limited condition, so that carbon addition results in an increase in algal productivity (Park *et al.,* 2011; Sutherland *et al.,* 2015). For microalgae, N and P may co-limit production over a wide range of N:P ratios ranging from 10 to 30, with ratios above 30 indicating P limitation and below 10 indicating N limitation (Dodds, 2003). Typical N:P ratios (16:1) in wastewater indicate that phosphorus is seldom restricting algal development, while nitrogen may become limiting under specific situations (Sutherland *et al.,* 2015).

CONCLUSION

Wastewater treatment using microalgae and bacteria showed great potential, especially under the context of sustainable development in combination with biomass production. The role of microalgae in wastewater treatment was soon recognized in conventional waste stabilization ponds as the primary source of dissolved oxygen (DO) production. Besides, microalgae was also known to effectively remove nutrients, pathogens and various heavy metals. On the other side, bacteria has been widely applied in biological wastewater treatment include stabilization of dissolved and particulate organic matter present in wastewater, oxidation of ammonia to nitrite and nitrate (nitrification), and reduction of nitrite and nitrate to nitrogen gas (denitrification). Under some specific conditions, some bacteria also can absorb and store inorganic phosphorus at large amount. When cultured together, microalgae and bacteria can cooperate effectively for wastewater treatment as well as biomass flocculation although some natural antagonistic interactions are existed. It was showed that various environmental and operational factors have significant influences on microalgae and bacteria in wastewater individually or in combination with others. Therefore, those factors should be carefully monitored for improving performance of the system. Moreover, the impacts of different types of wastewater on the interactions between microalgae and bacteria as well as their applications for treatment also deserve more insight investigation.

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