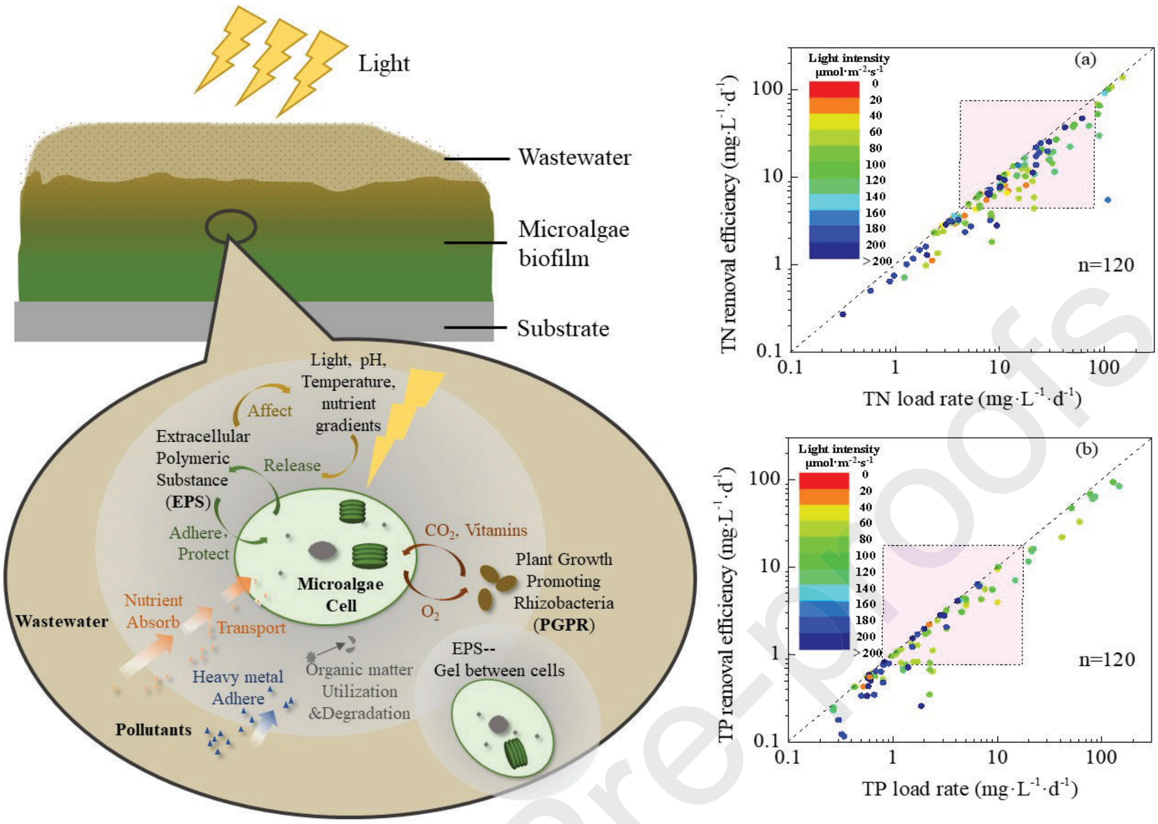

Non-suspended microalgae cultivation for wastewater refinery and biomass production

Lin-Lan Zhuang[a], Mengting Li[a], Huu Hao Ngo[a,b1]

[a] Shandong Key Laboratory of Water Pollution Control and Resource Reuse, School of Environmental Science & Engineering, Shandong University, Qingdao 266237, China

[b] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia

Abstract

Non-suspended microalgae cultivation technology coupled with wastewater purification has received more scientific attention in recent decades. Since the non-suspended microalgae cultivation is quite different from the suspended ones, the following issues are compared in this study such as advantages and disadvantages, pollutant removal mechanisms and regulations,

[1] Correspondence: ngohuuhao121@gmail.com; Tel.: + 61 2 95142745

influential factors, and microalgae biomass accumulation. The analysis aims to support the further application of this technology. The median removal rates of COD, TN, TP, NH_4^+-N and NO_3^--N were 91.6%, 78.2%, 87.5%, 93.2% and 81.7%, respectively, by non-suspended microalgae under the TN & TP load rates up to 150 $mg \cdot L^{-1} \cdot d^{-1}$. The main pathway for TN & TP removal is microalgae cell absorbance. Light intensity, pollutant composition and microalgae metabolic types are the major factors that influence pollutant removal and the lipid content of microalgae. Meanwhile the mechanism concerning how macro-outer conditions influence the micro-environment and further growth of non-suspended microalgae requires more investigation.

Keywords: attached microalgae cultivation; immobilized microalgae cultivation; wastewater purification; extracellular polymeric substance; nitrogen and phosphorus transfer.

1 Introduction

Microalgae need to absorb nutrients (mainly nitrogen and phosphorus) from the ambient environment for their cell component synthesis. Wastewater contains certain amounts of nutrients and water, to make microalgae growth possible (Sawayama et al., 1998; Gupta & Pawar, 2018). The cultivated microalgae biomass can be converted into animal feed, biodiesel, bio-char, fertilizer, and other products (Bharti et al., 2017; Daneshvar et al., 2019; Farooq et al., 2015). Therefore, microalgae biomass production technology coupled with wastewater purification has been recently proposed (Bakonyi et al., 2018; Garbowski et al., 2017; Kube et al., 2019; Orfanos & Manariotis, 2019). This technology has much potential due to the fast growth rate of microalgae, fixation of greenhouse gas CO_2, efficient removal of pollutants in wastewater and pollutants being transformed into valuable biomass products (Liu et al., 2019b; Shi et al., 2007; Yadavalli et al., 2014).

As microalgae cells are quite small and the achievable microalgae density is limited, it is difficult and energy-intensive to recover microalgae biomass from culture broth during the traditional suspended microalgae cultivation (Wang et al., 2018a; Yin et al., 2020). Hence, non-suspended microalgae cultivation will accelerate the application of this microalgae biomass production coupled with wastewater purification technology (Bharti et al., 2017; Ruiz-Marin & Mendoza-Espinosa, 2008; Sukacova et al., 2015). Non-suspended microalgae cultivation can be sub-divided into two types, i.e. immobilization cultivation and attached microalgae cultivation. The former is realized by trapping microalgae in the middle of gels (e.g. chitosan or alginate) (Eroglu et al., 2012), while the latter is realized by attaching microalgae on the surface of solid material in the form of biofilm. Furthermore, the microalgae are surrounded by their own extracellular polymeric substance (EPS) (Shukla et al., 2017). Given that the microalgae in non-suspended cultivation are quite different from traditional suspended cultivation, the following topics were compared here in this review to assess the viability of this technology: advantages and disadvantages; pollutant removal mechanisms and regulations; influencing factors; and dynamic process of pollutant removal during the non-suspended microalgae cultivation.

2 Advantages and disadvantages of non-suspended microalgae cultivation

Microalgae are immobilized in the gel balls or attached on the surface of substrate in non-suspended microalgae cultivation, so microalgae grow as an aggregation. The obtained microalgae biomass in non-suspended microalgae cultivation is denser than the suspended microalgae cultivation (Katarzyna et al., 2015; Roostaei et al., 2018), making the harvesting and dewatering process easier, saving more energy and cheaper (Miranda et al., 2017; Palma et al., 2017). In the non-suspended microalgae cultivation system with well-organized structure, the utilization of light source could be more efficient, enabling the footprint biomass productivity to be better than the suspended ones (Lee et al., 2014; Wang et al., 2018b). As reported some years ago, the attached microalgae cultivation achieved 2.8-times higher biomass productivity than the suspended system (Lee et al., 2014).

From the perspective of wastewater treatment, non-suspended cultivation separates the water retention time from the “sludge” (i.e. microalgae biomass) resistance time. Consequently, this system could help the process of microalgae biomass recovery, and tolerate a relatively higher pollutant load compared with the suspended microalgae cultivation system (Boelee et al., 2012; Gross et al., 2015; Wang et al., 2018b). The self-excretion polymerics or exotic polymeric materials (alginate) surrounding microalgae protect the microalgae from harm caused by exotic toxic pollutants or bacteria, the over-high loaded nutrients or photo-inhibition caused by high light intensity (Boelee et al., 2014b; Gurunathan et al., 2018; Kapdan & Aslan, 2008; Munoz et al., 2009; Roostaei et al., 2018).

There are also disadvantages of non-suspended microalgae. The polymeric surrounding microalgae inhibits the mass transfer and light transmission (Pathak et al., 2018; Ruiz-Gureca & del Pilar Sanchez-Saavedra, 2016; Zeng et al., 2015). The over-shading between cells, and CO_2/nutrient limitation both slow down the autotrophic growth of non-suspended microalgae, especially the immobilized microalgae (Eroglu et al., 2015; Gurunathan et al., 2018; Mallick, 2006; Shukla et al., 2017; Zamani et al., 2012; Zeng et al., 2013). It should be noted that the leakage of microalgae or chemicals applied for microalgae fixation in the immobilization cultivation systems and the sloughing of microalgae biofilm in the attached cultivation systems usually occur when operations take place for a long time (Shi et al., 2007; Zamora-Castro et al., 2008). Based on the description above, the advantages and disadvantages of non-suspended microalgae cultivation are summarized as Table 1 below.

There is no consistent conclusion about whether non-suspended microalgae are better at removing pollutants compared to suspended microalgae according to various studies (Table 2). In Ruiz et al.'s analysis, immobilized microalgae demonstrated worse phosphorus removal than suspended microalgae, yet Wang et al. documented the opposite (Ruiz-Gureca & del Pilar Sanchez-Saavedra, 2016; Wang et al., 2019). This kind of contradictions has been found with other pollutant items (e.g. ammonia) in other research papers (Aguilar-May & del Pilar Sanchez-Saavedra, 2009; Tam & Wong, 2000). Microalgae cultivation conditions varied from study to study, which also had great influence on pollutant removal. That may explain the contradictions in the pollutant removal rate.

Non-suspended microalgae showed different physicochemical properties with suspended microalgae owing to the different growing environments. Lipid content is an important item that evaluates the potential of biodiesel production. Attached microalgae could show a smaller lipid content than suspended algae, and the carbon chain length and saturated degree also showed diversity (Wu et al., 2019). However, the lipid contents in non-suspended microalgae and suspended microalgae are comparable in one recent study (Tsolcha et al., 2018). Therefore, the mechanism concerning how cultivation conditions influence the non-suspended microalgae growth and the further wastewater pollutant removal needs more investigation.

3 Mechanism of pollutant removal by non-suspended microalgae

In the attached microalgae cultivation systems, microalgae cells are surrounded by their released extracellular polymeric substance (EPS) (Palma et al., 2017; Shen et al., 2014). In the immobilized cultivation systems, there are artificial polymers (e.g. alginate or chitosan) around the microalgae besides EPS. EPS and exotic polymers function as glue, sticking microalgae cells together, depriving the movability of cells. In some microalgae biofilms, filamentous cyanobacteria could act as a matrix that captures other microalgae cells (Srinuanpan et al., 2018; Stauch-White et al., 2017). Besides, authigenic (i.e. EPS) and exotic polymers function as buffer media, forming a concentration gradient from the outside environment to the fixed microalgae cells (Fig. 1). Pollutants firstly are absorbed and stored in this polymeric matrix, then transferred and slow-released to microalgae (Lee et al., 2013; Zamora-Castro et al., 2008). Hence, the sharp variance of pollutant concentration in the bulk liquid media will not influence the non-suspended microalgae growth immediately. The microalgae response to the changes in the outside environment is commonly delayed. It explains why pollutant removal is less efficient in non-suspended microalgae cultivation according to some studies (Ruiz-Gureca & del Pilar Sanchez-Saavedra, 2016). Non-suspended microalgae indicated less damage than suspended microalgae when attacked by heavy metal ions (Wang et al., 2013). So, the robustness of non-suspended microalgae cultivation for wastewater treatment emerges as superior to that involving suspended ones.

Different pollutants showed a variety of pathways in the polymeric matrix. Referring to nitrogen and phosphorus, these elements are adsorbed, transferred and finally absorbed by microalgae. However, the form of existence and ion valence strongly influence the transport and utilization rate of nutrients. Heavy metal ions were mainly adsorbed by the various sites (e.g. OH^-, SH^-, COO^-) provided by porous polymer matrix other than stored in microalgae cells (Eroglu et al., 2015; Rinanti et al., 2017; Shen et al., 2018; Travieso et al., 2002; Zeraatkar et al., 2016). This explained why better heavy metal removal was very evident in non-suspended microalgae cultivation (Kiran et al., 2007; Saeed & Iqbal, 2006; Soltmann et al., 2010; Zamani et al., 2012). However, in other researchers' analyses, the pollutant removal function of the agent could be ignored (Kumar et al., 2018). Some organic molecules may be degraded by the exo-enzyme or degraded in cells (e.g. phenol, biophenol A, PAH) (Pathak et al., 2018; Zeng et al., 2015).

The micro-environment (mass gradient, light intensity) of non-suspended microalgae influences microalgae growth (the microalgae autotrophic/ heterotrophic type, cell division, EPS release, cell components) directly (Stauch-White et al., 2017). The microalgae growth and EPS release in turn influence the nutrient/light transport (Bharti et al., 2017; Liu et al., 2017). Hence, non-suspended microalgae and wastewater have a complex interaction bonded by EPS (Osorio et al., 2019; Shen et al., 2017b). In some studies, plant growth promoting rhizobacteria (PGPR) and symbiotic bacteria were introduced in the non-suspended microalgae cultivation systems (Cruz et al., 2013; de-Bashan et al., 2005; Safonova et al., 2004; Wang et al., 2019). PGPR and microalgae could cooperate in aspects of gas exchange and phytohormones/vitamin supplement, enhancing the pollutant removal process by non-suspended microalgae (Eroglu et al., 2015; Wang et al., 2018b).

4 Wastewater pollutant removal properties by non-suspended microalgae cultivation

4.1 Overall removal of pollutants in wastewater by non-suspended microalgae cultivation

Wastewater pollutant removal properties by non-suspended microalgae cultivation are investigated in this section. 120 groups of pollutant removal rate data from more than 60 papers are summarized in Fig. 2. All pollutant removal rates ranged hugely from 0% to 100%, while the distribution patterns were different as shown in Fig. 2. The medians of COD, TN, TP, NH_4^+-N and NO_3^- -N removal rates were 91.6%, 78.2%, 87.5%, 93.2% and 81.7%, respectively. COD, TP, and NH_4^+-N revealed a centralized distribution around a high removal rate (90%-100%),. This means it was easier to remove COD, TP, and NH_4^+-N, other than NO_3^- -N in most studies and non-suspended microalgae had a predilection of different nitrogen. Thus, how to optimize the NO_3^- -N seems to be a key point for the further study.

Non-suspended microalgae can also removal heavy metal ions, other inorganic salts, dyes, surface active substance, polycyclic aromatic hydrocarbons (PAHs), and endocrine disrupting compounds (EDCs) (El-Sheekh et al., 2017; Kesaano et al., 2015b; Pathak et al., 2018; Sole & Matamoros, 2016). Information concerning pollutant initial concentration, removal performance, and microalgae species is listed in Table 3. Adsorption, as the key heavy metal removal pathway, is always a fast process, in which 60%-80% can be completed in less than one day (Shen et al., 2019).

The variable performances of pollutant removal among different studies demonstrated that the cultivation system configuration, the wastewater type (including the composition, chemical properties), cultivation conditions (e.g. light, CO_2), microalgae species and amount all influenced pollutant removal process (Orandi et al., 2012; Palma et al., 2017)., which would be discussed in section 4.2 - 4.5.

4.2 Influential factor of pollutant load rate on microalgae growth and wastewater purification

The wastewater type influenced pollutant removal rate and pollutant removal efficiency by non-suspended microalgae cultivation. More than 10 kinds of artificial or real wastewaters, i.e. dairy wastewater (Castro-Cesena et al., 2016), raw/secondary effluent of wastewater treatment plant (Posadas et al., 2014; Zhang et al., 2018b), winery wastewater (Danaee et al., 2018), aquaculture wastewater (Ruiz-Marin et al., 2010), piggery wastewater (Tsolcha et al., 2018), human urine (Liu et al., 2012), shrimp culture wastewater (Kumar et al., 2016; Ruiz-Gureca & del Pilar Sanchez-Saavedra, 2016), mine tailing water (Zhang et al., 2018a), industrial wastewater (Perez-Martinez et al., 2010), anaerobically digested mixed sludge (Posadas et al., 2014), oilfield wastewater (Zamora-Castro et al., 2008; Zheng & Ke, 2017), were verified for their availability to help the growth of non-suspended microalgae. The properties of various kinds of wastewater had huge differences from the perspectives of pH value, high/low COD content, high/low ammonia/phosphorus concentration, bacterial content, biotoxicity, or different bio-availabilities. It could be concluded that non-suspended microalgae had a high robustness for many kinds of wastewater, which highlighted a great potential for scaled-up application for various kinds of wastewater purification and resource conversion.

Different wastewaters led to different nitrogen and phosphorus loads and N/P ratios, which influenced the non-suspended microalgae growth and pollutant removal performance. Fig. 3 summarizes the relationship between TN & TP load and relative removal efficiency. It shows that TN & TP load rates both ranged widely from 0.1 $mg\cdot L^{-1}\cdot d^{-1}$ to 150 $mg\cdot L^{-1}\cdot d^{-1}$ (Kumar et al., 2018; Lee et al., 2013; Perez-Martinez et al., 2010; Zamani et al., 2012), and specifically, the maximum TN & TP removal efficiency did not show a decreasing trend along with the increasing load rate. Even when TN or TP load rates were around 100 $mg\cdot L^{-1}\cdot d^{-1}$, the removal efficiency could achieve above 90% in some studies (Rajendran et al., 2018; Shen et al., 2017b; Zhang et al., 2008). It could be concluded that non-suspended microalgae could tolerate high loaded nitrogen and phosphorus. However, the pollutant removal could be low with 20% removal in some other studies under different culture conditions (Boelee et al., 2014a; Liu et al., 2012; Liu et al., 2019a; Posadas et al., 2014). In Boelee's study, microalgae were cultured outdoor with a relative low temperature of 13-20 °C, which could inhibit microalgae growth (Boelee et al., 2014a). In Posadas's study, real wastewater was applied instead of the artificial wastewater most studies used. Hence, the bacterial negative effects on microalgae growth should be considered (Posadas et al., 2014). All these culture parameters varied with different studies, forming the diversity of pollutant removal performance. Thus, to keep a high-level pollutant removal, the culture condition, as a critical parameter, should be optimized. If the culture condition and system were fixed in one study, the removal efficiency would no longer maintain at a high level with the increasing of load rate, especially when the pollutant was over-loaded (Kapdan & Aslan, 2008). As 150 $mg \cdot L^{-1} \cdot d^{-1}$ was the upper limit of TN or TP load according to the existing reports, whether above 150 $mg \cdot L^{-1} \cdot d^{-1}$ is over-loaded for non-suspended microalgae needed further investigation.

4.3 Influential factor of light intensity on microalgae growth and wastewater purification

Light intensity is a critical factor for microalgae autotrophic growth. According to Sukacova et al.'s study, nitrate uptake rate increased with solar irradiance ranging from 5 to 23 $MJ \cdot m^{-2} \cdot d^{-1}$ (Sukacova et al., 2017). As this foundation was obtained based on one non-suspended microalgae cultivation system with a narrow light intensity range, the universality should by testified for all kinds of non-suspended microalgae cultivation systems. Hence, the influence of light intensity on wastewater purification by non-suspended microalgae was summarized based on more than 60 papers and shown in Fig. 3. Points with blue color represented the non-suspended microalgae cultured under high light intensity (more than 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$), while the points with green/red color represented the microalgae cultured under low light intensity (less than 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$). As can be seen from the relative location of blue points to red points in Fig. 3, it could be deduced that non-suspended microalgae tended to achieve better pollutant removals in terms of TN and TP under high illumination other than low illumination, especially with a moderate pollutant load rate (shown as the pink area in Fig. 3). As reported, strong illumination could damage the surface microalgae cells (Wang et al., 2015) and slow down the nitrogen and phosphorus absorbance. However, the pollutant removal efficiency kept positive correlation with the increasing of illumination intensity according to the statistic results. At the meanwhile, it was possible to obtain a high TN removal efficiency under a relative low illumination less than 100 μmol·m^{-2}·s^{-1} (Osorio et al., 2019; Zhang et al., 2008) and achieve a poor TN removal efficiency under high illumination (Boelee et al., 2014a; Palma et al., 2017). The reasons were described as follows: 1) The light intensity provided was the external light level, which could not represent the real light intensity microalgae biofilm received, especially the bioreactors with complex structures (Shen et al., 2017b); 2) The process of wastewater purification by non-suspended microalgae cultivation is a complex one and it is affected by many other factors. The suitable setup of other parameters (e.g. relatively high temperature) could make up for the shortage of illumination (Shi et al., 2007); while the unbefitting parameters (e.g. too-high ammonia concentration) could inhibit the non-suspended microalgae growth and the subsequent pollutant removal process (Wu et al., 2019). Therefore, it is an integrated function of all the factors on non-suspended microalgae growth and pollutant removal.

Some microalgae have a heterotrophic metabolic ability. For some microalgae cells in the biofilm's deep layer, they may also absorb nitrogen and phosphorus for their heterotrophic growth without light (Fica & Sims, 2016; Roostaei et al., 2018). Perez Garcia et al. proved that immobilized *Chlorella vulgaris* removed ammonia better under heterotrophic cultivation conditions with organic carbon added in comparison to autotrophic condition with light (Perez-Garcia et al., 2010). *Chlorella sorokiniana* also performed better at removing both TN and TP under dark conditions with heterotrophic growth in contrast to autotrophic growth (Liu et al., 2012). However, very different results were reported by other researchers. The phosphorus or nitrogen concentration in the media for non-suspended microalgae cultivation increased during the night, which meant non-suspended microalgae absorbed nutrient under light, but released some under dark conditions (Boelee et al., 2014a; Tsolcha et al., 2018). More investigations should be carried out for how light/dark condition affects nutrient removal. To be noticed, for microalgae of the mixotrophic metabolic types, *Chlorella* and *Scenedesmus*, these both showed significantly double to ten-fold lipid content compared to autotrophic conditions (Roostaei et al., 2018).

4.4 Influential factor of carbon/nitrogen sources on microalgae growth and wastewater purification

Removing pollutants by non-suspended microalgae cultivation mainly depends on microalgae growth. Hence, any factors that influence microalgae growth (e.g. the carbon sources, nitrogen sources, C/N ratio, among others) will affect pollutants' removal. The removal of ammonia, phosphorus, and nitrate ions were all enhanced by the addition of bicarbonate during the attached microalgae cultivation (Kesaano et al., 2015a). Besides inorganic carbon source, the addition of organic matter (in terms of chemical oxygen demand, i.e. COD) also: firstly, enhanced immobilized microalgae's mixotrophy; and secondly, significantly improved the removal rate of ammonia from 40% to about 90% with the original ammonia concentration of 30 mg/L (Liu et al., 2019a).

Nitrogen in wastewater takes different forms. Ammonia is the favorite for some microalgae, e.g. *Chlorella vulgaris*, followed by nitrite and nitrate (Kumar et al., 2018; Wang et al., 2018b; Zhang et al., 2018b), which could lead to different nitrogen sources having varied removal rates

(Castro-Cesena et al., 2016; Wu et al., 2019). In Kumar et al.'s study, the removal efficiency of ammonia, nitrate and nitrite were 37.5%, 33.3% and 18.8%, respectively, after 4 h contact with immobilized microalgae (Kumar et al., 2018). In Zhang et al.' study, attached microalgae consumed 80% ammonia (from 19 mg/L to 4 mg/L) in two days, while only 15% nitrate (from 13 mg/L to 11 mg/L) were reduced in wastewater (Zhang et al., 2018b). However, nitrate showed a better rate of removal than ammonia in one study. Nitrate concentration decreased from 10 mg/L to 2 mg/L in three days, while ammonia just decreased from 30 mg/L to 24 mg/L during the batch cultivation of attached cyanobacteria (Singh & Thakur, 2015). Hence, the feeding preference varies with algae species. Nevertheless, both nitrate or ammonia were utilized by microalgae under conditions of limited nitrogen (Boelee et al., 2012). Nitrogen sources also influence the removal of phosphorus. With a preferable nitrogen source, both nitrogen and phosphorus were quickly and simultaneously removed (Zhu et al., 2018). Usually, an increase in the nitrogen or phosphorus concentration would improve the other's utilization. However, this phenomenon was not apparent in a recent paper (Shen et al., 2019), so the mechanism of nutrient interaction requires further research.

5 Dynamic process of non-suspended microalgae growth and pollutant removal

A series of mass balance calculation showed that 70%-90% nitrogen and above 90% phosphorus removed from wastewater were transferred to the microalgae biomass during non-suspended microalgae cultivation (Kapdan & Aslan, 2008; Piltz & Melkonian, 2018; Shi et al., 2014; Zamalloa et al., 2013). This means that microalgae biomass accumulation is the major pathway for nitrogen and phosphorus removal from wastewater. It is evident that the dynamic process of non-suspended microalgae growth and pollutant removal is closely linked (Li et al., 2016). The pollutant removal rate was reported to rise with the non-suspended microalgae growth rate (Kesaano et al., 2015a). It is essential to acquire the non-suspended microalgae growth regulations.

5.1 The dynamics of non-suspended microalgae growth

(1) Immobilized microalgae growth

Since the experimental periods for immobilized microalgae growth and wastewater treatment were commonly short (less than 3 days) (Kumar et al., 2018; Tam & Wong, 2000), the growth of immobilized microalgae has rarely been studied. In some research, the immobilized microalgae beads were re-used for three batch experiment rounds. Due to limitations of space, microalgae could not keep growing (Liu et al., 2012). Consequently, it is not possible to estimate the efficiency or consistency of high pollutant removal (Chevalier & Delanoue, 1985; Shen et al., 2017a).

(2) Attached microalgae growth

In this section, the attached microalgae growth and the influential factors are discussed. Microalgae biofilm thickness affects microalgae growth. With the original microalgae biofilm thickness increasing from 130 μm to 2000 μm, the average biomass production rate rose from 4.5 g dry weight·m^{-2}·d^{-1} to 9.9 g dry weight·m^{-2}·d^{-1}. However, when the biofilm thickness achieving a certain level, the relatively thick or thin biofilm would no longer influence the microalgae biomass accumulation rate (Boelee et al., 2014b).

Microalgae cells have an initial N/P ratio of around 5.42 (according to the common molecular formula of microalgae $CH_{1.78}N_{0.12}O_{0.36}P_{0.01}$) (Praveen & Loh, 2015), which changes with microalgae species and culture conditions at a certain level (Boelee et al., 2012). The wastewater fraction (e.g. N/P ratio) would also affect microalgae growth and their cell composition. N/P ratio in non-suspended microalgae cell tended to be similar with the N/P ratio in the wastewater (Whitton et al., 2016). That is, a non-appropriate ratio of N/P may lead to poor microalgae growth and equally poor pollutant removal efficiency.

As microalgae biofilm, the growth properties of microalgae cells from the upper layer would be different from the cells at the bottom layer. The upper layer microalgae cells prioritize their search for exterior light, nitrogen and phosphorus. According to Boelee et al.'s calculations, nitrate had a penetration depth of around 400 μm, and phosphorus could penetrate only 260 μm (Boelee et al., 2014a; Boelee et al., 2014b). The maximum oxygen concentration was obtained at around 200 μm (Kesaano et al., 2015a). Therefore, only a layer of upper microalgae could absorb nutrients and light for growth (Posadas et al., 2014). The microalgae cells under 400 μm in the biofilm would have no chance to get the required nutrients from the bulk liquid media, so in effect they could not remove pollutants. It explains why the over-thick biofilm could not achieve a relatively faster growth rate and a faster pollutant removal rate subsequently.

As the EPS matrix could quickly absorb nutrients from wastewater and release them slowly to the surrounding microalgae cell, the upper layer microalgae biofilm could be nutrient-saturated after contact has been made with highly concentrated wastewater. Hence, scraping part of upper layer biofilm at a 2 - 3 days interval may expose the deep layer starved biofilm and enhance the removal of nutrients (Boelee et al., 2014b). This is a solution to the mass transfer problem. Hence, the removal of pollutants would be accelerated.

5.2 The dynamics of pollutant removal

Many researchers have documented the dynamics of pollutant concentration in wastewater along with the non-suspended microalgae cultivation. The main types of pollutant removal curves are shown in Fig. 4 (a). Pollutant concentration in wastewater could go through a plateau phase (type 1 in Fig. 4 (a)) or directly enter a fast-decline phase (type 2 in Fig. 4 (a)) once the water contacts with non-suspended microalgae. For example, COD and phosphorus in dairy wastewater went through a 3-day plateau phase before declining rapidly, while these two items in the other groups simply entered the fast-decline phase (Tsolcha et al., 2018). The plateau phase could be caused by the adjustment of non-suspended microalgae to a new environment. It might be several days long or shorter than one day which can be ignored (Shi et al., 2007; Tsolcha et al., 2018). The second reason for the plateau phase could be the small initial inoculated amount of non-suspended microalgae, which made the pollutant removal trend not obvious at the beginning. Subsequently, the fast-decline phase occurred with the fast growth of non-suspended microalgae and the increasing number of microalgae. Another reason for the plateau phase is that the non-suspended microalgae would choose their favorite nutrient source if there are alternative choices. For example, the nitrate concentration indicated a plateau during the fast-decline phase of ammonia in attached microalgae cultivation in one recent study (Zhang et al., 2018b).

For attached microalgae cultivation, when the amount of microalgae is limited under a certain level (biofilm depth, i.e. $D_b < $ the depth that a pollutant could transfer, i.e. D_t in Fig.4 (c)), the pollutant removal rate would increase with the growth of attached microalgae owing to rising amount of functional microalgae (Shi et al., 2007; Tsolcha et al., 2018). When the biofilm depth $D_b >$ the depth of that pollutant could transfer D_t, the section thickness that implemented pollutant removal, i.e. the upper layer with thickness of D_t, would be constant. Hence, attached microalgae would retain a fast rate of diminishing pollutants, which is in fact the fast-decline phase. Commonly, Day 2-Day 7 represents the fast-decline phase for pollutant removal during the batch experiment of attached microalgae cultivation (Cheng et al., 2017; Tsolcha et al., 2018;

Wu et al., 2019). The concentration of pollutants in wastewater is gradually decreased, which in turn slows down the non-suspended microalgae growth. A consequence of this is the further reduction in pollutants until they are all absorbed (type 4 in Fig. 4 (a)) (Johnson & Wen, 2010).

For immobilized microalgae cultivation, the batch experiment period is commonly short, which is counted in hours rather than days (Chevalier & Delanoue, 1985; Tam & Wong, 2000). Unlike the microalgae biofilm which has an unlimited upper space for growth, immobilized microalgae are limited in beads. So, microalgae growth would be quickly inhibited by the limited space (Fig. 4 (b) and (c)) (Shen et al., 2017a). Usually, the pollutant removal process by immobilized microalgae entered the fast-decline phase owing to the fast pollutant absorbance by alginate matrix (Ruiz-Gureca & del Pilar Sanchez-Saavedra, 2016; Sole & Matamoros, 2016; Wang et al., 2016). Other studies have indicated no effect of blank alginate or chitosan bead on pollutant removal (Kaya & Picard, 1996; Kumar et al., 2018; Liu et al., 2012). In this case, the pollutant removal is total conducted by immobilized microalgae assimilation, which is a relatively slow process than alginate absorbance. With the growth of immobilized microalgae, more microalgae could involve in the process of pollutant assimilation, thus, the pollutant removal rate accelerates. However, the increasing microalgae density brings more obstructions for mass transfer into the inner side of microalgae beads (Fig. 4 (b)). Commonly, the rapid pollutant removal process stopped in 48 h-72 h. The saturated absorbance of pollutants and limited microalgae growth led to the type 3 outcome in Fig. 4 (a), i.e. “unable to use” (Castro-Cesena et al., 2016; Shen et al., 2017a; Tam & Wong, 2000).

6 Problems and prospects for non-suspended microalgae cultivation

The problems and prospects of non-suspended microalgae cultivation are summarized as follows. Firstly, since attached microalgae cultivation for wastewater treatment is still a relatively new concept, present-day cultivation systems are typically simple with a plate-like substrate (Cheng et al., 2017; Osorio et al., 2019). Hence, light utilization efficiency and biomass footprint productivity could be enhanced by modifying the system structure. Secondly, understanding how light and pollutants transfer in immobilized microalgae beads or attached microalgae biofilm (Boelee et al., 2014a), is important for explaining the mechanism of pollutant removal and microalgae biomass production. This is an issue that has not yet been thoroughly explained. Thirdly and lastly, leakage of immobilized microalgae or attached microalgae slough commonly occur during the last period of cultivation (Perez-Martinez et al., 2010; Schnurr et al., 2013; Shi et al., 2007). Finding out why this happens and adjusting the system from season to season so that the system operations remain stable is another aspect of this topic that needs further exploration.

7 Conclusion

Non-suspended microalgae cultivation coupled with wastewater treatment has great advantage of easy microalgae recovery from treated wastewater, while the pollutant removal efficiency is comparable with suspended cultivation. Pollutants in terms of COD, TN and TP in most studies could be further improved by optimizing culture conditions (including light intensity, temperature and wastewater fractions, et al.). The continuous and fast microalgae growth is the key point for high pollutant removal efficiency. Therefore, the construction and robustness optimization of non-suspended microalgae cultivation system and the mechanism concerning how pollutant transfer and microalgae growth require more investigation in the future.

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Highlights

- Non-suspended microalgae achieve easy biomass recovery and high stress resistance.

- TN and TP could get a 90% removal with load rate higher than 150 mg·L^{-1}·d^{-1}.
- Above 70% TN and TP were transferred from wastewater to microalgae biomass.
- The dynamic growth of non-suspended microalgae determined the pollutant removal rate.
- The mechanism of pollutant transfer and microalgae growth require investigation.

Table 1 Advantages and disadvantages of non-suspended microalgae cultivation

Advantages	Disadvantages	N
· Making harvesting/dewatering easier and cheaper · High cell density · Potential higher microalgae productivity · Increasing the microalgae retention time in system Resistance to growth stresses or toxicity · Overcome photo-inhibition during high light intensities	· Over-shading, CO_2 and nutrients transfer limitation · Special for immobilization systems: Producing secondary pollution	· ·

Table 2 Wastewater pollutant removal by non-suspended and suspended microalgae

Non-suspended cultivation type	Pollutant item	Pollutant Removal	Pollutant Removal in suspended microalgae cultivation systems	Microalgae species	Culture
Immobilized type	phosphorus	8.35mg/L→5.67mg/L RR 32.07%	8.35mg/L→3.93mg/L RR 52.93%	*Chlorella vulgaris*	23±2℃; con 174 μmol·s-1·… waste
	Ammonia	44 mg/L→22 mg/L RR 50%	44 mg/L→27 mg/L RR 38.6%	*Nannochloropsis oculata*	synthetic
	Phosphorus	7.3 mg/L→1.6 mg/L RR 78.1%	7.3 mg/L→4.5 mg/L RR 38.4%	*Nannochloropsis oculata*	synthetic
	Copper	RR 54%X	RR X	*Pseudomonas putida*	
	Ammonia	RR 78%	RR 40%	*Scenedesmus obliquus*	25±1℃; light 2; urban
	Phosphorus	RR 94%	RR 59%	*Scenedesmus obliquus*	25±1℃; light 2; urban
	Ammonia	25 mg/L→18 mg/L RR 28%	25 mg/L→11 mg/L RR 56%	*Synechococcus elongatus*	27±1℃; light artificial
	Nitrate	23 mg/L→14.5 mg/L RR 58.6%	23 mg/L→13 mg/L RR 43.5%	*Synechococcus elongatus*	27±1℃; light artificial
	Phosphorus	6.8 mg/L→1.6 mg/L RR 76.5%	6.8 mg/L→0.7 mg/L RR 89.7%	*Synechococcus elongatus*	27±1℃; light artificial
	Ammonia	30 mg/L→1 mg/L RR 96.7%	30 mg/L→4 mg/L RR 86.7%	*Scenedesmus obliquus*	25℃; light: da h; artificial
Attached type	TP	4 mg/L→0 mg/L RR 100%	4 mg/L→2.4 mg/L RR 40%	*Scenedesmus vacuolatus*	light: dark ra artificial
	nitrate	14 mg/L→8 mg/L RR 42.9%	14 mg/L→11.5 mg/L RR 17.9%	*Scenedesmus vacuolatus*	light: dark ra artificial
	COD	RR 95.67%	RR 90.14%	*Scenedesmus obliquus, Chlorella vulgaris and Oscillatoria tenuis*	28 ± 2℃; light 2;light: dark ra primary set
	TP	RR 64.40%	RR 56.03%	*Scenedesmus obliquus, Chlorella vulgaris and Oscillatoria tenuis*	28 ± 2℃; light 2;light: dark ra primary set

	TN	RR 69.55%	RR 43%	*Scenedesmus obliquus, Chlorella vulgaris and Oscillatoria tenuis*	28 ± 2℃; light [2];light: dark ra primary set
	Ammonia	RR 91.24%	RR 64.78%	*Scenedesmus obliquus, Chlorella vulgaris and Oscillatoria tenuis*	28 ± 2℃; light [2];light: dark ra primary set
	Cadmium	RR 121%X	RR X	*Synechococcus sp.*	

*RR is short for removal rate
*Data with underline meant the better removal performance in two groups
*"RR X" means that the removal rate is nor reported in referred paper.

Table 3 Other pollutant removals by non-suspended microalgae cultivation

Category		Initial concentration (mg/L)	Removal rate (%)	microalgae species	non-suspensi type
Heavy metal	Cu (II)	300	43%	*Ankistrodesmus braunii, Chlorella sp.*, and *Scenedesmus quadricauda*	Immobilize
	Cu (II)	15	93%	*Chlamydomonas sp.*	Attached
	Zn	3.5	94.30%	*Stichococcus bacillaris*	Attached
	Co	3	94.50%	*Scenedesmus obliquus*	Immobilize
	Co	0.31	10.5%	*Chlorella*-like microalga	Attached
	Cd (II)	10	92.45%	*Chlorella sp.*	Immobilize
	Ni	2.14	24.8%	*Chlorella*-like microalga	Attached
	Mn	22.7	24.8%	*Chlorella*-like microalga	Attached
	Sr	0.62	26.4%	*Chlorella*-like microalga	Attached
	Cr (VI)	50-60	82%	L. putealis	Immobilize
Acidic Sulfate		1000-4000	35%-45%	*Chlorella* vulgaris	Attached
Sodium salicylate					
phenol and biophenol A				*Ocrhomonas danica* and *Monoraphidium braunni*	
Indigo blue dye			33%	*Chlorella vulgaris*	Immobilize
Phenols		0.48	85%	*Chlorella* sp., *Scenedesmus* sp. et al.	Attached
Anionic surface active substance (SAS)		22.25	73%	*Chlorella* sp., *Scenedesmus* sp. et al.	Attached
Oil spill		40	96%	*Chlorella* sp., *Scenedesmus* sp. et al.	Attached
Oil		30	73.67%	Scenedesmus obliquus	Immobilize
Polycyclic Aromatic Hydrocarbons (PAHs)		-	-	-	Immobilize
Endocrine disrupting compounds (EDCs)	BPA	0.01	46%	*Chlorella sp.* and *Nitzschia acicularis*	Immobilize
	BPAF	0.01	80%	*Chlorella sp.* and *Nitzschia acicularis*	Immobilize
	BPF	0.01	87%	*Chlorella sp.* and *Nitzschia acicularis*	Immobilize
	2,4 DCP	0.01	76%	*Chlorella sp.* and *Nitzschia acicularis*	Immobilize
	EE2	0.01	98%	*Chlorella sp.* and *Nitzschia acicularis*	Immobilize
	4-OP	0.01	93%	*Chlorella sp.* and *Nitzschia acicularis*	Immobilize