



Received: 23 September 2016
Accepted: 16 December 2016
Published: 29 December 2016

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ENVIRONMENTAL CHEMISTRY, POLLUTION & WASTE MANAGEMENT | REVIEW ARTICLE

Removal of nitrogen from wastewater using microalgae and microalgae–bacteria consortia

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Abstract: Exceeding nitrogen discharge into natural water bodies can lead to eutrophication in natural aquatic environments, as well as the decline in shellfish habitat and aquatic plant life. Currently, bacterial biological treatment process is the most common process employed in wastewater treatment plants, which requires extensive oxygen. The large demand for oxygen provided by mechanical aeration is costly and can strip out volatile compounds. Microalgae are photosynthetic micro-organisms, which can be a good source of oxygen in the wastewater treatment process. The effect of using microalgae, either solo or in consortia systems along with other micro-organisms (mainly bacteria) have been studied by researchers to improve their contaminant removal efficiency. In a consortia system, microalgae generate oxygen through photosynthesis to satisfy the oxygen requirement of bacteria. Simultaneously, they also remove contaminating nutrients throughout their growth cycle. Various factors affect the performance of the consortia systems such as lighting, pH, and species of microalgae and bacteria. Since microalgae are suspended and dispersed in the media, harvesting is crucial to achieving a high-quality effluent. This paper presents an overview on nitrogen removal from wastewater using different types of systems including microalgae solo and microalgae–bacteria consortia systems. The parameters that affect system performance as well as biomass harvesting methods are also discussed.

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Qiuyan Yuan has 10 years research and development experience in sustainable nutrient removal and recovery processes applied to wastewater and biosolids. Since 2014, her research area has expanded to solid waste treatment and management. Currently, she is focusing on: (1) innovative wastewater nutrient removal and recovery, including landfill leachate treatment; (2) developing technology to produce high value product form solid waste including organic waste, glass, and tires; (3) landfill methane mitigation using innovative bio-cover.

PUBLIC INTEREST STATEMENT

Conventional wastewater nitrogen removal process is an energy intensive process due to the high aeration demand. The mandate of sustainable development has motivated the development of innovative treatment process to reduce the energy demand. Microalgae, which can produce oxygen through photosynthesis has been studied intensively in the past decade in the application of wastewater treatment process. It has been demonstrated that microalgae can serve as “aeration device” to provide oxygen for the treatment process. This paper presents an overview on nitrogen removal from wastewater using different types of systems including microalgae solo and microalgae–bacteria consortia systems. The parameters that affect system performance, as well as biomass harvesting methods are also discussed.

Subjects: Water Engineering; Water Science; Pollution

Keywords: wastewater treatment; biological nitrogen removal; microalgae; microalgae–bacteria consortia

1. Introduction

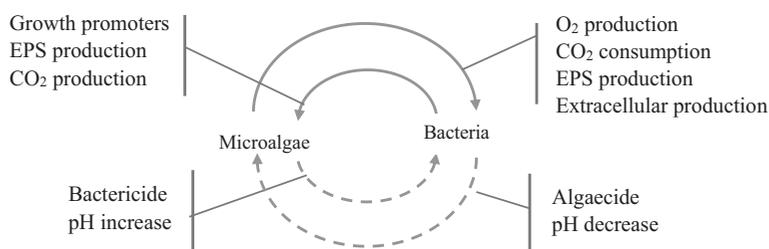
City, industry, and agriculture operations create a large volume of wastewater every year. Natural water bodies receive the major proportion of various wastewater streams. The water body is eutrophic when the nitrogen concentration is higher than 1.9 mg/L (Brown & Simpson, 2001), which can result in algal blooms. Many microalgae grow on the surface of the water that blocks the sunlight and exerts oxygen from aquatic bodies. Habitats of aquatic life decrease due to the decline in oxygen thereof.

Biological (activated sludge) treatment is the most commonly used process for nitrogen removal in wastewater treatment plants (WWTPs). There are two steps for removing nitrogen in biological treatment: nitrification and denitrification. In this process, nitrifiers, including ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), convert total ammonia (free ammonia and unionized ammonia) to nitrate. Denitrification happens in an anoxic environment in which denitrifiers reduce nitrate and nitrite to nitrogen gas. It takes 4.57 grams of O₂ to oxidize per gram of total ammonia to nitrate. In WWTPs, mechanical aeration supplies a large amount of oxygen and costs 45–75% of total energy demand of the plant (Oilgae, 2010). The aeration has the risk of stripping out volatile compounds (Muñoz, Köllner, Guieysse, & Mattiasson, 2004). In addition, waste-activated sludge (WAS) is the major by-product of biological treatment. The production of WAS is corresponding to the amount of wastewater treated (Metcalf and Eddy, 2003). To treat 1 million liters of wastewater, the activated sludge process generates about 70–100 kg dry WAS. With the continued increase in wastewater generation, the amount of WAS is also increasing (Athanasoulia, Melidis, & Aivasis, 2012). To treat and dispose of WAS, significant energy and a large land area are required.

Microalgae are primarily oxygen-releasing photosynthetic organisms with a simple cell having no roots, stems, or leaves. Microalgae grow fast, produce ubiquitously, and can double their biomass within 24 h. They can grow exponentially within 3.5 h. They are ubiquitous in the environment and can thrive in almost any habitat as long as the required nutrients are available. Some microalgae grow on rocks, in soils, and/or in symbiotic relationships with plants. Most commonly, however, microalgae grow in fresh and marine aquatic systems, as well as in wastewater streams from a variety of sources (Zhou, 2014). Microalgae can produce a wide range of valuable metabolites including fats, sugars, and bioactive compounds (Andersen, 2013). Generally, lipid content is in the range of 20 to 50% of their dry weight. Depending on the group of algae, such as *Botryococcus* (green algae), the content could be as high as 90% (Metting, 1996). After lipid extraction, the protein-rich residue is ideal for feeding animals and fertilizing crops (Cai, Park, & Li, 2013). There have been many studies conducted on microalgae for applications in biotechnology. So far, microalgae have been implemented in industry for different purposes, such as synthesis of supplements, food additives, and other bioactive compounds for the cosmetic and pharmaceutical industries (Priyadarshani & Rath, 2012). In natural aquatic systems, microalgae act as tiny aeration devices that produce oxygen for other bacteria and serve as carbon dioxide sinks that fix CO₂. Other than oxygen–carbon dioxide exchange, the interactions between microalgae and bacteria also include other aspects (Figure 1), which indicates the potential of microalgae–bacteria consortia system on wastewater treatment.

Figure 1. Interactions between microalgae and bacteria.

Notes: Plain line: positive interactions; Dashed line: negative interactions.
Source: Adapted from Rivas and Riquelme (2012) and Muñoz and Guieysse (2006).



In terms of wastewater, studies on microalgae-based treatments have been conducted for more than a half-century. Waste stabilization pond systems (WSPs) and high-rate algal ponds (HRAP) are two currently available technologies. A WSP is an open system where microalgae and heterotrophic bacteria form a symbiotic relationship as shown in Figure 1. In this system, microalgae assimilate nitrogen and phosphorus, and bacteria remove organic matter. An HRAP is a shallow, paddlewheel-mixed open raceway pond. In this system, microalgae grow rapidly and produce extensive oxygen, driving aerobic treatment and the assimilation of wastewater nutrients in algal biomass. The end products from the microalgae-based treatments can be used as animal feed or crop fertilizer (Oilgae, 2010). The advantages of microalgae-based wastewater treatment as stated by the US Department of Energy (DOE) National Algal Biofuels Technology, included the “potential to treat agricultural drainage and eutrophic water bodies, wastewater treatment revenue that offsets microalgae production costs, lower capital and operation and maintenance costs than conventional wastewater treatment, [and] lower energy intensity than conventional wastewater treatment (a green-house gas benefit)” (US DOE, 2010).

The use of high-quality effluent is important for wastewater treatment. Harvesting of suspended growing microalgae or microalgae–bacteria consortia is expensive (Zhang & Hu, 2012). The efficiency of common industrial processes, such as filtration, centrifugation, and micro-straining, have worked poorly at removing microalgae. Adding chemicals such as lime and alum are efficient but costly. Biomass auto-flocculation has been observed in the laboratory condition, however, the mechanism is still unclear. Other methods including biomass immobilization and dosing edible chitosan are promising strategies for harvesting (Muñoz & Guieysse, 2006).

This review will focus on microalgae-based treatments: solo microalgae system and microalgae–bacteria consortia on nitrogen removal in wastewaters. The pertinent factors and microalgal harvesting are also discussed.

2. The application of microalgal treatment in different wastewater streams

Profiles of wastewater streams are significantly different from each other, with no distinguishing chemical characteristics or physical properties. Wastewater pollution comes from three sources: municipal systems, agricultural systems, and industrial systems.

In general, there are three types of municipal wastewater in WWTPs: primary effluent, secondary effluent, and reject water generated from the dewatering of anaerobic-digested activated sludge (Constantine, 2006). Compared to primary and secondary effluents, reject water contains much higher nitrogen concentrations, which have become a treatment issue for the WWTP. Current reject water treatments include physical–chemical treatments and biological treatments (Constantine, 2006). Some researchers have used reject water as cultivation media for algal biomass and lipid production (Cai et al., 2013; Min et al., 2011; Rusten & Sahu, 2011), which shows the potential of microalgae in the treatment of reject water.

Agricultural wastewaters, especially from dairy and swine production, have high turbidity and nutrient concentrations, and high concentrations of insoluble organic compounds. The contaminants with high turbidity can block light and decrease photosynthetic efficiency, which obstructs algal treatment applications in agricultural wastewaters. Therefore, in related studies, streams from agriculture wastewater were commonly diluted before algal-based treatments to decrease the turbidity and nutrient concentration (González et al., 2008; Woertz, Feffer, Lundquist, & Nelson, 2009; Zhu et al., 2013).

Industrial wastewaters also have complex constituents and high levels of toxic compounds. Generally, industrial wastewaters are treated using a variety of hazardous chemicals for pH correction, sludge removal, as well as color and odor removal (Oilgae, 2010). Based on published reports,

industrial wastewaters are not suitable for microalgae growth. However, a few studies have demonstrated the feasibility of microalgae-based remediation of some specific industrial wastewaters (Chinnasamy, Bhatnagar, Claxton, & Das, 2010; Zhou, 2014).

Many benefits support the potential of microalgae-based treatments, such as low operating costs, ability to reduce atmospheric CO₂ level and/or capture of CO₂ from industrial flue gases, and production of valuable end bio-products (Oilgae, 2010). Studies of microalgae-based treatments on nitrogen removal include the microalgae solo system and the consortia system.

3. Microalga treatment systems

3.1. Microalgal solo systems

In natural aquatic systems, microalgae assimilate large amounts of nutrients and trace metals during the growing season (Gangstad, 1979). Microalgae can also digest inorganic nitrogen sources, such as nitrate, nitrite, and ammonium. Among those, ammonium is preferred since microalgae can assimilate it by consuming less energy than the other two forms. Microalgae show advantages in many applications: they can grow rapidly, produce many bio-valuable by-products, the residue of waste microalgae can be used as animal feed and crop fertilizer, and do not need a large amount of land for waste disposal (Cai et al., 2013). The aforementioned advantages make this system a great candidate for wastewater treatment. Microalgae solo systems here refer to the methods using only microalgae in wastewater treatment (nitrogen removal) without the assistance of other organisms. Many experiments have been conducted to investigate the performance of microalgae solo systems on wastewater streams and these are summarized in Table 1.

3.2. Microalgal–bacteria consortia systems

In microalgae–bacteria consortia systems (Figure 1), microalgae can produce various organic substances that bacteria can assimilate. However, the relationships between microalgae and bacteria are very complex. Some species of bacteria can release hormones to promote algal growth (Mouget, Dakhama, Lavoie, & Noüe, 1995). For instance, when co-immobilizing *Chlorella vulgaris* with *Azospirillum brasilense* in alginate beads, populations of algae, pigments in algal cells, and size of micro-algal colonies were significantly increased (Gonzalez & Bashan, 2000). However, the experiment also showed that *Pseudomonas vesicularum* improved algal performance without producing any

Table 1. Experiments on microalgal solo systems

Medium source	Microalgal species	Experimental settings	References
Secondary effluent	<i>C. vulgaris</i>	Batch tests (6 d); LI = 6,000 lux; L/D: 16/8	Kim et al. (2013)
Secondary effluent	Four species of green microalgae	Batch tests (7 d); LI = 7,000 lux; L/D: 8/16	Su, Mennerich, and Urban (2012a)
Secondary effluent	<i>Scenedesmus obliquus</i> <i>C. vulgaris</i>	Batch test (2.5 d), LI = 7,290 lux; semi-continuous (35 h/cycle); LI = 10,800 lux; cycle was not mentioned	Ruiz-Marin et al. (2010)
Secondary effluent	Mixed green algal culture	Batch test (14 d); LI = 7,000 lux; L/D: 16/18	Su et al. (2012b)
Primary settled wastewater	<i>C. vulgaris</i>	Batch test (10 d); LI = 4,300 lux; L/D: 16/8	Lau, Tam, and Wong (1995)
Primary settled wastewater	<i>C. vulgaris</i>	Batch tests (15 d); LI = 14,580–16,740 lux; L/D: 8/16	Choi and Lee (2015)
Source-separated urine	<i>Chlorella sorokiniana</i>	Continuous (HRT = 1 d); LI = 27,000–97,200 lux; illuminating continuously	Tuantet et al. (2014)

Notes: d = days; LI = light intensity; L/D = lighting to dark cycle; HRT = hydraulic retention time.

plant hormones (Mouget et al., 1995). Classification of consortia systems can be based on the effect of microalgae in systems, which are microalgae-assistant systems and algae-dominant systems. Microalgae can also act as an oxygen producer for other organisms in the former system and remove nutrients in the latter system.

3.2.1. Microalgae-assistant systems

WWTPs demand intensive oxygen for nitrification, which is accomplished with mechanical aeration. However, it is costly and can strip out volatile contaminants (Muñoz, Jacinto, Guieysse, & Mattiasson, 2005). In algae-assistant systems, microalgae mainly supply dissolved oxygen (DO) for bacteria to remove nutrients and actively uptake nutrients as well (Karya, van der Steen, & Lens, 2013). Rapid growth rates of microalgae correspond to sufficient oxygen release. In conventional oxidation (stabilization) ponds or suspended algal pond systems that have been developed such as HRAP, microalgae are combined with heterotrophic aerobic bacteria, which provides sufficient oxygen for bacteria to remove organic and inorganic pollutants (Pittman, Dean, & Osundeko, 2011). Karya et al. (2013) had successfully cultivated a bio-flocculent alga-activated sludge which could remove up to 100% of NH_4^+ (50 mg/L) in a semi-continuous reactor. According to experiments carried out by Wolfaardt, Lawrence, Robarts, and Caldwell (1994), the degradative efficiency of bacteria increased by 37% in the presence of microalgae. They hypothesized that algal products enhanced the performance of bacteria. Table 2 presents a summary of the experimental results related to ammonia removal and oxygen production.

3.2.2. Microalgae-dominant systems

In this type of system, microalgae play the key role in nutrient removal and are essentially required to produce adequate biomass for sufficient uptake. For this reason, strategies for improving microalgae density have been investigated. *A. brasilense* is known as plant-growth-promoting bacterium as it produces several phytohormones *in vitro* (Gonzalez & Bashan, 2000). According to research conducted by Mouget et al. (1995), the microbial strains *Pseudomonas diminuta* and *Pseudomonas vesicularis* promoted the growth of green microalgae *Chlorella sp.* and *Scenedesmus bicellularis* without releasing any growth-promoting substance. In these studies, it was assumed that stimulation happened because of photosynthetic oxygen tension reduction (air suction) by bacteria.

Table 2. Experiments with microalgae–bacteria consortia

Medium source	Consortia content	Experiment settings	DO mg/L	References
Artificial medium containing acetonitrile	<i>C. sorokiniana</i> , <i>C. vulgaris</i> , <i>Sc. Obliquus</i> , <i>Se. capricornutum</i> (separately tested)/ bacteria mixed culture	Continuous; HRT = 3.5 d; Continuous lighting, Li = 18,500 lux	1.66 mg/L	Muñoz et al. (2005)
Artificial wastewater	<i>Scenedesmus quadricauda</i> /nitrifier enriched activated sludge	Semi-continuous; HRT = 1 d/2 d; SRT = 30 d/15 d; Continuous illumination, LI = 2,300 lux	12 mg/L after 4 h running (daily supply rate: 0.46 kg/m ³ /day)	Karya et al. (2013)
Artificial wastewater	Mixture of <i>Scenedesmus quadricauda</i> , <i>Anabaena variabilis</i> , <i>Chlorella sp.</i> , <i>Chlorococcus sp.</i> , <i>Spirulina sp.</i> / activated sludge	Continuous; HRT = 1 d; SRT = 15 d;	6.5 ± 2.1 mg/L (daily supply rate: 0.156 kg/m ³ /day)	Vandaele et al. (2000)
Pre-treated swine manure	<i>Chlorella sorokiniana</i> /mixture bacteria	Continuous; HRT: 10 d; Continuous illumination; LI = 10,000 lux	10 mg/L	González et al. (2008)
Pre-treated municipal wastewater	Wastewater born microalgae/ activated sludge	Batch test; HRT = 10 d; L/D = 12/12; LI = 7,000 lux	Stay below 0.33 mg/L at the first week, gradually increased to 5 mg/L	Su et al. (2012c)

Notes: DO = dissolved oxygen; d = days; LI = lighting intensity; HRT = hydraulic retention time; SRT = solids retention time.

4. Efficiency of solo algal systems and consortia systems

Nitrogen is essential for building up the algal cells' components, such as genetic material, enzymes, proteins, hormones, vitamins, alkaloids, amides, and energy transfer molecules. Nitrogen is the second most abundant element making up 6–10% of dry weight of green algae *Chlorella*. Nitrogen content ranges from 1–10% of the cell dry weight (Grobbeelaar, 2013). Carbon is the predominant element of *Chlorella* and accounts for approximate 50% of cell dry weight (Andersen, 2013). Most species of microalgae can utilize both organic and inorganic nitrogen. For inorganic nitrogen, eukaryotic microalgae can only assimilate nitrite, nitrate, and ammonium/ammonia. A cyanobacterium is a prokaryote that can convert atmospheric nitrogen to ammonia by fixation (Cai et al., 2013). To assimilate the inorganic nitrogen, nitrate and nitrite must be reduced to ammonium by nitrate reductase (NR) and nitrite reductase (NiR), respectively. Microalgae assimilate ammonium to glutamine and release the hydrogen ion. Since the assimilation of ammonium does not require the redox reaction, it consumes less energy than the assimilation of nitrite and nitrate. Besides, in most microalgae species, NR activity is fully repressed in the cell when sufficient ammonium is supplied. Therefore, ammonium is one of the most preferred source of inorganic nitrogen (Cai et al., 2013; Zhou, 2014).

In activated sludge, removing nitrogen requires two groups of micro-organisms namely nitrifiers and denitrifiers. Nitrifiers are autotrophic bacteria, which do not need an organic carbon source but consume large amounts of oxygen. Inorganic nitrogen sources are electron donors in the nitrification process. Nitrification process has two steps: oxidation of ammonia to nitrite by ammonia-oxidized bacteria (AOB) and oxidation of nitrite to nitrate by nitrite-oxidizing bacteria (NOB). Through these two steps, both AOB and NOB obtain energy for assimilation. In the denitrification process, nitrate or nitrite is reduced by denitrifiers to accept electrons and provide energy for the assimilation of organic matter. The reduction involves four steps (Equation (1)):



Table 3. Efficiency of algal treatments on nitrogen removal in different wastewater streams

	Medium source	Algal species	Initial nitrogen (mg/L)	Removal efficiency (%)	Reference
Microalgae solo system	Secondary effluent	<i>C. vulgaris</i>	N-NH ₄ ⁺ : 8.05 ± 0.16; 18.31 ± 0.53	100% within 2 d	Kim et al. (2013)
	Secondary effluent	<i>Phormidium sp.</i> , <i>C. reinhardtii</i> , <i>C. vulgaris</i> and <i>S. rubescens</i> separately	TKN: 26.4 ± 0.7	100% within 4 d for <i>Phormidium sp.</i> , and <i>C. reinhardtii</i> ; within 6 d for the other two	Su et al. (2012b)
	Piggery wastewater	<i>C. zofingiensis</i>	TN: 63.96–82.7	65–80% within 4 d	Zhu et al. (2013)
	Source-separated urine	<i>Chlorella sorokiniana</i>	TN: 4,300–7,100	20–30% per day	Tuantet et al. (2014)
	Reject water	Microalgae consortium	TN: 220	65% per day	Halfhide et al. (2015)
Consortia (microalgae assistant)	Artificial wastewater	<i>Scenedesmus quadricauda</i> /nitrifier enriched activated sludge	N-NH ₄ ⁺ : 50	100% per day nitrification	Karya et al. (2013)
	Artificial wastewater	Mixture of <i>S. quadricauda</i> , <i>A. variabilis</i> , <i>Chlorella sp.</i> , <i>Chlorococcus sp.</i> , <i>Spirulina sp.</i> /activated sludge	N-NH ₄ ⁺ : 66	85% per day	Vandaele et al. (2000)
	Pre-treated swine manure	<i>Chlorella sorokiniana</i> /mixture bacteria	N-NH ₄ ⁺ : 250–120	99% per day	González et al. (2008)
	Pre-treated municipal wastewater	Wastewater born microalgae/activated sludge	N-NH ₄ ⁺ : 39.4 ± 5.5	91.0 ± 7.0% in 10 d	Su et al. (2012c)
	Raw leachate	Mixed culture of bacteria and algae	N-NH ₄ ⁺ : 80	11% per day	Sniffen, Sales, and Olson (2016)
Consortia (microalgae dominant)	Modified OECD medium	<i>C. vulgaris</i> and <i>Bacillus licheniformis</i>	N-NH ₄ ⁺ : 20	78% within 6 d	Liang et al. (2013)

Notes: TKN = total Kieldahl nitrogen; d = days; TN = total nitrogen.

Few studies have evaluated different parameters to increase the efficiency of solo microalgae and consortia systems for nitrogen removal. Table 3 lists the efficiency results of experiments carried on solo microalgae and consortia (algae-assistant and microalgae-dominant) systems, respectively.

5. Factors affecting the microalgal system

Both microalgae and bacteria are sensitive to their living environment. Temperature, pH, light intensity and pattern, nutrient concentrations, DO, and some other factors can influence their activity and performance in different ways. Therefore, it is necessary to take them into consideration and optimize the treatment environment. For microalgae solo systems, lighting intensity, pH value, biomass density, and different types of species should be considered. Since microalgae are providing oxygen and organic substance for bacteria, factors that affect microalgae performance are similar for consortia systems.

5.1. Light supply

Light is important for activity and growth of photosynthetic organisms. Microalgae can capture light very efficiently using chlorophyll and convert light energy into chemical energy (i.e. Adenosine triphosphate-ATP). This is also known as photosynthesis. During this process, oxygen and reducing agents which convert inorganic carbon (i.e. CO₂) to organic molecules are also produced (Masojidek, Koblizek, & Torzillo, 2004). Photosynthetic processes happening inside algal cells provide energy to build their cell structures and to reproduce. The production of more algal biomass results in more oxygen being produced. Adequate DO is required for efficient ammonia-nitrogen removal. In another way, if algal density is too high, photons cannot penetrate deeply into the culture broth, causing inefficient light utilization (Park & Lee, 2001). To avoid such problems, photo-bioreactors should be properly designed with an ample surface to volume ratio for light capture, appropriate mixing for homogeneity to support sufficient mass and photon transfer, and adequate biomass density to prevent self-shading (Muñoz & Guieysse, 2006).

5.1.1. Light wavelength and intensity

Pigments in microalgae cells including chlorophylls, carotenoids, and phycobilins are absorbed in a narrow spectrum range (Raaman, 2006). The pigments existing in microalgae are different depending on the species. Green microalgae have *chlorophylls a* and *b*, and most of them do not have accessory light-harvesting pigments, which could help to extend the absorption wavelength. Red microalgae contain *chlorophyll a* and phycobilisomes. Most cyanobacteria, also known as blue-green algae, possess *chlorophyll a*, phycocyanin, and phycoerythrin as light-harvesting molecules (Masojidek et al., 2004). These studies reported that algal biomass production and nutrient removal are influenced by wavelength and light intensity (LI). Kim, Liu, Lee, and Lu (2013) reported a 50% higher biomass production of microalgae *Scenedesmus sp.* using red and blue lights than the culture cultivated under white light. In addition, the same study also showed better nutrient removal by mixed lights with specific ratios than white light. Another study conducted with different monochromatic lights and white light on *C. vulgaris* presented a higher growth rate with blue light as compared to the wavelengths of white, red, and green light (Blair, Kokabian, & Gude, 2014). Ho, Chen, and Chang (2012) reported that LI was related to algal growth, carbohydrate/lipid production, and CO₂ fixation efficiency. They tested LIs ranging from 140 to 540 μmol/m²/s, which exhibited that biomass productivity and CO₂ fixation rate increased with the increase in LI until the peaks reached an LI value of 420 μmol/m²/s. Light inhibition happened after that stage, resulting in the decrease in both CO₂ fixation and biomass productivity. Liao, Li, Chen, and Zhu (2014) developed a novel tubular photo-bioreactor forming periodic light and dark regions. This study stated that algal specific biomass production increased with increasing LI values ranging from 120 to 240 μmol/m²/s; however, a decrease in production was reported for LI values from 240 to 300 μmol/m²/s at 100 Hz frequency of light/dark cycle.

Light wavelength and intensity also influence the performance of nitrifiers. Both ammonium and nitrite oxidizing activities can be inhibited by strong light in the ocean ecosystem. In these systems, the most efficient nitrification happens at the bottom of the euphotic zone where the light intensity

is only 5–10% of that on the surface (Ward, 2011). Photoinhibition on nitrifiers is species dependent. According to the active zone, AOB work in more shallow area than the NOB. Therefore, it was proposed that NOB are more sensitive to photoinhibition than the ammonia oxidizers (Olson, 1981). Guerrero and Jones (1996) studied light inhibition on marine AOB and NOB. Their study revealed that photoinhibition is species-specific and dependent on dosage (light intensity and lighting period) and wavelength. The results showed that AOB were more sensitive to blue light than NOB. Cool-white fluorescent light inhibited AOB activity but did not influence NOB. Light inhibition on AOB and NOB were also reported for soil nitrifying bacteria (Guerrero & Jones, 1997). According to Ward (2011), the possible reason for photoinhibition on nitrifiers is that cytochromes of both AOB and NOB that are involved in the energy transduction pathways of nitrification can be damaged by the light.

5.1.2. Lighting period

In addition to wavelength and LI, the frequency of light also influences algal biomass production and nitrogen removal. It has been proved that dark periods between short flashes of light can increase photosynthesis efficiency, especially under high LI (Liao et al., 2014; Park & Lee, 2001). The average biomass productivity increased by $21.6 \pm 2.1\%$ at the artificial light/dark cycle frequency of 100 Hz. After photoinhibition, the dark phase proved effective for recovering nitrifiers' activity (Guerrero & Jones, 1996; Yoshioka & Saijo, 1984). A study carried out by Yoshioka and Saijo (1984) showed that the light activity could also inhibit AOB and NOB; however, they were able to recover in dark condition in 10 days, which means that the dark phase was necessary for proper functioning of nitrifiers.

5.2. pH of algal and bacterial growth media

The pH plays an important role in many cellular processes, which include energy metabolism, structure and function of organelles, enzymes, and proteins. For most algae, the pH range of culturing media is from 7 to 9. Extreme pH may cause the disruption of many cellular processes which could lead to culture collapse (Cautteau, 1996). Some components are required in algal cultivation media to maintain a stable pH by avoiding metal ion precipitation, retarding contaminants' growth (microbial inhibitor), or serving other functions (Brand, Andersen, & Nobles, 2013). The pH tolerance ranges vary among different algal species. Some species could only live in a narrow range; however, others could thrive in a wide range (Moss, 1973). *Chlorella ellipsoidea* grows in a wide pH range between 4 and 10. The best growth performance of *C. vulgaris* was observed at pH 10 (Gong, Feng, Kang, Luo, & Yang, 2014). The pH also impacts nutrient uptake. Zhou, Wu, Zhao, and Wang (2015) studied the optimal pH ranges for *C. vulgaris* on nutrient removal and found the optimal pH range for ammonia and nitrogen removal was 7–8. Different results were established by Liang et al. (2013). In a co-cultured system of *Bacillus licheniformis* and *C. vulgaris*, it was shown that the optimal pH for N-NH_4^+ removal was 7, while for phosphorus removal pH did not have significant impact (Liang et al., 2013; Zhou et al., 2015). However, the concentration of nutrients could be affected by pH. A high pH value could result in an increase in free ammonia concentration and phosphorus precipitation in the form of calcium phosphate (Cai et al., 2013). Meanwhile, excessive free ammonia concentration affects algal photosynthesis, depressing their growth (Abeliovich & Azov, 1976). On the other hand, the activities of algal cells such as CO_2 consumption and N-NH_4^+ uptake could also induce pH variations. It is known that dissolved CO_2 consumption by photosynthetic process increases OH^- concentration and N-NH_4^+ uptake by microalgae while releasing H^+ (Knud-Hansen, 1998). In addition to algae, nitrifying bacteria also contributes to pH decrease due to the nitrification process. Low pH inhibits both groups of AOB and AOA, but their activity can be restored by pH adjustment (Ward, 2011).

5.3. Microalgal and bacterial species

Selection of microalgae and bacteria species depends on the role they play in the system and their performance in different kinds of wastewaters.

In solo algal systems, *Chlorella sp.*, is one of the most common species used in wastewater treatments. Alcántara, Muñoz, Norvill, Plouviez, and Guieysse (2015) confirmed the presence of nitrate reductase (an enzyme involved in the bacterial denitrification pathway) in axenic cultures of *C.*

vulgaris. Moreover, *Scenedesmus sp.* is also widely used in studies. They are equipped with flotation spines making their colonies buoyant, which contribute to the efficient uptake of light and nutrients. Both of the species have similar performances on nutrient removal (Cai et al., 2013). They can also grow under autotrophic (without organic substrate) and heterotrophic conditions (with organic substrate and without light), but these species were inhibited when they grew in the mixotrophic mood (with organic substrate under light/dark cycle) (Combres, Laliberte, Reyssac, & Noue, 1994; Perez-Garcia, Escalante, de-Bashan, & Bashan, 2011). Su, Mennerich, and Urban (2012b) studied four species on the effectiveness of nutrient removal. The species were three green microalgae and one cyanobacterium. In this study, ammonia concentration was 25 mg/L and it was completely removed in 6 days by all four species. Among these species, *Phormidium sp.*, a cyanobacterium, and *Chlamydomonas reinhardtii* removed all ammonia on the fourth day. On the fourth day, a peak of NO_3^- , which was less than 6 mg/L was observed in the *Phormidium sp.* treatment. It took another 3 days for *Phormidium sp.* to remove the nitrate.

In marine ecosystems, except providing oxygen, microalgae can also provide organic substrates for bacteria. In this mutual relationship, microalgae can provide proteins, organic carbon, and some other carbohydrates for bacteria. In exchange, bacteria supply inorganic nutrients and other metabolic compounds including vitamins, hormones, and EPS (extracellular polymeric substances), which contributes to bio-flocculation. However, this mechanism is not universal among all microalgae and bacteria species. It is species-specific as the microenvironment of each alga is different (Ramanan, Kim, Cho, Oh, & Kim, 2016). In studies about wastewater treatment, shown in Table 2, green microalgae species are prevalent in experiments and bacteria are commonly collected from activated sludge or wastewater (Karya et al., 2013; Ruiz-Marin, Mendoza-Espinosa, & Stephenson, 2010; Su, Mennerich, & Urban, 2012c).

In algal dominant systems, some bacteria (such as *pseudomonas*) were found to be effective at promoting algal growth. The appropriate selection of strains of plant growth promoting bacteria (PGPB) is important. As mentioned before, *P. diminuta* and *P. vesicularis* effectively enhanced the algal production while Dakhama, de la Noüe, and Lavoie (1993) found that *Pseudomonas aeruginosa* depressed the growth rate of various green microalgae and cyanobacteria by releasing anti-algae substance. According to Gonzalez and Bashan (2000), when co-immobilized with nitrogen-fixing bacteria *Phyllobacterium myrsinacearum*, pigment production in algal cells increased significantly while nutrient removal by microalgae was reduced. *A. brasilense* is the most studied PGPB, which is effective for numerous crops (De-Bashan, Hernandez, Morey, & Bashan, 2004) including *C. vulgaris* (De-Bashan, Antoun, & Bashan, 2008). Studies showed that the addition of phytohormones produced by *A. brasilense* increased the algal population. Co-culturing with *A. brasilense*, *C. vulgaris* can grow well under unfavorable aquatic conditions (high pH and presence of toxic substances). Moreover, when co-cultivated with *Bacillus*, the nutrient removal efficiency of *C. vulgaris* also improved significantly (Liang et al., 2013).

5.4. Dissolved oxygen in aquatic media

Since DO is required by AOB, AOA, and NOB, they can only be active in aerobic environments. To oxidize each gram of ammonia, nitrifying bacteria need 4.7 gram of oxygen. However, denitrification happens under anoxic conditions, which means the DO concentration should be less than 0.5 mg/L. The DO level is commonly considered as a key factor for determination of nitrification and denitrification rates. The nitrification process stops when the DO value drops below 0.2 mg/L. Other studies stated that complete simultaneous nitrification and denitrification occurred at the DO value of 0.3 mg/L. Below 0.3 mg/L, denitrification will prevail over nitrification (Li et al., 2008; Pochana & Keller, 1999). Oxygen saturation coefficients of Monod kinetics is 0.3 mg/L for nitritation and 1.1 mg/L for nitrification (Wang & Yang, 2004). According to the coefficients, nitrite accumulation can occur when the DO value is maintained below 1.1 mg/L, meaning that NOB could become a rate-limiting factor for nitrogen removal. A fully aerobic membrane bioreactor was operated to treat black water under three low DO levels below 0.5 mg/L. In this study, the optimal DO range for denitrification was 0.15–0.35 mg/L; however, ammonia could only be reduced to 40 mg/L (Hocaoglu, Insel, Cokgor, & Orhon, 2011).

5.5. Organic matter

Denitrifiers are a group of heterotrophic bacteria, which use nitrite or nitrate as the electron acceptor in the respiration process and obtain energy from organic substances. In WWTPs, dosing organic matter is necessary to provide sufficient electron donors for nitrate removal. In general, to reduce each gram of nitrate, 4 grams of biochemical oxygen demand (BOD) is required. The amount of BOD can change due to the varying carbon source and operational conditions (Metcalf and Eddy, 2003). For example, with the same amount of chemical oxygen demand (COD), denitrifiers consume more nitrate using acetate than methanol; the amount of nitrate removed by denitrifiers can vary even using the same source of COD due to the different operational processes or conditions (Ahn, 2006). Meng, Yang, Liu, and Meng (2008) explored effects of different COD/N ratio on nitrogen removal. The results indicated that a high COD/N ratio of 15 limited nitrification performance, whereas a low COD/N ratio of 5 was preferred by AOB; however, the low COD/N ratio limited the denitrification process.

Some microalgae can grow heterotrophically without light when organic substrates are available. In this way, microalgae grow without light and take up organic carbon to obtain energy. Three growth conditions, heterotrophic, autotrophic, and mixotrophic were tested on *C. vulgaris* by Perez-Garcia, De-Bashan, Hernandez, and Bashan (2010). In this study, microalgae performance on biomass production and nutrient removal was superior under heterotrophic conditions than the other two. Zhu et al. (2013) performed 10-day batch tests with different COD concentrations in piggery wastewater under continuous illumination (24 h per day). The results showed that microalgae *Chlorella zofingiensis* had better performance on total nitrogen (TN) removal in higher COD-concentrated wastewater. They removed 81% TN with an initial concentration of 148 mg/L TN in the wastewater which had a COD concentration of 3,500 mg/L; and removed approximately 69% TN with an initial concentration of 17 mg/L TN in the wastewater which had a lower COD concentration of 400 mg/L.

6. Harvesting of biomass

Both solo microalgae system and microalgae-based consortia systems showed considerable potentials in treating wastewater, however, the cost of biomass harvesting might be a barrier for their practical applications (Karya et al., 2013).

In solo algal systems, harvesting efficiency depends on aspects such as algal species, cell density, and culture conditions. Microalgae are typically small sized (1–30 µm), have a low concentration (0.3–5 g/l), are negatively charged, and have a specific density that is close to that of their culture medium. These characteristics make them dispersedly suspended in media (Molina Grima, Belarbi, Ación Fernández, Robles Medina, & Chisti, 2003). To capture all of the algae, the harvesting process could be very complex, involving one or more steps through several physical, chemical, or biological processes (Franchino, Comino, Bona, & Riggio, 2012). The available industrial methods for harvesting include filtration, centrifugation, gravity sedimentation, and micro-straining. However, none of them has been proven to be cost-effective and suitable for large-scale microalgae removal (Muñoz & Guieysse, 2006). For large-scale microalgae removal, membrane filtration is costly because of extensive maintenance and the high-energy requirement for pumping. Conventional filtration processes are not reliable for small-size microalgae. Centrifugation methods require intensive energy (Muylaert, Vandamme, Foubert, & Brady, 2015) and the efficiency of the process depends on the microalgal species and centrifugation speed (Molina Grima et al., 2003). Gravity settling is suitable only to harvest large-sized microalgae cells but the process can be enhanced if it is followed by flocculation.

Coagulation and flocculation harvest the biomass by adding chemical coagulants and flocculants or raising pH over 10. Although these processes are effective at harvesting, they are costly and can increase the effluent's salinity (Muñoz & Guieysse, 2006). The flocculant type and microalgae species are factors that affect the harvesting efficiency. Abomohra, El-Sheekh, and Hanelt (2014) studied four different flocculants, NaOH, MgSO₄·7H₂O, FeCl₃·6H₂O, and NaCl, with different dosing concentrations, 0, 50, 150, 250 mg/L, on green microalgae *Scenedesmus obliquus*. The results showed that NaOH with a dosing concentration of 250 mg/L performed the best as it removed 85% microalgae cells within 2 h. Other flocculants including MgSO₄·7H₂O and NaCl did not show any flocculent

activity. Beach, Eckelman, Cui, Brentner, and Zimmerman (2012) conducted research on other flocculants such as chitosan biopolymer, ferric sulfate, and alum at concentrations of 50, 75, 100, 125, 150 mg/L. In this research, chitosan was found to be the optimal flocculant for green microalga *N. oleoabundans* at the concentration of 100 mg/L. Chitosan is polymer flocculant and it is regarded as edible and nontoxic. Polymer flocculants are attractive because they are easily manufactured and typically have good performance with less dosage producing large and stable flocs. However, the salinity can affect the harvest efficiency (Molina Grima et al., 2003). In addition, for different species, microalgae require different chitosan dosages. Polymer flocculants are often less effective for harvesting marine microalgae (Muylaert et al., 2015). For *Tetraselmis chui*, *Thalassiosira pseudonana* and *Isochrysis sp.*, the optimal dosage was 40 mg/L; *Chaetoceros muellari*; however, required 150 mg/L to get the desired flocculation (Heasman, Diemar, O'connor, Sushames, & Foulkes, 2000). Chen et al. (2012) used aqueous ammonia as the flocculant and tested it on marine microalgae and freshwater microalgae. The results showed that marine microalgae were removed easier than freshwater algae. To reach a pH above 10, marine microalgae species take less amounts of aqueous ammonia. The initiating flocculation was achieved by dosing Ca(OH)_2 at a pH value of 7.97 for wild-type *Chlamydomonas reinhardtii* and pH value of 10 for *Nannochloris*. Except the aforementioned flocculants, filamentous fungi (Xie, Sun, Dai, & S.Yuan, 2013; Zhang & Hu, 2012) were also studied at lab scale. Xie et al. (2013) co-cultured fungi *C. echinulate* with green microalgae *C. vulgaris* and formed pellets with diameters ranging from 2 to 10 mm, which enabled the complete removal of microalgae by simple filtration. Without dosing flocculants, auto-flocculation can happen when the pH rises due to the photosynthetic carbon dioxide depletion (Muylaert et al., 2015).

For consortia systems, the methods used for harvesting are the same as for microalgae solo systems. To obtain a simple sedimentation by gravity, bioflocculent algae–bacteria biomass was developed (Gutzeit, Lorch, Weber, Engels, & Neis, 2005; Karya et al., 2013; Su, Mennerich, & Urban, 2011). The formation of bioflocculent biomass is commonly achieved by continuously mixing and illumination followed by 1 h sedimentation. After settling, the supernatant is discarded and the algae–bacteria biomass could be obtained after the settling period of 1 month. The formation process is expected to be influenced by cell surface properties of the algae and EPS (extracellular polymeric substances), and by other factors (e.g. amount of calcium) (Gutzeit et al., 2005); however, the responsible mechanisms are still unknown.

Using an immobilization system is another possible solution for harvesting. Immobilization to a polymeric material such as carrageenan, chitosan, or alginate has been reported by various researchers (Eroglu et al., 2012; Eroglu, Smith, & Raston, 2015; Gonzalez & Bashan, 2000). Advantages of immobilization include protecting microalgae and/or bacteria that are resistant to harsh environments, recycling the biomass in an economical way, and reducing the damage to cells before recovering (Eroglu et al., 2015). However, there are some disadvantages, such as limited oxygen and nutrient transfer, weak and costly matrices, and difficulties for implementation of large-scale applications (Eroglu et al., 2015; Muñoz et al., 2005).

A reasonable harvest process is supposed to be cost-effective and can be applied to large volumes for treatment. It is complicated to develop a universal method for microalgae or algae–bacteria harvest due to the various affecting factors, like microalgae species, salinity, etc. This area is actively researched, and it targets different biomass types to develop a specific and economical harvesting system that is important for practical applications.

7. Perspective

In conventional nitrification process in WWTPs, providing DO for nitrifiers typically required high energy and state-of-the-art equipment to create very fine air bubbles. As a tiny photosynthetic cell, microalgae can serve as an aeration device to provide oxygen for the bacteria. Using microalgae to treat wastewater give us a promising way to save energy and develop a sustainable treatment process. According to Tables 1 and 2, microalgae–bacteria consortia systems have been mostly studied in artificial wastewater and real wastewater with low ammonia concentrations. However, studies on

wastewater with high ammonia concentrations, such as concentrate, are rare. To treat reject water, a high rate of oxygen production is necessary for the removal of high concentrations of ammonia. Among the studies that have been reported, best result was obtained using the bio-flocculent biomass that produced 0.48 kg O₂/m³/d. Illumination is essential for microalgae photosynthetic activity. In the studies listed in Tables 1 and 2, a typical photoperiod for consortia systems was 24 h a day and LI values were strong (above 4,000 lux). However, continuous illumination allows microalgae to continuously produce oxygen, which can inhibit the denitrification process. Thus, further research on finding optimal conditions for simultaneous removal of ammonia and nitrate is necessary.

Funding

This research was financially supported by Natural Sciences and Engineering Research Council of Canada (NSERC) [grant number RGPIN-2014-05510].

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Citation information

Cite this article as: Removal of nitrogen from wastewater using microalgae and microalgae–bacteria consortia, Huijun Jia & Qiuyan Yuan, *Cogent Environmental Science* (2016), 2: 1275089.

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