Journal of Environmental Management 217 (2018) 845–857

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Review

Advances in algal-prokaryotic wastewater treatment: A review of nitrogen transformations, reactor configurations and molecular tools

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ARTICLE INFO

Article history:
Received 23 October 2017
Received in revised form
19 March 2018
Accepted 4 April 2018
Available online 24 April 2018

Keywords:
Algal-prokaryotic consortia
Biological nitrogen removal
Reactor configurations
N metabolic pathways
Full-scale applications

ABSTRACT

The synergistic activity of algae and prokaryotic microorganisms can be used to improve the efficiency of biological wastewater treatment, particularly with regards to nitrogen removal. For example, algae can provide oxygen through photosynthesis needed for aerobic degradation of organic carbon and nitrification and harvested algal-prokaryotic biomass can be used to produce high value chemicals or biogas. Algal-prokaryotic consortia have been used to treat wastewater in different types of reactors, including waste stabilization ponds, high rate algal ponds and closed photobioreactors. This review addresses the current literature and identifies research gaps related to the following topics: 1) the complex interactions between algae and prokaryotes in wastewater treatment; 2) advances in bioreactor technologies that can achieve high nitrogen removal efficiencies in small reactor volumes, such as algal-prokaryotic biofilm reactors and enhanced algal-prokaryotic treatment systems (EAPS); 3) molecular tools that have expanded our understanding of the activities of algal and prokaryotic communities in wastewater treatment processes.

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https://doi.org/10.1016/j.jenvman.2018.04.021
0301-4797/

1. Introduction

Algae can improve biological wastewater treatment processes through synergistic interactions with prokaryotic microbial communities (including bacteria and archaea). In particular, algae can provide the dissolved oxygen (DO) required for aerobic heterotrophic metabolism and nitrification through photosynthesis. Water and wastewater treatment accounts for approximately 4% of electrical energy use in the US (EPRI, 2002), with the greatest electricity demand (30–60%) for aeration (WRF and EPRI, 2013). In addition, algal-prokaryotic biomass can be harvested and used for biodiesel or valuable chemical production or anaerobically digested for biomethane production (Kesaano et al., 2015; Park et al., 2011a; Pittman et al., 2011; Wang et al., 2013; Wang and Park, 2015). Therefore, algal-prokaryotic wastewater treatment processes have the potential to reduce costs, increase energy security for municipalities and reduce emissions of secondary pollutants from power plants, such as CO_2, CH_4, sulfur hexafluoride, and oxides of nitrogen (NO_x) (EPA, 2017).

Algae have been applied in waste stabilization ponds (WSPs) since the 1950s (Oswald, 1963; Oswald and Gotaas, 1957). Besides nutrient and organics removal, WSPs also remove pathogens from wastewater (Oragui et al., 1987; van der Steen et al., 1999; Verbyla and Mihelcic, 2015). More recently, engineered algae-based wastewater treatment systems have been developed to reduce the footprint and improve nutrient removal efficiency and effluent quality, including high rate algal ponds (HRAPs), closed photobioreactors (e.g. tubular or flat plate), algal-prokaryotic biofilm reactors and enhanced algal-prokaryotic systems (EAPS) (Christenson and Sims, 2012; Garcia et al., 2000; Gross et al., 2015a; Hoffmann, 1998; Karya et al., 2013; Naumann et al., 2012; Park et al., 2011b; Posadas et al., 2013). These systems commonly use algae to assimilate nutrients, provide DO for degradation of organic matter and nitrification, improve biomass settling ability, mitigate CO_2 and increase pH to enhance ammonia (NH_3) volatilization and phosphate precipitation (Craggs et al., 1996; Kumar et al., 2010). Algae have also been applied for treatment of reverse osmosis reject waters containing high salinity and nutrients (Wang et al., 2016). Additionally, algal-prokaryotic processes have been shown to enhance the removal of antibiotics, such as cephalosporin, through production of reactive oxygen species, such as the superoxide radical, hydrogen peroxide and singlet oxygen, as the by-products of algal photosynthesis (Guo and Chen, 2015).

Microbial communities in algal-prokaryotic wastewater treatment systems are complex and a greater understanding of the symbiotic interactions between algae and prokaryotes is needed to optimize system performance. Several prior review articles have been published on algal-prokaryotic interactions (Cole, 1982; Muñoz and Guieysse, 2006; Ramanan et al., 2016) and algal-based wastewater treatment processes (Abinandan and Shanthakumar 2015; de-Bashan and Bashan, 2010; Gross et al., 2015b; Hoffmann, 1998; Razzak et al., 2013); however, no recent review focuses on N removal in algal-prokaryotic wastewater treatment systems. The aim of this review article is to provide an overview of the algal-prokaryotic interactions, reactor design and configurations, and metabolic N transformation pathways. Molecular methods used for understanding microbial community structure and the outlook for full-scale algal-prokaryotic wastewater treatment applications are included in this paper.

2. Algal-prokaryotic interactions

The “phycosphere” has been used to describe the zone where algal exudates influence other co-occurring microorganisms (Cole, 1982). Algal surfaces provide a favorable micro-niche for opportunistic prokaryotes (Goecke et al., 2010). The interactions between algae and prokaryotes (Fig. 1) are of ecological and biochemical importance at the micro-niche level and affect nutrient cycling and biomass productivity at the system level (Halfhide et al., 2014; Liu et al., 2017).

2.1. CO_2/O_2 exchange

One of the most important algal-prokaryotic interaction is CO_2/O_2 exchange. Aerobic prokaryotes use O_2 produced through algal photosynthesis and produce CO_2 for algal growth. Balanced CO_2/O_2 exchange will avoid accumulation of high DO concentrations, which are toxic to both algae and prokaryotes. Nitrifying prokaryotes also reduce concentrations of free NH_3 and decrease pH to levels that do not inhibit algal growth (de Godos et al., 2009; Gonzalez et al., 2008a). Algal-prokaryotic interactions can also be antagonistic. For example, prokaryotes and algae will compete for available nutrients under nutrient limited conditions (Choi et al., 2010; Risgaard-Petersen et al., 2004; Su et al., 2012; Zhang et al., 2011). The competition for common substrates (CO_2, HCO_3^-, NH_4^+ and O_2) will also shift the microbial communities and N metabolism pathways (de Godos et al., 2016). Sparging CO_2 has been shown to promote nitrification in HRAPs (de Godos et al., 2016; Park et al., 2011b).

2.2. Light intensity

Light is a key factor affecting algal growth; however, light intensity can affect algae and prokaryotes differently. Normally, algal activity increases with increasing light intensity up to the saturation point for the photosynthesis (~200 $\mu mol\ m^{-2}s^{-1}$; Ogbonna and Tanaka, 2000); however, the light saturation level for algae is species-specific (Park et al., 2011a; Torzillo et al., 2003; Bouterfas et al., 2002). For example, freshwater green algae *Selenastrum minutum* showed the highest growth rate at 420 $\mu mol\ m^{-2}s^{-1}$, while the light saturation range for *Scenedesmus obliquus* was 180–540 $\mu mol\ m^{-2}\ s^{-1}$ (Bouterfas et al., 2002; Singh and Singh, 2015). Nitrifiers have been shown to be more sensitive to light than algae (Yoshioka and Saijo, 1984; Vergara et al., 2016).

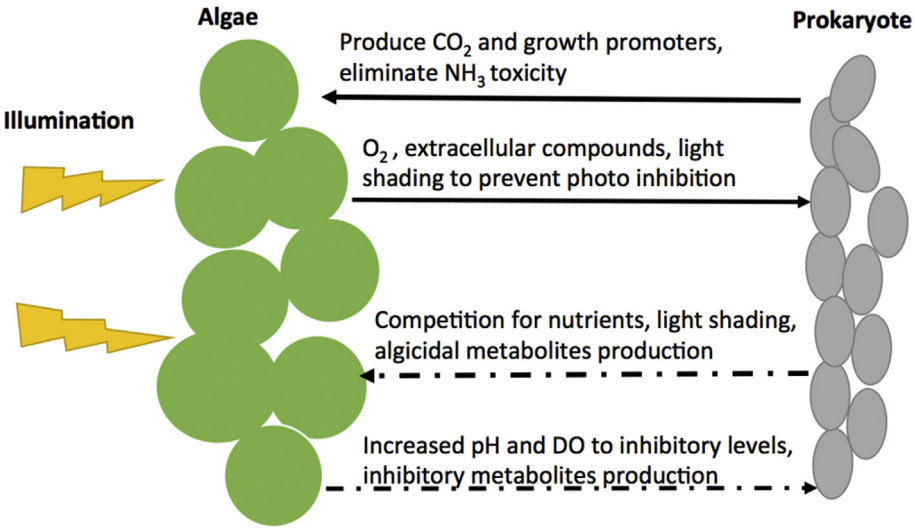

Fig. 1. Algal-prokaryotic interactions. Solids lines indicate positive impacts and dashed lines indicate negative impacts.

Illumination of dilute cultures at only 75 μmol $m^{-2}s^{-1}$ was shown to inhibit the growth of NH_3 oxidizing microorganisms (AOM) and nitrite oxidizing bacteria (NOB) under 12 h light/12 h dark conditions (Yoshioka and Saijo, 1984) and nearly complete nitrification inhibition was observed at 300 μmol $m^{-2}s^{-1}$ (Lipschultz et al., 1985). NOB are more sensitive to light than AOM (Vergara et al., 2016; Yoshioka and Saijo, 1984). Photo-inhibition of AOM and NOB can affect N removal in algal-prokaryotic systems, especially under outdoor conditions where solar illumination can be as high as 600–2000 μmol $m^{-2}s^{-1}$ (Park et al., 2011a; Halfhide et al., 2015). However, shading of AOM and NOB by algae may prevent photo-inhibition. Vergara et al. (2016) did not observe significant nitrification inhibition in an algal-prokaryotic consortium when the incident irradiance was lower than 250 μmol $m^{-2}s^{-1}$.

2.3. Metabolites

Algal and prokaryotic metabolites and their interaction mechanisms are summarized in Table 1. Prokaryotes can benefit algae by releasing growth promoters such as phytohormones (e.g. indoleacetic acid and cytokinins) and vitamin B_{12} (Croft et al., 2005; Goecke et al., 2010; Lau et al., 2009; Tarakhovskaya et al., 2007; Vance, 1987). More than half of algae species require external vitamin B_{12} for vitamin B_{12}-dependent methionine synthase (Croft et al., 2005). Prokaryotes, such as *Halomonas* sp., can produce vitamin B_{12} to support the growth of algae (Croft et al., 2005). Bacterium *Mesorhizobium* sp. was found to support the growth of B_{12}-dependent green algae, *Lobomonas rostrata* (Kazamia et al., 2012). Prokaryotic activity can be enhanced by the presence of extracellular compounds produced by algae, including carbohydrates, transparent exopolymer particles (TEPs), and proteins (Croft et al., 2005; Goecke et al., 2010). Production of extracellular polymeric substances (EPS) by prokaryotes assists in formation of biofilms in algal-prokaryotic biofilm reactors (Hoh et al., 2016), which facilitates biomass retention. Algae can also produce species-specific inhibitory metabolites, which are harmful to prokaryotes (Muñoz and Guieysse, 2006). Prokaryotic growth may also inhibit algal activity by producing algicidal metabolites (Fukami et al., 1997).

Most laboratory studies of algal-prokaryotic interactions have focused on one or two species. However, little is known about the synergistic interactions of the varied species in algal-prokaryotic wastewater treatment systems or how wastewater characteristics, reactor configurations and operating conditions affect community structure, physiology and metabolite production. Research is also needed on the impact of metabolites that remain in the effluent on effluent quality and water reuse potential.

3. Bioreactors for algal-prokaryotic wastewater treatment

Reactor configurations used in algal-prokaryotic wastewater treatment systems include open ponds (WSPs and HRAPs), closed photobioreactors and biofilm (or attached growth) reactors. Simple schematics of these configurations are shown in Fig. 2 and commercial systems available are summarized in Table 2. In general, WSPs and HRAPs have lower initial costs (except for land area requirements) but system control is poor (Muñoz and Guieysse, 2006). Closed systems (tubular or flat plate photobioreactors) have higher initial costs but growth conditions can be well controlled and greater biomass production can be achieved in a smaller area. Therefore reactor selection will be highly dependent on land costs, effluent quality requirements and intended biomass use (Muñoz and Guieysse, 2006). Recently, algae have also been integrated in bioelectrochemical systems to produce electricity and remove nutrients; however, these systems have only be operated at laboratory scale (Luo et al., 2017; Rashid et al., 2013; Xiao and He, 2014).

Insert Fig. 2. Examples of reactor configurations for algal-prokaryotic wastewater treatment. (A) High rate algal pond; (B) closed photobioreactor; (C) flat airlift reactor; (D) airlift reactor; (E) revolving algae biofilm reactor; (F) rotating disk biofilm reactor.

3.1. High rate algal ponds (HRAPs)

HRAPs are typically shallow (0.2–1 m) open raceway ponds lined with PVC, clay or asphalt to reduce infiltration into surrounding soil and groundwater (Abeliovich, 1986; Hoffmann, 1998; Park et al., 2011a). Paddle wheels are normally used to provide turbulence and enhance algal productivity and wastewater treatment efficiency (Craggs et al., 2012). Large scale application of HRAPs for wastewater treatment was first proposed by Oswald and Golueke (1960), and these systems have since been used for treatment of municipal, industrial and agricultural wastewater (Hoffmann, 1998; Park et al., 2011a). HRAPs have been shown to

Table 1
Algal and prokaryotic metabolites and their interaction mechanisms.

Mechanism	Nature of Relationship	Produced by:	Detailed biochemical description	References
Phtyo-hormone production	Positive to algae. No effect on prokaryote.	Prokaryote	Indole- 3-Acetic Acid (IAA) and cytokinins, chelators and other growth factors promote cell division in *Chlorella*.	Lau et al. (2009); Tarakhovskaya et al. (2007); Vance (1987).
Primary metabolite production	Positive for prokaryote. Effect on algae dependent on whether prokaryotes are hosts or scavengers.	Algae	Prokaryotes benefit from production of primary metabolites by algae such as carbohydrates, transparent exopolymer particles (TEPs), amino acids, peptides and proteins. Heterotrophic prokaryotes provide vitamin B_{12} and other metabolites, such as vitamins, which support algal growth. May be negative for algae by causing floc formation and reduced photosynthetic surface area (may be beneficial to harvesting). Prokaryotes entering algal membranes may be detrimental if they penetrate the tissue.	Croft et al. (2005); Goecke et al. (2010); Kazamia et al. (2012).
Bioactive metabolites or exotoxin production	Negative for prokaryote and algae if no defense response is elicited.	Both algae & prokaryote	Prokaryote and algae produce secondary metabolites in an effort to gain a competitive advantage. Algae produce antimicrobial secondary metabolites to reduce microbial attack. Chlorellin, a mixture of fatty acids that can be isolated from *Chlorella*, found to inhibit gram positive and gram negative bacteria. Some cyanobacteria excrete cyanotoxins that may inhibit nitrifiers.	Goecke et al.(2010); Makarewicz et al. (2009); Pratt et al., (1944); Unnithan et al.(2014).
Lysis	Positive for prokaryote negative for algae.	Prokaryote	Algal lysis by bacteria can occur through production of extracellular products or cell-to-cell contact mechanisms. Gram-negative myxobacteria attack and cause lysis of algal cells.	Graham and Wilcox (2000); Kato et al.(1998).
Small-molecule auto-inducer production and detection	Variable, dependent on the specific interaction.	Variable	Alga *Delisea pulchra* coats its surface with a mixture of halogenated furanones that are structurally similar to acyl homoserine lactones (AHLs) and are responsible for community responses in prokaryotes and algae such as flocculation and biofilm formation.	Camilli and Bassler (2006)

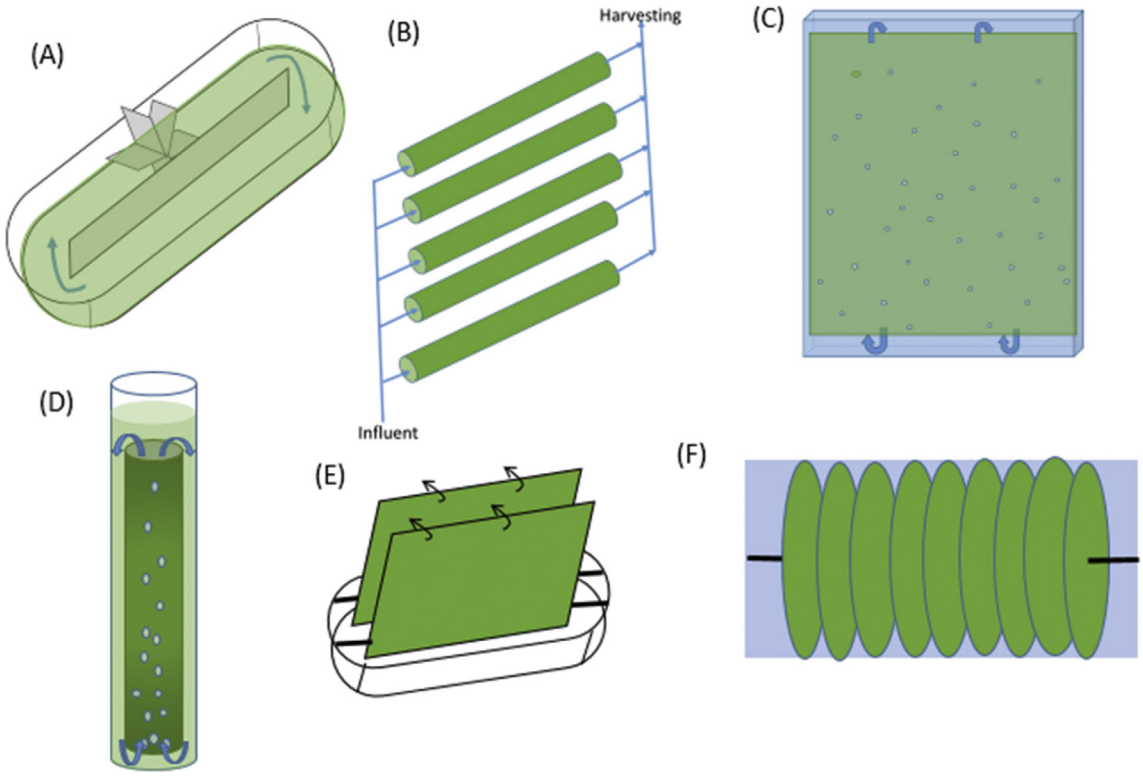

Fig. 2. Examples of reactor configurations for algal-prokaryotic wastewater treatment. (A) High rate algal pond; (B) closed photobioreactor; (C) flat airlift reactor; (D) airlift reactor; (E) revolving algae biofilm reactor; (F) rotating disk biofilm reactor.

provide improved N and phosphorous (P) removal efficiencies compared with WSPs (Garcia et al., 2000). HRAPs have also been suggested as appropriate for sanitation in small rural communities because of their simplicity of operation in comparison to conventional technologies such as activated sludge (García et al., 2006). In addition, Deviller et al. (2004) applied a HRAP for treatment of high nitrate concentration wastewater in a recirculating aquaculture system (RAS) used to produce sea bass (*Dicentrarchus*

Table 2
Commercial algal-prokaryotic wastewater treatment systems.

Reactor Configuration	Algae growth types	Company	Website
Algae Raceway	Suspended growth	MicroBio Engineering	http://microbioengineering.com
Closed tubular Photobioreactor	Suspended growth	SCHOTT North America, Inc.	http://microsites.schott.com/us-pbr/english/index.html
Algae turf scrubbers	Attached growth	HydroMentia	https://hydromentia.com
Algadisk photobioreactor	Attached growth	ALGADISK	http://algadisk.eu
Algaewheel	Attached growth	OneWater	http://www.algaewheel.com
Revolving algal biofilm reactor	Attached growth	Gross-Wen Technologies	http://www.gross-wen.com

labrax). Fish survival rates in a RAS integrated with a HRAP were higher during months when photosynthesis was at the maximum rate (Deviller et al., 2004).

Although there is no generally accepted design manual for HRAPs, Azov and Shelef (1982), Craggs et al. (2014), de Godos et al. (2016), Oswald (1988) and Posadas et al. (2015) provided useful information for large-scale HRAPs design. HRAP depths typically range from 0.2 to 1 m, depending on the wastewater clarity for light penetration (Craggs et al., 2014). Horizontal water velocities between 0.09 and 0.3 m s^{-1} are typically recommended for HRAPs to provide good mixing (Garcia et al., 2000; Park et al., 2011a, 2011b). Recommended hydraulic residence times (HRTs) range from 3 to 15 days (Posadas et al., 2015). HRAP areas range from 1000 to 50,000 m^2 in full-scale applications (Abeliovich, 1986; Craggs et al., 2012).

Disadvantages of HRAPs include high evaporation rates and poor settling biomass (Posadas et al., 2015; Uduman et al., 2010). High evaporation rates (3–10 L m^{-2} d^{-1}) can be partially mitigated by controlling turbulence (Posadas et al., 2015). Commercial technologies, such as chemical flocculation, sedimentation, dissolved air floatation (DAF), filtration and centrifugation have been applied for large-scale microalgae harvesting from HRAPs (Hoffmann, 1998; Udom et al., 2013). Coagulation and sedimentation is the most common and least complex method used in large-scale applications (Udom et al., 2013). DAF with coagulant addition has also been shown to be effective (Green et al., 1995; Uduman et al., 2010; Wiley et al., 2009) but have high energy requirements. Solids separation efficiency in HRAPs can be improved by recycling settled biomass and species control (Park et al., 2011a). An algal-bacterial clay reactor (ABCT) process with added fine clay particles was shown to enhance BOD removal and solids separation in HRAPs (Carberry and Greene, 1992).

3.2. Closed photobioreactors

Compared with WSPs and HRAPs, closed photobioreactors have higher areal biomass productivities and photosynthetic efficiencies, less risk of pollutant volatilization and lower water evaporation losses (Tredici and Zittelli, 1998). Molinuevo-Salces et al. (2010) found that tubular photobioreactors had had similar organic matter removal efficiencies (50–60%) as open ponds when treating anaerobically digested swine slurry; however, effluent from closed photobioreactors had lower total suspended solids (TSS) concentrations.

Closed photobioreactors can be operated in any open space because they are isolated from ambient conditions (Torzillo et al., 1986). However, closed photobioreactors are more expensive to construct and require transparent materials, such as glass and acrylic, for their construction. The high temperatures that develop in closed photobioreactors can adversely affect biomass growth; therefore, cooling systems may be needed to maintain cultures below 40 °C (Torzillo et al., 1986). Another drawback of closed photobioreactors is DO accumulation (Molina et al., 2001). DO concentrations in closed systems can reach as high as 400% of saturation at peak solar irradiance, which is detrimental for both algae and prokaryotes (Molina et al., 2001). Oxygen consumption by prokaryotes in mixed algal-prokaryotic biomass has been shown to decrease negative effects of high DO on algal growth (Bilanovic et al., 2016).

Closed photobioreactor modules can be arranged in a number of ways, including horizontal, inclined, vertical or spiral (Tredici, 2002). Flat plate and tubular photobioreactors are the most common designs due to their large illuminated surfaces and high algal productivity (Ugwu et al., 2008). Compared with tubular photobioreactors, flat plate photobioreactors have been shown to have lower DO accumulation but more problems with temperature control. Scale-up of flat plate photobioreactors is more difficult than for tubular photobioreactors (Borowitzka, 1999; Ugwu et al., 2008). Tubular photobioreactors can be scaled up by increasing the length of the tubular modules or by connecting modules in various configurations (Borowitzka, 1999).

3.3. Algal-prokaryotic biofilm reactors

Challenges in harvesting suspended algae from HRAPs and closed photobioreactors have stimulated interests in the development of algal-prokaryotic biofilm reactors, which produce an effluent with a lower TSS concentration than suspended growth systems (Hoffmann, 1998). In these systems, wastewater passes through the bioreactor while the biomass remains attached to a stationary or moving support medium; therefore, the residence time of the algae and prokaryotes (or mean cell residence time [MCRT]) is much longer than the HRT. This allows algal-prokaryotic biofilm reactors to be operated at higher organic and ammonium loading rates and shorter HRT than suspended growth systems because communities with slow growth rates (e.g. nitrifying bacteria) are retained in the reactor. In addition, the attached growth system has the potential to be operated with greater depth, which decreases reactor footprint. Biomass in these systems must be harvested by scraping the biomass from the support medium (Kesaano and Sims, 2014). Several bench-, pilot- and full-scale studies have been carried out with algal-prokaryotic biofilm reactors (Boelee et al., 2011; Kesaano et al., 2015; Posadas et al., 2013); however, additional research is needed to identify support materials and operating strategies for optimal biofilm formation and wastewater treatment efficiency. Long-term pilot and demonstration studies of algal-prokaryotic biofilm wastewater treatment processes are also needed (Gross et al., 2015b).

3.3.1. Supporting materials

Materials used to support the biofilm growth include polyethylene, polystyrene, polyurethane, loofah, nylon sponges, cardboard and cotton (Gross et al., 2015a; Hoffmann, 1998; Wilkie and Mulbry, 2002). Cotton canvas or cord was shown to be a good support medium (Christenson and Sims, 2012; Gross et al., 2013); however, the cotton needed to be replaced every 2–3 months (Gross et al., 2015b). Wilkie and Mulbry (2002) used polyethylene screen as a support medium for dairy manure waste treatment and

achieved 51–93% total P (TP) removal, 39–62% total N (TN) removal and a biomass productivity of 5.3–5.5 g m^{-2} d^{-1}. Johnson and Wen (2009) found that polystyrene foam provided a good attachment surface for *Chlorella* sp. for treatment of dairy manure wastewater and achieved 62–93% TP removal, 61–79% TN removal and a biomass productivity of 2.57 g m^{-2} d^{-1}.

3.3.2. Algal-prokaryotic biofilm reactor configurations

Algal-prokaryotic biofilms can be divided into stationary or mobile biofilms depending on the motion of the supporting materials. The layout of stationary algal-prokaryotic biofilm reactors can be horizontal or vertical. Algae turf scrubbers (ATS) are stationary biofilm reactors that have been used for both wastewater and surface water treatment (Muñoz et al., 2009). In these systems, flat sheets of material are placed horizontally in a reactor to support the biofilms. ATS have fewer moving parts and lower capital costs than mobile biofilm reactors; however, they require large land areas. Naumann et al. (2012) and Shi et al. (2014) developed a vertical twin layer system consisting of two layers of glass fiber covered with plain print paper for attached growth of biofilms. The flow of the medium was equally distributed to the top of each module using a drip irrigation system. The vertical twin layer system had a smaller footprint than horizontal ATS.

Rotating algal biofilm reactors (RABRs) are mobile biofilm systems in which solid braid cotton cord is coiled around a cylinder for biofilm growth (Christenson and Sims, 2012). The coiled cylinders are partially submerged (40%) in a raceway pond and gear motors are used to drive the rotation of the cylinder. RABRs have achieved dissolved TP and TN removal rates as high as 2.1 and 14.1 g m^{-2} d^{-1}, respectively. Biomass production rates were 5.5 g m^{-2} d^{-1} at bench scale, and as high as 31 g m^{-2} d^{-1} in a pilot-scale reactor treating wastewater effluent. Gross and Wen (2014) described a revolving algal biofilm (RAB) reactor, which consisted of a vertical rotating belt made of cotton duct canvas and a wastewater reservoir. The maximum biomass production rate in a pilot-scale RAB was 19 g m^{-2} d^{-1}.

Algal-prokaryotic biofilm reactors can be purged with CO_2 to increase the biomass productivity or combined with other treatment process to improve wastewater treatment efficiency. Zhang et al. (2015) reported a biomass productivity of 60 g m^{-2} d^{-1} with synthetic wastewater purged with 0.5% CO_2 enriched air in outdoor algal-prokaryotic biofilm reactors. Combining an algal-prokaryotic biofilm reactor with other reactors, such as a membrane reactor, can be used to polish secondary wastewater effluent and further reduced total suspended solids concentrations to less than 0.5 mg L^{-1} (Gao et al., 2015).

3.4. Algal-prokaryotic communities integrated with bioelectrochemical systems

Bioelectrochemical systems are alternative methods for energy neutral wastewater treatment. The basic bioelectrochemical system is the microbial fuel cell (MFC), where heterotrophic bacteria directly transfer electrons from oxidation of organic matter via anodes to the cathode, where electron acceptors (e.g. O_2, NO_3^-) are present, to complete the circuit and generate electricity. The integration of algae into bioelectrochemical systems can generate electricity while removing N, P and metals from wastewater. Algae have been investigated for two different functions in bioelectrochemical systems (Cui et al., 2014; Strik et al., 2008; Xiao and He, 2014): 1) as a substrate at the anode, 2) assisting the cathode process by DO production. Depending on their function, algae cultivation can either be placed in an external photobioreactor or as an internal bioelectrochemical system component. The external photobioreactor can be placed upstream or downstream of the bioelectrochemical system (González del Campo et al., 2013; Strik et al., 2008). The internal algae cultivation can be placed at the anode or cathode.

Despite the promising benefits of integrated algae and bioelectrochemical systems, there are challenges associated with this application. Algal cell walls are difficult to biodegrade (Xiao and He, 2014) and the complex composition of the algal product after pretreatment or hydrolysis are not favorable for energy recovery by MFCs (Xiao and He, 2014). The competition between algae and other microorganisms for nutrients and space are additional challenges for system stability (Xiao and He, 2014). Illumination requirements associated with algal photosynthesis should also be considered in reactor design.

4. Nitrogen transformation processes in algal-prokaryotic systems

Combining microalgae with prokaryotes for wastewater treatment can greatly increase the efficiency of N removal from wastewater through multiple means (Fig. 3). Prokaryotes are responsible for the steps in nitrification and denitrification necessary to completely remove N as nitrogen gas (N_2). Algae and prokaryotes also remove N through assimilation (Stein and Klotz, 2016). As mentioned previously, when illuminated, algae produce O_2, which can facilitate nitrification while diminishing energy inputs associated with aeration (Chae and Kang, 2013).

4.1. Nitrogen transformation pathways

The steps in converting NH_3 to N_2 involve a series of oxidations, followed by reductions, and are catalyzed primarily by prokaryotes utilizing these compounds as respiratory electron donors and electron acceptors (Fig. 3). Nitrification involves the aerobic oxidation of the most reduced form of N, NH_3, to NO_2^- and subsequently to nitrate (NO_3^-). Denitrification (primarily anaerobic) has four major reactions: the reduction of NO_3^- to NO_2^-, NO_2^- to nitric oxide (NO), NO to nitrous oxide (N_2O), and finally N_2O to N_2 (Zumft, 1997). The sequential steps of N oxidation and reduction are catalyzed by different organisms. Oxidation of NH_3 to NO_3^- is separated to two groups; AOM, which oxidize NH_3 to NO_2^-, and NOB, which oxidize NO_2^- to NO_3^- (Nunes-Alves, 2016). Denitrification is carried about by a wide phylogenetic array of bacteria (see below) under anaerobic or microaerophilic conditions.

This 'classic' view of the N cycle has been complicated by recent discoveries of alternatives to some of the steps in the cycle. Recently, it was discovered that some organisms are capable of completely oxidizing NH_3 to NO_3^- (Daims et al., 2015). Shortcut N removal includes nitrification of NH_3 to NO_2^- followed by denitrification beginning from NO_2^-, skipping the steps that produce and consume NO_3^- (Zanetti et al., 2012). NH_3 can also be anaerobically oxidized by anammox (anaerobic ammonia oxidizing) bacteria, which produce N_2 by combining NH_3 with NO_2^- if oxygen tensions are very low. A small amount of NO_3^- will also be produced in anammox systems (Sonthiphand et al., 2014). Dissimilatory NO_3^- reduction to NH_3 (DNRA), instead of through NO to N_2O to form N_2, is also a relevant process in wastewater treatment (Kraft et al., 2011).

In addition to being used as respiratory electron donors or acceptors, N compounds can be assimilated into biomass. NH_3, NO_2^- and NO_3^- are readily assimilated by microorganisms from all three domains of life. Both algae and prokaryotes can assimilate and reduce NO_2^- and NO_3^- to NH_3, which is incorporated into amino acids, nucleotides, and other nitrogenous biomolecules. In algae, NO_3^- is transported into the cytoplasm, where cytoplasmic NO_3^- reductase reduces it to NO_2^-, which is then transported into the

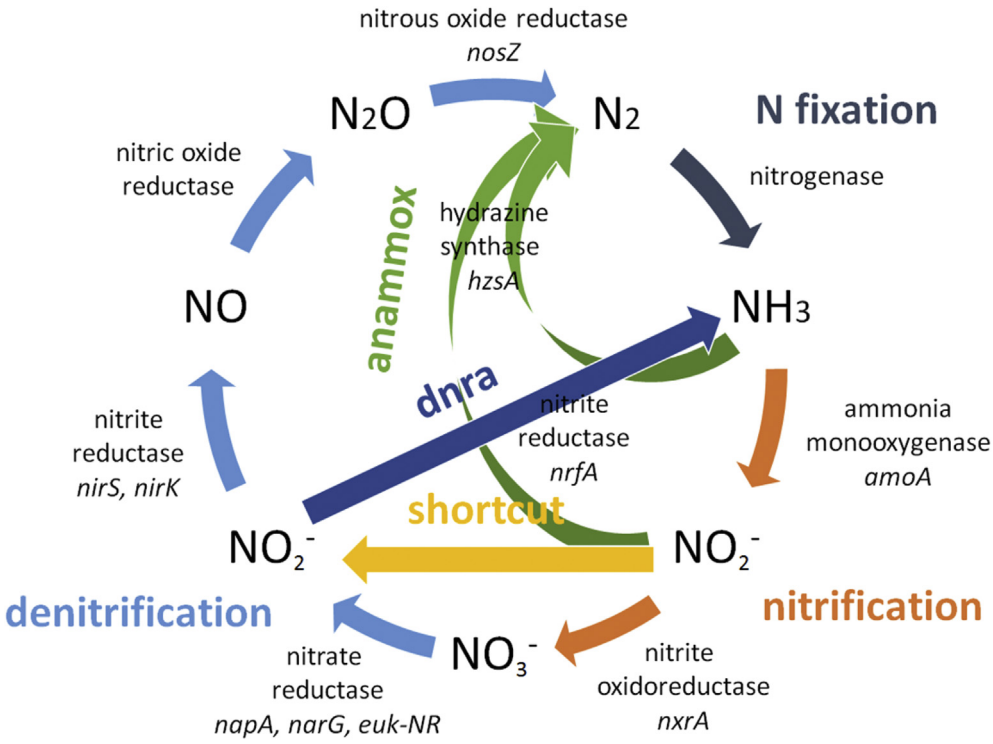

Fig. 3. Steps of the N cycle, with genes (in italic) used to verify the genetic potential for, and activity of, each step.

chloroplast, where it is further reduced to NH_3, which is assimilated into biomass (reviewed in Sanz-Luque et al., 2015).

Chlorella vulgaris is capable of assimilating multiple forms of dissolved inorganic N. If multiple forms are available, it will first assimilate NH_3, then NO_2^-, and then NO_3^-, because NH_3 does not need to be reduced for amino acid synthesis (Barsanti and Gualtieri, 2014). NH_3 is moderately lipid soluble and therefore diffuses through the membrane, while NH_4^+ is taken up by energy requiring transport mechanisms (Nokkaew et al., 2013). Although NH_3 is readily assimilated, free NH_3 is toxic to microalgae at high concentrations, with optimal growth between 20 and 250 mg L^{-1} of total ammonia N (TAN). *C. vulgaris* is capable of growth at TAN concentrations from 10 to 1000 mg L^{-1}, but grows poorly with less than 10 mg L^{-1} or greater than 750 mg L^{-1} (Tam and Wong, 1996).

Alternative algal N metabolisms are currently being uncovered. A few eukaryotes, including representatives of fungi, foraminifers, and diatoms, are capable of growing under anaerobic conditions, and physiological measurements indicate that they can respire NO_3^- (Kamp et al., 2015). Some diatoms appear to respire NO_3^- to NH_3 (similar to DNRA), while some benthic foraminifers and fungi release N_2O; foraminifers also release N_2 (Kamp et al., 2015). Of particular concern when considering application of *Chlorella* in 'green' biotechnologies is the formation of N_2O by *C. vulgaris*, as N_2O is a potent greenhouse gas (Weathers, 1984). This observation was initially disputed because of the possibility of bacterial contamination of cultures used in the study. However, when the cultures were treated with antibiotics, and PCR-assayed to verify the absence of bacterial contamination, N_2O formation was still detected (Guieysse et al., 2013). Production of this gas was stimulated under conditions that favor NO_2^- accumulation in *C. vulgaris*, including incubation in the dark (when the chloroplast-localized NO_2^- reductase, which is normally supplied with reductant generated via photosystems, cannot operate), and after NO_2^- addition to cells treated with photosynthesis inhibitors. Consistent with a requirement for NO_2^-, N_2O production ceased when NO_3^- reductase activity was inhibited. Nitrate reductase can use NO_2^- as a substrate to form NO, and this possibility is consistent with these observations. However, the mechanism of formation of N_2O from NO cannot be elucidated from these observations (Guieysse et al., 2013). Further research on the mechanism of N_2O production is needed to optimize engineered algal-prokaryotic wastewater treatment systems to assure that N_2O emissions are not a concern.

4.2. Nitrogen transformation pathways in algal-prokaryotic bioreactors for wastewater treatment

The metabolic pathways and fate of N in algal-prokaryotic wastewater treatment systems depend on the wastewater characteristics, reactor operating regime and environmental conditions. Su et al. (2011) estimated that 40–53% and 17–20% of NH_3 removal was due to assimilation and nitrification, respectively, when municipal wastewater was treated in a stirred tank photobioreactor. González-Fernández et al. (2011) reported that ammonium (NH_4^+) was mainly removed by nitrification, followed by assimilation and denitrification when anaerobically digested swine slurry was treated in open ponds. García et al. (2000) found that NH_3 stripping accounted for most of the N removed in the HRAPs with pH ranging from 7.63 to 9.93.

Several authors have investigated N removal in wastewaters with varying NH_4^+ concentrations. He et al. (2013) reported that 47–63% of the N supplied was assimilated by algal and prokaryotic biomass when NH_4^+ concentrations were between 29 and 174 mg N L^{-1}, while simultaneous nitrification/denitrification was observed at 656 mg N L^{-1}.

Operational factors, such as MCRT and food to microorganism ratio (F/M) can also affect the N removal pathway in algal-prokaryotic bioreactors. For example, HRAPs operated at shorter MCRTs had higher bacteria/algae ratios, resulting in higher nitrification rates (Park and Craggs, 2011). Medina and Neis (2007) found well settling flocs along with high nutrient removal efficiency were

achieved at HRT of 4 days and low F/M ratio of 0.15 d^{-1}.

Seasonal variations of N metabolism pathways were observed during outdoor operation of a HRAP (de Godos et al., 2016). The proportion of NH_4^+ removal through nitrification increased during summer resulting in lower NH_4^+ concentrations. Percentage of N loss through volatilization was higher in winter, because of the relatively high reactor NH_4^+ concentration (de Godos et al., 2016).

4.3. Enhanced algal-prokaryotic wastewater treatment systems (EAPS) for nitrogen removal

Recently, enhanced algal-prokaryotic wastewater treatment systems (EAPS) have been developed to enhance N removal efficiency by providing environmental conditions required to optimize the functions of different groups of microorganisms. EAPS take advantage of high N and COD removal rates of bacteria and DO production by algae. In addition, EPS produced by both algae and prokaryotes promote the formation of flocs with good settling ability, which allows for separation of HRT and MCRT (Van Den Hende et al., 2016; Wang et al., 2014, 2015b). Most EAPS are still in the bench-scale research stage.

Combining algae with an activated sludge system (photo-activated sludge) has been shown to improved nutrient removal efficiency, especially under lower aeration conditions (Medina and Neis, 2007; van der Steen et al., 2015). Karya et al. (2013) investigated a photo-activated sludge sequencing batch reactor (SBR) for the treatment of artificial municipal wastewater without aeration. In this system, approximately 81–85% of NH_4^+ was removed due to nitrification rather than biomass uptake. The oxygen production of the algae (0.46 kg m^{-3} d^{-1}) was significantly higher than that of HRAPs (0.3–0.38 kg m^{-3} d^{-1}). Well settling biomass allowed the SBR to be operated at short HRT and long MCRT, which allowed microorganisms with low growth rates to be retained in the bioreactor. Anbalagan et al. (2016) operated a photo-activated sludge process with varying HRTs and found that TN removal was 76%–86% and 48–81% at HRTs of 6 and 4 days, respectively. In the combined system, the activated sludge was responsible for COD removal and the addition of a small amount of algae (9% by mass) improved N removal efficiency (Roudsari et al., 2014).

de Godos et al. (2014) applied a two-stage anoxic-aerobic photobioreactor with an algal-prokaryotic consortium to simultaneously remove organic carbon (C) and N from synthetic wastewater. The long MCRT (20–30 days) promoted the development of both nitrifiers and denitrifiers. The system removed 95% of organic C and 90% of N when treating synthetic wastewater containing 200 mg L^{-1} of organic C and 140 mg N L^{-1} of NH_4^+. The biomass also had good settling ability, with a sedimentation rate of 0.28–0.42 m h^{-1}.

Taking advantage of the fact that different organisms with different growth requirements catalyze different N transformation steps, environmental conditions can be controlled to select for different populations in the algal-prokaryotic consortia. For example, shortcut N removal can be enhanced by controlling DO concentrations to inhibit NOB while favoring AOM (Peng and Zhu, 2006). The wastewater then enters an anoxic phase, where denitrifiers (and potentially anammox bacteria) reduce NO_2^- to N_2. Skipping the oxidation of NO_2^- to NO_3^- also results in skipping the reduction of NO_3^- to NO_2^-, therefore less electron donor is needed to facilitate denitrification (Ge et al., 2015).

NO_2^- accumulation has been observed in studies of algal-prokaryotic consortia treating high NH_4^+ strength wastewater (Gonzalez et al., 2008a, 2008b). However, TN removals were relatively low in these studies because an optimized anoxic stage was not applied. Wang et al. (2015b) developed a shortcut N removal process using an algal-prokaryotic consortium for the treatment of centrate from anaerobically digested swine manure in a photo-sequencing batch reactor (PSBR) operated in a 12 h light/12 h dark cycle. Oxygen for nitritation was mainly provided by algal photosynthesis during the light period. NOB were inhibited by alternating high NH_4^+ and NO_2^- concentrations and low DO concentrations. With addition of acetate during the dark period, > 90% TN removal was achieved. The PSBR produced algal-prokaryotic flocs with sizes of up to 1 mm and good settling ability, with an average sludge volume index (SVI) < 70 mL g^{-1}. Similar nutrient removal efficiency and the formation of well-settled floc were observed by Arashiro et al. (2017) using a PSBR with SRTs of 7 days and 11 days.

The ALGAMMOX (algal anaerobic ammonium oxidation) process developed by Manser et al. (2016) is similar to the algal-prokaryotic shortcut N process but replaced heterotrophic denitrification with anammox. The system combined algae and AOM with anammox granules in a PSBR with alternating light and dark periods. NH_3 conversion to NO_2^- increased by addition of anammox to a mixed culture of algae and AOB from 4.5 to 7.0 mg NH_4^+–N L^{-1} h^{-1} during the light period. Meanwhile NO_2^- was completely removed by anammox activity during the dark period. No C source addition was needed for this process.

4.4. Molecular methods used to analyze N transformations

Molecular tools can be used to evaluate the activities of algal-prokaryotic consortia by targeting key steps of N metabolism. The traditional method is analysis of 16S rRNA (Rowan et al., 2002; Srithep et al., 2014) such as fluorescence *in situ* hybridization (FISH), terminal restriction fragment length polymorphism (T-RFLP) (Saikaly et al., 2005), and denaturing gradient gel electrophoresis (DGGE) (Boon et al., 2001; Juretschko et al., 1998; Nicolaisen and Ramsing, 2002). 16S rRNA analysis provides taxonomic information about microbial communities, but does not provide clear indications about metabolic activities since biochemical capabilities do not match 16S phylogenies. Therefore, a purely taxonomical approach targeting 16S or 18S ribosomal subunits would not give an accurate representation of the metabolic functions. Instead, metabolic activities (e.g. N cycling activity) can be tracked using molecular techniques targeting an essential component (e.g., enzyme), for each pathway. Genes encoding enzymes can be targeted to detect the functional potential in genomic DNA (PCR) or transcriptional activity via RNA presence (RT-PCR). Quantitative PCR (qPCR) can be used to compare the relative number of gene copies encoding these steps in different samples. Quantitative reverse transcriptase PCR (qRT-PCR) is then used to measure transcript abundance, which indicates that the target genes are likely being expressed.

Functional genes encoding enzymes that are required to catalyze N transformations can be used to evaluate the potential presence and activity of the different steps in the N cycle (Supplementary Table 1). For nitrification, ammonia monooxygenase is responsible for NH_3 oxidation to NO_2^-. Ammonia monooxygenase (*amoCAB)* is expressed by AOM, which include proteobacteria, *Nitrospira,* as well as *Thaumarchaeans* (Calvó et al., 2005; Gao et al., 2014; Kowalchuk et al., 2000; Rotthauwe et al., 1997; O'Mullan and Ward, 2005; Purkhold et al., 2000). NH_3 oxidizing proteobacteria (AOB) are comprised primarily of beta- (*Nitrosomonas* and *Nitrosospira*) and gamma-proteobacteria (*Nitrosococci*) (Calvó et al., 2005); NH_3 oxidizing archaea (AOA) include *Nitrososphaera* and *Nitrosopumilus*. A single primer pair cannot be designed to amplify all *amoA* genes because of the level of gene sequence divergence among these distinct lineages; therefore, multiple primer pairs have been designed to target AOA and AOB *amoA* genes individually (Gao et al., 2014). Nitrite

oxidoreductase can be used to identify the potential for NO_2^- oxidation, and is encoded by the *nxrAXB* operon (Starkenburg et al., 2006), formerly called *nor* operon (Kirstein and Boek, 1993). The known NOB (*Nitrobacter*, *Nitrococcus*, *Nitrospina* and *Nitrospira* genera) are distributed among four phylogenetic groups (Alpha-, Gamma-, *Deltaproteobacteria*, and *Nitrospira*, respectively) (Bock et al., 1990; Watson and Waterbury, 1971). To identify the genetic potential for, or transcript abundance related to, NO_2^- oxidation, the *nxrA* gene is targeted (Wang et al., 2015a).

Denitrification begins with NO_3^- reduction via nitrate reductase. A phylogenetically broad range of microorganisms are capable of using NO_3^- as a terminal electron acceptor (Supplementary Table 1), and this diversity is reflected in a variety of forms of this enzyme. There are three forms of nitrate reductase; two are dissimilatory: periplasmic (encoded by *napEDABC*), and membrane bound (encoded by *narGHJI*). The last is assimilatory (encoded by *nasABC*) (Zumft, 1997). Genes encoding subunits of the dissimilatory nitrate reductases, *napA* and *narG,* are used for tracking this pathway (Wang et al., 2015a). Nitrite reductase catalyzes the reduction of NO_2^- to NO and has two forms, copper-dependent type K and cytochrome cd_1 type S. The presence of either type of nitrite reductase is determined by targeting *nirS* and *nirK* genes (Braker et al., 2000). Nitric oxide and nitrous oxide reductases complete denitrification, reducing NO to N_2. Nitrous oxide reductase is found in all classes of proteobacteria, as well as in a few Gram-positive bacteria, and *nosZ* is frequently used as a functional biomarker for nitrous oxide reductase presence (Pauleta et al., 2013).

DNRA is catalyzed by a very broad group of microorganisms including members of the gamma-, delta-, and epsilonproteobacteria, as well as *Bacteriodetes*. The genes encoding the initial reduction of NO_3^- to NO_2^- are not unique to this process. However, the pentaheme cytochrome c nitrite reductase encoded by *nrfA*, which is responsible for reducing NO_2^- directly to NH_3, is a good marker for DNRA, though it is important to note that the relatively few *nrfA* sequences available to use to design PCR primers compromises the ability to detect this gene in nature (Kraft et al., 2011).

The anaerobic alternative to NH_3 oxidation is the anammox pathway. This pathway has been detected in the order *Brocadiales* of the phylum *Planctomycetales* (Harhangi et al., 2012). Five genera are currently known to be capable of anammox: "*Candidatus Brocadia*," "*Candidatus Kuenenia*," "*Candidatus Scalindua*," "*Candidatus Anammoxoglobus*," and "*Candidatus Jettenia*" (Kartal et al., 2007, 2011; Quan et al., 2008; Schmid et al., 2007). The anammox process is catalyzed by hydrazine synthase (encoded by *hzsCBA),* which forms hydrazine from NH_3 and NO_2^-, and hydrazine oxidoreductase, which oxidize hydrazine to N_2. This pathway is best earmarked by hydrazine synthase (*hzsA*) because this gene is unique to anammox bacteria (Harhangi et al., 2012).

For algae, the only N transformations for which genes have been characterized include assimilatory N metabolism (e.g., those encoding assimilatory nitrate reductase *euk-NR*, as well as nitrite reductase). The amino acid sequence of eukaryotic nitrate reductase is divergent from prokaryotic, and it may be possible to design primers capable of distinguishing it from the prokaryotic versions of this enzyme (Stolz and Basu, 2002). So far, the genes encoding eukaryotic dissimilatory nitrate reduction have only been identified in fungi (Kamp et al., 2015), and the molecular mechanism for N_2O formation by *C. vulgaris* is unknown (Sanz-Luque et al., 2015).

5. Challenges and future trends of full-scale algal - prokaryotic wastewater treatment systems

The challenges of full-scale application of algae-based wastewater treatment systems include: varying environmental conditions, varying wastewater compositions, high land area requirements, and difficulty in harvesting and downstream processing of the algae to produce valuable products (Acién et al., 2016; Novoveská et al., 2016; Van Den Hende et al., 2014).

Changes in environmental conditions will shift the microbial communities and affect system stability. Operational strategies, such as harvesting frequency, reactor design, and inoculum size, can be adapted to optimize conditions and adjust environmental variables (Novoveská et al., 2016). Up-scaling from indoor reactors to a 12 m^3 outdoor reactor shifted the dominant algal species from filamentous cyanobacteria (*Phormidium* sp.) to filamentous (*Ulothrix* sp. or *Klebsormidium* sp.), which contained high amounts of antibiotics, chlorophyll, and carotenoids (Van Den Hende et al., 2014). Novoveská et al. (2016) found that the dominant algal community shifted from *Scenedesmus dimorphus* to a diverse polyculture of the genera *Chlorella*, *Cryptomonas,* and *Scenedesmus* in a large-scale application (50,000 gal d^{-1}). Up-scaling reactors may also reduce light penetration (photosynthetic photon flux density), which favors the growth of bacteria. Van Den Hende et al. (2014) found a decrease in nutrient removal efficiency and biomass productivity when up-scaling SBRs to an outdoor raceway pond. Biomass productivity is directly affected by temperature. Temperatures as low as 0 °C will be detrimental to algae growth (Novoveská et al., 2016). Heating with waste heat can be considered in the winter (Van Den Hende et al., 2014).

Wastewater composition, such as the presence of heavy metals, trace organic compounds and nanoparticles, may inhibit algal growth. High organic matter concentrations tend to induce greater proportions of bacteria (Acién et al., 2016). Phenanthrene concentrations of 10 mg L^{-1} were shown to inhibit the growth of *Chlorella sorokiniana* (Acién et al., 2016). TiO_2 – nanoparticles, which have been detected in soil and surface water, were also shown to inhibit algal growth (Li et al., 2015). The small particles can pass through the algal cells and produce oxidative stress caused by reactive oxygen species accumulation inside the chloroplasts.

Up-scaling under outdoor conditions was shown to increase pH (de Godos et al., 2016). pH control is especially important when treating wastewater with high ammonia concentrations, as high pH increases free NH_3 concentrations. High pH levels can also decrease bacteria and microalgae grazer populations and trigger lipid accumulation (Tan et al., 2016). Sparging CO_2 or flue gas was recommended for pH maintenance but will increase costs by about 20% (de Godos et al., 2016).

The robustness of biological and engineering aspects of the process is critical for full-scale applications. The process could become more economically competitive with conventional wastewater treatment processes by: 1) increasing the robustness of the process by means, such as adding an equalization tank, to ensure that effluents meet discharge limits under changing environmental conditions and wastewater characteristics, 2) reducing HRTs from 7 to 11 days to approximately 0.3 days so that it is comparable to conventional wastewater treatment processes, and 3) reducing power consumption below 0.5 kWh m^{-3} of wastewater (Acién et al., 2016). The developed process should be operated under real conditions for an extended period that includes different geographic regions, seasons and shock loads to validate the system stability (Cai et al., 2013; Zhu et al., 2013).

Nutrient concentrations also need to be considered when designing and operating algal-prokaryotic wastewater treatment processes. When treating secondary effluent with low nutrient concentrations, membrane reactors have been shown to be appropriate to retain algal cells (Acién et al., 2016). When treating high strength wastewaters, such as anaerobic digester centrate, dilution may be needed, which reduces the net flow capacity and the reliability of the overall process (Acién et al., 2016). Recently, Wang et al. (2017) reported a hybrid algal photosynthesis and ion-

exchange (HAPIX) process where natural zeolite was used as NH_4^+ adsorbent to decrease the toxicity of high NH_4^+ strength wastewater to algae (>1000 mg L^{-1} as N). The HAPIX eliminated the need for dilution.

High land requirements are an impediment that can be alleviated by improving algal photosynthetic efficiency. Offshore cultivation can also minimize land requirement (Novoveská et al., 2016). Reducing the surface area from approximately 6-10 m^2 per person equivalent to 2–3 m^2 per person equivalent would be required to improve the economic balance (Acién et al., 2016). The profit from biomass production can enhance the economic sustainability, especially in small cities.

The harvesting and the processing of algae to produce valuable products also poses a challenge. The harvested biomass can be used for anaerobic co-digestion, biofertilizer, pigments and biostimulants, which produce not only nutrients but also phytohormones and growth promoters for agricultural purposes. The utilization of biomass for bioplastics, animal feed or biofuels are still in the conceptual stages due to the regulatory and technical obstacles (Acién et al., 2016).

6. Conclusions

Algal-prokaryotic wastewater treatment systems have the potential to improve the environmental and economic sustainability of wastewater treatment. The following is a summary of the key points of this review and associated research needs:

- Algae provide DO through photosynthesis that can be used by aerobic heterotrophs and nitrifiers, significantly reducing the aeration cost of wastewater treatment. Interactions between algae and prokaryotes also affect light, inorganic and organic C availability, the formation of stable biofilm communities, and the presence of toxics (e.g. oversaturated DO, free NH_3), growth promoters and growth inhibitors in the medium. More work is needed to understand these complex interactions in wastewater treatment systems.
- A number of reactor configurations, such as HRAPs, closed photobioreactors, and algal-prokaryotic biofilm reactors have been developed to take advantage of algal-prokaryotic interactions and to separate HRT and MCRT to retain slow-growing prokaryotes, such as nitrifiers. These systems have resulted in improved effluent quality, reduced biomass harvesting costs and reduced reactor volume requirements.
- EAPS have been developed to use natural diurnal light fluctuations to provide periods of aerobic (light) and anaerobic (dark) conditions. This promotes the activity of prokaryotes that catalyze key steps in the N cycle, resulting in high TN removal efficiencies. However, most of these systems are still in the bench-scale stage of research; pilot and full-scale demonstrations are needed to evaluate the stability of these processes during different seasons and in different geographic locations.
- Molecular tools have expanded our understanding of the activities of prokaryotic communities in wastewater treatment processes; however, few studies were identified that have used these methods to investigate algal-prokaryotic consortia in wastewater treatment systems. Further, the primers necessary for most molecular queries of microbial communities (e.g., via PCR) need to be regularly updated as new pathways are elucidated, more organisms are discovered, and more genomes are sequenced.
- The critical issues for full-scale application of algal-prokaryotic wastewater treatment systems are the system stability and robustness under varying environmental conditions, high land requirements and high cost of biomass harvesting and downstream processing to produce valuable products. Biological and engineering aspects to address these issues, such as improving algal photosynthetic efficiencies are needed.

Acknowledgements

This material is based upon work supported by the National Science Foundation under Grants Nos. 1511439 and 1243510. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2018.04.021.

References

Abeliovich, A., 1986. Algae in wastewater oxidation ponds. In: Handbook of Microalgal Mass Culture, vol. 35, pp. 331–338.
Abinandan, S., Shanthakumar, S., 2015. Challenges and opportunities in application of microalgae (Chlorophyta) for wastewater treatment: a review. Renew. Sustain. Energy Rev. 52, 123–132.
Acién, F.G., Gómez-Serrano, C., Morales-Amaral, M.M., Fernández-Sevilla, J.M., Molina-Grima, E., 2016. Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? Appl. Microbiol. Biotechnol. 100 (21), 9013–9022.
Anbalagan, A., Schwede, S., Lindberg, C.-F., Nehrenheim, E., 2016. Influence of hydraulic retention time on indigenous microalgae and activated sludge process. Water Res. 91 (15), 277–284.
Arashiro, L.T., Rada-Ariza, A.M., Wang, M., van der Steen, P., Ergas, S.J., 2017. Modelling shortcut nitrogen removal from wastewater using an algal–bacterial consortium. Water Sci. Technol. 75 (4), 782–792.
Azov, Y., Shelef, G., 1982. Operation of high-rate oxidation ponds: theory and experiments. Water Res. 16 (7), 1153–1160.
Barsanti, L., Gualtieri, P., 2014. Algae: Anatomy, Biochemistry, and Biotechnology. CRC Press Taylor & Francis Group, Boca Rotan, USA, p. 345.
Bilanovic, D., Holland, M., Starosvetsky, J., Armon, R., 2016. Co-cultivation of microalgae and nitrifiers for higher biomass production and better carbon capture. Bioresour. Technol. 220, 282–288.
Bock, E., Koops, H.-P., Moiler, U.C., Rudert, M., 1990. A new facultatively nitrite oxidizing bacterium, Nitrobacter vulgaris sp. nov. Archives Microbiol. 153, 105–110.
Boelee, N.C., Temmink, H., Janssen, M., Buisman, C.J.N., Wijffels, R.H., 2011. Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms. Water Res. 45, 5925–5933.
Boon, N., Windt, W.D., Verstraete, W., Top, E.M., 2001. Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. Microbiol. Ecol. 39, 101–112.
Borowitzka, M.A., 1999. Commercial production of microalgae: ponds, tanks, and fermenters. Prog. Ind Microbiol. 35, 313–321.
Bouterfas, R., Belkoura, M., Dauta, A., 2002. Light and temperature effects on the growth rate of three freshwater [2pt] algae isolated from a eutrophic lake. Hydrobiologia 489 (1), 207–217.
Braker, G., Zhou, J., Wu, L., Devol, A.H., Tiedje, J.M., 2000. Nitrite reductase genes (nirK and nirS) as functional markers to investigate diversity of denitrifying bacteria in pacific northwest marine sediment communities. Appl. Environ. Microbiol. 66 (5), 2096–2104.
Cai, T., Park, S.Y., Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae: status and prospects. Renew. Sustain. Energy Rev. 19, 360–369.
Calvó, L., Cortey, M., García-Marín, J.-L., Garcia-Gil, L.J., 2005. Polygenic analysis of ammonia-oxidizing bacteria using 16S rDNA, amoA, and amoB genes. Int. Microbiol. 8, 103–110.
Carberry, J.B., Greene, R.W., 1992. Model of algal bacterial clay wastewater treatment system. Water Sci. Technol. 7/8, 1697.
Chae, K.-J., Kang, J., 2013. Estimating the energy independence of a municipal wastewater treatment plant incorporating green energy resources. Energy Convers. Manag. 75, 664–672.
Choi, O., Das, A., Yu, C.P., Hu, Z., 2010. Nitrifying bacterial growth inhibition in the presence of algae and cyanobacteria. Biotechnol. Bioeng. 107 (6), 1004–1011.
Christenson, L.B., Sims, R.C., 2012. Rotating algal biofilm reactor and spool harvester for wastewater treatment with biofuels by-products. Biotechnol. Bioeng. 109 (7), 1674–1684.
Cole, J.J., 1982. Interactions between bacteria and algae in aquatic ecosystems. Annu. Rev. Ecol. Syst. 13, 291–314.
Craggs, R., Park, J., Heubeck, S., Sutherland, D., 2014. High rate algal pond systems for low-energy wastewater treatment, nutrient recovery and energy

production. New Zealand J. Bot. 52 (1), 60–73.
Craggs, R., Sutherland, D., Campbell, H., 2012. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. J. Appl. Phycol. 24 (3), 329–337.
Craggs, R.J., Adey, W.H., Jenson, K.R., St. John, M.S., Green, F.B., Oswald, W.J., 1996. Phosphorus removal from wastewater using an algal turf scrubber. Water Sci. Technol. 33 (7), 191–198.
Croft, M.T., Lawrence, A.D., Raux-Deery, E., Warren, M.J., Smith, A.G., 2005. Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature 438 (7064), 90–93.
Cui, Y., Rashid, N., Hu, N., Rehman, M.S.U., Han, J.I., 2014. Electricity generation and microalgae cultivation in microbial fuel cell using microalgae-enriched anode and bio-cathode. Energy Convers. Manag. 79, 674–680.
Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R.H., Bergen, M.V., Rattei, T., Bendinger, B., Nielsen, P.H., Wagner, M., 2015. Complete nitrification by Nitrospira bacteria. Nature 528 (7583), 504–509.
de-Bashan, L.E., Bashan, Y., 2010. Immobilized microalgae for removing pollutants: review of practical aspects. Bioresour. Technol. 101 (6), 1611–1627.
de Godos, I., González, C., Becares, E., García-Encina, P., Muñoz, R., 2009. Simultaneous nutrients and carbon removal during pretreated swine slurry degradation in a tubular biofilm photobioreactor. Appl. Microbiol. Biotechnol. 82 (1), 187–194.
de Godos, I., Vargas, V.A., Guzmán, H.O., Soto, R., García, B., García, P.A., Muñoz, R., 2014. Assessing carbon and nitrogen removal in a novel anoxic–aerobic cyanobacterial–bacterial photobioreactor configuration with enhanced biomass sedimentation. Water Res. 61 (0), 77–85.
de Godos, I., Arbib, Z., Lara, E., Rogalla, F., 2016. Evaluation of high rate algae ponds for treatment of anaerobically digested wastewater: effect of CO_2 addition and modification of dilution rate. Bioresour. Technol. 220, 253–261.
Deviller, G., Aliaume, C., Nava, M.A.F., Casellas, C., Blancheton, J.P., 2004. High-rate algal pond treatment for water reuse in an integrated marine fish recirculating system: effect on water quality and sea bass growth. Aquaculture 235 (1–4), 331–344.
EPA, 2017. Sources of Greenhouse Gas Emissions. https://www.epa.gov/ghgemissions/sources-greenhouse-gas-emissions. Accessed in September 2017.
EPRI, 2002. Water & Sustainability (Volume 4): U.S. Electricity Consumption for the Water Supply & Treatment–the Next Half Century. EPRI.
Fukami, K., Nishijima, T., Ishida, Y., 1997. Stimulative and inhibitory effects of bacteria on the growth of microalgae. Hydrobiologia 358 (1), 185–191.
Gao, F., Yang, Z.H., Li, C., Zeng, G.M., Ma, D.H., Zhou, L., 2015. A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent. Bioresour. Technol. 179, 8–12.
Gao, J., Luo, X., Wu, G., Li, T., Peng, Y., 2014. Abundance and diversity based on amoA genes of ammonia-oxidizing archaea and bacteria in ten wastewater treatment systems. Appl. Microbiol. Biotechnol. 98 (7), 3339–3354.
García, J., Green, B.F., Lundquist, T., Mujeriego, R., Hernández-Mariné, M., Oswald, W.J., 2006. Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. Bioresour. Technol. 97 (14), 1709–1715.
Garcia, J., Mujeriego, R., Hernandez-Marine, M., 2000. High rate algal pond operating strategies for urban wastewater nitrogen removal. J. Appl. Phycol. 12 (3–5), 331–339.
Ge, S., Wang, S., Yang, X., Qiu, S., Li, B., Peng, Y., 2015. Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: a review. Chemosphere 140, 85–98.
Goecke, F., Labes, A., Wiese, J., Imhoff, J., 2010. Chemical interactions between marine macroalgae and bacteria. Mar. Ecol. Prog. Ser. 409, 267–299.
González-Fernández, C., Molinuevo-Salces, B., García-González, M.C., 2011. Nitrogen transformations under different conditions in open ponds by means of microalgae–bacteria consortium treating pig slurry. Bioresour. Technol. 102 (2), 960–966.
Gonzalez, C., Marciniak, J., Villaverde, S., Garcia-Encina, P.A., Munoz, R., 2008a. Microalgae-based processes for the biodegradation of pretreated piggery wastewaters. Appl. Microbiol. Biotechnol. 80 (5), 891–898.
Gonzalez, C., Marciniak, J., Villaverde, S., Leon, C., Garcia, P.A., Munoz, R., 2008b. Efficient nutrient removal from swine manure in a tubular biofilm photobioreactor using algae-bacteria consortia. Water Sci. Technol. 58 (1), 95–102.
González del Campo, A., Cañizares, P., Rodrigo, M.A., Fernández, F.J., Lobato, J., 2013. Microbial fuel cell with an algae-assisted cathode: a preliminary assessment. J. Power Sources 242, 638–645.
Green, F.B., Lundquist, T.J., Oswald, W.J., 1995. Energetics of advanced integrated wastewater pond systems. Water Sci. Technol. 31 (12), 9–20.
Gross, M., Henry, W., Michael, C., Wen, Z., 2013. Development of a rotating algal biofilm growth system for attached microalgae growth with in situ biomass harvest. Bioresour. Technol. 150, 195–201.
Gross, M., Jarboe, D., Wen, Z., 2015a. Biofilm-based algal cultivation systems. Appl. Microbiol. Biotechnol. 99 (14), 5781–5789.
Gross, M., Mascarenhas, V., Wen, Z., 2015b. Evaluating algal growth performance and water use efficiency of pilot-scale revolving algal biofilm (RAB) culture systems. Biotechnol. Bioeng. 112 (10), 2040–2050.
Gross, M., Wen, Z., 2014. Yearlong evaluation of performance and durability of a pilot-scale revolving algal biofilm (RAB) cultivation system. Bioresour. Technol. 171, 50–58.
Guieysse, B., Plouviez, M., Coilhac, M., Cazali, L., 2013. Nitrous oxide (N_2O) production in axenic *Chlorella vulgaris* microalgae cultures: evidence, putative pathways, and potential environmental impacts. Biogeosciences 10, 6737–6746.
Guo, R., Chen, J., 2015. Application of alga-activated sludge combined system (AASCS) as a novel treatment to remove cephalosporins. Chem. Eng. J. 260, 550–556.
Halfhide, T., Dalrymple, O.K., Wilkie, A.C., Trimmer, J., Gillie, B., Udom, I., Zhang, Q., Ergas, S.J., 2015. Growth of an indigenous algal consortium on anaerobically digested municipal sludge centrate: photobioreactor performance and modeling. Bioenergy Res. 8, 249–258.
Halfhide, T., Åkerstrøm, A., Lekang, O.I., Gislerød, H.R., Ergas, S.J., 2014. Production of algal biomass, chlorophyll, starch and lipids using aquaculture wastewater under axenic and non-axenic conditions. Algal Res. 6, 152–159. Part B(0).
Harhangi, H.R., Le Roy, M., van Alen, T., Hu, B.L., Groen, J., Kartal, B., Tringe, S.G., Quan, Z.X., Jetten, M.S., Op den Camp, H.J., 2012. Hydrazine synthase, a unique phylomarker with which to study the presence and biodiversity of anammox bacteria. Appl. Environ. Microbiol. 78 (3), 752–758.
Hoh, D., Watson, S., Kan, E., 2016. Algal biofilm reactors for integrated wastewater treatment and biofuel production: a review. Chem. Eng. J. 287, 466–473.
He, P.J., Mao, B., Lü, F., Shao, L.M., Lee, D.J., Chang, J.S., 2013. The combined effect of bacteria and Chlorella vulgaris on the treatment of municipal wastewaters. Bioresour. Technol. 146 (0), 562–568.
Hoffmann, J.P., 1998. Wastewater treatment with suspended and nonsuspended algae. J. Phycol. 34 (5), 757–763.
Johnson, M.B., Wen, Z., 2009. Development of an attached microalgal growth system for biofuel production. Appl. Microbiol. Biotechnol. 85 (3), 525–534.
Juretschko, S., Timmermann, G., Schmid, M., Schleifer, K.-H., Pommerening-Roser, A., Koops, H.-P., Wagner, M., 1998. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: nitrosococcus mobilis and Nitrospira-like bacteria as dominant populations. Appl. Environ. Microbiol. 64 (8), 3042–3051.
Kamp, A., Hogslund, S., Risgaard-Petersen, N., Stief, P., 2015. Nitrate reduction by eukaryotic microbes. Front. Microbiol. 6, 1–15.
Kartal, B., Maalcke, W.J., de Almeida, N.M., Cirpus, I., Gloerich, J., Geerts, W., Op den Camp, H.J., Harhangi, H.R., Janssen-Megens, E.M., Francoijs, K.J., Stunnenberg, H.G., Keltjens, J.T., Jetten, M.S., Strous, M., 2011. Molecular mechanism of anaerobic ammonium oxidation. Nature 479 (7371), 127–130.
Kartal, B., Rattray, J., van Niftrik, L.A., van de Vossenberg, J., Schmid, M.C., Webb, R.I., Schouten, S., Fuerst, J.A., Damste, J.S., Jetten, M.S., Strous, M., 2007. Candidatus "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. Syst. Appl. Microbiol. 30 (1), 39–49.
Karya, N., van der Steen, N.P., Lens, P.N.L., 2013. Photo-oxygenation to support nitrification in an algal-bacterial consortium treating artificial wastewater. Bioresour. Technol. 134, 244–250.
Kazamia, E., Czesnick, H., Nguyen, T.T.V., Croft, M.T., Sherwood, E., Sasso, S., Hodson, S.J., Warren, M.J., Smith, A.G., 2012. Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. Environ. Microbiol. 14 (6), 1466–1476.
Kesaano, M., Sims, R.C., 2014. Algal biofilm based technology for wastewater treatment. Algal Res. 5, 231–240.
Kesaano, M., Smith, T., Wood, J., Sims, R.C., 2015. Applications of algal biofilms for wastewater treatment and bioproduct production. In: Singh, B., Bauddh, K., Bux, F. (Eds.), Algae and Environmental Sustainability. Springer, India. New Delhi, pp. 23–31.
Kirstein, K., Boek, E., 1993. Close genetic relationship between *Nitrobacter* hamburgensis nitrite oxidoreductase and *Escherichia coli* nitrate reductases. Archives Microbiol. 160, 447–453.
Kowalchuk, G.A., Stienstra, A.W., Heilig, G.H.J., Stephen, J.R., Woldendorp, J.W., 2000. Changes in the community structure of ammonia-oxidizing bacteria during secondary succession of calcareous grasslands. Environ. Microbiol. 2 (1), 99–110.
Kraft, B., Strous, M., Tegetmeyer, H.E., 2011. Microbial nitrate respiration - genes, enzymes and environmental distribution. J. Biotechnol. 155, 104–117.
Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, F.X., van Langenhove, H., 2010. Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions. Trends Biotechnol. 28 (7), 371–380.
Lau, S., Shao, N., Bock, R., Jürgens, G., De Smet, I., 2009. Auxin signaling in algal lineages: fact or myth? Trends plant Sci. 14 (4), 182–188.
Li, B., Huang, W., Zhang, C., Feng, S., Zhang, Z., Lei, Z., Sugiura, N., 2015. Effect of TiO2 nanoparticles on aerobic granulation of algal–bacterial symbiosis system and nutrients removal from synthetic wastewater. Bioresour. Technol. 187, 214–220.
Lipschultz, F., Wofsy, S.C., Fox, E.L., 1985. The effects of light and nutrients on rates of ammonium transformation in a eutrophic river. Mar. Chem. 16 (4), 329–341.
Liu, H., Lu, Q., Wang, Q., Liu, W., Wei, Q., Ren, H., et al., 2017. Isolation of a bacterial strain, Acinetobacter sp. from centrate wastewater and study of its cooperation with algae in nutrients removal. Bioresour. Technol. 235, 59–69.
Luo, S., Berges, J.A., He, Z., Young, E.B., 2017. Algal-microbial community collaboration for energy recovery and nutrient remediation from wastewater in integrated photobioelectrochemical systems. Algal Res. 24, 527–539. Part B.
Manser, N.D., Wang, M., Ergas, S.J., Mihelcic, J.R., Mulder, A., van de Vossenberg, J., van Lier, J.B., van der Steen, P., 2016. Biological nitrogen removal in a photosequencing batch reactor with an algal-nitrifying bacterial consortium and anammox granules. Environ. Sci. Technol. Lett. 3 (4), 175–179.
Medina, M., Neis, U., 2007. Symbiotic algal bacterial wastewater treatment: effect of food to microorganism ratio and hydraulic retention time on the process

performance. Water Sci. Technol. 55 (11), 165–171.
Molina, E., Fernández, J., Acién, F.G., Chisti, Y., 2001. Tubular photobioreactor design for algal cultures. J. Biotechnol. 92 (2), 113–131.
Molinuevo-Salces, B., García-González, M.C., González-Fernández, C., 2010. Performance comparison of two photobioreactors configurations (open and closed to the atmosphere) treating anaerobically degraded swine slurry. Bioresour. Technol. 101 (14), 5144–5149.
Muñoz, R., Guieysse, B., 2006. Algal–bacterial processes for the treatment of hazardous contaminants: a review. Water Res. 40 (15), 2799–2815.
Muñoz, R., Köllner, C., Guieysse, B., 2009. Biofilm photobioreactors for the treatment of industrial wastewaters. J. Hazard. Mater. 161 (1), 29–34.
Naumann, T., Çebi, Z., Podola, B., Melkonian, M., 2012. Growing microalgae as aquaculture feeds on twin-layers: a novel solid-state photobioreactor. J. Appl. Phycol. 25 (5), 1413–1420.
Nicolaisen, M.H., Ramsing, N.B., 2002. Denaturing gradient gel electrophoresis (DGGE) approaches to study the diversity of ammonia-oxidizing bacteria. J. Microbiol. Meth. 50, 189–203.
Nokkaew, A., Triampo, W., Amornsamankul, S., Pimpunchat, B., Modchang, C., Triampo, D., 2013. Ammonium uptake by unicellular green microalgae: mathematical modeling and parameter optimization. Southeast-Asian J. Sci. 2, 41–51.
Novoveská, L., Zapata, A.K.M., Zabolotney, J.B., Atwood, M.C., Sundstrom, E.R., 2016. Optimizing microalgae cultivation and wastewater treatment in large-scale offshore photobioreactors. Algal Res. 18, 86–94.
Nunes-Alves, C., 2016. Microbial ecology: do it yourself nitrification. Nat. Rev. Microbiol. 14 (2), 61.
O'Mullan, G.D., Ward, B.B., 2005. Relationship of temporal and spatial variabilities of ammonia-oxidizing bacteria to nitrification rates in Monterey Bay, California. Appl. Environ. Microbiol. 71 (2), 697–705.
Ogbonna, J.C., Tanaka, H., 2000. Light requirement and photosynthetic cell cultivation – development of processes for efficient light utilization in photobioreactors. J. Appl. Phycol. 12 (3), 207–218.
Oragui, J.I., Curtis, T.P., Silva, S.A., Mara, D.D., 1987. The removal of excreted bacteria and viruses in deep waste stabilization ponds in Northeast Brazil. Water Sci. Technol. 19 (3–4), 569–573.
Oswald, W., 1963. The high-rate pond in waste disposal. Dev. Ind Microbiol. 4, 112–125.
Oswald, W.J., 1988. Large-scale Algal Culture Systems (Engineering Aspects), Microalgal Biotechnology. Cambridge University Press, Cambridge [Cambridgeshire] ; New York.
Oswald, W.J., Golueke, C.G., 1960. Biological transformation of solar energy. Adv. Appl. Microbiol. 11, 223–242.
Oswald, W.J., Gotaas, H.B., 1957. Photosynthesis in sewage treatment. Trans. Am. Soc. Civ. Eng. 122 (1), 73–97.
Park, J.B.K., Craggs, R.J., 2011. Nutrient removal in wastewater treatment high rate algal ponds with carbon dioxide addition. Water Sci. Technol. 63 (8), 1758–1764.
Park, J.B.K., Craggs, R.J., Shilton, A.N., 2011a. Recycling algae to improve species control and harvest efficiency from a high rate algal pond. Water Res. 45 (20), 6637–6649.
Park, J.B.K., Craggs, R.J., Shilton, A.N., 2011b. Wastewater treatment high rate algal ponds for biofuel production. Bioresour. Technol. 102 (1), 35–42.
Pauleta, S.R., Dell'Acqua, S., Moura, I., 2013. Nitrous oxide reductase. Coord. Chem. Rev. 257 (2), 332–349.
Peng, Y., Zhu, G., 2006. Biological nitrogen removal with nitrification and denitrification via nitrite pathway. Appl. Microbiol. Biotechnol. 73 (1), 15–26.
Pittman, J.K., Dean, A.P., Osundeko, O., 2011. The potential of sustainable algal biofuel production using wastewater resources. Special Issue Biofuels - II Algal Biofuels Microb. Fuel Cells 102 (1), 17–25.
Posadas, E., García-Encina, P.-A., Soltau, A., Domínguez, A., Díaz, I., Muñoz, R., 2013. Carbon and nutrient removal from centrates and domestic wastewater using algal–bacterial biofilm bioreactors. Bioresour. Technol. 139, 50–58.
Posadas, E., Muñoz, A., García-González, M.-C., Muñoz, R., García-Encina, P.A., 2015. A case study of a pilot high rate algal pond for the treatment of fish farm and domestic wastewaters. J. Chem. Technol. Biotechnol. 90 (6), 1094–1101.
Pratt, R., Daniels, T., Eiler, J.J., Gunnison, J., Kumler, W., Oneto, J.F., Strait, L.A., Spoehr, H., Hardin, G., Milner, H., 1944. Chlorellin, an antibacterial substance from Chlorella. Am. Assoc. Adv. Sci. Sci. 351–352.
Purkhold, U., Pommerening-Roser, A., Juretschko, S., Schmid, M.C., Koops, H.P., Wagner, M., 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: implications for molecular diversity surveys. Appl. Environ. Microbiol. 66 (12), 5368–5382.
Quan, Z.X., Rhee, S.K., Zuo, J.E., Yang, Y., Bae, J.W., Park, J.R., Lee, S.T., Park, Y.H., 2008. Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor. Environ. Microbiol. 10 (11), 3130–3139.
Ramanan, R., Kim, B.-H., Cho, D.-H., Oh, H.-M., Kim, H.-S., 2016. Algae–bacteria interactions: evolution, ecology and emerging applications. Biotechnol. Adv. 34 (1), 14–29.
Rashid, N., Cui, Y.-F., Saif Ur Rehman, M., Han, J.I., 2013. Enhanced electricity generation by using algae biomass and activated sludge in microbial fuel cell. Sci. Total Environ. 456, 91–94.
Razzak, S.A., Hossain, M.M., Lucky, R.A., Bassi, A.S., de Lasa, H., 2013. Integrated CO_2 capture, wastewater treatment and biofuel production by microalgae culturing—a review. Renew. Sustain. Energy Rev. 27, 622–653.
Risgaard-Petersen, N., Nicolaisen, M.H., Revsbech, N.P., Lomstein, B.A., 2004. Competition between ammonia-oxidizing bacteria and benthic microalgae. Appl. Environ. Microbiol. 70 (9), 5528–5537.
Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene amoA as a functional marker; molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63 (12), 4704–4712.
Roudsari, F.P., Mehrnia, M.R., Asadi, A., Moayedi, Z., Ranjbar, R., 2014. Effect of microalgae/activated sludge ratio on cooperative treatment of anaerobic effluent of municipal wastewater. Appl. Biochem. Biotechnol. 172 (1), 131–140.
Rowan, A.K., Snape, J.R., Fearnside, D., Barer, M.R., Curtis, T.P., Head, I.M., 2002. Composition and diversity of ammonia-oxidising bacterial communities in wastewater treatment reactors of different design treating identical wastewater. Microbiol. Ecol. 43, 195–206.
Saikaly, P.E., Stroot, P.G., Oerther, D.B., 2005. Use of 16S rRNA gene terminal restriction fragment analysis to assess the impact of solids retention time on the bacterial diversity of activated sludge. Appl. Environ. Microbiol. 71 (10), 5814–5822.
Sanz-Luque, E., Chamizo-Ampudia, A., Llamas, A., Glavan, A., Fernandez, E., 2015. Understanding nitrate assimilation and its regulation in microalgae. Front. Plant Sci. 6, 1–17.
Schmid, M.C., Risgaard-Petersen, N., van de Vossenberg, J., Kuypers, M.M., Lavik, G., Petersen, J., Hulth, S., Thamdrup, B., Canfield, D., Dalsgaard, T., Rysgaard, S., Sejr, M.K., Strous, M., den Camp, H.J., Jetten, M.S., 2007. Anaerobic ammonium-oxidizing bacteria in marine environments: widespread occurrence but low diversity. Environ. Microbiol. 9 (6), 1476–1484.
Shi, J., Podola, B., Melkonian, M., 2014. Application of a prototype-scale twin-layer photobioreactor for effective N and P removal from different process stages of municipal wastewater by immobilized microalgae. Bioresour. Technol. 154, 260–266.
Singh, S.P., Singh, P., 2015. Effect of temperature and light on the growth of algae species: a review. Renew. Sustain. Energy Rev. 50, 431–444.
Sonthiphand, P., Halland, M.W., Neufeld, J.D., 2014. Biogeography of anaerobic ammonia-oxidizing (anammox) bacteria. Front. Microbiol. 5 (399), 1–14.
Srithep, P., Khinthong, B., Chodanon, T., Powtongsook, S., Pungrasmi, W., Limpiyakorn, T., 2014. Communities of ammonia-oxidizing bacteria, ammonia-oxidizing archaea and nitrite-oxidizing bacteria in shrimp ponds. Ann. Microbiol. 65 (1), 267–278.
Starkenburg, S.R., Chain, P.S., Sayavedra-Soto, L.A., Hauser, L., Land, M.L., Larimer, F.W., Malfatti, S.A., Klotz, M.G., Bottomley, P.J., Arp, D.J., Hickey, W.J., 2006. Genome sequence of the chemolithoautotrophic nitrite-oxidizing bacterium Nitrobacter winogradskyi Nb-255. Appl. Environ. Microbiol. 72 (3), 2050–2063.
Stein, L.Y., Klotz, M.G., 2016. The nitrogen cycle. Curr. Biol. 26 (3), R94–R98.
Stolz, J.F., Basu, P., 2002. Evolution of nitrate reductase: molecular and structural variations on a common function. ChemBioChem 3, 198–206.
Strik, D.P.B.T.B., Terlouw, H., Hamelers, H.V.M., Buisman, C.J.N., 2008. Renewable sustainable biocatalyzed electricity production in a photosynthetic algal microbial fuel cell (PAMFC). Appl. Microbiol. Biotechnol. 81 (4), 659–668.
Su, Y., Mennerich, A., Urban, B., 2011. Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. Water Res. 45 (11), 3351–3358.
Su, Y., Mennerich, A., Urban, B., 2012. Synergistic cooperation between wastewater-born algae and activated sludge for wastewater treatment: influence of algae and sludge inoculation ratios. Bioresour. Technol. 105 (0), 67–73.
Tam, N.F.Y., Wong, Y.S., 1996. Effect of ammonia concentrations on growth of Chlorella vulgaris and nitrogen removal from media. Bioresour. Technol. 57 (1), 45–50.
Tan, X.-B., Zhang, Y.-L., Yang, L.-B., Chu, H.-Q., Guo, J., 2016. Outdoor cultures of *Chlorella pyrenoidosa* in the effluent of anaerobically digested activated sludge: the effects of pH and free ammonia. Bioresour. Technol. 200, 606–615.
Tarakhovskaya, E.R., Maslov, Y.I., Shishova, M.F., 2007. Phytohormones in algae. Russ. J. Plant Physiol. 54 (2), 163–170.
Torzillo, G., Pushparaj, B., Bocci, F., Balloni, W., Materassi, R., Florenzano, G., 1986. Production of Spirulina biomass in closed photobioreactors. Biomass 11 (1), 61–74.
Torzillo, G., Pushparaj, B., Masojidek, J., Vonshak, A., 2003. Biological constraints in algal biotechnology. Biotechnol. Bioprocess Eng. 8 (6), 338–348.
Tredici, M.R., 2002. Bioreactors, Photo. Encyclopedia of Bioprocess Technology.
Tredici, M.R., Zittelli, G.C., 1998. Efficiency of sunlight utilization: tubular versus flat photobioreactors. Biotechnol. Bioeng. 57 (2), 187–197.
Udom, I., Zaribaf, B.H., Halfhide, T., Gillie, B., Dalrymple, O., Zhang, Q., Ergas, S.J., 2013. Harvesting microalgae grown on wastewater. Bioresour. Technol. 139 (0), 101–106.
Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A., 2010. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. J. Renew. Sustain. Energy 2 (1), 012701.
Ugwu, C.U., Aoyagi, H., Uchiyama, H., 2008. Photobioreactors for mass cultivation of algae. Bioresour. Technol. 99 (10), 4021–4028.
Vance, B.D., 1987. Phytohormone effects on cell division in *Chlorella pyrenoidosa* chick (TX-7-11-05)(chlorellaceae). J. Plant Growth Regul. 5 (3), 169–173.
Van Den Hende, S., Beelen, V., Bore, G., Boon, N., Vervaeren, H., 2014. Up-scaling aquaculture wastewater treatment by microalgal bacterial flocs: from lab reactors to an outdoor raceway pond. Bioresour. Technol. 159, 342–354.
Van Den Hende, S., Beelen, V., Julien, L., Lefoulon, A., Vanhoucke, T., Coolsaet, C., Rousseau, D.P., 2016. Technical potential of microalgal bacterial floc raceway

ponds treating food-industry effluents while producing microalgal bacterial biomass: an outdoor pilot-scale study. Bioresour. Technol. 218, 969–979.
van der Steen, P., Brenner, A., van Buuren, J., Oron, G., 1999. Post-treatment of UASB reactor effluent in an integrated duckweed and stabilization pond system. Water Res. 33 (3), 615–620.
van der Steen, P., Rahsilawati, K., Rada-Ariza, A.M., Lopez-Vazquez, C.M., Lens, P.N.L., 2015. A new photo-activated sludge system for nitrification by an algal-bacterial consortium in a photo-bioreactor with biomass recycle. Water Sci. Technol. 72 (3), 443–450.
Verbyla, M.E., Mihelcic, J.R., 2015. A review of virus removal in wastewater treatment pond systems. Water Res. 71, 107–124.
Vergara, C., Muñoz, R., Campos, J.L., Seeger, M., Jeison, D., 2016. Influence of light intensity on bacterial nitrifying activity in algal-bacterial photobioreactors and its implications for microalgae-based wastewater treatment. Int. Biodeterior. Biodegrad. 114, 116–121.
Wang, H., Ji, G., Bai, X., He, C., 2015a. Assessing nitrogen transformation processes in a trickling filter under hydraulic loading rate constraints using nitrogen functional gene abundances. Bioresour. Technol. 177, 217–223.
Wang, M., Kuo-Dahab, W.C., Dolan, S., Park, C., 2014. Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae, Chlorella sp. and Micractinium sp., in wastewater treatment. Bioresour. Technol. 154 (0), 131–137.
Wang, M., Park, C., 2015. Investigation of anaerobic digestion of Chlorella sp. and Micractinium sp. grown in high-nitrogen wastewater and their co-digestion with waste activated sludge. Biomass Bioenergy 80, 30–37.
Wang, M., Payne, K., Tong, S., Ergas, S., 2017. A hybrid algal photosynthesis and ion-exchange (HAPIX) process for side stream wastewater treatment: experimental and modeling studies. In: Water Environment Federation 90th Annual Technical Exhibition and Conference (WEFTEC 2017), Chicago, IL.
Wang, M., Sahu, A.K., Rusten, B., Park, C., 2013. Anaerobic co-digestion of microalgae Chlorella sp. and waste activated sludge. Bioresour. Technol. 142, 585–590.
Wang, M., Yang, H., Ergas, S.J., van der Steen, P., 2015b. A novel shortcut nitrogen removal process using an algal-bacterial consortium in a photo-sequencing batch reactor (PSBR). Water Res. 87, 38–48.
Wang, X.-X., Wu, Y.-H., Zhang, T.-Y., Xu, X.-Q., Dao, G.-H., Hu, H.-Y., 2016. Simultaneous nitrogen, phosphorous, and hardness removal from reverse osmosis concentrate by microalgae cultivation. Water Res. 94, 215–224.
Watson, S.W., Waterbury, J.B., 1971. Characteristics of two marine nitrite oxidizing bacteria, *Nitrospina gracilis* nov. gen. nov. sp. and *Nitrococcus mobilis* nov. gen. nov. sp. Archives Microbiol. 77, 203–230.
Weathers, P.J., 1984. N_2O evolution by green algae. Appl. Environ. Microbiol. 48, 1251–1253.
Wiley, P.E., Brenneman, K.J., Jacobson, A.E., 2009. Improved algal harvesting using suspended air flotation. Water Environ. Res. 81 (7), 702–708.
Wilkie, A.C., Mulbry, W.W., 2002. Recovery of dairy manure nutrients by benthic freshwater algae. Bioresour. Technol. 84 (1), 81–91.
WRF, EPRI, 2013. Electricity Use and Management in the Municipal Water Supply and Wastewater Industries. Water Research Foundation (WRF), Electric Power Research Institute (EPRI).
Xiao, L., He, Z., 2014. Applications and perspectives of phototrophic microorganisms for electricity generation from organic compounds in microbial fuel cells. Renew. Sustain. Energy Rev. 37, 550–559.
Yoshioka, T., Saijo, Y., 1984. Photoinhibition and recovery of NH_4^+ oxidizing bacteria and NO_2^-oxidizing bacteria. J. General Appl. Microbiol. 30 (3), 151–166.
Zanetti, L., Frison, N., Nota, E., Tomizioli, M., Bolzonella, D., Fatone, F., 2012. Progress in real-time control applied to biological nitrogen removal from wastewater. A short-review. Desalination 286, 1–7.
Zhang, L., Chen, L., Wang, J., Chen, Y., Gao, X., Zhang, Z., Liu, T., 2015. Attached cultivation for improving the biomass productivity of Spirulina platensis. Bioresour. Technol. 181, 136–142.
Zhang, Y., Noori, J.S., Angelidaki, I., 2011. Simultaneous organic carbon, nutrients removal and energy production in a photomicrobial fuel cell (PFC). Energy & Environ. Sci. 4 (10), 4340–4346.
Zhu, L., Wang, Z., Takala, J., Hiltunen, E., Qin, L., Xu, Z., Qin, X., Yuan, Z., 2013. Scale-up potential of cultivating Chlorella zofingiensis in piggery wastewater for biodiesel production. Bioresour. Technol. 137, 318–325.
Zumft, W., 1997. Cell biology and molecular basis of denitrification. Microbiol. Mol. Biol. Rev. 62, 533–536.