Nitrate and Nitrite Removal from Wastewater Using Algae

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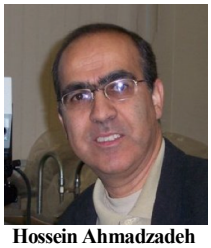

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Abstract: *Background*: Both land-based agriculture and aquatic algae culturing systems require a steady supply of macronutrients, primarily nitrogen (N) and phosphorus (P), in addition to a variety of micronutrients for biomass production. The use of commercial fertilizer for large-scale algae production significantly increases the cost of algae production. Microalgae have a high capability to remove combined nitrogen compounds, ammonia, nitrate and nitrite, from wastewaters.

Methods: The algae assimilates inorganic nitrogen and converts nitrogen into biomass, thus providing an opportunity for efficient recycling of nutrients in wastewater. Furthermore, the microalgae can be a feedstock for biodiesel and other valuable by-products including pigments, proteins and lipids. Combined nitrogen is assimilated in different forms and at different rates that vary among the phylogenetically diverse strains of microalgae.

Results: In this review, we summarize nitrate removal rates and biomass production of different microalgae species reported in the literature.

Conclusion: A comparison of the literature suggests that *Chlorella vulgaris*, *Neochloris oleoabundans* and *Dunaliella tertiolecta* are able to remove nitrate more effectively than other strains studied. Moreover, important parameters influencing nitrate removal, including initial nitrate concentration, light intensity, pH and temperature, are discussed. Alternative culture methods, immobilization and biofilm formation for nitrate remediation, are introduced which are able to lower costs of the harvesting process.

Keywords: Biomass concentration, microalgae, immobilization, nitrate removal rate.

1. INTRODUCTION

Population growth, industrialization and rapid urbanization have led to excessive nitrogen (N) pollution, often in the form of nitrate, presenting a water-quality problem of growing concern [1]. Excessive fertilizer use in urban and agricultural regions has caused serious problems of nitrate and phosphate (P) pollution in surface waters, groundwaters and the marine environments. Nitrate fertilizer, not taken up by plants, is leached from soils and can percolate into ground waters and/or be washed into freshwater reservoirs and the ocean through urban storm water systems. Municipal wastewater discharge [2], sewage waste and septic tanks [3], livestock farms, processed food plants, dairy and meat processing facilities and decomposition of decaying organic matter also release significant amounts of N into aquatic environments [2]. While the question of whether N or P input is the major factor in eutrophication is questioned [4], and generally biologists favor nitrogen as the limiting nutrient while geochemists favor phosphate limitation [5], there is little debate that increased N input into our waterways presents a major perturbation to aquatic ecosystems [6]. Eutrophication of surface waters including lakes, streams and drinking water reservoirs has resulted in algae blooms. What were once occasional algae blooms occurring as a regional phenomenon are now appearing on a global basis with greater frequency. The immediate consequences of these blooms include the degradation of recreational lakes and total oxygen consumption that result in major fish kills. Certain cyanobacteria such as *Microcycstis* produce neurotoxins (cyanotoxins) that can persist in the water column long after the algae bloom has faded [7].

Nitrogen goes through a biogeological cycle producing compounds with different oxidation states that are available to plants, algae and microbes: Nitrate, nitrite, ammonium, organic nitrogen including amino acids, urea and proteins. While ammonium is energetically more favorable and is the preferred nitrogen source when it is available [8, 9], in many waterways nitrate concentrations are generally much higher than ammonium concentrations. For example, many industrial wastewaters often contain more than 200 mg NO_3^- N while effluents from industries producing explosives, fertilizers, pectin, cellophane, and metal finishing, contain greater than 1000 mg NO_3^- N. The nuclear industry also produces nitrate loaded wastes in extremely high concentrations at many points during the nuclear fuel cycle (up to 50,000 mg NO_3^- N/L). Therefore, in this review

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article, we focus mainly on NO_3^- and NO_2^- removal. While almost all algae can grow on low to high concentrations of nitrate, many strains have acute sensitivity to high concentrations of ammonium/ammonia. The review article by Collos and Harrison [10] showed a ranking of sensitivity to high levels of ammonium/ammonia (39-1.2 mM) where the order of tolerance was*: Chlorophyceae > Cyanophyceae, Dinophyceae, Diatomophyceae,* and *Raphidophyceae*.

Most wastewater treatment systems have two levels of treatment, primary (physical settling of solids), secondary (various forms of oxidation e.g. activated sludge or trickling filters). Where regional or government regulations mandate higher effluent quality, tertiary treatment is used for nutrient removal and disinfection. In primary wastewater treatment the major forms of nitrogen are organic-N and ammonium. During secondary treatment the two major forms of nitrogen are rapidly converted to nitrate by nitrifying bacteria, such as *Nitrosomonas, Nitrosococcus, Nitrobacter,* and *Nitrococcus* [11]. Nitrite is the most ephemeral form of nitrogen in the environment. In both wastewater treatment systems and in surface waters, it occurs as the least prevalent form of inorganic nitrogen.

Although nitrate is one small component of the nitrogen cycle, the focus of this review is nitrate assimilation by algae. There are many biogeochemical and physical processes within the nitrogen cycle. The daily shifts in pH in surface waters due to algal photosynthesis facilitate the conversion of ammonium ions to ammonia gas and its subsequent volatilization into the atmosphere [12]. A microscopic examination of almost any alga taken from an oxidation pond will show hundreds of bacteria attached to the outer surface of the alga. The bacterial-algal interactions play a key role in nutrient processing. The main aspects of this synergistic relationship include the photosynthetically generated oxygen, which fuels bacterial mineralization of organic material producing inorganic nutrients for algal growth [13]. Table **1** below summarizes the forms of nitrogen in surface waters and their impact on water quality [14].

The removal of nitrate by bacterial dissimulatory nitrate reduction plays a major role in the conversion of nitrate to nitrogen gas in anaerobic sediments in lakes, ponds and wetlands. Incomplete denitrification results in the release of nitrous oxide, which has been shown to be 300 times more potent a greenhouse gas than carbon dioxide [15] and is considered the most active compounds in ozone depletion in the 21st Century [16]. Nitrous oxide emissions into the atmosphere are in part due to human activities including agricultural fertilization and livestock feedlots [17]. The contributions of nitrous oxide to the atmosphere by natural and anthropogenic sources are a topic of the on-going debates [18]. Recent studies by Guieysse *et al*. and Alcántara *et al*. have shown that algae in high-rate production ponds can also contribute to the production of nitrous oxide [19].

Many groundwater basins have been historically underused for human consumption due to high nitrate concentrations that leads to health consequences. Thus, direct use of groundwater resources for human consumption has been prohibited in many parts of the world. Nitrate may be reduced to nitrosamines in the stomach which, as known carcinogens, may be a factor causing gastric cancer [20, 21]. Nitrate reacts with hemoglobin in the blood to form methemoglobin, leading to an overall reduced ability of the red blood cells to release oxygen to the tissues. This lack of oxygen results in methemoglobinemia (blue-baby syndrome) [22].

Groundwaters contaminated with nitrate above the United States Environmental Protection Agency (EPA) and World Health Organization (WHO) maximum level (10 mg L^{-1} NO_3^- N), must be treated before use as drinking water. Europe also set a maximum of 12 mg L^{-1} NO_3^- N in drinking water for the same concern [23]. Methods for nitrate and nitrite removal in water resources are a controversial issue that has attracted a good deal of attention.

Generally, there are two basic types of treatments for removing nitrate from water or wastewater: physicochemical and biological methods. Physicochemical methods include reverse osmosis (RO) [24], ion exchange (IE) [25], electrodialysis (ED) [26] and activated carbon adsorption in conjunction with pH adjustment [27]. While IE and RO are well developed, both are energy intensive processes and are not highly efficient, producing brine waters that are frequently discharged into adjacent waterways [28]. Recently, researchers have developed new methods for nitrate removal, including metallic iron-aided abiotic nitrate reduction (also known as zero-valent iron or ZVI) [29, 30]. Many have sought biological solutions to cost effective and sustainable treatment processes that can be as effective as the conventional physicochemical processes [20].

A variety of biological methods are available for the denitrification of surface and ground waters based on plant and microbial metabolic processes. The best described mechanisms are assimilation of nitrate by plants, algae and microbes and microbial respiratory denitrification where nitrate and its reduction products serve as alternate electron acceptors under anaerobic conditions resulting in the conversion of nitrate to N_2 gas (dissimilatory nitrate reduction, DNR). Nitrate and ammonia assimilation, in contrast to the respiratory nitrate reduction, results in N being converted to biomass rather than being released to the atmosphere as the relatively inert N_2 gas. Other less known microbial processes include a dissimilatory nitrate reduction to ammonium [31], and anaerobic ammonium oxidation **(**anammox**)** [32, 33]. In addition, denitrification can be coupled to sulfide or iron oxidation [34-37].

Cyanobacteria and microalgae have been reported to be more efficient for N bioremediation [38] than higher plants, due in part to higher rates of biomass production but also because algae lack the large stores of structural carbon (ie. cellulose) characteristic of land plants. Thus, the C/N ratio of higher plants ranges from 18-120 (by atoms) while microalgae range from 5 to 20 [39] indicating that water reclamation and nutrient recovery can be accomplished more rapidly, and in a smaller area, using algae rather than terrestrial plants. Mass-culture of algae on manure N and P is an alternative to land spreading of manure effluents, particularly in the case of confined animal feed operations (CAFOs). Groundwater contamination is problematic in these operations and many CAFOs do not have affordable access to large tracts of land for manure application to soils. A highly productive crop is needed to remove manure N and P in smaller land areas than are required by crops such as

Table 1. Overview of the primary forms of N found in surface waters and associated concerns.*

Nitrogen Parameter	General Description	When Found	Sources to Surface Waters	Health and Environmental Concerns
Nitrate-N (NO_3^-)	Main form of N in groundwater and high-N surface waters. Dissolved in water and moves readily through soil.	Present as a common form of nitrogen, since most other N forms can transform into nitrate in N cycle.	Transformed into nitrate from other N forms found in fertilizer, soil N, atmosphere and human and animal waste.	Methemoglobinemia in infants and susceptible adults. Toxic to aquatic life, especially freshwaters Eutrophication and low oxygen (hypoxia), especially in coastal waters.
Nitrite-N (NO_2^-)	Low levels in waters – typically measured in the lab together with nitrate.	Less stable intermediary form of N found during N transforming processes.	Same as nitrate.	Methemoglobinemia in infants and susceptible adults. Toxic to aquatic life.
Ammonia-N (NH_3)	Unionized Ammonia – low levels in most waters.	Most of NH_3 NH_4^+ is in the NH_4^+ form. But NH_3 increases with higher temps and pHs (potential of Hydrogen).	Human and animal waste discharges.	Toxic to aquatic life.
Ammonium-N (NH_4^+)	Measured in the lab together with ammonia – usually higher than ammonia but less toxic	Usually found at low levels compared to nitrate and organic N. Found near waste sources.	Human and animal waste discharges.	Can convert to more highly toxic ammonia in high pH and temperature waters.
Organic-N	The main form of N in low-N surface waters (where nitrate is low).	Living and dead organisms/algae. Found naturally in water and is supplemented by human impacts.	Algae; soil; organisms; human and animal waste.	Can convert to ammonium and ultimately nitrate under certain conditions.
Inorganic N	The sum of Nitrite, Nitrate, ammonia, and ammonium.			See separate parameters above.
Total Kjeldahl N (TKN)	Lab measurement which includes organic-N, ammonia and ammonium.	Useful to determine organic-N when ammonia ammonium is also determined separately and subtracted from TKN.		See separate parameters above.
Total N	Sum of TKN, nitrite and nitrate.			See separate parameters above.

*Adapted from [14].

corn. At least 70 percent of the cost of municipal wastewater treatment can be attributed to secondary and tertiary treatment. Much of this is due to the energy costs of oxygen transfer in biological secondary treatment and to the chemical requirements in tertiary treatment [40]. Microalgae have been used for over 50 years in municipal wastewater treatment where photosynthetically generated O_2 is consumed by bacterial populations that decompose organic wastes to simple inorganic nutrients and in tertiary treatment to remove inorganic nutrients before discharge to receiving waters [41].

While there are approximately 4,000 known species of microalgae and cyanobacteria [42], for this review, the efficiency of N uptake and biomass production by selected algal strains common to eutrophic waters were compared (Section 3). Furthermore, experimental factors including initial nitrate concentration and the ratio of ammonia to nitrate (Section 4.1), light/dark cycle and light intensity (Section 4.2), pH (Section 4.3), and temperature (Section 4.4), are also discussed. Finally, we provide a survey of alternative culturing technologies, including immobilization (Section 5.1) and biofilm formation (Section 5.2) aimed at harvesting the biomass at a low cost.

2. PHOTOTROPHIC NITRATE ASSIMILATION

Phytoplankton are responsible for ~ 70% of global nitrogen assimilation on earth with about 65% consumed as reduced nitrogen (ammonia and organic nitrogen), approximately 10% *via* nitrogen fixation and the balance as nitrate [43]. Because N and P tend to be limiting nutrients for algal growth and because phytoplankton grow in very dilute nutrient solutions in natural waters, algae have developed extremely efficient mechanisms for nutrient uptake. In many waterways, especially estuarine and marine systems, nitrate concentrations are generally much higher than ammonium concentrations. Nitrate is tolerated at rather high levels by both plants and algae while there are toxicity issues associated with ammonium. *Many algal strains show a high tolerance for ammonium but others have a distinct sensitivity to even low concentrations of ammonium [44]. This*

sensitivity/toxicity to ammonia is in part due to the pH shift that occurs when carbon dioxide becomes limited and algae begin to take up bicarbonate ions. The ion is broken down to carbon dioxide and hydroxyl ions. The carbon dioxide is used for photosynthesis and the hydroxyl ions are excreted back into the water. This causes a rise in pH resulting in ammonium ions (NH_4^+) being converted to ammonia (NH_3) [45]. In a study of six classes of microalgae, Cyanophycea (blue-green algae) had the highest tolerance to ammonium while the Dinophyceae (dinoflagellates) had the least [46].

Ammonia is more toxic than nitrate to both plants and animals because it dissipates transmembrane proton gradients needed for both respiratory and photosynthetic electron transport mechanisms. Thus, upon uptake, or conversion of nitrate to ammonia, ammonia is incorporated rapidly into amino acids. However, in polluted waters including dairy [47-49], swine [50] and municipal wastewaters [51, 52], ammonia and organic N are the predominant forms of combined N, while nitrate and nitrite are generally found at trace levels. Oxidation-reduction potential can predict the oxidation state of N compounds and their interconversion by nitrification and denitrification reactions [53].

Nitrate reduction to ammonium takes place through sequential reactions involving 2-electron and 6-electron reductions catalyzed by nitrate reductase and nitrite reductase. The ammonium produced is incorporated into amino acids *via* glutamate dehydrogenase (at high concentrations of ammonium) or the glutamine synthetase/glutamate synthase cycle (at low levels). Plants and algae assimilate nitrate and immediately reduce nitrate to nitrite *via* the enzyme nitrate reductase (NR) using either nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor. Because nitrite is highly reactive and more toxic than nitrate, in higher plants it is immediately transported from the cytoplasm into the chloroplasts of the leaves or plastids of root tissues. In higher plants, two different forms of nitrite reductase both containing an iron-sulfur cluster and a specialized heme prosthetic group [54], are found in the chloroplasts and mitochondria where nitrite reductase reduces nitrite to ammonia without intermediate forms of varying redox levels. In the chloroplasts, reduced ferredoxin produced by photosynthetic electron transport is used as reducing agent for nitrite reductase while plastids use NADPH derived from the oxidative pentose pathway [55]. Because of the high energetic cost of the process and because nitrate reduction competes for reducing equivalents with photosynthetic carbon fixation, nitrate reduction is highly regulated [55]. While it is generally thought that the presence of ammonium inhibits nitrate uptake, there is evidence that in phytoplankton the uptake and assimilation mechanisms are not as simple or as tightly coupled as previously thought. Under various environmental conditions, especially light and temperature, and among different microalgal groups and even species, there is more flexibility in the mechanisms regulating N assimilation [56].

Although many aspects of nitrate assimilation in microalgae are similar to those of higher plants, differences are seen due in part to the evolutionary diversity of algae and to structural differences between these major taxonomic divisions. In the cyanobacteria and unicellular algae there are no storage vacuoles or transport systems seen in higher plants. Thus, in some green algae, including *Chlorella* [57], *Chlamydomonas* [58] and *Monoraphidium braunii* [59], nitrate uptake and reduction is tightly coupled and stimulated by blue light. However, nitrate uptake in *Hydrodictyon*, a large vacuolated coenocytic alga (Characeae) is not as closely coupled and is regulated more directly by energy supply [60]. In algae, the major reductant used is NADPH of photosynthetic origin and there are differences in the structure, reducing agent and location of nitrate and nitrite reductases [61]. Nitrate uptake and reduction to nitrite and ammonium are driven in cyanobacteria by photosynthetically derived ATP and reduced ferredoxin [62]. Nitrate reductase (NR) from *Chlorella* sp. can utilize both NADH and NADPH for nitrate reduction [63] presumably due to light:dark cycles and because there is intense competition for energy and reductant between photosynthetic carbon fixation and other energy intensive processes. While nitrate reductase is argueably [64, 65] localized in the cytoplasm of higher plants, immuno-specific electron microscopy of NR localized the enzyme in the pyrenoids, structures associated with the chloroplasts of eukaryotic algae of *Monoraphidium braunii* [66], *Chlamydomonas reinhardii*, *Chlorella fusca*, *Dunaliella salina*, and *Scenedesmus obliquus* [66]. Starch grains associated with the pyrenoids and enzymes, including phosphoribulokinase, phosphoriboisomerase [67], and ribulose bisphosphate carboxylase-oxygenase [68] suggest a functional role for this structure in photosynthetic carbon metabolism.

3. NITRATE BIOREMEDIATION BY MICROALGAE

3.1. Comparison of Various Algal Strains Toward Nitrate Removal and Biomass Production

Previous studies have shown that species of microalgae have different capabilities in N uptake and assimilation and biomass production. However, variations in experimental procedures make the data difficult to interpret and compare. These include the use of different algae species and strains, media composition including both defined inorganic media or wastewater effluents, the ratios of reduced *vs* oxidized forms of N and BOD levels, CO_2 enrichment, light intensity, diurnal light regimes and temperature. For example, Sacristán de Alva *et al*. (2013), cultivated *Scenedesmus accutus* in municipal wastewater after settling (primary treatment) and after undergoing activated sludge treatment (secondary treatment). The primary effluent had approximately twice the levels of COD, nitrates and reduced N and supported twice the biomass than effluent from secondary treatment. They obtained a low nitrate removal rate of 0.59 mg L^{-1} d^{-1} [69]. However, Doria *et al*. (2012) used an outdoor photobioreactor with high light intensity (from 100 to 1500 μmol m^{-2} s^{-1}) under natural light/dark cycles and improved nitrate removal efficiency for *S. accutus* over 10-fold (6.26 mg L^{-1} d^{-1}). The ten-fold difference in nitrate uptake rate observed using the same species could be attributed to the composition of wastewater and culture methods. In their work [70], secondary treated municipal wastewater also led to lower biomass concentration (0.74 g L^{-1}) than primary treated wastewater (1.1 g L^{-1}) (Table **2**) [69]. Compared with secondary wastewater, the primary

wastewater used in the Sacristán de Alva experiment had higher levels of inorganic phosphate, organic nitrogen and ammonia that potentially supported the higher algal growth.

Nunez *et al*. (2001) used artificial wastewater (essentially, tap water, nitrate, ammonia and phosphate at 11.8, 40 and 4.5 mg L^{-1}, respectively, and supplemented with vitamins and trace minerals) for the cultivation of *Scenedesmus obliquus* with 50% and 70% daily dilution in continuous culture [71]. Nitrate uptake rates were 6.5 and 8.3 mg L^{-1} d^{-1}, respectively, higher than the rate obtained by Doria *et al*. (2012), and comparable to rates observed by Sacristán de Alva *et al*. (2013) (Table **2**). *S. obliquus* was grown in continuous culture, replacing a fraction of growth medium with fresh medium, which could account for higher rates of nitrate uptake. But the influence of high BOD and reduced N levels used in Sacristán de Alva's work, compared to the low BOD and relatively high levels of nitrate to ammonia ratio of the Nunez study is unclear, along with the question of whether the specific algae strains used, also played a role in nitrate uptake.

Su *et al*. (2012a) compared three common green algae species (*Chlorella vulgaris, Scenedesmus rubescens, Chlamydomonas reinhardtii* and the cyanobacterium *Phormidium sp*.) using effluent from a secondary clarifier with a COD of 30 mg L^{-1}, and total Kjeldahl nitrogen of 95.5 mg L^{-1} comprised of ammonia (95.5%) and the balance by nitrate and nitrite. Cells were cultured in photobioreactors with a light/dark cycle of 12:12 h, with 7000 lux. As depicted in Fig. (**1**) and tabulated in Table **2**, *C. reinhardtii* removed nitrate in 4 days as compared with *S. rubescens* and *Phormidium sp* that removed nitrate in 6 and 7 days, respectively. In terms of removal capacity, *C. reinhardtii* had the highest nitrate removal rate (0.16 mg L^{-1} d^{-1}) while *S. rubescens* had the highest biomass productivity (6.56 g m^{-2} d^{-1}) (Table **2**) [72]. Sydney *et al*. (2011) compared nitrate uptake by 20 different strains of microalgae cultured under identical conditions. They showed that *Botryococcus braunii* and *Chlorella vulgaris* had the maximum nitrate removal efficiency with an uptake rate of 22.2 and 20.28 mg L^{-1} d^{-1}, respectively (Table **2**) [73]. While *Botryococcus braunii*is is known for its unusually high lipid content, its potential as a source for biofuel production is limited due to its slow growth rate [74], yet in this study showed a very high rate of nitrate uptake.

Complete nitrate removal from primary treated sewage was observed with the growth of *Haematococcus pluvialis* [75]. Initial nitrate concentration was 42.4 mg L^{-1} and an uptake rate of 8.48 mg L^{-1} d^{-1} was observed (Table **2**). The significant improvement in nitrate removal rates observed for the same strain (40 mg L^{-1} d^{-1}) [76] may be due to the higher light intensity utilized (100 µmol photon m^2 s^{-1} compared to the 50 µmol photon m^2 s^{-1}) in the earlier study. Therefore, as Sacristán de Alva suggested [69], light intensity can be considered an important factor for nitrate removal efficiency in some species (see section 3.2).

Another key to nitrate uptake variation in the literature may reflect the fact that nitrate uptake is influenced by the presence of other nitrogen sources especially ammonium ion. For example, *Chlorella vulgaris* removed 62.5% of NO_3^- with a removal rate of 2.65 mg L^{-1} d^{-1} and nitrite uptake at a rate of 0.01 mg L^{-1} d^{-1} (Table **2**) from effluent without ammonium ion [77]. However, in wastewater containing 205 mg L^{-1} NH_4^+ in combination with initial nitrate concentrations ranging from 1.5 to 198.3 mg L^{-1} NO_3^-, lower rates of nitrate removal by *C. vulgaris* were observed [78]. In these experiments, ammonium ion was the preferred nitrogen source and nitrate uptake did not begin until the ammonium ion was consumed [78]. This has been attributed to the observation that ammonium ion assimilation does not involve a redox reaction and it requires less energy [79]. Corey *et al*. (2013) measured nitrate uptake using five different ratios of nitrate and ammonium in cultures of *Palmaria palmate* and *Chondrus crispus*. Total N concentration was 300 µM (with NO_3^-/NH_4^+ ratios of 300:0, 270:30, 150:150, 30:270, 0:300). *P. palmate* showed the highest NO_3^- uptake (4.39 µmol NO_3^- gDW^{-1} h^{-1}) at 270:30 NO_3^-/NH_4^+. For *C. crispus* nitrate uptake was equivalent at 300:0 NO_3^-/NH_4^+, 270:30 NO_3^-/NH_4^+, and 150:150 NO_3^- /NH_4^+ with a mean uptake rate of 6.57 µmol NO_3^- gDW^{-1} h^{-1} (Table **2**) [80]. Therefore, determining strain-specific optimal ratios of ammonium and nitrate in the medium can result in more efficient nitrate removal.

While clearly environmental parameters can affect nitrate removal efficiency, there is little in the literature that assesses the importance of each parameter and compares their relative effect on nitrate removal efficiency. However, finding species with significantly higher rate of nitrate uptake relative to other species in comparable conditions can provide researchers with valuable information. For example, *Neochloris oleoabundans* was able to completely remove nitrate with an initial concentration of 452 mg L^{-1} with an uptake rate of 150 mg L^{-1} d^{-1} [81]. *Dunaliella tertiolecta* and *Chlorella vulgaris* also have high nitrate uptake rates of 155 and 103.3 mg L^{-1} d^{-1}, respectively [82]. Therefore, *Neochloris oleoabundans*, *Dunaliella tertiolecta* and *Chlorella vulgaris* are excellent candidates for nitrate bioremediation.

4. EXPERIMENTAL PARAMETERS AFFECTING REMOVAL OF NITRATE

The principle parameters affecting nitrate and nitrite removal include, but are not limited to, initial nitrate concentration, light intensity, pH and temperature. Data for nitrate uptake by algae related to these parameters is summarized in Tables **2-4**.

4.1. Initial Nitrate Concentration

Initial nitrate concentrations reported in the literature for algae growth experiments range from 45 to 1914 mg L^{-1} (summarized in Table **3**) producing contradictory results for the effect of initial nitrate concentration on biomass production and nitrate removal rates. For example, Wang and Lan (2011) grew *Neochloris oleoabundans* in media containing 45 to 218 mg L^{-1} of NO_3^-. Their data showed that increasing initial nitrate concentration increased nitrate uptake rates, reaching a maximum of 1.82 mg L^{-1} h^{-1} at 140 mg NO_3^-. However, further increase in nitrate concentration to 218 mg L^{-1} resulted in reduced cell growth [83]. It has

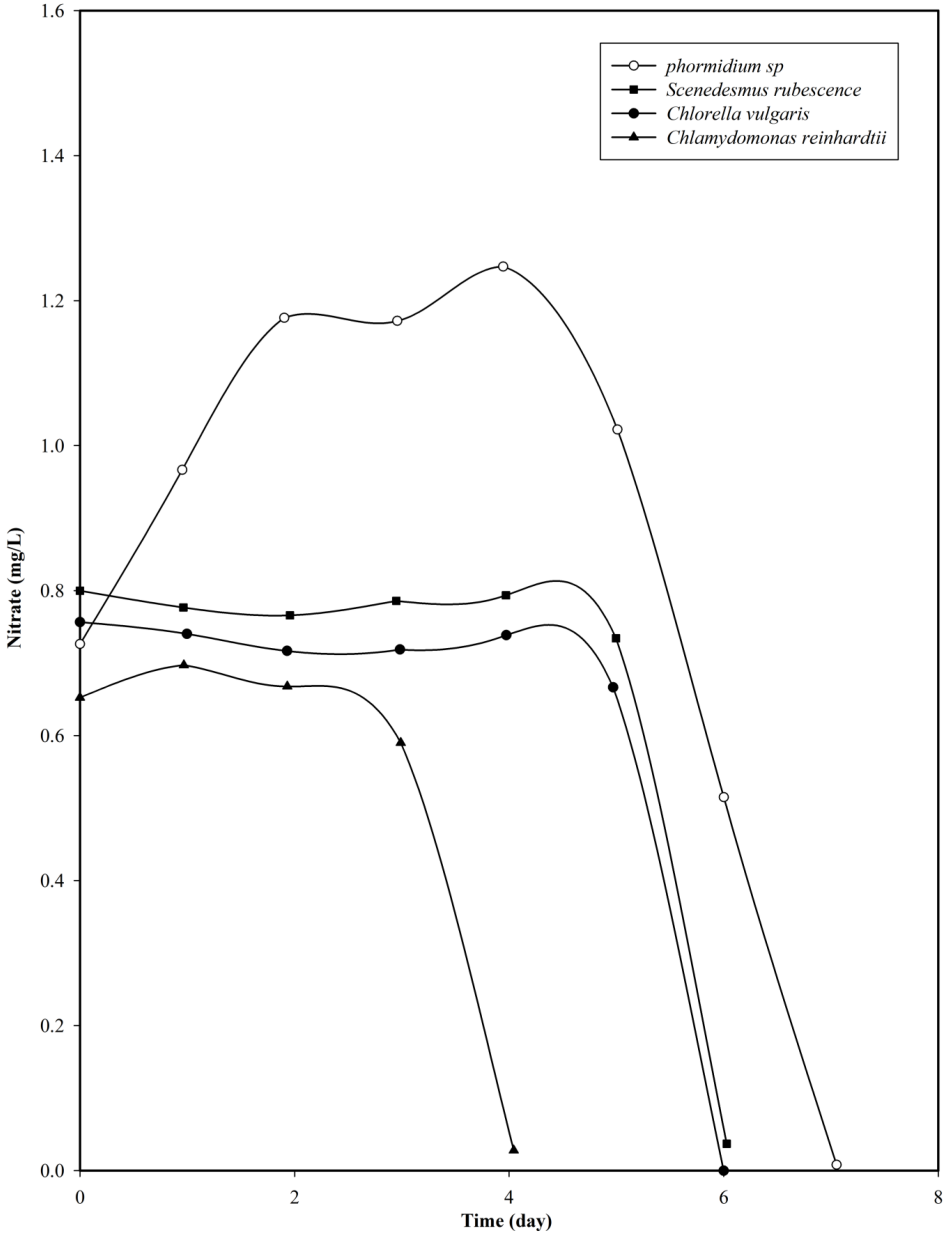

Fig. (1). Nitrate removal of four different unicellular microalgae. (*Chlamydomonas reinhardtii, Chlorella vulgaris, Scenedesmus rubescence and phormidium sp*). (Reproduced from [72] with permission).

been suggested that increasing nitrates in the medium stimulates NR activity leading to NH_4^+ accumulation and toxicity [84]. A significant increase in biomass concentration (from 4.5 to 6.4 g L^{-1}) was also observed for *Chlorella vulgaris*, when initial nitrate concentration was increased from 124 to 1798 mg L^{-1} and nitrate uptake rates also increased (Table **3**) [84]. *Haematococcus pluvialis,* a freshwater Chlorophyta, grown with nitrate concentrations ranging from 32 to 1600 mg L^{-1}, showed growth inhibition at nitrate concentrations higher than 80 mg L^{-1} [76]. Continuous feeding of nitrate at 40 mg L^{-1} to high density cultures of *H. pluvialis* alleviated growth inhibition. Observations that increasing nitrate concentrations result in an increase in nitrate removal rates suggests that nitrate stimulates cellular nitrate reductase activity at moderate nitrate levels [84]. *Nannochloropsis gaditana,* a heterokont in the family *Eustigmataceae,* uses nitrate as the sole nitrogen source at low concentrations (54 mg L^{-1}) [85]. Nitrate removal decreased by 60% when initial nitrate concentration was increased to 1914 mg L^{-1} (Table **3**). Nitrogen limitation is a key factor that initiates lipid accumulation in many groups of algae. The final lipid content in *Nannochloropsis gaditana* decreased 15% when nitrate levels were significantly increased [85]. Ogbonna *et al*. (2000) showed no significant effects on growth or nitrate uptake by a photosynthetic bacterium *Rhodobacter sphaeroides*, a green algae *Chlorella sorokiniana* and *Spirulina platensis*, a cyanobacteria, with nitrate concentrations of 700 mg L^{-1} [86]. Thus, variation in initial nitrate concentrations can have different effects on nitrate removal efficiency, assimilation and growth in a taxa specific fashion.

Table 2. Nitrate removal rate by microalgae species extracted from original references.

Algal Species	Days for Assimilation	PH	T(°C)	Light Intensity ($\mu MOL\ M^{-2}S^{-1}$)	Removal Ratio (%)		Uptake Rate ($MG\ L^{-1}D^{-1}$)		Biomass Concentration ($GDWL^{-1}$)	Reference
					NO_3^-	NO_2^-	NO_3^-	NO_2^-		
Chlorella. Sp	4	-	25	200	62	82	2.65	-	-	[77]
Scenedesmus accutus pvuw12	3	7.4	25	50	100	-	6.26	-	0.74	[70]
Phormidium sp	7	-	-	112	100	100	0.1	0.02	$2.71G/M^2/D$	[72]
Chlamydomonas reinhardtii	4	-	-	112	92.7	22.2	0.16	0.005	$6.06G/M^2/D$	[72]
Chlorella vulgaris	6	-	-	112	100	100	0.13	0.008	$6.28G/M^2/D$	[72]
Scenedesmus rubescence	6	-	-	112	97.5	-	0.13	-	$6.56G/M^2/D$	[72]
Rhodobacter sphaeroides	3	7	30	100	0	-	0	-	-	[86]
Chlorella sorokiniana	3	6	30	100	45.4	-	3.3	-	-	[86]
Spirulina platensis	3	9.2	30	100	100	-	7.2	-	-	[86]
Palmaria palmate	1	-	10	125	26	-	4.96	-	-	[80]
Chondrus cripus	1	-	10	125	56.35	-	11.21	-	-	[80]
Haematococcus pluvialis	5	7.5	23	50	100	-	8.48	-	-	[75]
Scenedesmus obliquus	50%DILLUTION 1	9.71	23	92	-	6.56	6.56	-	0.003	[71]
	70%DILLUTION 1	9.35	23	88.9	-	8.3	8.3	-	0.17	[71]
Botryococcus braunii	14	7.2	25	56	79.74	-	22.21	-	-	[73]
Chlorella vulgaris	14	7.2	25	56	73.7	-	20.28	-	-	[73]
Haemotoccus pluvialis	2	7.5	23	100	-	-	40	-	0.6	[76]
Neochloris oleobundans	28	8	21.5	147	-	-	150	-	0.68	[81]
Chlorella vulgais	6	-	26	350	100	-	103.3	-	4	[82]
Dunaliella tertiolecta	4	-	26	350	100	-	155	-	3.3	[82]
Neochloris oleobundans	3	6.8	-	1280 (LUMEN)	100	-	43.7	-	3.15	[83]
Scenedesmus accutus	16	8.3	27	592	71	-	0.59	-	1.1	[69]

4.2. Light/Dark Cycle and Light Intensity

Light intensity and diurnal light cycles are important factors affecting nitrate uptake [79]. In the natural environment, these are not controllable, however, under laboratory conditions, continuous light increases the rate of nitrate uptake in some algae species. Data on nitrate removal under light/dark cycles and light intensities is summarized in Table **4**. Comparisons of *Chlorella kessleri* grown under 12 h (L/D) lighting and continuous illumination [87], demonstrated that continuous illumination lead to a higher nitrate uptake rate (10.5 mg L^{-1} d^{-1}) than 12 h (L/D) light cycle (4.6 mg L^{-1} d^{-1}) (Table **4**). In contrast, no significant difference in the nitrate and nitrite uptake was reported between alternating (12:12 light/dark) and continuous illumination (24 h light) by a mixed algae culture (*Chlamydomonas reinhardtii, Scenedesmus rubescens* and *Chlorella. vulgaris*), although a higher biomass production capability was achieved for the continuous illumination (Table **4**) [88]. Biomass generation rates with 0, 12 and 24 h illumination per day were 0.93, 7.51 and 9.38 g m^{-2} d^{-1}, respectively (Table **4**). Lower productivity under a 12:12 hour light regime could be attributed to the loss of biomass through respiration in the dark [88].

Light intensity also affects microalgae growth and nitrate removal efficiency. Increasing light intensity is usually accompanied by an increase in nitrate removal rates in microalgal systems. Increasing light intensity from 400 to 1000 µmol photons m^{-2} s^{-1} led to an increase in nitrate uptake rate from 2.2 to 6.3 mg L^{-1} d^{-1} by *Chlamydomonas reinhardtii* (Table **4**) [89]. Increasing light intensity to the point of light saturation, the point which photosynthetic activity reaches its maximum, increases microalgae growth rates. However, at light intensities above the saturation point, photoinhibition occurs, the photosynthetic capacity decreases and growth is inhibited [90]. For example, in light intensities ranging from 5 to 50 µmol photons m^{-2} s^{-1}, maximum removal of nitrate was found at 10 µmol photons m^{-2} s^{-1} for *Trentepohlia aurea*. Nitrate removal rates measured in this system were 0.94, 1.10, 1.02, and 0.80 mg L^{-1} d^{-1} when grown with 5, 10, 20, and 50 µmol photons m^{-2} s^{-1}, respectively (Table **4**) [91]. The data showed that nitrate

Table 3. Effect of initial nitrate concentration on nitrate removal efficiency extracted from original references.

Algal Species	Initial NO^-_3 (mg L^{-1})	Biomass Concentration (g L^{-1})	Residue NO^-_3 (mg L^{-1})	Removal Time (d)	NO^-_3 Removal Rate (mg L^{-1} d^1)	Removal Ratio (%)	pH	T(˚C)	Light Intensity (µmol $m^{-2}s^{-1}$)	Reference
Neochloris oleabundans	45	1.85	0	2	22.6	100	6.8	-	1280 lumen	[83]
	70	2.37	0	2	34.8	100	6.8	-	1280	[83]
	144	3.15	0	3	43.7	100	6.8	-	1280	[83]
	218	2.91	1.4	5	42.5	99.3	6.8	-	1280	[83]
Nannochloropsis gaditana	54	0.72	0	7	7.71	100	7	24	220	[85]
	674.6	-	434	9	26.73	45	7	24	220	[85]
	1294.68	-	806	9	54.22	44	7	24	220	[85]
	1914.68	-	1240	9	74.88	40	7	24	220	[85]
Chlorella vulgaris	124	4.5	94	17	0.45	24.19	-	25	600	[84]
	248	6.1	186	17	0.57	25	-	25	600	[84]
	372	11.5	298	17	0.60	19.89	-	25	600	[84]
	744	10.5	653	17	0.85	12.23	-	25	600	[84]
	1798	6.4	1667	17	0.95	7.28	-	25	600	[84]
Neochloris oleabundans	135.67	1.85	0	1	135.67	100	-	30	360	[81]
	226.11	2.37	0	2	113	100	-	30	360	[81]
	452.23	3.15	0	3	150.74	100	-	30	360	[81]
	678.35	2.91	4.52	6	112.30	99.3	-	30	360	[81]
	904.47	2.70	224.76	6	113.28	75.15	-	30	360	[81]

removal decreased at 20 and 50 µmol photons m^{-2} s^{-1}, light levels, which may exceed the saturation point for this green alga adapted to growth on the trunks and branches of Monterey cypress.

4.3. pH

The pH in microalgal cultivations media is altered by uptake of inorganic carbon from the medium, nitrification in ammonium treatment processes, and microalgal uptake of nitrogen compounds [82]. Eustance *et al*. (2013), showed pH increases in unbuffered growth media with nitrate as the nitrogen source and air as carbon source during growth of two Chlorophyte strains. Enriched CO_2 concentrations can help to prevent pH increases. For example, *Scenedesmus sp*. 131 and *Monoraphidium sp*. 92 exhibited no significant fluctuation in pH when they were grown under 5% CO_2 while growth under ambient air increased pH from 8.5 to 11. Without CO_2 enrichment, nitrate removal rates were 26.3 mg L^{-1} d^{-1} and 17 mg L^{-1} d^{-1} for these strains, respectively, while enrichment with 5% CO_2 resulted in higher nitrate uptake rates which reached 33 and 49.5 mg L^{-1} d^{-1} for the *Scenedesmus* strain and *Monoraphidium,* respectively (Table **5**) [92].

pH fluctuations can also be minimized by the high buffering capacity of saline media or by use of organic buffers. Buffers, including HEPES (pKa 7.4), CHES (pKa 9.3), and CAPS (pKa 10.4) have been used to stabilize pH in culture media. Gardner *et al*. (2011) compared several buffers on nitrate removal rates by *Scenedesmus sp*. and *Coelastrella sp*. The unbuffered system had the highest nitrate removal rate with 22.5 mg L^{-1} d^{-1} for *Scenedesmus sp*. while *Coelastrella sp*. in media buffered by CHES had a maximum nitrate uptake rate with 8.75 mg L^{-1} d^{-1} (Table **5**) [93]. Buffering solutions are not favored for large-scale algal production because of the high cost of commercial organic buffers and are thus confined to laboratory scale experiments.

4.4. Temperature

The growth of microalgae is influenced by temperature *via* effects on enzyme kinetics, changes in catalytic rate and also unfolding/inactivation of enzymes [94, 95]. Additionally, temperature influences metabolite degradation and biosynthesis and changes in conformation of vital structures such as cell membranes [96]. As the temperature drops, kinetic movement of phospholipids in the membrane decelerate making the membranes more rigid; but as the temperature increases, movements accelerate and membranes become more fluid [94]. While most microalgae can adapt to short-term as well as long-term changes in temperature, each strain has a characteristic optimum temperature [97, 98]. For example, the optimum growth temperature for polar microalgae is usually below 10°C [99], for temperate algae is around 10-25°C [100], for tropical strains is around 25°C [97] and for desert algae is between

Table 4. Effect of Light/Dark cycle and light intensity on nitrate removal efficiency and biomass production extracted from original references.

Algal Species	L/D Cycle	Light Intensity ($\mu mol\ m^{-2}s^{-1}$)	Initial NO^-_3 ($mg\ L^{-1}$)	Final NO^-_3 ($mg\ L^{-1}$)	Nitrate Removal Rate ($mg\ L^{-1}\ d^1$)	Removal Time (d)	Biomass Concentration ($g\ L^{-1}$)	T(°C)	pH	Reference
Chlorella kessleri	12h(L+D)	-	168.1	154.1	4.6	3	-	30	-	[87]
	24h(L)		168.1	136.5	10.5	3	-	30	-	[87]
Mixed algae (*Chlamydomonas reinhardtii, Scenedesmus rubescence, Chlorella vulgaris*)	12h(L+D)	112	7	0	0.7	10	7.51 $g/m^2/d$	22.3	-	[88]
	24h(L)		7	0	0.7	10	9.38 $g/m^2/d$	22.3	-	[88]
Trentepohlia urea	-	5	182	-	0.94	4	0.001	25	8	[91]
		10	182	-	1.1	4	0.002	25	8	[91]
		20	182	-	1.02	4	0.001	25	8	[91]
		50	182	-	0.8	4	0.002	25	8	[91]
Chlamydomonas reinhardtii	-	400	-	-	2.2	4h	-	25	6.5-7.5	[89]
		800	-	-	5.8	4h	-	25	6.5-7.5	[89]
		1000	-	-	6.3	4h	-	25	6.5-7.5	[89]

Table 5. Effect of pH on nitrate removal rate extracted from original references.

Species	pH Buffer	Sparge	pH	Initial NO_3^- ($mg\ L^{-1}$)	Nitrate Uptake Rate ($mg\ L^{-1}\ d^{-1}$)	Biomass Concentration ($g\ L^{-1}$)	T(°C)	Light Intensity ($\mu mol\ m^{-2}s^{-1}$)	Removal Time (Day)	Reference
Monoraphidium sp. 92	-	5% CO_2	-	198	49.5	1.89	24	350	6	[92]
	-	Air	-	204.6	17.05	1.13	24	350	12	[92]
Scenedesmus sp. 131	-	5% CO_2	-	198	33	2.41	24	350	6	[92]
	-	Air	-	210.8	26.35	1.23	24	350	8	[92]
Scenedesmus sp.	Unbuffered	-	8.4–11.1	90	22.5	0.83	27	75	4	[93]
	Unbuffered	-	-	180	22.5	0.72	27	75	8	[93]
	Unbuffered	-	-	360	25.7	1.56	27	75	14	[93]
	HEPES	-	7.5	180	12.85	0.97	27	75	20	[93]
	CHES	-	9.4	180	15	1.08	27	75	20	[93]
	CAPS	-	9.4-10.5	90	22.5	1	27	75	4	[93]
	CAPS	-	-	180	21.25	0.54	27	75	8	[93]
	CAPS	-	-	360	25.7	1.60	27	75	14	[93]
Coelastrella saipanensis	Unbuffered	-	6.5–10	180	7.83	0.71	27	75	8	[93]
	HEPES	-	7.5	180	7.5	0.86	27	75	14	[93]
	CHES	-	9.0	180	8.75	0.96	27	75	12	[93]
	CAPS	-	9.7–10.0	180	7.23	0.71	27	75	8	[93]

25 and 35°C [101]. Fluctuations in optimal temperatures can affect microalgae growth. For example, the growth rate of *C. vulgaris* decreased at 35°C by 17% when compared with growth at 30°C. Further increase in temperature (38°C) resulted in a cell death [102]. In another study, 20°C was reported as optimal for growth of *Nannochloropsis oculata.* At temperatures below 20°C, the growth rate dropped 50%, falling from 0.13 to 0.06 day^{-1}. A rapid decrease in the microalgae growth rate was also found at higher temperatures (25°C) [102].

Adaptation to changes in temperature involves a variety of responses in microalgae. Rising temperature increases photosynthetic carbon fixation [95], although not the light dependent reactions of photosynthesis. As photosynthetic rates are enhanced, nutrient assimilation and other energy and reductant requiring processes, including nitrate uptake, also increase with temperature [103]. A maximum nitrate uptake rate of 7.2 μmol mg^{-1} Chl per h in *Chlamydomonas reinhardtii* was reported at 30°C [89] while arctic species have optimal temperatures for both growth and nitrate assimilation at temperatures near freezing [104]. Specific affinity for inorganic N was studied in several algae and bacterial strains in chemostat cultures. While specific affinity for nitrate was strongly dependent on temperature (Q10 = 3, where Q10 is the proportional change with a 10°C temperature increase) and decreased below the optimum temperature, the specific affinity for ammonium exhibited no clear temperature dependence. This work implies that at low temperatures, there is an increased dependence on ammonia rather than nitrate as a N source [104].

4.5. Other Parameters

There are many other parameters affecting nitrate removal rate and growth, including mixing velocity which can act to optimize light exposure and nutrient availability in culture media. Different mixing velocities of 0, 100 and 300 rpm were used for mixed algal cultures (*Chlamydomonas reinhardtii*, *Scenedesmus rubescens* and *Chlorella vulgaris*) [88]. The reactor with 300 rpm mixing velocity had the maximum nitrate and nitrite removal efficiency. The initial concentrations of nitrate and nitrite were 7.1 and 1.2 mg L^{-1} respectively, for the three mixing velocities. Both NO_3^- and NO_2^- were removed above 99% at the end of the each experiment [88]. Provision of both macro- and micro-elements are also critical for growth and nitrate uptake. An increase in nitrate removal rates by *Scenedesmus accutus,* was observed by adding $FeSO_4$ to wastewater. All of the nitrate in the wastewater was removed after 48 h in comparison to control media (with no $FeSO_4$ addition) that consume nitrate in 72 h [70] suggesting that Fe limited algal growth in the wastewater used.

5. ALTERNATIVE TECHNOLOGIES FOR NITRATE BIOREMEDIATION

Cell immobilization and biofilm systems have been suggested as cost effective systems for wastewater treatment including nitrate bioremediation [105].

5.1. Cell Immobilization

Chevalier and Noue (1985) were among the first to immobilize microalgae in carrageenan beads for nutrient removal. Since then, the entrapment of microalgae in gel beads has been explored for nutrient removal from wastewater as a method providing ease of harvest, one of the major technical problems constraining algal systems [106]. Entrapment of microalgae in alginate or carrageenan beads is the most common immobilization techniques [107], while chitosan and polyvinyl foams are inexpensive polymers with a long term performance [108]. Nitrate and nitrite removal rates by *Chlamydomonas reinhardtii* in alginate beads were reported at 5.3 and 4 μmol mg^{-1} chl h^{-1} respectively. A sequential consumption of nitrite and then nitrate occurred after ammonium was completely consumed, suggesting that nitrite inhibits nitrate uptake in this system. The authors suggested that photorespiration in the entrapped cells, due to an in increased O_2/CO_2 ratio, lead to the local accumulation of ammonium [109]. In freely suspended cells of *C. reinhardtii*, nitrate and nitrite were consumed simultaneously and at higher rates (6.1 and 5.8 μmol mg^{-1} chl h^{-1} respectively) [109].

Several studies have reported higher nitrate removal efficiencies by immobilized cells in comparison with free living microalgae. Chitosan immobilization of *Scenedesmus sp*. cells resulted in a 70% nitrate removal within 12 h, at a rate significantly higher than free living cells (20% nitrate removal within 36 h of treatment) (Fig. **2**) [110]. However, the percentage of nitrate removal was lower than that reported by Lau *et al*. (1998a), where complete consumption of nitrate from its initial value of 11.5 mg L^{-1} by immobilized *C. vulgaris* was observed [111]. The higher initial nitrate concentration (44 mg L^{-1}) of the *Scenedesmus sp.* experiments may have influenced the rate [110].

Variations in immobilization methods, specific algae strains and culture conditions, appear to have led to the inconsistent rates found in the literature. The type of immobilized bead was important parameter influencing nitrate and nitrite removal efficiency in a study by Mallick and Rai (1994). They compared nitrate and nitrite uptake rates of *Anabaena doliolum* and *Chlorella vulgaris* in immobilized beads composed of chitosan, agar, alginate, carrageenan and free-living cells. They reported chitosan immobilized cells had the maximum efficiency in term of nitrate and nitrite removal (Table **6**). The nitrate uptake rates were 3.66 and 2.86 [μg NO_3^- (per mg dry wt^{-1}) h^{-1}] for *Anabaena doliolum* and *Chlorella vulgaris,* respectively, while nitrite removal rates were lower at 1.3 and 1.6 [μg NO_2^- (mg dry wt^{-1}) h^{-1}], respectively [112].

A major problem in immobilization technology is that microalgae may be released from the beads when the maximum holding capacity is surpassed [113]. A twin layer system was developed to separate microalgae from their growth medium and allow diffusion of nutrients from the media to the cells. A twin layer system was used to remove nitrate from municipal wastewater by two green microalgae (*Chlorella vulgaris* and *Scenedesmus rubescens*). Nitrate concentrations at day 4 were 0.09 mg L^{-1} and 0.10 mg L^{-1} for *C. vulgaris* and *S. rubescens*, respectively with initial value of 4 mg L^{-1} by both algae [114].

5.2. Biofilm formation

Microalgal biofilm systems have some advantages allowing short hydraulic retention times [115, 116] and requires less energy input because stirring is not needed compared to suspended microalgal systems. Nitrate removal using a microalgal biofilm investigated at low, intermediate, and high nutrient loads, showed that the higher loading rates lead to lower nitrate removal. In the minimum loading rate (0.18 g m^{-2} d^{-1}), nitrate was completely removed from initial concentration of 9 mg L^{-1} in 6 days [117].

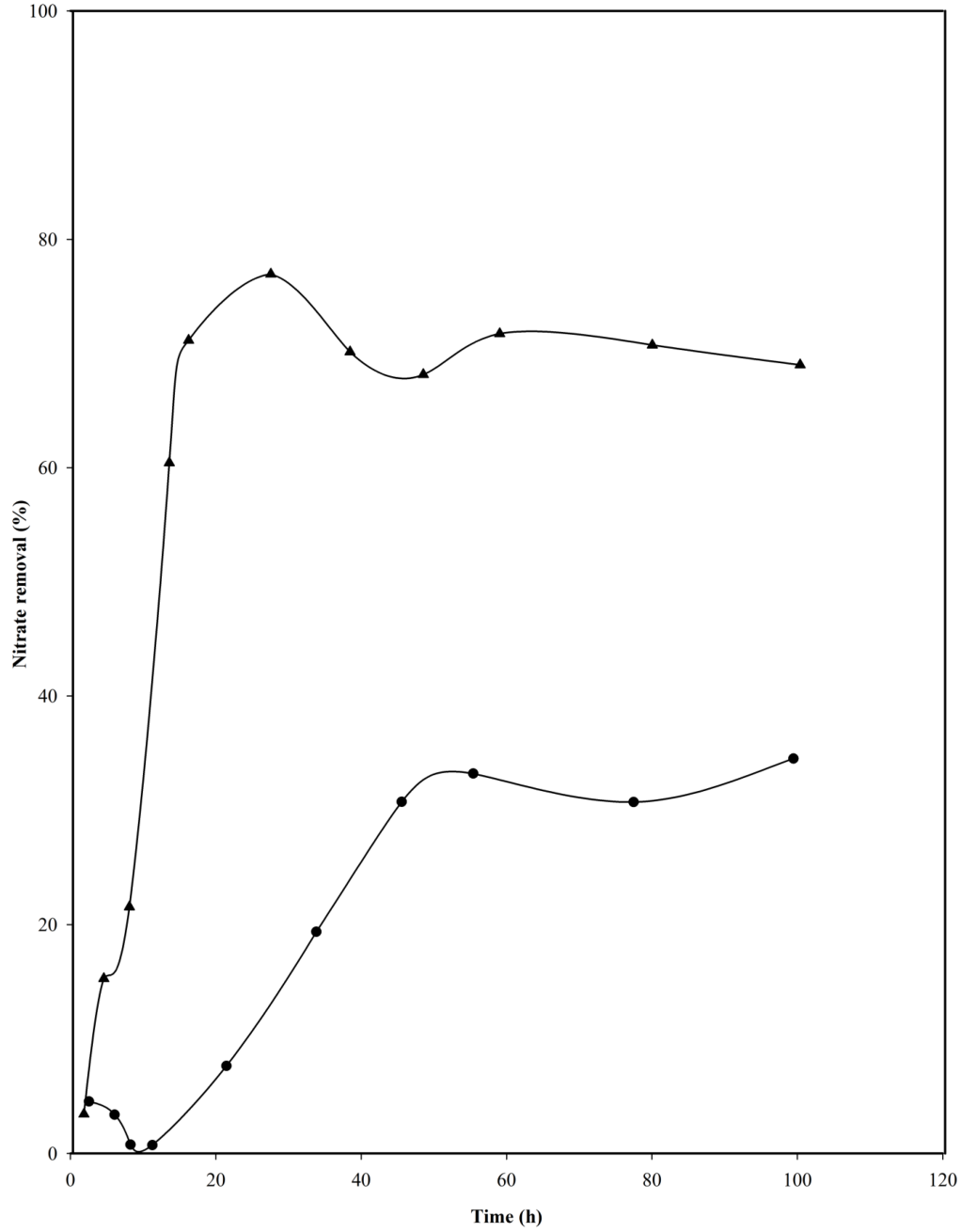

Fig. (2). Nitrate uptake from medium as percentage removed by free-living cells (●) and immobilized *Scenedesmus sp.* cells (▲). (Reproduced from [110] with permission).

CONCLUSION

The use of microalgae for nitrate and nitrite removal from wastewater has potential as an alternative technique to conventional nutrient removal methods when coupled to biomass production. Wastewater can be considered as a cost effective and available medium for microalgae. By assimilation of nitrate and nitrite and conversion into biomass, algae growth provides an efficient means of reclaiming the nutrients in wastewater and purifying water. The conclusions from this review are that: a) The most favorable strains for nitrate removal and biomass production are *Neochloris oleoabundans, Duniella tertiolecta and Chlorella vulgaris*, b) Higher initial nitrate concentrations result in higher nitrate removal rates in many, but not all species, c) Increasing light intensity to the saturation point leads to maximum nitrate removal and when light intensity surpasses that point, photosynthetic efficiency along with nitrate uptake decreases, d) Optimal growth temperatures of 10-35˚C have been reported for different species and nitrate uptake but ammonia uptake is not strongly influenced by temperature, e) Using buffers or enriched CO_2 concentrations help to prevent major fluctuations of pH when nitrate is used as the nitrogen source, f) Entrapment of microalgae in alginate or carrageenan beads for wastewater treatment shows promise and avoids the high cost of harvesting free-living microalgae, and g) In addition to their bioremediation capabilities, the microalgae feedstock can be used to produce

Table 6. Removal of NO_3^- and NO_2^- by free and immobilized *Anabaena doliolum* and *Chlorella vulgaris* over three cycles (I. II and III). (Copied from [112] with permission).

Cells and Method of Immobilization	NO_3^- Uptake [µg NO_3^- (mg dry wt^{-1}) h^{-1}]			NO_2^- Uptake [µg NO_2^- (mg Dry wt^{-1}) h^{-1}]		
	I	II	III	I	II	III
Anabaena doliolum						
Alginate	3.4	2.4	2.0	1.3	1.1	0.6
Agar	3.9	2.5	1.8	1.5	1.1	0.7
Carrageenan	2.5	2.0	1.0	1.2	1.0	0.5
Chitosan	4.3	3.7	3.0	1.6	1.5	0.9
Free cells	2.5	1.9	0.9	1.1	0.9	0.5
Chlorella vulgaris						
Alginate	3.5	2.1	1.7	1.5	1.0	0.4
Agar	3.7	2.3	1.7	1.6	1.2	0.4
Carrageenan	2.9	1.7	1.5	1.4	0.9	0.3
Chitosan	3.8	2.6	2.2	1.7	1.2	0.6
Free cells	2.9	2.1	1.2	1.4	1.1	0.4
Standard error	**0.02- 0.08**			**0.01- 0.05**		

a variety of products including feed, biofuels, nutraceuticals, high value chemicals and hydrogen in an integrated system. Therefore, algal-based biotechnology is an environmentally and economically sound approach to reduce nitrate and nitrite level in wastewater while generating valuable co-products. In the near future, wastewater engineers and scientists from the algae biomass industry will retrofit wastewater treatment plants to integrate wastewater treatment and CO_2 mitigation, such that environmental water quality standards are maintained and multiple sources of revenue can be generated from the production of algal biomass. All of this is dependent on a fundamental understanding of the physiology and growth characteristics of the individual stains of algae.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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