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Removal of Ammonia from Wastewater Effluent by *Chlorella Vulgaris*[*]

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Abstract: The capability of *Chlorella vulgaris* to remove nitrogen in the form of ammonia and/or ammonium ions from wastewater effluent in a local wastewater treatment plant (i.e., the Mill Creek Plant in Cincinnati, Ohio, U.S.A.) was studied. The wastewater effluent leaving the plant was found to include high concentrations of nitrogen (7.7±0.19 mg/L) (ammonia (NH_3) and/or ammonium ion (NH_4^+)) and total inorganic carbon (58.6±0.28 mg/L) at pH 7, and to be suitable for growing *Chlorella vulgaris*. When *Chlorella vulgaris* was cultivated in a batch mode under a closed system, half of the nitrogen concentration was dramatically removed in 48 h after a 24-h lag-phase period. Total inorganic carbon concentration also concomitantly decreased during the rapid growth-phase. The total biomass weight gained during the entire cultivation period balanced out well with the total amount of inorganic carbon and nitrogen removed from the culture medium. These results indicate that wastewater can be synergistically used to polish residual nutrients in wastewater as well as to cultivate microalgae for biofuel production.

Key words: microalgae; *Chlorella vulgaris*; wastewater effluent; nitrogen removal

Introduction

Diminishing petroleum resources along with increased demand for petroleum by emerging economies are driving our society to search for new renewable and carbon neutral liquid transportation fuels. Biofuels such as bioethanol and biodiesel derived from agricultural and forestry residues and oil crops have been considered to be candidates for renewable liquid fuels. Recently, microalgae-derived biodiesel production has received great attention due to their ability to produce substantial amounts of triacylglycerols (e.g., 20%-50% dry cell weight)[1], as a feedstock for biodiesel[2,3], and to utilize a large fraction of solar energy (up to 10%)[4] and carbon dioxide (CO_2), and to grow at much faster rates than terrestrial biomass[5]. In addition, sugars generated as metabolites have potential to produce bioalcohols[6-10].

Nitrogen and phosphorous discharged through agricultural, sewage, and industrial effluent are the major contributors to ecological eutrophication[11,12]. In general, treated wastewater still include nitrogen and phosphorous in the form of nitrate, nitrite, ammonia/ammonium, and phosphorus[12]. Microalgae require nitrogen, phosphorous, CO_2, and light for their autotrophic growth. It is estimated that there are 2×10^5-10×10^5 different microalgal strains in nature, but only about 3×10^4 strains have been described[13]. Among the strains, *Chlorella vulgaris* belongs to a genus of single-celled green algae, and is known as one of the fastest growing microalgae, and typically includes 14%-22% of lipid, 51%-58% of protein, 12%-17% of carbohydrates, and 4%-5% of nucleic acid[14].

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In previous studies, a variety of wastewaters from urban, industrial, or agricultural sources[15-17] were used to remove nitrogen and phosphorus by using *Chlamydomonas reinhardtii*[18], *Botryococcus braunii*[19], *Chlorella pyrenoidosa*[20], *Haematococcus pluvialis*[21], *Spirulina maxima*[22], *Spirulina* (*Arthrospira*)[23], *Scenedesmus rubescens*[24], *Scenedesmus abundance*[25], *Scenedesmus quadricauda*[25], *Scenedesmus acuminatus*, *Scenedesmus obliquus*[26-28], *Scenededesmus dimorphus*[29], and *Haematococcus plurialis*[21]. *Chlorella vulgaris* has been reported to be able to readily uptake nitrogen from ammonium ion and ammonia and phosphorus through the cell membrane from the wastewater[24,25,29-32]. It was also reported that algae-derived biofuels could compensate for environmental burden using wastewater as a source for CO_2 and fertilizer according to previous life cycle assessment (LCA) studies[33,34].

The Mill Creek plant located in Cincinnati, Ohio, U.S.A. is a major wastewater treatment facility where ~70% and 30% of the wastewater is typically collected from industrial and domestic sources, respectively. A sample collected from the wastewater effluent contains nitrogen at the concentration level of 7.7±0.19 mg/L in the form of ammonia (NH_3) and/or ammonium (NH_4^+) with low concentrations (undetectable by ion chromatography) of nitrate, nitrite, and orthophosphate. In this study, the removal of nitrogen (i.e., ammonia (NH_3)/ammonium (NH_4^+)) by *Chlorella vulgaris* has been explored for potential wastewater treatment and biofuels production.

1 Materials and Methods

1.1 Culture media

Wastewater effluent was collected from the Mill Creek wastewater plant in Cincinnati, OH, U.S.A., and was used as a culture medium. The plant monitors the concentrations of ammonia/ammonium, nitrite/nitrate, and total phosphate on a daily basis, and their annual average concentrations were 14.67±3.65 mg/L, 1.95±1.10 mg/L, and 0.90±0.31 mg/L, respectively, during 2008 and 2009 (Lachat's QuikChem 8500 Series 2 Flow Injection Analysis System). A 30-mL sample of *Chlorella vulgaris* suspended in shuisheng-4 medium[35] was added to the wastewater effluent. The cell density of *Chlorella vulgaris* in the wastewater was found out to be 253 mg/L.

1.2 Light condition

Fluorescent lamps with 6500 K color temperature similar to natural sunlight were used as the source of light, and the incoming light intensity to beakers was set to 6000 lux (100.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$) by controlling the distance between the beaker and lamp. The light intensity was measured using a light intensity meter (HQRP digital lux meter, LX1010BS, Osprey-Talon Company), and a 16-h light and 8-h dark cycle was applied to the culture.

1.3 Determination of cell density of *Chlorella vulgaris*

The cell density of *Chlorella vulgaris* was determined by measuring the optical density of a 15-mL sample at 682 nm[36] for every 24 h by using UV-vis spectrophotometer (Cary 50, Varian, Inc.). Here, the absorbance of UV spectrophotometer at 682 nm was calibrated by measuring the weight of dried *Chlorella vulgaris*. Then, the weight of dried biomass was obtained from the prepared calibration curve.

1.4 Determination of nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentration

The concentrations of nitrate and phosphate ions were determined with Dionex DX-500 ion chromatography system equipped with IonPac AS14 anion-exchange column. The flow rate of sodium carbonate/sodium bicarbonate effluent (1.8 mmol/L Na_2CO_3 and 1.7 mmol/L $NaHCO_3$ in 1.0 L of deionized water) was 1.2 mL/min.

1.5 Determination of total inorganic carbon concentration

An acid-base titration method[37] was used to determine the inorganic carbon species in the aqueous phase. This titration method determines total inorganic carbon concentration (carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), and aqueous carbon dioxide (CO_2 (aq))) using a 0.01 mol/L hydrochloric acid solution as a titrant.

1.6 Determination of nitrogen concentration in the form of ammonia (NH_3) and ammonium ion (NH_4^+)

Nitrogen concentrations in the form of ammonia and ammonium ions present in the wastewater were measured using a commonly used ammonia probe (Model: 9512HPBNWP Orion Thermo Scientific)[38,39]. All ammonium ions were converted into ammonia by raising the pH of the sample solution of the culture medium (i.e., wastewater effluent) above 12 while monitoring its pH (Oakton pH 11 series pH meter), and the resultant ammonia concentration was determined by the ammonia probe. Then the concentration distribution of ammonia and ammonium ions was determined by the equilibrium at the original pH of the sample solution. The standard ammonia solution required for the calibration of the ammonia probe was prepared according to the EPA Method 350.3[40]. 10 mol/L NaOH (reagent grade, Fischer Scientific) was required for raising the pH of the solution above 12. Distilled and deionized water from a MilliQ water purification system was used for preparing the standard and NaOH solutions.

The probe was re-calibrated every 5 samples according to the EPA Method 350.3[40] over a range of 1000 mg/L to 1 mg/L of NH_4Cl solution. 25 mL of a sample was taken in a container with an equal surface to volume ratio as recommended by the EPA Method 350.3. Then the sample was stirred using a flat micro-magnetic stirrer placed on a magnetic stirrer base (Fischer Scientific magnetic stirrer base). The probe was immersed in the sample at an inclination angle of 45 degrees with continuous stirring. A strong base solution (10 mol/L NaOH) was added to reach a pH higher than 12. A reading was taken after the voltage signal in mV reached a constant value after a 1-min time interval. After each measurement, the probe was washed thoroughly using deionized water and wiped with a soft tissue. When it was not in use, the ammonia probe was stored in a 0.1 mol/L ammonium chloride solution.

A 25-mL sample withdrawn from the batch culture at a 24-h interval was used to determine the nitrogen concentration. The sample was filtered out using a syringe filter (0.45 μm nominal pore with 25 mm diameter, Whatman filter) in order to avoid potential blockage of the membrane of the ammonia probe. All the measurements were carried out in triplicate to ensure the validity of the data.

2 Results and Discussion

The wastewater effluent collected from the Mill Creek plant contained large amounts of total inorganic carbon (58.6±0.28 mg/L) and nitrogen (7.7±0.19 mg/L) in the form of ammonia (NH_3)/ammonium (NH_4^+) with the small amounts of nitrate (NO_3^-) and nitrite (NO_2^-) (1.95±1.10 mg/L on annual average) and phosphate (PO_4^{3-}) (0.90±0.31 mg/L on annual average). In addition, the initial pH of the wastewater effluent was almost constant at pH 7. *Chlorella vulgaris* can grow using carbon dioxide and light, known as the photosynthetic process: $6CO_2 + 12H_2O + \text{light (energy)} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$. Microalgae can utilize bicarbonate ions (HCO_3^-) as a carbon source for photosynthesis with the help of an enzyme called carbonic anhydrases (CA) as well as carbon dioxide. In addition, inorganic nutrients, especially nitrogen and phosphate are required for their growth.

The growth rate of *Chlorella vulgaris* and the uptake rate of total inorganic carbon are shown in Fig. 1. The lag-phase period for adaptation to the wastewater condition was found to be short (24 h), and total inorganic carbon was not reduced during the period. During the growth phase, the cell density of *Chlorella vulgaris* dramatically increased, and the total inorganic carbon concentration sharply decreased until 96 h, indicating active photosynthetic reaction. The pH of the wastewater continued to increase from the lag phase through the growth phase as shown in Fig. 2. Microalgae are known to produce hydroxyl ions (OH^-) when a bicarbonate ion is consumed for photosynthesis within the algal cell by following the reaction: $HCO_3^- \rightarrow CO_2 + OH^-$[41-43]. The pH started to level off as the uptake rates of total inorganic carbon and nitrogen were discontinued after 144 h.

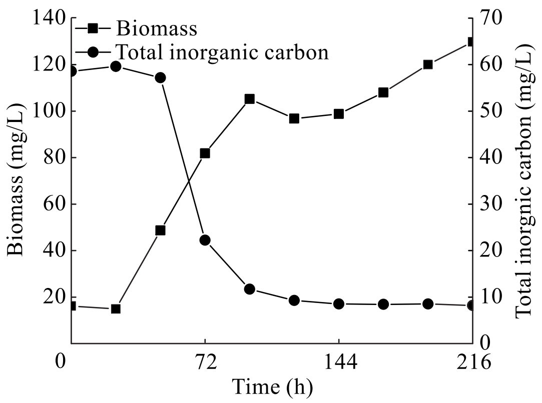

Fig. 1 Growth rate of *Chlorella vulgaris* and uptake rate of total inorganic carbon

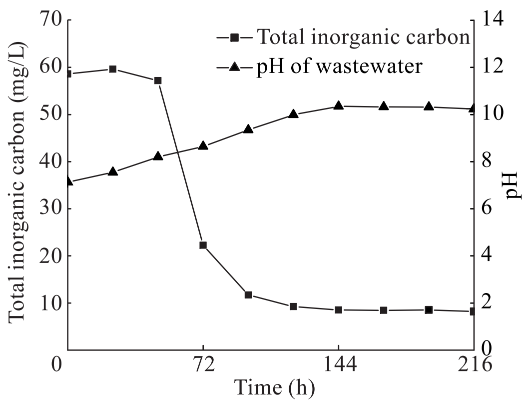

Fig. 2 Total inorganic carbon uptake and pH increase

The removal rate of nitrogen (ammonia (NH_3)/ammonium ion (NH_4^+)) is shown in Fig. 3. During the lag phase, the nitrogen removal efficiency varied little from 7.6±0.24 mg/L to 7.7±0.19 mg/L matched with no growth of the *Chlorella vulgaris* or uptake of total inorganic carbon. As with the uptake of the inorganic carbon, the nitrogen concentration did not decrease during the initial lag phase, but then sharply decreased from 7.6±0.24 mg/L to 3.4±0.17 mg/L at the same time as the decrease in total inorganic carbon concentration during the rapid growth-phase (i.e., 24 to 96 h). It is interesting to note that the total biomass weight gained during the entire cultivation period is well balanced by the total amount of inorganic carbon and nitrogen removed from the culture medium as clearly shown in Fig. 1.

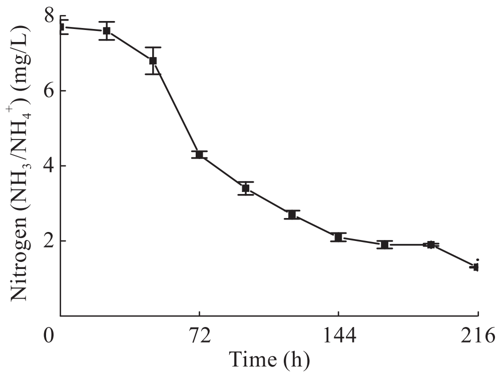

Fig. 3 Removal of nitrogen in the form of NH_3/NH_4^+ from wastewater effluent by *Chlorella vulgaris*

3 Conclusions

In this study, *Chlorella vulgaris* was used to remove nitrogen in the form of ammonia and ammonium ions from the wastewater effluent collected from the Mill Creek plant in Cincinnati, OH, U.S.A. The mass balances taken for inorganic carbon and nitrogen show that the weight gained by biomass is almost comparable to the amount of inorganic carbon and nitrogen removed from wastewater. The experimental results indicated that *Chlorella vulgaris* has potential to remove nitrogen (i.e., ammonia and ammonium ion) at a reasonable uptake rate from wastewater while being cultivated using wastewater effluent. As a result of consuming bicarbonate ions, the pH of the culture medium increased. This can open up an opportunity to utilize wastewater in order to cultivate microalgae for the dual purpose of removing nutrients and producing biofuels.

References

[1] Hu Q, Sommerfeld M, Jarvis E, et al. Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. *Plant Journal,* 2008, **54**(4): 621-639.

[2] Liu Z Y, Wang G C, Zhou B C. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresource Technology*, 2008, **99**(11): 4717-4722.

[3] Hill J, Nelson E, Tilman D, et al. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proceedings of the National Academy of Sciences of the United States of America*, 2006, **103**(30): 11 206-11 210.

[4] Huber G W, Iborra S, Corma A. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chemical Reviews*, 2006, **106**(9): 4404-4498.

[5] Chisti Y. Biodiesel from microalgae. *Biotechnology Advances*, 2007, **25**: 294-306.

[6] Ginzburg B Z. Liquid fuel (oil) from halophilic algae: A renewable source of non-polluting energy. *Renewable Energy*, 1993, **3**(2-3): 249-252.

[7] Dote Y, Sawayama S, Inoue S, et al. Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. *Fuel*, 1994, **73**(12): 1855-1857.

[8] Miao X, Wu Q. Biodiesel production from heterotrophic microalgal oil. *Bioresource Technology*, 2006, **97**(6): 841-846.

[9] Milne T A, Evans R J, Nagle N. Catalytic conversion of microalgae and vegetable oils to premium gasoline, with shape-selective zeolites. *Biomass*, 1990, **21**(3): 219-232.

[10] Minowa T, Yokoyama S-Y, Kishimoto M, et al. Oil production from algal cells of Dunaliella tertiolecta by direct thermochemical liquefaction. *Fuel*, 1995, **74**(12): 1735-1738.

[11] Liu W, Zhang Q, Liu G. Lake eutrophication associated with geographic location, lake morphology and climate in China. *Hydrobiologia*, 2010, **644**(1): 289-299.

[12] Abe K, Imamaki A, Hirano M. Removal of nitrate, nitrite, ammonium and phosphate ions from water by the aerial microalga Trentepohlia aurea. *Journal of Applied Phycology*, 2002, **14**(2): 129-134.

[13] Sheehan J, Duahay T, Benemann J, et al. A look back at the U.S. Department of Energy's aquatic species program-biodiesel from algae. Golden, CO: National Renewable Energy Laboratory, 1988.

[14] Becker E W. Microalgae Biotechnology and Microbiology. New York: Cambridge University Press, 1994.

[15] Fallowfield H J, Garrett M K. The treatment of wastes by algal culture. *Journal of Applied Bacteriology Symposium Supplement*, 1985, **14**: 187S-205S.

[16] Aragón A B, Padilla R B, Ros de Ursinos J A F. Experimental study of the recovery of algae cultured in effluents from the anaerobic biological treatment of urban wastewaters. *Resources*, *Conservation and Recycling*, 1992, **6**(4): 293-302.

[17] Noüe Jdl, Laliberté G, Proulx D. Algae and waste water. *Journal of Applied Phycology*, 1992, **4**(3): 247-254.

[18] Kong Q-X, Li L, Martinez B, et al. Culture of microalgae Chlamydomonas reinhardtii in wastewater for biomass feedstock production. *Applied Biochemistry and Biotechnology*, 2010, **160**(1): 9-18.

[19] An J Y, Sim S J, Lee J S, et al. Hydrocarbon production from secondarily treated piggery wastewater by the green alga Botryococcus braunii. *Journal of Applied Phycology*, 2003, **15**(2-3): 185-191.

[20] Sánchez S, Martínez M E, Espejo M T, et al. Mixotrophic culture of Chlorella pyrenoidosa with olive-mill wastewater as the nutrient medium. *Journal of Applied Phycology*, 2001, **13**(5): 443-449.

[21] Kang C D, An J Y, Park T H, et al. Astaxanthin biosynthesis from simultaneous N and P uptake by the green alga Haematococcus pluvialis in primary-treated wastewater. *Biochemical Engineering Journal*, 2006, **31**(3): 234-238.

[22] Kosaric N, Nguyen H T, Bergougnou M A. Growth of Spirulina maxima algae in effluents from secondary waste-water treatment plants. *Biotechnology and Bioengineering*, 1974, **16**(7): 881-896.

[23] Olguín E J, Galicia S, Mercado G, et al. Annual productivity of Spirulina (Arthrospira) and nutrient removal in a pig wastewater recycling process under tropical conditions. *Journal of Applied Phycology*, 2003, **15**(2-3): 249-257.

[24] Shi J, Podola B, Melkonian M. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: An experimental study. *Journal of Applied Phycology*, 2007, **19**(5): 417-423.

[25] Kassim T I. Possible use of microgreen algae to remove phosphate and nitrate from wastewater. In: Proceedings of International Symposium on Environmental Pollution Control and Waste Management. 2002, **7-10**: 628-632.

[26] Hodaifa G, Martínez M E, Sánchez S. Daily doses of light in relation to the growth of Scenedesmus obliquus in diluted three-phase olive mill wastewater. *Journal of Chemical Technology & Biotechnology*, 2009, **84**(10): 1550-1558.

[27] Martínez M E, Sánchez S, Jiménez J M, et al. Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. *Bioresource Technology*, 2000, **73**(3): 263-272.

[28] Voltolina D, Gómez-Villa H, Correa G. Nitrogen removal and recycling by Scenedesmus obliquus in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle. *Bioresource Technology*, 2005, **96**(3): 359-362.

[29] González L E, Cañizares R O, Baena S. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae Chlorella vulgaris and Scenedesmus dimorphus. *Bioresource Technology*, 1997, **60**(3): 259-262.

[30] Schlee J, Komor E. Ammonium uptake by Chlorella. *Planta*, 1986, **168**(2): 232-238.

[31] Yun Y S Y, Lee S B, Park J M P, et al. Carbon dioxide fixation by algal cultivation using wastewater nutrients. *Journal of Chemical Technology & Biotechnology*, 1997, **69**(4): 451-455.

[32] Bich N N, Yaziz M I, Bakti N A K. Combination of *Chlorella vulgaris* and *Eichhornia crassipes* for wastewater nitrogen removal. *Water Research*, 1999, **33**(10): 2357-2362.

[33] Clarens A F, Resurreccion E P, White M A, et al. Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environmental Science & Technology*, 2010, **44**(5): 1813-1919.

[34] Lardon L, Helias A, Sialve B, et al. Life-cycle assessment of biodiesel production from microalgae. *Environmental Science & Technology*, 2009, **43**(17): 6475-6481.

[35] Powell E E, Mapiour M L, Evitts R W, et al. Growth

kinetics of *Chlorella vulgaris* and its use as a cathodic half cell. *Bioresource Technology*, 2009, **100**(1): 269-274.

[36] Widjaja A, Chien C C, Ju Y H. Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *Journal of the Taiwan Institute of Chemical Engineers*, 2009, **40**(1): 13-20.

[37] Fischer R B, Peters D G. Basic Theory and Practice of Quantitative Chemical Analysis. Philadelphia, London, Toronto: W. B. Saunders Company, 1968.

[38] McKenzie L R, Young P N W. Determination of ammonia-, nitrate- and organic nitrogen in water and waste water with an ammonia gas-sensing electrode. *The Analyst*, 1975, **100**(1194): 620-628.

[39] Association A P H. Standard Methods for the Examination of Water and Wastewater. New York: American Water Works Association and Water Pollution Control Federation, 2005.

[40] Thomas R F, Booth R L. Selective electrode measurement of ammonia in water and wastes. *Environmental Science and Technology*, 1973, **7**(6): 523-526.

[41] Shiraiwa Y, Goyal A, Tolbert N E. Alkalization of the medium by unicellular green algae during uptake dissolved inorganic carbon. *Plant and Cell Physiology*, 1993, **34**(5): 649-657.

[42] Azov Y. Effect of pH on inorganic carbon uptake in algal cultures. *Applied and Environmental Microbiology*, 1982, **43**(6): 1300-1306.

[43] Prins H B A, Snel J F H, Helder R J, et al. Photosynthetic HCO_3^- utilization and OH—excretion in aquatic angiosperms. *Plant Physiology*, 1980, **66**: 818-822.