# Improved Ammonium Removal from Industrial Wastewater through Systematic Adaptation of wild type *Chlorella pyrenoidosa*

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## Abstract

A single step process for ammonium removal from nitrogenous industrial effluents with a concomitant generation of algal biomass, which can be used for producing biofuels and other value added products is proposed. A microlagal strain found in the effluent treatment plant of a fertilizer industry in Mumbai, India was systematically adapted to remove up to 700 ppm of ammoniacal nitrogen from industrial wastewater, which is nearly four times higher than the ammonium tolerance reported in the literature as well as other algal strains tested in our laboratory. 18S rRNA sequencing revealed the strain to be *Chlorella pyrenoidosa*. Effects of process parameters such as pH, temperature and light intensity on cell growth and ammonium removal by the adapted cells were studied. Optimal conditions were found to be pH of 9, temperature of 30$^{o}$C and a light intensity of 3500 Lux for the adapted cells.

## Keywords

Nitrogenous wastewater treatment, Microalgae, Ammonium removal, *Chlorella pyrenoidosa*, Fertilizer industry effluent

## 1. Introduction

The fertilizer industry generates large amounts of wastewater containing high amounts of nitrogen in the form of ammonium or nitrate. Other industries related to tannery, explosives and metal finishing also generate nitrogenous wastewater. Discharging nitrogenous effluents into water bodies can cause eutrophication, and can impact health by causing diseases such as methaemoglobinaemia, also known as blue baby syndrome (Vitousek, Aber, et al., 1997). Therefore, it is essential to remove nitrogen before discharging the effluent in the environment.

Conventional treatment of ammoniacal nitrogen is a two-step process: nitrification or oxidation followed by biological denitrification or reduction. During nitrification, ammonia is oxidized to nitrate which requires high amount of oxygen. The effluent from this step is then denitrified via an anoxic process wherein nitrate is reduced to nitrogen gas in a series of reactions (Kuba, Van Loosdrecht, et al., 1997; Park, Craggs, et al., 2011; Yoo, Ahn, et al., 1999). The reduction step requires addition of organic carbon source, if not present in the effluent. Therefore, the major factors contributing to the cost of the overall process are (i) energy requirement for aeration in nitrification and (ii) cost of organic carbon source in biological denitrification. Moreover, the denitrification process produces $CO_2$, which is a greenhouse gas.

The use of microalgae to treat nitrogenous wastewater can significantly improve process economics by eliminating the aeration and carbon source costs and by generating useful biomass which can subsequently be converted to biofuels or value added products (Shyam Kumar and Saramma, 2012; Wang and Lan, 2011; Chen, Pan, et al., 2012; Hoa Binh aus Hanoi, 2013; Singh, Nigam, et al., 2011b; Singh, Nigam, et al., 2011a; John, Anisha, et al., 2011; Pittman, Dean, et al., 2011). As microalgae are autotrophs capable of using carbon dioxide as the primary carbon

source, flue gases containing $CO_2$ can also potentially be incorporated into the process(Woertz, Feffer, et al., 2009).

Wastewater from the fertilizer industry contains very high concentrations of ammoniacal nitrogen, $NH_4^+$-N (around 1500-2000 ppm). Algal species grow at relatively lower concentrations of ammonium (below 100 ppm) (Abeliovich & Azov, 1976; Markou, Vandamme, & Muylaert, 2014). While ammonium is an excellent source of nitrogen, at higher concentrations it can inhibit cell growth by hampering photosynthesis and carbon metabolism. Ammonia uncouples the electron transport in Photosystem II by breaking down the proton gradient required to drive photophosphorylation (Azov & Goldman, 1982). Azov and Goldman reported a 50% reduction in photo-assimilation of $^{14}C$ by various freshwater algae at an ammonia concentration as low as 20 ppm. Park et al. reported a 70% inhibition of cell growth of *Scenedesmus* sp. at ammonium concentrations above 100 ppm (Park, Jin, et al., 2010). Markou et al tested the effects of various levels of ammonia on *Arthospira* and *Chlorella* and found that ammonia had multiple effects on the photosynthetic apparatus of both algae including photosystems I and II, electron transport chain as well as the oxygen evolution complexes (Markou, Depraetere, et al., 2016). Experiments with cyanobacterium *Synechocystis* sp treated with varying concentrations of $NH_4Cl$ also indicated a PS II photo-inhibition (Synechocystis, Oa, et al., 2008). Most of the studies using microalgae have used wastewater with relatively low concentrations of ammoniacal nitrogen, in the range of 20-100 ppm (Ruiz-Marin, Mendoza-Espinosa, et al., 2010; Lincoln E.P., Wilke, A.C., French, 1996; Ogbonna, Yoshizawa, et al., 2000). Therefore, in order to use microalgae for removing high concentrations of ammoniacal nitrogen, either genetic modification or acclimatization of the cells to high ammonium levels is required (Collos and

Harrison, 2014). A recent study with "ammonium-rich" synthetic wastewater was carried out with up to 150 ppm of $NH_4Cl$. The authors proposed a three-stage process with varying ratios of $NH_4^+$-N and organic loads in each stage for effective removal of ammonium using an indigenous algal strain (Wang, Zhou, et al., 2016).

In our lab, we have isolated a microalgal species from a water source close to the effluent treatment plant of a fertilizer industry and adapted the cells to high levels of ammonium. These cells showed much higher tolerance to ammoniacal-nitrogen (up to 700 ppm) compared to other species tested in our lab as well as those reported in the literature. We also evaluated three critical process parameters, namely pH, temperature and light intensity for their effect on cell growth as well as ammonium removal. Temperature and pH are known to impact the ammonia-ammonium equilibrium in the wastewater as well as the ammonium metabolism in cells (Chen, Pan, et al., 2012). Effect of light/dark cycles has also been studied on cell growth and nitrogen removal by *Chlorella kessleri* and it was found that continuous illumination resulted in better nitrogen removal compared to when light/dark cycles were used (Lee and Lee, 2001). Furthermore, light intensity was also found to impact the toxicity of ammonia in recent studies (Markou, Depraetere, et al., 2016). This also agrees with our findings as will be discussed later.

The work presented here focuses on (1) adaptation of the selected microalgal strain to high levels of ammonium and (2) optimizing the process for cell growth and ammonium removal. A process developed using these cells can be used to treat industrial nitrogenous wastewater and subsequently utilize the algal biomass generated to produce biofuels and value added products.

## 2. Methods

### 2.1 Culture medium and wastewater samples

Nitrogenous wastewater effluent was procured from the Rashtriya Chemicals and Fertilizers (RCF), Mumbai, India. This will henceforth be referred to as 'RCF effluent'. The effluent contained approximately 1800 ppm of ammoniacal nitrogen. For each experiment, the RCF effluent was diluted to obtain the desired ammonium content, after which 16.8 g/l $NaHCO_3$ and 0.5 g/l of $K_2HPO_4$ were added as carbon, phosphorous and potassium sources. Process optimization studies were carried out using synthetic medium, which was a modified version of Zarrouk's medium (Gami, Naik, et al., 2011), after comparability was established with RCF effluent.

Synthetic medium was prepared by replacing salts containing nitrogen and with ammonium chloride to obtain a final ammonium concentration comparable to that in RCF effluent. The composition of the synthetic medium was: 16.8 g/l $NaHCO_3$, 0.5 g/l $K_2HPO_4$, 1 g/l $K_2SO_4$, 1 g/l NaCl, 0.2 g/l $MgSO_4.7H_2O$, 0.04 g/l $CaCl_2.2H_2O$, 0.01 g/l $FeSO_4.7H_2O$. 1ml of micronutrient solution is added to 1 litre of the media. The composition of the Micronutrient stock solution was 286 mg/ml $H_3BO_3$, 18 mg/ml $Mncl_2.4H_2O$, 22 mg/ml $ZnSO_4.7H_2O$, 39 mg/ml $Na_2MoO_4.2H_2O$, and 8 mg/ml $CuSO_4.5H_2O$.

### 2.2 Microorganisms

Two types of cultures were tested for the ability to grow in wastewater containing ammoniacal nitrogen: (1) Pure cultures of *Chlorella vulgaris*, *Botryococcus braunii*, *Scnedesmus acuminatus* and *Arthospira platensis* procured from SAG cultures, Germany and (2) Sample obtained from the

effluent treatment plant of RCF, Mumbai, India. This sample was suspended in Zarrouk's medium initially, and once growth was observed visibly, the cells were passaged to RCF effluent.

### 2.3 Identification of Microorganisms obtained from the pond near RCF effluent treatment plant

The algal sample from the effluent treatment plant of RCF, Mumbai, India was sent to Chromous Biotech Pvt. Ltd. for identification of the microorganism to its nearest species using the following steps: (i) The isolation of genomic DNA from the sample (ii) Amplification of DNA using high-fidelity PCR Polymerase (iii) Sequencing of the PCR product bi-directionally and (iv) Analysis of sequence data to identify the culture and its closest neighbours. As per the report the sample was found to be closest to the *Chlorella pyrenoidosa* gene for the 18S rRNA partial sequence.

### 2.4 Biomass measurement and growth rate calculation

Five independent experiments were carried out to obtain a correlation between Optical Density (OD) of the culture broth at 600nm and dry cell weight. In each experiment, samples of different biomass concentrations were diluted to various values of OD. The $OD_{600}$ was then recorded using a UV-Visible Spectrophotometer (Hitachi, U-2900) and the samples were dried and weighed. The values of OD and dry weight were then plotted to obtain a straight line ($R^2$>0.97). The average correlation obtained from all five experiments was 1OD= $0.47\pm0.047$ g/l. Subsequently, cell growth was measured using $OD_{600}$ daily, keeping the OD values between 0.2 and 0.9 by appropriate dilutions.

Maximum growth rate ($\mu_{max}$) was calculated by plotting $\ln(X/X_0)$ versus time in days and then measuring the slope of the linear portion, where $X_0$ is the initial biomass concentration, and X is the biomass concentration at different time points.

## 2.5 Ammonium estimation

Ammonium estimation in the culture medium was carried out using Nessler's reagent. The protocol used for estimating the ammonium content is applicable to a range of 0-30 ppm of ammonium as per the standard curve obtained. As per the protocol, 0.5 mL of the sample was taken in a test tube after centrifugation to avoid any solid particulate matter. 1mL of freshly prepared 10% sodium potassium tartarate and 0.25 mL of Nessler's reagent were added to the test tube. The test tubes were vortexed vigorously to ensure thorough mixing and then incubated at room temperature for 30 minutes. The OD measurements were then carried out at 410 nm for the sample and the concentration of ammonium was correspondingly determined from the standard curve.

159 2.6 Evaluation of process parameters

160 All experiments were carried out in 250 ml conical flasks containing 100 ml of
161 medium. Adaptation studies were carried out using RCF effluent initially and
162 once comparability was established between the effluent and the synthetic
163 medium, the latter was used for subsequent studies. Cells from a well-grown flask
164 were centrifuged and suspended into fresh medium at the desired OD value. pH
165 was maintained by sparging with $CO_2$ every day after measuring the pH. The
166 flasks were sampled daily for measuring cell growth and at regular intervals to
167 measure ammonium using the methods described above. Culture pH was
168 measured using a pH meter (Systronics pH system 362) and light intensity was
169 measured using a Lux meter (HTC). For the temperature studies alone, the flasks
170 were incubated in a water bath while the flasks at room temperature were kept on
171 a tissue culture rack fitted with tube lights.

172 **3. Results and Discussion**

173 3.1 Screening and selection of algal strain

174 Pure cultures of *B. braunii* and *S. acuminatus* were grown in Bold's Basal
175 Medium (Al-hothaly, Mouradov, et al., 2015) and *C. vulgaris, A. platensis* and
176 wild type *C. pyrenoidosa* (from RCF) were grown in Zarrouk's medium initially.
177 Once the cells reached an OD of around 1.0, all the cultures were transferred to
178 RCF effluent diluted to contain 100 ppm of ammonium. Except for *A. platensis*
179 and *C. pyrenoidosa* none of the other strains were able to grow at this
180 concentration of ammonium. Therefore only these two strains were taken forward
181 for subsequent experiments.

Figure 1 shows the cell growth profiles of *A. platensis* and *C. pyrenoidosa* at different ammonium levels. Increasing levels of ammonium resulted in growth inhibition in both cases although the extent of inhibition was different. Another experiment was also carried out for *A. platensis* in 50 ppm of ammonium. A 50% inhibition in cell growth was observed in *A. platensis* between 50 and 100 ppm of ammonium and the cells could not grow at all in 200 ppm. This is also in line with the findings of Giorgos et al (Markou, Vandamme, et al., 2014) that *A. platensis* undergoes inhibition at ammoniacal nitrogen concentrations above 150 ppm. Belkin and Boussiba (1991) found that *A. platensis* undergoes 50% inhibition in cell growth at a total ammoniacal nitrogen concentration of 140 ppm (Belkin and Boussiba, 1991).

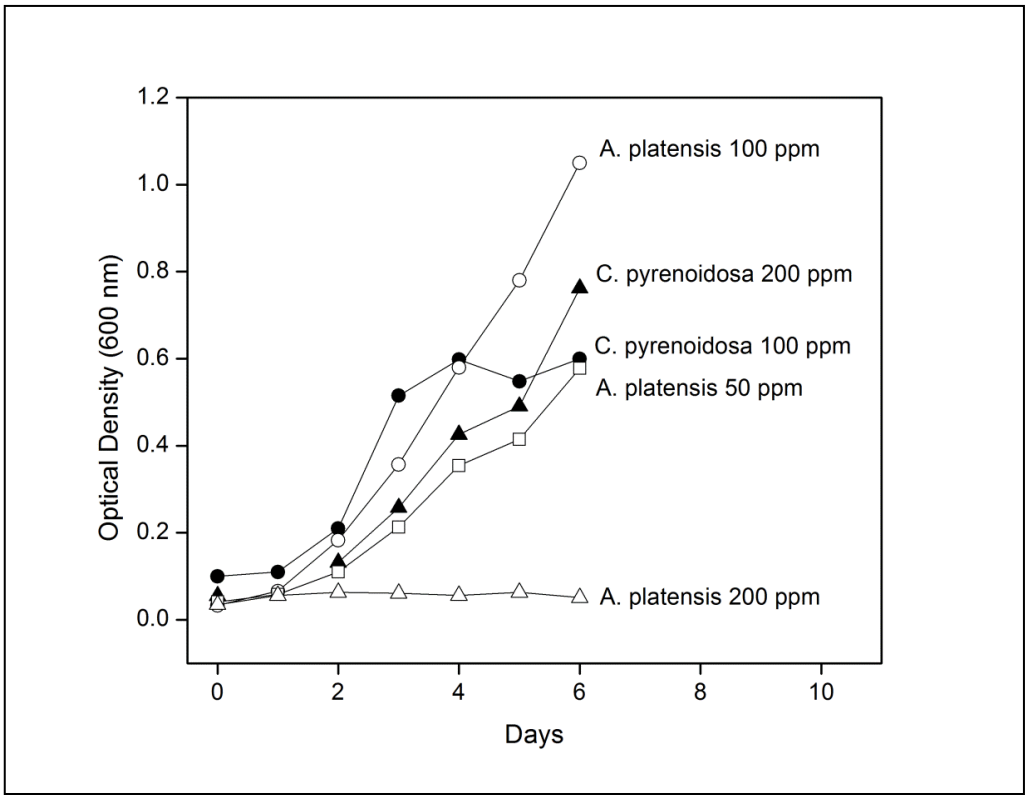



Figure 1. Effect of ammonium concentration on cell growth of *A. platensis* and *C. pyrenoidosa*.

In case of *C. pyrenoidosa* the effect of ammonium was not as drastic. Although there was a growth inhibition at 200 ppm compared to 100 ppm, the cells were still able to grow and reached the same OD two days later. The literature reports studies carried out with much lower concentrations (20-30 ppm) of ammonium using *Chlorella sp.* (Kim, Lingaraju, et al., 2010; Woertz, Feffer, et al., 2009).

Based on its ability to tolerate much higher concentrations of ammonium compared to what is reported in the literature and the results from our lab, *C. pyrenoidosa* was selected as the algal species for the adaptation studies.

## 3.2 Adaptation to ammoniacal nitrogen

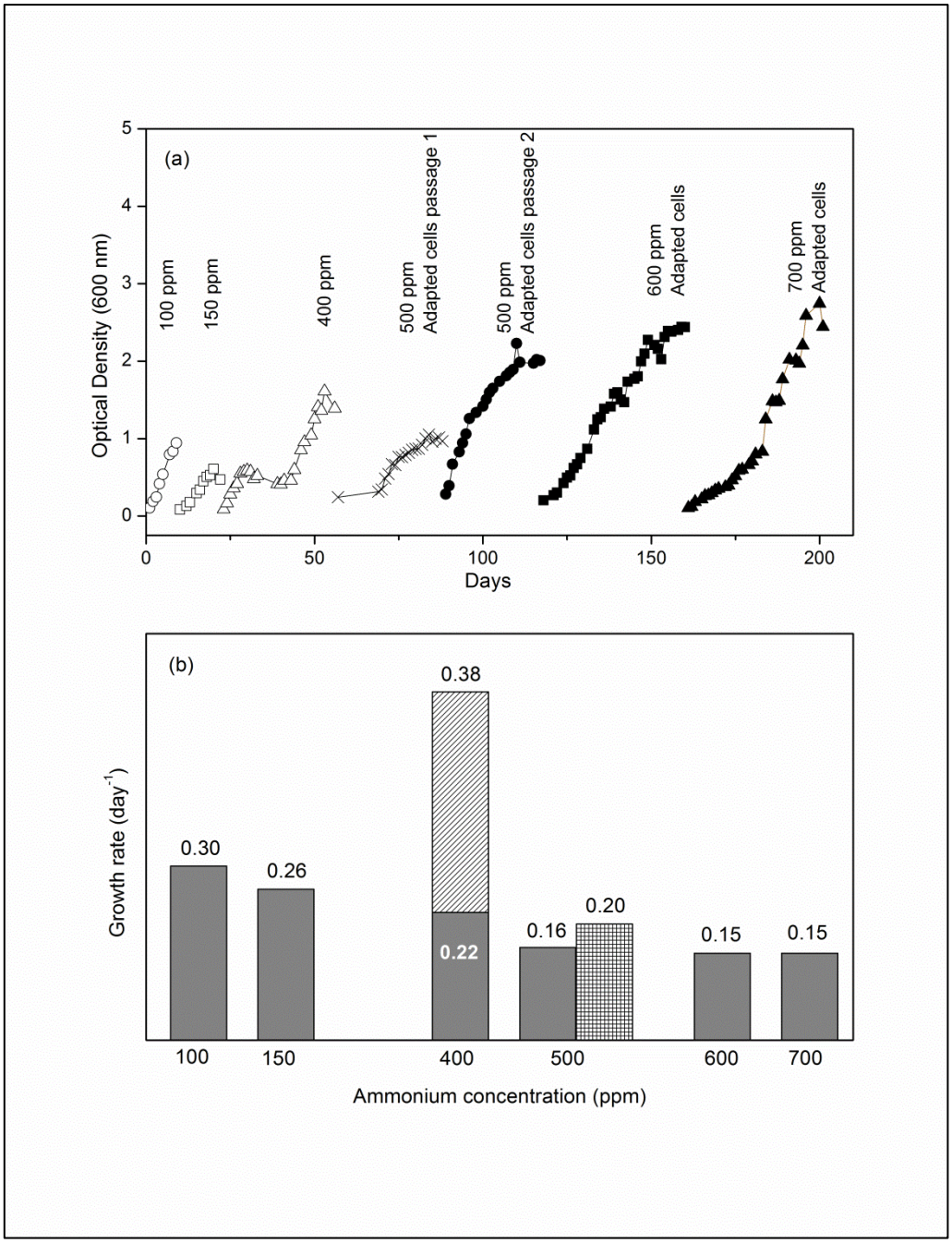
Preliminary studies showed that cell growth of *C. pyrenoidosa* was severely inhibited at ammonium concentrations beyond 200 ppm. To overcome this, cells were adapted sequentially to increasing concentrations of ammonium. Cells were initially grown in RCF effluent at 100 ppm of ammonium. As shown in figure 2a, the OD of cells increased from 0.1 to 0.95 over a period of nine days. When these cells were inoculated in RCF effluent containing 150 ppm, cell growth slowed down and the maximum OD achieved was only 0.6. Cells from 150 ppm ammonium concentration were then taken to 400 ppm ammonium and allowed a prolonged exposure at this concentration. While the OD tapered off at about 0.5 initially, the cells eventually adapted and began to grow again after 20 days. The final OD reached was about 1.5.

These cells were then inoculated into RCF effluent containing 500 ppm of ammonium. In this experiment, much better cell growth was observed and the OD reached about 1.0 in 15 days. These cells were passaged one more time at 500 ppm ammonium.

The growth profile further improved and an OD of 1.5 was achieved within 13 days. However, at even higher concentrations (600 and 700 ppm), growth inhibition was observed resulting in a 7-day delay in reaching an OD of 1.5 with each 100 ppm increase in ammonium concentration. Interestingly, the maximum OD achieved by cells increased with increasing ammonium concentration. This also shows that the cells seem to have adapted to ammonium and have developed increased tolerance and ability to metabolize ammonium.

Figure 2b shows the maximum growth rate of cells at different ammonium levels. As expected the growth rate decreased with increasing ammonium concentration.

Prolonged exposure to higher levels of ammonium seemed to help the cells acclimatize which can be seen by the increased growth rate in the second phase of growth observed at 400 ppm. Similarly, when cells were passaged a second time in 500 ppm of ammonium, the growth rate was found to increase slightly. The final growth rate at 700 ppm was about half of that seen at 00 ppm, although the final OD was significantly higher at 700 ppm.




235

236 Figure 2a. Adaptation study: cell growth profiles at increasing concentrations of ammonium.
237 Figure 2b. Effect of ammonium concentration and adaptation to ammonium on maximum
238 growth rate of *C. pyrenoidosa*

### 3.3 Comparison between RCF effluent and synthetic medium

A comparability study was carried out between RCF effluent and synthetically prepared Zarrouk's medium which was modified to remove the existing nitrogen sources and supplemented with ammonium chloride. Both media contained approximately 600 ppm of ammonium at the beginning of the experiment. Cell growth and ammonium removal were monitored with time and comparable profiles were obtained in both cases as shown in figure 3. Synthetic medium was therefore used for subsequent studies with pH, temperature and light intensity optimization.

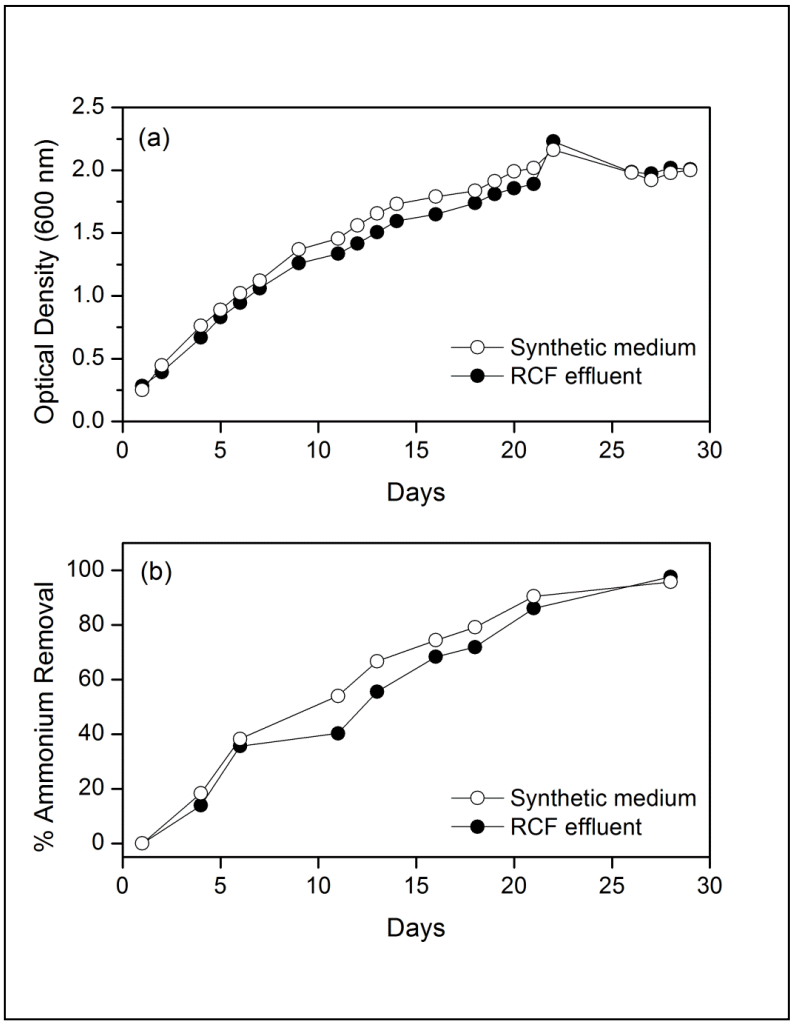



Figure 3. Cell growth and ammonium removal profiles with synthetic medium and RCF effluent.

### 3.4 Effect of pH

The culture pH naturally increases with time due to photosynthesis. However, it was seen that allowing the pH to increase inhibited cell growth beyond a pH of 9.5. Cells did not grow in the absence of pH control. Cell growth profiles were similar when the pH was controlled at 8 and at 9. However, adjusting the pH to 9 increased the ammonium depletion by about 10-20% compared to when pH was adjusted to 8 as seen in figure 4. As a result, there was nearly a 10-day delay in ammonium depletion at pH 8. This is due to the fact that at lower pH, the ammoniacal nitrogen stays in its protonated form ($NH_4^+$) and does not strip easily from the medium. At higher pH it is in the free $NH_3$ form which is easily stripped off (Farrell, Frauson, et al., 2016; Park, Jin, et al., 2010). As the cell growth was not impacted by pH, keeping the ammonium removal in mind, a pH of 9 is better than a pH of 8 for the process.

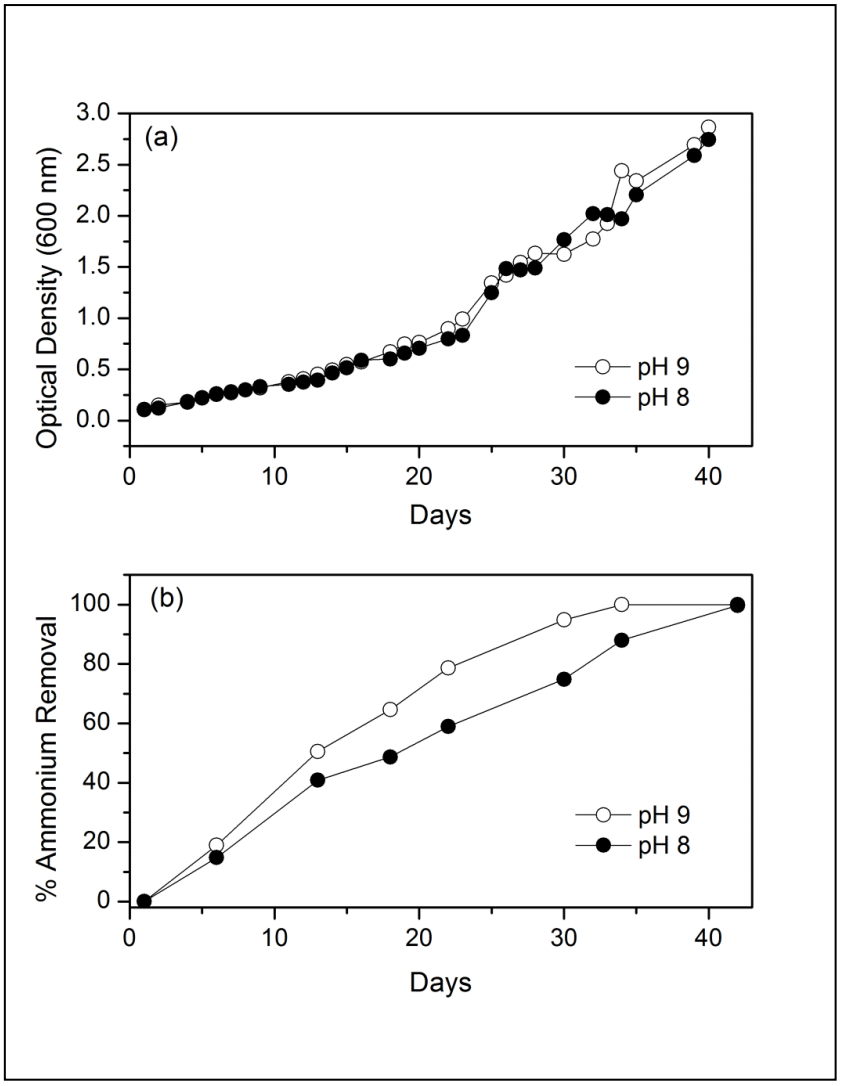



Figure 4. Cell growth and ammonium removal profiles at different pH conditions

## 3.5 Effect of temperature

Results showed that the optimum temperature for cell growth is 27$^{o}$C. At 30$^{o}$C the initial growth rate was comparable to that at 27$^{o}$C but the final OD reached was lower by a factor of around 1.6. Cells grown at room temperature (RT), which averaged around 25$^{o}$C had a lower initial growth rate, although the final OD reached was higher compared to cells grown at 30$^{o}$C.

However ammonium consumption was the highest at room temperature and at 30$^{o}$C, reaching about 90% by day 33 whereas at 27$^{o}$C the consumption was only

75% as shown in figure 5. The optimum temperature for both growth and ammonium consumption seems to be 30$^{o}$C as the end result of the process should be to remove the ammonium and also obtain a high biomass concentration that can be utilized for biofuels and value added products.

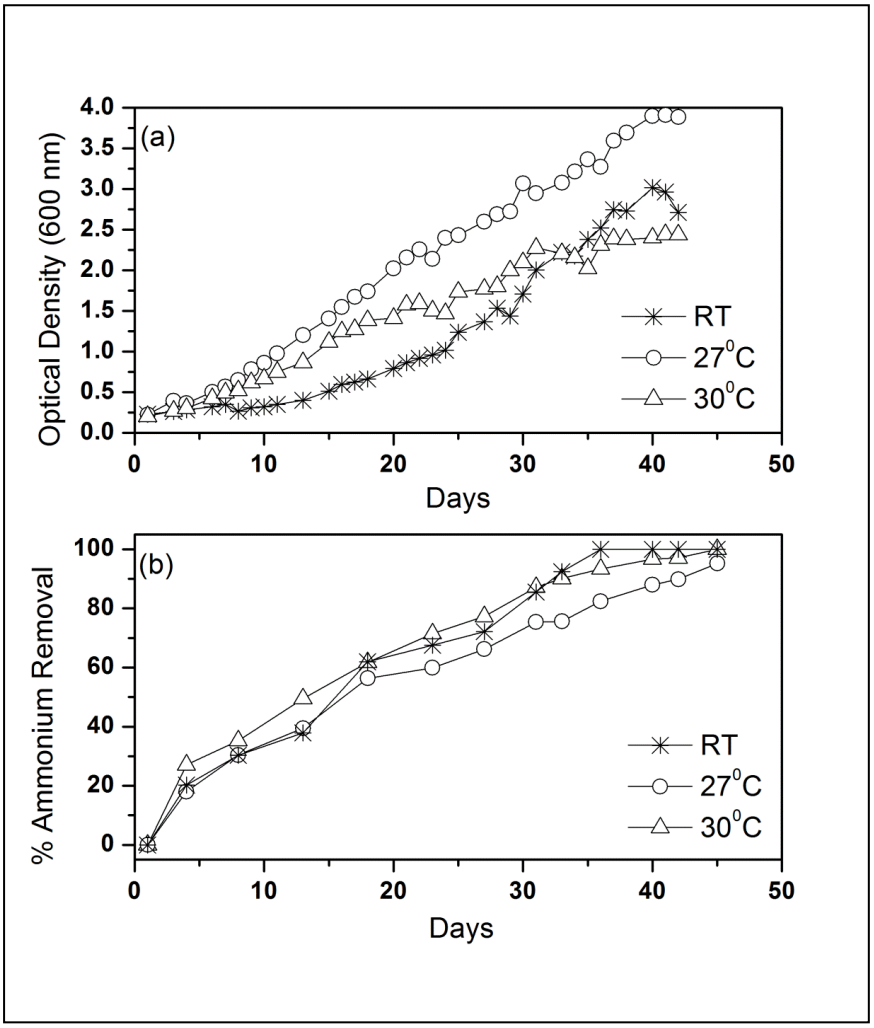



Figure 5. Cell growth and ammonium removal profiles at different temperatures

## 3.6 Effect of light intensity

Light intensity was varied from 200 to 3500 Lux. Cells did not grow at 200 Lux. As seen in figure 6, at 500 and 1200 Lux, cell growth was inhibited. Cell growth was highest at 3500 Lux, followed by 2700 Lux. Ammonium depletion profiles were similar at 500, 1200 and 2700 Lux. However, at 3500 Lux, ammonium

consumption was significantly higher, reaching nearly 100% in about 25 days while the other flasks were still at 70-80% consumption. As expected, increasing the light intensity improved cell growth as well as ammonium consumption. At 3500 Lux, it appears that the inhibitory effect of ammonium is reduced and also the assimilation of ammonium seems to be higher as the ammonium depletion was higher from the very beginning, despite the cell growth being similar till day 15 or so in all cases.

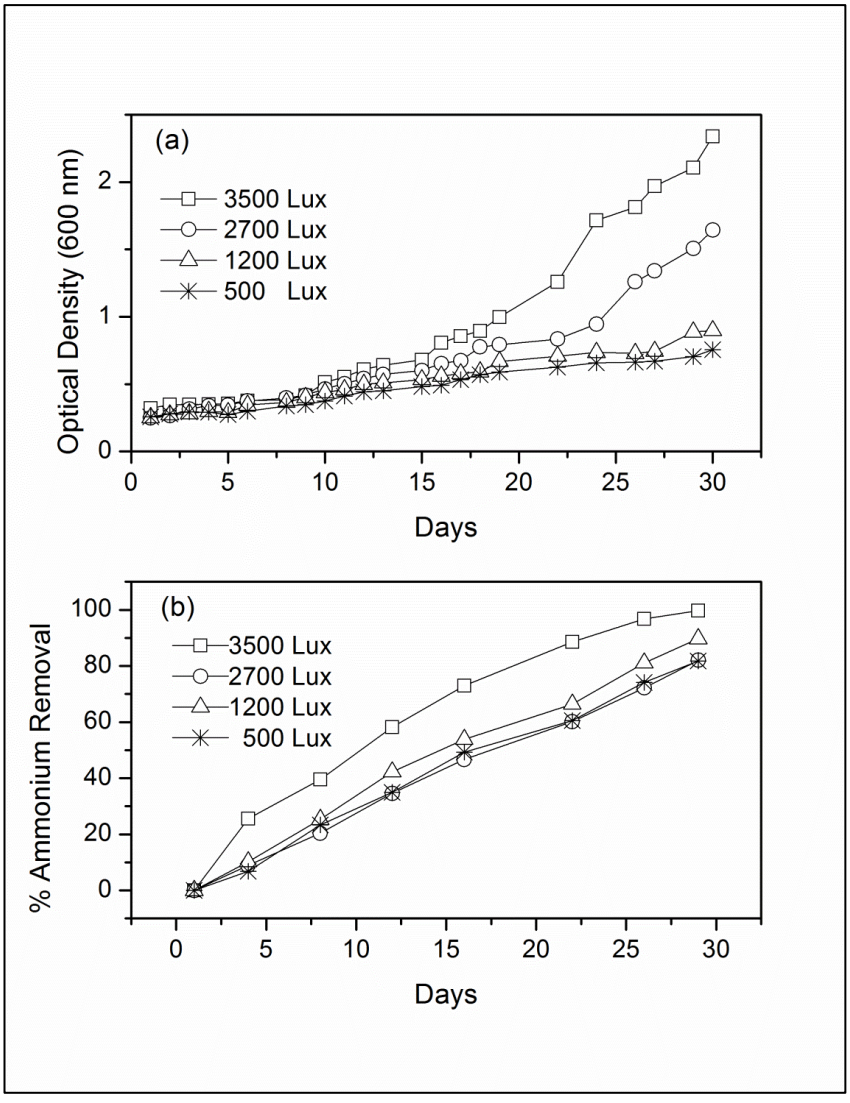



Figure 6. Cell growth and ammonium removal profiles at different light intensities

## 4. Conclusion

Wild type *C. pyrenoidosa* was successfully adapted to high ammonium levels and was able to remove 700 ppm of ammonium from wastewater within 25 to 30 days. Effects of pH, temperature and light intensity were evaluated on the adapted cells and optimal values were found to be a temperature of $30^{o}$C, pH of 9 and light intensity of 3500 Lux. These cells can be used to remove ammonium from industrial effluents and then harvested to produce biofuels and value added products. Future studies will involve characterization of the algal biomass and subsequent conversion of the biomass to value added products based on the composition.

## 5. Acknowledgements

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## 6. References


Abeliovich, A. and Azov, Y. (1976) Toxicity of ammonia to algae in sewage oxidation ponds. Applied and Environmental Microbiology, **31**(6), 801–806.

Al-hothaly, K. A., Mouradov, A., Mansur, A. A., May, B. H., Ball, A. S., and Adetutu, E. M. (2015) The Effect of Media on Biomass and Oil Production in Botryococcus braunii Strains. , (February), 11–22.

Azov, Y. and Goldman, J. C. (1982) Free Ammonia Inhibition of Algal Photosynthesis in Intensive Culturest. Applied and environmental microbiology, **43**(4), 735–739.

Belkin, S. and Boussiba, S. (1991) Resistance of Spirulina platensis to ammonia at high pH values. Plant and cell physiology, **32**(7), 953–958. [online] http://pcp.oxfordjournals.org/content/32/7/953.abstract\nhttp://pcp.oxfordjournals.org/content/32/7/953.short.

Chen, S. Y., Pan, L. Y., Hong, M. J., and Lee, A. C. (2012) The effects of temperature on the growth of and ammonia uptake by marine microalgae. Botanical Studies, **53**, 125–133.

Collos, Y. and Harrison, P. J. (2014) Acclimation and toxicity of high ammonium concentrations to unicellular algae. Marine Pollution Bulletin, **80**, 8–23.

Farrell, T. P. O., Frauson, F. P., Cassel, A. F., Bishop, D. F., Farrell, T. P. O., Frauson, F. P., Cassel, A. F., and Bishop, D. F. (2016) Nitrogen removal by ammonia stripping. , **44**(8), 1527–1535.

Gami, B., Naik, A., and Patel, B. (2011) Cultivation of Spirulina species in different liquid media. Journal of Algal Biomass Utilization, **2**(3), 15–26.

Hoa Binh aus Hanoi, T. (2013) Improving the nitrogen removal in algal wastewater stabilization ponds.

John, R. P., Anisha, G. S., Nampoothiri, K. M., and Pandey, A. (2011) Micro and macroalgal biomass: A renewable source for bioethanol. Bioresource Technology, **102**, 186–193.

Kim, J., Lingaraju, B. P., Rheaume, R., Lee, J. Y., and Siddiqui, K. F. (2010) Removal of ammonia from wastewater effluent by chlorella vulgaris. Tsinghua Science and Technology, **15**(4), 391–396.

Kuba, T., Van Loosdrecht, M. C. M., Brandse, F. A., and Heijnen, J. J. (1997) Occurrence of denitrifying phosphorus removing bacteria in modified UCT-type wastewater treatment plants. Water Research, **31**(4), 777–786.

Lee, K. and Lee, C.-G. (2001) Effect of Light/dark Cycles on Wastewater Treatments by Microalgae. Biotechnology and Bioprocess Engineering, **6**, 194–199.

Lincoln E.P., Wilke, A.C., French, B. T. (1996) Cyanobacterial Process for Renovating Dairy Wastewater. Biomass and Bioenergy, **10**(I), 63–68.

Markou, G., Depraetere, O., and Muylaert, K. (2016) Effect of ammonia on the photosynthetic activity of Arthrospira and Chlorella : A study on chlorophyll fl uorescence and electron transport. ALGAL, **16**, 449–457. [online] http://dx.doi.org/10.1016/j.algal.2016.03.039.

Markou, G., Vandamme, D., and Muylaert, K. (2014) Ammonia inhibition on Arthrospira platensis in relation to the initial biomass density and pH. Bioresource Technology, **166**, 259–265.

Ogbonna, J. C., Yoshizawa, H., and Tanaka, H. (2000) Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. , 277–284.

Park, J. B. K., Craggs, R. J., and Shilton, A. N. (2011) Wastewater treatment high rate algal ponds for biofuel production. Bioresource Technology, **102**, 35–42.

Park, J., Jin, H. F., Lim, B. R., Park, K. Y., and Lee, K. (2010) Ammonia removal from anaerobic digestion effluent of livestock waste using green alga Scenedesmus sp. Bioresource Technology, **101**(22), 8649–8657. [online] http://dx.doi.org/10.1016/j.biortech.2010.06.142.

Pittman, J. K., Dean, A. P., and Osundeko, O. (2011) The potential of sustainable algal biofuel production using wastewater resources. Bioresource Technology, **102**, 17–25.

Ruiz-Marin, A., Mendoza-Espinosa, L. G., and Stephenson, T. (2010) Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresource Technology, **101**, 58–64.

Shyam Kumar, S. and Saramma, A. V (2012) Nitrate and Phosphate uptake by immobilized cells of Gloeocapsa gelatinosa. Journal of Marine Biological Association of India, **54**, 119–122.

Singh, A., Nigam, P. S., and Murphy, J. D. (2011a) Mechanism and challenges in commercialisation of algal biofuels. Bioresource Technology, **102**, 26–34.

Singh, A., Nigam, P. S., and Murphy, J. D. (2011b) Renewable fuels from algae: An answer to debatable land based fuels. Bioresource Technology, **102**, 10–16.

Synechocystis, C., Oa, P. C. C., Drath, M., Kloft, N., Batschauer, A., Marin, K., Novak, J., and Forchhammer, K. (2008) Ammonia Triggers Photodamage of Photosystem II in the. , **147**(May), 206–215.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., and Tilman, D. G. (1997) Vitousek et al. 1997. Ecological Applications, **7**(3), 737–750.

Wang, B. and Lan, C. Q. (2011) Biomass production and nitrogen and phosphorus removal by the green alga Neochloris oleoabundans in simulated wastewater and secondary municipal wastewater effluent. Bioresource Technology, **102**, 5639–5644.

Wang, J., Zhou, W., Yang, H., and Ruan, R. (2016) Application of nitrogen sufficiency conversion strategy for microalgae-based ammonium-rich wastewater treatment. , **3330**(April).

Woertz, I., Feffer, A., Lundquist, T., and Nelson, Y. (2009) Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. Journal of Environmental Engineering, **135**(11), 1115–1122.

Yoo, H., Ahn, K. H., Lee, H. J., Lee, K. H., Kwak, Y. J., and Song, K. G. (1999) Nitrogen removal from synthetic wastewater by simultaneous nitrification and denitrification (SND) via nitrite in an intermittently-aerated reactor. Water Research, **33**(1), 145–154.

## Figure Captions

Figure 1. Effect of ammonium concentration on cell growth of *A. platensis* and *C. pyrenoidosa*.

Figure 2a. Adaptation study: cell growth profiles at increasing concentrations of ammonium. Figure 2b. Effect of ammonium concentration and adaptation to ammonium on maximum growth rate of *C. pyrenoidosa*

Figure 3. Cell growth and ammonium removal profiles with synthetic medium and RCF effluent.

Figure 4. Cell growth and ammonium removal profiles at different pH conditions

Figure 5. Cell growth and ammonium removal profiles at different temperatures

Figure 6. Cell growth and ammonium removal profiles at different light intensities

## Figures

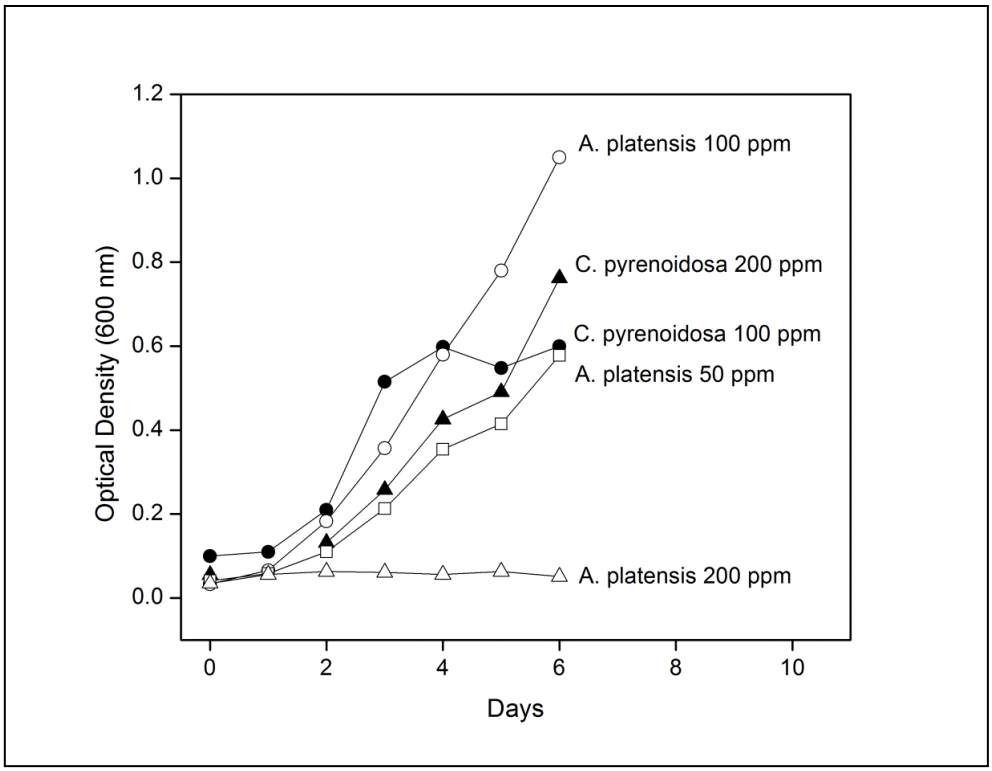
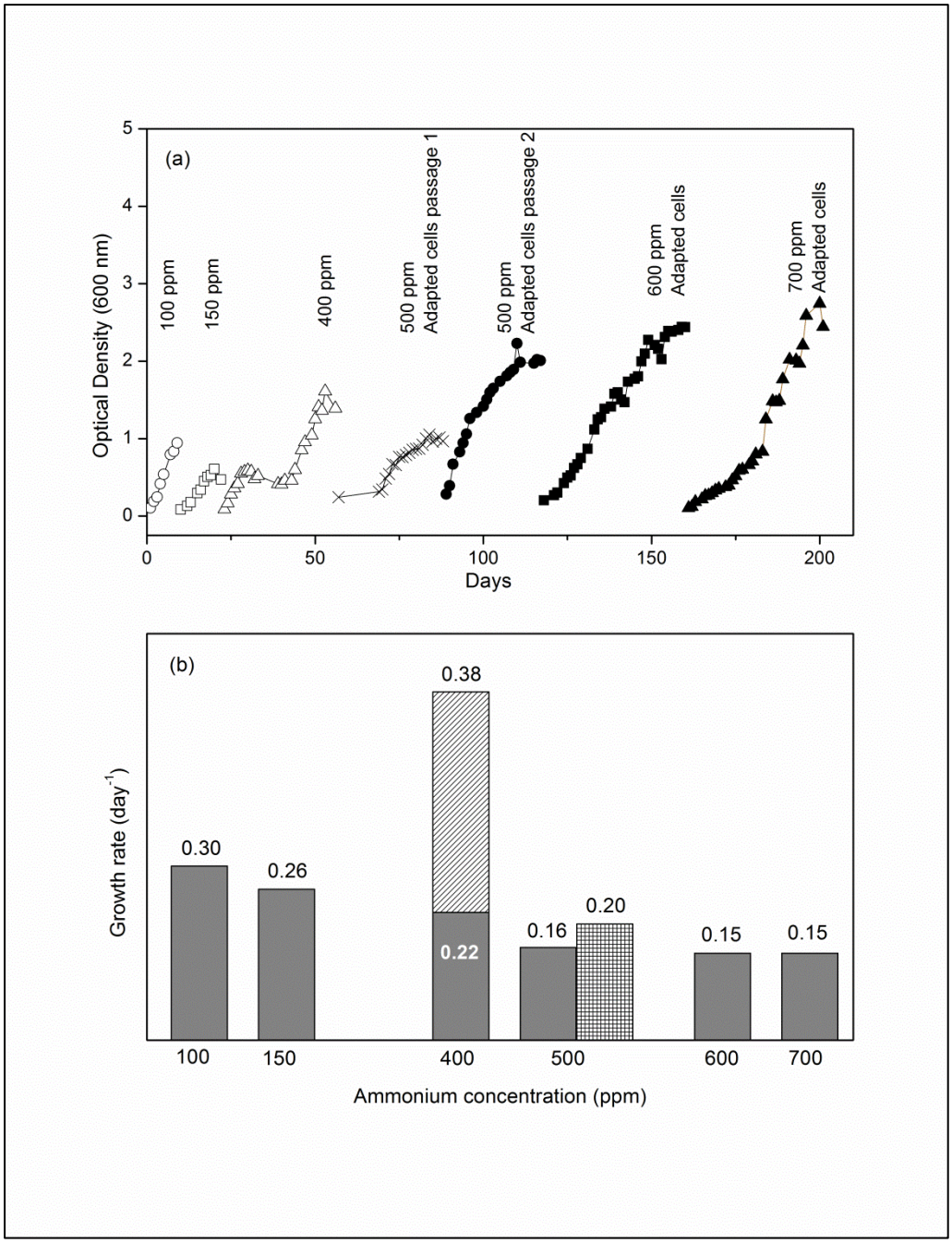



Figure 1. Effect of ammonium concentration on cell growth of *A. platensis* and *C. pyrenoidosa*.

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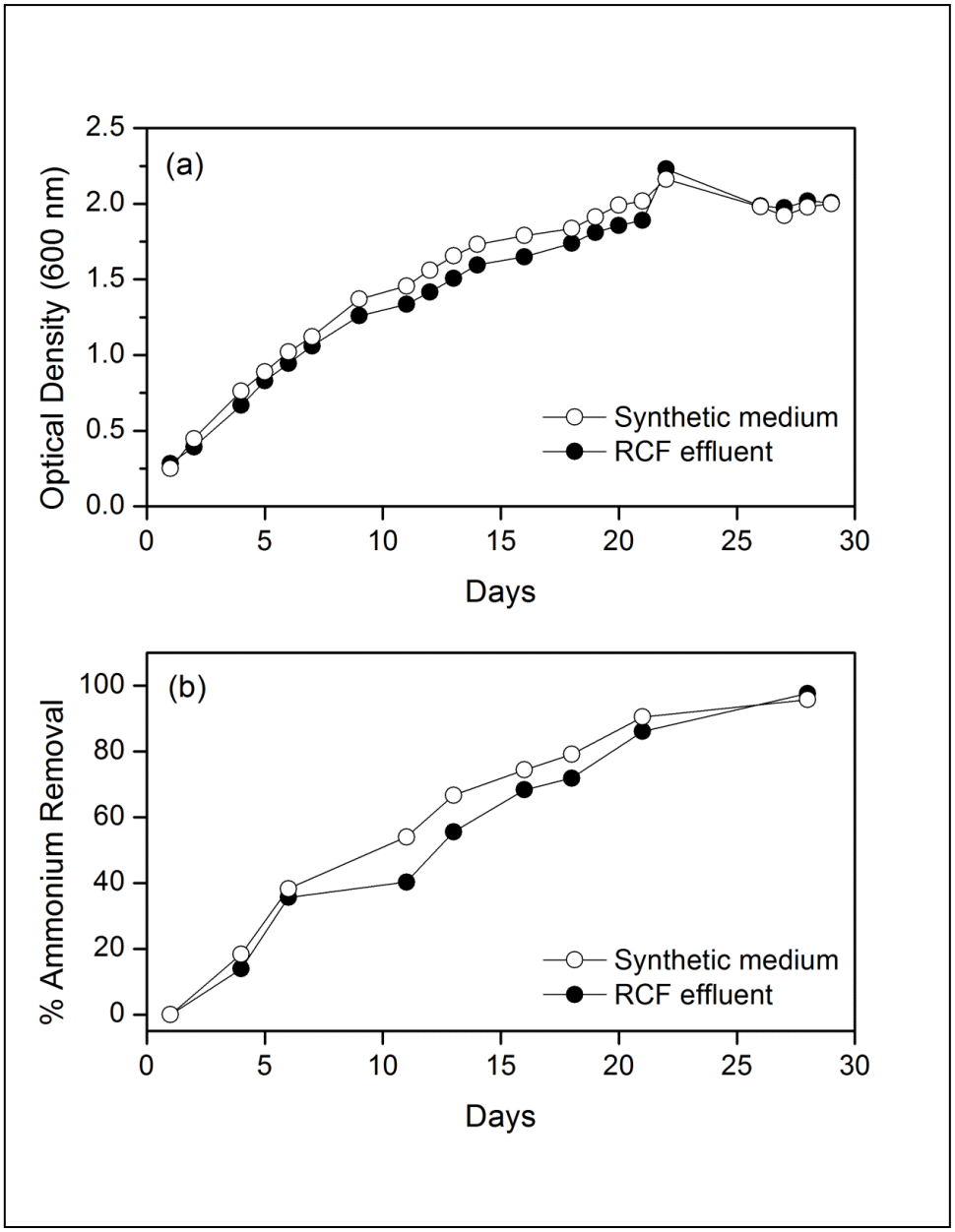
445 Figure 2a. Adaptation study: cell growth profiles at increasing concentrations of
446 ammonium

447 Figure 2b. Effect of ammonium concentration and adaptation to ammonium on
448 maximum growth rate of *C. pyrenoidosa*

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450 Figure 3. Cell growth and ammonium removal profiles for cells growth in synthetic medium
451 and RCF effluent.

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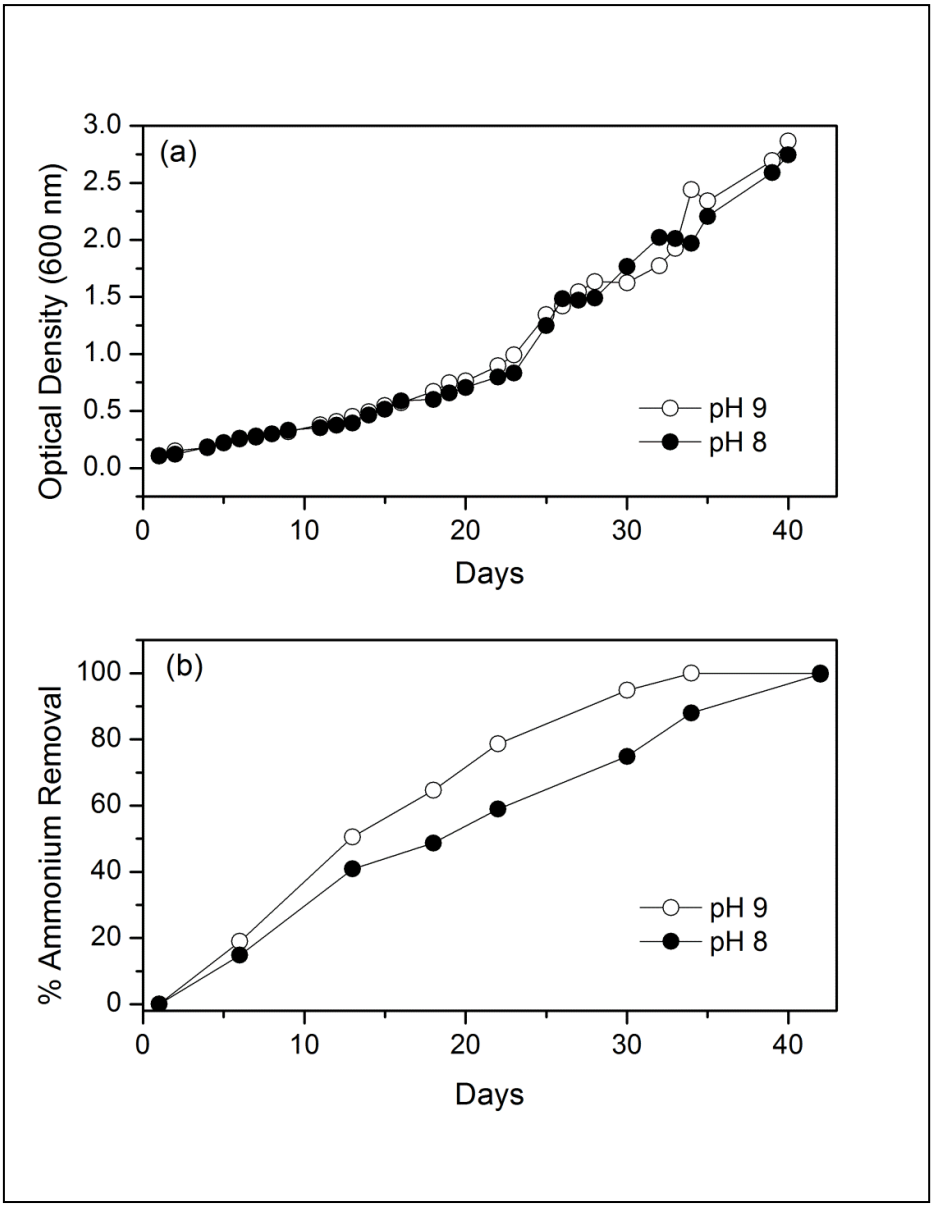


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454 Figure 4. Cell growth and ammonium removal profiles at different pH conditions

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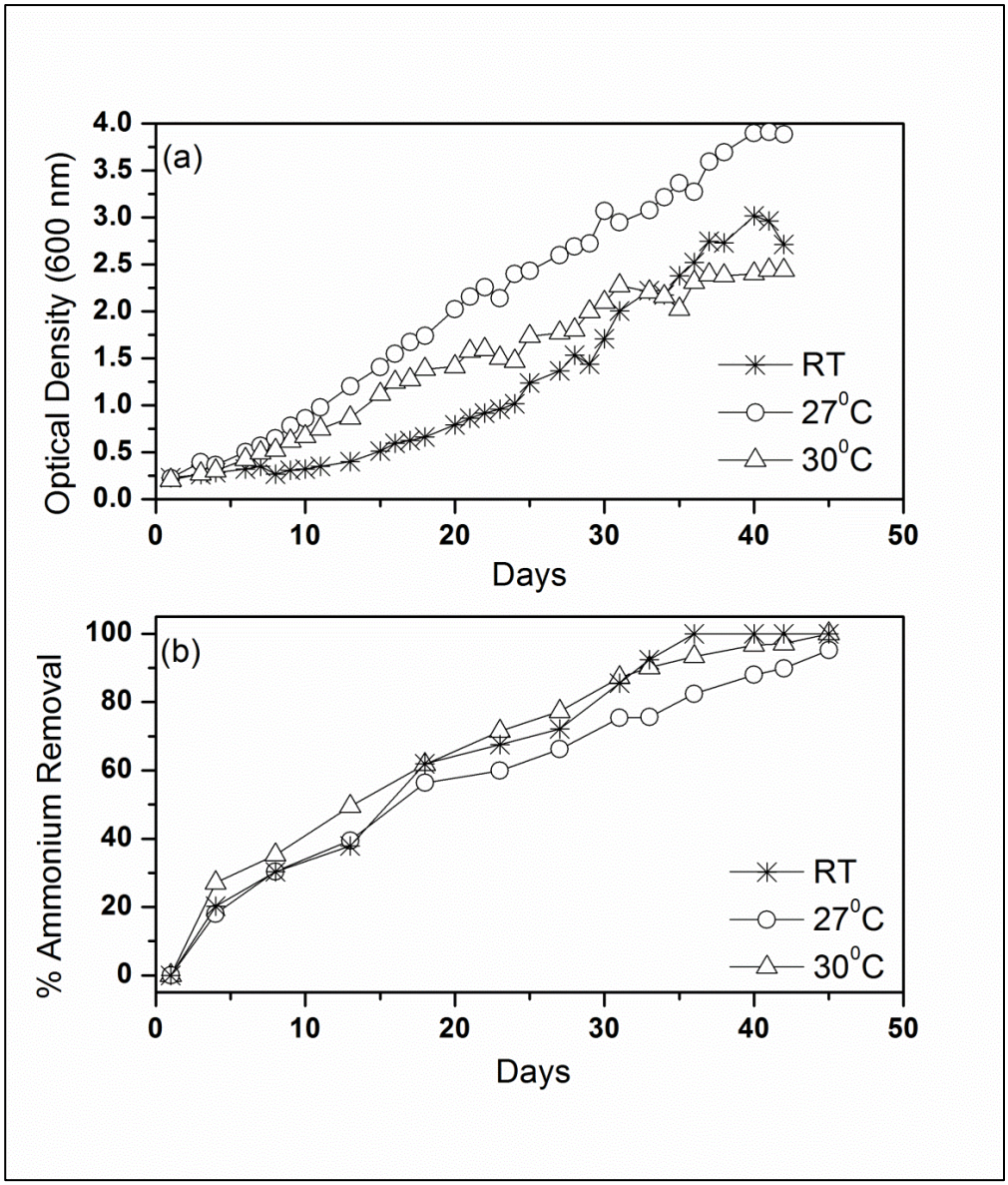


Figure 5. Cell growth and ammonium removal profiles at different temperatures

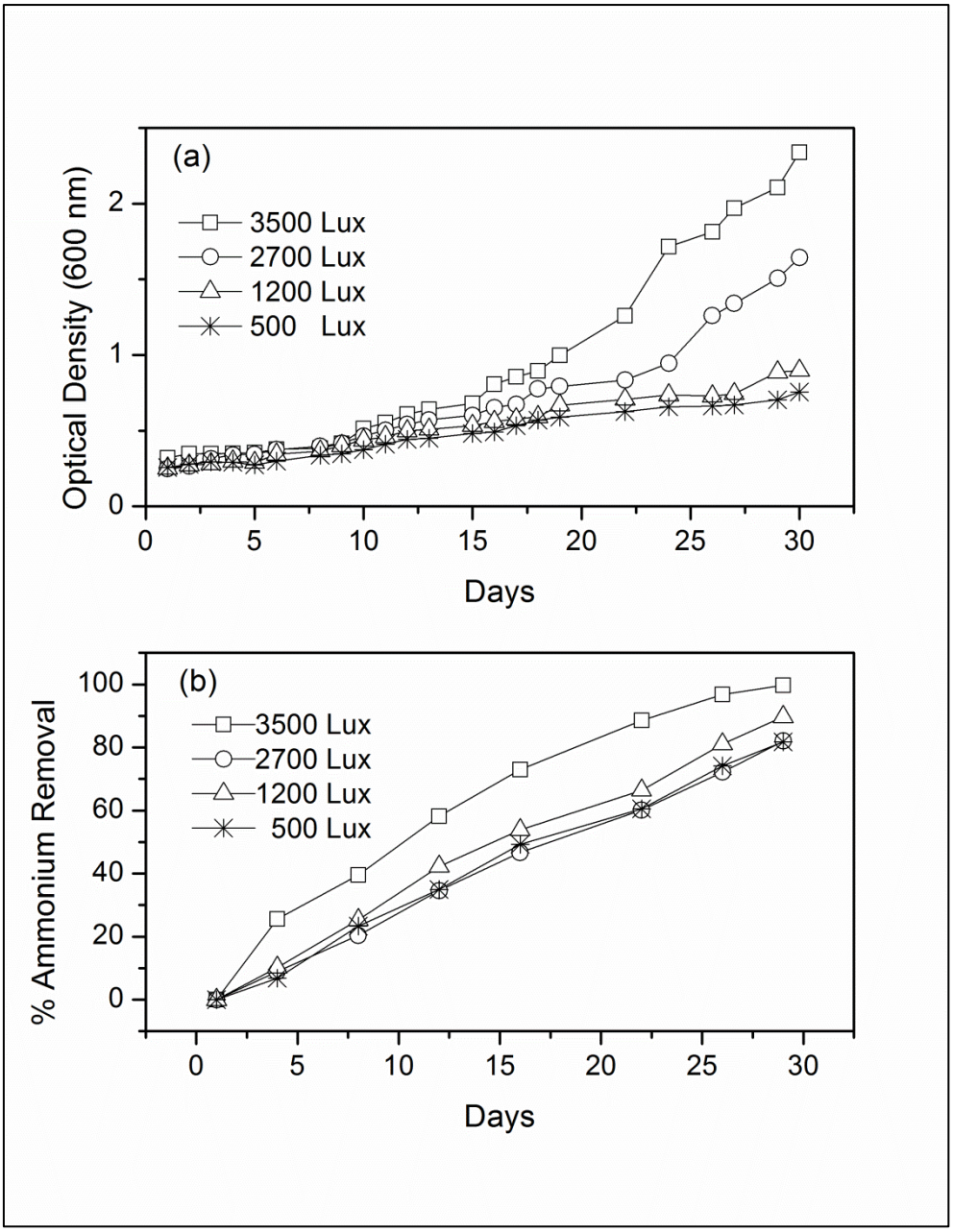


Figure 6. Cell growth and ammonium removal profiles at different light intensities