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Uptake of total ammonia nitrogen(TAN) by marine microalgae Isolated from Mediterranean Sea coast Alexandria, Egypt.

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Background: Microalgae are able to assimilate various types of nitrogen. The interaction of nitrogen on their removal by microalgae should be of great concern when microalgae are used as a biological treatment in marine culture systems.Materials and methods: Two marine cyanobacteria isolates *Phormodium formosum and Oscillatoria simplicissima* were evaluated for their ability to remove total ammonia nitrogen (TAN) from aqueous solution. The algal cells were cultured in medium F/2 with different TAN concentration (0.1, 0.5, 1.0 and 1.5 mg /l). Experiments were conducted at pH 7.5 ± 0.3, temperature 25°C ± 1°C and illuminated with cool white fluorescent lamps at an intensity 100 µE/m2/s. continuously for 20 days. Results: The results, 0.1, 0.5 and 1 mg/l TAN concentration enhanced the algal growth (chlorophyll a), whereas elevated concentration (1.5mg/L) was inhibitory to growth in case of both two algae. The total ammonia nitrogen removal efficiency by *P. formosum* was 99% at TAN concentration of 1.0 mg/l after 10th days of incubation period but removal efficiency by *O. simplicissima* was 96 % after 12th days of incubation period at the same concentration. TAN decreased to less than 20% by *P. formosum* when its concentration exceeded 1.5 mg/l. Conclusion: Our results suggest that *P. formosum* and *O. simplicissima* are a good choice for removing ammonia from marine culture systems.

Keywords: Removal, Total ammonia nitrogen, Marine microalgae, Chlorophyll (a)

INTRODUCTION

Ammonia is formed as the principal product of protein metabolism in aquatic organisms. Ammonia exists in two forms that are convertible in water, NH3 and NH4+, the sum of which is called total ammonia nitrogen (TAN). Wastewater derived from Blackwater and greywater potentially contains nitrogen (Ghunmi et al., 2011), which is a greater contributor to the decline in the quality of a body of water when compared to wastewater coming from industrial facilities (Nourmohammadi et al., 2013). Therefore, a disinfection process must be performed (jiawen et al., 2016). Generally, this process is carried out at the stage of tertiary treatment. The chemical compound used most frequently in the disinfection process is chlorine (Zhang et al., 2015). The presence of NH_4^+-N led to breakpoint chlorination (Cl2 NH_4^+-N weight ratio=7.6÷15:1) and formation of trihalomethana as a by-product of disinfection (Patroescu et al., 2015; Liu et al., 2012). Excessive nitrogen concentration in the environment can lead to changes in the natural nitrogen cycle between living beings, soil, water, and atmosphere (Obaja et al., 2003). Nitrogen contamination in water body can create serious problems, such as deterioration of water quality, eutrophication of dam, and potential hazard to animal and human health (Khan and Mohammad, 2014). The nitrogen compound is a nutrient that can cause algal bloom, which reduces the amount of oxygen in the water, while the ammonia-nitrogen (NH3-N) is toxic to aquatic life (Gupta et al., 2015). Nitrogen in domestic wastewater is in the form of organic nitrogen (± 40%) and ammonium nitrogen (± 60%) (Schwarzenbach et al., 2010). According to (Gupta et al., 2015), the most widely used nitrogen-ion ammonium removal technology is ion exchange, adsorption, and biological technology. The proportion of un-ionized ammonia (NH3), the more-toxic form to aquatic organisms, increases with water pH (Renaud et al., 2002; Ip and Chew, 2010). Ammonia can be removed by bacterial biofilters (Tseng et al. 2006, Grommen et al., 2002), heterotrophic bacterial biofilters (Avnimelech 1999), seaweed biofilters (Neori et al., 2000), and microalgae (Voltolina et al., 2005; Godos et al., 2010). Uptake rates of nitrate and ammonium were studied as a function of nitrate or ammonium concentrations with cultures of *Chaetoceros gracilis* and *Isochrysis galbana* (Eppley et al., 1969). Since free ammonia is toxic to algae, it does not accumulate in their cells but is stored in the system through an ammonia binding reaction. Glutamate dehydrogenase and glutamate synthetase activities are higher in green algae, which are peculiar in having a highly effective pathway to detoxify ammonia (Klochenko et al., 2003). Microalgae exhibit an active, and most likely a passive, ammonium uptake. The proportion of ammonia in marine systems can increase to > 10% of TAN. The aim of this study was to evaluate the efficiency of ammonia uptake by these two species of microalgae and finally, to select a candidate for removing ammonia from marine culture systems.

MATERIALS AND METHODS

Reagents

All solvents and reagents were of analytical or laboratory grade. Deionized water was used throughout.

Algal Isolation

The algal species used in this study were isolated from Eastern harbor of Mediterranean Sea coast of Alexandria, Egypt. Samples were grown separately in 500 ml culture flasks containing F/2 medium (Guillard and Ryther, 1962; Guillard, 1975). The medium was then autoclaved at 120 °C for 30 minutes. The culture was incubated at pH 7 ± 0.3, temperature 25°C ± 1°C and illuminated with cool white fluorescent lamps at an intensity 100 µE/m2/s. continuously for 20[th] days. The algae were kept under optimum conditions. The isolated strain was identified according to available literature (Tomas et al., 1996; Prescott, 1968; Cronberg et al., 2006)

Growth rate of microalgae and their TAN uptake

In order to apply this approach in the field, only ammonia was added in the experiment on microalgal growth and TAN uptake. Fifty milliliters of microalgae were inoculated in 1-L flasks with 500 mL of F/2 medium containing different concentration of ammonium ion (0.1, 0.5, 1.0 and 1.5 mg /l). The determination of algal growth as a chlorophyll (a) Strickland and Persons 1972). Harvesting took place by centrifugation at 5000 rpm for 15 min. The pigment content in filtered extract was determined by the absorbance at 663 and 645 nm in a 1cm quartz cell against a blank of 80% aqueous acetone. Samples for ammonia determination were fixed and determined using indophenol blue technique (Intergovernmental 1983). Specific TAN uptake was calculated by using the equation below.

Efficiency calculation of concentration reduction

Reduction efficiency (RE) = $(C_{in}-C_{out})\ C_{in}\times 100$ %

Where:

Reduction efficiency = Concentration reduction efficiency (%)

C_{in} = Inlet sample concentration (mg/l)

C_{out} = Outlet sample concentration (mg/l)

Statistical Analysis

In all measurements was carried out in triplicate. Statistical analyses were performed using one-way Analysis of Variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple range tests. Differences at $P<0.05$ were considered statistically significant. The results were presented as mean values (±SD, Standard Deviations).

RESULTS

Isolation of the microalgae

The algal strain was identified as *Phormidium* *formosum* and *Oscillatoria simplicissima*. The growth of *P. formosum* increased and reached to maximum value at stationary phase after 10th days then, phase started to decrease but in case of *O. simplicissima*, the growth increased and reached to maximum value at stationary phase after 12th days (Figure1).

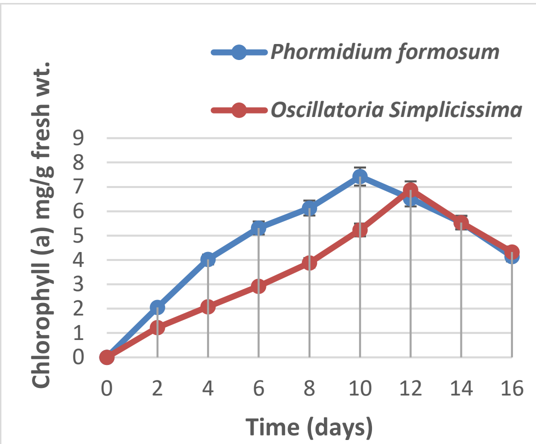

Figure 1: The growth of *P. formosum* and *O. Simplicissima* measured as chlorophyll (a) mg/g fresh wt.

Growth of microalgae by chlorophyll (a) with different concentration of NH4-N.

The total Chl *a* content (mg/g fresh wt.) was obtained and is represented in Figure 2 for *P. formosum* and Figure 3 for *O. simplicissima* at different concentration of ammonia. It was noted that the total Chl *a* content increased gradually with the incubation time in all cultures, with the highest Chl *a* content of *P. formosum* increased and reached to maximum value at stationary phase after 10th days then, phase started to decrease but in case of *O. simplicissima*, the Chl *a* content increased and reached to maximum value at stationary phase after 12th days clearly demonstrates that the final Chl *a* content of the culture significantly increased at concentration (0.1, 0.5 and 1.0 mg/l) for tow microalgae . However, it died at concentration (1.5 mg/l) after 2 days. This result suggests that at low NH4-N concentrations, Chl *a* formation was limited by NH4-N supply, while excessive NH4-N concentration does not favor the additional synthesis of Chl *a*.

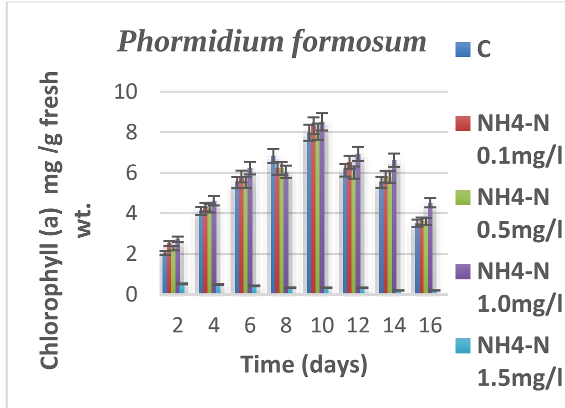

Figure 2: The growth of *P. formosum a* measured as chlorophyll (a) mg/g fresh wt. under different concentration of NH^4-N. (C) represents algal treatment without NH4-N.

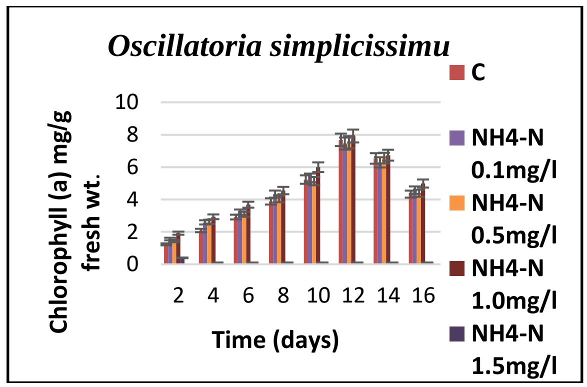

Figure 3: The growth of *O. simplicissmu a* measured as chlorophyll (a) mg/g fresh wt. under different concentration of NH^4-N. (C) represents algal treatment without NH4-N.

Removal Efficiency of Total Ammonia Nitrogen (TAN)

P. formosum and *O. simplicissima* are effective in removing TAN at different concentration (0.1, 0.5, 1 and 1.5 mg/l) of ammonia (Figure 4).

The maximum NH4-N removal efficiency was obtained after 10th days of incubation for *P. formosum*, and the values were found to be 99.5%, 99.2%, 99% and 12% at different concentration 0.1, 0.5,1 and 1.5 mg/l of NH_4-N respectively (Figure 5). NH_4-N was removed by *O. simplicissima,* when the initial concentration was 0.1 mg/L recorded 98% after12th days of incubation period (Figure 6).

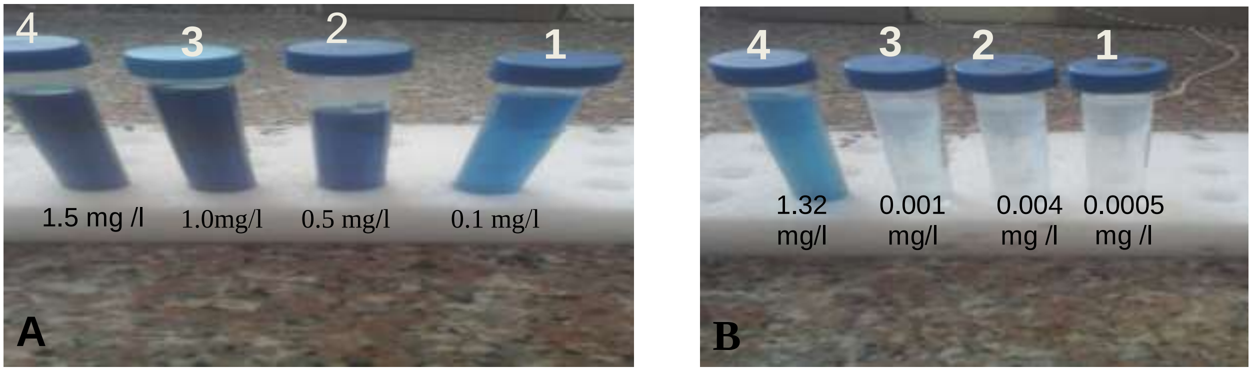

Figure 4: The different concentration of ammonia (NH4-N) before treatment with microalgae(A); low in concentration of ammonia after treatment with *P. formosum* at 10th days of incubation period(B).

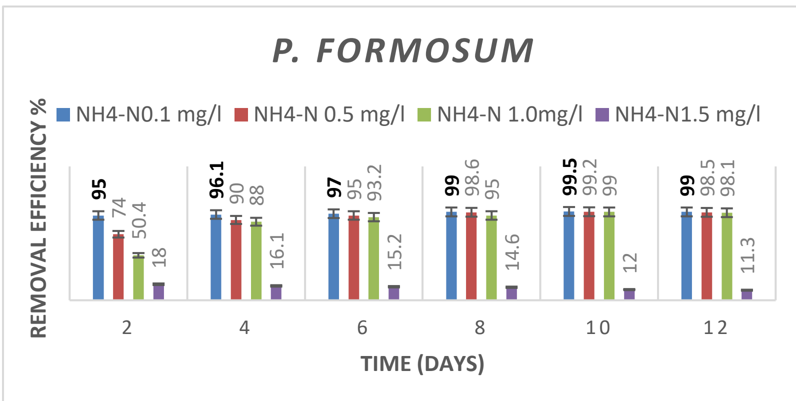

Figure 5: The removal efficiency of total ammonia nitrogen (TAN) by *Phormodium formosum*

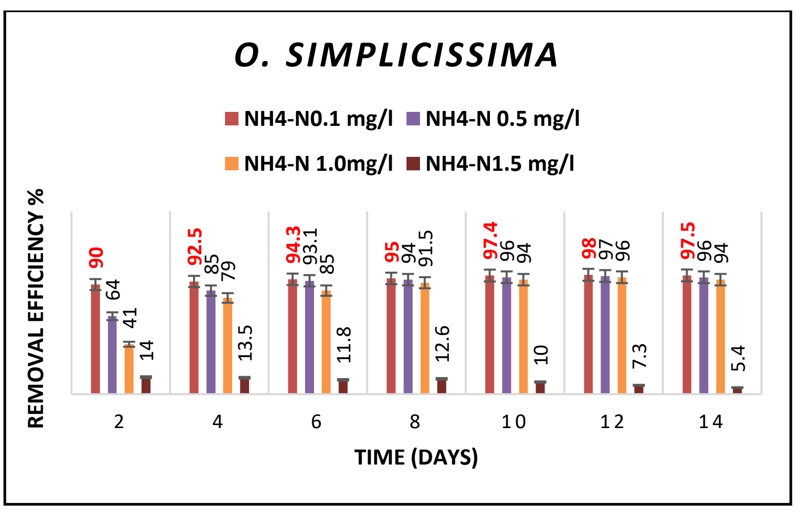

Figure 6: The removal efficiency of total ammonia nitrogen (TAN) by *Oscillatoria simplicissima*

However, the TAN removal was 96% when TAN concentration ~~of~~ was increased to 1.0 mg/L, However, with further increase in the TAN levels to 1.5 mg/L.TAN removal efficiency decreased to less than 10%.

DISCUSSION

Most studies on the biochemical production of algal and their analysis were carried out in stationary phase of growth period (Becker, 1994). Nitrogen is the major constituent of proteins, chlorophyll, and enzymes involved in photosynthesis. Therefore, nitrogen affects the photosynthesis of microalgae. The nitrogen absorbed by microalgae mostly includes NO3-N and NH4-N, and their uptake, deposition, and assimilation in microalgae are different. Chlorophyll is an extremely important biomolecule that is critical in photosynthesis, and that allows plants to absorb energy from light. The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystem (Voltolina et al., 2005). Chl *a* ($C_{55}H_{72}O_5N_4Mg$) is important in the energy phase of photosynthesis. Two electrons are needed for the electron acceptors to proceed in the photosynthesis process. Within the reaction centers of both photosystems is a pair of Chl *a* molecule that transfer electrons to the transport chain through redox reactions. Chl *a* is a common pigment found in algae. This pigment is what algae use to trap energy from light to promote algal growth. Some species, e.g., *Hillea* sp. and *Prorocentrum minimum*, failed to grow with ammonium-N because of ammonia's toxic effects in high concentrations (Lourenco et al., 2002). Although nitrogen is available to microalgae in various forms, nitrate, ammonium ions, and urea are the dominant forms (Syrett, 1981). Among these, ammonium ions are preferentially taken up, followed by nitrate and urea (Levasseur et al., 1993). The reported NH_4-N removal efficiencies varied, depending on the media composition and algae species (de-Bashan et al., 2010). The NH_4-N removal efficiency achieved in this study was higher, compared to that of other studies; an average of 72% nitrogen removal was reported for *C. vulgaris* NH4-N/L containing diluted ethanol and citric acid production effluent (Valderrama et al., 2002). Martinez et al., (Martinez et al., 2000) reported over 97% nitrogen removal by *Scenedesmus obliquus* Olguin [Olguin, 2003] obtained a maximum of 96% NH4-N removal by *Spirulina* in an outdoor raceway as a result of treatment with 2% diluted anaerobic effluents from pig wastewater containing almost the same amount of nitrogen as in the experiment carried out by Martinez et al. (Martinez et al., 2000). Nevertheless, few reports showed higher or more efficient NH4-N removal, even at higher concentrations of nitrogen. (Shi et al., 2007) investigated the effect of the initial nitrogen and phosphorus concentrations on the nutrient removal performance of the algae *Botryococcus braunii* from secondary treated piggery wastewater. Aslan and Kapdan (Aslan and Kapdan, 2006) investigated the batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by *C. vulgaris*.

CONCLUSION

The total ammonia nitrogen (TAN) removal efficiency by *P. formosum* was 99% at concentration 1.0 mg/l after 10^{th} days of incubation period but removal efficiency by *O. simplicissima* was 96% after 12^{th} days of incubation period. Two marine microalgae isolates can be a potential biological treatment for aquaculture water. The preferential uptake of ammonia by two microalgae solves the problems of ammonia toxicity in culture system.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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