Biomass Production and Ammonia and Nitrite Removal from Fish Farm Effluent by Scenedesmus Quadricauda Culture

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Introduction

One of the serious problems in human communities will be the shortage of drinking water resources in future. The development of fresh water fish culture is a deteriorating factor to this problem. Ammonia (NH_3) and nitrite (NO_2) are toxic chemicals resulting from fish culture, especially in intensive fish culture systems. Low concentrations of Ammonia make physiological and morphological changes in aquatic organisms, while high levels of it may cause mortality. NO_2 may cause methemoglobina in fish and gut and intestine cancer in human. To overcome some of these problems, one of the solutions for NH_3 and NO_2 reduction (or removal) is the use of biological processes. Microalgae have potential use to remove the excess nutrients and other contaminants because of their high capacity nutrient uptake. Most of literature stated that green algae, *Scenedesmus quadricuada* could be used for NH_3 removal due to suitable growth, high tolerance and low cost of culture technology. The purpose of this study was to determine NH_3 and NO_2 removal efficiency of green algae, *S. quadricuada* from fish farm wastewater effluent as well as algal biomass production.

Materials and Methods

S. quadricuada was collected from Karasgan Farms, Isfahan. It was cultured on agar-agar medium followed by successive algal culture on test tube, 250 ml and 2000 ml flasks, using bold basal medium (BBM). To evaluate the effect of *S. quadricuada* on wastewater rich in nitrogenous compound (NH_3 and NO_2), an experiment was carried out with six treatments (Table 1) in triplicates with quite random design. Wastewater was collected from Pouyankasra Rainbow Trout Farm effluent, located in Ardal, Chaharmahal-va-Bakhtiari Province, Iran. Treatments (Table 1) were prepared in three types, with raw effluent (M), diluted effluent with distilled water (L), and concentrated effluent with NH_4Cl and NO_3 (H), all in two forms of with (+BBM) and without (-BBM) BBM medium.

Table1: Treatment types used in this experiment.

Treatments	NH_3-N (mg/L)	NO_2-N (mg/L)	BBM	Symbol
Diluted wastewater (L)	0.108	0.623	with	L+BBM
	0.072	0.587	without	L-BBM
Raw wastewater (M)	0.213	1.239	with	M+BBM
	0.166	1.204	without	M-BBM
Concentrated wastewater (H)	0.444	2.477	with	H+BBM
	0.322	2.412	without	H-BBM

Each treatment type was cultured in a 2-liter flask for 21 days under 22 ºC, 60 µmol photons/m^2/s light intensity and with 12 hours light: 12 hours dark periods. Algal density, dry biomass, specific growth rate (SGR) and population doubling time (DT), chlorophyll *a*, and NH_3 and NO_2 concentrations were determined according to standard methods.

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Data analyses were carried out using one way ANOVA, following by Duncan's test at the signification level of 0.05 with the contribution of SPSS software.

Results

Results of this study are presented in Fig 1. The average maximum of *S. quadricuada* biomass was obtained in the 1st week (wk) from L+BBM (1.01 g/l), and in the 2nd and 3rd wks from M+BBM (1.33 and 1.04 g/l, respectively) (Fig. 1-A). The highest algal number was estimated in the 1st wk from L+BBM (2.56×10^6 cells/ml), the 2nd wk from H+BBM (4.08×10^6 cells/ml) and the 3rd wk from L+BBM (9.04×10^6 cells/ml) (Fig.1-B). The highest chlorophyll *a* content was observed in H+BBM (1.70 and 2.45 mg/l in the 1st and 2nd wks respectively) (Fig. 1-C). The maximum SGR and minimum D_T were obtained from L+BBM (0.27 day^{-1} and 2.61 day) in the 1st wk (Fig. 1-D and E). Results showed that the NH_3 and NO_2 removal efficiency of *S. quadricuada* was different in examined the treatment types, although the maximum removal rates of both NH_3 and NO_2 were approximately 90% in L+BBM (Fig. 2-F and G). In addition, there was no significant correlation between chlorophyll *a* and NH_3 (and NO_2) removal rate ($r=0.2$, $p>0.05$). However, there is significant correlation between NH_3 (and NO_2) concentrations, biomass ($r=0.47$) and SGR ($r=0.53$).

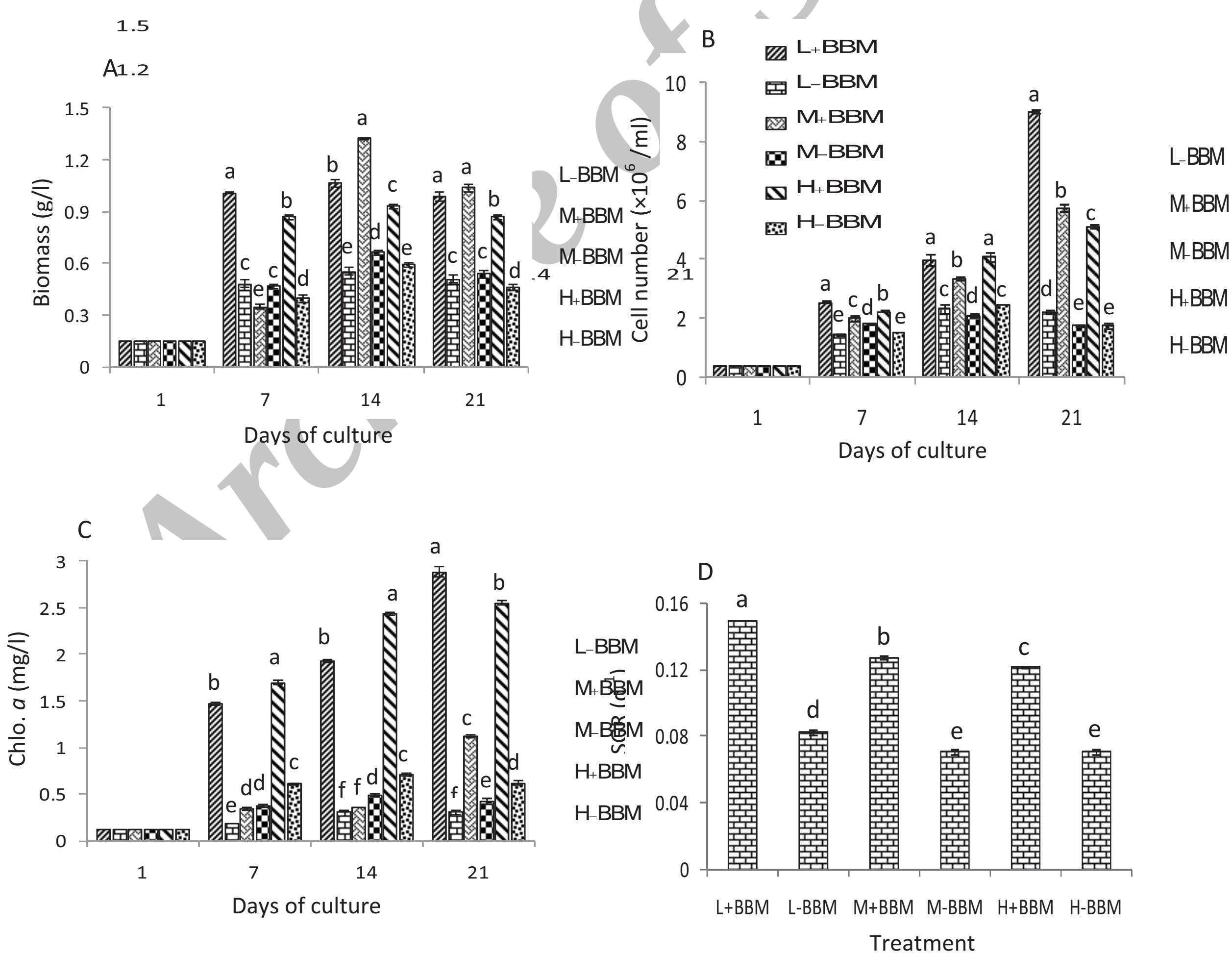

Fig 1: Biomass, Algal cell density, chlorophyll *a* , and specific growth rate (SGR) of *S. quadricauda* during experiment. Data are mean ±SE. Bars in the same letters are not significantly different (P>0.05).

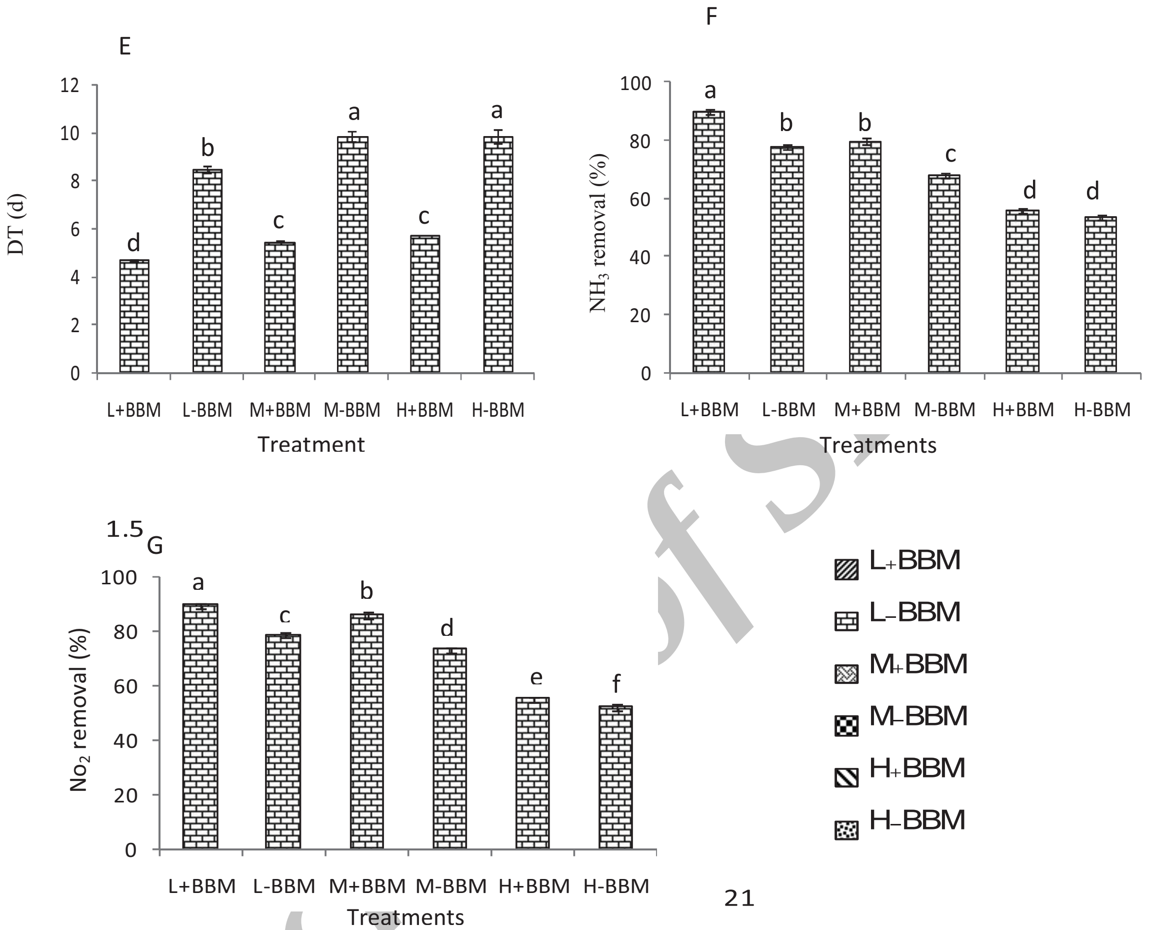

Fig 2: Population doubling time (D_T) , NH_3 and NO_2 removal rates of *S. quadricauda* during experiment. Data are mean ±SE. Bars in the same letters are not significantly different (P>0.05).

Discussion

Results showed that *S. quadricuada* could be used for NH_3 and NO_2 removal as well as biomass production from fish farm wastewater effluent. *S. quadricuada* grown on M-BBM had NH_3 and NO_2 removal rate of 68% and 73%, respectively. Previous researches reported 33% of NH3 removal rate in *S. quadricuada* and *S. dimorphus*. They showed that *S. dimorphus* had better NH3 removal efficiency compared to *Chlorella vulgaris*. Similar researches supported the results obtained by this study. For example, NH_3 removal rate of 99.1% by *Scenedesmus* and 90% by *Chlorella*. In general, most researches stated that *Scenedesmus* and *Chlorella pyrenoidosa* were suited for reduction of Nitrogenous and Phosphorous compounds from wastewater effluents. Today, with the development of aquacultures, especially intensive systems, wastewater effluents rich in NH3 and NO2 are released to natural freshwater ecosystems. These may be controlled or reduced by the application of *S. quadricuada* in treatment tanks after pond outlet. In addition, biomass produced from *S. quadricuada* using different wastewater effluents, could be used as live food for fish larvae and mollusks or in other industries such as biofuel or biodiesel as renewable energy resources.

Key words: *Scenedesmus quadricauda*, Biomass, Ammonia, Nitrite, Fish Farm Effluent, Green algae