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 (NH₃) and nitrite (NO₂) 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. NO₂ may cause methemoglobinemia in fish and gut and intestine cancer in human. To overcome some of these problems, one of the solutions for NH₃ and NO₂ 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 quadricauda could be used for NH₃ removal due to suitable growth, high tolerance and low cost of culture technology. The purpose of this study was to determine NH₃ and NO₂ removal efficiency of green algae, S. quadricauda from fish farm wastewater effluent as well as algal biomass production.

Materials and Methods
S. quadricauda 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. quadricauda on wastewater rich in nitrogenous compound (NH₃ and NO₂), 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 NH₃Cl and NO₃ (H), all in two forms of with (+BBM) and without (-BBM) BBM medium.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NH₃-N (mg/L)</th>
<th>NO₂-N (mg/L)</th>
<th>BBM</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted wastewater (L)</td>
<td>0.108</td>
<td>0.623</td>
<td>with</td>
<td>L+BBM</td>
</tr>
<tr>
<td></td>
<td>0.072</td>
<td>0.587</td>
<td>without</td>
<td>L-BBM</td>
</tr>
<tr>
<td>Raw wastewater (M)</td>
<td>0.213</td>
<td>1.239</td>
<td>with</td>
<td>M+BBM</td>
</tr>
<tr>
<td></td>
<td>0.166</td>
<td>1.204</td>
<td>without</td>
<td>M-BBM</td>
</tr>
<tr>
<td>Concentrated wastewater (H)</td>
<td>0.444</td>
<td>2.477</td>
<td>with</td>
<td>H+BBM</td>
</tr>
<tr>
<td></td>
<td>0.322</td>
<td>2.412</td>
<td>without</td>
<td>H-BBM</td>
</tr>
</tbody>
</table>

Each treatment type was cultured in a 2-liter flask for 21 days under 22 °C, 60 μmol photons/m²/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 NH₃ and NO₂ 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 DT 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 NH₃ and NO₂ removal efficiency of *S. quadricuada* was different in examined the treatment types, although the maximum removal rates of both NH₃ and NO₂ were approximately 90% in L+BBM (Fig. 2-F and G). In addition, there was no significant correlation between chlorophyll a and NH₃ (and NO₂) removal rate (r=0.2, p>0.05). However, there is significant correlation between NH₃ (and NO₂) 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. quadricuada* during experiment. Data are mean ±SE. Bars in the same letters are not significantly different (P>0.05).](image-url)
Discussion

Results showed that *S. quadricauda* could be used for NH$_3$ and NO$_2$ removal as well as biomass production from fish farm wastewater effluent. *S. quadricauda* grown on M-BBM had NH$_3$ and NO$_2$ removal rate of 68% and 73%, respectively. Previous researches reported 33% of NH$_3$ removal rate in *S. quadricauda* and *S. dimorphus*. They showed that *S. dimorphus* had better NH$_3$ 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 NH$_3$ and NO$_2$ are released to natural freshwater ecosystems. These may be controlled or reduced by the application of *S. quadricauda* in treatment tanks after pond outlet. In addition, biomass produced from *S. quadricauda* 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