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Bioremediation of Aquaculture Wastewater Using Microalgae *Chlorella vulgaris*

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Abstract

Microalgae have received increasing attentions as an alternative treatment approach to remediate wastewater for both nutrient removal and biomass production. Wastewater from aquaculture industry contains high levels of nitrogen and phosphorus, which affect plant growth. Conventional methods for treating aquaculture wastewater generally are inefficient and unprofitable. In this work, microalgae *Chlorella vulgaris* growth was studied in aquaculture effluent medium in order to reduce its contents of NO_3 and PO_4. Furthermore, the effect of $NaHCO_3$ and Na_2CO_3 concentrations and addition time on biomass productivity was evaluate to determine the most suitable conditions for biomass growth. It was found that highest biomass content (0.3 g/L) was achieved at 3.4 g/L of sodium bicarbonate concentration and 19 h of addition time.

Keywords: Biomass, microalgae, growth, treatment, wastewater

1 Introduction

Wastewaters from various industrial sources remain a major concern due to its high amount of pollutants such as organic loading, heavy metals and chemicals, which cause public health and environmental problems [1]. Conventional methods (e.g. chemical precipitation, membrane filtration, electrolytic processes and adsorption) have been widely used for decontamination of effluents, however these techniques exhibit disadvantages such as high reagent cost, energy requirements and sludge generation [2]. Microalgae have been reported to remove CO_2, nitrogen, phosphorus, and toxic metals from a different type of wastewaters, becoming an attractive alternative as wastewater treatment [3]. Because of being source of nutrients, wastewater can greatly reduce the production cost of microalgae; hence, growth of microalgae in wastewater provides biomass and simultaneous bioremediation of wastewater [4]. Microalgae are single-celled or colonial photosynthetic organisms that are available in large amount and in many different environments including rivers, lakes, oceans and soils [5, 6]. These organisms synthesize lipids, carbohydrate and proteins, which are used for producing value-added products [7]. In addition, they are attractive feedstock for biofuels production due to its high photosynthetic efficiency, biomass productivity and oil content [8]. Among a wide variety of microalgae, *Chlorella* sp. is frequently applied in wastewater treatment because of its enhanced ability in removing nitrogen, phosphorus, and chemical oxygen demand (COD) [9]. This work aims to study microalgae *Chlorella vulgaris* growth in aquaculture wastewater medium in order to determine the viability of this technique for both reducing pollutants and promote biomass productivity.

2 Materials and Methods

Culture methods

Chlorella vulgaris #1803 (UTEX culture collection, University of Texas at Austin, TX, USA) samples were grown in modified Bold Basal medium [10]. The culture volume of 0.35 L was coupled to bubbling aeration system with a flow air injection of 0.18 L/min in conditions of 12 h of light and 12 h of dark.

Aquaculture wastewater

The wastewater used as culture medium was obtained from farming ponds of *Pesquera de Santander,* which is located in Pinchote, Santander. For microalgae cultivation, this wastewater was filtered twice using qualitative paper membrane (pore size = 50-100 μm and 0.45 μm). Then, it was sterilized by autoclave equipment (120 psi, 60 min).

Experimental design

In order to determine the effect of carbon source ($NaHCO_3$ and Na_2CO_3) and addition time on biomass production, a central factorial 2^3 experimental design was implemented as shown in Table 1. The design of experiments was developed using STATISTICA 7.0 software.

Table 1. Experimental design for both sources of carbon

Reactor	$NaHCO_3$ (g)	Na_2CO_3 (g)	Addition time (h)
1	1	1.01	19
2	1	1.26	36
3	0.72	1.51	36
4	1.2	1.26	48
5	1	1.62	53
6	1	1.26	36
7	1.28	1.26	36
8	0.8	0.9	24
9	1.2	1.01	24

Biomass quantification

To quantify biomass amount, 10 mL of culture medium was taken once per 5 days during 15 days, which was filtered using Whatman GF/C filters pre-combusted for 1 hour at 100 ° C. After this procedure, filters were sent to oven for 1 hour at 100 ° C followed by 12 hours of drying until achieving constant weight [11].

Total nitrogen and PO_4 quantification

Phosphates (PO_4) and nitrogen concentrations in wastewater before and after treatment were determined by spectrophotometric method of vanadate-molybdate [12] and Nitrogen Total HR Test'N tube method [13], respectively.

3 Results and Discussion

Biomass quantification

Biomass productivity from *Chlorella vulgaris* in presence of $NaHCO_3$ and Na_2CO_3 is shown in Figure 1. These results suggested that sodium bicarbonate significantly increases biomass concentration with values higher than 0.13 $g/L{\cdot}d^{-1}$ for all experiments. On the other hand, sodium carbonate is not a source of carbon that improves biomass concentration, since most experiments did not exceed productivity values above 0.10 $g/L{\cdot}d^{-1}$. Srinivasan et al. [14] reported similar results concluding that sodium bicarbonate is the most efficient source of carbon for biomass production due to its contribution for pH stabilization and control of contaminants in the crop, which facilitate the growth of microalgae.

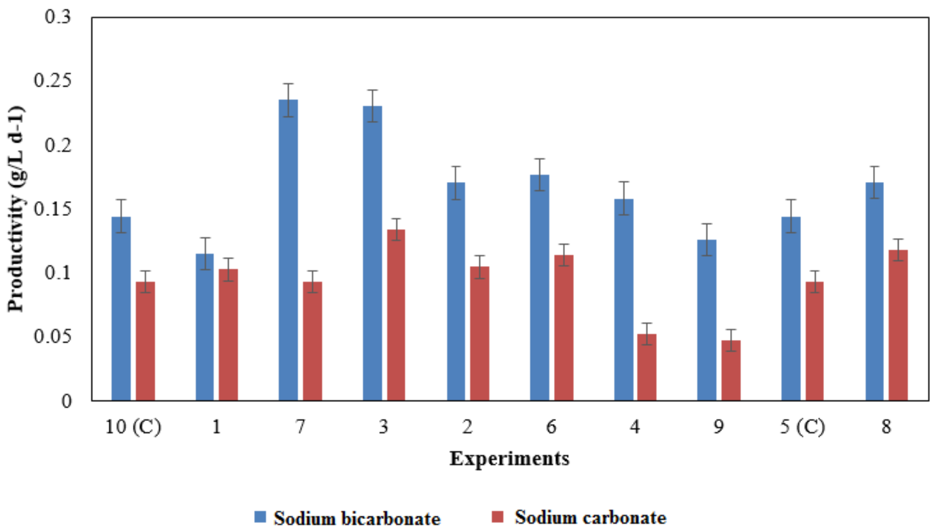

Figure 1. Biomass production in presence of $NaHCO_3$ and Na_2CO_3 in microalgal culture of *C. vulgaris*.

According to Figure 1, the experiment that exhibited highest productivity of biomass (0.235 g/L) in presence of sodium bicarbonate was number 7, which corresponded to 1.79 g/L of $NaHCO_3$ and addition time of 36 hours. For sodium carbonate, highest concentration of biomass (0.134 g/L) was achieved in experiment number 3, under culture conditions of 3.15 g/L of Na_2CO_3 and addition time of 48 hours. Pareto diagram shown in Figure 2 indicated that the most significant variable in all experiments was sodium bicarbonate concentration.

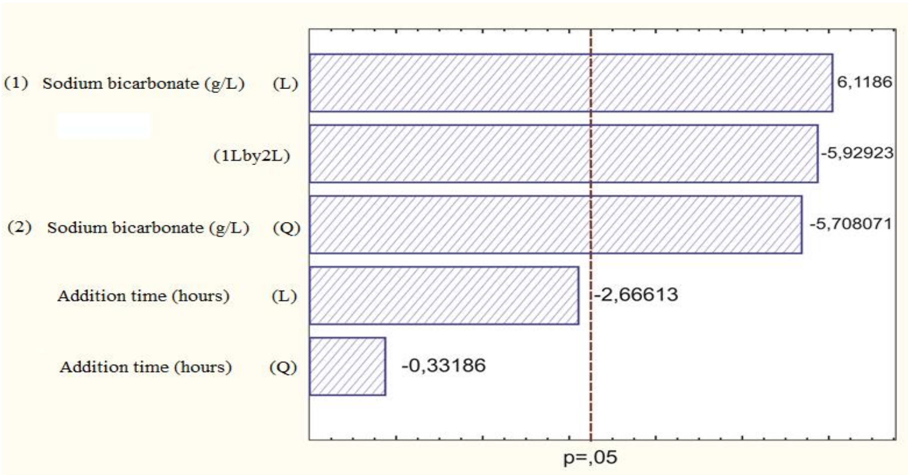

Figure 2. Pareto diagram of sodium bicarbonate and addition time effects on biomass productivity.

A response surface graph was performed for both sodium bicarbonate concentration (g/L) and addition time (h) and is represented by Figure 3, which suggested that biomass production efficiency was achieved at high concentrations of sodium bicarbonate (> 3.4) with a shorter time of addition (5-15 hours).

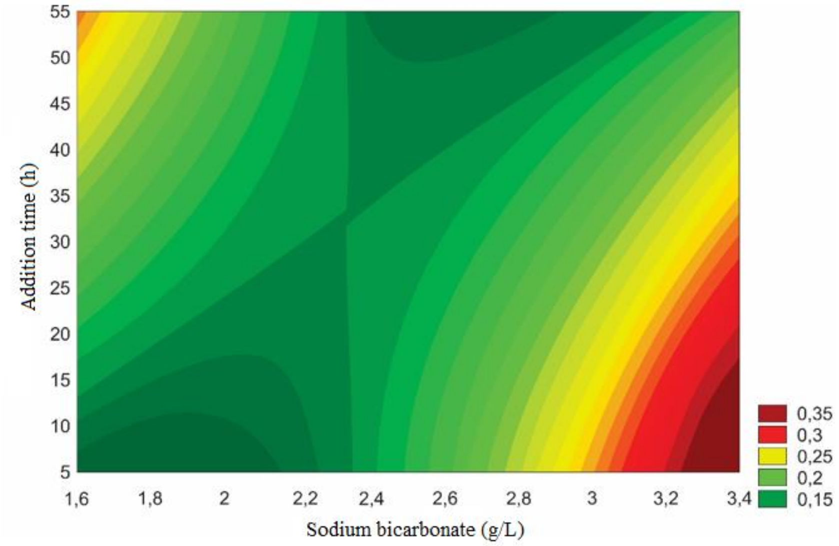

Figure 3. Response surface of sodium bicarbonate concentration and addition time.

Total nitrogen and PO_4 quantification

Due to low concentrations of Total Nitrogen (0.081 mg/L) and PO_4 (6 mg/L) in wastewater before microalgal treatment, an additional source of nutrients was added to each reactor in amounts summarized in Table 2.

Table 2. Source of nutrients added to culture medium

Nutrient	Concentration (g/L)	Amount (mL/L)
K_2HPO_4	7.5	10
KH_2PO_4	17.5	10
KNO_3	25	10

Figure 4 displays percentage of PO_4 and total Nitrogen consumed by microalgae culture in presence of $NaHCO_3$ and Na_2CO_3. It is observed that a consumption of 99.8% of PO_4 is commonly obtained by adding sodium bicarbonate to the crop. As is known, one of the main essential macronutrients in microalgal growth is Nitrogen. For nitrogen consumption, these percentages are greater than 99.9% using both sodium carbonate and sodium bicarbonate.

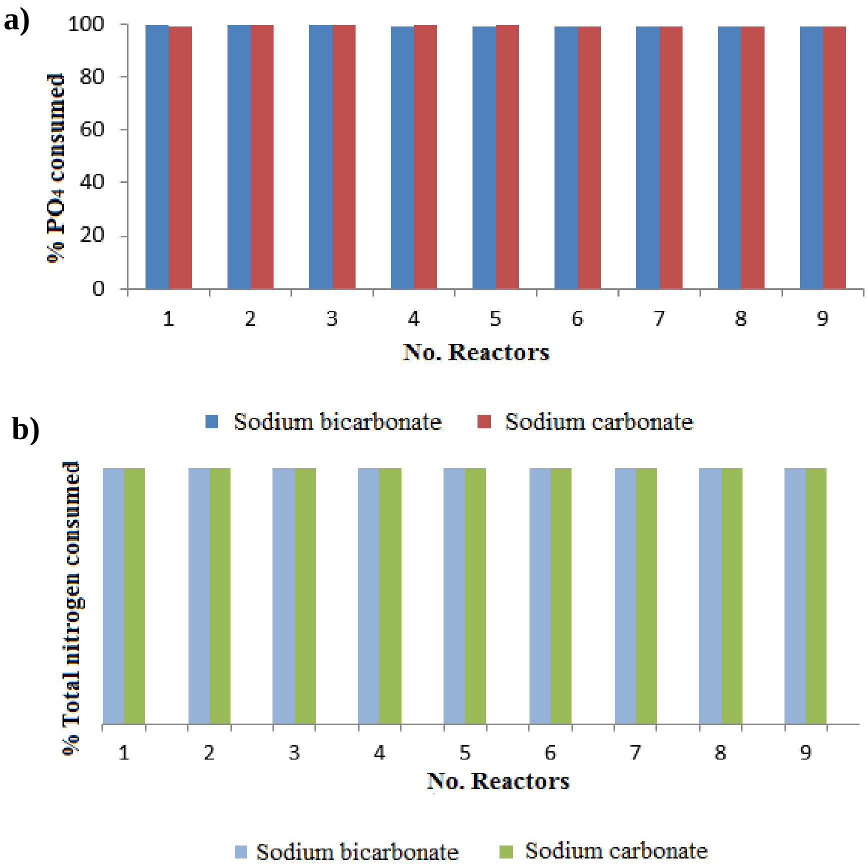

Figure 4. Percentage of PO_4 (a) and total Nitrogen (b) consumed in cultures with the addition of $NaHCO_3$ and Na_2CO_3

Optimization

To perform optimization of biomass production, sodium bicarbonate and addition time were selected as variables and a factorial 2^2 experimental designed was carried out in STATISTICA 7. Experiments took place on two bottle type reactors during 15 days.

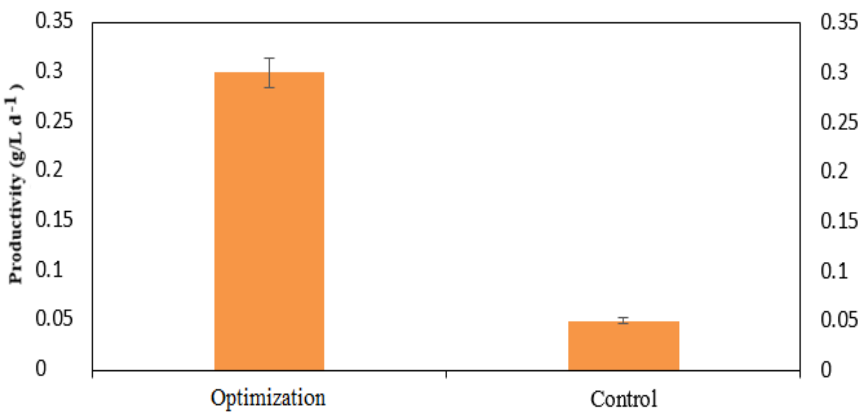

Figure 5. Optimization of biomass productivity in presence of $NaHCO_3$

It was found that the best conditions for biomass production by *Chlorella vulgaris* is the use of 3.4 g/L of sodium bicarbonate concentration and 19 hours of addition time. At the end of optimization procedure using the best conditions of concentration and addition time, an increase in biomass production respected to control experimentation was evidenced with increments between 0.05 g/L and 0.3 g/L as is shown in Figure 5.

4 Conclusions

This research attempted to evaluate the effect of $NaHCO_3$ and Na_2CO_3 concentrations and addition time on biomass productivity from *Chlorella vulgaris* when aquaculture wastewater is used as medium of culture. The optimum conditions to achieve highest biomass production was 3.4 g/L of sodium bicarbonate and 19 hours of addition time. In addition, results for phosphorous and total nitrogen consumption suggested that this microalga could be used for reducing pollution in aquaculture wastewater.

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