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Cultivation of *Chlorella vulgaris* in Aquaculture Wastewater for Protein Production

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Abstract

Microalgae have emerged as environment friendly alternative source of valuable products for energy, pharmaceutical and cosmetic industries. These microorganisms have been also studied in wastewater treatments due to its ability to remove CO_2, nitrogen, phosphorus, and toxic metals. In this work, cultivation of microalgae Chlorella vulgaris was carried out in aquaculture wastewater in order to reduce its contents of NO_3 and PO_4. In addition, different concentration of inorganic

carbon sources ($NaHCO_3$ and Na_2CO_3) and addition times were considered for determining suitable conditions in microalgae culture to produce proteins. It was found that highest protein content (45 % w/w) was achieved at 3.4 g/L of $NaHCO_3$ and 19 h of addition time.

Keywords: Microalgae, growth, protein, treatment, wastewater

1 Introduction

Nowadays, anthropogenic activities, such as agricultural practices, urbanization and industrialization have caused large amounts of wastewaters [1]. The pollutants discharged from industrial wastewaters negatively affect all aspects of the environment, such as water, air and land [2]. Wastewater generated in aquaculture industry needs treatment prior to its reuse or release in environment to avoid the eutrophication [3]. In general, these treatments are difficult because the wastewater contains various pollutants, high organic matter contents, and poorly biodegradable components [2]. It have been reported that algae cultivation using various wastewaters offers simultaneous bioremediation of water and reduce the biomass production cost [4]. In addition, microalgae has recently been highlighted as source of valuable products (e.g. proteins, carbohydrates, lipids and pigments) [5, 6]. They accumulate diverse nutraceuticals and pharmaceutical compounds in their cell bodies that can be used in food, cosmetic, energy and pharmaceutical industry [7, 8]. The selective cultivation of microalgae has gained attention because of their high productivity, which is related to their high growth rate and photosynthetic efficiency [9]. One of the main advantage of using these microorganisms is its ability to grow in unsuitable conditions for other crops, needing merely some nutrients and sunlight [10]. Microalgae biomass is rich in proteins that compete favourably, in terms of quantity and quality, with conventional food proteins such as soybeans, eggs, and fish [11]. Therefore, algal biomass shows promising qualities as another unconventional protein source [12]. Depending on the microalga, feeding, and cultivation conditions, protein contents of up to 71% (in dry matter) have been reported [9]. This paper aims to cultivate microalgae *Chlorella vulgaris* for both protein production and bioremediation of aquaculture wastewater. Furthermore, the effect of carbon source concentration and addition time on protein production is evaluated in order to determine optimal conditions for cultivating these microorganisms, which provide highest amount of protein.

2 Materials and Methods

Culture methods: *Chlorella vulgaris* (UTEX culture collection #1803) strain was obtained from University of Texas at Austin, which were grown in modified Bold Basal medium [13]. A bubbling aeration system was implemented with air injection of 0.18 L/min. The culture volume was 0.35 L and photoperiod of 12 h light/12 h dark.

Aquaculture wastewater: Wastewater from aquaculture activities was acquired from farming ponds in *Pesquera de Santander* (Pinchote-Santander). Before cultivating microalgae, the wastewater was filtered twice by qualitative paper membrane (pore size = 50-100 μm and 0.45 μm) and sterilized using autoclave equipment at 120 psi during 60 min.

Experimental design: To determine the effect of carbon source ($NaHCO_3$ and Na_2CO_3) and addition time on protein production, a central non-factorial 2^3 experimental design was carried out using STATISTICA 7.0 software. Table 1 summarized values selected to perform experiments for both parameters (carbon source concentration and addition time).

Table 1. Experimental design for protein production

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

Protein quantification: To determine amount for protein, procedure based on Bradford method was carried out. Hence, a buffer solution of potassium phosphate was prepared and filters from previous stage were submerged in this solution and performed according to the methodology proposed by Moheimani et al. [14].

Optimization: A non-factorial 2^2 experimental designed was implemented to optimize protein production. These experiments took place on two bottle type reactors during 15 days with conditions shown in Table 2. The experimental data was entered to STATISTICA 7.0 software.

Table 2. Experimental design for optimizing protein production

Reactor	$NaHCO_3$ (g)	Addition time (h)
1	1.7	19
2	1.7	19

3 Results and Discussion

Protein quantification: Protein production using *Chlorella vulgaris* in presence of $NaHCO_3$ and Na_2CO_3 is shown in Figure 1. Protein productivity remained stable in reactors with sodium bicarbonate. Highest amount of protein (35 % w/w) was achieved in reactor 7. On the other hand, reactor 1 reported lowest protein concentration (25 % w/w). For sodium carbonate, protein production was lower in comparison to sodium bicarbonate. Reactor 3 and 9 exhibited highest (30% w/w) and lowest (12% w/w) percentage of proteins, respectively.

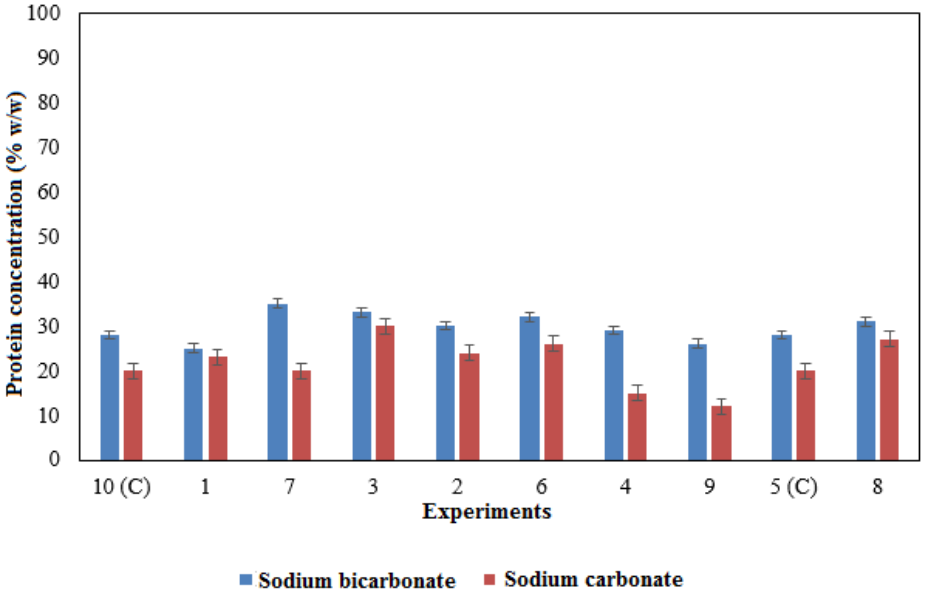

Figure 1. Protein production in presence of $NaHCO_3$ and Na_2CO_3 in microalgal culture of *C. vulgaris*.

Total nitrogen and PO_4 quantification: Wastewater from aquaculture activities reported low concentration of total nitrogen and PO_4 for cultivation of microalgae. Hence, other source of nutrients were added to culture medium (K_2HPO_4: 7.5 g/L, 10 mL/L; KH_2PO_4: 17.5 g/L, 10 mL/L; KNO_3: 25 g/L, 10 mL/L).

According to phosphate curve (R^2=0.991), the following equation was deduced to determine concentration of phosphate in culture medium:

$$C_{PO_4}\left(\frac{g}{L}\right) = \frac{Abs^{470\,nm} - 0{,}0238}{5{,}7045} \quad (1)$$

Results from Equation 1 were used to calculate amount of PO_4 and total nitrogen remaining after microalgal treatment, which is shown in Figure 2.

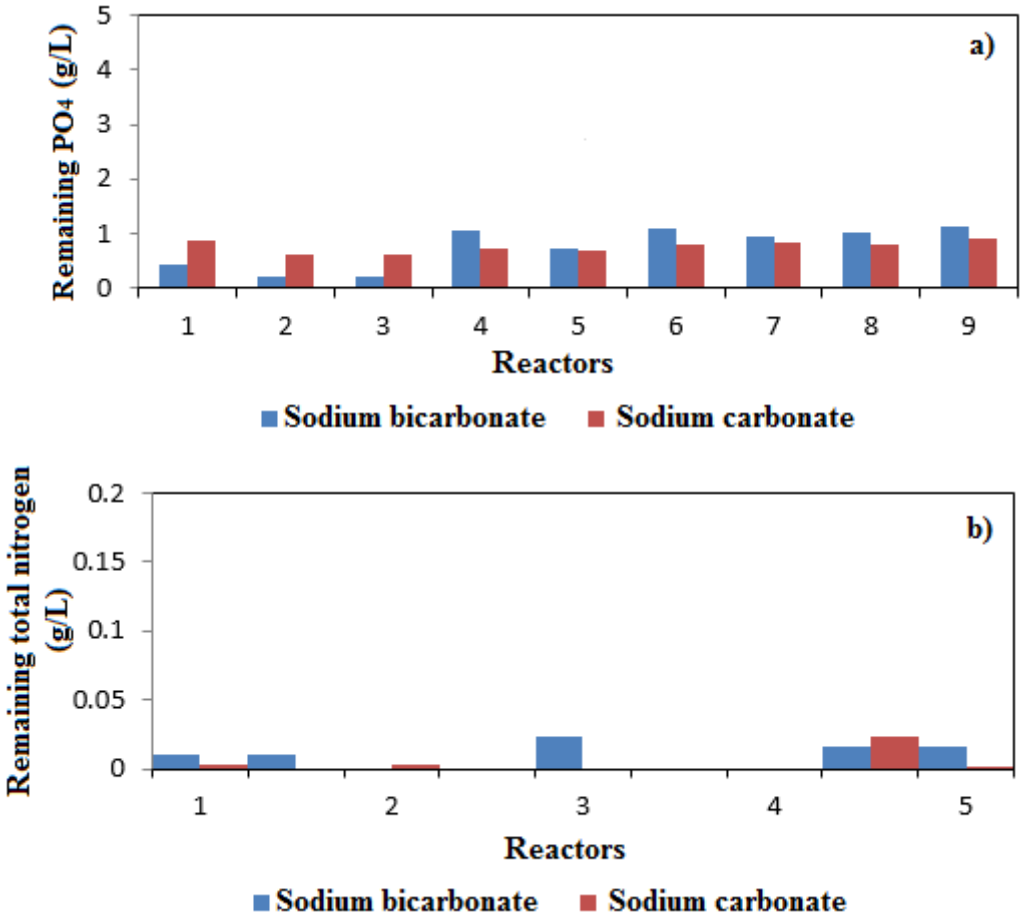

Figure 2. Percentage of PO_4 (a) and total Nitrogen (b) remaining in cultures with the addition of $NaHCO_3$ and Na_2CO_3

Figure 2-a) shows the concentration of PO_4 in reactors after microalgae treatment. It is observed that remaining PO_4 is lower in presence of sodium bicarbonate compared to sodium carbonate. The low concentrations for phosphate suggested that microalgae *Chlorella vulgaris* could reduce the amount of this pollutant from aquaculture wastewater. The results obtained in determination of final total nitrogen concentration is represented in Figure 2-b), where it can be highlighted that final concentration is significantly lower than initial concentration in presence of two forms of inorganic carbon.

Optimization: After analyzing the results of the first experimentation and performing statistical study, response surface was obtained for the best source of inorganic carbon (sodium bicarbonate) as is illustrated in Figure 3. For producing proteins, the optimal conditions were 3.4 g/L of sodium bicarbonate and addition time of 19 h or 1.6 g/L and 55 h. It was selected conditions for protein production in minimum time, which increased from 13 % w/w to 45% w/w (Figure 4).

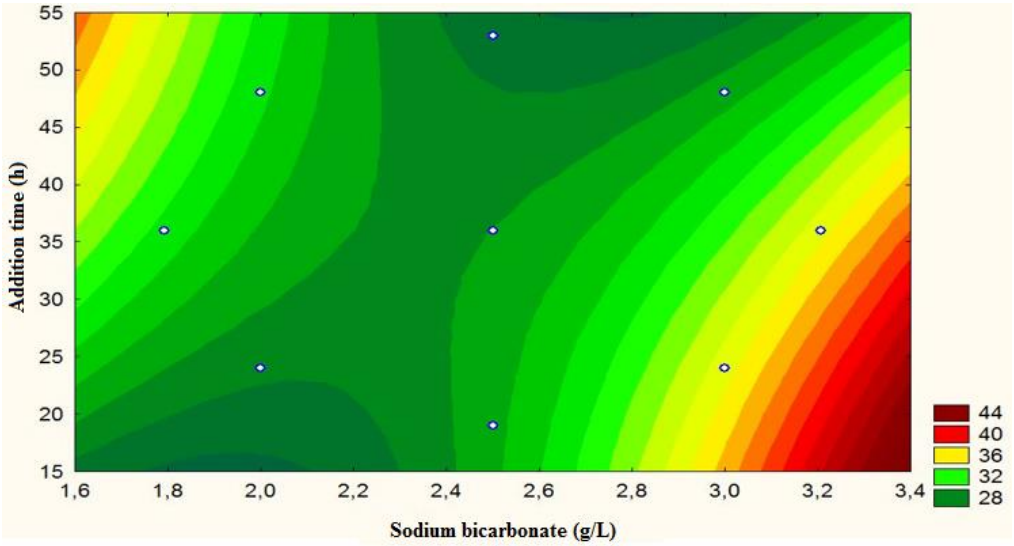

Figure 3. Surface response of protein productivity optimization in presence of $NaHCO_3$

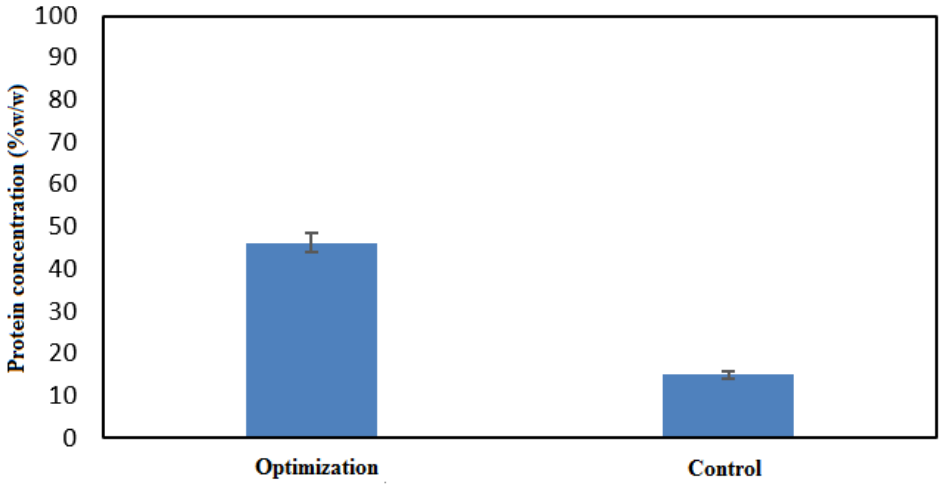

Figure 4. Optimization of protein productivity in presence of $NaHCO_3$

4 Conclusions

As this research has demonstrated, the best source of inorganic carbon for microalgae cultures of *C.vulgaris* in effluents from aquaculture activities is sodium bicarbonate providing higher protein concentration than sodium carbonate. The optimal conditions for achieving highest protein production (45 %w/w) was 3.4 g/L sodium bicarbonate and 19 h of addition time. In addition, low remaining concentration of phosphate and total nitrogen suggested that chlorella vulgaris could be applied in remediation of aquaculture wastewater; however, it is necessary to add macro and micronutrients such as N and P for microalgae growth.

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