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BIOMASS PRODUCTION AND WASTEWATER TREATMENT FROM AQUACULTURE WITH *CHLORELLA VULGARIS* UNDER DIFFERENT CARBON SOURCES

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Abstract

Algae are sustainable sources of biomass for fuel, food, feed and essential for their growth are light, CO2, and inorganic nutrients like nitrogen and phosphorous. The aim of our study was to explore the effect of different carbon sources on biomass accumulation in microalgae Chlorella vulgaris and its ability to remove N and P compounds during its cultivation in aquaculture wastewater. Microalgae cultivation was initiated in bioreactor from 500ml Erlenmeyer flask containing 250ml wastewater from semi closed recirculation aquaculture system. The cultures were maintained at room temperature (25-27ºC) on a fluorescent light with a light:dark photoperiod of 12 h: 12 h. The microalgae were cultivated in wastewater with two different carbon sources: carbon dioxide (2%, v/v), and sodium bicarbonate ($NaHCO_3$) (1.125g.l^{-1}). The growth of strain was checked for 96 hours period. In our study C. vulgaris showed better growth in wastewater from aquaculture with bicarbonate utilization as carbon source during the experiment.

Keywords*:aquaculture, biomass, carbon sources,Chlorella vulgaris, wastewater.*

INTRODUCTION

Algae are sustainable sources of biomass for fuel, food, feed and essential for their growth are light, CO_2, and inorganic nutrients like nitrogen and phosphorous. Algae are fast growing and can be cultivated in systems that don't require agricultural land and thereby it doesn't compete with another culture (Mercer and Armenta, 2011). Wastewater treatment is another possible use of algae since they grow well on the nutrients present in the water (Larsdotter, 2006). The uptake of dissolved nutrients by microalgae is the primary way to remove nitrogen in aquaculture systems (Attasat et al., 2013; Sirakov et al., 2013). Many authors have studied the use of microalgae to treat wastewater from aquaculture. Aquaculture systems involving microalgae production and wastewater treatment seems to be quite promising for microalgae growth combined with biological cleaning (Mata et al., 2010).

Chlorella vulgaris Beyerinck (Beijerinck) is a robust and fast growing microalgae species commonly cultivated and interesting regarding the production of secondary metabolites (Mansson, 2012).*C. vulgaris* is highly valued for its protein content, as it can be used for is potential biomass. The factors like CO_2, intensity of light, wavelength affects the growth rate of the *C. vulgaris* (Sankar and Ramasubramanian, 2012).

Often carbon and nitrogen are the most important nutrients contributing to the biomass produced (Prabakaran and Ravindran, 2012). Carbon is the most important element found in algal biomass and it constitutes over 50% in typical algal biomass (Becker, 1994). Using captured CO_2 for microalgae growth is limited by the high cost of CO_2 capture and transportation, as well as significant CO_2 loss during algae culture. The algae grow poorly at night, but CO_2 cannot be temporarily stored until sunrise. For these reasons, it is necessary a more efficient and a cheap source of carbon such as sodium bicarbonate. Microalgae have the ability to use organic carbon as an energy source and this is important because it can minimize the inhibitory effects of seasonal and diurnal light limitation on growth in outdoor cultures.

The aim of our study was to explore the effect of different carbon sources on biomass accumulation in microalgae *C. vulgaris* and

itsability to remove N and P compounds during its cultivation in aquaculture wastewater.

MATERIALS AND METHODS

Microalgae strain, medium and cultivation

C. vulgaris (SKU: 100-CVC00-50) which is a green alga belonging to the division *Chlorophyt*a and class *Trebouxiophyceae* was purchased from Algae depot – USA (www.algaedepot.com). The wastewater used like a media for tested algae cultivation originate from semi closed recirculation aquaculture system (semi – closed RAS), before it cleaning at mechanical and biological filters.

Algae cultivation was initiated in bioreactor from 500ml Erlenmeyer flask containing 250ml wastewater. The experiment was conducted in variants with carbon dioxide (2%, v/v) and sodium bicarbonate ($1.125 g.l^{-1}$).Three luminescent lamps Sylvania Aqua Star – 18w, 10 000 K were placed at a distance of 30 mm from flasks. Light regime was adjusted at 12:12 h light:dark cycle in an illumination incubator until the end of experiment. The temperature was kept between 25 and 27°C. The strains were checked for 96 hours growth period. In the laboratory, the samples of wastewater were filtered through a 25mm, 3µm glass microfiber filters (GF/C) mounted on a Millipore filtration unit. The cells in exponential period were inoculated (10%, v/v) in a liquid medium.

Optical density, chlorophyll and carotenoid content of microalgae culture

Optical densities of microalgae cultures were measured at 0, 24, 48, 72 and 96 hours after the start of the experiment in three replicates. The sample with volume one ml was appropriately diluted with deionised water and the average value was recorded by absorbance at 450 nm with the help of spectrophotometer DR 2800 (Hach Lange).

The isolation of pigments from algae cells included the following procedures: harvesting 2 ml of microalgae cells by centrifugation at 10000 rpm, two times for 3 min and discarding the supernatant, suspension of cells in 2 ml methanol/water 90:10 v/v and mixing of Vortex for 1 min., heating of the suspension for half an hour in a water bath at 60ºC, cooling of the samples at room temperature, centrifugation the suspension (10000 rpm for 3 min) and discarding the supernatant with dissolved pigments. The absorbance of the pigments extract (665, 652 nm for chlorophyll content (a+b) and 470, 666nm for carotenoids content) was recorded by using spectrophotometer. The chlorophyll content was computed ($mg.l^{-1}$) according Porra et al. (1989) and carotenoid content was computed ($mg.\ l^{-1}$) according Lichtenthaler (1987).

Hydrochemical analysis

Samples for hydro chemical analysis were taken at the beginning of the trial, 24, 48, 72 and 96 hours after the start of the experiment in three replicates. The samples were centrifuged at 300 rpm for 10 min, for freeing them from algal cells (Lee and Lee, 2002).

The measurement of pH was made with a portable combined meter and with a pH probe (Hach Lange).

Other analyzed hydro chemical parameters were measured spectrophotometrically with spectrophotometer DR 2800 (Hach Lange). The methods and range of tests which were used during the experiment are shown it table 1.

Table 1. Methods and range of tests used to monitor the water quality parameters during experiment

Quality parameters	Determination method	Measuring range ($mg.l^{-1}$)
Ammonia	Indophenol blue	0.015 – 2
Nitrate - nitrogen	2.6 dimethylphenol	5 - 35
Total nitrogen	Koroleff digestion + 2.6 dimethylphenol	5 - 40
Phosporus (ortho + total)	Phosphormolybdenum blue	0.15 – 4.5

Data analyses were conducted by using one-way Analysis of Variance ANOVA (MS Office, 2010).

RESULTS AND DISCUSSIONS

The algae need the carbon for the photosynthesis. About 50% of the alga biomass is made up of carbon and is therefore needed to a large extent for good growth (Becker, 1994). The natural CO_2 present in the air is not sufficient to maintain optimal growth. In our study the optical density of *C. vulgaris* was 1.25 in wastewater with carbon source – $NaHCO_3$ used like a growing media for 96hour (Figure 1). It was with 12% higher optical

density compared to wastewater with carbon dioxide for the same strain. Jeong et al. (2003) receive a similar result in the culture of *C. vulgaris* that showed the highest growth rate on 5 day (1.17 optical densities). Elvira-Antonio et al. (2013) was reached higher productivity in *C. vulgaris* cultures with 1g.l^{-1} sodium carbonate. Goswami et al. (2012) observed that CO_2 gas in algal culture has a poor dissolving capacity and most of it tend to lost in the air, so it is convenient to use bicarbonate form instead of CO_2 gas.

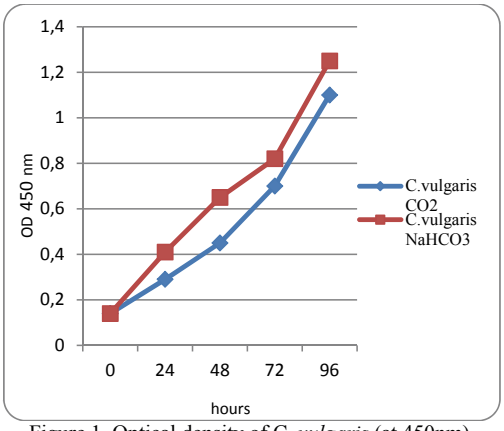

Figure 1. Optical density of C. *vulgaris* (at 450nm) cultivated in wastewater from aquaculture with different carbon source

Ahmad et al. (2013) established that *Chlorella* grow well in wastewater as well as in the nutrient medium. In our experiment the highest chlorophyll content was determinate in *C.vulgaris* cultivated in wastewater with carbon source – NaHCO3 (9.6 mg.l^{-1}) (Figure 2). Our results correspond with Chinnasamy et al. (2009), which growth response of *C. vulgaris* in terms of biomass and total chlorophyll showed a similar pattern. Šoštarič et al. (2009) cultivate *C.vulgaris* in bicarbonate concentration of 1.05 g.l^{-1} in the pure solution from the modified Solvay process and determined 5.2 mg.l^{-1} chlorophyll.

The microalgae *C. vulgaris* is known to produce pharmaceutically important carotenoids: canthaxanthin and astaxanthin (Mendes et al. 2003). Increased production of carotenoids in presence of higher amounts CO_2 might add to the economic utility of this algal strain.

In our study the quantity of carotenoids in *C. vulgaris* were higher (2.1 mg.l^{-1}) in cultures grown in wastewater with $NaHCO_3$ carbon source, compared with the carotenoids of wastewater with carbon dioxide - 1.89 mg.l^{-1} (Figure 3). Chinnasamy et al. (2009) cultivated *C.vulgaris* in 6% $CO_2$and established 2.0 mg.l^{-1} carotenoids. We achieve the same results with at less 2% CO_2 added in wastewater from aquaculture.

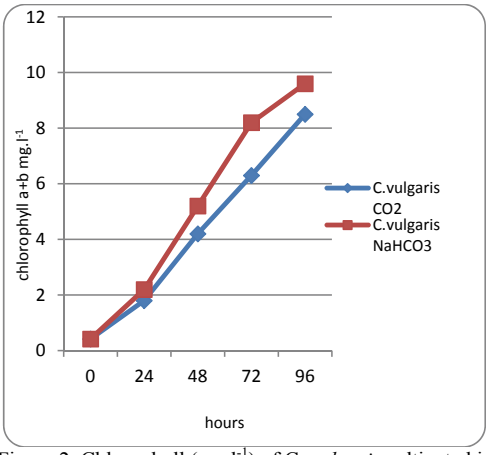

Figure 2. Chlorophyll (mg.l^{-1}) of C. *vulgaris* cultivated in wastewater from aquaculture with different carbon source

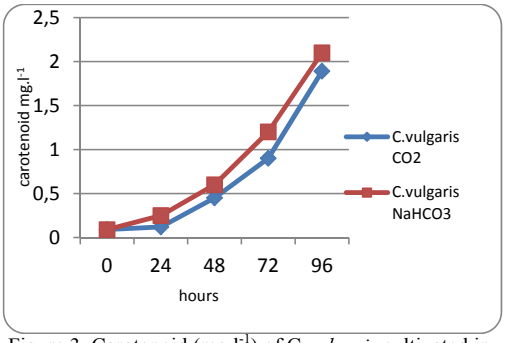

Figure 3. Carotenoid (mg.l^{-1}) of C.*vulgaris* cultivated in wastewater from aquaculture with different carbon source

The pH will rise as the algae grow and consume the carbon dissolved in the water. This indicates that *Chlorella* can be flocculated efficiently by increasing the pH of the culture to 11 (Mansson, 2012). During our trial the measured pH varied from 6.08 to 9.94 in tested algae strain and the pH value increased mostly in cultivation with $NaHCO_3$ (Figure 4). This is in compliance with Sorensen et al.(1996), who

maintained that, increase of pH is due to the use of CO_2(aq) from bicarbonate to compensate the lack of CO_2 from gas supply. It is important to maintain pH within an adequate range to avoid the loss of carbon dioxide existing in the media.

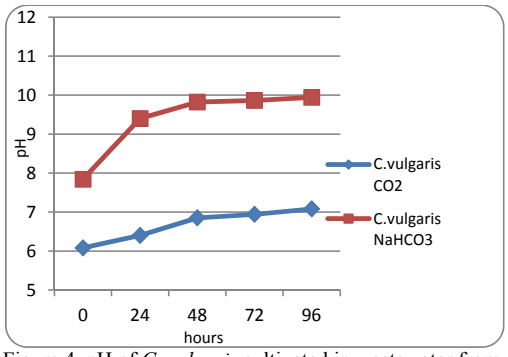

Figure 4. pH of *C. vulgaris* cultivated in wastewater from aquaculture with different carbon source

The concentration of nitrate at the beginning of the experiment was 4.43 mg.l^{-1}. In the end the most effective reduce of nitrate was in *C. vulgaris* cultivating in wastewater from RAS with $NaHCO_3$ carbon source (0.1mg.l^{-1}) and the differences were statistically proven ($P \leq 0.05$) (Table 2). This result obtained for *C. vulgaris* with $NaHCO_3$was with 13.5% better compared with the same of *C. vulgaris* with 2% CO_2. Elvira-Antonio et al. (2013) obtained 85.13% nitrogen removable growing *C. vulgaris* in the presence of 1 g.l^{-1} $NaHCO_3$. Shi et al. (2007) reported that the nitrate was removed from synthetic wastewater by the *C. vulgaris* after 4 days, like in our experiment.

At the beginning the ammonium was 14.3 mg.l^{-1}, and after 96 hour their quantity reduced up to 0.42 mg.l^{-1} for *C. vulgaris* with $NaHCO_3$ carbon source and 0.43 mg.l^{-1} for *C. vulgaris* with 2% CO_2. In our experiment, after 96 hours the ammonium decreased with 97% in wastewater from RAS with $NaHCO_3$ carbon source. Our results about ammonium removal efficiency was comparable to studies conducted by Martínez et al. (2000), who described elimination of NH4+ (between 79% and 100%) after188.25 h, and with González et al. (1997), who reported ammonium removal efficiencies of 90% from agro-industrial wastewater after 216 h. Shi et al. (2007) established that ammonium removal efficiency by *C. vulgaris* in wastewater was 78% in the first 3 days.

The better uptake total nitrogen (7.69%) was observed again in *C. vulgaris* with $NaHCO_3$ carbon source compared with 2% CO_2. At the end of the trial after the cultivation of our strain in wastewater with sodium bicarbonate the total nitrogen decreased with 71.2%.

Table 2. Hydrochemical parameters during the experiment (Data are expressed as mean ± standard error).

Parameters	*C. vulgaris* CO_2	*C. vulgaris* $NaHCO_3$
Nitrate	1.5±0.04*	1.33±0.04*
Amonium	4.81±1.55ns	4.38±1.51ns
Total nitrogen	7.03±0.91ns	6.51±0.96ns
Total phosphorus	1.51±0.03*	1.36±0.04*

*p≤0,05

The efficiency in total phosphorus removal from wastewater was better in wastewater with $NaHCO_3$ used as carbon source used for *C. vulgaris* cultivation, compared to 2% CO_2. In the beginning was measured a level of phosphorus compounds 3.6 mg.l^{-1}. At the end of the experiment for *C. vulgaris* cultivation in wastewater with $NaHCO_3$ carbon source phosphorus decreased with 91.1%, while with 2% CO_2 – 90.2%. Shi et al. (2007) demonstrated result that about 90% of the phosphate was removed from synthetic secondary wastewater within 2 days by *C. vulgaris* microalgal strains.

Aslan and Kapdan (2006) used *C. vulgaris* for nitrogen and phosphorus removal from wastewater with an average removal efficiency of 72% for nitrogen and 28% for phosphorus. Shi et al. (2007) performed experiments with *Chlorella* to remove nitrate from municipal wastewater and reduce levels of phosphate, ammonium and nitrate in synthetic secondary wastewater. In their experiment ammonium was removed less rapidly by the algae than phosphate. The specifics depend on the type of wastewater, the type of algae and their growth conditions, and most importantly on the relationship between the amount of biomass applied and the hydraulic loading of the wastewater (Shi et al., 2007).

CONCLUSIONS

Our results showed that wastewater from aquaculture with carbon source sodium bicarbonate promote better algal growth of *C. vulgaris* compared in wastewater with carbon source 2% CO_2.

Higher purification effect for tested hydrochemical parameters was observed in the wastewater with added sodium bicarbonate like carbon source for cultivation of the algae species.

C. vulgaris can be used for biological treatment of wastewater originate in aquaculture.

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