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Lipid production in mixotrophic cultivation of *Chlorella vulgaris* in a mixture of primary and secondary municipal wastewater

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ABSTRACT

Microalgae's biomass productivity and oil content depend heavily on the method of its cultivation. In this study, nutrient removal from municipal wastewater by *Chlorella vulgaris* in batch culture was investigated. Carbon dioxide was supplied from sodium hydrogen carbonate. Effect of parameters including *light intensity*, *sodium hydrogen carbonate concentration*, and *daily illumination time* on the productivity of biomass and lipid was investigated. Lipid and biomass production of *C. vulgaris* increased at higher concentration of *sodium hydrogen carbonate concentration* and higher *light intensity* until a certain value and then decreased, but longer *daily illumination time*, increased both biomass and lipid productivity. Cultivation of *C. vulgairs* in mixotrophic mode was also studied in a mixture of primary and secondary wastewater with different ratios (25, 50 and 75 volume percent of the primary wastewater). It was observed that using 25% of the primary wastewater results in 100% COD removal, 100% ammonium removal and 82% nitrate elimination. Biomass productivity and lipid productivity of *C. vulgaris* in a mixture of primary (25%) and secondary wastewater were 138.76 mg/L/d and 45.49 mg/L/d, respectively.

1. Introduction

Fossil fuels are known as unsustainable energy sources because of their greenhouse gas release into the atmosphere [1]. In order to reduce fossil fuel consumption, production of renewable energies such as biofuels and especially biodiesel has attracted a high attention in recent years [2,3]. Vegetable oil-originated biodiesel demands a competition between the resources used for fuel and food, and thus is not a suitable replacement for fossil fuels [3]. Among other feedstock for biodiesel production, microalga is a mighty valued candidate because of its high lipid content and short duration of cultivation time [1,2,4–7]. However, microalgae cultivation in conventional systems demands a high amount of water and nutrients and consequently turns it to an ineffective process for producing biodiesel [8]. On the other hand, wastewater effluents are rich in nutrients including nitrogen, phosphorus and carbon. Therefore, by cultivation of microalgae in wastewaters, enough nutrients will be available for biomass growth [9]. From another point of view, microalgae need carbon dioxide for their photosynthetic metabolism that can be provided from industrial flue gases. This leads to the reduction of greenhouse gas emission to the atmosphere and carbon fixation at the same time [7]. Microalgae are commonly cultivated under autotrophic growth condition where light energy is harvested by the cells and carbon dioxide is utilized as a carbon source. In the heterotrophic culture, organic carbon is used as the carbon source. However, in mixotrophic growth regime, CO_2 and organic carbon are both simultaneously assimilated and the metabolisms of respiratory and photosynthetic, operate at the same time.

In earlier studies, a variety of wastewaters including municipal, industrial and agricultural sewage were used to investigate nitrogen and phosphorus removal by microalgae cultivation [10]. Different types of microalgae such as *Chrollela vulgaris*, *Scendesmus obliques*, *Spirulina maxima*, *Botrycoccus braunii* and *Chlamydomonas reinhardtii* have been used for autotrophic, heterotrophic and mixotrophic cultivation in sewage [9]. Among these species, *C. vulgaris* has been known as one of the fastest growing microalgae and found to be able to readily uptake nitrogen and phosphorus from wastewater through its cell membrane [10].

Algae cultivation is often carried out in secondary sewage or synthetic wastewaters. Wang et al. (2009) evaluated the growth of a green algae *Chlorella* sp. in wastewaters of a local municipal wastewater treatment plant. The removal rate of NH_4–N, phosphorus and COD was 74.7%, 90.6% and 56.5%, respectively for a wastewater before the primary settling stage. They also reported a high removal efficiency of NH_4–N and phosphorus from secondary

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wastewater [11]. Aslen et al. (2006) investigated the effect of the initial nitrogen and phosphorus concentration on the performance of *C. vulgaris* for nutrient removal in synthetic wastewater. Ammonium concentration varied between 13.2 and 410 mg/L while phosphorus concentration was between 7.7 and 199 mg/L [12]. Sreesai et al. (2007) focused on nutrient removal and growth of *C. vulgaris* in wastewater. They evaluated the influence of light intensity and retention time on the growth and nutrient removal efficiency [13].

Many studies demonstrated that *C. vulgaris* has a great potential for the treatment of wastewater and capable to be cultivated in two cultivation modes: autotrophic and heterotrophic.

In this study, variety of conditions was investigated for the autotrophic cultivation of *C. vulgaris* in municipal wastewater. The optimum value of parameters including *light intensity*, *sodium hydrogen carbonate concentration* (microalgae can fix CO_2 in the form of soluble carbonate) and *daily illumination time* were determined to gain maximum biomass, lipid productivity and high-efficiency of nutrient removal.

Furthermore, mixotrophic cultivation of this microalga was also investigated in a mixture of primary and secondary wastewater. The key aim of the present research was to compare the autotrophic and mixotrophic cultivation of *C. vulgaris* in primary and secondary wastewater and study its potential for wastewater treatment and lipid production.

2. Material and methods

2.1. Microalgae strain, media and algal cultivation

The used algal strain, *Chlorella vulgaris*, was obtained from Materials and Energy Research Center, Iran. The microalga was cultivated in BBM medium containing (g/L): 0.175 KH_2PO_4, 0.025 $CaCl_2.2H_2O$, 0.075 $MgSO_4.7H_2O$, 0.25 $NaNO_3$, 0.075 K_2HPO_4, 0.025 NaCl, 0.01 $Na_2EDTA.2H_2O$, 0.00498 $FeSO_4.7H_2O$, 0.00805 H_3BO_3 and 1 ml of the trace element solution including (g/L): 2.86 H_3BO_3, 1.81$MnCl_2.4H_2O$,0.222 $ZnSO_4.7H_2O$, 0.390 $Na_2MoO_4.2H_2O$, 0.079 $CuSO_4.5H_2O$, 0.0494 $Co(NO_3)_2.2H_2O$. It was stored in solid BBM slants at 4 °C until use. The wastewater from primary and secondary municipal plant was provided from Shahrak-e-gharb wastewater treatment plant, Tehran, Iran and used as culture medium. The wastewater was filtered before use to remove solid particles. The properties of the secondary and primary wastewater are listed in Table 1.

C. vulgaris was cultivated in flasks containing 2.0 L of the wastewater with initial inoculum concentration of 0.1 g/L of microalgae. Agitation of the culture broth was provided by air-bubbling (0.5 vvm) at room temperature. The cultivation flasks were exposed to 20 W fluorescent lamps as the light source. The emission spectrum of fluorescent lamps was about 400 nm–700 nm with a peak wavelength at 450 nm. The incoming light intensity to flasks was set at 2000–10000 lx ($\mu mol.m^{-2}.s^{-1}$) by controlling the distance between flasks and fluorescent lamps. The *light intensity* was measured by light intensity meter (Lutron-YK-10LX lux meter, Taiwan).

Microalgae cultivation was conducted in 2 modes: autotrophic and mixotrophic cultivation. In autotrophic cultivation, effect of parameters including *light intensity*, *sodium hydrogen carbonate concentration*, and *daily illumination time* on biomass and lipid productivity was investigated. Autotrophic cultivation was carried out using secondary municipal wastewater. In mixotrophic cultivation, the effect of organic carbon source on the biomass and lipid productivities was investigated by adding a specified volume of the primary municipal wastewater to the secondary wastewater.

Table 1
Characteristics of secondary and primary municipal wastewater.

Primary effluent	Concentration (mg/L)	Secondary effluent	Concentration (mg/L)
NH_4^+–N	43.31	NH_4^+–N	0.63
NO_3^-–N	56.19	NO_3^-–N	224.78
PO_4^{-3}-P	0.63	PO_4^{-3}-P	0.53
COD	256	COD	96
pH	7.2	pH	7.1

2.2. Analytical methods

Biomass concentration was determined every 24 h by measuring the optical density at 680 nm using a UV–visible spectrophotometer. The dry mass of *C. vulgaris* was calculated from the calibration curve between absorbance and weight of the dried biomass [13].

All samples were filtered using 0.45 μm filter prior to the measurement of ammonium, nitrate, phosphate and COD. Ammonium concentration was determined by the colorimetric salicylate method by measuring the absorbance of ammonium at 685 nm [14]. Amount of nitrate-nitrogen was determined using ultraviolet spectrophotometric standard method [15]. Phosphate content was determined by vanadomolybdophosphoric acid colorimetric method at 420 nm [16]. The closed reflux method was used for COD measurement according to the Standard Method [15].

Lipid determination was conducted according to Bligh and Dyer method. Lipid was extracted from *C. vulgaris* using a mixture of methanol, chloroform and water and measured, gravimetrically [17].

2.3. Evaluation of effective parameters on biomass and lipid production

C. vulgaris was cultivated autotrophically in the secondary wastewater under different values of *sodium hydrogen carbonate concentration* (as a source of carbon dioxide), *light intensity* and *daily illumination time*.

Similar to CO_2, *sodium hydrogen carbonate* forms carbonate ion in the water which is consumed by the algae.

Response surface methodology (RSM) coupled with central composite design was employed to investigate the effect of these parameters on biomass and lipid production that were considered as two responses. RSM is a combination of mathematical and statistical methods suitable for the analysis and modeling of problems in which a response of interest is influenced by several variables where optimization of this response is the objective.

In this Study, *sodium hydrogen carbonate* was used because of unavailability of reliable CO_2 source. Concentration range of $NaHCO_3$ was based on information in the literature and our preliminary experiments. Solar illumination intensity at the earth surface during a day time is between 2000 and 9000 lx.

Table 2
Level of parameters affecting on *C. vulgaris* cultivation.

Factors	Factor amount +1	0	−1
Light intensity (lx)	10,000	6000	2000
Daily illumination time (h)	24	8	12
Sodium hydrogen carbonate (g/L)	2	1	0