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ORIGINAL ARTICLE

Studies on reduction of inorganic pollutants from wastewater by *Chlorella pyrenoidosa* and *Scenedesmus abundans*

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KEYWORDS

Chlorella pyrenoidosa;
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Abstract The aim of this study was to identify the potential for cultivation of *Chlorella pyrenoidosa* and *Scenedesmus abundans* in raw and autoclaved domestic wastewater (sewage) for nutrient removal, in a batch process. The growth was observed by measuring chlorophyll content. The inoculum size of 10% and 20% was used and the growth of microalgae and nutrient removal was monitored on daily basis. The maximum removal of ammonium nitrogen, phosphate and nitrates by *Chlorella pyrenoidosa* in raw samples was observed as 99%, 96% and 80%, respectively, whereas the maximum removal of ammonium nitrogen, phosphate and nitrates by *Scenedesmus abundans* in raw samples was observed as 98%, 95% and 83%, respectively. The maximum chlorophyll content was observed as 11.33 mg/l and 7.23 mg/l for *C. pyrenoidosa* and *S. abundans,* respectively, in raw samples. The experimental results reveal that both the microalgae are capable to grow and remove the nutrients from domestic wastewater.

1. Introduction

An alternative source for producing biofuel in present scenario helps in reducing environmental impact across the globe. Facilitating photosynthetic process of harnessing CO_2 for its growth and storing as energy source for biofuel production, compared to other microbes such as bacteria and fungi, establish algae as sustainable products for biofuel production [1]. Also, bio-energy production based on photosynthesis requires less water for cultivation of microalgae compared to other sources [2–4]. However, cultivating microalgae in freshwater for biodiesel production at large scale is still a challenging part as there is an increase in demand for freshwater for various purposes. The ideology of cultivating microalgae in wastewater is to reduce the growth media components for cultivation and also it helps in cleaning up (i.e., nutrient removal) the wastewater [5].

Although several other photosynthetic groups exist such as phototrophic bacteria, cyanobacteria, and diatoms, green microalgae are importantly used for cultivation in various wastewater sources. Microalgae also utilize inorganic nutrients such as Chlorides and sulfates that are needed to be removed from wastewater. Nitrogen and phosphates are important

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compounds that play a major role for algal growth in wastewater systems. Nitrates, Nitrites and Ammonium are the important nitrogen sources for microalgae in wastewater. Phosphates are also another major nutrient that is adequately present and plays a major role as intermediates in carbohydrate metabolic activity [6]. The main challenge in cultivating microalgae in raw wastewater is the presence of other microbes that affect the biomass productivity [7,8]. The wastewater produced from urban areas consists of major eutrophic compounds that affect other water bodies when discharged without meeting its desirable limits [9].

Present scenario using biological treatment has a setback in treating wastewater as the bacteria require fixed N:P ratio [10]. The effluent from the secondary treatment unit of domestic wastewater treatment plant can be used for large scale cultivation of microalgae for biofuel production [11,12]. Chlorophyll *a* is the photosynthetic pigment that is presented only in the green microalgae and contains four nitrogen molecules for each compound. Usually, all microalgae are photoautotrophs that absorb light and convert into Adenosine triphosphate (ATP), and Nicotinamide adenine dihydrogen phosphate (NADPH) to carry out photosynthesis [13]. In general, microalgae convert inorganic nitrogen into organic nitrogen and assimilate as chloroplastic nitrogen [6,14]. Reducing phosphate to lowest level in wastewater treatment plants is important to reduce chances of water pollution [15]. Green algae are prominently used for domestic wastewater treatment [5,8,12,16–20]. Although previously several studies have been conducted on microalgae for wastewater treatment, not many studies have been carried out on *Chlorella pyrenoidosa* and *Scenedesmus abundans* with emphasis on ability for inorganic pollutant reduction in raw and autoclaved sample with respect to inoculum sizes. Within this view an attempt has been made to study the effectiveness of *C. pyrenoidosa* and *S. abundans* for removal of pollutants in domestic wastewater (raw and autoclaved) by conducting batch studies and monitoring on daily basis.

2. Materials and methods

2.1. Culture preparation

C. pyrenoidosa (NCIM 2789) and *S. abundans (NCIM 2897)* were collected from National Collection of Industrial Microorganisms (NCIM), Pune, India. The cultures were separately grown in 250 ml Erlenmeyer flasks containing 100 ml of BG 11 medium [21].

2.2. Sample collection and its characteristics

The wastewater sample was collected from the aeration tank of sewage treatment plant in a pre-cleaned, sterilized polyethylene bottles. The collected samples were labeled, taken to the laboratory and stored in a laboratory refrigerator at 4 °C. All the chemicals used in the study are purchased from Thomas baker Ltd. (Mumbai, India) and the reagents were prepared using double distilled water. The analysis of various characteristics of the sample has been carried out as per standard procedure prescribed by American public health association [22] and the results are presented in Table 1.

Table 1 Characteristics of sewage wastewater sample.

Parameters[a]	Values
Turbidity (NTU)	379
pH	7.7
Alkalinity	365
Biological Oxygen Demand	236
Total Hardness	19
Chemical Oxygen Demand	286
Sulfates	32
Total solids	3500
Ammoniacal nitrogen	992
Nitrates	197
Phosphates	286
Chlorides	268

[a] All parameters (except pH and turbidity) are in mg/l.

2.3. Experimental studies

Batch scale experiments were conducted on daily basis to understand the ability of microalgal cultures for reduction of inorganic pollutants. The main parameters such as Ammoniacal Nitrogen (NH_3-N), Phosphates (PO_4^{3-}), Nitrate (NO_3-N), and Chloride (Cl^-) were considered for nutrient removal study and measure of Chlorophyll *a* for growth measurement. All the experiments were conducted in 500 ml conical flasks containing 300 ml of the wastewater sample under continuous illumination of 1800 lux measured using TES Lux meter (TES CORP).

2.3.1. Chlorophyll estimation

The growth of microalgae was monitored on daily basis by determining its chlorophyll content. The chlorophyll content of microalgae is determined by methanol extraction followed by spectrophotometric analysis [23]. 3 ml algae culture was centrifuged at 10,000 rpm for 10 min. The supernatant was drained off and the sample was re-suspended in ethanol/diethyl ether and kept boiling for 5 min. After boiling, the sample was made up to 5 ml with ethanol/diethyl ether. The optical density was measured at 660 nm and 642.5 nm with solvent as a blank. The chlorophyll content was determined using the following formula:

$$\text{Chlorophyll (mg/l)} = (9.9 * OD_{660}) - (0.77 * OD_{642.5})$$

3. Results

The chlorophyll profile of *C. pyrenoidosa* and *S. abundans* with different inoculum size is presented in Fig. 1. It can be noted from Fig. 1(a) that the maximum chlorophyll content in raw sewage sample for *C. pyrenoidosa* with inoculum concentration of 10% and 20% was observed as 11.4 mg/l and 7.73 mg/l in 4th day and 7th day, respectively. Similarly, from Fig. 1(b), it can be noted that the maximum chlorophyll content in raw sewage sample for *S. abundans* with inoculum concentration of 10% and 20% was observed as 7.23 mg/l and 7.12 mg/l in 6th day and 7th day, respectively.

3.1. Nutrient removal from sewage

3.1.1. Nitrogen removal

Nitrogen is one of the important compounds that are mainly present in wastewater that exists in different forms viz., nitrite,