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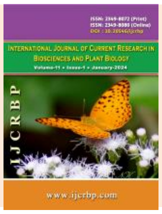

Original Research Article

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Extracellular polymeric substance extraction from *Nannochloropsis* species in treated wastewater

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Abstract

Microalgae have emerged as a desirable source for biofuel production because of high biomass and lipid productivity from waste water sources. An efficient method for treating waste water, producing bioenergy and byproducts on a sustainable basis is to cultivate microalgae in waste water. The cultivation of Extracellular Polymeric Substance (EPS) production has getting more attention as a result of high hydrocarbon biosynthesis. In this context EPS were produced from Nannochloropsis species. To produce the most biomass and lipid production the microalgae Nannochloropsis species was grown under stress conditions. Further both lipid and biomass productivity was also recorded. The biomass characteristics are carbohydrates, proteins and lipids were determined by phenol sulphuric acid method, Bradford method and Bligh and dyer method.

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Introduction

The waste water is an excellent medium for algal growth by providing sufficient nutrients. Wastewater can stimulate a rapid growth rate, and it has a higher lipid productivity. Microalgae have the scope of large scale mass cultivation. It has the potential to be a source of biofuel (Wang et al., 2019). The use of wastewater in algal cultivation has a role in the reduction of wastewater pollution load and utilization of the microalgae for biomass and energy production (Aravantinou et al., 2013). However, the integration of photosynthetic organisms into wastewater treatment processes offers a dual benefit: the efficient removal of pollutants and the conversion of CO_2 into biomass (Maheshwari et al., 2020). Microalgae can effectively remove various pollutants such as organic matter, nutrients, heavy metals, and even emerging contaminants like pharmaceuticals and personal care products (Wu et al., 2012). By harnessing the inherent capabilities of microalgae and the nutrient-rich environment provided by treated wastewater, this approach holds promise for sustainable biofuel production, CO_2 sequestration, and wastewater treatment (Maheshwari et al., 2020). Optimizing microalgae growth is essential for maximizing biomass and lipid production, particularly in species like *Nannochloropsis* used for biofuel application (Peng et al., 2020). Overall, the unique characteristics of *Nannochloropsis* species, such as tolerance to high CO_2 levels, high temperature tolerance, and efficient lipid production, highlight their potential for sustainable biofuel production and CO_2 sequestration applications (Peng et al., 2020). This lipid-rich composition, coupled with their rapid growth rate, makes *Nannochloropsis* an attractive option for renewable energy sources (Paramasivam et al., 2021). Similarly, many studies focused on the EPS production and characterization of algae. Organic and inorganic substituents usually exist in EPS, affecting its biological properties. Identifying microbial strains with high EPS yield and flocculation activity is crucial for cost-effective and industrial applications (Nouha, 2016).

Additionally, the passage highlights the importance of quantifying EPS components, such as polysaccharides and proteins, using established colorimetric and spectroscopic methods (Gong et al., 2009). Therefore, understanding the lipid content and production potential of microalgae, particularly in the context of wastewater treatment, is crucial for advancing sustainable solutions for energy production and environmental remediation (Kumar Singh et al., 2021). The lipid from microalgae could be used in different processes from energy exploitation (Converti et al., 2009).

The quantification and analysis of proteins are important in biological research, biotechnology, and various fields of science and medicine (Slocombe et al., 2013). The aim of the project is to extract extracellular polymeric substances from *Nannochloropsis* species in sewage wastewater.

Materials and methods

Sample collection

The samples were sewage treated wastewater and reverse osmosis water. RO water is considered as control. Sewage treated wastewater were collected from Sewage treatment plant, Karpaga Vinayaga College Of Engineering Technology, Chengalpattu, Tamil Nadu, India.

Analysis of sewage wastewater

The parameters like pH, total dissolved solids (TDS), total suspended solids (TSS), electrical conductivity (EC), salinity, turbidity, chloride, alkalinity, hardness, biological oxygen demand (BOD) and chemical oxygen demand (COD).

Total dissolved solids

The total dissolved solids in sewage treated wastewater and Reverse osmosis water were measured by using TDS meter.

Total suspended solids

Tem milliliter of sample was filtered using in Whatman filter paper and kept in the oven at 105°C for 1 h. After drying and cooling, the weight of the sample was measured. Whatman filter paper was weighed before the sample was poured.

$$\text{TSS} = \text{mg of solid paper/volume of sample} \times 1000$$

Where, mg of solid paper = Before weight - After weight of Whatman filter paper

Electrical conductivity

Minerals, chemicals and other dissolved materials were estimated in water using an electrical conductivity meter.

Salinity

It was measured by using Handheld Refractometer. To get an accurate reading, the refractometer was calibrated. The plate was then exposed to the prism. The samples were pipetted out couple of drops of the samples being tested onto the prism was added. The plate was gently closed observed through the round end of the meter to read the salinity level.

Turbidity

Turbidity was measured by using Turbidometer after calibration. The samples were poured to a clean transparent cuvette and placed into the turbidometer chamber, ensuring it was properly aligned. Then the chamber was closed securely to avoid any light leakage. The light emitted through the sample was recorded to observe turbidity level of the samples, usually in NTU (Nephelometric Turbidity Units).

Chloride

The sample of 25 ml was added with a pinch of potassium chromate indicator followed by the addition of 0.1N of silver nitrate to the burrette. It turns yellow into brownish colour. It can be calculated by using formula:

Chloride=Volume of silver nitrate × Normality of silver nitrate × 33.5 ×1000/Volume of sample.

Alkalinity

Take 20 ml of sample in conical flask, add 3 drops of methyl orange indicator. Add 0.02N of sulphuric acid to the burette. It turns yellow to orange color. It can be calculated using this formula:

Alkalinity = Volume of sulphuric acid ×1000/Volume of sample

Hardness

Take 20 ml of sample, add 1 ml of ammonium buffer. To this, add 1 pinch of EBT indicator. In burette take 0.1N OF EDTA. It turns wine red to blue color. It can be calculated by: Hardness=Volume of EDTA× Normality of EDTA×1000×100/ Volume of sample

Biological oxygen demand

Take 4 BOD bottles (2 blank and 2 samples).Add 300 ml of distilled water in 2 blank. Add 10 ml of sample +290 ml of distilled water in 2 sample bod bottles. Add 2 ml of manganese sulphate to another two bottles without forming bubbles. To that add 2 ml of alkali-iodide azide reagent. Precipitation will occur (brownish color). Add 2 ml of conc. sulphuric acid and close the bottle and mix it well to dissolve the precipitate.1 blank and 1 sample bottle are kept in an incubator at 20℃ for 5 days.Take 0.025N of sodium thiosulphate in burette .Add 203 ml of sample water in conical flask and add 2 ml of starch solution and then titrate until blue colour to colourless. It can be calculated by using formula:

$$BOD=(D_1-D_2)-(B_1-B_2)f/P$$

Where,

D_1: Dissolved oxygen of diluted sample immediately after preparation, mg/l
D_2: Dissolved oxygen of diluted sample immediately after 5 days incubation at 20℃, mg/l
B_1: Dissolved oxygen of blank before incubation, mg/l
B_2: Dissolved oxygen of blank after incubation, mg/l
f: Fraction of diluted water volume in a sample to volume of dilution water in blank.
p: Fraction of sample taken to total combined volume.

Chemical oxygen demand

Take 10 ml of sample into a round bottom flask. Add some glass beads, to that add 1 ml of mercury sulphate and mix it by swirling the flask. Add 5 ml of potassium dichromate solution. Slowly add 15ml silver sulphate. Connect with reflux condenser using a hot plate for 2h.After that cool the flask and rinses the condenser with 25 ml of distilled water collecting in same flask. Add 2 to 3 drops of ferroin indicator to the flask and titrate with ferrous ammonium sulphate solution. Ma ke the blank preparation in the same manner as sample as distilled water instead of a sample. It can be calculated as:

$$COD=8*1000*M(VB-VS)/Volume\ of\ sample$$

Where,

VB: ml FAS used for blank
VS: ml FAS used for sample
M: Molarity of FAS
8000: Milliequivalent weight of oxygen

Cultivation of microalgae

Preparation of medium

Microalgae were cultured in BG 11 medium consisting the composition shown in Table 1.

Table 1. Composition of BG-11 medium.

Substance	Volume
$NaNO_3$	1.5 g/l
K_2HPO_4	0.04 g/l
$MgSO_4$	0.075 g/l
$CaCl_2.2H_2O$	0.036 g/l
Citric acid	0.006 g/l
Ferric Ammonium Citrate	0.006 g/l
EDTA (Disodium salt)	0.001 g/l
Na_2CO_3	0.02 g/l

Cultivation of microalgae in different concentrations

The medium was dissolved in 1 litre of RO water. 20 ml of inoculum was inoculated in the culture. Each strain was grown in a 1L conical flask. All media were sterilized in an autoclave at 121°C for 20 minutes. The Cultures were grown in different concentrations.

Sample A: Sewage waste water is T1 (250 ml SWW + 750 ml RO), T2 (500 ml SWW+500 ml RO), T3 (750 ml SWW +250 ml RO), T4 (1 L SWW).

Sample B: RO is T1 (1 L)

Extraction of extracellular polymeric substances (EPS)

After the end of fermentation the media were centrifuged at 14,000 rpm for 10 minutes. Protein precipitation was carried out by adding (12% w/v) trichloroacetic acid (TCA) to the supernatant. And the solution was kept at 4°C for 24 hours. EPS were precipitated by adding (2 v/w) of cold ethanol to the supernatant, which was further kept at 4°C overnight. Then the solution was centrifuged at 14,000 rpm for 10 minutes.

The partially purified EPS dry powder was collected. The dry weight of pellets was measured by drying the precipitation at 60°C to a constant weight. For the determination of carbohydrates, lipids, and proteins of microalgae, the phenol-sulfuric acid method, the Bligh and Dyer method and the Bradford method, were carried out respectively.

Results and discussion

Growth of microalgae

The microalgae were grown in sewage treated wastewater and reverse osmosis water for 20 days.

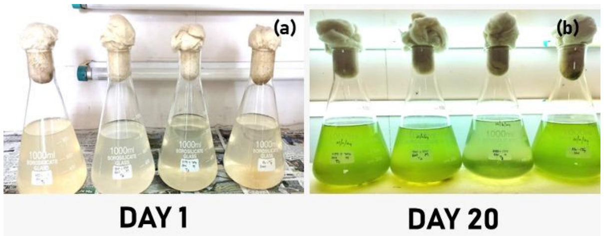

Fig. 1: Sewage treated wastewater

Analysis of water

The sewage wastewater and RO water were analyzed by using different parameters before the cultivation of microalgae.

Table 2. Analysis of sewage waste water.

Parameters	Sewage waste water
pH	6.35
Total Dissolved Solids	610 ppm
Total Suspended Solids	0.036
Electrical conductivity	0.451 mS/cm
Salinity	6.8%
Turbidity	3.8 NTU
Chloride	870 mg/l
Alkalinity	379 mg/l
Hardness	1000 mg/l
Biological Oxygen Demand	45.2 mg/l
Chemical Oxygen Demand	300 mg/l

Extraction of EPS

The amount of EPS produced by microalgae during the cultivation is shown in Table 3. In sewage treated wastewater T_1 has a high EPS content compared to all others.

Table 3. Amount of EPS extracted from microalgae.

Water samples	Concentration	EPS (mg/l)
Sewage wastewater	T1	0.25
	T2	0.17
	T3	0.14
	T4	0.11
RO	T1	0.24

Biomass characterization

The results of microalgal biomass characterization are shown in Table 4. Carbohydrate, protein, and lipid content of Nannochloropsis species were calculated.

Table 4. Microalgal biomass characterization.

Samples	Sewage waste water				RO
Concentration	T1	T2	T3	T4	T1
Carbohydrates (%)	10.1	6.1	`3.8	6.2	11.1
Proteins (%)	12.1	10	11.1	12.3	13.1
Lipids (mg/l)	0.5	0.2	0.3	0.4	0.5

From wastewater, the cultivation of microalgae leads to an efficient process that is used for industrial purposes and also for other applications. In this study, the growth of microalgae was observed for 20 days with continuous air supply through a motor and continuous illumination. After 20 days, the EPS can be extracted from all the concentrations.

The result of EPS from T_1 was higher compared to the other which is 0.24 mg/l. The biomass characterization of carbohydrate, protein, and lipid are also estimated. Carbohydrate content was found to be 3-11%, protein content found to be 10-12% and lipids content found to be 0.2-0.5 mg/l.

Conclusions

Microalgae are essential living things that collect metabolites from both inside and outside of cells. In this sense, important metabolites like EPS produced from micro-algae would be beneficial in a range of applications and provide a large economic contribution to the respective nations as commercial items. As a result of this study, microalgae from Nannochloropsis species have higher biomass productivity and lipid content. The growth of algae occurs under continuous illumination. Using wastewater as a medium for the growth of microalgae, it accumulates EPS. Since microalgae are suitable for microorganisms for EPS biosynthesis, it yields a greater amount and it is a low cost, and efficient process. Further results from EPS can be converted into biofuels and many other applications.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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