Integrated growth potential of *Chlorella pyrenoidosa* using hostel mess wastewater and its biochemical analysis  
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ABSTRACT

Wastewater from hostel mess endorses the potential for the sustainable growth of microalgae culture, as it contains necessary nutrients at high concentration. In this paper, the potential growth of microalgae species *Chlorella pyrenoidosa* has been studied using the wastewater generated from Ilango Adigal hostel mess at Pondicherry University. The experiments were conducted to analyze the physico-chemical parameter of hostel mess wastewater, and its utilization at different concentration for microalgae cultivation. The results showed the effective growth and biomass production of microalgae species *Chlorella pyrenoidosa* when the growth media contains at higher than 50% concentration of wastewater. At 100% hostel mess wastewater concentration, the growth reached 1.369 g/L dry wt. biomass on the 30th day growth cycle. The biochemical estimation of biomass was around 26% carbohydrate, 39% protein, 8% lipid, 13% chlorophyll pigment, ash 4%, and remaining moisture. The calorific value of the dried biomass was estimated ~ 19,400 KJ/g. The study indicates that the hostel mess wastewater has sufficient nutrient for the sustainable growth of *Chlorella pyrenoidosa* for potential biomass resource for production of biofuels and other valuable products.

Keywords:  Wastewater, Biomass, Biochemical components, Microalgae, *Chlorella pyrenoidosa*.

1. Introduction

Environment friendly and sustainable clean energy development are the prime concern. Globally, water and wastewater management has given top priority. Phytoremediation is an economically viable and sustainable approach for the wastewater treatment as well as biomass production by using microalgae. It has been reported that the various source of wastewater containing organic and inorganic supplements for microalgae growth endorses a cleaner and cheaper technology (Bhatt *et al.*, 2014). Microalgae draw high research attention due to testament of its compelling potential for food, cosmetics, antioxidants, fatty acids, enzymes, polymers, pigments or bioactive compounds industrial chemicals and pharmaceuticals as well as in the field of biofuel production (Hidalgo *et al.*, 2013, Mata *et al.*, 2010). Microalgae comprise a vast group of single-celled photoautotrophic organism with ability to grow and survive in diverse environmental conditions. It performs photochemical reaction in the presence of solar energy and nutrients, way for CO2 sequestration from environment and production of cellular metabolic products, during this process oxygen is released. The micro algal cultivation for sustainable fuel production has gained more interest due to its rapid growth rate and non-polar triacylglycerol synthesis- which are the major substrate to produce biodiesel (Sharma *et al.*, 2011). For sustainable bioenergy and biofuels, researchers are pointing towards the utilization of animal waste/residue like fish waste, and slaughter house waste for bioenergy *e.g.* biogas, biodiesel purpose but microalgae biomass
shows more creditable suitability comparatively (Jaiswal et al., 2014). Microalgae biomass progression for sustainable food and fuels production includes no competition for land with crops in view of ability to grow in marine as well as wastewater, without additional demand of freshwater. It is a unique opportunity to treat wastewater by bioremediation and providing nutrients to microalgae for biomass production using nutrient-rich wastewater effluents.

Coupling of wastewater treatment with microalgae cultivation offers an economical and environment friendly implementation for sustainable biomass production. Wastewater-based cultivation system save enormous amount of freshwater and required nitrogen, phosphorus nutrients for microalgae growth. There is a great potential for exploitation of various wastewater sources like domestic, industrial, municipal and agricultural sources for harnessing of algal diversity for biofuel feedstock (Zhou et al., 2014, Mostafa et al., 2012). In terms of economic viability and sustainability of microalgae biomass production, integration of microalgae mediated wastewater treatment has been introduced by many researchers (Raouf et al., 2012). Wastewater production from institutional kitchen/mess and other commercial food service activities are unavoidable on daily basis. The higher content of organic pollutants in hostel mess wastewater comes from the washing of food items, meat, vegetables, dishes and kitchenware. The quantity and quality of wastewater from institutional kitchens are influenced by cooking of food varieties and its leftover washings. Kitchen waste contains a significant amount of organic matter which traditionally ends up in wastewater (Baskar et al., 2009, Huelgas et al., 2009). Institutional kitchens play daily life role in mainly educational and professional organizations which generates large quantity of liquid organic waste and polluting water bodies’ receivers. The integration of hostel-mess wastewater with microalgae cultivation is an efficient means of nutrients utilization for phytoremediation and biomass production. Microalgae biomass can be used for varieties of purpose for food as well as bio-fuel sources. The unicellular green microalga Chlorella spp. reported for utilizing various types of wastewater for treatment and biomass production. The present study aimed to evaluate the growth potential of Chlorella pyrenoidosa at various concentrations of hostel mess wastewater (HMW) and analysis of various biochemical profile of biomass for carbon-neutral economical and sustainable technology to commercialize in future.

2. Material and methods

2.1 Microalgae and culture conditions

The freshwater unicellular green microalgal strain Chlorella pyrenoidosa was obtained from Department of Environmental Sciences, B.B.A. University, Lucknow. The inoculated medium was incubated under florescent illumination (white light, intensity 2000 lux) with photoperiod of 12 h/12 h light-dark cycles in air condition room at 25±2°C temperature for 30 days. The culture was grown in 1000 ml Erlenmeyer flasks with 500 mL medium (BG-11 media) and hand shaken 2-3 times in a day to prevent settling. The culture media and glassware were sterilized prior to inoculation with log phase of fresh microalgae cells.

2.2 Characterization of Hostel-Mess Wastewater (HMW)

The influents (untreated wastewater) of kitchen were collected from mess of Ilango Adigal Hostel, Pondicherry University, Puducherry. This hostel-mess produces significant amount of wastewater- which generated due to the cooking and washing activities of food items and the waste food leftovers in utensils. The influents were collected in sterilized sampling bottles and stored at 4°C before use. Physico-chemical parameters of the initial water sample of HMW were analyzed in triplicate by following the Standard Methods of APHA (21st edition).
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2.3 Optimization of microalgae growth on HMW and biomass cultivation

The microalgae C. pyrenoidosa were grown at various concentrations of influent of HMW (0%, 25%, 50%, 75% and 100%) in distilled water in appropriate combination of Standard BG-11 medium. The homogenous microalgal suspension with optical density ~ 1.525 was inoculated at 20% (V_inoculation/V_medium) in each 1000 ml of Erlenmeyer flasks containing 400 ml of varying concentrations of influents and standard media. The microalgae growth was observed in terms of optical density at 686 nm on each day by using UV-VIS Spectrophotometer. The experiment was carried out in triplicate and average values were recorded. After the growth, biomass were harvested by centrifugation and dried at 60°C in oven for biochemical analysis.

2.4 Biochemical characterization

The C. pyrenoidosa biomass was harvested by centrifugation at 5000 rpm, 4°C for 10 min after 30 days of cultivation cycle, i.e. at the end of stationary phase. The biomass pellet was dried at 60°C in a hot air oven until obtaining constant weight. The dried biomass of microalgae was further grind with the help of mortar and pestle into powdered form for its biochemical estimation and calorific analysis.

2.4.1 Estimation of carbohydrates

The intracellular and extracellular carbohydrate of dried biomass was estimated by Phenol-Sulphuric acid method. The analysis was based on complex sugars and derivatives form yellow-brown complexes with phenol and concentrated sulfuric acid that absorption maximum at 490 nm.

2.4.2 Estimation of protein

The crude protein was determined by Lowry protein assay for total protein concentration in solution which exhibited by a color change of sample solution in proportion to protein concentration and measured by colorimetric techniques (Lowry et al., 1951).

2.4.3 Estimation of lipids

Lipid extraction from dried and powdered biomass of C. pyrenoidosa was carried out by using solvents methanol: chloroform (2:1, v/v) (Bligh and Dyer, 1959). The mixer was kept for 24 hours at room temperature for exposure of lipids to solvents system properly. Then, the mixer was vortex and centrifuged for 10 min at 3000 rpm by three repetitions with centrifuged pellet. Supernatants layer were collected to stand for 2-3 hours. Lower supernatant layer containing lipids were pipette out for evaporation of solvent at 80°C in hot air oven and lipid content was calculated in % dry cell weight.

2.4.4 Estimation of chlorophyll pigment

Freshly harvested biomass was homogenized in 90% (v/v) acetone for chlorophyll content. The sample container was covered with aluminum foil to ensure the absence of light and stored in dark for 24 hours at 4 °C. Then, extract was centrifuged at 3000 rpm for 10 min and the clear supernatant was taken for pigment determination by spectrophotometry (Strickland et al., 1972).

2.4.5 Estimation of dry cell weight
The known volume of microalgal culture was centrifuged at 5000 rpm for 10 min and washed three times with distilled water. The biomass pellets were collected and dried in hot air oven at 80°C until dried completely. Dried biomass calculated by gravimetric in g/L dry cell weight.

2.4.6 Estimation of moisture content

Moisture content of microalgal biomass was determined by complete drying of sample in hot air oven at 105 ±5°C until obtaining constant weight as per AOAC (2000) method.

2.4.7 Estimation of ash contents

Ash content of microalgal biomass i.e. inorganic residue or mineral content was determined after the removal of water and organic matter by heating at 575°C in furnace by standard method of AOAC (2000).

2.4.8 Estimation of calorific value

The completely dried and powdered biomass of *C. pyrenoidosa* was used for analysis of calorific value by the instrument IKA5000 bomb calorimeter. Standard benzoic acid tablets were used for standardization of instrument. All the calculation was made by system inbuilt Calvin-software.

3. Results and discussion

Microalgae is an important organism to utilize nutrients mainly nitrogen and phosphorous from wastewater generated from various sources. The microscopic image of green unicellular microalgae *C. pyrenoidosa* has been taken by Petrological Microscope (CENSICO, Model – 082933) and it appears spherical to oval in shape (Figure 1).

![Microscopic image of Chlorella pyrenoidosa](image)

Figure 1: Microscopic image of *Chlorella pyrenoidosa*

The analysis of HMW generated on daily basis shows the presence of 3.47 mg/l of total nitrogen, 1.40 mg/l of total phosphorus and also the presence of K, Cu, Fe and Zn in the amount of 7.3 mg/l, 0.027 mg/l, 0.6 mg/l and 1.146 mg/l respectively as shown in Table 1. The presence of efficient nutrients in hostel mess wastewater and previous studies supports the growth of *C. pyrenoidosa* biomass. Bhaskar *et al.*, 2009 investigated the feasibility of institutional kitchen wastewater treatment by using plant *Phragmites australis* on a constructed wetland.
**Table 1:** Physico-chemical characterization of hostel-mess wastewater

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Unit</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH</td>
<td>---</td>
<td>7.8</td>
</tr>
<tr>
<td>2.</td>
<td>Electrical conductivity (EC)</td>
<td>ds/m</td>
<td>0.63</td>
</tr>
<tr>
<td>3.</td>
<td>Total suspended solids (TSS)</td>
<td>mg/l</td>
<td>55</td>
</tr>
<tr>
<td>4.</td>
<td>Total dissolved solids (TDS)</td>
<td>mg/l</td>
<td>848</td>
</tr>
<tr>
<td>5.</td>
<td>Biological oxygen demand (BOD)</td>
<td>mg/l</td>
<td>65</td>
</tr>
<tr>
<td>6.</td>
<td>Chemical oxygen demand (COD)</td>
<td>mg/l</td>
<td>433</td>
</tr>
<tr>
<td>7.</td>
<td>Total nitrogen (TN)</td>
<td>mg/l</td>
<td>3.47</td>
</tr>
<tr>
<td>8.</td>
<td>Total phosphorus (TP)</td>
<td>mg/l</td>
<td>1.40</td>
</tr>
<tr>
<td>9.</td>
<td>Potassium (K)</td>
<td>mg/l</td>
<td>7.30</td>
</tr>
<tr>
<td>10.</td>
<td>Copper (Cu)</td>
<td>mg/l</td>
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</tr>
<tr>
<td>11.</td>
<td>Iron (Fe)</td>
<td>mg/l</td>
<td>0.6</td>
</tr>
<tr>
<td>12.</td>
<td>Zinc (Zn)</td>
<td>mg/l</td>
<td>1.146</td>
</tr>
</tbody>
</table>

The experimental results of *C. pyrenoidosa* growth on 25%, 50%, 75% and 100% concentration of HMW with blank and standard BG-11 media clearly indicates efficient growth on above 50% HMW concentration. BG-11 media (standard media) used as control to compare the growth of microalgae with HMW and growth in 100% HMW approximately equal with BG-11 media. The blank (no nutrient) tested for the selected species show ineffective growth as shown in Graph 1.

**Graph 1:** Growth of *C. pyrenoidosa* at different concentration of wastewater and BG-11 media.

The microalgae harvested on 30th day of its full cyclic growth. The dry weight biomass yield at blank, standard BG-11 media, 25%, 50%, 75% and 100% (V/V) are 0.209 g/L, 1.397 g/L, 0.299 g/L, 0.539 g/L, 0.933 g/L and 1.336 g/L respectively. In these results, microalgae dry weight biomass production on 100% of HMW is approximately equal to the biomass yield on standard BG-11 media. It also indicates that more than 50% concentration of HMW shows effective biomass yield compared to 100% as depicted in Graph 2. The experimental study conducted by Sharma et al., 2014 demonstrated that BG-11 was found most suitable growth media for *C. pyrenoidosa* compare to Fogg’s media, BBM and Bristol. The calculated biomass yield on dry cell weight basis was 0.266 mg/l in BG-11 medium. The microalgae
growth showed that the hostel-mess wastewater can be used directly for biomass production without treatment and could be sources for growth of microalgae as well as wastewater treatment.

![Graph 2: Dry biomass yield of C. pyrenoidosa at 30th day.](image)

**Table 2: Biochemical analysis of C. pyrenoidosa biomass**

| Sr. No. | Biochemical composition | Obtained value  
|---------|------------------------|----------------|
| 1.      | Dry cell weight        | 20.40 ±0.12%  
| 2.      | Carbohydrate           | 26.01 ±0.58%  
| 3.      | Protein                | 38.67 ±0.17%  
| 4.      | Lipids                 | 08.02 ±0.37%  
| 5.      | Chlorophyll            | 13.34 ±0.29%  
| 6.      | Moisture               | 84.39 ±1.51%  
| 7.      | Ash content            | 03.85 ±0.38%  
| 8.      | Calorific value        | 19.426 ±0.598 KJ/g|

The biochemical composition of *C. pyrenoidosa* biomass showed high amount of protein compare to carbohydrate as shown in Table 2. The observed maximum amount of carbohydrate and protein in the 7th week of cultivation was around ~ 16% and 46% respectively and decrease at the end of 8th week and agrees with the reported literature (Verma *et al.*, 2015). In a previous study (Sharma *et al.*, 2014), the biochemical compositions of biomass was estimated as 26% carbohydrate, 50% protein and 11% lipids in BG-11 medium cultivation. The above studies and related investigations clarify the wastewater generated from food waste has enough sufficient nutrient for the growth of microalgae *C. pyrenoidosa*.

4. Conclusion

The integration of microalgae cultivation with hostel-mess wastewater proved the efficient biomass yield for the cultivation of *C. pyrenoidosa* strain. The biochemical composition of *C. pyrenoidosa* biomass was analyzed for qualitative and quantitative components. The presence of rich quantity of carbohydrate, protein and lipids in the algal biomass prove its potential.
feedstock for food and possible bio-fuel purpose. The study clearly supports the utilization of wastewater for algal growth as it serves two purposes; wastewater treatment to maintain a cleaner environment as well as generation of valuable biomass from algae.

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5. References


