ABSTRACT

The use of fossil fuels is unsustainable due to limited supply and also due to large emissions of Carbon dioxide due to the effect of global warming. Biofuel is a viable option but can, as produced today, only provide a limited amount of fuels needed. Biofuels are presently derived from terrestrial plants, which require large amounts of arable land. Biofuels from microalgae on the other hand do not necessarily require arable land and can theoretically replace fossil fuels absolutely. Biofuels from microalgae could use industry waste water as growth medium particularly paper industry waste water is an interesting potential provider due to its high nitrogen and phosphorus in waste water. In this research work marine microalgae Nannochloropsis Salina was cultivated using f/2 medium using modified air lift photo-bioreactor along with the paper industry effluent waste water. The doubling time calculated from optical density attained at 48 hrs the cell count almost doubled during this period. Since the marine species is sensitive to pH we need to maintain the pH at 7 below 7 indicated the decreased biomass levels in culture. The lipid extraction was studied using solvent methods. The functional compounds in lipids FAME were studied using GC-MS analysis, the Nannochloropsis salina showed qualities of growing in fresh water and brackish water apart from the marine water which is a desirable characteristic for algal phycoremediation.

Key words: f/2 medium, Nannochloropsis salina, GC-MS, microalgae, FAME.
1. INTRODUCTION

The exploitation of fossil fuels is unsustainable due to the insufficient availability and the consequent widespread green-house gas emissions Chisti (2008); Ekendahl, et al. (2010). The production of renewable biofuels such as ethanol and biodiesel are today chiefly produced from terrestrial plants such as sugar cane, rapeseed, and palm that require enormous amounts of arable land John, et al. (2011); Chisti (2008); Griffiths and Harrison (2009); Ma and Hanna (1999); Xu, Miao and Wu (2006). The use of arable land for the manufacturing of biofuels is contentious since this competes with food production Chisti (2008); Griffiths and Harrison (2009).

Microalgae on the other hand, can be cultivated without the need of arable land and are also in general much more efficient than the terrestrial plants in capturing solar energy Malcata (2011). According to Li et al (2008a) switchgrass, which is the best growing terrestrial plant, can acclimatize less than 0.5% of the total solar energy received in a characteristic midlatitude location whereas microalgae may adapt up to 10% Li, et al. (2008a). The doubling time and water requirements are also factors that significantly favour microalgae. The distinctive doubling time for algae is approximately 24 hours Chisti (2008); Malcata (2011). According to Kliphuis et al (2010) microalgae requires around 1.5 liters of water to produce 1 liter of biodiesel compared to 10 000 liters for land crops Kliphuis, et al. (2010). Chisti (2008) has approximated that the USA would need to use over 60% of its agricultural cropping land to be able to cover its need for biodiesel using palm. This can be compared to approximately 3% for a microalga with 30% oil content (Chisti 2008).

Microalgae requires huge amounts of CO₂, it takes approximately 1.8 ton CO₂ to generate 1 ton of microalgal biomass Chisti (2008). To be able to attain the high cell densities that are required to make algal cultivation profitable CO₂ needs to be supplied in concentrations higher than in the air. To supplementary improve the prospects of microalgae cultivations industrial flue gas and waste water could be used. Several types of waste waters include phosphorous, nitrogen and trace metals which are essential for microalgal development, and the exploit of this would diminish mutually costs and eutrophication effects Chisti (2008); Kim (2013); Huang, et al. (2010); Kong (2009); Patil, et al. (2008); Gurumoorthy (2016,). With soaring oil prices and increased environmental awareness microalgae seems very promising. The thriving application of algal biomass for biofuel manufacturing will basically depend on the development and harvesting of algal biomass at cheaper costs. The current study is an effort to decrease the waste water pollution and produce the algal biomass and biofuels from nutrient rich paper industry effluents.

*Nannochloropsis* is a genus with non-flagellate small cells, 2-4 μm in diameter Tomaselli (2007). Like the other known species of Eustigmatophyceae the cells are green cocoid that are either single, in pairs or in colonies Barsanti and Gualtieri (2005). *Nannochloropsis* has a cell wall of polysaccharides and do not accumulate starch Tomaselli (2007). It has received a great deal of interest as a source of polyunsaturated fatty acids, since it can accumulate large amounts of eicosapentaenoic acid. *Nannochloropsis* is commonly cultivated in fish hatcheries as feed for rotifers and to enhance growth in larvae tanks Tredici (2009). The optimum pH for growth for *N. salina* is 9, but it can grow in the range of pH 5-10.5 (SERI Microalgal Technology Research group 1986). The temperature range for growth lies between 17-32°C with optimum at around 28°C (SERI Microalgal Technology Research group 1986).

2. COLLECTION OF MICROALGAE AND PAPER INDUSTRY EFFLUENTS

Marine microalgae *Nannochloropsis salina* culture samples were delivered from Central marine fisheries research institute, Chennai, Tamilnadu. The paper industry waste effluents
where collected in and around Karur district, Tamil nadu. The industrial effluents were collected in aseptic bottles and transported to the laboratory

3. BIOMASS PRODUCTION USING SEMICONTINUOUS AIRLIFT PHOTOBIOREACTOR

The algal cultures were maintained in air conditioned room and 100 ml *Nannochloropsis salina* samples were inoculated in 1000 ml conical flask containing sea water was enriched with paper industry waste water effluents and f/2 medium and the seawater pH is maintained at 7 after inoculation, a compressor is attached to the flask the air from the compressor will be utilized for aeration and mixing process for light source 19v led bulb was fitted near the photo-bioreactor for lightening purpose in the night since the algae grows in dark for photosynthesis we fit the 19v led bulb so the algae can utilize the light source in night for biomass production Figure 1. The pH is maintained correctly to increase the biomass when there is decrease in pH leads to less biomass production. Periodically the algal growth was examined in terms of the increase in the optical density at 550 nm. The results showed algal growth increased and attained exponential phase in 72 hours and the f/2 medium was used sparingly since the waste effluents contain more nitrogen and phosphorous source which is suitable for algal biomass production. The results showed that the algal growth continued till the 30th day and then it started declining due to environmental conditions and decrease in pH levels.

![Figure 1 Airlift photo-bioreactor used for algal biomass production](image)

4. ANALYSIS OF ALGAL BIOMASS

Algal biomass was measured by taking the dry weight of algae at different intervals. From 100 ml of algal broth biomass was extracted at day 5, 10, 15, 20 and 25. The measured algal biomass of the entire sample is shown in Table1. These values are the mean of three determinations ± Standard deviation. There was a progressive increase in growth of the algal sample. By the end of 25 days *Nannochloropsis salina* showed high dry biomass i.e. 5.3±0.26 (g/100ml±SD). The dry biomass is further used for lipid extraction
The data obtained in Table 1 were statistically analyzed using Analysis of variance two way classification to determine whether there is significant difference between the species and there is significant effect of different times.

### Table 1 Algal biomass measurements

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>DAYS</th>
<th>DRY WEIGHT MEASUREMENTS (g/100ml±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nannochloropsis salina</em></td>
<td>5th</td>
<td>0.66±0.00</td>
</tr>
<tr>
<td></td>
<td>10th</td>
<td>1.45±0.30</td>
</tr>
<tr>
<td></td>
<td>15th</td>
<td>2.49±0.07</td>
</tr>
<tr>
<td></td>
<td>20th</td>
<td>4.50±0.20</td>
</tr>
<tr>
<td></td>
<td>25th</td>
<td>5.3±0.26</td>
</tr>
</tbody>
</table>

5. **ALGAL LIPID EXTRACTION**

To harvest the algal dry mass cultures were filtered using Whatman No. 41 filter paper and the biomass was dried in hot air oven for 24 hours at 80°C Bagchi et al., (2015). Two hundred grams of the dry algal mass Figure 2 was wrapped in Watman’s filter paper, sealed and kept in the middle chamber of the soxhlet extractor to run extraction. The round-bottomed flask of the soxhlet was filled with 200ml ethanol and the soxhlet was placed on a hot plate at a temperature of 70°C Redfern et al., (2014) and the Soxhlet apparatus was kept running for 36 hours for ten cycles to complete the lipid extraction process and the lipid content obtained from 200 grams of algal biomass is 38ml lipid content.

6. **TRANSESTERIFICATION**

Biodiesel molecules are mixture of fatty acids methyl esters (FAMEs) produced from transesterification reaction between triglycerides esters (vegetable oil or animal fat) and alcohol (methanol) in presence of alkalis that acts as a catalyst. In a transestrification process, each mole of triglyceride is converted into a mole of fatty acids methyl esters (FAMEs) using three moles of methanol. The algal lipids are with 2 grams of NaOH and 110 ml of methanol and the mixture was stirred continuously for 20 min on a magnetic stirrer and kept in an incubator for 58 hrs at 60 rpm to separate the biodiesel and sedimento layers the uppermost layer that is the biodiesel was separated carefully and was measured the algal biodiesel obtained from transestrification is 20ml. Biodiesel was washed using 5% water a number of times until a clear solution is obtained. Biodiesel was stored at room temperature for 12 hours.

**Figure 2** Dry algal biomass inside Soxhlet apparatus

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Final volume of biodiesel was measured and pH was checked Sander and Murthy, 2010. Use of methanol resulted in soap formation because the small methyl group surrounds the fatty acids, while the free fatty acids form long chains Stavarache et al., 2007.

7. GC-MS ANALYSIS OF FAME

GCMS was employed to study the Algal FAME using JEOL GC MATE II data system was used the time range was 60 to 600 ionizations and it generated three different peaks the figure 3 Shows the GCMS peak values and their respective retention time Gurumoorthy et al (2016). Table 2 Shows the composition of fatty acid methyl esters in microalgae Nannochloropsis salina

![Figure 3](image)

Figure 3 Shows the GC-MS peak values for Fatty Acid Methyl Esters

<table>
<thead>
<tr>
<th>Peak No</th>
<th>Retention Time (min)</th>
<th>Description of the ester</th>
<th>Chemical formula</th>
<th>Scan</th>
<th>Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.16</td>
<td>Tetradecanoic acid, methyl ester (Myristic acid cetane number C14:0)</td>
<td>C_{14}H_{26}O_{2}</td>
<td>1376</td>
<td>2171</td>
</tr>
<tr>
<td>2</td>
<td>8.76</td>
<td>Octadecanoic acid, methyl ester (Oleic acid cetane number C18:1)</td>
<td>C_{18}H_{36}O_{2}</td>
<td>1492</td>
<td>2016</td>
</tr>
<tr>
<td>3</td>
<td>9.18</td>
<td>Oxiranedodecanoic acid, 3 octyl cis</td>
<td>C_{22}H_{42}O_{3}</td>
<td>1573</td>
<td>1899</td>
</tr>
<tr>
<td>4</td>
<td>9.65</td>
<td>Tetradecanoic acid, methyl ester (Myristic acid cetane number C14:0)</td>
<td>C_{14}H_{26}O_{2}</td>
<td>1623</td>
<td>2181</td>
</tr>
<tr>
<td>5</td>
<td>9.94</td>
<td>Pentadecanoic acid, 14 methyl, methyl ester</td>
<td>C_{15}H_{30}O_{2}</td>
<td>1718</td>
<td>1330</td>
</tr>
<tr>
<td>6</td>
<td>10.26</td>
<td>1,2 benzedicarboxylic acid, bis(2methylpropyl) ester</td>
<td>C_{8}H_{16}O_{4}</td>
<td>1780</td>
<td>1543</td>
</tr>
<tr>
<td>7</td>
<td>10.5</td>
<td>Hexadecanoic acid (Palmitic acid Cetane number C16:0)</td>
<td>C_{16}H_{32}O_{2}</td>
<td>1880</td>
<td>2061</td>
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<tr>
<td>8</td>
<td>11.12</td>
<td>10-octadecenoic acid, methyl ester (Oleic acid cetane number C18:1)</td>
<td>C_{18}H_{36}O_{2}</td>
<td>1946</td>
<td>1148</td>
</tr>
</tbody>
</table>
8. CONCLUSIONS

The main goal of this study was to determine whether marine microalgae are able to grow in waste water from paper industry effluents. The results indicate that they can thrive on these environments. The GC-MS fatty acid profile of *Nannochloropsis salina* displayed high percentage of C18:1 Oleic acid which has been suggested as a compound for enrichment in biodiesel and the presence C14:0 Myristic acid which is the chief component present in biodiesel and C16:0 Palmitic acid which is also a major biodiesel component present in this fatty acid profile which eventually proves that the *Nannochloropsis salina* species is an apt able candidate for biodiesel production and also it is useful for phycoremediation this study reveals that this marine algae can be well utilized for treating paper industry waste water and as well as for the production of biodiesel.

REFERENCES


Biofuel Production from Marine Microalgae *Nannochloropsis Salina* Using Paper Mill Effluents


