Cultivation of microalgae using municipal wastewater as a nutritional source


Key words: Wastewater, Treatments, Microalgae

Abstract

Using wastewater to grow algae is probably the most promising way to reduce biodiesel production cost associated with nutrients and water. In the present study, three municipal waste water (MWW) from different effluents were collected and subjected to physical and chemical analysis. From about 21 isolates, only two promising microalgal isolates, Chlorella vulgaris and Scenedesmus quadricauda were tested for their ability to grow on the collected waste waters. Different treatments were performed for the collected (MWW) to determine the most suitable one for cultivation of microalgae. The results showed that supplementation with NaNO3 is the best treatment at least for Chlorella vulgaris.

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1. Introduction

Biodiesel production from microalgae has been expected to displace the petroleum-based energy sources because of its high aerial productivity, lipid contents and the higher energy yield: which is about 7–31 times higher than palm oil [1] and hundred times than other oily plants such as corn, soybean, canola, jatropha, coconut and palm oil [2]. However, the cultivation of algae on large-scale for bioenergy production should be significantly effective to compete with the cost of energy production from other resources, especially petroleum based fuel. The main spending would have to cover the costs of large amount of water with nutrients supplement, biomass harvest, and lipid extraction from biomass. In order to reduce the cost for nutrients and freshwater, it has been suggested to mix algal cultivation with wastewater treatment [3], [4]. Many species of microalgae are capable of effectively growing in wastewater conditions through their ability to utilize abundant organic carbon and inorganic N and P in the wastewater. Microalgae are efficient in removing N, P and toxic metals from wastewater [5], [6] and therefore have potential to play an important remediation role particularly during the final (tertiary) treatment stage of wastewater. The significant advantage of algal processes in wastewater treatment over the conventional chemical-based treatment methods is the potential cost saving and the lower level technology that is utilized, therefore making this approach more attractive to developing countries. The efficient growth of microalgae in wastewater depends on a variety of variables. As with any growth medium, critical variables are the pH and temperature of the growth medium, the concentration of essential nutrients, including N, P and organic carbon, and the availability of light, O2 and CO2. For example, increased temperature decreased algal biomass [7]. Some biotic factors like pathogenic bacteria or predatory zooplankton may have negatively effect on algal growth. Moreover, other microorganisms in the waste- water might out-compete the microalgae for essential nutrients. The starting density of microalgae in the wastewater is also expected to be a critical factor for the growth of the whole population [8]. These variables will clearly differ depending on the wastewater type and from one wastewater treatment site to another. Additionally, there will be variation in the ability of different algal species to tolerate a particular wastewater condition. Unicellular chlorophytic microalgae have been shown to be particularly tolerant to many
wastewater conditions and very efficient at accumulating nutrients from wastewater [9], [10]. A broad range of studies have analyzed the growth of microalgae under a variety of wastewater conditions, mainly growth in municipal sewage wastewater and agricultural manure wastewater. These studies have mainly been focused on evaluating the potential of algae for removing N and P, and in some cases metals from wastewater [11]. The ability of microalgae to grow well under definite wastewater conditions, as described above, has showed the potential of these resources as suitable sustainable growth medium for biofuel feedstock. In this study we will briefly evaluate how effective wastewater resources can provide significant algal biomass and which wastewater treatment will improve generation of high amounts of algal biomass.

2. Materials and Methods
2.1. Microalgal isolates collection and identification
Two algal stains Chlorella vulgaris and Scenedesmus sp were isolated from brackish water in Cairo and Qalubia, Governorates, Egypt, respectively. Algal identification has been done according to the keys of identification by Komárek and Fott [12]. A further confirmation on the identification were done using 18s rRNA. All identification steps were carried out at Sigma Scientific Services Co., Cairo, Egypt. Genomic DNA was isolated from all algal species via a phenol chloroform method on a pellet obtained by centrifugation of 10 mL of algal culture at the late-log phase. DNA amplification from genomic DNA containing a partial 18S ribosomal RNA region was performed by PCR using the following primers:

Forward: 5ʹ-GCGGTAATTCAGCTCCAATAGC-3ʹ
Reverse: 5ʹ-GACCATACTCCCCCGGAAAC-3ʹ.

Briefly, DNA was denatured at 94°C for 5 min and amplified by 30 cycles of denaturation at 95°C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min. There was a final extension period at 72 °C for 10 min prior to a 4 °C hold. The PCR product was isolated using a Gel PCR Clean-Up Kit (Qiagen). For sequencing reactions, 25 ng of PCR product was used as template with 10 pmol of the above primers in separate reactions in a final volume of 12 μL. The samples were then sent to the Biosearch tec. Company in USA for sequencing. Ribosomal RNA nucleotide sequences from the isolates were obtained from the NCBI database based on the BLAST results of each microalga sequenced in this study. When sequences from multiple isolates of a species were available, two nucleotide sequences were chosen: (i) highest maximum score sequence, (ii) highest maximum score sequence with identified genus and species.

2.2. Wastewater
The wastewater used in this study were collected from three different effluent lines of a final settling tank. The first sample was collected from oxidation station in Saadat city, Meonufia (OS), where, the second was collected from yellow Mountain station in El salaam city, Cairo Governorate (Y) and the last sample was collected from zenin station, Giza Governorate (Z). All are Sewage treatment plants. The samples were pre-filtrated using Whatman No.1 filters before analysis. They were subjected to complete physical and chemical analysis at Central Lab, Egyptian Petroleum Research Institute. Anions and cations were determined according to ASTM D-4327 and 6919, respectively using ion chromatography. The instrument used was Dionex IC model ICS 1100 equipped with high capacity columns (AS9 and CS12) for anion and cations, respectively.

Heavy metals were determined using Flame Atomic Absorption Spectrophotometer model Zenit 700p according to ASTM D4691.

Physical properties including density, specific gravity, pH, etc., were measured according to the following standards procedures:

- Total dissolved solids (TDS) were determined according to ASTM D-1888.
- Density and specific gravity were determined according to ASTM D-1429
- pH was determined according to ASTM D-1293 using digital pH meter model meter Toledo-Seven Go.
- Alkalines (CO3, OH, and HCO3) were measured according to ASTM D-3875. Calculations were done using Alkalinity calculator Ver. 2.10 (USGS).

2.3. Microalgal cultivation in different wastewaters with different treatments.
The two selected microalgal isolates were cultivated on the three tested waste waters S, Y and Z with ten different treatments. (1) 100% waste water. (2) 100% waste water + complete BG11 elements (A+B) without fresh water. (3)100% waste water + B solution only. (4) 100% waste water + 1 g/l NaNO3. (5) 100% waste water + soil extract. (6) 100% waste water + 5g/l glucose. (7) 50% waste water + 50% BG11. (8)50% waste water + 50% tap water + 100% BG11 components. (9) 70% waste water + 30% BG11. (10) 70% waste water + 30% tap water + 100%BG11 components.
All Flask cultures were completed to 250 mL in Erlenmeyer flasks containing the wastewater of 100 mL as a working volume at a shaking incubator of 25 ± 1°C, rotation speed of 150 rpm and continuous illumination with 60 µmol cool-white fluorescent light for about 20 day. The initial pH was controlled to pH 6.5 ± 0.1. The growth of the two isolates were compared with their growth on BG11 medium. The microalgae growth was determined by measuring optical density at a wavelength of 685 nm [11] (denoted as OD 685) using a spectrophotometer (model jenway 6300, Eu). The dry cell weight (DCW) of microalgae biomass was also obtained by filtering 50 ml aliquots of culture through a cellulose acetate membrane filter (0.45 µm pore size, 47 mm in diameter). Each loaded filter was dried at 105°C until the stability of weight. The dry weight of the blank filter was subtracted from that of the loaded filter to obtain the microalgae dry cell weight.

2.4. Cultivation profile on wastewater
In order to evaluate the growth performance of microalgae in waste water, the selected isolates were cultivated on it for maximum growth time (about 22 day). Cell counts were conducted on 0, 2, 4, 6 day and every two days until 22 day.

3. Results and discussion
3.1. Phylogenetic characterization of candidate microalgal strains.
Two water samples were collected from diverse aquatic habitats from Cairo and Qalubia, Egypt. Visual microscopy confirmed the isolation of axenic cultures. Morphological comparisons to other described microalgae suggested that these strains belonged to the genera *Chlorella* and *Scenedesmus*. To specify the identity of the microalgae strains used in our experiments, a partial 18S region of the ribosomal RNA gene was amplified by PCR and sequenced. The obtained sequences were then compared to existing sequences in the NCBI database. Sequence identity searches confirmed a close relationship of the isolated candidate strains to *Chlorella vulgaris* strain KCTC AG10191 and *Scenedesmus sp CCAP 217 7* as shown in (Figs.1 and 2).

![Fig. 1: phylogenetic tree of 18S rRNA gene sequences from microalgae *Chlorella vulgaris* strain KCTC AG10191](image1)

![Fig. 2: phylogenetic tree of 18S rRNA gene sequences from microalgae *Scenedesmus* sp CCAP 217 7](image2)
2.2. Wastewater samples collection and analysis

The samples were collected from three different water stations in three different areas. All of them were subjected to complete physical and chemical analysis to determine the elements content ratio in each one (Table 1). In aquatic ecosystems, nitrogen and phosphorus are the most important, as they are most often in short supply relative to the needs of plants, algae and microorganisms. Other elements, like iron, manganese and copper, are needed in small amounts [13]. Aforesaid output data showed the variation of nitrate and phosphate percentages from sample to another which were affected the Microalgal isolates biomass. One of the most important factors for algal growth is the nitrogen source and its concentration, so, it has been investigated through the previous analysis. The results show the absence of nitrogen in (OS) sample and presence in low levels in (Y) and (Z) samples. For this reason, the supplementation of wastewater with external nitrogen source often will be very useful. This result agree with the studies showing that the combination of several waste water would be a choice for optimization of wastewater for microalgae cultivation [14] and [15]. Phosphorus is an essential nutrient in biological wastewater treatment, it was found only in OS station and absent in the two other stations. The results also show nearly the absence of most heavy metals which enhance algal growth. Furthermore, some physical properties such as pH and TDS, were suitable for algal growth.

Table (1): Physical and chemical properties of the collected water samples

<table>
<thead>
<tr>
<th>Water sample Analysis</th>
<th>OS</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>physical properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dissolved solids (TDS) mg/l</td>
<td>2080</td>
<td>1220</td>
<td>746</td>
</tr>
<tr>
<td>pH at 25˚C</td>
<td>7.32</td>
<td>7.58</td>
<td>7.87</td>
</tr>
<tr>
<td>Salinity mg/l</td>
<td>1133.3</td>
<td>449.9</td>
<td>433.1</td>
</tr>
<tr>
<td>Hardness mg/l</td>
<td>612.1</td>
<td>410.0</td>
<td>274.9</td>
</tr>
<tr>
<td>Chemical properties(mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>Nil</td>
<td>18.12</td>
<td>19.6</td>
</tr>
<tr>
<td>Phosphate</td>
<td>60.99</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Chloride</td>
<td>686.84</td>
<td>272.65</td>
<td>262.5</td>
</tr>
<tr>
<td>Sodium</td>
<td>414.21</td>
<td>211.68</td>
<td>116.38</td>
</tr>
<tr>
<td>Magnesium</td>
<td>20.51</td>
<td>26.28</td>
<td>15.95</td>
</tr>
<tr>
<td>Heavy metals (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>Nil</td>
<td>Nil</td>
<td>10.68</td>
</tr>
<tr>
<td>Copper</td>
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</tr>
<tr>
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<td>Nil</td>
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<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Nickle</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

3.3 Application of the previously mentioned treatments for three different waste waters on Chlorella vulgaris strain KCTC AG10191 and Scenedesmus sp. CCAP 217 7

Ten treatments were applied on all collected wastewater. The results indicated that (OS) water is the best one for microalgal cultivation and the supplementation with nitrogen source (NaNO₃) is the best treatment. The growth of the two promising algal strains were enhanced by this addition but the yield was different due to the wastewater used. The growth limitation in the wastewaters of Chlorella vulgaris strain KCTC AG10191 (C.V) and Scenedesmus sp. CCAP 217 7 (S.S) could be partially explained by nutrients such as nitrogen and phosphorus depletion, but it seemed that there were other factors besides the N and P limitation in the culture because the nutrients used were enough during the total cultivation. However, the highest biomass production was achieved in the culture of (OS) wastewater supplemented with nitrogen source. Therefore the results indicate that the best waste water is (OS) as a nutrient source.
and the most promising treatment is treatment (4) (fig.3). This result was similar to that studies which reported the importance of nitrogen source for microalgal growth [16], [17], [18] and [19]. As shown in fig. 4 and 5, the biomass yield is nearly similar in all treatments and the supplementation with external nitrogen source have no promising increasing effect which mean that the algal growth on (Y) and (z) wastewaters with all treatments is not the best in compare with BG11 media. Also we noted that the treatment 6 (supplementation with glucose) have a bad effect on both microalgal isolates. This result was not agree with Liang et al., 2009, [20] who reported the ability of many algae to assimilate a variety of organic carbon sources.

Fig.3: Application of the ten treatments on (OS) water and the two promising microalgal strains Chlorella vulgaris strain KCTC AG10191 and Scenedesmus sp CCAP 217 7

Fig.4: Application of the ten treatments on (Y) water and the two promising microalgal strains Chlorella vulgaris strain KCTC AG10191 and Scenedesmus sp CCAP 217 7

Fig.5: Application of the ten treatments on (Z) water and the two promising microalgal strains Chlorella vulgaris strain KCTC AG10191 and Scenedesmus sp CCAP 217 7
3.4. Growth profile of microalgal strains on waste water

Algal growth in terms of optical density O.D680 on the (OS) wastewater under axenic condition were plotted in (Figs. 6 and 7). Short lag phase was observed specially for the organism (C.V.) indicating that this algal isolate could adapt well in OS. waste water. Moreover, the algal growth was significantly enhanced in the OS + (O.S waste water supplemented with NaNO₃ as a nitrogen source) because of its higher levels of nitrogen. The final algal biomass reach to 6.2 O.D in *Chlorella vulgaris* strain KCTC AG10191 and 2.6 O.D in *Scenedesmus* sp CCAP 217 7 under the condition of OS+. In the treatment OS- (O.S waste water without NaNO₃) the final algal biomass reach to 3.2 O.D in *Chlorella vulgaris* strain KCTC AG10191 and 1.82 O.D in *Scenedesmus* sp CCAP 217 7. The algal growth enhanced by the addition of nitrogen source in the ten day but the treatment OS+ still the best. These results were matched with the studies which proved that the algal growth is directly affected by the availability of nutrients [21].

![Growth curve on (os+)](image1)

**Fig.6:** Growth curve of the two promising microalgal strains *Chlorella vulgaris* strain KCTC AG10191 and *Scenedesmus* sp CCAP 217 7 growing on (OS) water supplemented with NaNO₃

![Growth curve on(OS-)](image2)

**Fig.7:** Growth curve of the two promising microalgal strains *Chlorella vulgaris* strain KCTC AG10191 and *Scenedesmus* sp CCAP 217 7 growing on (OS) water without NaNO₃
Conclusion

- Based on current tools, algal cultivation for biofuel production alone is unlikely to be economically viable or provide a positive energy yield.
- Dual-use microalgae cultivation for wastewater treatment coupled with biofuel generation is therefore an attractive route in terms of reducing the nutrient (fertilizer) and freshwater resource costs of biofuel generation from microalgae.
- The high biomass productivity of wastewater-grown microalgae suggests that this cultivation method offers real potential as a viable means for biofuel generation and is likely to be one of many approaches used for the production of sustainable and renewable energy.
- The results show that the supplementation of waste water with nitrogen source is the best treatment give high biomass production.
- The algal growth rate enhanced by the addition of nitrogen source from the starting of cultivation.
- For further investigation, the lipid content and fatty acid profile of lipid extracted from *Chlorella vulgaris* strain KCTC AG10191 and *Scenedesmus* sp CCAP 217 7 when growing on wastewater will be investigated in the following research paper.

References


