Alternatives for energy production in aerobic wastewater treatment facilities

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ABSTRACT

Using technologies such as anaerobic digestion for energy generation from wastewater demands a change in infrastructure that several treatment works are not prepared to immediately implement. This works explores the use of energy production technologies to increase the sustainability of conventional aerobic wastewater treatment plants. The first option considered sludge (a by-product from wastewater treatment) as raw material for biodiesel production as Fatty Acid Methyl Esters (FAME). The second option consisted of the addition of microalgae during aerobic wastewater treatment and subsequent harvesting of combined microalgae–sludge to produce biodiesel. Results showed that microalgae were able to grow in aerobic wastewater treatment reactors, reaching maximum growth after 6 days. The use of microalgae did not statistically affect chemical oxygen demand removal but provided benefits on ammonia removal (100% removal vs 68 ± 9% when microalgae were not added). Activated sludge contained fewer lipids (13 ± 3%, by dry weight) than the microalgae–sludge mixture (20.8 ± 4.5%). Hence, FAME production when using microalgae–sludge was higher (51.12 ± 12 mg of FAME/g of dry microalgae–sludge) than when using activated sludge (25.6 ± 7 mg of FAME/g of dry activated sludge). This work showed that producing biodiesel from microalgae grown in conjunction with bacteria during aerobic wastewater treatment can reduce energy use and carbon emissions produced by 18.6 and 26.5%, respectively.

Key words | aerobic activated sludge treatment, biodiesel, in situ transesterification, microalgae, wastewater

INTRODUCTION

Aeration has been the most traditional method to achieve wastewater treatment. However, an analysis of the gas emissions released when treating domestic wastewater by conventional aeration indicates that one megalitre of treated wastewater could produce approximately 340 g of methane and 335 kg of carbon dioxide (Czepiel et al. 2002). Additionally, using aeration for wastewater treatment requires a considerable amount of electricity to operate. Ataei (2010) reported an energetic consumption of 500 kWh per megalitre of wastewater treated, which further increases carbon dioxide emissions. Reliable and sustainable technologies are needed in order to decrease carbon dioxide emissions produced by wastewater treatment and take advantage of the organic matter and nutrients contained in wastewater. Figure 1 shows some mature and new technologies that could be used to accomplish energy retrieval during wastewater treatment. As can be seen, one of the energy ports that these technologies enable is production of transport fuels, such as hydrogen, methane and biodiesel.

Wastewater can be used to produce hydrogen using microbial electrolysis cells, a technology that needs considerable changes in aerobic treatment infrastructure. The same occurs with methane production, which is derived as biogas from wastewater treatment under non-oxygen conditions. The fact that wastewater treatment using anaerobic digestion and microbial cells requires a change in treatment infrastructure and operation increases the investment needed. This can deter technology intake, especially in wastewater treatment plants that are already built. More immediate actions could be increasing the efficiency of current treatment works operations, or the use of subsequent technologies for producing energy or biomaterials. Microalgae use and biodiesel production could be alternative technologies as their use does not necessarily require a change of aerobic wastewater treatment infrastructure.
Microalgae have the potential to be grown in aerobic wastewater treatment tanks to produce combined microalgae–sludge liquors. It has been reported that microalgae developed using wastewater can produce lipid productivities up to 505 mg/(L·d) (Kong et al. 2010; Pittman et al. 2011). In addition, microalgae can offer wastewater treatment benefits described in the literature years ago (Golueke & Oswald 1962; Benemann et al. 1977). Recent publications mainly consider the growth of microalgae in a separate treatment system. Examples of single treatment systems are high-rate algal ponds (Park et al. 2011), corrugated raceways (Craggs et al. 1997) and photobioreactors (Jacob-Lopes et al. 2010). Although these single treatment systems aim to benefit lipid yields during microalgae cultivation, using a combined microalgae–sludge biomass in aerated tanks could also be an effective and low-cost method for increasing treatment efficiencies and to reduce carbon emissions.

This work compared the use of activated sludge and combined microalgae–sludge to produce biodiesel using conventional and in situ transesterification. Additionally, the treatment effectiveness of microalgae addition to aerobic reactors was measured to quantify its potential to improve wastewater treatment. Finally, the savings in energy use and carbon emissions were calculated from this innovative approach that can help conventional aerobic treatment be more sustainable.

**METHODS**

**Sampling of wastewater, sludge and microalgae**

Returned activated sludge and settled wastewater were collected from a municipal wastewater treatment plant located in Cramlington, UK, and stored in a fridge at 5°C. It was observed than the sludge produced in the treatment works is transported to a facility located in Bran Sands, UK, where it is pre-treated using thermal hydrolysis and then partially converted to methane by anaerobic digestion. As thermal hydrolysis may facilitate the extraction of lipids, pre-treated sludge was also collected to evaluate its potential for biodiesel production before its conversion to methane. Microalgae used were a mixed culture of green microalgae taken from a pond located in the north-east of England.

![Figure 1](https://iwaponline.com/wst/article-pdf/67/12/2856/440480/2856.pdf)

**Operation of aerobic reactors and microalgae harvesting**

Initial experiments to evaluate wastewater treatment were conducted using cylindrical glass bioreactors with 1 L of working volume. Six bioreactors were used: two contained settled wastewater and four had settled wastewater plus green microalgae inoculated with 100 mL (two with a 1/10 v/v dilution) or 10 mL (two with a 1/100 v/v dilution) of a concentrated solution. Bioreactors were kept at room temperature (14 ± 4°C) and artificially illuminated using four hydroponic plant lights, each providing an intensity of 20,000 lux. Bioreactors were saturated with oxygen by bubbling air using a glass tube linked to a pump. When the bioreactors started to operate, volumes of 2 mL were sampled daily to measure optical density. For this measurement a spectrophotometer was calibrated with distilled water at 595 nm, as this corresponds to the wavelength of *Chlorella a*. Wastewater was characterised before treatment and after biological treatment plus the removal of total solids by centrifugation.

After this initial study, a set of larger experiments were done using green microalgae grown in a 20 L photosynthetic bioreactor made of polypropylene. A working volume of 10 L of wastewater was inoculated with a 1/10 dilution of...
green microalgae. Experiments were stopped once the maximum optical density observed in the glass bioreactors was reached ($\lambda = 1.22$). A calibration curve was derived to correlate the absorbance values to an amount of algae cells and the total suspended solids (TSS) derived from microalgae. For this last parameter it was determined that $219.4 \times 10^6$ cells corresponded to 2.3 g/L of total suspended solids from microalgae.

At the end of each reactor experiments the total solids were harvested by centrifugation for 1 hour using a Thermo Scientific Heraus Cryofuge 5500i centrifuge set at the maximum speed (4,400 rpm). After centrifugation, the solids harvested were stored in 45 mL tubes and dried in an oven at 100 °C for over 4 hours. Once the solids started to become a viscous paste, the temperature of the oven was decreased to 45 °C to achieve complete drying. Final powder products were stored in desiccators before analysis.

**Conventional and in situ transesterification**

Transesterification reactions were performed in three samples of biomass: activated sludge, pre-treated activated sludge and microalgae plus sludge. Before transesterification, the amount of total lipids was obtained by following the Bligh & Dyer (1959) method, as explained in Velasquez-Orta et al. (2012). Conventional transesterification experiments were conducted after lipid extraction while in situ transesterification experiments were done directly in dried biomass. Transesterifications were done using a high catalyst mole ratio equivalent to 0.8 mole sulphuric acid per mole of lipid or to 22% $\text{H}_2\text{SO}_4$ by weight. This ensured that the catalyst was not limiting the reaction. A high methanol molar ratio of 600 moles per mole of lipid was also used. As the transesterification of microalgae using sulphuric acid is slow, reactions were undertaken for 16 hours at 60 °C and a mixing rate of 380 rpm. This temperature was selected as this is the standard used in industry.

Fatty acid methyl esters (FAME) were obtained from the transesterification reactions. FAME constitute biodiesel and are derived from the esterification of long chain fatty acids with a range of 14–24 carbons. When reactions concluded, a known quantity of FAME sample was preserved for further analysis.

**Wastewater analyses**

Colour, turbidity, acidity, alkalinity, total suspended solids, total Kjeldahl nitrogen (TKN) and ammonia were measured for the different wastewaters according to standard methods.

**FAME analyses**

Analysis of total FAME yields was performed using gas chromatography (GC, Hewlet Packard 5890) adjusted to the following conditions: carrier gas: helium, 7 psi; air pressure, 32 psi; hydrogen pressure, 22 psi; a capillary column was used with a head pressure of 4.5 psi. FAME samples obtained after the reaction were mixed with a standard solution (C17, 10 mg/mL) in 2 mL vials. One microlitre of the mixture was injected to the gas chromatograph and data were collected using DataApex Clarity software, UK. The milligrams of FAME obtained in the biodiesel-rich phase from experiments was calculated by multiplying the weight of the final biodiesel mixture obtained by the FAME concentration measured by GC.

**Calculations**

The amount of energy recovered by producing biodiesel from the sludge–microalgae biomass was calculated using a reported energy content of biodiesel of 38.4 MJ/kg or 9.8 KWh/L (Mallick et al. 2012). Data published by Sydney et al. (2010) were used to calculate the fixation of CO$_2$ by microalgae biomass. In their report they show that 144 g of CO$_2$ can be fixed per kilogram of dry *Chlorella* sp., and different species of microalgae have fixation ranges of 136–186 g CO$_2$/kg (Sydney et al. 2010). It is important to note that recoveries mentioned in this work do not take into account the energy required for processing biomass to biodiesel, which should be considered in an overall life cycle assessment. Statistical analyses were done using Minitab® 15.1.
RESULTS AND DISCUSSION

Microalgae growth

As can be seen in Figure 2, microalgae inoculated to aerobic bioreactors with wastewater had a lag phase of 24 hours followed by linear growth. No significant differences \((p > 0.05)\) were observed between reactors inoculated with the two selected dilutions of microalgae \((1/10 \text{ or } 1/100, \text{v/v})\). The maximum absorbance \((\lambda = 1.2)\), obtained after 6 days of inoculation, corresponded to \(40 \times 10^6\) algae cells/mL and 420 mg/L of total suspended solids from microalgae. Measured total suspended solids increased from 318 to 440 mg/L in the 6-day period. From these results we can note that there is not only an increase in the total suspended solids but also an exchange on the type of organic biomass. The TSS measured at the beginning of the aeration corresponded to a biomass mainly composed of bacteria while, after 6 days of aeration, microalgae constituted 95% of the biomass by weight \((420 \text{ mg/L of the } 440 \text{ mg/L measured})\). This denotes the potential of microalgae to grow in activated aeration tanks in the presence of other types of microorganism. Figure 2 also shows that, after the 4th day, there is an observed increase in absorbance in wastewater without inoculation, which may be due to contamination and a start of microalgae growth favoured by lighting.

Microalgae required 6 days to achieve maximum growth; however, aerobic activated sludge reactors normally operate at hydraulic residence times \((\text{HRT})\) between 6 and 24 hours \((\text{Noyes } 1994)\). It is therefore unlikely that considerable yields of microalgae would be obtained if grown in aerobic activated sludge reactors for 24 hours; instead microalgae could be potentially grown in surface-aerated vessels that have conventional \(\text{HRT}\) between 3 and 10 days \((\text{Metcalf & Eddy Inc. et al. } 2005)\).

Wastewater treatment

Initial and final results obtained from the characterisation of wastewater are depicted in Table 1. Four conditions were compared: ‘raw settled wastewater’ which corresponds to the initial effluent before treatment, ‘centrifuged wastewater’ to compare the treatment provided only by centrifugation, ‘treated wastewater by aeration’ and ‘treated wastewater by aeration plus microalgae’. As expected, centrifugation of wastewater helped decrease the total and volatile suspended solids, achieving 64 and 70% removals, respectively. Thanks to the removal of suspended solids there was also a slight decrease in COD, nitrogen \((\text{TKN})\) and phosphate concentrations by 30, 33 and 13%, respectively. However, when including wastewater treatment by aeration with or without microalgae the removal of COD, nitrogen \((\text{as TKN or ammonia})\) and phosphorous \((\text{as phosphate})\) was further improved from centrifugation alone. Additionally, after aerobic treatment an increased separation of the total suspended solids by centrifugation was obtained as is shown in Table 1.

As can be seen in Figure 3 there was no significant difference in COD, TKN or suspended solids \((\text{SS})\) removal between wastewater treatment using only aeration and treatment with aeration plus microalgae. However, when treating wastewater using aeration plus microalgae the amount of ammonia removed \((100\%)\) was greater than when using only aeration \((68 \pm 9\%)\). This is due to the fact that microalgae are able to take ammonia, as nutrient, directly from wastewater. This is an advantage of microalgae over bacteria, as the latter first break down ammonia into nitrates and nitrites, which are then converted to nitrogen by a denitriﬁcation process conducted in an additional vessel. Phosphate removal was also improved when using microalgae in aeration tanks from 70% removal without microalgae to 93% removal with microalgae \((\text{Table 1})\).

FAME production from mixed algae and sludge products

Approximately 4 g of dried microalgae were obtained from every 10 L of wastewater treated. The amount of lipid produced from activated sludge was significantly lower than from mixed microalgae plus sludge \((p = 0.028)\). Lipids obtained from mixed microalgae plus sludge were 20.8 ± 4.5% of total biomass by weight. Activated sludge contained
13 ± 3 wt% of lipids, and sludge collected after pre-treatment had 13 ± 6 wt% of lipids.

As can be seen in Table 2, there were no statistical differences in the FAME yield obtained from activated sludge when using in situ transesterification or lipid extraction plus transesterification (p > 0.05). The percentage of FAME contained in the total lipids was similar for activated sludge with or without microalgae. However the percentage FAME contained in the sludge after pre-treatment was significantly lower. This shows that biodiesel extraction from sludge should be done before pre-treatment, as hydrolysis could have degraded the fatty acids chains to free fatty acids. Final results obtained from the average values of FAME retrieved from each type of biomass showed a significant higher yield for sludge with microalgae than for sludge without microalgae with values of 51.12 ± 12 and 25.6 ± 7 mg/g of dry biomass, respectively.

### Energy and carbon dioxide recoveries

The use of microalgae in wastewater treatment plants offers advantages as it increases the amount of lipids present in sludge, enabling a higher FAME production. A current study by this author has estimated that an activated sludge plant generates 218 kg of dry sludge per megalitre of wastewater treated (Velasquez-Orta & Graham 2012b).

### Table 1 | Composition of raw and treated domestic wastewater samples used in experiments. Samples were taken from batch reactor systems after 6 days of aeration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw settled wastewater</th>
<th>Centrifuged wastewater</th>
<th>Treated wastewater by aeration</th>
<th>Treated wastewater by aeration and Inoc. microalgae</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7 ± 0.2</td>
<td>8.7</td>
<td>7.3 ± 0.1</td>
<td>8.4 ± 0.8</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>18.5</td>
<td>18.5</td>
<td>18.5</td>
<td>18.5</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>1,465</td>
<td>1,417</td>
<td>1,299</td>
<td>1,092 ± 70</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>58</td>
<td>18</td>
<td>5.4</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>Colour (Hazel)</td>
<td>600</td>
<td>300</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Alkalinity (ppm)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>12 ± 2 (OH⁻)</td>
<td>15 ± 4 (CO₃⁻)</td>
</tr>
<tr>
<td>Acidity (CO₂, ppm)</td>
<td>63 ± 10</td>
<td>63 ± 15</td>
<td>N.A.</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Total suspended solids (ppm)</td>
<td>225 ± 70</td>
<td>82.5 ± 11</td>
<td>50</td>
<td>39 ± 7</td>
</tr>
<tr>
<td>Volatile suspended solids (ppm)</td>
<td>205 ± 63</td>
<td>63 ± 11</td>
<td>48 ± 4</td>
<td>35 ± 8</td>
</tr>
<tr>
<td>Ammonia nitrogen (ppm)</td>
<td>41 ± 3</td>
<td>35 ± 1</td>
<td>14 ± 4</td>
<td>0</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen (ppm)</td>
<td>77 ± 2</td>
<td>52 ± 10</td>
<td>29 ± 2</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>COD (ppm)</td>
<td>322 ± 8</td>
<td>235 ± 11</td>
<td>71 ± 12</td>
<td>115 ± 30</td>
</tr>
<tr>
<td>Fluoride (ppm)</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
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<tr>
<td>Chloride (ppm)</td>
<td>253</td>
<td>250</td>
<td>284</td>
<td>270</td>
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<tr>
<td>Nitrate (ppm)</td>
<td>2.0</td>
<td>1.9</td>
<td>2.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Phosphate (ppm)</td>
<td>16.4</td>
<td>14.3</td>
<td>5.0</td>
<td>1.2</td>
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<tr>
<td>Sulphate (ppm)</td>
<td>87</td>
<td>87</td>
<td>107</td>
<td>95</td>
</tr>
</tbody>
</table>

[Figure 3] Percentage removal of organic matter, nutrients and suspended solids after different treatment methods. Samples were taken from batch reactor systems after 6 days of aeration.

13 ± 3 wt% of lipids, and sludge collected after pre-treatment had 13 ± 6 wt% of lipids.
Adding microalgae to aerobic treatment reactors not only increases nutrient removal (ammonia and phosphorus) but can also help reduce carbon dioxide emissions by 26.5%.

Considering that microalgae are grown in conjunction with sludge in aerobic tanks, a FAME production of 9.3 L per megalitre of wastewater is to be expected. This means an energy reduction of 91 KWh per megalitre of wastewater, which could offset the total energy used in the aerobic treatment of wastewater by up to 18.6% (not considering the energy use for biodiesel production).

Using microalgae can also decrease carbon dioxide emissions from wastewater treatment plants. The author has calculated, in an additional research, that the biological aerobic treatment of wastewater generates 207 kg CO2 per megalitre (Velasquez-Orta & Graham 2022b). The data obtained in this study suggest a yield of 380 kg of microalgae per megalitre of wastewater treated. It is therefore calculated that 54.7 kg of CO2 per megalitre of wastewater could be captured by using microalgae. This accounts to a 26.5% reduction of the total emissions produced during aerobic treatment.

**CONCLUSIONS**

This work showed that there is a fraction of lipids in activated sludge that can be transesterified to produce FAME and that this fraction can increase when using microalgae in aerobic reactors. Using mixed sludge–microalgae biomass for biodiesel production can help offset the energy use during wastewater treatment by 18.6%. Adding microalgae to aerobic treatment reactors not only increases nutrient removal (ammonia and phosphorous) but can also help reduce carbon dioxide emissions by 26.5%.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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**Table 2**  FAME production from activated sludge and sludge–microalgae using conventional and *in situ* transesterification

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Transesterification process</th>
<th>FAME (% of total lipids)</th>
<th>FAME (mg/g of dry biomass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalgae plus activated sludge</td>
<td><em>In situ</em> transesterification</td>
<td>29.5 ± 7</td>
<td>61.4 ± 14</td>
</tr>
<tr>
<td></td>
<td>Lipid extraction plus transesterification</td>
<td>19.6 ± 5</td>
<td>40.8 ± 10</td>
</tr>
<tr>
<td>Activated sludge</td>
<td><em>In situ</em> transesterification</td>
<td>17.9 ± 3</td>
<td>23.3 ± 6</td>
</tr>
<tr>
<td></td>
<td>Lipid extraction plus transesterification</td>
<td>21.5 ± 5</td>
<td>28.0 ± 11</td>
</tr>
<tr>
<td>Activated sludge with pre-treatment</td>
<td><em>In situ</em> transesterification</td>
<td>4 ± 2</td>
<td>5.2 ± 5</td>
</tr>
<tr>
<td></td>
<td>Lipid extraction plus transesterification</td>
<td>9 ± 4</td>
<td>12.0 ± 5</td>
</tr>
</tbody>
</table>
Sydney, E. B., Sturm, W., de Carvalho, J. C., Thomaz-Soccol, V., Larroche, C., Pandey, A. & Soccol, C. R. 2010


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