CULTIVATION OF INDIGENOUS ALGAE FOR INCREASED BIOGAS PRODUCTION

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ABSTRACT

There is an increased demand for biogas in the society and one way to meet this is to use cultivated microalgae as fermentation substrate. In the present study, a co-digestion experiment was established where municipal food waste was fermented with harvested microalgae cultivated in lake water. The experiment was carried out as a laboratory batch experiment with fermentation bottles, where 0, 12, 25 and 37% of the food waste was replaced with harvested microalgae, respectively. The results showed that the biogas production was generally improved after addition of microalgae. During the first 25 days of fermentation, replacement of 12% food waste with microalgae gave the highest biogas production rate. However, higher proportions of microalgae (25% and 37%) slightly decreased the gas production rate compared to 12% and compared to using food waste alone.

Keywords: algae cultivation; biogas production, indigenous, food waste; co-digestion

1 INTRODUCTION

In recent years, numerous ideas have been considered to develop environmentally friendly alternatives to fossil fuels. The interest for renewable energy sources is increasing rapidly and in Sweden there is an increasing demand for substrates for biogas production. Today, source separated organic waste in Sweden is usually digested for production of biogas. The gas supports busses, taxis and regular cars in the surrounding areas with biogas fuel. Lately, an increased demand for biogas fuel has led to a situation where the production of gas needs to be doubled in the near future. However, the available substrate is limited since the organic waste is used near to its full potential. Growing crops for biogas production is attractive to most biogas producers. However, recent calculations [1] of the energy balance in these systems raise question whether growing energy crops can actually provide any net environmental benefit.

One approach is to use fast-growing algae for production of biomass that can be harvested and used for energy, such as biogas production. Addition of algae biomass to the fermentation process could be part of the solution since algae are rich in both carbohydrates as well as nutrients. In addition, the photosynthetic efficiency of algae in engineered systems can reach 4–5% of the solar energy compared to 1–2% for terrestrial plants [2]. Cultivation of algae has several advantages compared to higher plants due to faster growth rates and the possibility of cultivation on non-arable areas such as small lakes or ponds [3],[4]. In addition, the extensive ability of algae to assimilate nutrients such nitrogen (N) and phosphorous (P) offers the opportunity to combine the cultivation of algae for biomass with simultaneous treatment of waste water and recirculation of the digestate to arable land [5]. Hence, large scale algae production, e.g. in waste water treatment, can increase the biogas production and meet the demand from society. Previous studies have found that recirculation of nutrients by cultivation of single species of micro algae, such as Chlorella Vulgaris [6] can be a cost effective biogas substrate production, depending on the electricity tariffs. Some optimization measures were however required for increased profitability.

One such idea could be to promote the already existing algae production systems available, e.g. an
eutrophicated lake like lake Mälaren. The aim of the present study was to investigate the potentials for cultivating indigenous algae from a lake in central Sweden, and use the harvested algae as a co-substrate in biogas production. The study was carried out as a laboratory experiment in batch fermentation bottles and the production of biogas was measured during a 36 day fermentation period.

2 MATERIALS AND METHODS

2.1 Algae cultivation

Fresh water was collected from Lake Mälaren in March (early spring/late winter). The water was used immediately in the experiment, without any preservation or storage. A batch experiment was set up with three 250 L glass aquariums, each containing about 100 L water. A modified nutrient mix of Jaworski’s Medium[5] was added to each aquarium. The aquariums were placed in a climate chamber with consistent light at 22±0.5ºC (s.d.). The algae biomass was harvested after three weeks of cultivation by centrifuging the water at 5000 G for 15 min.

Algal growth, i.e. biomass growth, was monitored by optical density values scanned with a spectrophotometer (Hitachi, U-2000) at 600 nm, using deionized water as the blank control. Approximately 3.5 ml of each sample was used and measured in 1 cm cuvettes. The water in the aquarium was stirred prior to the sampling in order to prevent settling of the algae. After 21 days of cultivation the predominant groups of algae species in each flask were characterized by conventional microscopy.

2.2 Food waste and inoculums

Food waste was collected from the large scale biogas plant in Västerås, central Sweden, where source-separated household waste is digested for production of biogas. The inoculums were collected from the anaerobic digester at the same biogas plant. The content of total solids (TS) and volatile solids (VS) in food waste were 7.07 ± 0.21% and 81.48 ± 0.82%, while for inoculums they were 3.79 ± 0.03% and 68.23 ± 0.68%, respectively.

2.3 Batch fermentation experiment

The batch experiment of biogas production was performed in 1000 mL conical flasks in duplicates. Total working volume of fermented material in each flask was 700 ml and the amount of added inoculums was 4.24 g VS (corresponding to 6 g L\(^{-1}\)). Four treatments were prepared by replacing different percentages of food waste with algae, as shown in Table 1. A control group contained inoculums only was run in parallel to obtain the background biogas production.

The initial pH values of all treatments were adjusted to 7.5 by adding 2M hydrochloric acid. The fermentation temperature was regulated at 32±0.5ºC. Gentle stirring with a speed of approximately 120 rpm was applied during the whole fermentation.

2.4 Analytic methods

Total solids (TS) and volatile solids (VS) were determined according to the standard methods (American Public Health Association, 1992). The volume of biogas produced was periodically determined by displacement of acidified (pH 2) and saturated NaCl solution [7], [8]. To analyze the content of CH\(_4\), 1.0 mL of gas sample was taken with a gas-tight pressure lock syringe (SGE analytical science, Australia), and then immediately measured by a gas chromatograph using a thermal conductivity detector (TCD) and a stainless steel column packed with ProParkQ 50/80 (3.0 mm diameter and 2.0 m length). Argon was used as the carrier gas. The inject port and TCD temperatures were set at 40 ºC and 90 ºC, respectively. The column was 50 ºC, and filament current was 70 mA.

Table 1 Experimental protocol for co-digestion experiments with algae and food waste.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Algae</th>
<th>% Food waste</th>
<th>Algae (A) VS (g L(^{-1}))</th>
<th>Amount g VS</th>
<th>Food waste (FW) VS (g L(^{-1}))</th>
<th>Amount g VS</th>
<th>A:FW VS:VS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>3.57</td>
<td>2.50</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>88</td>
<td>0.44</td>
<td>0.31</td>
<td>3.18</td>
<td>2.23</td>
<td>1:7.18</td>
</tr>
<tr>
<td>C</td>
<td>25</td>
<td>75</td>
<td>0.87</td>
<td>0.62</td>
<td>2.66</td>
<td>1.86</td>
<td>1:3.00</td>
</tr>
<tr>
<td>D</td>
<td>37</td>
<td>63</td>
<td>1.33</td>
<td>0.93</td>
<td>2.16</td>
<td>1.51</td>
<td>1:1.62</td>
</tr>
</tbody>
</table>
3 RESULTS AND DISCUSSION

3.1 Algae growth

Figure 1 presents the photos of the cultivated algae with different periods. After 16 d of cultivation, the algae showed good growth. Growing for another 5 d improved the amount of algae just slightly. Similar results have been shown in previous studies using water from Lake Mälaren [8], where an optimum growth was reached after approximately 15 days.

The optical density at the time of harvest (Table 2) showed that the growth of algae differed somewhat in the three aquariums, even though the same amount of nutrients and the same water was used. One reason for this may be that the original density of algae may differ slightly in the water. Normally, algae enter a dormant stage during wintertime and may hence be less visible in the water. It is therefore possible that different concentrations of algae actually were amended to the aquariums. Also, although thoroughly mixed, different composition of microalgae could be present in the aquarium. These results suggest that the different species of algae responded differently to the light and nutrients.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Photographs of cultivated algae after 7, 11, 16 and 21 days, respectively.

<table>
<thead>
<tr>
<th>Aquarium No</th>
<th>Optical density (OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.396±0.004</td>
</tr>
<tr>
<td>2</td>
<td>0.334±0.012</td>
</tr>
<tr>
<td>3</td>
<td>0.136±0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>0.289</td>
</tr>
</tbody>
</table>

According to the estimate by [9] an OD of 1 unit is roughly equal 1 g L$^{-1}$ biomass. Using this method, the algal growth was estimated at approximately 0.289 g L$^{-1}$ over the 21 day period.

Several species of algae were identified under the microscope. The species include three types of cyanobacteria, namely *Anabaena* sp., *Lyngbya* sp. and *Microcystis* sp. Cyanobacteria are oxygenic phototrophs (often misleadingly called green-blue algae), but belong to the eubacteria group, and are therefore distinct from algae [10]. Cyanobacteria appear to be resilient to so called photo-inhibition, i.e. the growth can be significantly enhanced by light and may therefore potentially be enhanced by indoor solar irradiation [9].

In addition, several algae were found of the one cell green algae type (*Chlorella* sp.). Autotrophic microalgae can utilize carbon dioxide as their
carbon source and sunlight for energy for oil accumulation under certain conditions. It has been found that many autotrophic microalgae, such as *Chlorella* can accumulate oil [10], [11]. According to [12], the oil content of *Chlorella vulgaris* is 40%.

Other than cyanobacteria and green algae, the water also contained large populations of diatoms. Diatoms have a faster growth rate compared to other algae. However, diatoms may not be desirable for energy utilization due to their low lipid content.

### 3.2 Biogas production

Generally, the biogas production was improved by addition of algal substrate. These results are in agreement with other reports [10], [13], [14] that have shown that it is possible to convert algal biomass to gas via anaerobic fermentation. De Schampheelaere and Verstraete [15] used a closed loop system to feed algae into an anaerobic digestion plant and achieved good results. Algae consist of polysaccharides and have a low lignin content, which make them easy material to convert to methane by anaerobic digestion process.

![Figure 2](image)

*Figure 2* The biogas production measured periodically during 36 days of fermentation and substitution of 0, 12, 25 and 37% of food waste with algae.

All treatments followed more or less the same pattern over the fermentation period (Fig.2), with a moderate increase up until day 25, followed by a drop and then further increase. The highest production during the first 25 days was achieved by the treatment where 12% of food waste was replaced by the harvested algae. After this, solely food waste produced equal amounts as 12% algae. The higher proportions of algae (25% and 37%) generated slightly lower amounts of biogas during the entire fermentation period.

Algal cells contain polysaccharides, proteins and cellulose which makes them easy material to convert to methane by anaerobic digestion processes. It was therefore expected that addition of algal biomass would improve the biogas production. Algal biomass contains more easy degradable carbon than organic waste, and therefore stimulates the activity of the bacterial community responsible for fermentation. The drop in biogas production after 25 days suggest remains unexplained, however it may be due to temporary setbacks of the fermentation process due to experimental conditions. After about 20-25 days solely food waste produced equal amounts of biogas as 12% algae. One reason for this could be that most algal biomass was consumed at this time, and other more hemicellulloses and lignin based food material served as bacterial feed. The beneficial effect of algae could therefore no longer be seen.

The cumulative production and yield of biogas after 36 days of fermentation is shown in Figure 3. The results followed the same patterns as for the daily measurements shown in Figure 1 with highest biogas production at 12% of algal biomass. A further increase of algae feed (25% and 37%) decreased the biogas production. Similar results have been shown by for example [16] who found that an algae proportion of 18.9% gave an increased biogas yield whereas an algal biomass of 40% of the proved to inhibit the methanogenic activity. The same author suggested that this decrease could be caused by an increased production of H₂S reducers who are known to inhibit the fermentation process.
Figure 3 The cumulative production (A) and yield (B) of biogas after 36 days of fermentation and substitution of 0, 12, 25 and 37% of food waste with algae.

The results presented in Figure 3 could be explained by the fact that a too large proportion of algal biomass may lead to high pH levels and excess ammonia production due to degradation of the protein rich algal cells. Similar results have been found by [17]. Therefore, large amounts of algae may eventually inhibit the fermentation process. Also, since the cultivated algae showed to contain relatively large amounts of diatoms, a large proportion of algal biomass automatically supported the digester with high amounts of silica shells that are resistant to microbial attack.

Figure 4 The proportion of methane (A) and yield of methane (B) in the biogas after 36 days of fermentation and substitution of 0, 12, 25 and 37% of food waste with algae.

The main components of biogas are methane and carbon dioxide. The relative amount of methane determines the biogas quality and depends on the substrate and the fermentation conditions [18]. Figure 4 (A and B) shows the proportion and yield of the biologically produced methane after 36 days of fermentation. The proportion of methane was highest for 25% of algae, whereas the yield, measured as mL g\textsuperscript{-1} VS, was highest for 12% followed by 25% of algae. These results are in agreement with [19] who found that all microalgae tested in their experiment contained higher specific methane content than the standard fermentation substrate.

Altogether, the increased production of biogas as well as the increased proportion of methane indicates the potential of algal substrates as a complement to the traditionally used food waste based on higher plants. In addition, if properly managed, algae cultivation can also be considered as more sustainable [20] than plant cultivation because uncultivable land can be used, significantly less water is needed, and the risks of nutrient
runoff and biodiversity loss can be controlled. However, specific energy requirements must be taken into account, such as continuous mixing, CO₂ supply and costs for harvesting of the biomass.

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