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Microalgal growth in municipal wastewater treated in an anaerobic moving bed biofilm reactor

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HIGHLIGHTS

- The effluent from anaerobic treatment of wastewater is suitable for algal treatment.
- Discharge limits were met after 3 days of microalgal growth.
- No effect of the anaerobic treatment on microalgal lipid production was observed.
- Combining these treatments creates a sustainable wastewater treatment strategy.

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ABSTRACT

Nutrient removal from the effluent of an anaerobic moving bed biofilm reactor (AnMBBR) treated with microalgae was evaluated. Algal treatment was highly efficient in removal of nutrients and discharge limits were met after 3 days. Extending the cultivation time from 3 to 5 days resulted in a large increase in biomass, from 233.3 ± 49.3 to 530.0 ± 72.1 mg L^{-1}, despite nutrients in the water being exhausted after 3 days (ammonium 0.04 mg L^{-1}, orthophosphate <0.05 mg L^{-1}). Biomass productivity, lipid content and quality did not differ in microalgal biomass produced in wastewater sampled before the AnMBBR. The longer cultivation time resulted in a slight increase in total lipid concentration and a significant decrease in linolenic acid concentration in all treatments. Differences were observed in chemical oxygen demand, which decreased after algal treatment in wastewater sampled before the AnMBBR whereas it increased after algal treatment in the effluent from the AnMBBR.

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1. Introduction

The world's population is increasing rapidly, bringing increased production of human waste. Low-strength waste streams such as municipal wastewater are produced in large quantities (van Lier, 2008). At the same time, there is a growing need for energy and it is important to reduce the use of fossil fuels (Patil, 2007). In this perspective, conventional aerobic wastewater treatment technologies have some drawbacks for treatment of the abundant but low-strength municipal wastewaters, with a major problem being high energy consumption (McCarty et al., 2011). There is thus interest in developing more energy-efficient technologies and also in developing sustainable technologies for municipal wastewater treatment that allow recirculation of resources such as plant nutrients.

One alternative is anaerobic wastewater treatment, which has the potential to be a net energy producing technology through production of methane during anaerobic digestion of the wastewater (Shin et al., 2014). Treatment of low-strength wastewater with anaerobic membrane bioreactors has been shown to result in a great reduction in chemical oxygen demand (COD), even with a hydraulic retention time as short as 2–4 h (Bae et al., 2014). The effluent is generally low in suspended solids, but rich in nutrients such as nitrogen and phosphorus (Shin et al., 2014). It thus provides a suitable medium for cultivation of microalgae as a final step in removal and collection of nutrients from wastewater, as demonstrated by Ruiz-Martinez et al. (2012).

As previously mentioned, energy production is a central issue for sustainable development and microalgae are of interest in this

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regard (Islam et al., 2013). Overall, increased production of biofuels can decrease the dependence on fossil fuels, and various resources have been tested as a substrate for biofuel production (Patil, 2007). There is currently much focus on microalgae, since they have a high lipid content compared with e.g. oilseed crops and do not require arable land for biomass production (Brennan and Owende, 2010). Furthermore, using wastewater for production of algal biomass removes the costs of obtaining nutrients and water for production and obviously strengthens the economic potential of using microalgae for biofuel production.

Combining anaerobic treatment of municipal wastewater with microalgal treatment of the effluent obviously has potential as a sustainable technology. The present study examined nutrient removal by microalgae from the effluent of an anaerobic moving bed biofilm reactor (AnMBBR) in which primary-clarified low-strength municipal wastewater was treated. The fatty acid profile of the algal biomass produced was analysed and compared with the profile in microalgal biomass in wastewater sampled before the AnMBBR. Two different populations of green microalgae was tested, one dominated by *Chlorella* sp. and one dominated by *Scenedesmus* sp. Both are fast-growing microalgal species commonly used for wastewater treatment and of interest for biofuel production (Arita et al., 2015).

2. Methods

2.1. Wastewater

Primary-clarified municipal wastewater was collected at Källby wastewater treatment plant, Lund, Sweden, and filtered through a nylon cloth (mesh size 25 μm, Sintab, Sweden) in order to remove large particles. Wastewater batches were taken before and after treatment in an AnMBBR with reactor volume 0.43 m^3 filled with 50% K5 carriers (AnoxKaldnes, Veolia Water Treatment Technologies AB, Sweden), which had a protected area of 800 $m^2\,m^{-3}$. The temperature was set to 20 °C and the retention time was 5 h.

2.2. Selection of microalgae

Effluent from the AnMBBR was inoculated with water with a high concentration of microalgae or left untreated. Samples (500 mL) were kept in the greenhouse with stirring and aeration to stimulate algal growth. Inoculum from the 10 fastest growing samples, determined visually, was transferred to fresh batches of the effluent after 1 week. Re-inoculation was then performed once a week for 4 weeks in order to obtain a stable population. After this the samples were examined in an inverted microscope (Nikon). All samples were populated by green algae, with Scenedesmaceae dominating. Two different samples with populations dominated by either *Scenedesmus* sp. or *Chlorella* sp. (Fig. S1) were selected as inoculum for the experiments. Lately, there has been a major revision of the phylogeny of many algal groups following the development of molecular methods, with many of the species formerly belonging to the genus *Scenedesmus* having been moved to other genera, e.g. *Acutodesmus*, *Desmodesmus*. However, in this study we kept the designation *Scenedesmus* sp. throughout, since it is well established in the area of microalgal treatment of wastewater.

2.3. Experimental set-up

All experiments were performed in a greenhouse with a 16 h/8 h day/night regime with an added light intensity of 100 $\mu mol\,m^{-2}\,s^{-1}$ and a mean solar irradiance of 1287 ± 83 $Wh\,m^{-2}\,day^{-1}$ during the experimental period. The temperature was set to 25 °C. The experiments were performed as batch cultures and were continuously stirred at a speed of 100 rpm and aerated at a rate of 0.3 vvm. In all experiments, wastewater from the same batch, not inoculated with microalgae, served as the control.

The microalgal population dominated by *Scenedesmus* sp. (Fig. S1) was inoculated in wastewater collected before and after the AnMBBR. An inoculum amount of 5% v/v of a five-day-old microalgal, culture (see Section 2.2), in their late exponential phase, was added and corresponded to 43 ± 6 $mg\,L^{-1}$ DW of algal biomass. The effect of the algal treatments on concentrations of total nitrogen, ammonium, total phosphorus, orthophosphate and chemical oxygen demand was determined and the values compared against the initial concentrations. Biomass productivity, the amount of lipids in the biomass and lipid composition were also determined.

The effect of the two different microalgal populations (Fig. S1) was studied in the effluent from the AnMBBR. The inoculum corresponded to 35 ± 9 $mg\,L^{-1}$ (DW) for the *Scenedesmus*-inoculated treatments and 30 ± 4 $mg\,L^{-1}$ (DW) for the *Chlorella*-inoculated treatments. All replicates were examined under microscope before harvest to ensure that the microalgal populations were dominated by *Scenedesmus* sp. and *Chlorella* sp., respectively.

2.4. Analysis

2.4.1. Algal biomass and nutrient removal from the wastewater

The algal biomass was collected by filtration through a GF/C filter (Whatman 1822) and washed once with an equal amount of distilled water. Before and after filtration, the filters were dried in an oven at 105 °C until constant weight, in order to determine the dry weight of the algal biomass collected.

The concentration of total nitrogen in the wastewater from which the algal biomass had been removed was determined with Hach Lange LCK 138 (EN ISO 11905-1) and the concentration of ammonium was determined with Hach Lange LCK 304, LCK 303 (ISO 7150-1). Total phosphorus and orthophosphate was determined with Hach Lange LCK 350, LCK 349 (EN ISO 6878). COD was determined with Hach Lange LCK 114 (ISO 6060-1989).

2.4.2. Fatty acid methyl ester (FAME) content analysis

For analysis of fatty acid methyl esters (FAME), algal biomass was harvested by centrifugation (Avanti J-20, Beckman Coulter) at 3000 g for 20 min. The washed pellet was frozen at −80 °C and freeze-dried under vacuum. The lyophilised algal biomass was then treated with methanolic H_2SO_4 (2% v/v) for 60 min at 90 °C. The FAME were extracted with hexane and analysed by combined gas chromatography and mass spectrometry (GC–MS; Agilent 6890 GC and 5975 MS; Agilent Technologies, Santa Clara, CA, USA).

The GC was equipped with a 60-m fused silica capillary column (ID 0.25 mm) coated with HP-5MS UI (Agilent Technologies) and 2-μL aliquots of sample were injected by auto-injector (Agilent 7683B; Agilent Technologies) at 250 °C. The GC oven was programmed at 125 °C for 2 min, followed by an increase of 4 °C min^{-1} up to 250 °C and isothermal conditions for 10 min, and post-run cleaning of the column at 275 °C for one minute. The mass spectra were generated at 70 eV, acquiring data over m/z 29–400 at a scanning rate of 1.99 scans s^{-1}.

For quantification, 50 nmol heptadecanoic acid were added before esterification as an internal standard. The amounts of FAME in the samples were based on total ion chromatogram (TIC) peak area, with the exception of the non-separating C18:1, C18:2 and C18:3, which were based on quantification of molecular ions, using extracted ion chromatograms.

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