Bioresource Technology 169 (2014) 27–32

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Microalgal biomass and lipid production in mixed municipal, dairy, pulp and paper wastewater together with added flue gases

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HIGHLIGHTS

- Growth of microalgae on mixed municipal and industrial wastewater.
- High nitrogen and phosphorus removal was achieved in all treatments.
- *Selenastrum minutum* had the highest biomass and lipids yields.
- Lipid content was negatively correlated to the nitrogen concentration.
- Mixtures of wastewater have great potential to produce algal biomass and lipid.

ARTICLE INFO

Article history:
Received 1 April 2014
Received in revised form 17 June 2014
Accepted 18 June 2014
Available online 26 June 2014

Keywords:
Algae
Nitrogen removal
Phosphorus removal
Total lipids
Wastewater

ABSTRACT

The aim of the study was to grow microalgae on mixed municipal and industrial wastewater to simultaneously treat the wastewater and produce biomass and lipids. All algal strains grew in all wastewater mixtures; however, *Selenastrum minutum* had the highest biomass and lipids yields, up to 37% of the dry matter. Nitrogen and phosphorus removal were high and followed a similar trend in all three strains. Ammonium was reduced from 96% to 99%; this reduction was due to algal growth and not to stripping to the atmosphere, as confirmed by the amount of nitrogen in the dry algal biomass. Phosphate was reduced from 91% to 99%. In all strains used the lipid content was negatively correlated to the nitrogen concentration in the algal biomass. Mixtures of pulp and paper wastewater with municipal and dairy wastewater have great potential to grow algae for biomass and lipid production together with effective wastewater treatment.

1. Introduction

The reclamation of wastewater, both municipal and industrial, is of pivotal importance to achieving sustainability in our society at the global level. Often in traditional and well established wastewater treatment techniques the reduction of nitrogen and phosphorus is energy demanding. On the one hand, traditional treatments efficiently reduce the concentration of N and P; on the other, the treatments waste these important and vital nutrients through denitrification or deposition in landfill. It has been estimated that nitrogen pollution alone costs the European Union between €70 billion and €320 billion per year (Sutton et al., 2011). Furthermore, the energy required to produce N and P fertilizers is high, 11.1 and 10 kWh/kg, respectively (Olsson, 2012). Phosphorus resources are limited and we are rapidly approaching production peak (Cordell et al., 2009); hence, recycling this vital element is a pivotal challenge of the 21st century. Wastewater reclamation together with nutrient recycling is crucial to achieving environmental sustainability.

The pulp and paper industry is the world's largest producer of plant-based wastewater (Reid et al., 2008). Even though the amount of water per ton of paper produced has been decreasing over time, between 10 and 50 m^3 of water are still needed to produce a ton of paper (Pizzichini et al., 2005; Buyukkamaci and Koken, 2010). Consequently, at the global scale the pulp and paper industries produce a great amount of wastewater that has to be treated before being released into the environment. Even though the wastewater from the pulp and paper industry is rich in carbon it is limited in nitrogen and phosphorus. Hence in conventional wastewater treatment processes in the pulp and paper industry nutrients addition has been carried out to ensure microorganisms

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http://dx.doi.org/10.1016/j.biortech.2014.06.061
0960-8524/

growth for treating the wastewater (Thompson et al., 2001; Slade et al., 2004). Algae have been successfully used to remove of COD (chemical oxygen demands), colour and organic xenobiotics from diluted pulp and paper wastewater, with nutrients added to support the algal growth (Tarlan et al., 2002).

The wastewater produced by the dairy industry is rich in nitrogen and phosphorus (Kothari et al., 2012). Generally the dairy industry produces a volume of wastewater ca 2.5 times the volume of the milk processed, resulting in large amounts of wastewater (Ramasamy et al., 2004) and sludge.

In a study dealing with agroindustrial wastewater from dairy and pig farming algal growth could efficiently remove ammonia and phosphorus (González et al., 1997).

Municipal wastewater is globally produced in huge amounts and conventional wastewater treatment is costly and energy demanding (Lundin et al., 2000). Urban wastewater is rich in nutrients such as nitrogen and phosphorus (Ruiz-Marin et al., 2010; Doria et al., 2012).

The use of algae for wastewater reclamation is of great interest. Algae were already being tested in the treatment of municipal wastewater during the 1950s (Oswald and Gotaas, 1957). However, more recently a considerable amount of work has focused on algal treatment of municipal wastewater (Hoffmann, 1998; Park et al., 2011; Pittman et al., 2011). In some cases reductions in nitrogen and phosphorus of up to 90–95% were achieved (Hoffmann, 1998; Ruiz-Marin et al., 2010).

The potential of microalgae to remove N and P during tertiary sewage treatment has already been extensively assessed (Pittman et al., 2011). Furthermore it has been shown that algae grown on wastewater yielded more biomass when additional CO_2 was bubbled into the algae culture (Craggs et al., 2012).

Even though the interest in using algae to reclaim both municipal and industrial wastewater is continuing to increase, very few if any studies have focused on mixtures of municipal and industrial wastewater, including pulp and paper and dairy.

The aims of the present work are the following: (1) the use of microalgae to reclaim mixed municipal and industrial wastewater or sludge together with CO_2 addition; (2) to investigate how mixtures of municipal and industrial wastewater or sludge could provide good substrates for algal growth without the need for dilution with clean fresh water or nutrients supplementation; (3) to quantify biomass and lipid yield of three algal strains grown on three wastewater mixtures.

2. Methods

2.1. Local strain isolation

Wastewater samples from the local municipal wastewater treatment plant (Umeva, Umeå northern Sweden 63°52′ N) were placed in a closed bottle near the laboratory window under continuous agitation by a magnetic stirrer. After a few weeks of incubation, algal growth was visible to the naked eye and then small aliquots were streaked on sterile agar plates with Bristol medium (Wang and Lan, 2011). Subsequently a single colony was transferred to a new sterile plate with Bristol medium to obtain an algal monoculture, albeit it was not axenic.

2.2. Morphology of the algae

Light microscopy was used to aid in identifying the local isolated algal strain in accordance with Bellinger and Sigee (2010). The strain isolated as described above and the strains purchased from the UTEX algal collection were observed under a light microscope (Optika B-353 LD2, Optika, Italy).

2.3. Growing conditions

Municipal influent wastewater was collected from the local wastewater treatment plant (Umeva, Umeå, Sweden) and pulp and paper influent was collected from SCA Obbola (Obbola, Sweden), which uses chemical pulping (sulphate process), while dairy final effluent and sludge was collected from the local dairy (Norrmejerier, Umeå). The following wastewater mixtures were prepared: (a) pulp and paper influent 4:1 dairy sludge; (b) pulp and paper influent 1:1 municipal influent; (c) pulp and paper influent 2:1 dairy final effluent. All the mixtures were left to settle in the coldroom over night before the supernatant was transferred into tubes. Sterile 50 ml plastic tubes each received 39 ml of wastewater mixture. Two microalgal strains, *Scenedesmus dimorphus* (417) and *Selenastrum minutum* (326) were purchased from UTEX, The Culture Collection of Algae at the University of Texas at Austin (in parenthesis is the strains UTEX id); while a third strain was isolated locally (see above) and identified as *Scenedesmus* sp. The three algal strains were grown for a couple of weeks on filtered and autoclaved municipal wastewater influent; then they were harvested by centrifugation at 3580*g* for 5 min, the supernatant was discarded and the pelleted cells were resuspended in tap water. Each tube containing 39 ml of wastewater mixture was inoculated with 1 ml of algal culture at a concentration of 0.1 g/l dry weight. The control tubes received only the wastewater mixtures without algal addition. The controls were coved by aluminium foil to avoid the growth of native algae.

The experiment was carried out at the local combined heat and power plant (Umeå Energi, Umeå), where the samples were continuously illuminated with fluorescent lamps at a PAR (photosynthetically active radiation) of 130 $\mu E\ m^{-2}\ s^{-1}$. The temperature was recorded every 5 min and ranged from 21.7 to 32.2 °C with a mean value of 27.5 °C. The flue gases from the combined heat and power plant, having approximately a 10% CO_2 concentration, were bubbled at a flow rate of 50 ml min^{-1}. The flue gases were bubbled into the tubes using a sterile plastic pipette with a volume of 1 ml. The experiment was ended 6 days after inoculation.

2.4. Chemical analyses, biomass quantification and lipid extraction

The pH of the wastewater mixtures was measured at the start of the experiment and again at the end of the experiment for all the samples. A Beckman 295 pH meter (Beckman Coulter, USA) with a Red Rod pH electrode (Radiometer Analytical, France) was used. At the beginning of the experiment total COD analyses were performed in duplicate samples of each non-inoculated non-centrifuged mixture (APHA, 1998). Furthermore, at the beginning of the experiment, duplicate samples of each non-inoculated wastewater mixture were centrifuged at 3580*g* for 5 min. Then the supernatant was analysed using a spectrophotometer (DR 3900 Hach Lange, Germany) following the manufacturer instructions (Hach Lange, Germany); while the pellets were transferred to a dry pre-weighed Eppendorf tube and dried at 70 °C for 24 h. The same procedure was performed at the end of the experiment 6 days after algal inoculation.

The algal removal of COD, NO_3, NH_4, PO_4, total N and total P in the different wastewater mixtures was calculated as follows: [(initial value − final value)/initial value] * 100. Total N removal was followed through the entire experiment by collecting two samples every 2 days for each treatment.

Algal biomass production was calculated by subtracting the initial total suspended solids of the wastewater mixtures (control at the beginning) and the algal inoculum from the final harvested biomass. All weight measurements were done with a high precision balance (Kern ABT 120-5DM; readout 0.01 mg; Kern, Germany). Nitrogen quantification analyses of the dry biomass at final harvest