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Biomass production and nutrient removal by *Chlorella* sp. as affected by sludge liquor concentration

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ABSTRACT

The use of microalgae for biomass production and nutrient removal from the reject water produced in the dewatering process of anaerobically digested sludge, sludge liquor, was investigated. The sludge liquor was characterized by a high content of total suspended solids (1590 mg L^{-1}), a high nitrogen concentration (1210 mg L^{-1}), and a low phosphorus concentration (28 mg L^{-1}). *Chlorella* sp. was grown in sludge liquor diluted with wastewater treatment plant effluent water to different concentrations (12, 25, 40, 50, 70, and 100%) using batch mode. The environmental conditions were 25 °C, a continuous lightning of 115 μmol m^{-2} s^{-1}, and a CO_2 concentration of 3.0%. The highest biomass production (0.42 −0.45 g dry weight L^{-1} Day^{-1}) was achieved at 40–50% sludge liquor, which was comparable to the production of the control culture grown with an artificial fertilizer. The biomass production was 0.12 and 0.26 g dry weight L^{-1} Day^{-1} at 12% and 100% sludge liquor, respectively. The percentage of nitrogen in the algal biomass increased from 3.6% in 12% sludge liquor and reached a saturation of ~10% in concentrations with 50% sludge liquor and higher. The phosphorus content in the biomass increased linearly from 0.2 to 1.5% with increasing sludge liquor concentrations. The highest nitrogen removal rates by algal biosynthesis were 33.6–42.6 mg TN L^{-1} Day^{-1} at 40–70% sludge liquor, while the highest phosphorus removal rates were 3.1–4.1 mg TP L^{-1} Day^{-1} at 50–100% sludge liquor.

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1. Introduction

At a wastewater treatment plant (WWTP), sludge treatment is a costly and complex operation. While many different options exist, anaerobic sludge digestion is the only form of sludge stabilization that produces bioenergy in the form of methane gas. The reject water derived from the dewatering process of anaerobically stabilized sludge, sludge liquor (SL), has a concentration of nitrogen in the order of 1000 mg L^{-1}, high chemical oxygen demand (COD), and high total suspended solids (TSS) content (Davis and Masten, 2009). The SL is usually returned to the head end of the WWTP and accounts for 15–25% of the total nitrogen load at the WWTP (Fux et al., 2006; Janus and van der Roest, 1997). Algae could potentially be integrated at a WWTP to treat the side-stream of SL and offers the combined benefits of nutrient removal, energy production, and CO_2 sequestration (Rusten and Sahu, 2011; Sahu et al., 2013; Yuan et al., 2012). In this process, algae recover waste nutrients from the SL and transform them into biomass that can be harvested and co-digested with sludge to produce more energy, in the form of biogas, at the WWTP. In addition, flue gas from burning of biogas in the combined heat and power unit (CHP), or CO_2 from the digesters may be used as a CO_2 source for the algal bioreactor, which can reduce the CO_2 footprint of the WWTP (Gronlund et al., 2004; Rusten et al., 2009). The main challenge of using SL for algal cultivation is the high TSS content, which can vary from 790 to 3700 mg L^{-1}, with corresponding light transmission values of 16 to 0.1% (670 nm, 1 cm light path) (Rusten and Sahu, 2011). In previous studies of reject water from digested sludge, different pretreatment methods were used to remove the TSS content (Rusten and Sahu, 2011; Udom et al., 2013; Yuan et al., 2012). Increasing the light transmission by diluting with WWTP effluent water may be a preferred method due to its simplicity. A disadvantage of using dilution is that it lowers the nutrient availability and therefore reduces the nutrient uptake rate which in turn may lead to a reduction in both nutrient removal and algal growth rates (Aksnes and Egge, 1991; Hessen et al., 2002; Marschner, 1986). This is the only study, to our knowledge, that uses a high strength reject water from anaerobically digested sludge with a high TSS content (>1 g L^{-1}) for autotrophic algae cultivation. The aim was to evaluate the efficiency

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of algal biomass production and nutrient removal in different concentrations of raw, unsterilized SL.

2. Methods

2.1. Strain selection

An initial experiment was carried out to find a suitable strain of algae for growth in SL. The genus *Chlorella* was chosen for its high protein content that implies a high nitrogen demand (Kay, 1991; Taub and Dollar, 1965). A screening of 8 strains of *Chlorella* isolated from locations in Norway was carried out. The strains were received from the Norwegian Institute for Water Research (NIVA) in Oslo, Norway, and were grown in a mixture of 30% SL and 70% effluent water from a rotating biological contactor (RBC) WWTP. The screening was carried out inside a greenhouse compartment using natural light (average PAR were 17 ± 9 mol m^{-2} Day^{-1}), a controlled temperature of 25 °C and an airflow of 3.0% CO_2. After 6 days of cultivation, the strain *Chlorella sp.* 137 had the highest NTU value and was chosen for this study (Table S1).

2.2. Experimental set-up and cultivation conditions

SL and effluent water were collected at Nordre Follo Renseanlegg, a municipal WWTP located in Vinterbro, Norway, with a population equivalent of 41 000. The SL was collected from the dewatering process of the sludge after mesophilic anaerobic digestion: centrifugation with the addition of a cationic polymer and tap water. The SL had a brown color and the following composition: 1590 mg L^{-1} TSS, 3780 mg L^{-1} COD, 906 mg L^{-1} ammonium (NH_4–N), and 28 mg L^{-1} total phosphorus (TP). The WWTP uses chemical precipitation for P-removal by in the liquid-handling part of the WWTP and results in a low TP concentration in the SL. Effluent water was collected at the outlet of the dissolved air flotation basin, which was the last step before discharge. The chemical compositions of SL and effluent water are shown in Table S1.

The experimental set-up is visualized in Fig. 1. The SL was diluted with effluent water to produce mixtures with 12, 25, 40, 50, and 70% SL. The light transmission of the SL was 2.8% at 100% SL and increased to 85, 71, 20, 15, and 8% for mixtures with 12, 25, 40, 50, and 70% SL, respectively. Growth was compared to a control culture in which *Chlorella* sp. was grown in a medium composed of a horticultural fertilizer (Superex vegetables, Kekkilä Oy, Finland), phosphate (KPO_4), nitrogen (Urea) and dissolved in tap water. The final nitrogen and phosphorus concentrations were 552 and 86 mg L^{-1}, respectively (Table S1).

Chlorella sp. was cultured in batch cultures, using glass cylinders with an inner diameter of 35 mm and a height of 400 mm. These were placed in a thermostatic water bath (25.0 ± 0.5 °C) and continuously illuminated by a panel of white fluorescence tubes (18 W, Narva, Germany), providing a photon flux density of 115 μmol m^{-2} s^{-1}. An airflow containing 3.0 ± 0.2% CO_2 (from pure liquid CO_2) was bubbled through the cultures after passing through a sterile filter (Acro® 37 TF Vent Devices, 0.2 μm). By experience, a concentration of 3% CO2 was needed for avoiding a rise in pH above 8. Before the experiment, the algae were adapted to their specific environmental conditions; they were grown in their respective SL concentrations using a semi-batch mode that included three dilutions. The concentration of algae at the start of the experiment was 0.01 g L^{-1} and corresponded to an optical density (OD) of 0.05 at 680 nm. OD was measured daily for an approximate estimate of growth and TSS. The experiment was carried out until the growth curve of OD leveled out for two days. The nutrient removal due to algal biosynthesis was separated from other mechanical or physical processes by analyzing the algal biomass for the various nutrient elements at the end of the cultivation period. The aluminum content was also measured to test for possible residues from the chemical P-removal process. All cultures were made in triplicates.

2.3. Analytical methods

The following parameters of the wastewater were analyzed, using Hach-Lange kits (Hach Lange, Germany): chemical oxygen demand (COD), total nitrogen (TN), ammonium nitrogen (NH_4–N), nitrate nitrogen (NO_3–N), total phosphorus (TP), and phosphate (PO_4–P). The elements S, K, Ca, Fe, Mg, Mn, Zn, Cu, and Al were analyzed with an Inductively Coupled Plasma Optical Emission Spectrometer (ICP) (Optima 5300 DV, Perkin Elmer, USA) after the addition of HNO_3 to 10% v/v and were decomposed by UltraClave (UltraClave III, MLS, Leutkirch) at 250 °C for 1.5 h. A calibrated pH-meter, Orion Aplus TM (Thermo Electron Corporation, USA), was used to measure pH. Turbidity (NTU) was measured with a HACH 2100AN turbidimeter (HACH Company, USA). Light transmission (%) was measured at 670 nm (Rusten and Sahu, 2011) and was compared with that of de-ionized water (using water for the zero-base measurements) (Unicam Heλios, UV–vis spectrometry) in 1 cm light path. The OD of the algae culture was measured at 680 nm and was compared with the respective SL dilution, without algae (L. Wang et al., 2010).

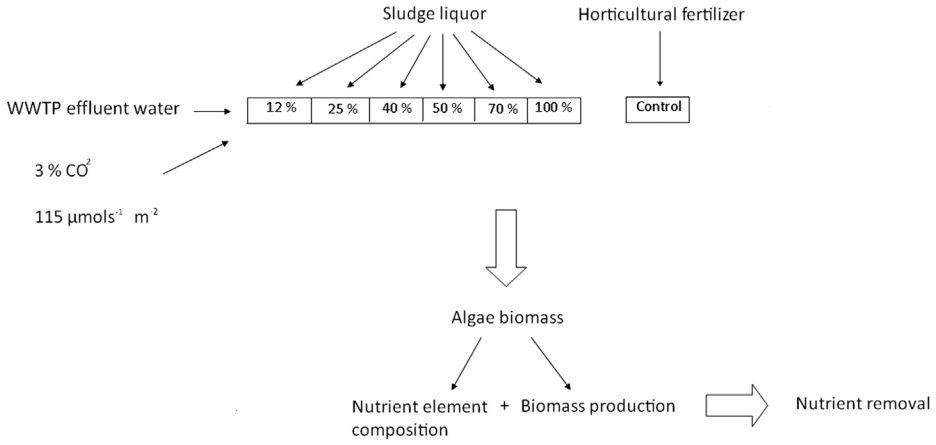

Fig. 1. The experimental set-up for evaluating the nutrient removal and biomass productivity of *Chlorella* sp. as affected by sludge liquor concentration (12, 25 40, 50, 70 and 100%) diluted with WWTP effluent water and compared to a control culture composed of a horticultural fertilizer.