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Harvesting microalgae using activated sludge can decrease polymer dosing and enhance methane production via co-digestion in a bacterial-microalgal process

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ABSTRACT

Third generation biofuels, e.g. biofuels production from algal biomass, have gained attention due to increased interest on global renewable energy. However, crop-based biofuels compete with food production and should be avoided. Microalgal cultivation for biofuel production offers an alternative to crops and can become economically viable when combined with the use of used water resources. Besides nutrients and water, harvesting microalgal biomass represents one of the major costs related to biofuel production and thus efficient and cheap solutions are needed. In bacterial-algal systems, there is the potential to produce energy by co-digesting the two types of biomass. We present an innovative approach to recover microalgal biomass via a two-step flocculation using bacterial biomass after the destabilisation of microalgae with conventional cationic polymer. A short solids retention time (SRT) enhanced biological phosphorus removal (EBPR) system was combined with microalgal cultivation. Two different bacterial biomass removal strategies were assessed whereby bacterial biomass was collected from the solid-liquid separation after the anaerobic phase and after the aerobic phase. Microalgal recovery was tested by jar tests where three different chemical coagulants in coagulation-flocculation tests ($AlCl_3$, PDADMAC and Greenfloc 120) were assessed. Furthermore, jar tests were conducted to assess the microalgal biomass recovery by a two-step flocculation method, involving chemical coagulants in the first step and bacterial biomass used in the second step to enhance the flocculation. Up to 97% of the microalgal biomass was recovered using 16 mg polymer/g algae and 0.1 g algae/g bacterial biomass. Moreover, the energy recovery by the short-SRT EBPR system combined with microalgal cultivation was assessed via biomethane potential tests. Up to 560 ± 24 mL CH_4/gVS methane yield was obtained by co-digesting bacterial biomass collected after the anaerobic phase and microalgal biomass. The energy recovery in terms of methane production obtained in the short-SRT EBPR system is about 40% of the influent chemical energy.

1. Introduction

Due to the challenges related to greenhouse gas emissions, decreasing fossil fuel reserves and global and national pressure, new solutions are sought to produce renewable energy including the use of biomass for biofuel production. However, first generation biofuels (derived from agricultural crops) are of questionable sustainability as they compete for land with food crops, thereby affecting the global food security [1,2]. Similarly, second generation biofuels, e.g. non-food energy crops (e.g. vegetative grasses or short rotation forests), agricultural and forest residues, compete for land use in some cases and there are technological difficulties related to the conversion processes [1]. Third generation biofuels such as microalgae have the advantages that they can be produced all year round, do not compete food production as they can be grown on non-arable land, have rapid growth rates and the biochemical composition can be manipulated by varying cultivation conditions and strains [1,3]. The cultivation of microalgae for biofuel production can be economically viable when coupled with wastewater treatment [3–6] which provides the water and nutrients (nitrogen and phosphorous) required for growth [7].

Conventional wastewater treatment has a high energy demand required mainly by the aeration process whereby organic carbon present in wastewater is oxidized to CO_2 and nitrification takes place under long sludge ages [8]. This leads to the loss of the energy potential of the activated sludge [9] together with the loss of nutrients (nitrogen and phosphorus) [8]. Short solids retention time (SRT) activated sludge systems offer a solution whereby rather than the oxidization of organic carbon, activated sludge preserves the organic carbon promoting higher potential for energy recovery [10].

In bacterial-algal used water treatment systems, nutrients and energy can be recovered [3]. In a novel wastewater resource recovery approach, Valverde-Pérez et al. [11] proposed an enhanced biological phosphorus recovery and removal (EBP2R) process, able to provide

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optimal culture media for downstream microalgal cultivation. The system referred to as TRENS, consists of a modified short-SRT EBP2R process where an additional solid-liquid separation is included after the anaerobic phase (Fig. S1, Supporting information, SI). Under anaerobic conditions, phosphorus accumulating organisms (PAO) take up the volatile fatty acids (VFA) from the wastewater and store them as polyhydroxyalkanoates (PHA) intracellularly while releasing intracellular phosphorus (poly-P) [12]. Under aerobic conditions the stored PHA are used to produce energy for biomass growth as well as phosphorus uptake and storage [12]. Thus, the effluent water of the solid-liquid separation after the anaerobic phase is rich in phosphorus, while the effluent from the solid-liquid separation after the aerobic phase is rich in nitrogen. The short-SRT EBP2R can provide optimal cultivation medium to a downstream photobioreactor (PBR) by mixing the phosphorus and nitrogen rich effluent streams in an optimal ratio.

When microalgal cultivation is coupled with wastewater treatment the lipid content of the microalgae is fairly low (4.9–11.3%) due to the relatively high nutrients supplied [3,13]. It is energetically favourable to apply anaerobic digestion when the lipid concentration is lower than 40% [14]. In addition, anaerobic digestion is applicable for biomasses with high moisture content (80–90%), which makes it suitable for microalgal biomass conversion [1,15]. Thus, anaerobic digestion is the preferred route over biodiesel production when energy recovery is considered from microalgae cultivated on wastewater resources [13]. The nutrient rich effluents of the anaerobic digestion can be used for further cultivation of microalgae [1]. Anaerobic digesters are in many cases available in the existing wastewater treatment plants and biogas production can be increased by co-digestion of microalgae and activated sludge [16]. Nonetheless, not all microalgal species are suitable for biogas production, mainly due to their cell wall structure and their high nitrogen content [14,17].

A C/N ratio of 20 (g/g) is suitable for optimal digestion conditions [4,18]. While, in freshwater microalgae it is typically around 10 [14,19]. Many studies proposed co-digestion with other biomass sources, e.g. activated sludge, to improve digestibility by balancing the C/N ratio, thereby providing optimal nutrient balance for enhanced methane yield [3,15,16,18]. Additionally, the co-digestion of various waste lines reduce costs by using a single anaerobic digester unit for digestion of multiple substrates [3].

The major bottleneck of microalgal cultivation for biogas production is the cost related to biomass harvesting [15,20,21]. Energy-intensive and expensive methods, e.g. centrifugation or membrane technologies [20], are only applicable when the biomass is used to produce high value products [21]. Thus simple harvesting methods are required for reliable and safe downstream applications [3].

Flocculation is an alternative and cheap harvesting method [20,22]. During coagulation the negative surface charge of microalgae, caused largely by the presence of carboxyl groups, is destabilised. This is followed by a second flocculation step whereby aggregates are formed, thus promoting more effective gravity sedimentation [21,23]. Iron or aluminium salts, which form positively charged hydroxides when dissolved in water, are successfully used as coagulants that neutralize the negative algal cells promoting aggregate formation [24]. $AlCl_3$ addition is a common method in wastewater treatment to enhance the coagulation-flocculation process [25]. Nevertheless, aluminium salts require high dosage and the downstream usage is limited due to toxicity [21]. Cationic polymers can induce flocculation of algal biomass by surface charge neutralization or by inter-cellular bridging [24]. The effectiveness of the polymers depends on their size and charge density. Compared to metal salts, polymers usually operate at lower dosages [21]. Flocculation efficiency by polymers declines at high dosages due to restabilisation [20,21]. Bioflocculation has also been proposed, whereby a specific bacteria, fungi or algae are added to the microalgal culture promoting flocculation [20,26].

Bacterial-algal systems have the potential to recover energy through biomass production. Thus, a cost-effective harvesting method is needed whereby the algal and bacterial biomass can be recovered. The objectives of this study are (i) to test the effect of different chemical flocculants on microagal recovery; (ii) to develop a cost-effective method of harvesting microalgae via a two-step flocculation using cationic polymer for destabilisation of microalgae and bacterial biomass from a short-SRT EBPR system to enhance the aggregation of the algae; (iii) to optimize the cationic polymer dosing; (iv) to assess the effect of different algae/bacterial biomass ratios and the effect of bacterial biomass settleability on algal biomass recovery; and (v) to assess the methane production potential by co-digestion of the harvested bacterial-algal biomass.

2. Materials and methods

2.1. Microalgal cultivation and EBPR operation

2.1.1. Algal biomass used for pre-testing different coagulants

We cultivated a mixed green microalgal consortium consisting mainly of *Chlorella sorokiniana* and *Scenedesmus* sp. (see Wágner et al. [27]). The consortium was cultivated with effluent water from the Lundtofte WWTP (Kgs. Lyngby, Denmark). Ammonium and phosphorus were spiked to reach 20 mg/L NH_4-N and 2.75 mg/L PO_4-P (16 N-to-P ratio) in the microalgal batch cultivation. 2 L glass reactors were used to cultivate the algae with constant stirring at 180 rpm using magnetic stirrers and with aeration with CO_2 enriched air (5% CO_2) at a flow rate of 10 L/h. Light was supplied from the two sides of the batches with fluorescent lamps (18 W, GroLux, Sylvania®, USA), providing 160 μmol photons m^{-2} s^{-1} continuously. The temperature in the room was regulated at 20 °C. 80% of the algal suspension was removed every 2–3 days from the batch reactor and the reactor was refilled with new effluent water. The pH of the algal culture varied between 6.84 and 7.95 during the experiments. The TSS of the algal suspension used for flocculation varied between 0.29 and 0.37 g/L. The algal TSS and OD values used for each flocculation experiment are reported in Table S1, SI.

2.1.2. Algal and bacterial biomass used for the two-step flocculation

The same mixed green microalgal consortium was used in the two-step flocculation experiments. The microalgal culture was grown on effluent water from a laboratory scale EBPR system [28] operated at 3–3.5 days SRT as a sequencing batch reactor (SBR) (fed with pre-clarified wastewater from Lundtofte WWTP, Kgs. Lyngby, Denmark). The ammonium and ortho-phosphate concentrations were adjusted to an N/P molar ratio of 17 in the beginning of each microalgal batch (adjusted to 23 mg/L NH_4-N and 3 mg/L PO_4-P). 1.5 L glass reactors were used to cultivate the algae with constant aeration with CO_2 enriched air (5% CO_2) at a flow rate of 10 L/h. Light was supplied from the top of the batch reactor continuously with a custom-built lamp, providing 500 μmol photons m^{-2} s^{-1}, with a metal-halide light bulb (OSRAM©, Germany). The reactors were kept at room temperature. The pH of the algal culture varied in the range of 7–8.5 during the experiments. 60% of the algal suspension was removed every 2–3 days and the batch reactor was refilled with new effluent water from the EBPR system (adjusted to N/P molar ratio of 17). The TSS of the algal suspension varied in the range of 0.27–0.52 g/L during the experiments. The algal TSS and OD values used for each flocculation experiment are reported in Table S1, SI. The bacterial biomass was taken from the short-SRT EBPR system using two biomass removal strategies: i) bacterial biomass removed at the end of the anaerobic phase; ii) bacterial biomass removed at the end of the aerobic phase. Samples for the biogas tests were taken during the course of 1 month, while the samples for the flocculation tests were taken throughout a 6 months period. Considering the use of real wastewater and the length of the experiments, results obtained can represent the effect of variability in used water resources, thereby allowing inferring experimental results more representative to real systems.