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Biogas production coupled to repeat microalgae cultivation using a closed nutrient loop

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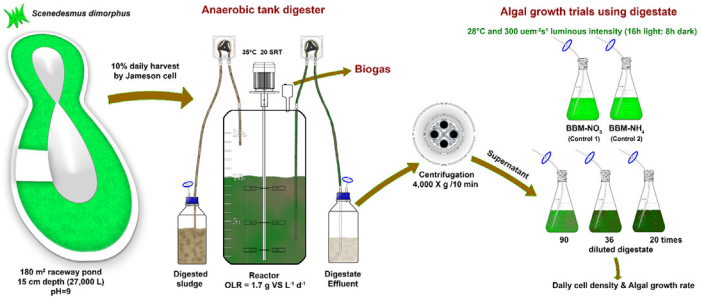
GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic digestion is an established technology to produce renewable energy as methane-rich biogas for which microalgae are a suitable substrate. Besides biogas production, anaerobic digestion of microalgae generates an effluent rich in nutrients, so-called digestate, that can be used as a growth medium for microalgal cultures, with the potential for a closed nutrient loop and sustainable bioenergy facility. In this study, the methane potential and nutrient mobilization of the microalga *Scenedemus dimorphus* was evaluated under continuous conditions. The suitability of using the digestate as culture medium was also evaluated. The results show that *S. dimorphus* is a suitable substrate for anaerobic digestion with an average methane yield of 199 mL g^{-1} VS. The low level of phosphorus in digestate did not limit algae growth when used as culture medium. The potential of liquid digestate as a superior culture medium rather than inorganic medium was demonstrated.

1. Introduction

The increased interest in the widespread adoption of cleaner alternatives to fossil fuels has driven the development and implementation of biofuel production (Correa et al., 2017). Anaerobic digestion (AD) has been widely implemented as a cost-effective technology to treat organic wastes and simultaneously produce renewable energy in the form of methane-rich biogas (Chen et al., 2008).

AD is a complex biochemical process which, in an oxygen-free environment, decomposes complex organic substances to simple

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compounds as methane and carbon dioxide (Kwietniewska and Tys, 2014, Ziemiński and Frąc, 2014). Studies on microalgae as feedstock for AD have shown comparable methane yields to other substrates such as sewage sludge and manures (Dębowski et al., 2013, Bohutskyi and Bouwer, 2013). Several microalgal species (e.g. *Isochrysis* spp., *Scenedesmus* spp.) have reported methane yields around 400 mL CH_4 g^{-1} VS (Tartakovsky et al., 2013; González-González et al., 2018). Furthermore, an improvement of methane production has been demonstrated when an adequate pre-treatment is applied (Keymer et al., 2013, Schwede et al., 2013, Passos et al., 2013).

AD of microalgae is particularly interesting when considering the potential to release and recycle nutrients to support microalgal growth (González-González et al., 2018). If used for biofuel production, such a system could be considered a 'solar power plant producing fuel'. The mineralization of nutrients during the degradation of organic matter generates an effluent rich in ammonium and phosphate (also known as digestate) that can partially or totally replace commercial culture media. This may significantly reduce microalgae production cost and a more sustainable biogas production (Möller and Müller, 2012, Uggetti et al., 2014).

The feasibility of using AD digestate nutrients to support microalgal growth has been proven by several studies (Cicci and Bravi, 2014, Cai et al., 2013). However, only a few studies researched the development of an integrated loop of microalgal AD and nutrient recycling to support microalgal growth (González-González et al., 2018) and most of the studies evaluated algal growth on digestates from other substrates (Budiyono et al., 2014, Cicci and Bravi, 2014, Franchino et al., 2013). However, the efficiency and advantages of a unified system for microalgae cultivation and anaerobic digestion has been demonstrated for a few studies (Sforza et al., 2017, De Schamphelaire and Verstraete, 2009).

As nutrient recycling is a suitable way to optimize biogas production by lowering production cost, it is essential to understand how the flow of nitrogen and phosphorous occurs inside the system. However, to date there is little knowledge related to mobilization of nutrients through anaerobic digestion of microalgae. Most of the studies on anaerobic digestion of other substrates generally show a high efficiency of nitrogen removal (60%–80%) while most of the phosphorous remains trapped in the solid phase (Zhang et al., 2014, Mehta and Batstone, 2013).

The aim of this study was to evaluate the feasibility of a closed loop integrating microalgae anaerobic digestion and cultivation since the literature lacks a study that integrates both processes in the same system and using the same microalgal species. In this study, the anaerobic digestion of *S. dimorphus* NT8c was evaluated in a continuous stirred tank reactor of 10 L where, among others, biogas production and nutrient mobilization were monitored. The fertilizer potential of the digestate for repeat algae growth was evaluated to assess the opportunities and obstacles of a closed system nutrient loop.

2. Materials and methods

2.1. Microalgal biomass cultivation and preparation

Microalgal sludge was obtained from the Algae Energy Farm of the University of Queensland located in Pinjarra Hills, Brisbane (Australia). The harvested strain *Scenedesmus dimorphus* NT8c, (Duong et al., 2015) was cultivated in 180 m^2 raceway ponds, 15 cm depth (27,000 L) to a total solids (TS) concentration of around 0.3–0.5 g TS L^{-1}. Nutrients were derived from fertilizers in the following concentrations: ammonium sulphate (3 mM), mono-ammonium phosphate (0.2 mM), magnesium chloride (0.2 mM), Aquasonic Ocean Nature sea salt 10 g/1000 L, chelated iron 1 g/1000 L, Rapisol Mi6 micronutrients (1 g/1000 L). Pond pH was adjusted to 9 with potassium hydroxide and 100% CO_2 was automatically added to the pond when pH increased above 9. Ammonia and phosphate levels were measured every 2–3 days and nutrients were added to the pond when the nutrient level was around half of the initial concentration. Approximately 10% of the pond was harvested daily through a Jameson cell which concentrated the microalgae from 0.3 to 0.5 g TS L^{-1} to 20–30 g TS L^{-1}. The algal slurry was then stabilized for flocculation using HCl to drop the pH from 9 down to 3. After overnight settling in a Vee-shaped pond the top two thirds of water were removed leaving a solids content of 60–90 g TS L^{-1}. Microalgae batches were stored at 4 °C until use. Prior to use, the top portion of the water was removed, leaving a final biomass content at around 70 g TS L^{-1}.

2.2. Reactor design and set-up

Anaerobic digestion of microalgal biomass was carried out in a mesophilic continuous stirred tank (CSTR) digester with a working volume of 10 L. The digester was operated at 35 °C and solids retention time (SRT) of 20 days. The digester content was intermittently mixed (45 min each hour) by a 4-blade stirrer connected to an overhead stirrer. The reactor was initially inoculated with digestate (20 g VS L^{-1}) from a wastewater treatment plant anaerobic digester treating a mixture of primary sludge and waste activated sludge at 35 °C and a SRT of 23 days. The digester was always fed with microalgal biomass; however operation during the first 3 SRT was considered the start-up period (data not shown). After the start-up, the reactor was operated under steady-state conditions for another 3 SRT (60 days) which allowed to collect reliable reactor performance data and enough digestate to perform the growth trials. Digester feedstock was prepared once per week by diluting with distilled water the harvested microalgal biomass to 40 g VS L^{-1} and adjusting the pH to 7 with a 4 M NaOH solution. The feedstock was stored at 4 °C to avoid uncontrolled microalgae degradation. The digester was automatically fed and drawn four times per day with a total of 500 mL. The average organic loading rate (OLR) of the digester was 1.7 g VS L^{-1} d^{-1}. Biogas production was measured using a tipping bucket gas meter. Biogas composition was determined by a Shimadzu GC-2014 gas chromatograph. Biogas and methane production are reported at standard temperature and pressure (0 °C and 1 bar).

2.3. Chemical analyses

Analyses of the total fraction were performed directly on the raw samples. For analyses of the soluble fraction, samples were centrifuged at 2500*g* for 5 min and the supernatant filtered through a 0.45 µm PES Millipore® filter. TS and VS were measured according to standard method procedures 2540G (Franson et al., 2005). Total and soluble chemical oxygen demand (tCOD, sCOD), were measured using a Merck COD Spectroquant® test kit (range 0.5–10 g L^{-1} and range 25–1500 mg L^{-1}, respectively) and a Move 100 colorimeter (Merck, Germany). Total Kjeldahl nitrogen (TKN), total phosphorus (TP), ammoniacal nitrogen (NH_4-N), and phosphate (PO_4-P) were measured using a LachatQuik-Chem 8500 Flow Injection Analyser following the manufacturer's protocol (Lachat Instrument, US). Biogas composition (CH_4, CO_2 and H_2) was determined using a Shimadzu GC-2014 gas chromatograph equipped with a Valco GC valve (1 mL sample loop), a HAYESEP Q 80/100 packed column (2.4 m length; 1/800 outside diameter, 2 mm inner diameter) and a thermal conductivity detector (TCD). The chromatograph injector, oven and detector temperatures were set at 75, 45 and 100 °C, respectively, and 28 mL min^{-1} of Argon at 135.7 kPa was used as a carrier gas (Astals et al., 2015).

2.4. Algal growth trials using digestate from the lab-scale reactor

The centrate (also known as supernatant) of the anaerobic digester effluent was obtained by centrifugation at 4000*g* for 10 min. Algal growth was evaluated in three different dilutions (20, 36 and 90 times diluted) of the liquid digestate by dilution with tap water. Two