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Anaerobic co-digestion of pig manure and algae: Impact of intracellular algal products recovery on co-digestion performance

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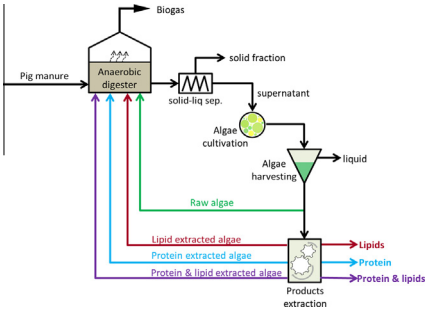
HIGHLIGHTS

- Pig manure was anaerobically co-digested with raw and processed algae.
- Processing increased algae biodegradability but not its degradation rate.
- Synergy between raw algae and pig manure increased methane yield of the mixture.
- There was no significant synergy between processed algae and pig manure.
- Concept was presented for a combined biorefinery processing pig manure and algae.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper investigates anaerobic co-digestion of pig manure and algae (*Scenedesmus* sp.) with and without extraction of intracellular algal co-products, with views towards the development of a biorefinery concept for lipid, protein and/or biogas production. Protein and/or lipids were extracted from *Scenedesmus* sp. using free nitrous acid pre-treatments and solvent-based Soxhlet extraction, respectively. Processing increased algae methane yield between 29% and 37% compared to raw algae (VS basis), but reduced the amount of algae available for digestion. Co-digestion experiments showed a synergy between pig manure and raw algae that increased raw algae methane yield from 0.163 to 0.245 m^3 CH_4 kg^{-1} VS. No such synergy was observed when algal residues were co-digested with pig manure. Finally, experimental results were used to develop a high-level concept for an integrated biorefinery processing pig manure and onsite cultivated algae, evaluating methane production and co-product recovery per mass of pig manure entering the refinery.

1. Introduction

Algae are an interesting feedstock for the production of biofuels, chemicals, cosmetics and animal feed (Milledge and Heaven, 2014; Passos et al., 2013). Advantages of algae include: (i) the capacity to grow on fresh, brackish, saline and wastewater streams; (ii) tolerance to a wide variety of environmental conditions; (iii) an ability to be cultivated on land not suitable for food production; and (iv) be produced all year round (Uggetti et al., 2014; Ward et al., 2014). Currently, most approaches for algae-based biorefineries (i.e. facilities to convert algae into multiple valuable products, including biofuels) are not economically viable, due to high costs of algae cultivation and valorisation (Milledge and Heaven,

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2014). Consequently, strong research efforts aiming to improve biofuels and/or biochemical production yields from algae are being made (Milledge and Heaven, 2014; Uggetti et al., 2014). One particular opportunity, which is the focus of this work, is value-adding to algae residue by using it as a feedstock for anaerobic (co-)digestion.

Anaerobic digestion (AD), which converts organic matter into biogas and a stabilised digestate, is a proven technology for the management of organic-rich streams (Mata-Alvarez et al., 2014). AD has been identified as a key process to make algae biorefineries commercially feasible (Milledge and Heaven, 2014; Uggetti et al., 2014), and can be used to treat either raw algal biomass or algal residue after extraction of valuable intracellular products (Keymer et al., 2013; Passos et al., 2013; Ramos-Suárez and Carreras, 2014; Sialve et al., 2009). The viability of algae AD is highly dependent on: (i) the organic concentration of the feedstock, since harvesting and concentrating algae biomass is a major cost; and (ii) the biochemical methane potential (B_0) of the algae (Uggetti et al., 2014). The latter depends on the algae culture strain and its cultivation conditions, which impact their composition (carbohydrate, protein and lipid content) as well as cell wall structure (Alzate et al., 2014; Sialve et al., 2009; Uggetti et al., 2014). Reported algae B_0, mostly mono-cultured, are highly variable ranging from 0.130 to 0.600 m^3 CH_4 kg^{-1} VS (Mussgnug et al., 2010; Ward et al., 2014). Unfortunately, the methane yield from natural mixed algae cultures grown in less controlled systems (real world application) are found in the lower range, rarely exceeding 0.300 m^3 CH_4 kg^{-1} VS (González-Fernández et al., 2011; Keymer et al., 2013; Passos et al., 2013). This fact has raised the interest on algae pre-treatment techniques, with and without co-products recovery, aiming to improve algae biodegradability through cell wall disruption (Milledge and Heaven, 2014; Ramos-Suárez and Carreras, 2014). Under this rationale, the feasibility of an algae-based biorefinery is mainly linked to: (i) the co-products economic value; (ii) biogas value as electricity and/or heat energy; (iii) algae harvesting and concentration, where harvested algae may not only be thickened, but also dewatered or even dried before processing for co-products extraction (Alzate et al., 2014; Sialve et al., 2009; Ward et al., 2014).

Anaerobic co-digestion (AcoD), the simultaneous anaerobic digestion of two or more substrates, improves economic viability of AD plants due to the potential for higher methane production than through digestion of single substrates (Mata-Alvarez et al., 2014). The increase in methane production from AcoD is mainly a result of increased organic loading rate; however, synergism (i.e. a complementary relationship between substrates that improves digestion performance) can further enhance methane production (Astals et al., 2014; Mata-Alvarez et al., 2014; Ramos-Suárez and Carreras, 2014). Beyond the implementation and operation expenses, onsite cultivation of algae presents some advantages over the use of other co-substrates (Mata-Alvarez et al., 2014). Such advantages include: (i) reduced or nullified co-substrate transport cost, which is one of the most important co-substrate selection criteria; (ii) minimising the effect of seasonality of some agro-industrial co-substrates, where supply can be variable or cease; and (iii) providing a co-substrate in regional areas where co-substrates are otherwise utilised or are not available. Taking into account these facts, algal biomass appears as a potential co-substrate for animal manure digester located in rural/remote areas.

The potential of using algae as a co-substrate has recently been reported in several publications; however, these studies focus on sewage sludge or carbon-rich waste as the main substrate (Cecchi et al., 1996; Mata-Alvarez et al., 2014; Ramos-Suárez and Carreras, 2014; Zhong et al., 2012), while few studies have evaluated AcoD of animal manure and algae (González-Fernández et al., 2011; Miao et al., 2014; Sarker et al., 2014). From a nutrient balancing perspective, AcoD of algae and manure does not seem obviously attractive, because both substrates are characterised by a relatively low carbon-to-nitrogen (C/N) ratio (<10) (Mata-Alvarez et al., 2011). However, González-Fernández et al. (2011) and Ramos-Suárez and Carreras (2014) observed that synergism is not always linked to the C/N ratio of the mixture when using algae as co-substrate and therefore AcoD of algae and pig manure warrants further investigation. Furthermore, previous algae AcoD studies have only tested raw algae in co-digestion mixtures and have not considered AcoD of algae residues after extraction of valuable co-products. There is a present need for a study on AcoD of algal residues to provide critical insights into AD plants aiming to process algae grown on anaerobic digestion supernatant.

The primary goal of this study is to evaluate anaerobic co-digestion of pig manure and algae (*Scenedesmus* sp.) with and without extraction of intracellular algal co-products. Algae processing targeted the extraction of lipids (solvent-based Soxhlet extraction) and/or protein (free nitrous acid pre-treatment) as high-value co-products. Biomethane potential tests were used to assess the effect of pre-treatment and co-product extraction on substrate biodegradability and degradation rate. Finally, the results of the study were used to evaluate a high-level concept for an integrated biorefinery treating pig manure and onsite cultivated algae.

2. Methods

2.1. Raw algae, manure and inoculum origin

Pig manure was collected as a representative composite sample of an entire direct-flush from a grown-out pig shed near Perth (WA, Australia). The sample was shipped immediately (chilled on ice-bricks) to The University of Queensland, and was received cold and stored at 277 K until use. Dry algal biomass was obtained from a pilot-scale open algal cultivation raceway (Pinjarra Hills, Australia). The raceway had a total volume of 30 m^3 with 1.5 m wide channels and 0.2 m depth. The green algae *Scenedesmus* sp. was cultured in an open pond and algal biomass was collected by filtration and dried in a solar collector and drying tunnel assembly. Microscope observation showed that the majority of the biomass was *Scenedesmus* sp. with small amounts of sand, grit and salt crystals.

Anaerobic inoculum was collected from the bottom (~2 m depth) of a partially covered anaerobic lagoon, which treats flush manure from a specialised breeder piggery located near Grantham (QLD, Australia). After collection, inoculum was stored at 277 K. Prior to commencement of the biomethane potential (BMP) tests, the inoculum was degassed at 310 K for one week. The specific methanogenic activity of the inoculum at 310 K was 0.09 kg COD-CH_4 kg^{-1} VS day^{-1}.

2.2. Algae high-value products extraction

Processed algal residues were prepared for the digestion/co-digestion testing. The processing steps extracted protein with free nitrous acid (FNA) (Section 2.2.1), and/or lipids via a solvent-based Soxhlet extraction (Section 2.2.2).

2.2.1. Protein extraction

FNA pre-treatment was carried out to release protein from algal cells. Dry algal biomass was re-suspended in deionized water at 47 g L^{-1}, and pH was adjusted to 5.5 using 0.1 M HCl. Sodium nitrite stock solution 30 g NO_2^--N L^{-1} was then added to the suspension resulting in an initial concentration of 0.3 g NO_2^--N L^{-1}. The FNA dose was selected from previous experiments (Bai et al., 2014), where 0.3 g NO_2^--N L^{-1} led to moderate cell disruption with