Waste Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

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

Co-digestion of chicken manure and microalgae *Chlorella* 1067 grown in the recycled digestate: Nutrients reuse and biogas enhancement

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ARTICLE INFO

Article history:
Received 31 July 2017
Revised 7 September 2017
Accepted 13 September 2017
Available online xxxx

Keywords:
Digestate
Chicken manure
Chlorella 1067
Co-digestion
Biogas

ABSTRACT

The present investigation targeted on a sustainable co-digestion system: microalgae *Chlorella* 1067 (*Ch.* 1067) was cultivated in chicken manure (CM) based digestate and then algae biomass was used as co-substrate for anaerobic digestion with CM. About 91% of the total nitrogen and 86% of the soluble organics in the digestate were recycled after the microalgae cultivation. The methane potential of co-digestion was evaluated by varying CM to *Ch.* 1067 ratios (0:10, 2:8, 4:6, 6:4, 8:2, 10:0 based on the volatile solids (VS)). All the co-digestion trials showed higher methane production than the calculated values, indicating synergy between the two substrates. Modified Gompertz model showed that co-digestion had more effective methane production rate and shorter lag phase. Co-digestion (8:2) achieved the highest methane production of 238.71 mL·(g VS)$^{-1}$ and the most significant synergistic effect. The co-digestion (e.g. 8:2) presented higher and balanced content of dominant acidogenic bacteria (*Firmicutes*, *Bacteroidetes*, *Proteobacterias* and *Spirochaetae*). In addition, the archaea community *Methanosaeta* presented higher content than *Methanosarcina*, which accounted for the higher methane production. These findings indicated that the system could provide a practicable strategy for effectively recycling digestate and enhancing biogas production simultaneously.

1. Introduction

Anaerobic digestion (AD) is an attractive treatment approach from livestock waste, mitigating the problem of disposal and reducing environmental impact (e.g., greenhouse gas emission), resulting in biogas and digestate production (Ebner et al., 2016; Costa Junior et al., 2015; Muscolo et al., 2017; Rodriguez-Verde et al., 2014). Digestate is rich in recalcitrant organic compounds and its management is considered the bottleneck for biogas industry. Especially the liquid digestate typically has a high content of nitrogen and would cause NH_3 volatilization and eutrophication of nearby water if it is used directly. There also existed risks to cause chemical (e.g., heavy metals) or biological (e.g., pathogens) pollution (Nkoa, 2014). Thus safe disposal of liquid digestate is extremely urgent.

Microalgae could utilize biodegradable matters in the liquid digestate as an alternative solution for its management. Microalgae cultivation could remove nitrogen (N) and phosphorus (P) from digestate by means of assimilation and precipitation. Moreover, the microalgae cultivation could also combine digestate management with bioenergy production if harvested biomass was downstream processed (Sole-Bundo et al., 2017). Golueke and Oswal made the first attempt to cultivate microalgae in digestate in the 1950s (Golueke and Oswald, 1959) and gained wide attention in future studies. Biomass productivities (dry weight) of the microalgae cultivated in animal manure-based digestate ranged from 14 to 670 mg·L^{-1}·d^{-1} (Xia and Murphy, 2016), which were comparable or even higher than those from photoautotrophic cultivation in synthetic medium (Zhu, 2015). While due to the potential impacts of digestate on the environment and human health (Nkoa, 2014), the applications of algae cultivated in digestate was limited. Unlike natural algae and medium-cultured algae, they could not be applied as food supplement and nutrients for human, livestock feed, fine chemicals for pharmaceuticals or various other applications. Algae-based biorefineries, including approaches to convert algae to multiple valuable products, such as biofuels, have been widely investigated and hold future promise. Yet, algal-biofuel approaches are hampered by the high cost of the algae feedstock production.

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http://dx.doi.org/10.1016/j.wasman.2017.09.016
0956-053X/

A sustainable utilization approach for microalgae cultivated in digestate is essential to be built. AD seems a simple and cost-effective strategy to convert algae biomass (Tan et al., 2015). However, the inherent deficiency of carbon, low biodegradability, potential ammonia inhibition and subsequently low methane yield may made the mono-digestion unfavorably economical. To improve the efficient biogas production from microalgae, different technologies have been explored. Previous studies about co-digestion of *Chlorella* with other biomass proved co-digestion an efficient approach for improving the AD process (Beltran et al., 2016; Wang et al., 2016b; Wang et al., 2013). Co-digestion means combining various substrates simultaneously and probably produces a synergistic effect because of the contribution of nutrient complement. It is considered one of the most effective approaches for increasing the efficiency of biotransformation (Dennehy et al., 2016; Li et al., 2013; Zhang et al., 2014; Khalid et al., 2011).

This study introduced a sustainable co-digestion system that integrated chicken manure (CM)-based liquid digestate management and co-digestion of algae biomass with CM. The purpose of this study is to (1) evaluate comprehensive effect of the system on recycling digestate and enhancing biogas production; (2) verify the existence of synergism during the co-digestion process; (3) demonstrate different microbial diversity in co- and mono-digestion mode using Illumina Miseq sequencing.

2. Materials and methods

The sustainable co-digestion system amplifies the algal biomass and biogas production via reuse of nutrients in the digestate. CM was firstly fed for AD process to produce biogas. The byproduct liquid digestate was recycled as media to cultivate *Chlorella* 1067 (*Ch.* 1067) and the harvested algal biomass was then added as co-feedstock to the CM based AD process (Fig. 1).

2.1. Substrate and inoculum

Ch. 1067 was cultivated in CM based liquid digestate after ultra filtration (UF) treatment in a 400 L open raceway pond. The raceway pond was equipped with a paddle wheel (HK-ZY051, SHUANGQIAO, China) with a diameter of 66 cm which was set at a constant speed of 20 r·min^{-1}. CO_2 flow rate and cycle were 1 L·min^{-1} and 12 h: 12 h. CM was a mixture of fresh chicken stool and urine collected from a commercial farm. The inoculated sludge, taken from an anaerobic digester operated for years, was enriched using glucose prior to experiment.

2.2. Test setup and design

In this study, substrate and inoculum were loaded in 250 mL flasks (hereafter called digesters) with a working volume of 200 mL, and incubated at 35 ± 1 °C in a time-controlled water-bath. Biogas produced was accumulated in an air pocket installed in each digester which was sampled once every 4 days during 1st day to 12th day, and once at 19th day, 29th day and 50th day respectively. The experiment was conducted for 50 days, when there was no biogas production for each digester. Total volatile solids (VS) of feed was 10 g in each digester with a constant feed (CM and *Ch.* 1067) to inoculum ratio of 0.5 and six CM to *Ch.* 1067 ratios (0:10, 2:8, 4:6, 6:4, 8:2 and 10:0) based on VS. The digesters were shaken twice per day through an oscillator equipped in the water bath. All the digesters were fixed by the crossing springs in the water bath. The shaking time (each time for one minute) and speed (60 r·min^{-1}) had been set in advance and automatically controlled. Assays with inoculums alone were also used as controls. The batch test was conducted in triplicates.

2.3. Analytical methods

2.3.1. Data analysis

The modified Gompertz equation (Eq. (1)) accurately describes and predicts cumulative methane yields through the AD process and has been widely applied in modeling methane production (Li et al., 2013).

$$P(t) = \mu + (P_{max} - \mu) \times \exp\left\{-\exp\frac{R \times e}{P_{max} - \mu}(\lambda - t) + 1]\right\} \quad (1)$$

where P (t) is the cumulative specific methane production (mL·(g VS)$^{-1}$) for a given time t (d). P_{max} is the specific methane production

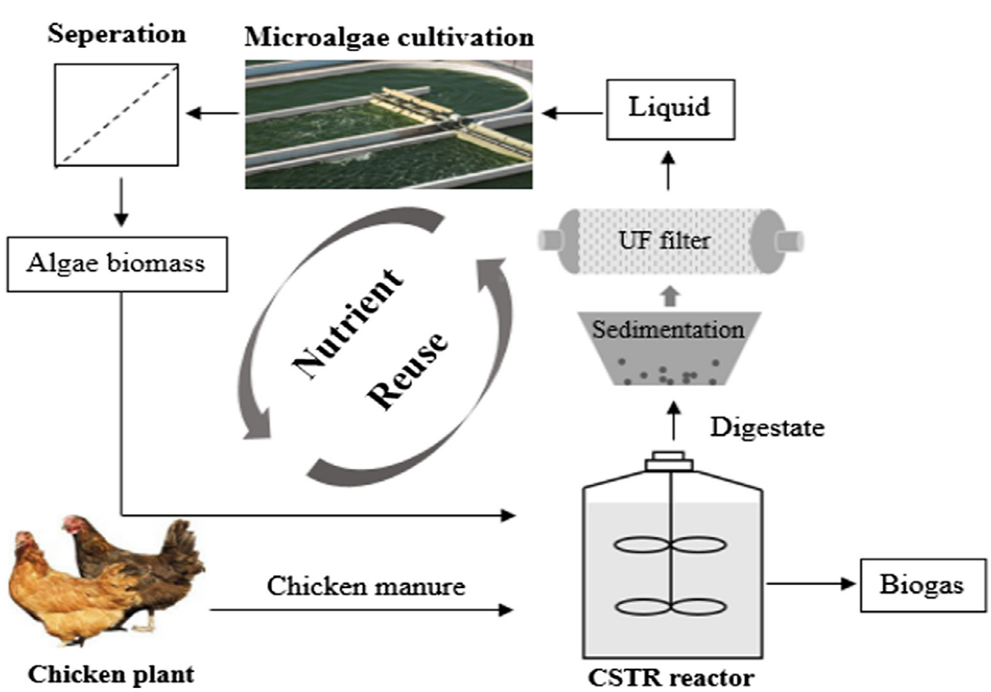

Fig. 1. Sustainable co-digestion system of chicken manure and *Chlorella* 1067 grown in recycled CM-based digestate.