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Effect of aerobic pre-treatment on hydrogen and methane production in a two-stage anaerobic digestion process using food waste with different compositions

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ABSTRACT

Aerobic pre-treatment was applied prior to two-stage anaerobic digestion process. Three different food wastes samples, namely carbohydrate rich, protein rich and lipid rich, were prepared as substrates. Effect of aerobic pre-treatment on hydrogen and methane production was studied. Pre-aeration of substrates showed no positive impact on hydrogen production in the first stage. All three categories of pre-aerated food wastes produced less hydrogen compared to samples without pre-aeration. In the second stage, methane production increased for aerated protein rich and carbohydrate rich samples. In addition, the lag phase for carbohydrate rich substrate was shorter for aerated samples. Aerated protein rich substrate yielded the best results among substrates for methane production, with a cumulative production of approximately 351 ml/gVS. With regard to non-aerated substrates, lipid rich was the best substrate for CH₄ production (263 ml/gVS). Pre-aerated P substrate was the best in terms of total energy generation which amounted to 9.64 kJ/gVS. This study revealed aerobic pre-treatment to be a promising option for use in achieving enhanced substrate conversion efficiencies and CH₄ production in a two-stage AD process, particularly when the substrate contains high amounts of proteins.

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1. Introduction

The use of renewable energy sources is a critical issue worldwide due to the serious negative environmental consequences caused by the use of fossil fuels, in addition to the proximate depletion of the latter in the near future. Anaerobic digestion (AD) is one of the most widely investigated methods used in the production of energy from different kinds of organic waste. During this process, strictly anaerobic bacteria and archaea are utilized to produce bio-fuels such as hydrogen and methane when growing on organic substrates. Hydrogen has been indicated as one of the most promising fuels for the future (Ozkan et al., 2010; De Gioannis et al., 2013). However, subsequent to anaerobic hydrogen production substrate conversion remains incomplete, with the majority remaining as a residue after the process. A promising system is represented by a two-stage AD process combining H₂ and CH₄ productions. During the first stage, organic compounds are hydrolysed and utilized by hydrogen producing bacteria to produce H₂

and volatile fatty acids (VFAs), whilst in the second stage, VFAs are used as substrates for CH₄ production by methanogens. Two-stage AD provides a positive energy yield (40–90% available energy), thus underlining the highly important process sustainability (Ruggeri et al., 2010). Several studies have demonstrated the ability of two-stage AD to improve CH₄ yields during the second stage, likely due to better hydrolysis (Liu et al., 2006; Pakarinen et al., 2011). Moreover, compared to one-stage AD, process control would be simpler and stability would be improved (Lim and Wang, 2013; Ariunbaatar et al., 2015).

During hydrolysis, the rate limiting step of anaerobic digestion, organic compounds including proteins, carbohydrates and lipids are broken down by hydrolytic bacteria into amino-acids, sugars and long chain fatty acids, respectively. Substrate pre-treatment methods are aimed at promoting and improving hydrolysis of high molecular weight compounds to readily-biodegradable constituents, and subsequently increasing the AD process product yields.

Hydrolysis occurs under both aerobic and anaerobic conditions; however, hydrolysis rates are significantly higher under aerobic conditions, likely due to the higher production of enzymes (Botheju et al., 2009). In addition, pre-aeration reduces accumulation

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of VFAs, resulting in a drop of pH during the process, thus improving the start-up stability of anaerobic digestion. Limited pre-aeration prior to anaerobic digestion has been shown to improve hydrolysis and biogas production (Charles et al., 2009; Zhu et al., 2009; Ahn et al., 2014; Cossu et al., 2016; Peces et al., 2016).

Composition of organic wastes varies according to the source from which the wastes are collected. Slaughterhouse wastes may be rich in proteins and lipids, while food wastes and organic fraction of municipal solid wastes (OFMSW) are rich in carbohydrates. An in-depth understanding of effective pre-treatment methods for each kind of waste is fundamental in improving biogas production.

To the best of the Authors' knowledge, no scientific reports have been published to date on the effects of aerobic pre-treatment on food waste with different compositions for either H₂ and/or CH₄ production in a two-stage AD process. Moreover, the effect of carbohydrate, lipid and protein content of food waste on pre-aeration efficiencies has not been addressed before. Therefore, the present work aims to study the aerobic pre-treatment effect of carbohydrate rich (C), protein rich (P), and lipid rich (L) food waste prior to two-stage anaerobic digestion on both H₂ and CH₄ production.

2. Materials and methods

2.1. Organic waste samples

Synthetic food waste samples were prepared in order to simulate industrial or municipal food waste with different compositions as indicated in a previous study (Alibardi and Cossu, 2016).

Three different substrates were prepared and classified as C, P, and L substrates. The composition of samples is shown in Table 1. The percentages are based on wet weight.

Food waste samples were shredded after preparation and characterized (Table 2) in order to have more detailed information for each substrate category.

2.2. Aerobic pre-treatment of the substrates

In order to compare the two-stage AD process with and without pre-aeration on the prepared substrates featuring different compositions, half the waste samples from each category were air injected using an aquarium pump (EIN WELTWEIT-Elite799) connected to a porous stone for better air diffusion. The air flow rate was fixed at 5 L/h using a flow meter (BROOKS SHO-RATE 1355). After 24 h, aeration was stopped. The inoculum was then added to each bottle with and without pre-treatment.

2.3. Two-stage AD – Hydrogen production

Laboratory scale tests were performed to evaluate Biochemical Hydrogen Potential (BHP) of the examined substrates. Batch tests were carried out using 1-L glass bottles which were subsequently

Table 1
Composition of synthetic food wastes (%W/W).

Ingredients	C	L	P
Tuna (%)	6.7	7.5	31.1
Butter (%)	5.5	22.3	5.5
Apple (%)	27.8	27	7.85
Banana (%)	27.8	27	7.85
Chicken breast (%)	6.7	7.5	31.1
Bread (%)	5.4	1.5	3.2
Pasta (%)	5.4	1.5	3.2
Minestrone soup (%)	14.7	5.5	10.2

Table 2
Average characteristics of food wastes with different compositions.

Parameters	C	L	P
TS (%)	28.56	30.72	43.2
VS (%TS)	95.4	96.1	97.3
TOC (%TS)	58.7	65.9	66.3
TKN (%TS)	3.34	3.05	7.98
Lipid (%TS)	16.1	41	17.3
Protein (%TS)	19.8	18.1	47.3
Glucose (%TS)	4.2	1.54	3.11
Fructose (%TS)	12.36	5.29	2.75
Sucrose (%TS)	15.56	7.42	2.78

sealed with silicon plug. Substrate concentration and food to microorganism ratio (F/M) were 5 gVS/l and 0.3 gVS/gVS, respectively. Granular sludge was used as inoculum for BHP and was collected from a full-scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy.

Heat treatment was carried out on granular sludge in a rotary water-bath incubator at a fixed temperature of 80 °C for 15 min in order to suppress methanogenic bacteria (Alibardi and Cossu, 2016). pH was set at 6.0 using phosphate buffer before the start of tests. Three main H₂-producing enzymes are used by anaerobic microorganisms: [Fe/Fe]/hydrogenases, [Ni/Fe]/hydrogenases and nitrogenases. These H₂-producing enzymes are generally all highly oxygen-sensitive and presence of oxygen may reduce their activities (Mathews and Wang, 2009). Accordingly, H₂ production should be carried out under strictly anaerobic conditions. Therefore, following aerobic pre-treatment, the bottles were flushed with N₂ gas for 3 min to ensure anaerobic conditions and incubated at a temperature of 35 ± 1 °C. All tests were performed in duplicate.

2.4. Two-stage AD – Methane production

After completing the H₂ production phase, the bottles were opened and pH, dissolved organic carbon (DOC) and VFAs were measured. Non-pre-treated granular sludge (at the same amount as the first stage) was then added to each bottle and all were sealed again, flushed with N₂ gas for 3 min, and incubated at the same initial mesophilic conditions of 35 ± 1 °C.

2.5. Analytical methods

Total solids (TS), volatile solids (VS), and TKN were analysed according to standard methods (APHA, 1999). Total organic carbon (TOC) values were calculated on the basis of the difference between total carbon and inorganic carbon present in the samples. Concentrations of carbohydrates, proteins, lipids and free sugars were obtained according to official methods (AOAC, 2003). The volume of biogas produced during the anaerobic digestion process was measured by means of the water displacement method. The produced gas composition in terms of H₂ first, and then CH₄, was analysed using a micro-GC (Varian 490-GC) equipped with an MS5A column to measure H₂ and CH₄, and a PPU column for CO₂ and two Thermal Conductivity Detectors. Argon was used as the carrier gas at a pressure of 60 kPa. Temperatures of column and injector were set to 80 °C.

VFAs concentrations were measured using a gas chromatograph (Varian 3900) equipped with a CP-WAX 58 WCOT fused silica column and a Flame Ionization Detector. Nitrogen was used as carrier gas with a flow of 4 ml/min in column. The oven temperature was set at 80 °C for the first minute and then increased at a rate of 10 °C/min to 180 °C for two minutes. Column and injector temperatures were set to 250 °C.

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