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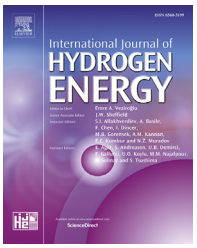

Effect of inoculum pre-treatment on mesophilic hydrogen and methane production from food waste using two-stage anaerobic digestion

Razieh Rafieenia [a], *Alberto Pivato* [b,*], *Maria Cristina Lavagnolo* [b]

[a] *Department of Industrial Engineering, University of Padova, Via Marzolo No.9, 35131, Padova, Italy*
[b] *Department of Civil, Environmental and Architectural Engineering, University of Padova, Via Marzolo No.9, 35131, Padova, Italy*

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ABSTRACT

Two-stage anaerobic digestion of food waste was performed using four different inoculum pre-treatment methods to enrich hydrogen (H_2) producing bacteria from sludge. The pre-treatments used in this study included heat shock, alkaline treatment, aeration, and a novel pretreatment using waste frying oil (WFO). Alkaline pretreatment and aeration did not completely inhibit methanogens in the first stage while no methane (CH_4) was detected in the reactors cultivated either with heat shock or WFO-pretreated inocula. The highest H_2 and CH_4 yields (76.1 and 598.2 mL/gVS, respectively) were obtained using the inoculum pretreated with WFO. The highest total energy yield (21.96 kJ/gVS) and total organic carbon (TOC) removal efficiencies (95.77%) were obtained using inoculum pretreatment with WFO. The total energy yield trend obtained using the different pretreatments was as follows: WFO > alkaline > heat > aeration > control.

Introduction

Hydrogen (H_2) has been regarded as a sustainable energy carrier and termed "fuel for the future" due to its carbon free nature that does not contribute to the emission of greenhouse gases when combusted. Furthermore, its energy yield (142 kJ/g) is 2.75 times greater than that of all carbon fuels [1]. H_2 can be produced by anaerobic bacteria in a process called dark fermentation (DF) using inexpensive organic wastes as a substrate. Subsequent to DF, a major fraction of the organic matter remains in the liquid phase in the form of organic acids that should be treated before disposal. A promising system to address this issue could be the integration of dark fermentation (DF) and anaerobic digestion (AD) which subsequently leads to waste reduction as well as enhanced bio energy recovery from organic wastes. Two-stage AD has been proven as a biological treatment method for waste stabilization and energy recovery from a wide variety of organic wastes [2–7]. The possibility of combining DF and AD is currently receiving growing interest due to the further conversion of DF effluents, shorter substrate retention time, improved bio-stabilization of organic wastes, and higher energy yields compared to single-stage AD [8–10].

Full scale studies have shown that the addition of sludge collected from wastewater treatment plants could increase the amount of energy recoverable from organic wastes [11,12]. However, pre-treatment of the sludge prior to DF is an

* *Corresponding author.*
E-mail address: alberto.pivato@unipd.it (A. Pivato).

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indispensable step to inhibit H_2 consuming populations and improve H_2 production using anaerobic mixed cultures. Different methods have been used to enrich H_2 producing bacteria and suppress H_2 consuming species include heat shock [13,14], acid or alkaline pre-treatment [15,16], addition of chemical inhibitors [17–19], ultrasonication [20], gamma irradiation [21], and aeration [2,22].

The inhibitory effect of long chain fatty acids (LCFAs) on methanogens have been shown previously [17,18,23,24] where the addition of various LCFAs to anaerobic mixed culture to suppress methanogenic H_2 consumption was studied. All of these studies showed that the inhibitory levels of LCFAs for H_2 producing bacteria are significantly higher than that for methanogens and therefore controlling the LCFAs levels in the reactor could be used as a strategy to suppress methanogenic activity. However, due to the presence of LCFAs in the DF effluents, they cannot be used for the subsequent ad. Recently, Rafieenia et al. [19], reported the inhibitory effect of LCFAs on hydrogenotrophic methanogens even after removing the LCFAs after pretreatment. These results showed that anaerobic microbiota could be pretreated with waste frying oil (WFO) as a source of LCFAs and after pre-treatment, the soluble phase containing solubilised LCFAs might be removed. LCFAs can be adsorbed on the cell wall on specific microbial populations and inhibit their growth by interfering with nutrient transfer into the cell and also facilitating the accumulation of toxic substances inside the cell [25,26].

Selection of the best inoculum pre-treatment for H_2 production depends on substrate and inoculum type. There are several studies that investigated different inoculum pre-treatments for H_2 production from glucose [16–18,27,28]. Also, there are few reports that compared different inoculum pre-treatment methods for H_2 production from complex wastes including potato and pumpkin waste [22], corn stover [16], waste ground wheat [29], and stale corn [30]. However, to the best of the authors' knowledge, no other studies have reported the effects of inoculum pre-treatment methods on two-stage AD for H_2 and CH_4 production from food waste (FW). Most of the two-stage AD studies have been performed using heat shock as the inoculum pre-treatment [31]. Therefore, in order to fill this knowledge gap, this study aims to:

1) Investigate the possibility of using anaerobic mixed cultures pretreated with WFO in two-stage AD for H_2 and CH_4 production.
2) Compare the two-stage AD of FW using WFO pretreated cultures with three common inoculum pre-treatment methods (aeration, heat shock and alkaline pre-treatment).
3) Evaluate the overall performance of two-stage AD in terms of energy yield and substrate degradation.

Materials and methods

Seed sludge and pre-treatments

Granular sludge, used as the inoculum, was collected from a full-scale Up-flow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy. Granular sludge was characterised by a Total Solids (TS) concentration of 15% and Volatile Solids (VS) concentration of 47% TS. Four different pre-treatments were used to enrich H_2 producing bacteria:

- Heat shock: Granular sludge was boiled at 90 °C for 30 min [32].
- Aeration: The granular sludge was aerated for 24 h using an aquarium pump with an air flow rate of 3 L/min [15].
- Alkaline pre-treatment: The pH of the granular sludge was adjusted to 12.0 ± 0.1 with 3 N NaOH and maintained for 24 h [6].
- Pre-treatment with WFO: a saponified WFO solution was prepared according to the method described by Rafieenia et al., [19]. Fifteen g/L of WFO was added to the granular sludge cultures (33 gVS sludge/L saponified WFO solution) and maintained for 24 h. After the treatment, the pre-treated cultures were washed three times with tap water. In order to wash the granular sludge, the pretreated cultures were remained stagnant for 30 min to precipitate the granular sludge. After which, the supernatant containing saponified WFO solution was removed using a syringe. The washing was repeated two additional times by adding tap water and removing the supernatant.

Control cultures were also prepared without any form of pre-treatment.

It should be mentioned that pre-treatment conditions for each method might vary from one study to another and optimal conditions depend on both the inoculum and substrate. Therefore, the pre-treatment conditions applied in the present study were chosen from the studies that used similar inoculum or substrate.

Substrate

Synthetic FW samples were prepared to simulate the FW composition in Italy [33]. FW samples were mixed and shredded in a kitchen mill to make a homogenous blend and then analysed. The composition and characteristics of the FW are shown in Table 1.

Two-stage anaerobic digestion tests

Two-stage batch AD tests were performed using 500 mL glass reactors with a working volume of 250 mL. In the first stage (H_2 production), pretreated cultures as well as the control received 5 gVS/L FW. Followed by a pH adjustment to 5.5 using NaOH (3 M) and HCl (3 M) [4]. Subsequently, the bottles were sealed with silicone rubber stoppers, purged with nitrogen for 3 min to ensure anaerobic conditions were achieved, and incubated in a water bath at 35 °C. After 96 h (when the biogas production ceased), the reactors were opened and samples (5 mL) were taken from each reactor and stored in a freezer (−20 °C) for further analysis. Before storage in the freezer, the samples were filtered using membrane filters with a pore size of 0.45 μm to be ready for dissolved organic carbon (DOC) and volatile fatty acid (VFA) analysis. In order to start the second stage (CH_4 production), 5 gVS/L of untreated granular sludge was added to the reactors [4]. The pH of all the reactors was

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