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## Waste Management

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# Hydrogen and methane production in a two-stage anaerobic digestion system by co-digestion of food waste, sewage sludge and glycerol

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## ABSTRACT

In this study, hydrogen and methane production from co-digestion of food waste (FW), sewage sludge (SS) and raw glycerol (GL) was evaluated in a two-stage acidogenesis-methanogenesis anaerobic system under mesophilic conditions (35 °C). The effect of glycerol addition (1 and 3% v/v) as co-substrate was assessed in ternary mixtures (FW + SS + GL), with the concentration of all substrates kept at 10 g VS/L. Besides contributing to reduce the lag phase of the acidogenic bacterial culture, the presence of GL increased the hydrogen production in all tested conditions and the maximum hydrogen yield was obtained for the FW + SS + 3%GL mixture (179.3 mL H<sub>2</sub>/g VS). On the other hand, the highest methane production (342 mL CH<sub>4</sub>/g VS) was achieved in the test supplemented with 1% GL. At 3% GL, abrupt reductions in the biogas CH<sub>4</sub> content and pH values resulting from instability in methanogenesis process were noticed over the experiment. By taking into account the hydrogen and methane production stages, the highest energy yield (i.e., 15.5 kJ/g VS) was obtained with the ternary mixture containing 1% GL. Overall, the results of this study demonstrate the feasibility of using glycerol as co-substrate to increase the H<sub>2</sub> and CH<sub>4</sub> production efficiency in a two-stage anaerobic co-digestion process, allowing simultaneous treatment of three residues (FW, SS and GL) and energy production.

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## 1. Introduction

The development of technologies for the production of clean energy from renewable sources is increasingly attracting the attention of the society nowadays. Biotransformation of organic waste into biogas can be considered an interesting alternative among the several approaches to accomplish that. In fact, many benefits can be gained from this method. From an environmental point of view, biodegradation reduces both the volume and the organic content of the waste, minimizing their impact on the ecosystem in the case of inadequate disposal. As regards to economic advantages, a high-energy renewable fuel is produced in the form of biogas while waste handling and disposal costs are reduced (Alibardi and Cossu, 2015; Han et al., 2005; Wong et al., 2014). Improvements in the management of the waste associated with the compliance with the local regulations also imply political benefits from the organic waste biodegradation.

Hydrogen (H<sub>2</sub>) has been widely recognized as a clean energy source with zero pollutant emissions and high specific energy content (142 kJ/g). Such characteristics make it an ideal alternative to fossil fuels (Levin et al., 2004). Among the biological methods to produce this gas, dark fermentation stands out because it is a simple technology and, at the same time, it allows to obtain H<sub>2</sub> from a great variety of substrates, enhancing the sustainability and viability of the production system (Das, 2009). Municipal and industrial wastes are among the most commonly used wastes in anaerobic fermentation processes due to their physical and chemical characteristics, low cost and great availability (Nathao et al., 2013). The attractiveness of municipal waste (e.g., food waste (FW), kitchen waste and paper waste) is justified by the fact that they consist essentially of organic matter. Therefore, anaerobic digestion is considered to be a feasible alternative method to both decrease the costs associated with treatment of such wastes and recover renewable energy in the form of biogas (Izumi et al., 2010).

Anaerobic digestion is a natural process that occurs in four consecutive biochemical stages in which distinct groups of microorganisms (bacteria and archaea) degrade complex organic compounds into simpler products in the absence of oxygen. In a simplified way, the anaerobic digestion process can be divided into

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two stages: fermentation and methanogenesis. The fermentation stage can be subdivided into three phases: hydrolysis, acidogenesis and acetogenesis (Palmisano and Barlaz, 1996). Hydrogen is an important intermediate in the anaerobic degradation of organic matter (Liu et al., 2006). Therefore, to enable obtaining a high hydrogen yield and consequently a gas rich in  $H_2$ , it is necessary to interrupt the anaerobic digestion process in the acidogenic phase. To achieve this, favorable operating conditions for acidogenic bacteria should be established while the activity of  $H_2$ -consuming microorganisms (both methanogenic archaea and homoacetogenic bacteria) should be prevented (Ntaikou et al., 2010). However, during the dark fermentation of organic wastes, typically only 10–20% of the energy contained in the substrate is converted into gaseous products as hydrogen and carbon dioxide, while 80–90% of the initial chemical oxygen demand (COD) such as soluble metabolic products, including volatile fatty acids (VFAs) and ethanol, remains in the liquid phase (Cooney et al., 2007; Schievano et al., 2012). Therefore, a second stage of treatment (i.e., anaerobic digestion for methane production) can be combined with the fermentative hydrogen production stage to optimize the use of the remaining organic matter and therefore increase energy recovery (Kyazze et al., 2007).

In this context, two-stage anaerobic digestion has been identified as a promising method because it allows the reduction of organic load and increases the overall energy conversion efficiency by generating two gases with high combustion power (Liu et al., 2013). The separation of hydrolysis/acidogenesis and methanogenesis phases could increase the stability of the whole process by controlling the acidification phase in the hydrogen production phase (first stage) and hence preventing the inhibition of the methanogenic population during methane generation phase (second stage) (Fu et al., 2017).

Recently, it has been shown that co-digestion of two or more substrates with complementary characteristics can result in synergistic effects, which may lead to improvements in biogas yield, process stability and costs reduction through the processing of different wastes in a single installation over traditional mono-digestion (Wu et al., 2016). Besides the optimization of anaerobic digestion reaction stoichiometry, co-digestion of different wastes can positively influence digestion performance with respect to sludge degradation. Xie et al. (2017) reported that synergistic effects can be reflected in increased biogas yields, accelerated biodegradation processes or a combination of both.

Crude glycerol is an industrial waste that has been attracting much attention worldwide due to the expansion of the biodiesel production. Consisting of a by-product of the production chain of this biofuel, the growing market for biodiesel leads to a higher amount of glycerol available in the market. Due to its high biodegradability ( $\approx 100\%$ ) and for being a carbon source easily assimilated by bacteria and yeasts under aerobic and anaerobic conditions (Mata-Alvarez et al., 2014; Thompson and He, 2006), glycerol is considered as an ideal substrate to be co-digested with other residues. Furthermore, the biological conversion of glycerol represents a promising path towards an economic viability in the biofuel sector (Fountoulakis and Manios, 2009).

Studies reported in the literature mainly focused on the sequential production of  $H_2$  and  $CH_4$  employing food waste as substrate (Chu et al., 2008; Han et al., 2005; Lee and Chung, 2010; Pisutpaisal et al., 2014; Wang and Zhao, 2009). Some have demonstrated the increase of  $H_2$  yield from the co-digestion of organic waste with sewage sludge (Kim et al., 2013a,b; Kim et al., 2004; Sreela-or et al., 2011; Tyagi et al., 2014; Zhou et al., 2013; Zhu et al., 2008a). One-stage  $H_2$  production from the digestion of organic waste or sewage sludge with crude glycerol as co-substrate were also evaluated (Fountoulakis and Manios, 2009; Rivero et al., 2014; Sittijunda and Reungsang, 2012; Zahedi et al.,

2016). Nevertheless, information on two-stage anaerobic systems for  $H_2$  and  $CH_4$  production using mixtures of more than two wastes is still scarce. The aim of this study was to perform a technical and energetic evaluation of a two-stage anaerobic co-digestion process for hydrogen and methane production employing a ternary mixture of food waste, sewage sludge and glycerol as substrate. Different glycerol concentrations (namely 1% and 3% v/v) were tested and their effect on biogas generation was assessed. Besides that, the overall energy recovery resulting from the anaerobic co-digestion process was also assessed.

## 2. Materials and methods

### 2.1. Feedstock samples

Three different wastes, namely food waste (FW), sewage sludge (SS) and raw glycerol (GL), were used as substrates in this study. Samples of FW were collected during an entire week at the university restaurant of the Federal University of Rio de Janeiro (UFRJ), Brazil. The composition of FW was as follows: Fruits and vegetables (57.4%); Grains (13.5%); Meat (bovine, poultry, fish) (26.3%) and rejected materials (bones and wastes not visually identified) (2.8%). The percentages are based on wet weight.

FW samples were shredded in a kitchen mill (Philips Walita – RI7630) and subsequently diluted with water to make a stock solution, which was then stored in a freezer at  $-20\text{ }^\circ\text{C}$  to avoid degradation that would possibly occur at room temperature. Such procedure was important to ensure that the substrate composition was maintained invariant during the experiments. After this preparation, the FW was characterized in order to have more detailed information about this substrate (Table 1). The sewage sludge (SS) used in this work was collected from the primary settler of a municipal sewage treatment plant located in Rio de Janeiro, Brazil. The sludge sample was stored in plastic containers and kept under refrigeration at  $4\text{ }^\circ\text{C}$ . Before each test, the sludge was removed from the refrigerator and maintained at room temperature.

Crude glycerol (GL) originated from the transesterification of soybean oil (60%) and animal fat (40%) was supplied by a research center (CENPES/PETROBRAS), located in Rio de Janeiro, Brazil. To prevent possible disturbances in the experiments related to overloading and microorganisms inhibition, the glycerol was diluted with water to make a stock solution of 10 g VS/L, which was then stored at room temperature. The characterization of the three substrates (FW, SS and GL) used in the several assays is shown in Table 1.

### 2.2. Inoculum

The inoculum used in this study consisted of anaerobic digester sludge obtained from a municipal sewage treatment plant, located in Rio de Janeiro, Brazil. The pH and volatile solids (VS) concentration of the inoculum were approximately 7 and 28.3 g/L,

**Table 1**  
Characteristics of different wastes used as feedstock in this study.

Parameter	Food waste	Sewage sludge	Crude glycerol
Moisture (%)	72.6	–	16.3
pH	4.0	6.5	6.3
COD (g/L)	93.9	38.7	1023.3
TS (g/L)	140.3	41.1	1000.7
VS (g/L)	135.9	22.3	947.7
Carbohydrate (%SV)	43.5	28.4	–
Protein (%SV)	18.4	11.0	–
TN (mg/L)	4210.0	476.0	–
$NH_4\text{-N}$ (mg/L)	200.7	83.7	–
Glycerol (%)	–	–	70

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