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# Effect of total solid content and pretreatment on the production of lactic acid from mixed culture dark fermentation of food waste

### Ahasa Yousuf, Juan-Rodrigo Bastidas-Oyanedel \*, Jens Ejbye Schmidt<sup>1</sup>

Department of Chemical and Environmental Engineering, Khalifa University of Science and Technology, Masdar Institute, Masdar City, P.O. Box 54224, Abu Dhabi, United Arab Emirates

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#### 1. Introduction

Excessive generation of the waste is currently a major issue worldwide due to urbanization and human population growth. The food waste composition of the world municipal solid waste is on average 40%, with a range from 23% to 67.5% (Hoornweg & Bhada-Tata, 2012). According to United Nations Food and Agriculture Organization (FAO), 1.3 billion tonnes of food were wasted in 2011 (FAO, 2011). If used in landfill, the high moisture and volatile solid content of food waste can cause environmental degradation by greenhouse gases emission, odor and ground water contamination. This situation calls for efficient resource recovery from the food waste to both minimize the environmental issues associated, and also create value from waste.

Dark fermentation (DF), a bioprocess, can convert the food waste into carboxylic acids, for example lactic, propionic, butyric, acetic, and valeric acid and solvents in the liquid phase, and bio-hydrogen and carbon dioxide in the gas phase (Bastidas-Oyanedel et al., 2015). The acids produced, when separated from the broth, can be used as platform chemicals for the production of alcohols, bio-hydrogen and bioplastics and can also be used as pure acids in various applications including food, pharmaceutical

<sup>1</sup> Post-publication corresponding author.

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#### ABSTRACT

Food waste landfilling causes environmental degradation, and this work assesses a sustainable food valorization technique. In this study, food waste is converted into lactic acid in a batch assembly by dark fermentation without pH control and without the addition of external inoculum at 37 °C. The effect of total solid (TS), enzymatic and aeration pretreatment was investigated on liquid products concentration and product yield. The maximum possible TS content was 34% of enzymatic pretreated waste, and showed the highest lactic acid concentration of 52 g/L, with a lactic acid selectivity of 0.6 g<sub>lactic</sub>/g<sub>totalacids</sub>. The results indicated that aeration pretreatment does not significantly improve product concentration or yield. Non-pretreated waste in a 29% TS system showed a lactic acid concentration of 31 g/L. The results showed that enzymatic pretreated waste at TS of 34% results in the highest production of lactic acid.

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and chemical industries (Agler et al., 2011; Bastidas-Oyanedel et al., 2015; Dahiya et al., 2015). Among the biological processes available, dark fermentation for carboxylic acid production shows the promise of practical viability due to its high market value in comparison to methane, animal feed and other products obtained from different bioprocesses (Bastidas et al., 2016; Vanwonterghem et al., 2015). Moreover, DF is a mixed culture fermentation (MCF), and therefore does not require sterile operating conditions and has the potential to consume a wide spectrum of substrates containing diverse organic compounds (Bastidas-Oyanedel et al., 2015; Jankowska et al., 2015).

Dark fermentation of food waste to carboxylic acids involves a series of biological mediated reactions under anaerobic conditions (Aceves-Lara et al., 2008; Boe et al., 2003; Jankowska et al., 2015). The mechanisms behind the product spectrum shifts of DF are not yet clear (Bastidas-Oyanedel et al., 2012; Bastidas-Oyanedel et al., 2008; Hoelzle et al., 2014; Temudo et al., 2007). A considerable body of research has already been conducted to control and optimize the product spectrum of MCF with the aid of modeling (Iwa et al., 2001). However, these models have been made on assumptions and require experimental support to be applied on a complex substrate such as food waste for optimization (Jankowska et al., 2015). Future interest in research is focused on the maximization of food waste utilization, without losing the specificity of produced carboxylic acid. In this regard, Chen et al. (2017a) have maximized the production of total volatile fatty acids with a maximum con-

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<sup>\*</sup> Corresponding author.

*E-mail addresses:* ahasa.yousuf@gmail.com (A. Yousuf), jbastidas@masdar.ac.ae (J.-R. Bastidas-Oyanedel), jschmidt@masdar.ac.ae (J.E. Schmidt).

centration of acetic acid of 2.7 g/L, at 55 °C mixed culture fermentation, when stepwise increasing the reaction pH from 7 to 9, using sewage sludge as feedstock with a concentration of 2.7 g/l of total suspended solids (approximately 0.3% TS). Chen et al. (2017b) have also obtained high purity propionic acid (16 g/L, with 90% purity) in a mixed culture fermentation of glucose using different levels of ammonium.

Another important topic to investigate is the purification and esterification of produced carboxylic acids, in order to produce alcohols. Esterification of carboxylic acids has been studied in organic solution using diverse catalysts then (Kaur & Ali, 2015; Liu & Wu, 2016; Park et al., 2010; Rezende & Pinto, 2016). The main challenge is that carboxylic acids produced by fermentation are in aqueous solution. These aqueous solutions then need to be purified and concentrated in an organic solvent, in order to be used in the available esterification technologies.

Total solid (TS) content is one of the parameters that can affect the MCF process. Several studies have been performed to evaluate the effect of TS content on the production of methane, yet to date, there have been no studies conducted for carboxylic acids. The other obstacle of this promising MCF technology is low product yield. Pre-treatment methods have been found to have a positive influence on the yield of carboxylic acid and thus, have become a point of interest for various researchers (Breton-Toral et al., 2016; Liu et al., 2016; Liang et al., 2014, 2015; Yin et al., 2014; Yu et al., 2014).

The objective of the study was to investigate the effect of TS content on the carboxylic acids spectrum, focusing on lactic acid from food waste. The study utilized industrial enzymes for waste degradation and investigated its effect on the product yield. It also investigated the effect of aerobic pretreatment, which involves the supply of air into the waste and is aimed at increasing the growth of microorganisms naturally present in the waste. Thus, in conclusion, the study investigated the effect of TS content, and enzymatic, and aerobic pretreatment on the product yield and product spectrum of the mixed culture fermentation of food waste. The experiments were performed in triplicate in a batch operation in a pH uncontrolled environment. pH uncontrolled fermentation was conducted as it results in an acidic environment at the end of fermentation, and makes the recovery of carboxylic acids feasible (Tang et al., 2016; Yousuf et al., 2016).

#### 2. Materials and methods

#### 2.1. Food waste

Synthetic food waste was used in order to reproduce experimental results. The synthetic food waste was prepared mimicking real food waste, based on the work of Nwobi et al. (2015). It contained 11% rice, 5% pasta, 7% potatoes, 2% corn, 4% bread, 1% pineapple, 1% apple, 1% carrot, 2% cucumber, 3% lemon, 1% pawpaw, 6% tomatoes, 3% pickles, 6% meat, 14% fish, 6% dairy, 2% cabbage, 2% lettuce, 2% okra, 2% eggplant, 2% cauliflower, 2% broccoli, 13% vegetable oil, 1% newspaper, 0.6% cardboard, and 0.4% A4 paper in terms of weight. The food was cut into small pieces, cooked, and then mixed thoroughly in the weight ratio mentioned above. Its composition, in g/100g\_TS has carbohydrates, 22.93; protein, 1.99; fats, 18.01; fibers, 2.49; ash, 4.03, as calculated by Nwobi et al. (2015). A large quantity of food was prepared and then stored at 4 °C, to prevent spoilage.

#### 2.2. Batch fermentation

Batch fermentation experiments were performed in triplicate and were carried in 500 mL glass bottles. After adding the corresponding food waste, the bottles were flushed with nitrogen gas to obtain anaerobic conditions. Bottles were capped with rubber stoppers and brewing airlocks. The initial pH for the food waste was found to be  $4.4 \pm 0.1$ . The bottles were kept at 37 °C, in an orbital shaker at a stirring speed of 120 rpm, without pH control, for 7 days. No external inoculum and nutrients were added.

#### 2.3. Effect of total solid content on food waste fermentation

The experiments were performed in semi-dry and dry conditions. In general, wet reactors have <10% TS, semi-dry with (10-20% TS) and dry with (>20% TS) (Pognani et al., 2009). The experimental conditions to investigate the effect of percentage of total solid were categorized into three sets: (1) Semi-dry fermentation with the total solids of  $19.0 \pm 1\%$ ; (2) Dry fermentation with the total solids in a range of 29.0 ± 1%; and (3) Extreme dry fermentation with the total solids of 34% ± 1%. This was the maximum possible TS content, as the food waste prepared had an approximate TS content of 34% and the remaining 64% was water present naturally in the waste. The extreme dry fermentation was only investigated for enzymatic pre-treated waste. The extreme dry fermentation condition was not possible to be tested for any other condition due to extreme dry environment, sampling was difficult because of absence of free fluids. A fixed amount of food waste, 100 g, was added in all the bottles, and then diluted with variable amount of water to achieve TS content of  $19 \pm 1\%$ ,  $29 \pm 1\%$  and  $34 \pm 1\%$ . The total solid concentration of the different TS fermentation was, in g TS/L: 180.0, 243.7 and 284.34 for 19.0 ± 1%, 29.0 ± 1% and 34.0 ± 1% of TS, respectively. The batch fermentation was performed as described in Section 2.2.

#### 2.4. Effect of enzymatic pretreatment

The enzymes were obtained from Novozymes A/S Bagsværd, Denmark. The mixture of enzymes contained in weight basis, 34.0% cellulase, 37.0% amalyse, 6.8% hemicellulose, 7.1% pectate lyase, 7.3% lipase and 7.4% protease. The enzymes codes as provided by the manufacturer are cellulase NS81210, amalyse NS81217, hemicellulase NS81233, pectate lyase NS81215, lipase NS81022 and protease NS81220 (Kolbl & Stres, 2016). For all the experiments performed, 1.631 g enzymes mixture/100 g of food waste was used, as suggested by (Nwobi et al., 2015). This ratio was selected based on the manufacturer's (Novozymes A/S, Denmark) recommended loading range.

The enzymatic hydrolysis was carried out in dry conditions with a TS content of 29% and extremely dry conditions with a TS content of 34%. Enzymatic hydrolysis was carried out at 50 °C, as per manufacturer's recommendation, for 24 h in an orbital shaker at 120 rpm. After enzymatic hydrolysis, batch fermentation was conducted as described in Section 2.2. The total process time was seven days, with enzymatic hydrolysis conditions for one day and batch fermentation for six days.

#### 2.5. Effect of aeration pretreatment

The effect of aeration pretreatment was performed using an air pump with a constant flow rate of 1.5 L/min. Air was sparged at the bottom of the glass bottles (as in Section 2.2) containing food waste for 24 h. During the aeration, the glass bottles were placed in a water bath at 37 °C. Aeration was performed without any addition of specific inoculum. Aerobic conditions would activate the growth of aerobic and/or facultative microorganisms present naturally in the food waste. Batch fermentation, as described in Section 2.2, was performed after the aeration pretreatment. The total process time was seven days, with aeration pretreatment con-

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