



# Acidogenic fermentation of food waste for volatile fatty acid production with co-generation of biohydrogen



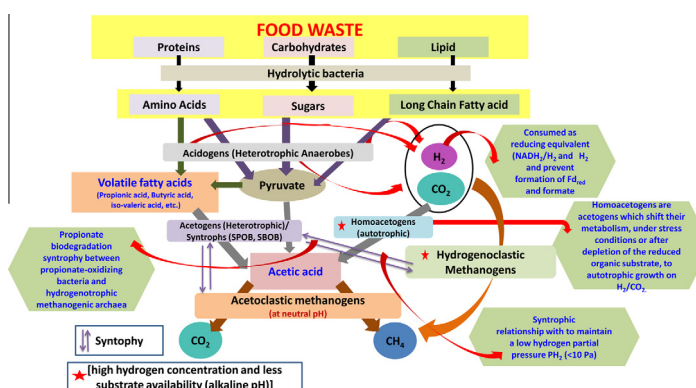
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## HIGHLIGHTS

- VFA production from food waste fermentation at variable redox conditions.
- Short chain carboxylic acids synthesis was higher at alkaline pH.
- Higher H<sub>2</sub> production was also observed at alkaline pH.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 16 October 2014  
 Received in revised form 29 December 2014  
 Accepted 3 January 2015  
 Available online 20 January 2015

### Keywords:

Degree of acidification  
 Short chain carboxylic acids  
 Volatile fatty acids (VFA) platform  
 pH  
 Buffering capacity

## ABSTRACT

Fermentation experiments were designed to elucidate the functional role of the redox microenvironment on volatile fatty acid (VFA, short chain carboxylic acid) production and co-generation of biohydrogen (H<sub>2</sub>). Higher VFA productivity was observed at pH 10 operation (6.3 g/l) followed by pH 9, pH 6, pH 5, pH 7, pH 8 and pH 11 (3.5 g/l). High degree of acidification, good system buffering capacity along with co-generation of higher H<sub>2</sub> production from food waste was also noticed at alkaline condition. Experiments illustrated the role of initial pH on carboxylic acids synthesis. Alkaline redox conditions assist solubilization of carbohydrates, protein and fats and also suppress the growth of methanogens. Among the carboxylic acids, acetate fraction was higher at alkaline condition than corresponding neutral or acidic operations. Integrated process of VFA production from waste with co-generation of H<sub>2</sub> can be considered as a green and sustainable platform for value-addition.

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## 1. Introduction

Among the biological routes, acidogenic fermentation (heterotrophic-dark) process for biohydrogen (H<sub>2</sub>) production shows the promise of practical viability due to its feasibility of utilizing different types of wastes as feedstock. In acidogenic microenvironment, monomers are formed from hydrolysis of organic compounds by

hydrolytic microorganisms and thus lead to the formation of H<sub>2</sub> along with a mixture of low-molecular-weight organic acids (volatile fatty acids (VFA) or carboxylic acids) and CO<sub>2</sub> as major fraction (Venkata Mohan, 2009). Bacterial hydrogen gas (H<sub>2</sub>) production is the consequence of transfer of cellular reduction equivalents, i.e. electrons (e<sup>-</sup>), onto protons (H<sup>+</sup>) and hence H<sub>2</sub> generation occurs with minimal energy requirement in the anaerobic process (Srikanth and Venkata Mohan, 2014). This H<sub>2</sub> when extracted from the system can be used as a fuel but when retained in the system it acts as an electron donor to produce both acetic acid (homoacetogenesis) and methane (hydrogenoclastic methanogenesis)

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(Noori and Saady, 2013) where CO<sub>2</sub> is the electron acceptor. Acidogenic bacteria facilitate the formation of acetic acid (C2), propionic acid (C3), butyric acid (C4) and valeric acid (C5), etc., and the higher chain fatty acids (C3 and above) are further oxidized to acetic acid through the action of syntrophic bacteria (H<sub>2</sub>-producing acetogenic bacteria). Acetic acid can also be produced by homoacetogens utilizing H<sub>2</sub> and CO<sub>2</sub>. H<sub>2</sub> and VFA are the two important value added products of acidogenic fermentation which if harvested properly in an integrated approach will make the whole process environmentally sustainable and economically viable. Acidogenic H<sub>2</sub> production was well studied and documented with various feedstock and at present it is at the stage of upscaling (Pasupuleti et al., 2014; Chandra and Venkata Mohan, 2014; Venkata Mohan et al., 2008). These short chain carboxylic acids can be utilized further as they are building blocks of various organic compounds including alcohols, aldehydes, ketones, esters, olefins, etc. (Singhania et al., 2013). By implementing appropriate methods, VFA can be also converted to alcohols (Uyar et al., 2009), biohydrogen (Srikanth et al., 2009) bioplastics (Cai et al., 2009; Venkata Mohan et al., 2010; Reddy et al., 2012; Amulya et al., 2014), microalgal lipids (Venkata Mohan and Devi, 2012), bioelectricity (Mohanakrishna et al., 2010), aldehydes (Spirito et al., 2014; Silva et al., 2013), etc. and are also used as preservatives in food and beverage industry and in the synthesis of pharmaceutical/chemicals.

Earlier reports documented anaerobic acidification of waste materials viz., urban organic waste, sludge, sugar beet processing waste, vinasse and vegetable waste as primary feed stocks for VFA production with different degrees of success (Singhania et al., 2013; Cai et al., 2004; Dinamarca et al., 2003). However, for the substrate to be viable as feedstock, it should be available in a reasonably good amount and should have high biodegradability and carbon load. Moreover, wastes containing the above conditions if used as a feedstock, shall contribute greatly to the ecological and economic efficiency of the process. In this realm, canteen based food waste which fulfils the above criteria can be thought of as potential feedstock for acidogenic fermentation. One third of the food produced globally for human consumption is wasted and lost accounting to around 1.3 billion tonnes. An attempt was made to study the viability of carboxylic acid synthesis under uncontrolled redox condition through fermentation of food waste coupled with the H<sub>2</sub> production towards hydrogen-carboxylate platform development. Open microbiome batch experiments were designed to assess the carboxylic acid synthesis and associated H<sub>2</sub> production at variable redox conditions (pH, 5–11). The major objective of this study was to establish optimum redox conditions for higher fatty acid productivity.

## 2. Experimental methodology

### 2.1. Anaerobic consortia

Anaerobic consortium collected from full scale anaerobic reactor treating sewage wastewater was used as parent inoculum. The sludge (3.6 g VSS/l; 40 ml) after removing grit was enriched with food waste (15 kg COD/m<sup>3</sup>-day; 48 h; pH, 7) for four cycles prior to inoculating the bioreactors.

### 2.2. Waste

Composite food waste was collected from institute canteen. The collected food waste after removing non-food particles was masticated using electrical blender and then filtered through a stainless steel sieve to remove coarse materials that may cause clogging problems. Oil present in the waste was separated using an oil-sep-

arating system that works based on the gravity. Oil interferes in the biological activities of the inoculum and covers the carbohydrate content of the food waste hence making it unavailable for further digestion. The oil free waste was used as a feedstock after adjusting pH and organic load (OL) by diluting with domestic sewage. Chemical oxygen demand (COD) of non-diluted waste has 4900 kg COD/l with a reasonably good biodegradable fraction (BOD/COD) of 0.72. All the experiments were performed at an organic loading rate of 15 kg COD/m<sup>3</sup>-day.

### 2.3. Experimental details

Seven identical bench scale anaerobic reactors were fabricated using borosilicate-glass bottles to have a total/working volume of 0.5/0.4 l and gas holding capacity of 0.1 l. The reactors were operated in suspended growth configuration in batch mode for 6 cycles. Each batch was operated with 48 h of retention time comprising of 20 min of FILL phase, 47 h of REACT (anaerobic) phase, 20 min of SETTLE phase and 20 min of DECANT phase in sequencing/periodic discontinuous mode. All the reactors were operated at an ambient temperature (28 ± 2 °C) with organic load rate 15 kg COD/m<sup>3</sup>-day to study the relative efficiency of VFA production as a function of pH. Prior to operation, the pH of each reactor was adjusted to 5, 6, 7, 8, 9, 10, and 11 using 1 N HCl or 1 N NaOH. Nitrogen gas was sparged into the reactor for 5 min after every feeding and sampling event to maintain anaerobic conditions. The reactors were kept in suspension mode during REACT phase by continuous mixing (100 rpm). Prior to startup, all the reactors were inoculated with 10% of inoculum.

### 2.4. Analysis

Carboxylic acid composition was analyzed using high performance liquid chromatography (HPLC; Shimadzu LC10A) employing UV-Vis detector (210 nm) and C18 reverse phase column (250 × 4.6 mm diameter; 5 μm particle size, flow rate: 0.6 ml/h; wave length: 210 nm). Mobile phase of (40% acetonitrile in 1 mM H<sub>2</sub>SO<sub>4</sub>; pH, 2.5–3.0) and 20 μl sample injection was used. Biogas composition was monitored using gas chromatography (GC; NUCON 5765) using thermal conductivity detector (TCD) with 1/8" X 2 m Heysep Q column employing Argon as carrier gas. The injector and detector were maintained at 60 °C each and the oven was operated at 40 °C isothermally. Chemical oxygen demand (COD-closed refluxing titrimetric method), VFA and pH were estimated by the standard methods. (APHA, 1998) Buffering capacity (β) was estimated based on the acid-base titrations employing auto-titrator (Mettler Toledo DL50). (Velvizhi and Venkata Mohan, 2014). The sample was divided into two parts of 3 ml each prior to the test. The first part was titrated with 0.1 N HCl till the end point at pH = 1.9 and the second part was titrated against 0.1 N NaOH till the end point of at pH = 12. The buffering capacity (β) was calculated using the equation, where, C is the concentration of acid or base (mol), V<sub>s</sub> is the volume of sample (ml), m is the slope of tangent on curve (Eq. (1))

$$\beta = \frac{C}{V_s \times m} \quad (1)$$

## 3. Results and discussion

### 3.1. Fatty acids

Experiments illustrated variation in total carboxylic acids (VFA) concentration and composition as a function of initial pH with respect to operation time (Fig. 1a). VFA production was analyzed at every 12th h of the operating cycles. It was observed that in

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