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Simultaneous hydrolysis and fermentation of unprocessed food waste into ethanol using thermophilic anaerobic bacteria

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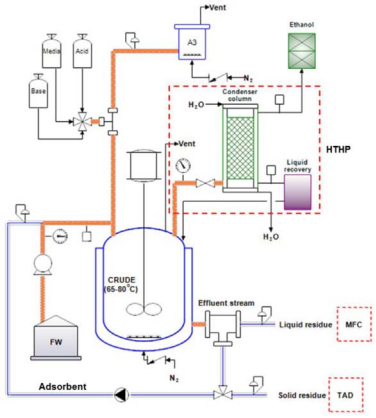
GRAPHICAL ABSTRACT

A3: *Thermoanaerobacter mathranii* A3; FW: Food waste; MFC: Microbial Fuel Cell; TAD: Thermophilic Anaerobic Digestion.



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ABSTRACT

The one-pot CRUDE (Conversion of Raw and Untreated Disposal into Ethanol) process was developed for simultaneous hydrolysis and fermentation of unprocessed food waste into ethanol using thermophilic (growing at 65 °C) anaerobic bacteria. Unlike existing waste to energy technologies, the CRUDE process obviates the need for any pre-treatment or enzyme addition. A High-Temperature-High-Pressure (HTHP) distillation technique was also applied that facilitated efficient use of fermentation medium, inoculum recycling, and in-situ ethanol collection. For material balancing of the process, each characterized component was represented in terms of C-mol. Recovery of 94% carbon at the end confirmed the operational efficiency of CRUDE process. The overall energy retaining efficiency calculated from sugars to ethanol was 1262.7 kJ dry weight kg^{-1} of volatile solids using HTHP. These results suggest that the CRUDE process can be a starting point for the development of a commercial ethanol production process.

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1. Introduction

The disposal rate of food scraps in the United States (US) has reached an alarming level of 35 million tons per year (FAO, 2013). The Food and Agriculture Organization has reported that nearly 50% of the food produced in the US was wasted either before or after reaching the consumer, accounting for over 1.3 billion tons per year of food globally produced (Gustavsson et al., 2011). With the growing population, it has been estimated that 27 Terawatt of energy will be required annually by 2050 for food production, disposal, and other anthropogenic activities across the globe (IEA, 2011). Per capita food waste in the US (0.30 kg/day) is comparable to developing countries such as Taiwan (0.27 kg/day), Singapore (0.30 kg/day), South Korea (0.35 kg/day), and Hong Kong (0.45 kg/day) (Chu & Majumdar, 2012; Peter and Peter, 2010).

Anaerobic decay of food waste in landfills results in 3.3 billion tons of greenhouse gases on an annual basis (Paritosh et al., 2017). The US alone accounts for 19% of the world's total greenhouse gas emissions. Uncontrolled release of greenhouse gases during food waste processing could worsen the global environmental balance. There is an urgent need to develop new methods for recycling food waste into energy with minimal emission of greenhouse gases. Development of any technology which could convert food waste into high energy density biofuel would have multiple advantages: (i) handling the food waste disposal issue; (ii) reducing dependence on fossil fuels by generating renewable energy from waste; and (iii) reducing greenhouse gas emissions.

Clostridium as well as *Thermoanaerobacter* spp. are garnering the attention of thermophilic industrial processes because of their natural potency to ferment a broad range of sugars into ethanol (Lynd et al., 2008). However, the ability of *Thermoanaerobacter* sp. to ferment hemicellulose constituent sugars and its ability to tolerate high titers of ethanol can make it a preferable choice over *Clostridium* sp. Moreover, the presence of three functionally characterized alcohol dehydrogenase (*adh*) genes i.e. *adhA*, *adhE*, and *adhB* for ethanol production and the evolutionary advantage of its carbohydrate transport system further makes *Thermoanaerobacter* sp. an ideal strain for developing a CRUDE process. Hence, we hypothesize that an one-pot CRUDE process can be developed using thermophilic *Thermoanaerobacter* sp., which obviates the need for pre-processing of food waste and enable high substrate loading to improve the economics of ethanol production. An ethanol recovery High-Temperature-High-Pressure (HTHP) technique is also demonstrated to make the entire process continuous and to reutilize the thermophilic inoculum. The detailed techno-economic analysis of the proposed process is also performed to evaluate its commercialization prospects.

2. Materials and methods

2.1. Characterization of the microorganism

Thermoanaerobacter mathranii A3 was routinely cultivated using the DSMZ media at 65 °C, pH 6.8, under shaking conditions (150 rotation per minute) (Sebayang et al., 2016). The purity of the culture was confirmed using 16S ribosomal DNA (rDNA) sequence analysis as described (Bhalla et al., 2015). The specific growth rate (μ) was determined using previously published method (Sawada & Nakamura, 1987). For the reactor scale CRUDE process, 1.8–2.2 g dry weight (DW) L^{-1} (volatile solids = 1.98 g L^{-1}) of actively growing *T. mathranii* A3 cells were inoculated.

The activities for endoxylanase, laccase, endoglucanase, beta-glucosidase, pectinase, amylase, lipase, protease and total protein concentrations were determined by using previously described methods (Dhiman et al., 2015; Dhiman et al., 2012).

Prior to elemental (carbon, hydrogen, and nitrogen) analyses, the cell mass was repeatedly washed with nanopure water (Barnstead water system with a resistivity of 17.6 MV cm) to remove any trace of media components. All the samples for elemental analyses were sent to Georgia Elemental Lab, (SD, USA).

Growth inhibition due to ethanol, lactic acid, or acetic acid were experimentally determined by incubating the growing *T. mathranii* A3 cells in DSM media with different concentration of ethanol, acid by-products. Growing *T. mathranii* A3 cells were incubated at 65 °C, for up to 48 h at 150 rotation per minute (RPM).

2.2. Characterization of food waste materials

Multiple food waste samples (~20 kg dry weight) were collected from six different locations in Rapid City, SD during a period of 16 months. These food waste samples were directly added to the reactor. The following characteristics of each waste sample were determined in series of tests: i) dry weight; ii) elemental analysis; iii) total carbohydrate composition using Anthrone reagent test (Urbansky, 2001), iv) volatile fatty acid (VFA) (Vohra et al., 2015), v) chemical oxygen demand (COD), vi) total organic carbon (American Public Health Association procedures), vii) total nitrogen through Kjeldahl method, viii) volatile solid (VS) contents (Passos et al., 2014), ix) ash content (Dhiman et al., 2017) and x) soluble sugars, polysaccharides, and insoluble sugars were individually determined according to standard National Renewable Energy Laboratory (NREL) procedures (Sluiter et al., 2011).

2.3. Ethanol production through CRUDE process

Initial CRUDE experiments were carried out in serum bottles (500 mL) at 65 °C, pH 7.0 under shaking conditions (50 rpm) for up to 13 days using food waste (5 g DW per 100 mL) as a source for carbon and energy. The serum bottles were crimped with aluminium seals and purged with pure N_2 for 15 min at 7 psi. After optimization of physicochemical parameters (e.g., pH, temperature, agitation, loading rates etc.) at serum bottle level, CRUDE experiments were performed using a Cell Ferm Pro, Eppendorf, 1 L capacity. During the fermentation process, nutrient supplementation and pH controls were optimized for enhanced ethanol concentration.

The ethanol productivity (Q) was calculated using the following equation:

$$Q(\mathrm{g\,L^{-1}h^{-1}}) = E_t - E_0/t$$

where E indicates total ethanol produced during fermentation (g L^{-1}), E_t is ethanol production after time "t" and E_0 is ethanol production at "0" time.

Ethanol specific productivity (q) was calculated using the following equation: (Mishra et al., 2016)

$$q = Q_t/X_t$$

where q: specific ethanol productivity in g g^{-1} h^{-1}; Q_t: ethanol productivity after time t; X_t: biomass (g L^{-1}) after time t.

Fermentation samples were collected every 24 h and analyzed to measure the concentrations of ethanol, sugars, VFA, and phenolic compounds (PCs). The Folin-Ciocalteau method was used to estimate total phenol, and results were expressed as grams of catechol equivalents per liter of liquid phase (Slinkard & Singleton, 1977). HPLC analysis was used to determine sugars and VFA using an Aminex column operated at 60 °C with 0.05 mM H_2SO_4 mobile phase at 0.45 mL min^{-1} flow rate (Vohra et al., 2015).

2.4. Ethanol distillation through high temperature high pressure (HTHP) technique

A glass column (50 × 8 cm) distillation assembly was combined with bench top reactors for distilling ethanol using a novel high temperature high pressure (HTHP) method. For distillation, 80 °C temperature and 8 psi pressure were maintained through continuous purging of industrial grade N_2 gas. A sterilized ceramic sparger was