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Anaerobic digestion of food waste using yeast

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ABSTRACT

Fermentative breakdown of food waste seems a plausible alternative to feeding food waste to pigs, incineration or garbage disposal in tourist areas. We determined the optimal conditions for the fermentative breakdown of food waste using yeast (*Saccharomyces cerevisiae*) in incubations up to 30 days. Yeast efficiently broke down food waste with food waste loadings as high as 700 g FW/l. The optimum inoculation was ≈46 × 10^6 cells/l of culture with a 40 °C optimum (25–40 °C). COD and BOD were reduced by ≈30–50%. Yeast used practically all the available sugars and reduced proteins and lipids by ≈50%. Yeast was able to metabolize lipids much better than expected. Starch was mobilized after very long term incubations (>20 days). Yeast was effective in breaking down the organic components of food waste but CO_2 gas and ethanol production (≈1.5%) were only significant during the first 7 days of incubations.

1. Introduction

Food waste from restaurants and hotels is an increasing environmental problem, particularly in tourist areas. Restaurant waste consists of restaurant discards, waste from food preparation, large amounts of oils and fats with some paper (serviettes) and wood (chopsticks and toothpicks) but would be largely free of heavy metal contaminants (lead, cadmium, mercury) (Han and Shin, 2002; Cirne et al., 2006; Forster-Carneiro et al., 2007). Food waste can be defined as any edible waste from food production, transportation, distribution and consumption. It is also referred to as garbage, swill and/or kitchen refuse, solid and liquid by-product wastes. They are generated throughout food production and processing sectors. In total, food waste generated from food preparation may constitute as much as 20% of the total human food supply from the stage of processing to the point of consumption (Westendorf et al., 1998). The low heavy metal content of restaurant waste distinguishes it from household garbage which often has high heavy metal levels rendering the breakdown residues of biological digestion processes too contaminated with heavy metals to be readily useable. Zhang et al. (2007) identified food waste as an excellent feedstock for fermentation digestion processes.

In Thailand a large component of the waste is cooked rice (as found for Korean restaurant waste, Han and Shin, 2002) which would be partially hydrolysed from cooking but would be expected to require further treatment to mobilize the starches. Davis (2008) used amylase treatment to mobilize the starch in food waste. Similar methods have been used for preparing corn paste wastes (Akpan et al., 2008) and sweet potato processing waste (Qian et al., 2008) but acid hydrolysis has also been used to mobilize both starches and cellulosic material such as corn cobs, peanut shells and newspaper (Akpan et al., 2005, 2008). Sawayama et al. (1997) used high temperature and pressure to thermally hydrolyse food waste prior to fermentation treatment.

The advantage of acid hydrolysis is that it mobilises both starches and some of the cellulose and many other organic compounds. The obvious drawbacks to acid hydrolysis are that it is expensive, involves bulk handling of acids which are highly corrosive and might mobilise undesirable toxic metals and finally the acid hydrolysate has to be neutralized before being inoculated with microorganisms. Han and Shin (2002) used microbial populations from ruminants as a source of acidophile microbes to break down cellulose and starches from Korean restaurant waste, however the main products from such fermentation were acetic and butyric acids. These are not high value products. Lipids of various kinds (fats and oils) are a major component of restaurant wastes (Cirne et al., 2006) and present particular problems for both physical reasons and in their metabolism. Yeasts and methanogen bacteria can

Abbreviations: FW/l, fresh weight per litre; BOD, Biochemical Oxygen Demand; COD, Chemical Oxygen Demand; TS, total solid; °C, degree celsius; BSA, bovine serum albumin; GC, gas chromatography; CO_2, carbon dioxide; mmol, millimoles; ml, millilitre; EtOH, ethanol.

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use hydrolysis products of fats and lipids but they are generally thought not to be able to rapidly ferment unprocessed lipids. There are also physical problems arising from fats and greases in food waste: fat saturated materials aggregate into grease balls and fats and greases deposit themselves on the walls of plumbing and storage vats. Physical problems arising from fats and grease balls may prove to be a major limitation to using microbial methods to process restaurant waste.

Food waste is a complex biomass containing various components such as starchy, fatty, and cellulosic materials. Without some sort of processing, these organic polymer materials may be difficult for ethanol producing microorganisms such as *Saccharomyces cerevisiae* (common yeast) to utilize. Food waste generated in Korea is rich in carbohydrate, as high as 65% of total solids due to its high proportion of cooked rice (Kim et al., 2008).

Food waste is difficult to dispose of by incineration. Most food waste has been placed in landfills together with other wastes (Han and Shin, 2002). Food waste is the major component of organic matter in garbage and so when it is disposed of in landfills it is the major source of methane gas produced in the landfills and a major contributor to the organic matter in leachates. Adding restaurant food waste to the waste stream exacerbates such problems.

Forster-Carneiro et al. (2007) studied improvements in the efficiency of semidry anaerobic digestion and dry fermentation (20–35% TS), where little or no water, or sludge is added to the organic fraction of the municipal solid waste to produce an inert biosolid final product with methane production from methanogenic bacteria. The anaerobic digestibility and biogas and methane yields of the food waste were evaluated using batch anaerobic digestion tests performed at 50 °C. The results of the study indicated that the food waste is a highly desirable substrate for anaerobic digesters with regards to its high biodegradability and methane yield.

Anaerobic digestion is the method of choice for the treatment of organic waste. This method has advantages of low-level sludge production, low-level energy consumption and potentially useful amounts of methane or ethanol production. However, solid organic materials such as kitchen garbage and sewage sludge are not digested quickly anaerobically. This is mainly a result of the unfavourable surface area/volume ratio limiting the surface area accessible to the microbes. Formation of grease balls worsens this surface area/volume problem and accessibility of the substrates to the bacteria. A liquidisation step is needed to speed up the anaerobic digestion. Analysis of the energy balance of thermal/-pressure liquidisation followed by anaerobic digestion treatment was better than direct incineration (Sawayama, 1997).

The physical and chemical characteristics of the organic waste are important information for designing and operating anaerobic digesters. They affect biogas/ethanol production and process stability during anaerobic digestion and the costs of handling the feedstock material (Zhang et al., 2007).

Yeasts on the other hand are extremely reliable organisms to grow in culture and backup cultures are easily maintained. This is a crucial advantage over methanogen digestion. Yeasts are the mainstay of the fermentation industry (Madigan et al., 1997). Food waste contains high enough levels of proteins and amino acids and ammonia from the hydrolysis and breakdown of foodstuffs to adequately supply fixed nitrogen for the growth of yeasts. Yeasts also have a requirement for phosphorus, which is assimilated as a dihydrogen phosphate ion, and sulphur, which can be assimilated as a sulphate ion or as organic sulphur compounds such as the amino acids methionine and cysteine. Some metals, like magnesium, iron, calcium, and zinc and potassium are also required for good growth of the yeast (Shimoda, 2004). Food waste is unlikely to lack the essential minerals required by yeasts or their vitamin requirements (Ritchie and Raghupathi, 2008). In previous studies we have shown that yeast very efficiently removed ammonia from food waste but was not efficient at removal of phosphate (Suwannarat and Ritchie, 2013a,b).

S. cerevisiae is used for stable ethanol fermentation around the world, but it is important that yeasts lack the full range of amylolytic enzymes (α-amylase, β-amylase and glucoamylase) required to fully break down starches to glucose. Yeast has only two genes for amylases, YIL099W (SGA1) and YIR019C (FLO11, MUC1 and STA4) (KEGG, 2013). These enzymes are both α-glucoamylases (EC:3.2.1.3) (KEGG, 2013). Watanabe et al. (2009) evaluated the culture conditions and material compositions for efficient ethanol production from rice washing drainage: they used rice bran as a cheap source of amylolytic enzymes (especially α-, β-amylase) because rice washing drainage from rice polishing contained only α-glucosidase. The alternative of digesting starches by acid hydrolysis has practical limitations already pointed out above. The efficiency of yeast in digesting bulk lipids and fats is not well documented but yeast is known to produce emulsifying agents which would help in breaking up aggregates of oils, greases and sludges (Barriga et al., 1999).

Watanabe et al. (2009) studied yeast-based anaerobic batch fermentation of rice waste (main culture: net volume 30–36 ml), using rice washing drainage (30 ml) as the substrate with lactic acid (final concentration: 100 mM) as the bactericidal agent. Different weights of rice bran were mixed in a 50 ml centrifuge tube, and then 1.0 ml of pre-culture yeast broth was inoculated. Fermentation processes were terminated after 14 days. The concentration of ethanol and sugars was analysed using an HPLC. The maximum ethanol concentration attained was 6.2% (V/V) (Watanabe et al., 2009).

Currently, "spent yeast" has a low value and is used as a protein supplement in animal feed (Barriga et al., 1999). The brewing industry is a ready bulk source of "spent yeast" which could be used to digest food waste at minimal cost. In using yeast to breakdown food wastes the incubations can be run at higher temperatures than used in brewing to maximize the digestion rate because the "flavor" of the product is not relevant in the case of using yeast to break down food waste (Fleet et al., 2009).

There are several additional reasons why yeast was selected for the study. Yeast is completely sequenced (KEGG, 2013). It can be genetically transformed allowing for either the addition of new genes or deletion through homologous recombination. A complete set of yeasts with single knockout mutations is available. The major advantage of yeast remains, however, its ease of cultivation and ready availability. Many different strains are available and standard procedures could be used to select a strain most suitable for anaerobic breakdown of food waste.

2. Materials and methods

2.1. Microorganism and culture conditions

S. cerevisiae, in this study was obtained from the Biology Laboratory of Prince of Songkla University, Phuket campus. It is a baker's yeast strain.

2.2. Inoculum yeast culture

Stocks of *S. cerevisiae* were maintained on agar plates in medium containing 6 g of peptone, 3 g of yeast extract, 6 g of dextrose, 15 g of agar added to 300 ml of distilled water. Liquid cultures were grown in the same medium with the agar omitted. The standard autoclaving routine was 121 °C for 15 min. Liquid cultures were grown out without aeration. The backup yeast cultures were maintained in the refrigerator at 4 °C until required (Akpan et al., 2008).