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Breakdown of food waste by anaerobic fermentation and non-oxygen producing photosynthesis using a photosynthetic bacterium

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ABSTRACT

Large volumes of food waste are produced by restaurants, hotels, etc generating problems in its collection, processing and disposal. Disposal as garbage increases the organic matter in landfills and leachates. The photosynthetic bacterium *Rhodopseudomonas palustris* (CGA 009) easily broke down food waste. *R. palustris* produces H_2 under anaerobic conditions and digests a very wide range of organic compounds. *R. palustris* reduced BOD by ≈70% and COD by ≈33%, starch, ammonia, nitrate, was removed but had little effect on reducing sugar or the total phosphorus, lipid, protein, total solid in a 7-day incubation. *R. palustris* produced a maximum of 80 ml H_2/g COD/day. A two-stage anaerobic digestion using yeast as the first stage, followed by a *R. palustris* digestion was tested but production of H_2 was low.

1. Introduction

Large amounts of food waste is generated from restaurants, food centers, hotels, schools, etc. Moreover, food waste has large numbers of used toothpicks and some chopsticks and paper as additional solid waste. Food waste is one of the main types of municipal solid waste (Zhang and Jahng, 2012). Food waste has presented many management problems in its collection, processing and disposal. Disposal of food waste is an increasing environmental problem. Phuket has a large tourist industry that is important economically and as a major source of food waste. Consequently, tourist industry has an increasing impact on the environment and generates environmental problems such as proper garbage disposal, water supply air pollution, etc. Further, Phuket has a lot of food trolleys and stalls selling food on the roadside and this adds to the already large amounts of food waste in Phuket. There are concerns about disposal of food waste discarded in bins and left to wash into drainpipes. Most food waste in Phuket like in many other countries was formerly used by pig farms (Westendorf et al., 1998). Today Phuket has few pig farms because of the increasing price of land and undesirability of pig farms in a major tourist area. This makes it necessary to develop alternative methods of disposal of food waste discarded in bins. As landfills have been closed at an alarming rate and fewer incinerators have been under construction in recent years, waste disposal has become a serious problem in many Thai cities. Since food waste has high moisture content, a high organics-to-ash ratio, and a loose physical structure, composting seems to be an ideal disposal method (Chang and Hsu, 2008). Disposal as garbage increases the organic matter in the landfill and the volume of leachate from landfills endangers primary sources of fresh water and causes ground water spoilage and the leachate is a major source of eutrophication in surface waters (Han and Shin, 2002). However, available sites for landfill are lacking and incineration of high-water-containing food wastes requires a large amount of energy. Treating food wastes in a waste water treatment plant site may be more effective due to the high water content instead of it being treated as solid waste (Gonzales et al., 2005).

Many different methods have been investigated for dealing with food waste including, anaerobic digestion, on-site composting, fermentation using a continuous-flow reactor, hybrid anaerobic solid–liquid, anaerobic digestion using bacteria, digestion using yeast, biological solubilization and mineralization etc. (Cirne et al., 2006; Hwang et al., 2002; Han and Shin, 2002; Stabnikova et al., 2008; Zhang et al., 2007; Gonzales et al., 2005). However, the treatment system needs to be very easy and very cheap. Microorganisms can be used to modify waste food to breakdown chemical compounds, reduce COD, BOD, the physical and chemical of characteristics of food waste (Cirne et al., 2006). Proposals have been made to feed food waste from cafeteria and canteens at PSU-Phuket into anaerobic digesters or anaerobic fermentation using methanogenic bacteria in sewage plants. Unfortunately, sewage is usually heavily contaminated with heavy metals

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and dangerous organic compounds and so the digest sludge may not be very useable for recycling projects. Hence, treating the food waste stream separately has the advantage that the digested sludge derived from food waste would have a very low heavy metal content and so would be potentially recyclable.

Purple non-sulfur bacteria have been used very successfully in treating swine waste water from piggeries (Kim and Lee, 2000; Kim et al., 2009). Studies show that these bacteria can remove or detoxify many organic compounds, organic acids, COD and phosphate (Kim et al., 2004; Larimer et al., 2004). They have a wider range of nutrition than nearly all other microbes and so are able to grow on virtually any organic material with the notable exception of cellulose (Larimer et al., 2004; KEGG, 2013). It is proposed in this project to investigate the use of photosynthetic bacteria to break down food waste under anaerobic conditions. Kim and Lee (2000) and Kim et al. (2009) found that *Rhodopseudomonas* grew very well photosynthetically in an open pond even though the surface was exposed to the atmosphere. The BOD of the piggery waste was so high that anaerobic conditions were maintained in the pond leading to a thriving rhodopseudomonad community.

Rhodopseudomonas palustris can produce hydrogen under anaerobic conditions and they can digest vegetable, starch, sugarcane juice and whey to produce hydrogen gas. Sterile food waste was used in this study to ensure that any effects on NH_3, NO_3^-, P, BOD, COD and gas production must have been due to the *Rhodopseudomonas* inoculum and not a result of the metabolism of any organisms already present. Raw food waste would contain many different and highly variable populations of microbes but Kim and Lee (2000) and Kim et al. (2009) found that *Rhodopseudomonas* were highly competitive in ponds of swine waste. A particular advantage of *Rhodopseudomonads* is that they are able to readily break down lipids. *R. palustris* can produce hydrogen gas from palm oil milling effluent which has a very high oil and fatty acid content (VFAs-Volatile Fatty Acids) (Suwansaard et al., 2009).

2. Materials and methods

2.1. Microorganism and medium

The common yeast *Saccharomyces cerevisiae*, in this study was obtained from the Biology Laboratory of Prince of Songkla University, Phuket campus. *S. cerevisiae* stock cultures were grown in liquid 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.

R. palustris (CGA009) was a kind gift from Prof Carrie Harwood, University of Washington, USA and grown in PM medium (Kim and Harwood, 1991). The simplified PM medium had the following composition: major components (in mol m^{-3}), Na_2HPO_4, 12.5; KH_2PO_4,12.5; $(NH_4)_2SO_4$, 7.57; $MgSO_4 7H_2O$, 0.238; $CaCl_2{\cdot}2H_2O$, 0.0454; $Na_2S_2O_3{\cdot}5H_2O$, 0.1; Sodium acetate, 10; Sodium benzoate, 10; *p*-aminobenzoic acid (PABA), 0.0146; Trace element mix (use 1 ml/l) (stock solution recipe in mol m^{-3}); Citric acid·H_2O, 105; $FeSO_4{\cdot}7H_2O$, 2.511; $ZnSO_4{\cdot}7H_2O$, 38.07; $MnSO_4{\cdot}H_2O$, 9.11; $CuSO_4{\cdot}5H_2O$, 1.570; $Co(NO_3)_2{\cdot}6H_2O$, 0.859; $Na_2MoO_4{\cdot}2H_2O$, 0.02139; H_3BO_3, 1.856. Growth of *R. palustris* cultures was followed by measuring optical density at 650 nm using a spectrophotometer (Ritchie, 2013) and by bacteriochlorophyll *a* assay (see below).

2.2. Feedstock characteristics

Food wastes were collected from the cafeteria in PSU-Phuket Campus, Phuket, Thailand. Toothpicks, Plastic, Tissues and Chopsticks and bones were first removed and then homogenized in a blender (2 kg of food waste was mixed with ≈1000 ml of tap water). Food waste was autoclaved at 121 °C for 30 min and anaerobic fermentation experimental set up is. Different components of the food: protein, carbohydrates, oil, total solids, COD, BOD, nitrate, total phosphorus were analyzed using standard methods (APHA, 1998), ammonia (Standard nitroprusside method: Solorzano, 1969), protein (Lowry method: Lowry et al., 1951).

2.3. Experimental setup

The experimental setup was similar to that described by Chen et al. (2007) and is illustrated in Mekjinda and Ritchie (2013a, 2013b). A 250 or 500 ml Schott bottle was used as the reactor and placed on a magnetic stirrer with a spin bar. A simple pneumatic trough was connected to the reactor to collect gas. Light sources were 2 × 20 W warm-white fluorescent lights.

2.3.1. Experimental procedure

The cultures were kept on agar slopes in 1% agar in PM medium. Complex anaerobic growth facilities were not required. Experimental cultures were grown out in 250 ml Schott bottles in PM medium.

(1) *Preparation of starter inocula*: Starter inocula were prepared by centrifuging 45 ml aliquots of cells at 500 rpm for 10 min. The supernatant was poured off and the pellet resuspended in autoclaved tapwater ready for inoculation of experimental cultures.
(2) *Preparation of food waste*: Food waste was collected from the cafeteria at PSU-Phuket campus, Phuket. Firstly Toothpicks, Plastic, Tissues, Bones and Chopsticks were removed and then homogenized in a blender (2 kg of food waste was mixed with ≈1000 ml of tap water). Mixed food waste was autoclaved after blending at temperature 121 °C, 15 min.
(3) *Two-stage fermentation protocol*: A two-stage process was also tried. *Rhodopseudomonas* was grown on spent media from digestion experiments using yeast.

First stage incubation was a 25 day anaerobic digestion using yeast. The common yeast *S. cerevisiae*, in this study was obtained from the Biology Laboratory of Prince of Songkla University, Phuket campus. *S. cerevisiae* stock cultures was grown in liquid 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. Three lots of 125 g FW/l food waste were inoculated with 10 ml of yeast inoculums (about 4.6×10^6 of cells/ml), under dark conditions, room temperature and shaken all the time.

The second stage incubation was where the spent yeast culture was inoculated with *Rhodopseudomonas*. The *Rhodopseudomonas* took over the cultures after inoculation. Hydrogen gas (H_2) was quantified using a gas chromatograph Shimadzu 8A Gas Chromatograph.

(4) The *Rhodopseudomonas* culture used for Experiment # 1 used 75 ml of cell culture, experiment # 2 used 70 ml of cell culture, and experiment # 3 used 65 ml of cell culture, experiment # 4 used 60 ml of cell culture, experiment # 5 used 55 ml of cell culture and experiment # 6 50 ml of cell culture. The *Rhodopseudomonas* culture used to inoculate the 2nd stage contained 5 μg/ml BChl a /ml of culture (see below). The *Rhodopseudomonas* treatments were incubated in the light.

2.3.2. Analysis of cell concentration during growth

We routinely used Bacteriochlorophyll *a* to monitor the amount of cells used in experiments. This is appropriate for a photosynthetic bacterium. The relationship between Bacteriochlorophyll *a* (BChl *a*) present and cell absorbance at 650 nm was measured using the Shimadzu double-beam spectrophotometer (Oh et al., 2004). Optical density of the cells was measured at 650 nm as