Anaerobic Fermentation of Food Waste in Phuket Province using Yeast

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Abstract

Phuket Province, Thailand is a major and still expanding tourist area. Restaurants, hospitals, hotels, residences, cafeterias and other industrial sites produce large volumes of food waste. Fermentative breakdown of food waste seems a viable alternative to feeding to pigs or garbage disposal. Food waste is difficult to dispose of by incineration because it has a high of moisture content and very high amounts of organic matter, such as carbohydrate, protein, lipid (fats and oils), cellulose etc. The aim of the project reported in this thesis is finally to determine the optimal fermentation condition of food waste using yeast (*Saccharomyces cerevisiae*). This included 1) Incubation time 2) Volume of food waste 3) Inoculums size 4) Fermentation temperature. The second aim was to compare the effectiveness of using autoclaved, pasteurised (80 ^{o}C) and acid pretreated food waste as feedstock. It was found that yeast readily fermented food waste producing CO_2 and alcohol and reduced COD and BOD_5 (COD, 89.7%; BOD_5, 85.6%) under anaerobic conditions. Yeast very effectively digested carbohydrate (measured as reducing sugar) and total sugar (>80%). It was able to slowly breakdown the partially hydrolyzed rice starch if being incubated long enough (about 75% after 30 day incubation). As expected, Yeast metabolized protein (68% removal) and reducing sugars (87.4%) in food waste but a surprising result was that yeast was much better at breaking down cooking oil and lipids than expected (> 85%). With 25 day incubation there is about 85% breakdown of Total sugar, Reducing sugar, Lipid and Protein. The optimum temperature was 40 ^{o}C and the optimum concentration of food waste was 175 g FW/l. The optimum inoculum rate for starting the cultures was 10 ml yeast inoculums (about 50×10^6 cells). Condition can digest of organic composition so best was autoclaved and pretreatment (80 ^{o}C) it had similar result but acid pretreated has poor digestion efficiency.

Keywords: Food Waste, Anaerobic Fermentation, Yeast (*Saccharomyces cerevisiae*)

ชื่อวิทยานิพนธ์ การหมักเศษอาหารในจังหวัดภูเก็ตแบบไม่ใช้ออกซิเจนโดยใช้ยีสต์
ผู้เขียน นางสาวจุฑารัตน์ สุวรรณรัตน์
สาขาวิชา เทคโนโลยีและการจัดการสิ่งแวดล้อม
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บทคัดย่อ

จังหวัดภูเก็ตเป็นเมืองแห่งการท่องเที่ยวมีสถานที่ประกอบการมากมาย อาทิเช่น ร้านอาหาร โรงพยาบาล โรงแรม สถานศึกษา สถานที่ประกอบการอุตสาหกรรม เป็นต้น เป็นที่มาของการเกิดเศษอาหาร การย่อยสลายเศษอาหารโดยการหมักเป็นทางเลือกอีกทางเลือกหนึ่ง นอกเหนือจากการใช้เป็นอาหารสัตว์หรือปล่อยเป็นขยะ เศษอาหารยากต่อการกำจัดโดยใช้เตาเผา เนื่องจากมีความชื้นสูงและมีสารอินทรีย์เป็นจำนวนมาก เช่น คาร์โบไฮเดรต โปรตีน ไขมัน (ไขมันและน้ำมัน) เซลลูโลส เป็นต้น เศษอาหารมีแนวโน้มการนำมารีไซเคิลมากกว่าการนำมาย่อยสลายภายในครัวเรือน องค์ประกอบหลักของเศษอาหารในประเทศไทยจะประกอบด้วยข้าว ผักและเนื้อหมูหรือเนื้อไก่ ซึ่งจะมีปริมาณไขมันสูงจากน้ำมันพืชที่ใช้ประกอบอาหาร วัตถุประสงค์ในการวิจัยครั้งนี้จะแบ่งเป็น 2 ส่วน ส่วนแรกจะทำการหาสภาวะที่เหมาะสมในการหมักโดยใช้ยีสต์ เช่น 1) หาเวลาที่เหมาะสมในการหมัก 2) หาปริมาณเศษอาหารที่เหมาะสมในการหมัก 3) ปริมาณยีสต์ที่เหมาะสมในการหมัก 4) อุณหภูมิที่เหมาะสมในการหมัก ส่วนที่สอง เปรียบเทียบสามสภาวะ คือ การฆ่าเชื้ออาหารก่อนเข้ากระบวนการ การให้ความร้อนที่ 80 ^{o}C และการปรับ pH อาหารก่อนเข้าระบบ จากผลการทดลองพบว่ายีสต์สามารถย่อยสลายเศษอาหารแล้วผลิตเป็นก๊าซและแอลกอฮอล์ และยังสามารถลดค่า COD และ BOD_5 ลงได้ 89.7% และ 85.6% ตามลำดับ ภายใต้สภาพไร้ออกซิเจน ยีสต์มีประสิทธิภาพในการย่อยคาร์โบไฮเดรต (วัดจากปริมาณน้ำตาลทั้งหมดและน้ำตาลรีดิวส์ได้มากกว่า 80% ย่อยสลายแป้งได้ 75% เมื่อบ่มเป็นเวลา 30 วัน ยีสต์ย่อยสลายโปรตีนได้ 68% และ น้ำตาลรีดิวส์ได้ 87.4% ส่วนของไขมันยีสต์สามารถย่อยได้มากกว่า 85% ระยะเวลาที่เหมาะสมในการหมักคือ 25 วัน ที่ปริมาณเศษอาหารที่ใช้คือ 175 g FW/ ปริมาณเชื้อที่เหมาะสมคือ 50×10^{6} cells ที่อุณหภูมิ 40 ^{O}C. สภาวะที่เหมาะสมในการปรับเศษอาหารคือ การฆ่าเชื้ออาหารก่อนเข้ากระบวนการหมัก

คำสำคัญ: เศษอาหาร, การหมักแบบไม่ใช้ออกซิเจน, ยีสต์ (*Saccharomyces cerevisiae*)

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CHAPTER 1

Introduction

1.1 Statement of the Problem

Food waste from restaurants and hotels is an increasing environmental problem. Most food waste from the tourist industry was formerly used by piggeries (Westendorf et al., 1998). 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, Perez et al., 2007).

In Thailand a major component of the waste is cooked rice (as found in Korean restaurant waste, Han and Shin 2002) which would have been partially hydrolysed from cooking but would require further treatment to mobilise the starches. Davis (2008) used amylase treatment to mobilise the starch in their study of yeast fermentation on waste consisting largely of starch. Similar methods have been used for preparing corn paste wastes (Akpan et al., 2008) but acid hydrolysis has also been used to mobilise both starches and cellulosic material such as corncobs, peanut shells and newspaper (Akpan et al., 2005; Akpan et al., 2008).

The advantage of acid hydrolysis is that it mobilises both starches and some of the cellulose and many other organic compounds but acid hydrolysis poses corrosion problems and the acid has to be neutralized before microbes are introduced. 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. Lipids of various kinds (fats and oils) are a major component of restaurant wastes (Cirne et al., 2006) and present particular problems in their metabolism. Yeasts and methanogen bacteria can use hydrolysis products of fats and lipids but generally it is thought that they cannot rapidly ferment unprocessed lipids. At the beginning of this study this was

thought to be a major limitation to using yeast to process restaurant waste but in the course of this project it was soon demonstrated that yeast efficiently broke down cooking oil lipids in restaurant waste (Suwannarat and Ritchie, 2013a, b). Yeast is known to be able to produce effective bioemulsifiers (Barraga et al., 1999) which help break down oily clumps (grease balls) in food waste and makes it possible for yeast to digest lipids. The bioemulsifiers would also make the carbohydrate in oil soaked rice grains accessible to digestion by the yeast.

Food waste is a complex biomass containing various components such as starchy, fatty, and cellulosic materials. The waste is also bulky, meaning that microbes have only a limited access to the material because of its low surface area to volume ratio. Without some more than of processing, these organic polymer materials are difficult for non-motile ethanol producing microorganisms such as *Saccharomyces cerevisiae* to utilize. Food waste generated in Korea is rich in carbohydrate, as high as 65% of total solids but unlike potato-based and bread-based starchy foods consumed in western countries in oriental countries the starch is mainly present as more or less intact rice grains (Kim et al., 2008).

In Korea, biological resources are not found in large quantity but approximately 82 million tons per year of organic wastes such as food waste, livestock manure, vegetable waste and waste sludge are produced each year that are potentially useable (Kim et al., 2008). Food waste contains carbohydrate as high as 65% of its total solid, is rich in carbon content and, therefore, is a good potential substrate for producing ethanol. But little information is available on the use of food waste as a feedstock for ethanol production.

Studies of the breakdown of waste material are often limited by the variability of the waste material. An effort has been made to find the optimum conditions for digestion of the food waste. The project will involve anaerobic digestion using yeast (*Saccharomyces cerevisiae*) fermentation. Glucose produced from enzymatic hydrolysis or acid hydrolysis of restaurant food waste will be used by the anaerobic yeast to produce ethanol: a large number of other sugars, proteins and lipids will also be used by the anaerobically growing yeast cells. In this study alcohol was measured using a gas chromatograph (Agilent 7890A GC). CO_2 can also give an estimate of ethanol production under anaerobic conditions using simple volumetric calculations.

1.2 Objectives

Characterize breakdown of food waste in Phuket Province using yeast (*Saccharomyces cerevisiae*) investigating the optimum conditions and determining what compounds in the food waste are readily digestible and what incubation times are optimal for digestion of most of the food waste

1.3 Scope

It was investigation on fermentation processes to manage food waste from food stalls and trolley sellers in Phuket. Manipulative experimental studies on yeast metabolism enabled us to do a useful study of the feasibility of using microbial digestion processes to specifically deal with food waste.

1.4 Expected Outcomes

1.4.1 It study has shown that treatment of food waste separately to domestic garbage leads to useful benefits.

1.4.2 Ethanol production will be a saleable and useful product from the digestion of food waste and standard fermentation methods can be used to deal with food waste.

1.4.3 Food waste will have low levels of toxic organic compounds such as herbicides and insecticides and also very low heavy metal content because almost no metals are present in food waste. Hence the digestion sludge could be safely used as fertilizer.

1.4.4 When the project began we anticipated that yeast would readily catabolise proteins and carbohydrates in food waste. A surprising result of the study has been that I found that yeast effectively broke down lipids in the food waste (Suwannarat and Ritchie, 2013a, b). Breakdown of lipids is a common environmental problem in treatment of food wastes (Arsova, 2010).

CHAPTER 2

Literature Review

2.1 Food waste

Food waste derived from many locations such as residences, commercial establishments (e.g. restaurants), street stalls, institutions (e.g. school, hospitals), cafeterias, canteens, lunchrooms and other industrial sites (Kreith, 1992; Haug, 1993). Food waste consists of various components such as protein, lipid, cellulose and high carbohydrate production 65% of its total solid, is rich in carbon and is a good substrate for produce ethanol (Kim et al., 2011). The high moisture content makes it a bulky commodity that is difficult to manage and transport large distances. Large amounts of food waste are used for fertilizer production and animal feeds (Kim et al., 2008; Masaaki et al., 2008; Moon et al., 2009). The incineration of food waste is unsuitable because of its high water content (making it difficult to burn) and the possibility of dioxin generation during combustion is also a potential problem (Wataru et al., 2004). Thus, it would be worthwhile to develop a method to change food waste to a high value product such as ethanol (Yan et al., 2011). Residues from digestion of food waste are unlikely to be a heavy-metal hazard (unlike domestic garbage) and so could be safely used as a soil conditioner and might be useable as animal feed (Westendorf et al., 2002).

Food waste includes uneaten food and food preparation scraps from residences or households, commercial establishments like restaurants, grocery stores, street stalls, cafeterias and industrial sources. Food waste is defined as any food substance, raw or cooked, which is discarded, or intended or required to be discarded. Food wastes are the organic residues generated by the handling, storing, sale, and preparation, cooking, and serving of foods (U.S Environment Protection Agency, 2012).

Food waste is a growing issue, and the disposal of it is controversial, given increased food prices and the space and resources required. Food waste makes up an estimated

8.4% by weight of municipal solid waste in the United States. The food waste includes uneaten food and food preparation leftovers from many locations. 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, this 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., 2002).

Food wastes are used as fertilizer or as feed ingredients for animal production. In Korea the government is encouraging food waste recycling and the exploration for bioengineering to transform food waste into useful product. Bioconversion process involving microbial metabolic process offer opportunities to transform food waste into a useful recycled product (Yang et al., 2006). Yan et al. (2011) studied optimization of conditions of enzymatic saccharification using food waste and transforming food waste hydrolysates to ethanol using *S. cerevisiae* H0558 but did not do detailed work on protein and lipid digestion.

The amount of food wasted in the United States is staggering. In 2010, more than 34 million tons of food waste was generated, more than any other material category except paper. Food waste accounted for almost 14 percent of the total municipal solid waste stream, less than three percent of which were recovered and recycled in 2010. The remaining 33 million tons was thrown away, making food waste the single largest component of MSW reaching landfills and incinerators (U.S Environment Protection Agency, 2012). Separation of food waste from domestic garbage after collection of the garbage is impractical.

Food waste from South Korea amounts to 11,577 tons per day. It comprises about 25% of municipal solid waste. Food waste is difficult to dispose of by incineration. Food waste is the main source of decay, obnoxious odor and environmentally dangerous leachates in the collection and transportation of garbage due to high volatile solids and moisture content. It is also obviously the main source of the high BOD and COD of garbage. Most food waste has been landfilled together with other wastes (Han et al., 2002). The large amounts of food wastes in landfills lead to the production of leachates with very high BOD and COD.

Where food waste is separated on site from garbage at the site of its production, then separate processing of food waste becomes a practicable proposition. Waste from processed

food is rich in nitrogen, phosphorus, potassium and other plant nutrients. With the exception of inedible animal parts, these wastes can be directly broken up and used as fertilizer without any additional processing. Food wastes have a large solid waste component from the meat and vegetable content and can be utilized as animal feed (usually for pigs). Potato peels can also be fermented into alcohol and cattle feed, but the peeling slurry requires a suitable reactor for the fermenting process (Yang et al., 2006). Piles of food waste, waiting for processing have a large amount of fermentation going on inside them. This releases strong organic leachate which may contain appreciable amounts of alcohol. The most common disposal method for this is to spread it onto fields or to feed it into the sewage system without attempting to recover a useable product from it.

Food waste is complex biomass containing various components such as starchy, fatty and cellulosic materials. Fermentation into useful amounts of alcohol and a useable sludge end product is not straightforward. These materials have a reputation for being difficult to readily utilize by ethanol producing microorganisms such as *S. cerevisiae* (Kim et al., 2011).

Food fermentation industries produce a variety of fermented foods and waste products from the fermentation process. The fermentative process not only creates different food products but also adds other attributes to the food such as preservation of the food and amends the food with desirable microbes. Also, the additions of microbial inoculants with or without enzymes are part of fermentation processes such as beer and wine making and cheese making. Food waste from fermentation processes are typically wet sludge and contain high levels of fermentable carbohydrate, the lactic fermentation process (using the bacterium *Lactobacillus*) appears to be the method of choice for processing such food wastes into animal feed (Yang et al., 2006). Lactic acid digestion has the advantage over alcoholic fermentation that CO_2 gas is not produced but alcoholic fermentation offers the advantage of producing commercially saleable ethanol.

Food waste, provided it is available as a separate waste stream and not mixed in with garbage, can thus be managed in many different ways for example, aerobic digestion, anaerobic digestion using bacteria, digestion using yeast, composting and use as animal feed, particularly for pigs (Han and Shin, 2002; Hwang et al., 2002; Gonzales et al., 2005; Crine et al., 2006; Zhang et al., 2007). Historically much of the food waste in Phuket was fed to pigs and an

efficient collection system was in place for its collection but the pig industry has largely disappeared from Phuket because of the cost of land and competition for land with the tourist industry. Piggeries are unpopular neighbors in tourist areas. The sludge from fermentation and distillation would find a ready use as fertilizer on local crops and oil palm and rubber plantations.

2.2 Ethanol

Increasing population and mobility of people has increased energy demand; petroleum and fossil fuel sources are limited in supply and cannot be renewed. Alternative fuel sources such as ethanol are attractive because ethanol production is not limited. There are lots of alternatives to ethanol such as methanol, methane, natural gas, propane, hydrogen, etc. Fermentation can produce ethanol from any feedstock rich in hydrolysable carbohydrate, not only more familiar sources such as sugarcane. Ethanol can be produced from cassava starch by *S. cerevisiae* (Akande et al., 2009). Asada and Kita (2011) studied ethanol production from chopsticks using delignification pretreatments. Fermentation of steam explosive hydrolysis of chopsticks (optimal condition at 25 atm for 5 minutes) produced a 79% theoretical ethanol yield (Asada et al., 2011). Asada and Kita (2011) used cane sugars and corn as standard carbon sources for yeast fermentation to develop efficient bioethanol production methods from cellulose biomass such as wood, bamboo, wheat straw and bagasse. The cellulose component of such substrates for bioethanol production, are generally covered with lignin in the cellulosic biomass. Lignin is not readily biodegradable and prevents the breakdown of cellulose mainly by acting as a physical barrier. The ball milling and steam explosion are effective pretreatments for the delignification of cellulosic biomass but requires considerable energy input. The cellulosic biomass is treated with high temperature and pressure saturated steam and then the pressure is reduced rapidly, which makes the cellulosic biomass undergoes an explosive decomposition (Asada et al., 2011).

Hydrolyzing enzymes work well on starchy and cellulosic materials to yield the monomeric unit, glucose, which can be subsequently fermented to produce ethanol. Utility cost of enzymatic hydrolysis is low compared to acid hydrolysis and heat treatment because it is usually conducted under mild conditions and does not present any corrosion problems. The cost of the enzymes has been reduced substantially by the advance of biotechnology (Kim et al., 2011).

Ethanol can be produced from many sources such as sucrose-based substrates such as sugar cane and sugar beet juice (Ergun et al., 2000), palm wine produced by fermentation processes from sugar palm (Akpan et al., 2005), and any organic material rich in hydrolysable carbohydrate such as cassava starch and potato starch (Akpan et al., 2008). Poor quality cereal grains unsuitable for sale can also be used as sources of starch. Oriental food waste made up largely of partially hydrolysed rice is a promising feedstock for digestion by yeast.

2.3 Fermentation

The food waste in South Korea amounts to about 4.5 million metric tons annually and major efforts have been made to recycle by means of various processes such as yeast fermentation (Yan et al., 2011), lactic acid fermentation (Yong et al., 2006), enzymatic hydrolysis (Yan et al., 2011) and acid hydrolysis (Akpan et al., 2005). Enzymatic hydrolysis is now used more than in the past because of low cost and it does not have the corrosion problems of acid hydrolysis (Kim et al., 2011). Yan et al. (2011) studied fermentation process effects of sterilisation (substrate was not sterilized) and sterilized (substrate was autoclaved) were compared to test the effects on ethanol production (Yan et al., 2011). Sterilization had a significant effect for microbial digestion because heat/pressure sterilization in the autoclave hydrolysed the carbohydrate chains of starch and would have also largely hydrolysed the proteins present enabling breakdown of much of the food waste by yeast. Yeast/hydrogen producing bacterium two-stage fermentation process can convert glucose to hydrogen but only 33.5% of theoretical yield (Song et al., 2011). Song et al. (2011) studied cogeneration of hydrogen from protein-mixed food waste by a two phase anaerobic fermentation from the defatted milk powder (DMP) rich in lactoprotein and lactose. The DMP pretreated by hydrolysis of lactoprotein was inoculated with hydrogen-producing bacteria to produce hydrogen in the first stage and second stage use residual H_2-producing solutions were reutilized by produce methane (Song et al., 2011).

Treatment of food waste by anaerobic digestion has an estimated total capacity of 3.5 million tonnes in South Korea (Bodik et al., 2010). Cho and Park (2010) determined the methane yields of different food wastes at 37 ^{o}C and 28 days of digestion time. They were 482,

294, 277 and 472 ml/g VSS (Volatile Solid Substances) for cooked meat, boiled rice, fresh cabbage and mixed food wastes, respectively (Bodik et al., 2010).

Kastner and Schnitzhofer (2010) studied a single step fermentation using two different types of bioreactor systems. On the one hand, the biogas production was performed in a conventional Continuous Stirred Tank Reactor (CSTR), which served as a reference for the second system which was fermentation carried out in a Fluidized Bed Reactor (FBR) with internal circulation.

Food waste creates many problems such as complaints by the public, emanating odor, attracting vermin, emission of toxic gases, potentially explosive amounts of methane, contamination of groundwater and drainage into drains and blockage of them with grease balls and wastage of landfill capacity. The fermentation of food waste is inherently acidogenic and so is inherently corrosive. Fermentation of particular substrates is affected by the fermentation constraints such as the biodegradability of the substrate, the degrading capability of microorganisms, surface/area volume considerations for oil droplets etc and the environmental conditions. The total biodegradability of food waste is mainly related to the relative amount of cellulosic materials, which comprise up to about 50% of food waste.

The fermentation pattern of cellulosic material by rumen microorganisms is comparable to that observed in the conventional anaerobic digesters, but it only involves the stages of hydrolysis and acidogenesis (Han et al., 2011). Rumen microbial communities typically produce butyrate rather than ethanol and so do not produce a very commercially attractive fermentation product.

Lactic acid fermentation processes have been investigated by several workers (Yang et al., 2006). Lactic acid silage techniques are applied to preserve forage for feeding to goats, sheep and cattle and in the dairy industry. Lactic acid starter bacteria are commonly used as inoculants by food fermentation industries to produce a variety of fermented foods. The fermentative process not only creates a different food product but also adds other attributes to the food such as preservation of the food (usually by high acidity) and amends the food with desirable microbes (Yang et al., 2006). Lactic acid bacterial digestion does not produce CO_2 and could be useful for the treatment of food waste if the major aims were reduction of BOD/COD and use of the digested product as a soil additive.

The major products of the fermentation process by yeast are carbon dioxide gas (CO_2) and ethyl alcohol. This process is carried out by yeast cells using a range of enzymes (Song et al., 2010). This is in fact a complex series of conversions that brings about the conversion of sugar to CO_2 and alcohol under anaerobic condition. The yeast cells gain energy from the conversion of the sugar into carbon dioxide and alcohol. The glycolytic step generates ATP and NADH but the formation of alcohol uses all the NADH produced in glycolysis. A by-product from alcoholic fermentation is carbon dioxide and glycolysis also produces large amounts of heat. This heat production allows fermentation to run at a high rate but in some circumstances reactors need cooling to prevent excessive heat from Glycolysis can kill the yeasts.

Fermentation can be divided into two phases. In the first phase, any oxygen present is rapidly used up by aerobic respiration by the yeast and the cells grow very rapidly converting sugars into CO_2 gas and water but not producing ethanol. Anaerobic fermentation will not begin until oxygen is completely consumed in the system and the second phase of anaerobic fermentation takes over. Anaerobic respiration is a slower activity and the yeast focuses on converting sugar to alcohol rather that increasing the numbers of cells (Song et al., 2010).

The enzymatic conversion of organic carbon into CO_2 by glycolysis has been shown to be mediated by the vitamin biotin in a number of reactions, namely, the synthesis of fatty acids, the carboxylation of pyruvic acid to oxaloacetic acid, the decarboxylation of oxaloacetic and succinic acids and the deamination of certain amino acids (Adler et al., 1981). This is important in fermentation processes because yeast requires biotin and other vitamins which might not necessarily be present in the feedstock. For example, acid hydrolysis will destroy any biotin present in a feedstock.

2.4 Aerobic and anaerobic fermentation

Waste water, garbage dump leachates and food waste can use an aerobic and/or anaerobic treatment system (Kim and Lee, 2000; Kim et al., 2004). Watanabe et al. (2009) studied anaerobic batch fermentation for production of bioethanol from rice washing drainage and rice bran (Watanabe et al., 2009). Organic wastes rich in protein such as milk, egg, meat and fish could be treated using similar methods. Anaerobic fermentation using a two-phase configuration

to produce hydrogen and methane from protein-mixed food waste is also feasible. The first stage produces hydrogen from defatted milk powder (DMP) and second stage produces methane by using the residual solution from the first stage and re-inoculation with a population of methanogens (Song et al., 2010).

Watanabe et al. (2009) studied anaerobic batch fermentation (main culture: net volume 30-36 ml), rice washing drainage (30 ml), lactic acid (final concentration: 100 mM) as the bactericidal agent, and 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 analyzed using an HPLC. The maximum ethanol concentration was 6.2% (V/V) (Watanabe et al., 2009).

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 (no expensive aeration required) and methane or ethanol production. However, solid organic materials such as kitchen garbage and sewage sludge are not digested quickly anaerobically; this is largely a surface/area volume effect (Arsova, 2010). A liquidisation step is needed to speed up the anaerobic digestion. The preliminary analysis of the energy balance of liquidisation followed by anaerobic digestion treatment was better than direct incineration (Sawayama, 1997).

Anaerobic digestion involves the degradation and stabilization of organic materials under anaerobic condition by microbial organisms and leads to the formation of biogas (Kelleher et al., 2000) or alcohol. Anaerobic treatment provides a method of reducing pollution from agricultural and industrial operations while at the same time offsetting the operations usage of fossil fuels (Chen et al., 2008). Anaerobic digestion has been widely used for the treatment of municipal sludge and limited application in the treatment of organic industrial waste including fruit and vegetable processing wastes, packinghouse wastes, and agricultural wastes (Parkin and Miller, 1983) and food waste (Arsova, 2010).

Many factors affect the design and performance of anaerobic digestion processes. Due to relatively high moisture content of food waste, bioconversion technologies such

as anaerobic digestion, are more suitable compared to thermo chemical conversion technologies, such as combustion and gasification. The physical and chemical characteristics of the organic waste are important information for designing and operating anaerobic digesters, because they affect biogas production and alcohol and process stability during anaerobic digestion and the costs of handling the feedstock material (Zhang et al., 2007).

Using biological methods for the treatment for organic fraction of municipal solid waste and optional recycling of biomass wastes is of great interest from an environmental viewpoint. Anaerobic digestion of different organic wastes such as market waste, fruit and vegetable, household waste, kitchen waste, biowaste, separated food waste and organic fraction of municipal solid waste are possible (Carneiro et al., 2007; Arsova, 2010).

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 product with higher methane productivity. The anaerobic digestibility and biogas and methane yields of the food waste were evaluated using batch anaerobic digestion tests performed at 50 ^{o}C. The nutrient content analysis showed that the food waste contained well balanced nutrients for many anaerobic microorganisms and so added vitamins were not required. The results of this study indicated that the food waste is a highly desirable substrate for anaerobic digesters with regards to its high biodegradability and methane yield. Perhaps a dry fermentation step would be useful as a pretreatment for a digestive process involving yeast fermentation in an aqueous system.

2.5 Yeast *(Saccharomyces cerevisiae)*

Yan et al. (2011) studied development of methods for transforming food waste hydrolysates using *S. cerevisiae* H 0558 co-cultures with a spore-forming *Clostridium* bacterium and compared non-sterilized and sterilized substrate as feedstock for ethanol and hydrogen production by fermentation. *S. cerevisiae*/*Clostridium* produced the highest hydrogen yield in a co-fermentation using protein-mixed food waste (de-fatted milk powder) as the feedstock. *S. cerevisiae* is a facultative anaerobe which can live aerobically or anaerobically. Unaerated

cultures of yeast will rapidly pull the oxygen concentration down to zero and will start anaerobic fermentation when the oxygen is depleted (Song et al., 2010) and when the oxygen levels reach zero the obligatory anaerobic *Clostridium* can start growing. The yeast provided the anaerobic environment needed for the activity of the hydrogen-producing bacteria and also produced proteinase aiding lactoprotein degradation to amino acids.

S. cerevisiae is a good cooperator with hydrogen-producing bacteria (Song et al., 2010) in promoting hydrogen production from DMP feedstock in a two-stage fermentation process, because it not only provides a better anaerobic condition for hydrogen-producing bacteria, but also produces some proteinase which facilitates lactoprotein degradation**.** In practice a yeast/*Clostridium* co-culture may be successful as a batch culture but continuous culture might not be possible because either the yeast or the *Clostridium* might completely take over the culture.

S. cerevisiae is a species of yeast (unicellular fungus). It is perhaps the most useful yeast; it is instrumental to winemaking, baking and brewing since ancient times. Growth on a massive industrial scale is well understood. It is believed that it was originally isolated from the skin of grapes (one can also see the yeast as a component of the thin white film on the skins of some dark-colored fruits such as plums); it lives among the waxes of the cuticle of fruits. It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* is used as the model bacterium. It is the microorganism behind the most common types of fermentation. *S. cerevisiae* cells are round to ovoid, 5–10 μm in diameter. It reproduces by a division process known as budding but it also has a sexual stage. Many proteins important in human biology were first discovered by studying their homologs in yeast; these proteins include cell cycle proteins, signaling proteins, and protein-processing enzymes (Bouquin et al., 2000). Many genes in plants also have direct homologues in yeast. Many fundamental problems in animal and plant cell biology can be studied using a yeast model.

Yeast typically breaks down substrates by secreting enzymes. Secretion of extracellular enzymes is an important means of utilizing compounds in their environment. *S. cerevisiae* is currently the only yeast cell that is known to have Berkeley bodies present, which are involved in particular secretory pathways (Manning et al., 1999).

All strains of *S. cerevisiae* can grow aerobically on glucose, maltose, and trehalose and fail to grow on lactose or cellobiose. However, growth on sugars other than glucose is variable. Galactose and fructose are shown to be two of the best fermenting sugars. The ability of yeasts to use some sugars can differ depending on whether they are grown aerobically or anaerobically. Some strains cannot grow anaerobically on sucrose and trehalose (Vogel et al., 2000). Thus to use sucrose in a feedstock when using such strains a two stage process is needed: an aerobic phase to hydrolyse the sucrose and a second anaerobic phase to use the fructose and glucose. It is important to note here that sucrose is hydrolysed to glucose and fructose (invert sugar) by autoclaving and so an apparent ability to use sucrose in laboratory culture can sometimes be misleading. In the last part of the thesis the digestibility of food waste that had been autoclaved and food waste that had been pasteurised (80 ^{o}C) or treated with acid (pH 3.5) will be examined. In industrial processes autoclaving food waste would not be practical but heating to 80 ^{o}C or treating with acid to minimize microbial contamination would be feasible.

All strains can use ammonia or urea as the sole nitrogen source, but cannot use nitrate, since they lack the ability to reduce it to ammonium ions. They can also use most amino acids, small peptides, and nitrogen bases as a nitrogen source. Some amino acids such as histidine, glycine, cystine, and lysine are, however, not readily used. *S. cerevisiae* secretes some proteases (Song et al., 2010) but typically its ability to hydrolyse proteins is limited (Donaldson et al., 1999), so it is generally thought that most extracellular proteins cannot be readily metabolized. In the present study, we found that this was not the case (Suwannarat and Ritchie, 2013a, b). Most amino acids from hydrolysis of extracellular proteins can be utilized. This is another instance where growth on an autoclaved feedstock could be potentially misleading because most of the present study was on feedstocks of autoclaved food waste.

Yeasts also have a requirement for phosphorus, which is assimilated as a dihydrogen phosphate ion, and sulfur, which can be assimilated as a sulphate ion or as organic sulfur compounds such as the amino acids methionine and cysteine. Some metals, like potassium, magnesium, iron, calcium, and zinc and potassium are also required for good growth of the yeast (Shimoda, 2004) but would be unlikely to be in limited supply in a food waste feedstock. The list

of essential elements for yeast is similar to that found for higher plants (Madigan et al., 1997) and again would be unlikely to be in short supply in digests of food waste.

Yeast (*S. cerevisiae*) is frequently used as a model system for plant cells as it shares many similar mechanisms for ion uptake (Stass et al., 2001). *S. cerevisiae* is used for stable ethanol fermentation around the world, but it is important that yeasts lack some amylolytic enzymes (α-amylase and glucoamylase) that help to rapidly break down starches. Rice polishings in rice washing drainage contains only α-glucosidase, so other amylolytic enzymes (especially α-, β-amylase) need to be added for a complete saccharification process of rice washing drainage waste. Watanabe et al. (2009) evaluated the culture conditions and material compositions for efficient bioethanol production from rice washing drainage and used rice bran as a source of amylolytic enzymes in their research.

S. cerevisiae has been developed as a model organism because it scores favorably on a number of the following criteria:

As a single celled organism *S. cerevisiae* is small with a short generation time (doubling time 1.25–2 hours at 30 °C) and can be easily cultured. Cells can easily be separated from the aqueous product by simple settlement, filtration or centrifugation. These are all positive characteristics in that they allow for the swift production and maintenance of multiple specimen lines at low cost and short experimental times and easy harvesting of a product.

S. cerevisiae is completely sequenced (KEGG, 2013). It can be genetically transformed allowing for either the addition of new genes or deletion through homologous recombination. Furthermore, the ability to grow *S. cerevisiae* as a haploid simplifies the creation of gene knockout strains. A complete set of yeasts with single knockout mutations is available.

As a eukaryote, *S. cerevisiae* shares the complex internal cell structure of plants and animals without the high percentage of non-coding DNA that can confound research in higher eukaryotes. The genome is small for a eukaryote.

Use of *S. cerevisiae* has a strong economic driver, at least initially, as a result of its established use in industry (Arsova, 2010). It grows on a broad range of substrates including, but not limited to: sugars, such as glucose, xylose, galactose, mannose, fructose, ribose, maltose, sucrose and lactose (Liang et al., 2010). Starches can be used, provided they are hydrolysed by acid digestion or enzymatically because *S. cerevisiae* has only a very limited range of amylase

enzymes. 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). These glucoamylases are only able to hydrolyse the straight-chain parts of starch and so are able to only partially hydrolyse native starches. However, it was found in the present study that yeast is much better at using starch than is generally believed (Suwannarat and Ritchie, 2013a, b).

S. cerevisiae is used in brewing beer, where it is sometimes called a top-fermenting or top-cropping yeast. It is also called a top-yeast because during the fermentation process its hydrophobic surface causes the flocs to adhere to CO_2 and rise to the top of the fermentation vessel. Top-fermenting yeasts are fermented at higher temperatures than lager (bottom) yeasts, and the resulting beers have a different flavor to the same beverage fermented with lager yeast. "Fruity esters" may be formed if the yeast undergoes temperatures near 21 °C, or if the fermentation temperature of the beverage fluctuates during the process. Lager yeast normally ferments at a temperature of approximately 5 °C, where *S. cerevisiae* stops dividing but continues to ferment sugars (Kaeberlein et al., 2005). The temperature and strain of yeast used is important in determining the nature of the fermentation product.

Despite the otherwise simple nutritional requirements of yeast, most strains of yeast require one or more vitamins. Adler et al. (1981) studied of the ability of various substances to replace the biotin requirement of *S. cerevisiae* and study the growth of aerobic and anaerobic cultures of *S. cerevisiae* under various conditions of medium supplementation. Food waste substrate was not likely to produce vitamin deficiency problems.

Yeasts produce a wide range of secondary metabolites. Special yeast strains are used in wine fermentation to ensure high wine quality and character. A good yeast strain produces a balanced array of flavor metabolites, without undesirable excess volatiles such as acetic acid, ethyl acetate, hydrogen sulphide and sulphur dioxide (Fleet, 2009). Currently, spent yeast has a low value and is used as a protein supplement in animal feed (Barrsiga et al., 1999) but it is important to know that it is a source of good quality protein and so its economic value is likely to increase (Arsova, 2010; Kim et al., 2011).

2.6 Gas Chromatography

Principles of Gas Chromatography

Gas Chromatography (GC) is a technique for extracting a sample mixture by changing the mixture into a vapor at a certain temperature. The vapor is then passed through a column containing a fixed phase (stationary phase or exchanger). The mobile phase (both the carrier gas and the sample vapour) interacts with the solid phase as they pass through it. Different compounds interact with the solid phase differently and so pass through the column at different rates and so emerge out of the column at different times as shown in Fig. 2.

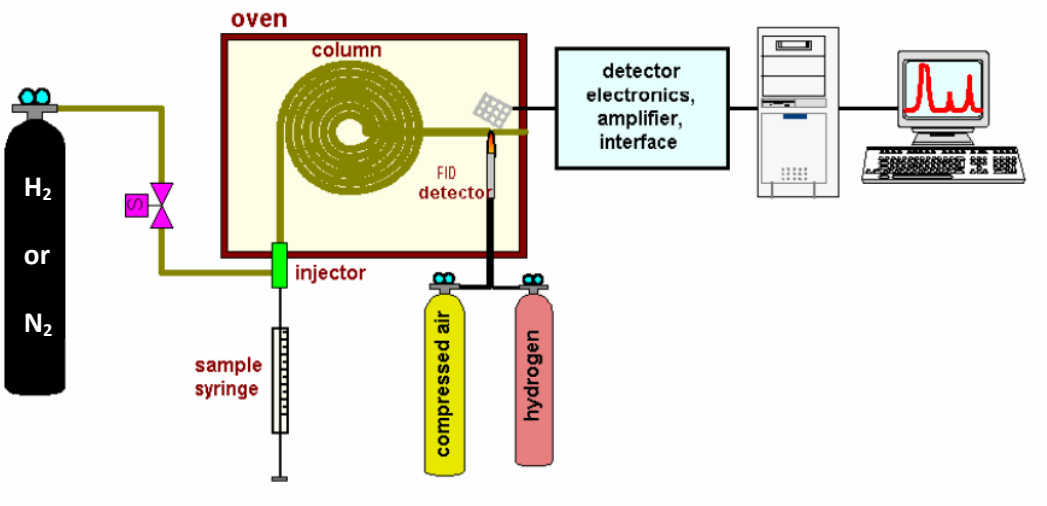
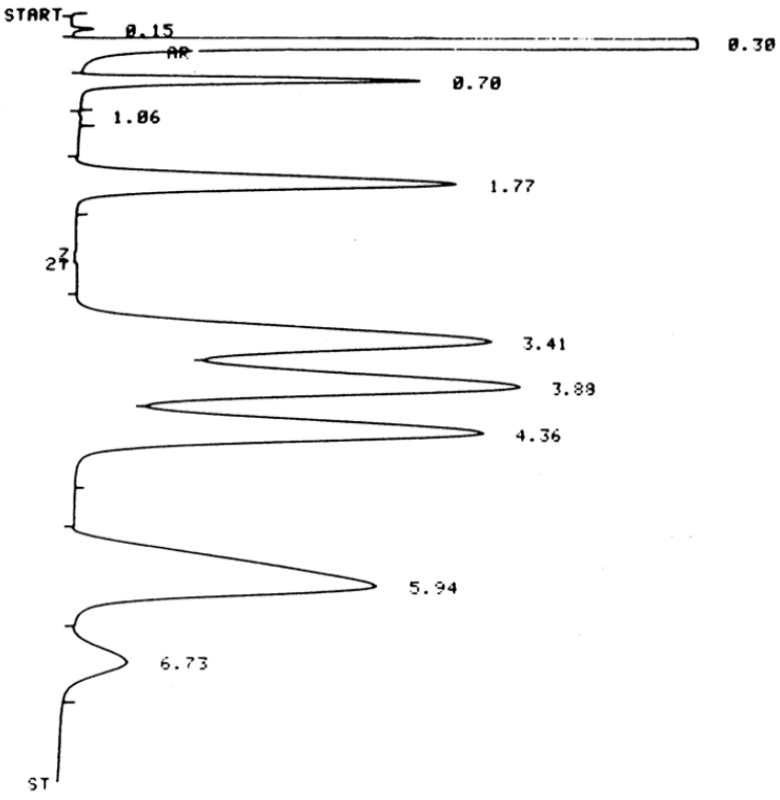


Fig. 1 Elements of the Gas Chromatography (Amonsit and Petsom, 1995)

For an analysis the sample is injected into the sample injection port (Fig. 1). It is vaporized by heating and then carried into the column with the mobile phase. Elements of the mixture will separate when moving through the column and are detected by the detector signal. Measurements from the detector are recorded and displayed in the form of chromatogram in Figure. 3



HP RUN # 2 MAR/28/76 TIME 23:51:05
ID:10-12-43 BOTTLE 19
ISTD

RT	EXP RT	AREA	CAL #	AMT
0.70	0.70	79380	2	10.069
1.77	1.76	195400	3	24.936
3.41	3.40	188100	4	24.555
3.88	3.87	195400	5	24.059
4.36	4.35	167800	6	19.927
5.94	5.93	195400	(R) 1	
6.73	6.73	31880	7	4.106

DIL FACTOR: 1.0000 E+ 0 ISTD AMT: 1.8400 E+ 1
SAMPLE AMT: 7.4250 E+ 1

Figure 2 shows and example of the chromatogram output data from a Gas Chromatograph. (from Amonsit and Petsom, 1995)

Qualitative analysis

Retention time (RT) is the time used for each type of material moving through the column from the start. The analysis of the position of the detector signal peak values (peak) of the measurement substances such as Fig. 4.

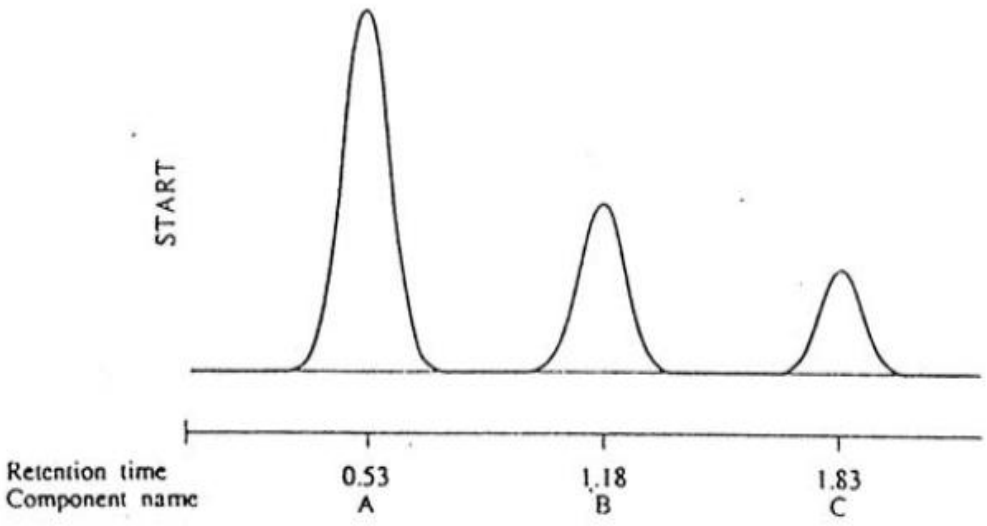


Fig. 3 Chromatogram shown Retention Time of Components A, B, and C. (from Amonsit and Petsom, 1995)

The retention time is characteristic of the chemical species in a particular analysis, the solvent, the type of column and temperature. The analysis conditions should be constant. The identity of any component in the sample mixture can be made by comparing the retention time between the chemical compositions of the mixed sample (unknown) with a standard component. The shape and width of the peak can also be important.

Column temperature is critical to separate the sample as shown in Figure 5

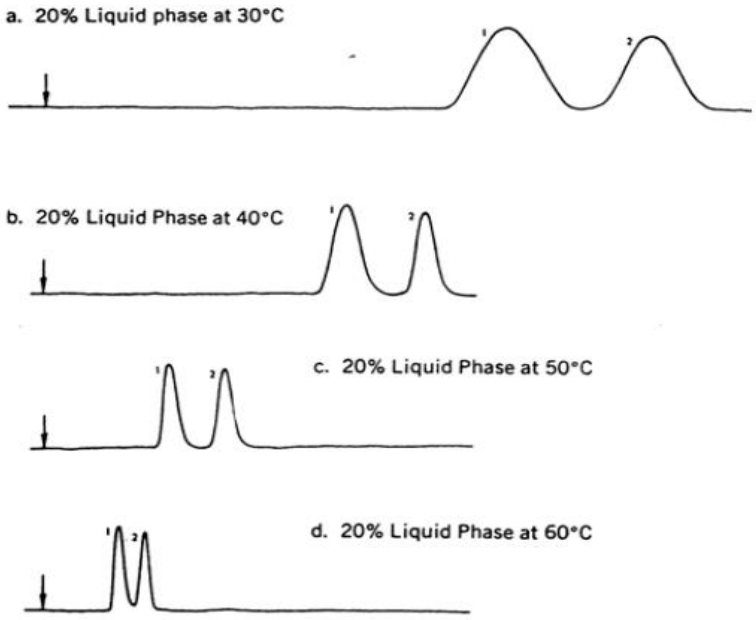


Fig. 4 shows the effect of temperature on Retention Time (from Amonsit and Petsom, 1995)

When the column temperature was increased, the substance moved faster and faster through the column. Therefore, a temperature gradient will help to separate the different components, and ensure that the retention time is not too long. Typically the boiling point of the substance of interest is the optimum temperature for the column, but care needs to be taken not to exceed the temperature tolerance of the column packing.

Quantitative analysis

Quantitative analysis by GC has been very popular in determining the amount of substance. Typical analytical approaches described by Amonsit and Petsom (1995) include (1) Normalization method, (2) External Standardization method and (3) Internal Standardization method. In the present study a standard external standardization method was used. A standard curve was prepared from a range of known concentrations of ethanol in water equilibrated in crimp-seal sample bottles equilibrated to a known temperature and the gas phase was sampled using a micro syringe. The ethanol standard curve is shown in Fig. 36 (Appendix).

CHAPTER 3

Materials and Methods

3.1 Materials

3. 1.1 Microorganism and culture conditions.

S. cerevisiae, in this study was obtained from the Biology Laboratory of Prince of Songkla University, Phuket campus and *S. cerevisiae* grown in liquid medium containing 6 gram of peptone, 3 gram of yeast extract, 6 gram of dextrose, 15 gram of agar added to 300 ml of distill water. The solution was heated to 121 ^{0}C for 15 minutes, and then cooled to room temperature. The yeast culture was maintained in the refrigerator at 20 ^{o}C until required (Akpan et al., 2008). Backup cultures were kept in the refrigerator at 4 ^{o}C.

3.1.2 Food Waste

Food waste used in this study was collected from stalls, trolley, restaurants, and canteens in Phuket province. Paper and pieces of wood and bones were removed. Samples of about 1 kg each from several sources were mixed together. The combined sample was then homogenized in a blender and used as feedstock for inoculums of yeast (*S. cerevisiae*).

3.2 Methods

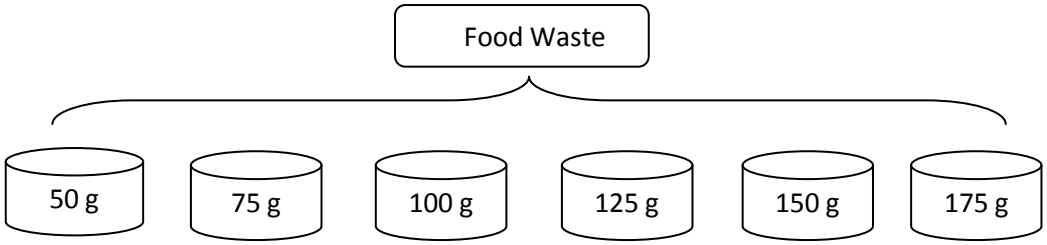
3.2.1 Characteristics of Food Waste

Before blending, estimates were made of the different classes of food present in the food waste by separating out the food into rice, vegetables, meat and bones and waste such as toothpicks, chops sticks and tissue paper and weighing each component. Wood, paper and bones were removed before homogenization in a blender. Characteristics of food waste such as pH, total

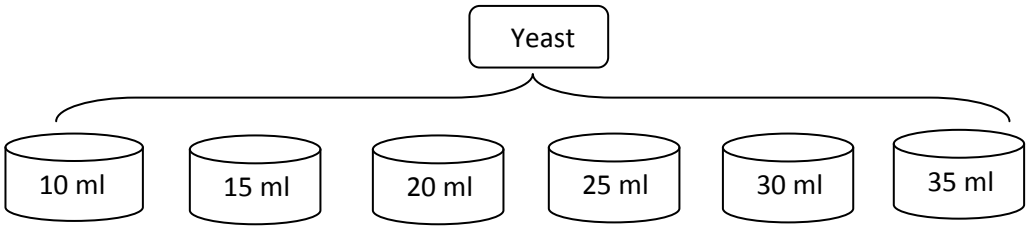
solid, moisture, total sugar, reducing sugar, starch, wet-dry basic, lipid, protein, carbohydrate, ammonia, COD, BOD_5 were determined.

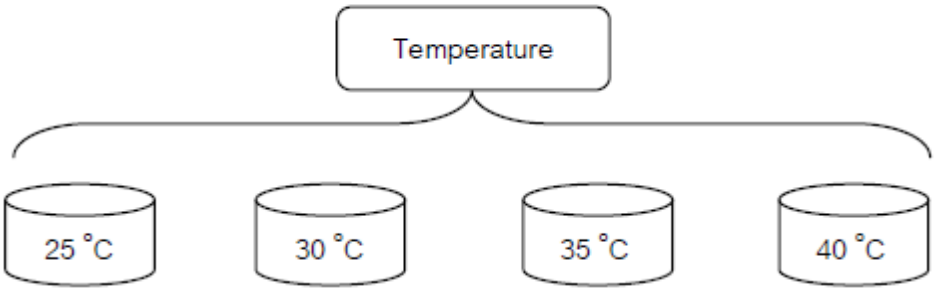
3.3 Fermentation

Initial experiments were set up with food waste volumes of 50, 75, 100, 125, 150, 175 g FW of food waste in 250 ml total volume, inoculated with 10, 15, 20, 25, 30, 35 ml of *S. cerevisiae* culture. The appropriate incubation time for breakdown was determined using incubations of 20, 25 and 30 days, and the appropriate inoculation volume of yeast to use and the optimum temperature for fermentation was determined in the range 25, 30, 35 and 40 ^{o}C using 7 day incubations.

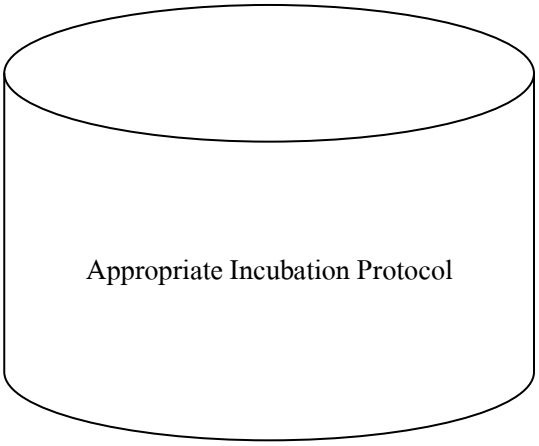


- Used food waste 6 bottle and added food waste to 50, 75, 100, 125, 150 and 175 g/FW in 250 ml total volume.
- Determine the breakdown of sugars, proteins, oils, starch and BOD_5 and COD at 20, 25 and 30 days.
- Select best combination of feedstock loading (FW of food waste), incubation time and temperature to optimise breakdown of the food waste.





- No aeration was used. Venting of CO_2 was measured to estimate CO_2 production.
- Yeast innoculation volumes used were 10, 15, 20, 25, 30 and 35 ml of cell suspensions containing about 5×10^6 cells per ml.
- Characteractics of incubated food waste and inoculums of yeast were checked periodically.
- The optimum combination of feedstock loading, innoculation volume and temperature was identified.



3. 4 Yeast Fermentation of Food Waste in Industrial Situations.

Autoclaving of food waste followed by yeast fermentation would be impractical in industrial situations. Partial sterilisation would be possible using HCl treatment (pH 3.5) which would discourage most non-yeast microbes from growing. Partial sterilization at 80 ^{o}C such as is used in the brewing and wine industries would also be practical.

- pH investigations. The effect of acidification on the fermentation efficiency was investigated using incubations adjusted to pH 3.5 using HCl compared to the control protocol.
- Pasteurisation of Food waste at 80 ^{o}C. Most experiments in the present study were done on autoclaved food waste under sterile conditions. To investigate the success of yeast fermentation under conditions similar to what would be used industrially some experiments were run where the food waste was pretreated at 80 ^{o}C before incubation with yeast.
- Production of ethanol by sterile culture on autoclaved food waste, fermentation of food waste treated at 80 ^{o}C and fermentation on acidified food waste were compared. Ethanol production was measured by Gas Chromatography (see below 3.9).

3.5 Cell Count by Spectrophotometer (turbidimetric method)

In a spectrophotometer light will pass through a cell suspension sample an the transmitted light is measured using a phototube and galvanometer. Themachine calculates the percentage transmittance (%T) and optical density (OD) (or Absorbance) which is OD = Log_{10} (100/%T): thus if a cell suspension transmits only 10% of the light then the OD is Log_{10} (100/10) = 1. The wavelength of light used needs to be specified. The most appropriate wavelength for following the growth of yeast is 650 nm (Ritchie and Raghupathi, 2008). Cell counts can be made on different dilution of cells to obtain a relationship between Optical density and cell numbers. Absorbance of cultures is only proprtional to cell numbers up to an absorbance of about 1.5 to 2.0. Thus to use absorbance to estimate cell number samples often have to be diluted.

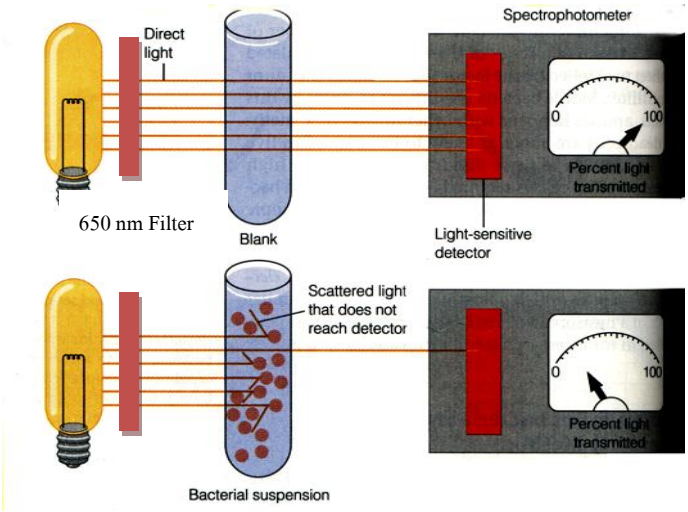


Fig. 6 Measurement yeast growth using spectrophotometer.

3.6 Using Gas Chromatography to Measure Alcohol

A gas chromatograph is basically a diffusion column with a detector unit to detect compounds as they migrate out of the exchange column. Pressured gas is pushed through the column after a sample is injected into a small gas space at the head of the column. The gas carrier is usually N_2 or Argon or Helium. In the present case He-carrier was used. The gas chromatograph used was an Agilent 7890A GC. The detector used was a UV detector.

To use the GC to measure ethanol samples of water with known concentrations of ethanol were placed in sealed vials and equilibrated to 60 ^{o}C. A sample needle was then inserted into the gas phase and injected into the GC. After about 2 minutes (the retention time) the ethanol had passed through the column into the detector unit. The retention time on the column is characteristic of the compound injected into the CG and the height and peak area recorded by the detector is proportional to the concentration of ethanol in the gas space in the sealed vial and that is proportional to the concentration of ethanol in the liquid phase for a given temperature. Using a range of different alcohol concentrations a standard curve can be prepared based either on peak height (or more accurately on peak area) vs. ethanol concentration (Amonsit et al., 1995).

In this study an Agilent 7890A GC was used. The carrier gas used was helium (He) and the software ChemStation was used to determine the area of ethanol peak.

3.7 Determination of ethanol concentration using gas chromatograph

The condition of gas chromatography for the analysis of ethanol from food waste were as follows.

Inlet

- Inlet mode : Split
- Inlet temperature : 150 ^{o}C
- Presseure : 5.10 psi
- Split ratio : 25:1
- Split flow : 99.9 ml/min
- Total flow : 103.6 ml/min
- Carrier gas : Helium gas

Capillary Column

- Oven temperature : 50 ^{o}C (1 min), 1 ^{o}C/min to 45 ^{o}C, 10 ^{o}C/min to 80 ^{o}C
- Colum mode : Constant flow
- Intial flow : 1.0 ml/min
- Nominal initial pressure 5.10 psi (39 kPa)

Detector

- Detector temperature : 300 ^{o}C
- Hydrogen flow : 30.0 ml/min
- Air flow : 300 ml/min
- Make up gas : Nitrogen gas
- Make up flow : 35.0 ml/min

CHAPTER 4

Results and Discussion

Initial experiments were carried out using experimental media consisting of different proportions of food waste. The fermentations were performed at different temperature using different level of yeast inoculation. Food waste volumes of 50, 75, 100, 125, 150, 175 ml in 250 ml total volume were inoculated with 10, 15, 20, 25, 30, 35 ml of *S. cerevisiae* culture ($\approx 4.6 \times 10^6$ cells/ml). Food waste was sterilized at 121 ^{o}C for 30 min before being inoculated with yeast culture and the incubation times used were 20, 25 and 30 days.

4.1 Using Absorbance to Monitor Growth of Yeast

Yeast was incubated at room temperature (25 ^{o}C). The number of cells was counted using a standard haemocytometer (Improved Neubauer) and the absorbance at 650 nm (A_{650}) was measured in a spectrophotometer (Shimadzu UV-1601 spectrophotometer (*Shimadzu*, Kyoto, Japan) (Ritchie and Raghupathi, 2008). Fig. 6 shows a curve of absorbance of the culture vs. yeast cell numbers. The relationship is linear only at absorbances below about $A_{650} = 2$. A standard curve can be constructed to convert A_{650} into cell numbers: the fitted line was $Abs_{650} = 0.6822$ (± 0.0118) million cells/ml, r = 0.996, n = 6, ±SE). To accurately monitor yeast cell numbers in cells suspensions with an absorbance more than 2 the samples have to be diluted to make them onto the linear range of the curve.

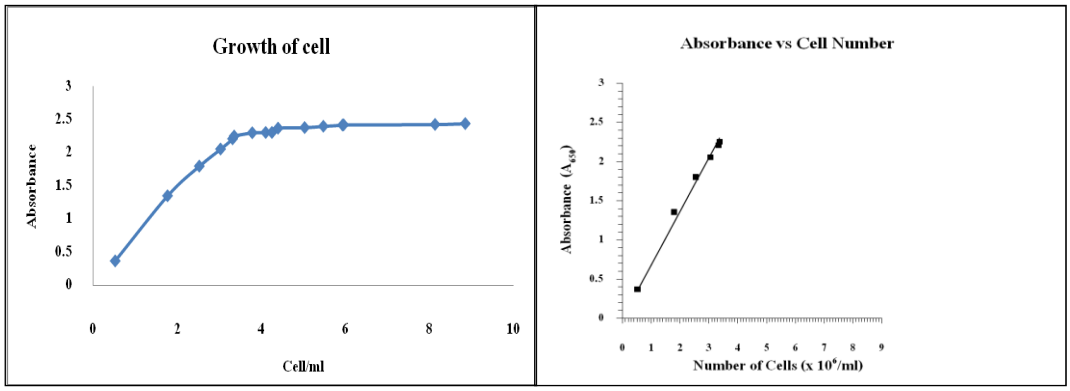


Fig. 7a. Absorbance vs. Number of cells.

Fig. 7b. Standard absorbance vs. Number of cells.

Growth of cells can also be monitored by the conversion of sugar to carbon dioxide gas and alcohol. Yeast grew rapidly in the first 2 days and continues to increase in numbers for up to 9 days. According to Yang et al. (2005) yeast can make more energy from carbohydrate during aerobic growth (respiration) than during anaerobic growth (fermentation). Fig. 1 in Suwannarat and Ritchie (2013b) shows an incubation experiment with a simple pneumatic trough to monitor gas production during incubation. The volume of gas produced each day could be easily monitored (Bonciu et al., 2010).

4.2 Composition of the Food Waste

The analyses of the food waste used in the present study were compiled and overall mean composition has been calculation and are shown in Table 1. The FW/DW ratio of the food waste was found to be 9.904 ± 0.315 (n = 5, ±SE).

Component	Total Sugar	Protein	Lipid	Reducing Sugar	Starch
Freshweight Basis (mg/gFW)	13.23±0.22	5.24±0.78	2.18±0.02	1.09±0.04	1.22±0.03
Dry Weight Basis (mg/gDW)	134.5±0.92	51.32±0.77	21.31±0.25	9.72±0.43	13.25±0.17

The major composition of food waste was total sugar, protein, lipid, starch and reducing sugar. Thai food waste had more carbohydrate of food waste. Korean foodwaste contains about 65% carbohydrate, mainly as rice (Kim et al., 2011).

4.3 Optimizing Incubation Time

Experiments were set up to determine the appropriate yeast inoculums, volume of food waste, and incubation time. Incubation times tested were 20, 25, 30 days with different volumes of food waste in from 50 to 175 gFW/250 ml. The results for the 20, 25 and 30 days experiments are shown in Figs 8, 9 and 10, respectively.

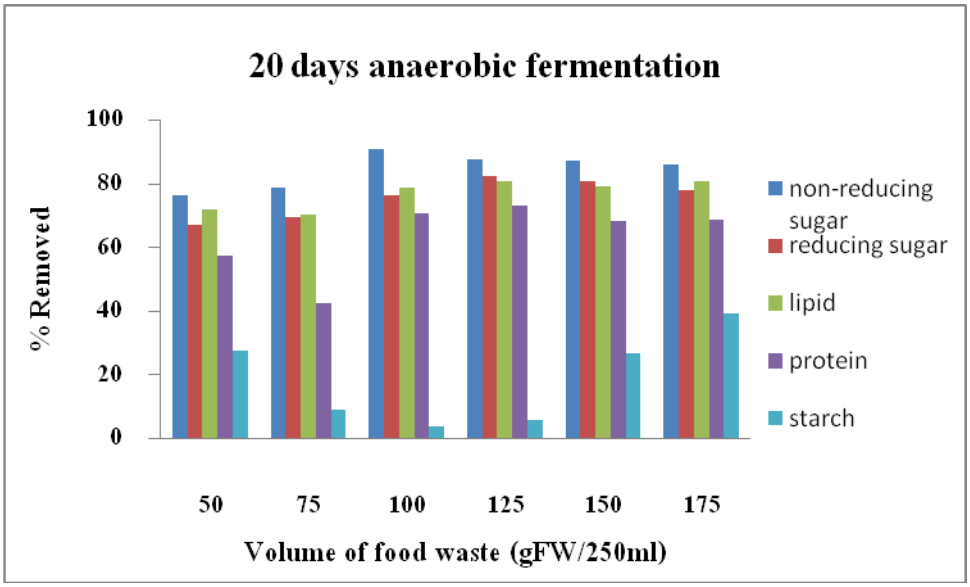


Fig. 8 Fermentation results for a 20 day incubation time with various loading of food waste from 50 gFW/250 ml to 175 gFW/250 ml (200 g/l to 700 g/l).

For the 20 day anaerobic fermentation incubation, the relative amounts of the 5 different components of the food waste (non-reducing sugar, reducing sugars, lipid, protein and starch) are shown in Fig. 8 Yeast can digest 100-175 g/250 ml of food waste very well: about 70-95% of non-reducing sugar, reducing sugar, lipid and protein are digested. In the lower feedstock loadings (50 and 75 gFW/250 ml) the non-reducing sugar, reducing sugar, lipid and protein were about 70% digested. In the case of starch yeast digested it well where the loading of food waste was low (50 gFW/250 ml) and high (175 g/250 ml) but at moderate loadings the yeast digested very little starch (100 gFW/250 ml). The yeasts were not hydrolyzing starch to any significant extent at the intermediate food waste loading rates. A surprising result of this digestion experiment was that over 70% of lipids were digested in all the different loadings of food waste. This result is similar to the results described previously (Fig. 3 in Suwannarat and Ritchie, 2013b) where higher loading of food waste were used. Suwannarat and Ritchie (2013b) found that COD and BOD_5 were reduced about 50% in the 20 day incubation.

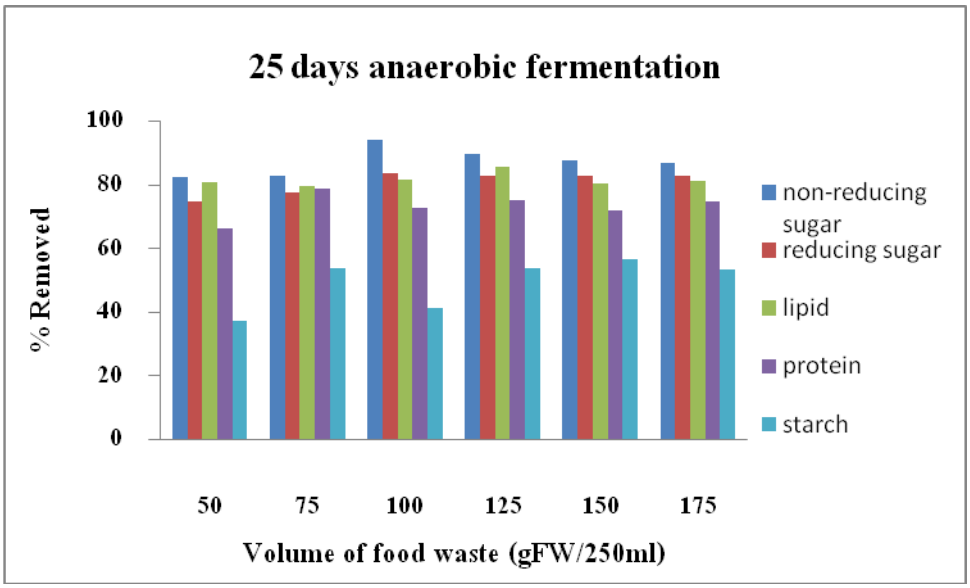


Fig. 9 Fermentation results for a 25 day incubation time with various loading of food waste from 50 gFW/250 ml to 175 gFW/250 ml (200 to 700 gFW/l).

A 25 day incubation shows a conspicuous difference to the 20 day incubation in that there was little or no increase in the total, non-reducing sugar, reducing sugar, lipid and protein digested ($\approx$ 80% removal) but there was a substantial increase in the amount of starch that had been removed. In the 20 day incubation some cultures had not begun to metabolize the starch but by 25 days all the cultures were mobilizing starch as a source of sugars. The 25 day incubation experiment described by Suwannarat and Ritchie (2013b) (Fig. 4) using food waste loadings ranging from 200 to 700 gFW/l also showed a great increase in mobilization of starch compared to the 20 day incubation and COD/BOD_5 removal values in exceeded 50%. Use of starch as a carbon source is only apparent after long incubation times and most of the other readily useable organic carbon had been used.

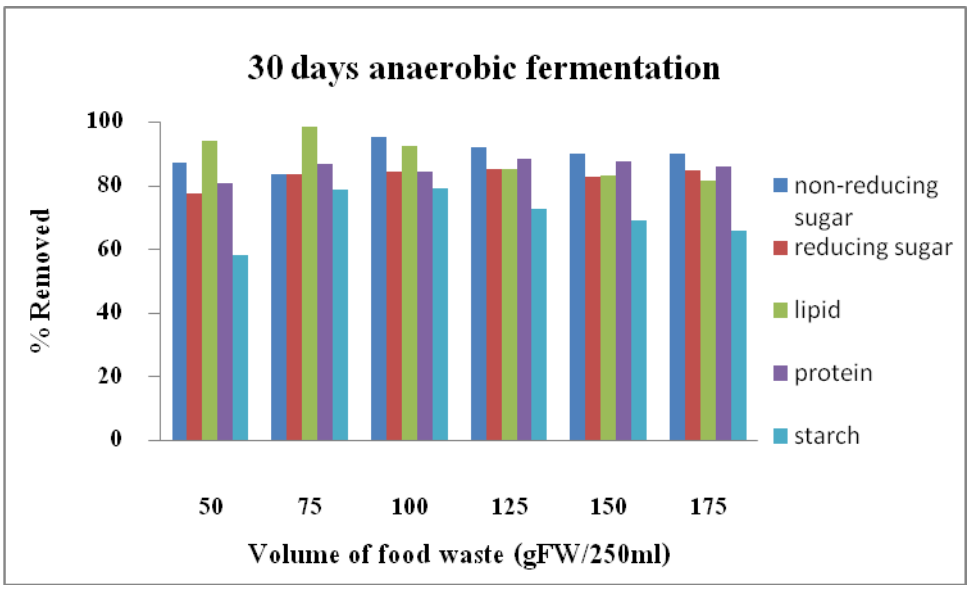


Fig. 10 Fermentation results for a 30 day incubation time with various loading of food waste from 50 gFW/250 ml to 175 gFW/250 ml (200 to 700 gFW/l).

The 30 day incubation shows nearly complete removal of non-reducing sugars, reducing sugars, lipids and proteins. Lipids, which were thought to pose problems for using yeast to digest food waste proved to be surprisingly digestible by yeast. In some of the treatments lipids had almost completely disappeared. The mobilization of starches that began in the 20 day incubation experiment and continued in the 25 day experiment led to at least 60% and up to 80% removal after 30 days. The overall results for the 30 day incubation described here confirm the experiments reported previously (Suwannarat and Ritchie, 2013b) but Suwannarat and Ritchie (2013b) had shown that a 30 day incubation did not substantially further reduce the BOD_5 and COD compared to the 25 day incubation experiment.

Overall, it was concluded that 25 day incubation is the optimum incubation time to achieve breakdown of most of the organic matter in food waste and achieve a large decrease in BOD_5/COD. The percentage removal of non-reducing sugar, reducing sugar, lipid, and protein was about 75-90 % of food waste. It is stated many times in the literature that yeast does not digest starch very well but being given enough time it does break down starch after it has used most of the other organic compounds. Yeast only has two amylases (KEGG, 2013). These are both α-glucoamylases (YIL099W and YIR019C) which only able to attack α-linked glucose units and so are not able to breakup branched starch chains (β-linked glucose). Hence, even after

30 days incubation the yeast was only able to break down about 60 to 80% of the starch. According to Carneiro et al. (2007) and Arsova (2010) the best organic matter removal efficiencies using yeast were for incubations less than 30 days.

4.4 Optimising food waste concentration

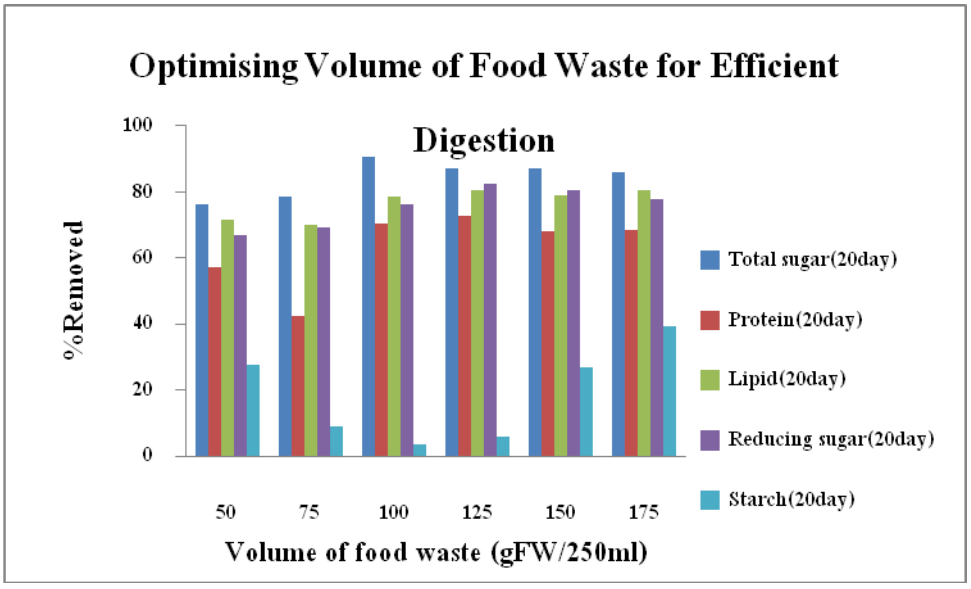


Fig. 11 20 day incubation experiment for optimizing volume of food waste for efficient digestion using 50 to 175 gFW/250 ml (200 to700 gFW/l).

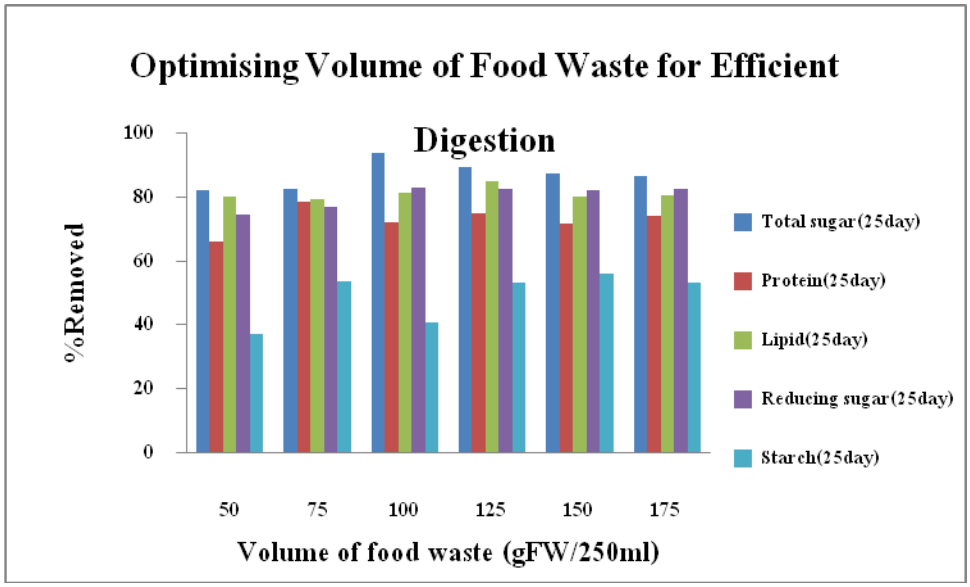


Fig. 12 25 day incubation experiment for optimizing volume of food waste for efficient digestion for loadings of 50 to 175 gFW/250 ml or 200 to 700 gFW/l.

In these experiments the use of autoclave-sterilized food waste had a significant effect on microbial digestion because the heat/pressure sterilization would have hydrolysed the carbohydrate chains of starch and would have also largely hydrolysed the proteins present enabling breakdown of the food waste by yeast (Figs 11, 12).

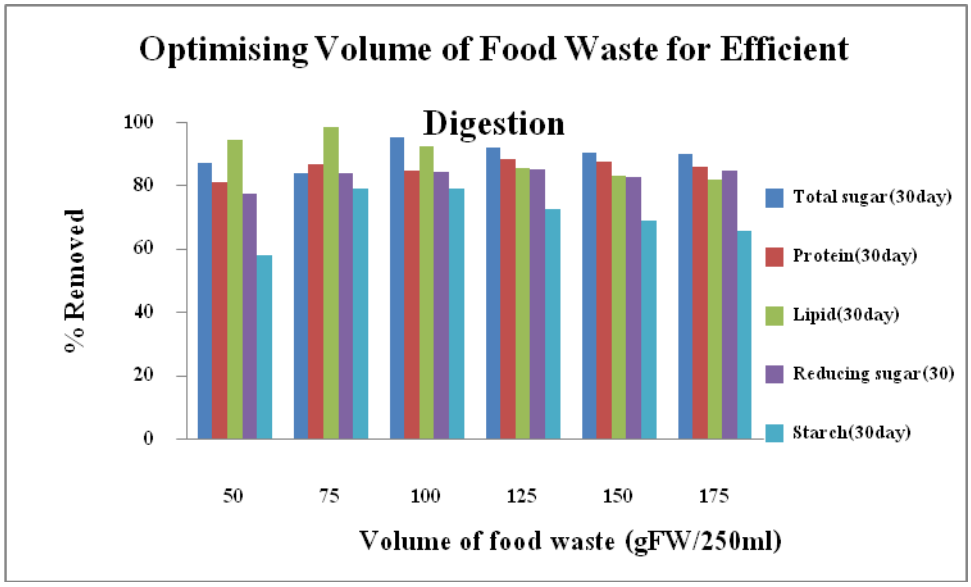


Fig. 13 30 day incubation experiment on optimization of feedstock volume of food waste for efficient digestion for loadings from 50 to 175 gFW/250 ml or 200-700 gFW/l.

It is important to note here that sucrose is hydrolyzed to glucose and fructose (invert sugar) by autoclaving and so an apparent ability to use sucrose in laboratory culture using autoclaved feedstock can sometimes be misleading (Yang et al., 2006).

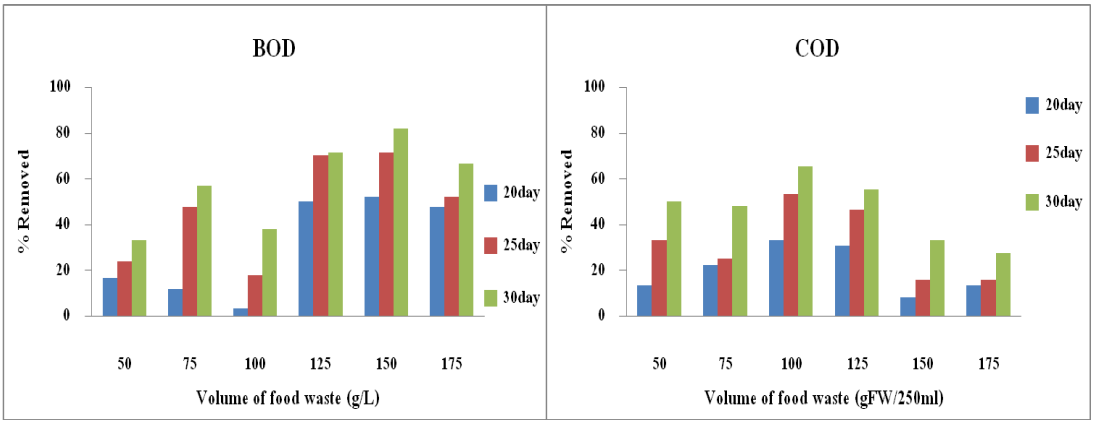


Fig. 14 BOD_5 and COD in digestion experiments to optimize food waste volume.

The BOD_5 results showed that the optimum incubation time is about 25 days with a feedstock loading of 125 to 175 gFW/250 ml (500 to 700 gFW/l). The COD results show that the best loading of feedstock is at an intermediate loading of 100 to 125 gFW/250 ml where the decrease in COD is about 60%. The decrease in COD is not satisfactory at low and high feedstock loadings (Fig. 14). The optimum feedstock loading in terms of both BOD_5 and COD is at about 125 gFW/250 ml or 500 gFW/l.

4.5 Carbon dioxide production

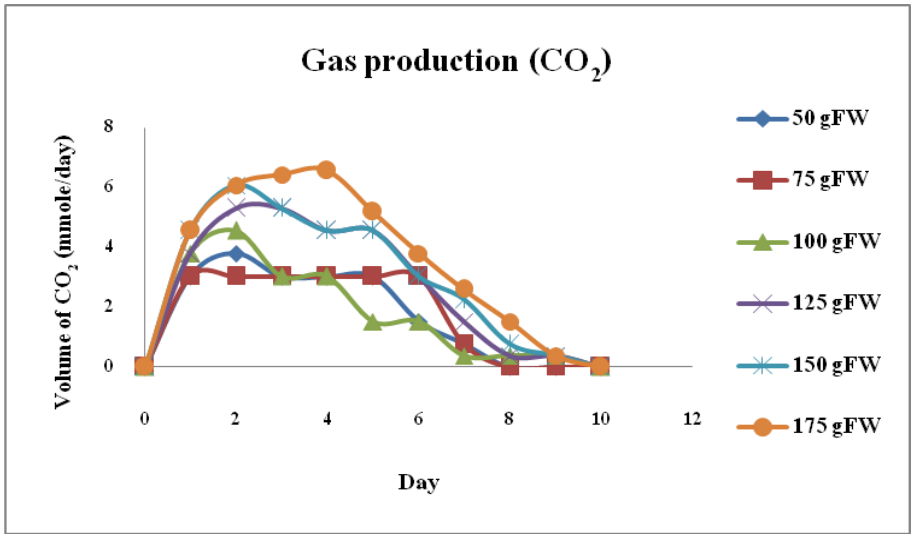


Fig. 15 Gas production from fermentation of different loadings of food waste (50 to 175 gFW/250 ml, 200 to 700 gFW/l) over 10 days.

Fermentation process by yeast converts sugar into carbon dioxide gas and ethanol (Song et al., 2010) and yeast cells get energy from the glycolysis steps (Fig. 15). Carbon dioxide gas production per day increases from the beginning to days 2. The highest CO_2 production was on day 3 (about 7 mmole) 175 g/L of food waste but had reached negligible daily gas production after days 8. Gas production was measured using a simple pneumatic trough device (Suwannarat and Ritchie 2013b) and converted to moles of CO_2 using the universal gas equation ($PV = nRT$ where, P is the pressure (kPa), V is the volume (m^3), n is the number of moles of ideal gas, R is the universal gas constant 8.3143 J K^{-1} $mol^{-1}$and T is the absolute temperature (K). One mole of CO_2 therefore occupies 24.5 l at 25 ^{o}C and normal air pressure. Gas

production was measured as cm of gas collected over water. The gas collection bottle was calibrated to convert cm of gas to mmoles of CO_2 by first working out the relationship between cm of gas collected over water and volume of gas (ml) and then converting it to mmoles of gas assuming an ideal gas. (see Fig. 35 in Appendix). Cumulative CO_2 could be calculated by summation (Fig. 16).

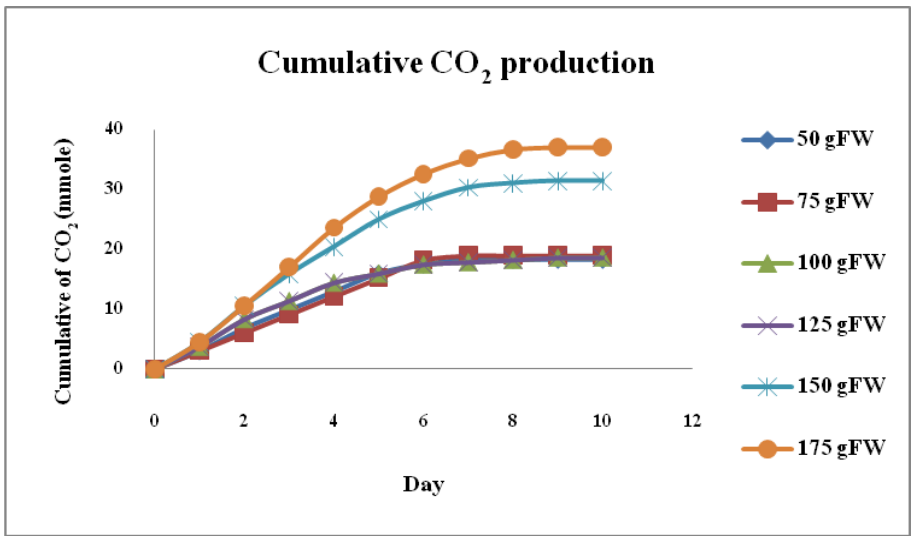


Fig. 16 Cumulative CO_2 production in fermentation with 50 to 175 gFW/250 ml or 200 to 700 gFW/l.

From fermentation results shown in Fig. 16, accumulative CO_2 production increased from the beginning of the experiment until day 8. Then it was constant until about day 10. As expected, yeast cells produced the maximum CO_2 gas when grown in media containing 175 gFW/250ml of food waste and incubated for 25 days. The experiments were run for 30 days but little or no extra gas was produced. Figs 11 to 13 show that the majority of the digestible sugars, protein and lipids had been digested after 20 days and there was little or no extra digestion after 25 or 30 days. However, as seen in Fig. 11 much of the starch had not been digested after 20 days but digestion of starch was more apparent after 25 (Fig. 12) or 30 (Fig. 13) days digestion. This late mobilization of starch did not appear to result in readily measureable CO_2 production. The glucose formed from the breakdown of the last remaining reserves of organic carbon useable by the yeast (starch) was apparently not used as a source of energy by glycolysis/fermentation

otherwise some resurgence of gas production would have been observed. The carbon dioxide gas from fermentation process was water saturated gas (CO_2+ water vapour) (Xu et al., 2010).

4.6 Digestion of Food Waste

Food waste was weighed on both wet and dry basis at the beginning of the experiments and at 20, 25 and 30 days for feedstock loadings of 50 to 175 gFW/250 ml (200 to 700 gFW/l). Five g of food waste/water suspension was weighed out in a beaker and dried at 105 ^{o}C for 2 hrs and then reweighed. The DW/FW ratio was then calculated as a percentage. The DW measurement for the food waste includes both matters in solution and insoluble matters.

Table 2. Organic matter remaining after 20, 25 and 30 days incubation.

Food waste (gFW/250 ml)	DW/FW ratio (0 day)%	DW/FW ratio (20 day)%	DW/FW ratio (25 day)%	DW/FW ratio (30 day)%
50	14.99	18.79	11.90	10.43
75	14.99	15.60	12.51	11.24
100	14.99	12.79	13.25	12.77
125	14.99	11.31	12.23	13.91
150	14.99	12.48	13.50	10.36
175	14.99	12.97	11.48	10.15

Table 2 shows that up to about 1/3 of the organic matter were converted to volatile CO_2 and water after 30 days incubation. About 2/3 of the dry weight of food waste does not appear to be readily broken down by yeast. This is probably mostly cellulose and some of the starch.

Wet basis was from fermentation process decreases over the long term. The first day was 14.99% (DW/FW × 100) and decreased to 10.15% after 30 days (Table2).

Food waste (g FW)	TS(0 day) (mg/l)	TS(20 day) (mg/l)	TS(25 day) (mg/l)	TS(30 day) (mg/l)
50	60.47	60.13	60.15	60.03
75	60.47	57.78	58.24	47.78
100	60.47	59.53	57.74	49.53
125	60.47	48.76	54.88	38.76
150	60.47	46.71	58.45	36.71
175	60.47	45.40	56.19	35.40

Table 3. Total Solids (TS) of food waste after 20, 25, 30 days of fermentation

Total solid was determined following APHA (1998) methods after autoclaving. Total solid value from fermentation on day zero was a very high value 60.47. Except in the case of the 50 gFW/250 ml experiment the TS went down during the incubation period, consistent with Wantana et al. (2011). Total solid is an indicator of dirt in the waste water, density of waste water and is used to demonstrate the efficiency of fermentation (Table 3).

4.7 The Effect of Yeast Inoculation Volume on Digestion of Food Waste

The starter yeast cultures used for inoculating the food waste had about 5×10^6 cells/ml. A range of inoculation volumes used was 10 to 35 ml per 250 ml of food waste suspension. Incubations were carried out for 7 days and the feedstock was 175 gFW/250 ml or 700 gFW/l because the experiments above showed that yeast could easily break down a food waste feedstock loading of 175 g FW/250 ml.

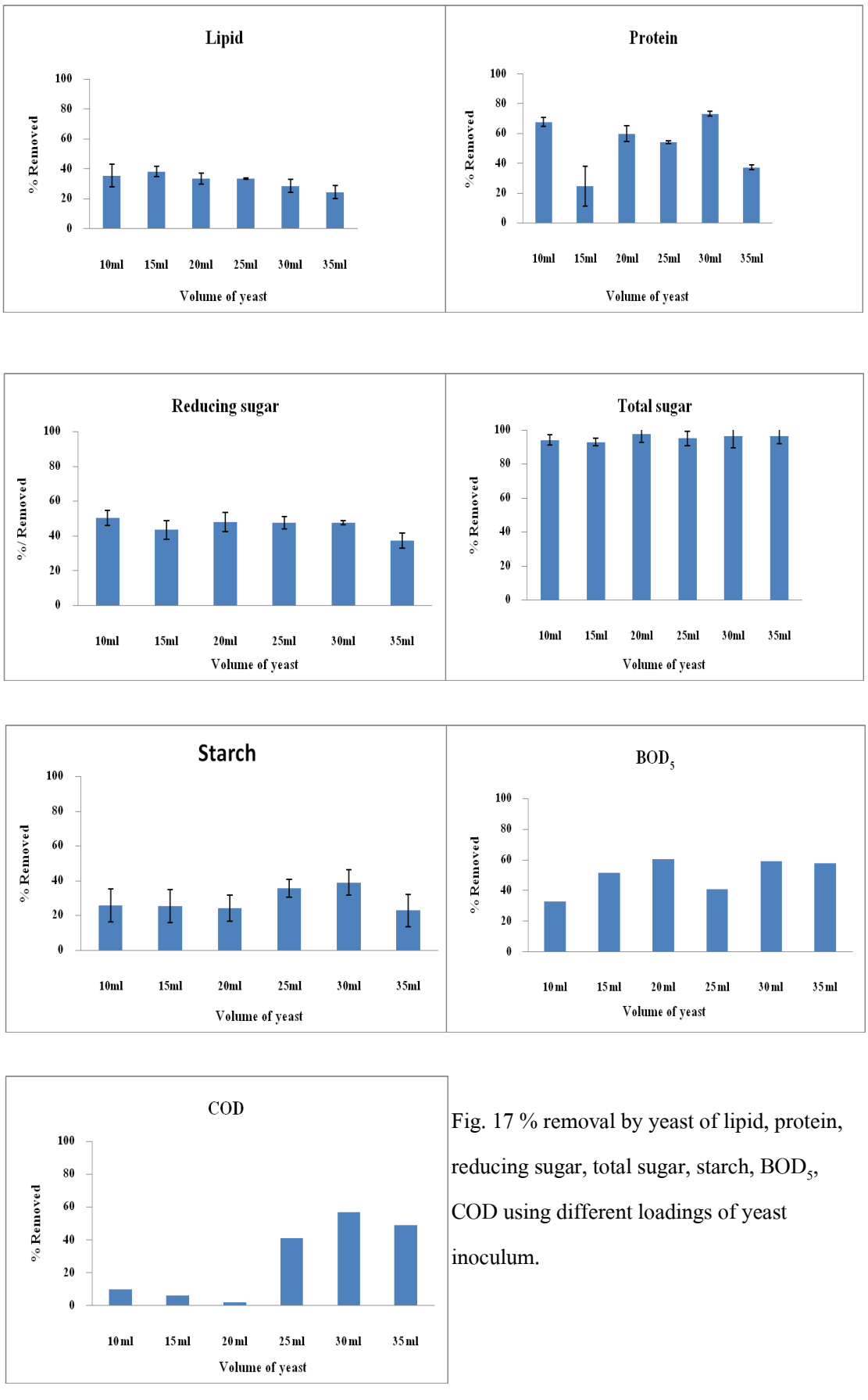


Fig. 17 % removal by yeast of lipid, protein, reducing sugar, total sugar, starch, BOD_5, COD using different loadings of yeast inoculum.

Fig. 17 shows the efficiency of digestion of food waste using different initial inoculations of yeast (10, 15, 20, 25, 30, 35 ml of yeast inoculum, $\approx 5 \times 10^6$ cells/ml). The results show that there was very little, if any, effect of the yeast inoculation volume on the digestion of lipid, protein, reducing sugar, total sugar or starch after 7 days incubation (the protein value for 15 ml inoculation volume appears to be erroneous). Digestion of starch was very limited after an incubation of only 7 days. This is consistent with the previous findings by Suwannarat and Ritchie (2013a, b) and Figs 11, 12 and 13 where it was found that mobilization of starch was increased considerably after incubations for about 25 days. The 7 day incubation used in the these yeast inoculation experiments showed that about 90-97% of the total sugar was removed from food waste in the first 7 days of incubation (Fig. 17). This is consistent with the finding shown in Fig. 16 that gas production had largely ceased by 7 days. Protein was also rapidly digested by yeast (about 70% removal at the highest from the food waste). Figs 11, 12 & 13 showed that lipid was largely removed after the samples were incubated for 20 or more days but the 7 day incubation (Fig. 17) showed that during the period when yeast was growing rapidly and producing large amounts of gas, lipids were not being digested very quickly. Reducing sugar remained relatively high because it was being replaced by reducing sugar (glucose) derived from starch and from total sugars. Figs 11, 12 & 13 show that by 20 days nearly all the available sugars had been used. The results of this inoculation experiment showed that overall the inoculation volume had little effect on total fermentation that had occurred after 7 days in terms of the food components measured (lipid, protein, sugars and starches). BOD_5 measurements showed that about 50% of metabolisable organic matter had been digested after 7 days. More COD was removed at heavier inoculations (25, 30 and 35 ml of yeast culture) compared to lower inoculations.

The results of this inoculation volume experiment might be affected by the use of autoclave sterilization (substrate was autoclaved) which probably had a significant effect on digestibility by the yeast because sterilization in the autoclave hydrolyses carbohydrate chains of starch and proteins making them readily available to the yeast (Yan et al., 2011). During the course of the experiment the volume of gas produced by the yeast was monitored on a daily basis (Fig. 18). The cumulative CO_2 production was also calculated and is shown in Fig. 19.

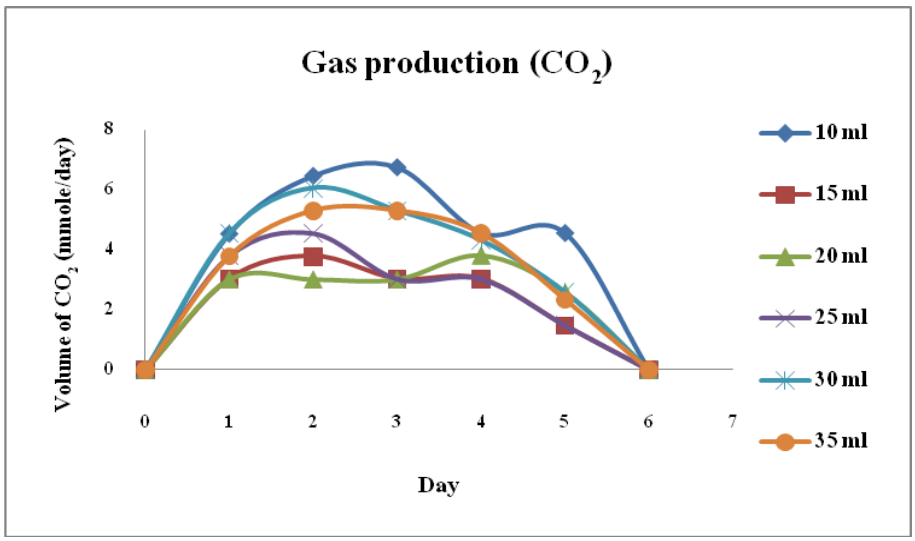


Fig. 18 Daily volume of CO_2 produced using different of inoculums of yeast.

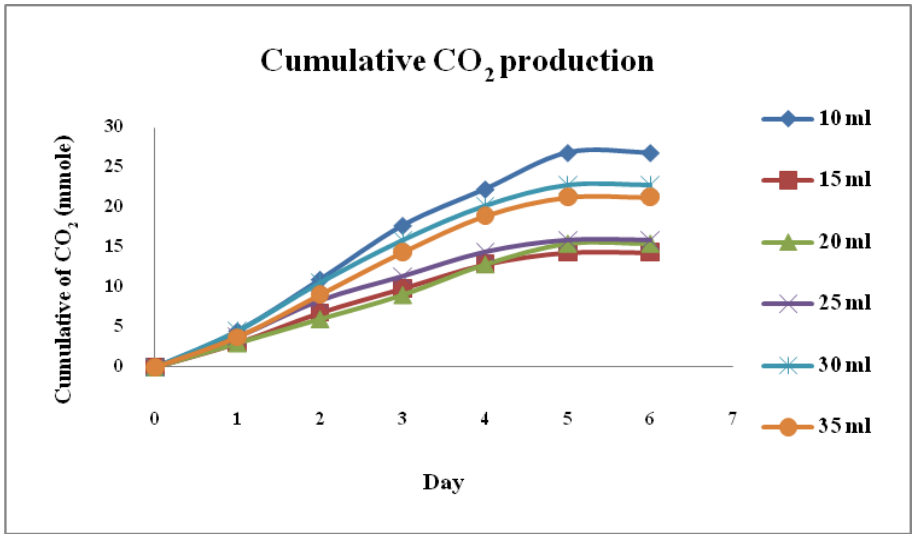


Fig.19 Total CO_2 production in experiments run with different volumes of yeast inoculum.

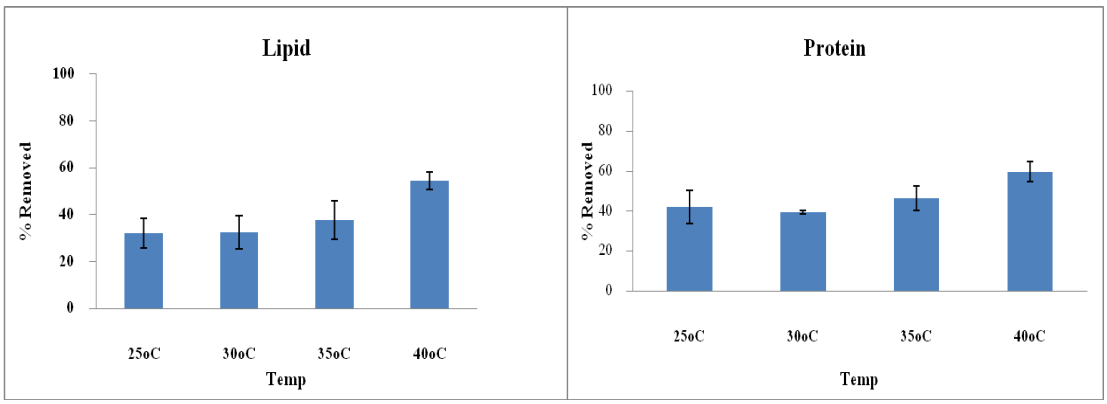
The gas production results shown in Figs 18 and 19 are basically similar to the incubation experiments shown in Figs 15 and 16. There is no obvious effect of inoculation volume on the total gas production over 7 days (The final production of CO_2 gas is not an obvious function of the yeast inoculum volume used, Fig. 19). The volume of carbon dioxide gas increased from the first day until days 6 of incubation. The maximum daily rate of gas production was on day 3, about 7 mmoles/day for the 10 ml yeast inoculation (Fig. 18). Carbon dioxide production is a result of sugar and starch conversion to CO_2. Fig. 19 shows that apparently the

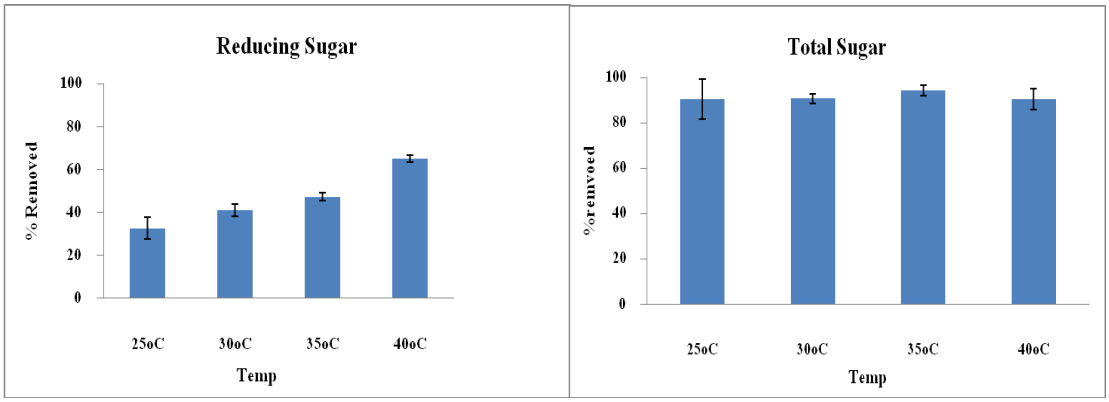
lowest inoculation rate (10 ml of yeast) gave the highest total CO_2 production of about 28 mmol of CO_2 gas. The apparent lack of effect of yeast inoculation volume on the results of the 7 day incubations most likely simply reflected the very rapid doubling times of yeast given plenty of food: doubling times are usually only a few hours in a yeast culture with unlimited food supply ($\approx$ 3 hr, Ritchie and Raghupathi, 2008).

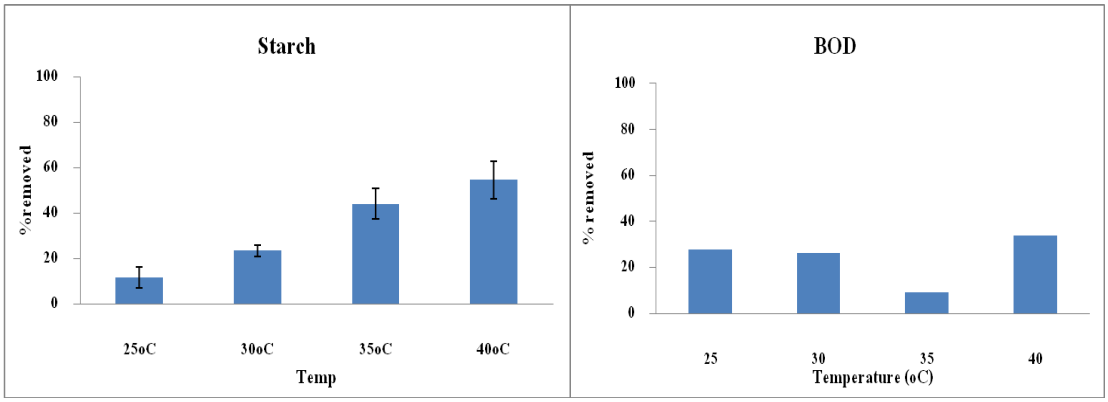
From the results of the 7 day incubation experiment it was concluded that there was no great advantage in using an inoculation volume greater than 10 ml (Figs 17, 18 & 19). It was there for decided to choose 10 ml of yeast ($\approx 5 \times 10^6$ total cells) for standard fermentation experiments.

4.8 Effect of Temperature on Efficiency of Digestion of Food Waste by Yeast

The effect of different temperatures (25 to 40 ^oC) on the efficiency of digestion of food waste was investigated using a feedstock of 175 gFW/250 ml (700 gFW/l) and a fixed standard yeast inoculation volume of 10 ml ($\approx 5 \times 10^6$ cells/ml). The temperature effect experiments were run for 7 days and then lipid, protein, reducing sugar, total sugar, starch and BOD_5 and COD were measured. Gas production was estimated as described previously using a simple pneumatic trough method (Suwannarat and Ritchie, 2013b) using the calibration curve for converting cm of gas collected over water to mmoles of ideal gas using the calibration curves shown in the Appendix (Fig. 35).







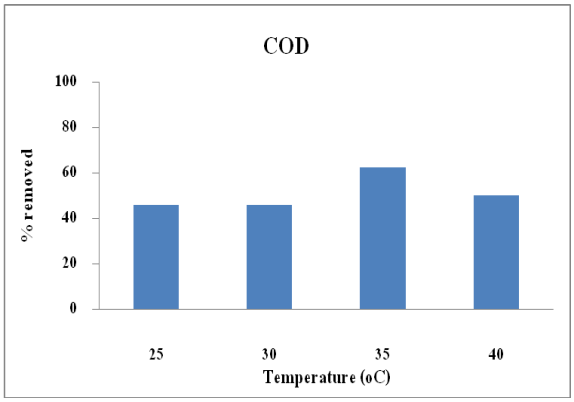


Fig. 20 % removal of food waste components by yeast incubated at different temperatures and fixed food waste starter feedstock and fixed yeast inoculation volume. BOD_5 and COD were also determined based on measurements made at t = 0 and t = 7 days.

Fermentation ran best at 40 ^{o}C (Fig. 20) particularly for lipids and starches which are digested more slowly compared to sugars and proteins. Digestion of lipids and starches seemed to be more temperature sensitive than digestion of sugars and protein. In previous experiments the standard temperature had been 25 ^{o}C. Total sugar data from Fig. 20 show that at 40 ^{O}C yeast can remove about 95% of the total sugar in 7 days. Digestion of lipid, protein, starch and reducing sugar reached more than 35-60% removal at 40 ^{O}C. BOD_5 and COD estimation found that about 30% to 45 % of organic material was removed at 40 ^{O}C. Davis (2008) found an optimum temperature of 27 ^{O}C but the optimum temperatures of various strains of yeast vary considerably.

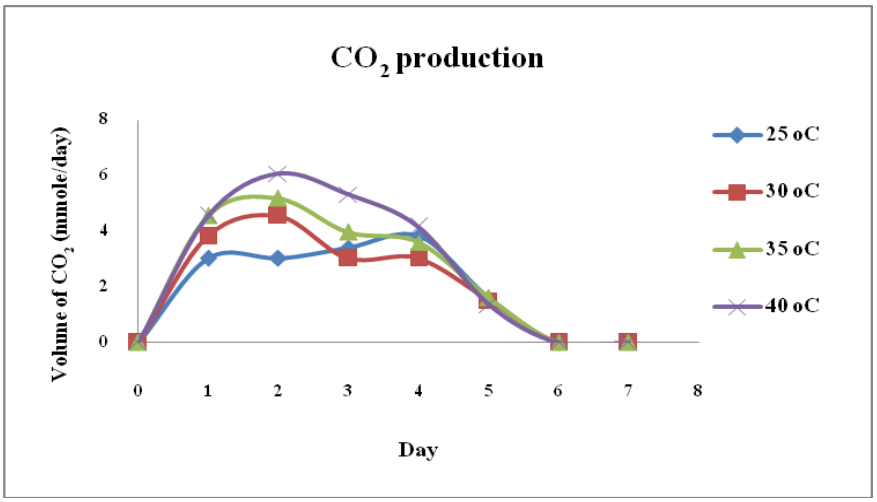


Fig. 21 Daily gas production from food waste shown incubated at different temperatures

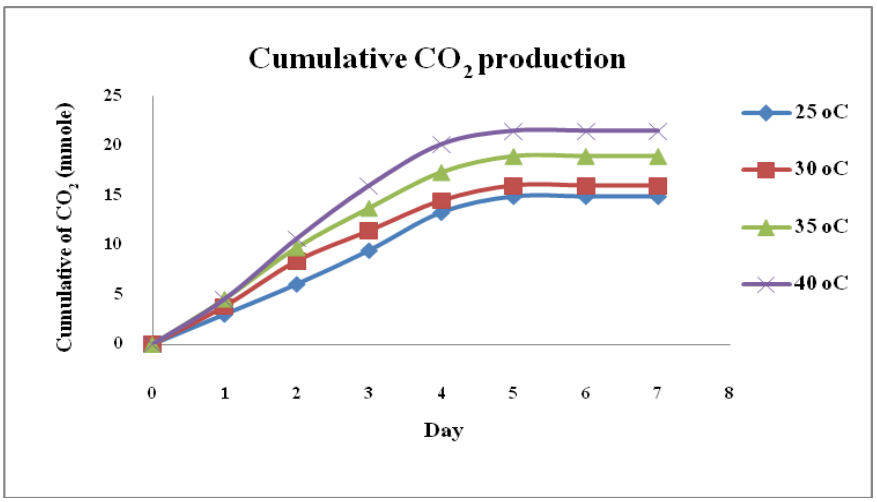


Fig. 22 Cumulative CO_2 production from food waste incubated at different temperatures

Figs 21 and 22 show CO_2 gas production per day and cumulative CO_2 production, respectively. Maximum gas production occurred after about 2-3 days and then rapidly declined. Higher incubation temperatures produced more gas but in all cases gas production ceased after 6 days. Fig. 21 shows maximum CO_2 production after 2-3 days, regardless of the incubation temperature. The result of the 25 ^{o}C incubation is similar to that found in Fig. 19. Yeast can produces optimal of CO_2 gas in 40 ^{o}C but high temperature increases the volume of gas produced but does not lengthen the duration of CO_2 production.

4.9 Comparison of the effect of different pretreatment methods on fermentation

Most of the present study was based on autoclaved media and sterile inoculation of yeast. This presents two difficulties. Autoclaving results in hydrolysis of carbohydrates and proteins providing simple sugars and amino acids for the yeast and so use of autoclaved food waste might overestimate how well yeast would be able to break down food waste where autoclaving was not used. In industrial situations it is not expensive to pretreat material at 80 ^{o}C and this pasteurisation protocol is often used in the food industry. Acid treatment has often been used to pretreat wastes but its possible negative effects on subsequent growth of yeast is not well documented (Akpan et al., 2005; Akpan et al., 2008; Arsova, 2010; Asada et al., 2011).

Three experimental fermentations were set up using the autoclaved food waste treatment as the control and the other pretreatments were pasteurization at 80 ^{o}C and pretreatment with HCl (pH 3.5). The obvious disadvantage of using non-sterile food waste as feedstock is that contaminating bacteria might take over the cultures and overwhelm the yeast. 175 gFW/250 ml (700 gFW/l) of food waste feedstock and 10 ml of yeast inoculum ($\approx 5\times10^{6}$ cells/ml) were used and the experiment was run for 25 days at 40 ^{o}C. The gas production was monitored for the first 7 days.

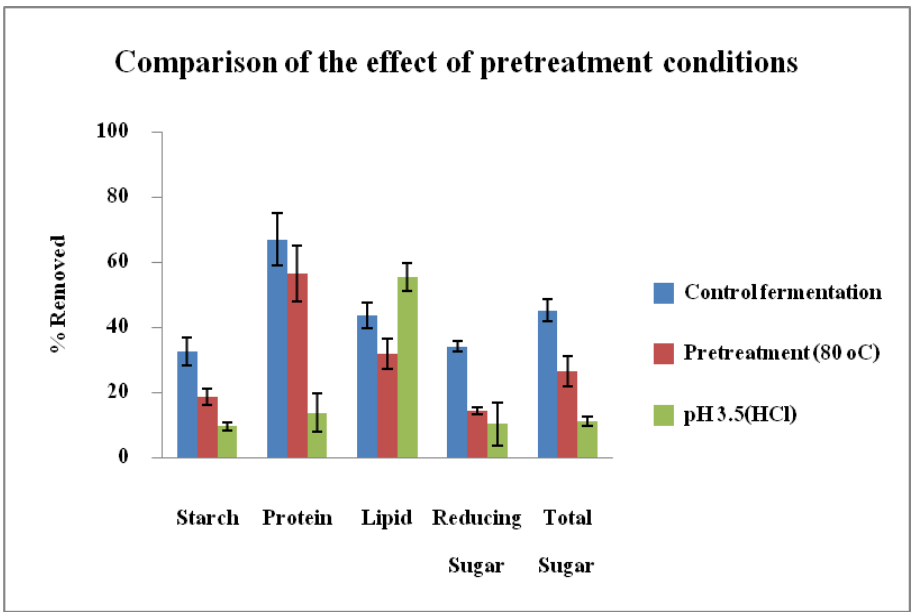


Fig. 23. Comparison of the effect of three pretreatment conditions. The control was autoclaved food waste and the two treatments were Pasteurisation at (80 ^{O}C) and acid (HCl) pretreatment (pH 3.5)

Fig. 23 shows that heat and acid treatment had significant effects on the efficiency of fermentation. Best results were found for the autoclaved food waste. And the results for pasteurised food waste were almost as good as for the autoclaved waste. Acid treated food waste was not a very successful feedstock. Reducing sugars were reduced by about 40% and total sugars by about 50% in the autoclaved food waste but results for the acid treated food waste were very poor (< 10%). Protein was reduced by about 65% after 25 days incubation in the control treatment but only about 10% in the acid treatment. The lipid results were exceptional: lipid in the acid treated food waste was reduced just as well as the control treatment. Breakdown of starch was very poor in the acid-treated food waste (< 10%).

In all the incubation treatments ammonia would have been a major N-source for the yeasts. Ammonia content was measured using the standard Solorzano method described in the Methods. Fig. 24 shows that in 25 days all the incubations removed most of the ammonia present. The incubation of pasteurized food waste achieved the highest degree of removal (about 95 %), followed by the incubation using autoclaved food waste ($\approx$ 90% removal) and $\approx$78% of the ammonia was removed in the acid treatment. The ammonia content of the food waste 175 gFW/250 ml was 0.0084 mg/ml or 8.4 mg/l which is equivalent to 0.49 mM. The food waste

therefore contained about 0.012 mg/g ammonia. This is about the ammonia content of standard culture media for growing yeast and so the yeast growing on food waste would not be N-limited (Ritchie and Raghupathi, 2008). Removal of ammonia from wastes is an important consideration in avoiding eutrophication in waterways. Ammonia is also an obnoxious odor leading to complaints from the public.

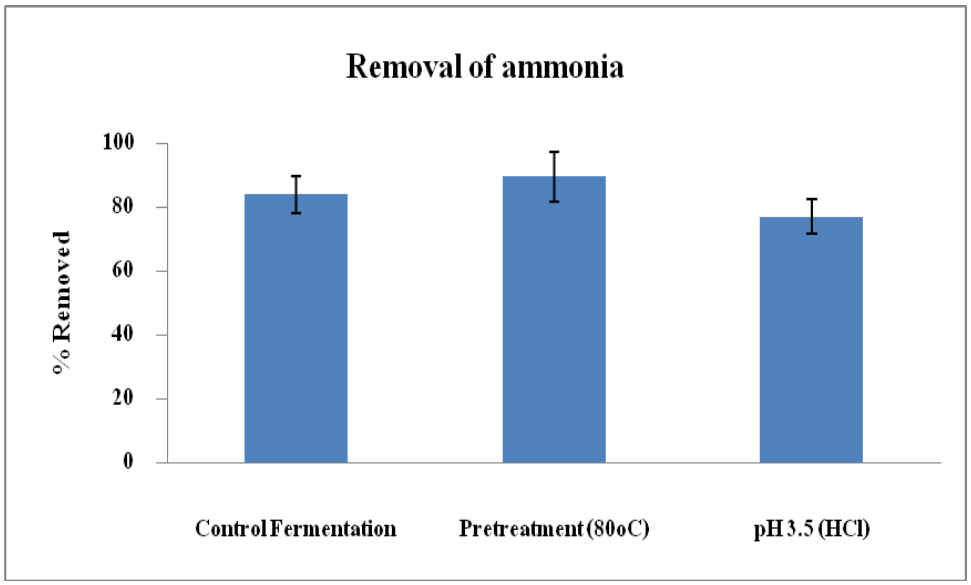


Fig. 24 Comparison of removal of Ammonia by yeast grown on Autoclaved (Control), Pasteurized (80 ^{O}C) and acid pretreatment pH 3.5 (HCl) of food waste

The results of BOD_5 and COD determinations are shown in Fig. 25. The results confirm the conclusion from the data on breakdown of sugars, protein, starches and lipids shown in Fig. 23. Pasteurization is a good alternative to autoclaving of food waste whereas digestion of acid treated food waste was very poor. In the incubation using autoclaved food waste the yeast could digest about 55-65% of the food waste. COD was decreased by 80% after 25 days of incubation for the autoclaved food waste but less than 40% in the case of the acid treated.

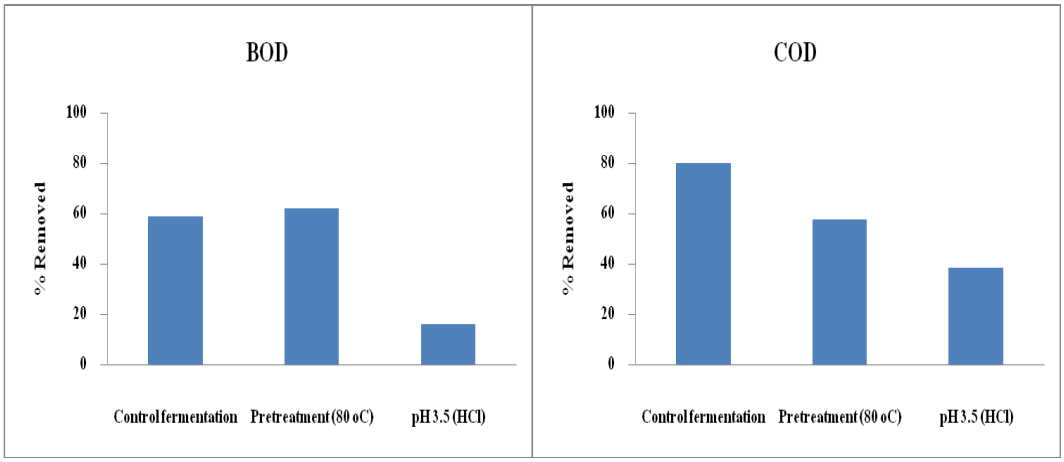


Fig. 25 Decrease in BOD_5 and COD values in three incubations conditions: control (autoclaved food waste), 80 ^{O}C pretreatment and pH 3.5 (HCl).

Fig. 26 through 28 shows the daily CO_2 production by incubations using autoclaved, pasteurized and acid treated food waste (pH 3.5) over the first 7 days of 25 days incubation. The incubation using autoclaved feedstock gave the highest total daily CO_2 production and the highest cumulative CO_2 production (Fig.26) followed by the pasteurized and acid treated feedstock respectively.

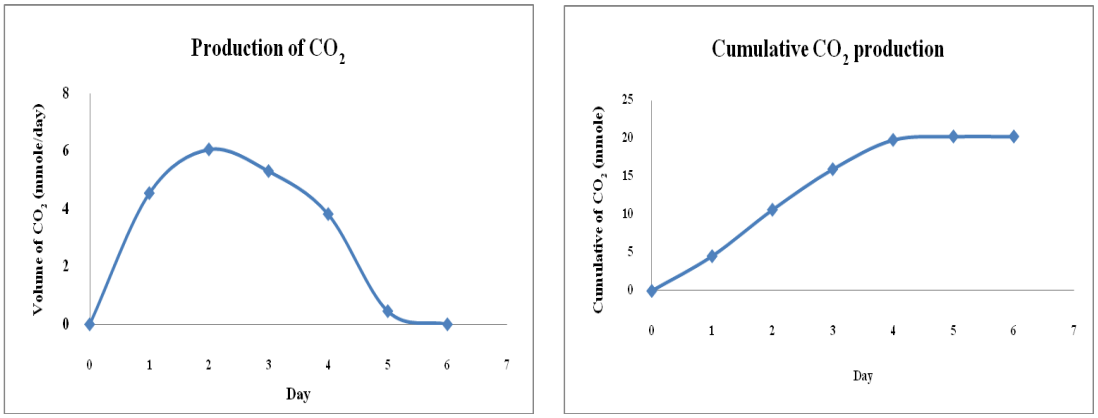


Fig. 26 Production and cumulative production of CO_2 by *S. cerevieiae* grown on food waste autoclaved at 40 ^{o}C during the first 7 days of incubation.

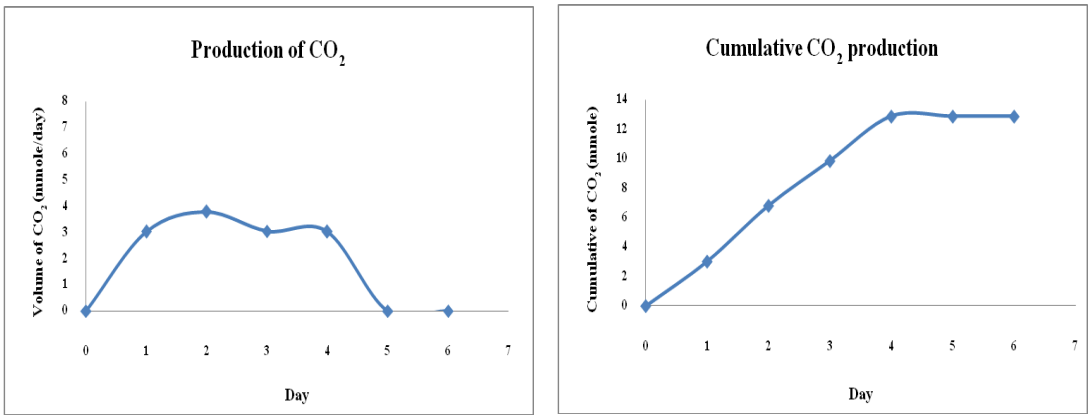


Fig. 27 Production and cumulative production of CO_2 by *S. cerevieiae* grown on food waste pasteurized food waste at 40 ^{o}C during the first 7 days of incubation

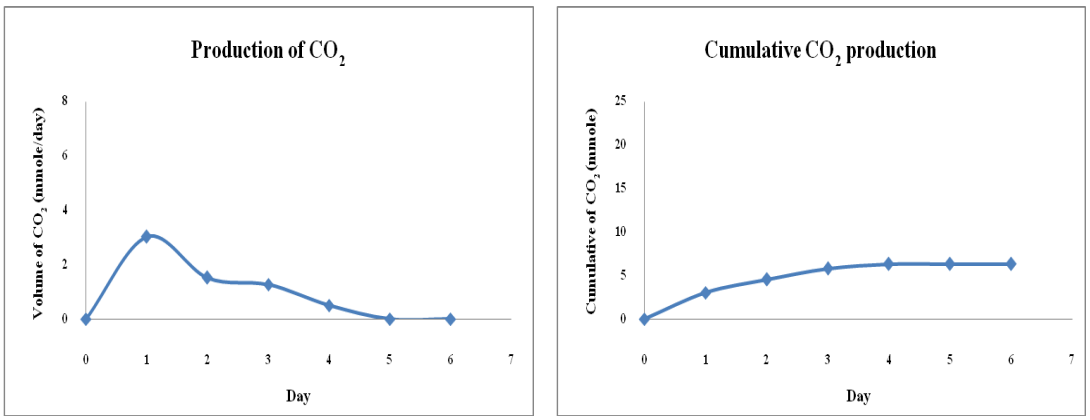


Fig. 28 Production and cumulative production of CO_2 by *S. cerevieiae* grown on acid treated food waste at 40 ^{o}C during the first 7 days of incubation

The results shown in Fig. 26 indicate that the maximum rate of CO_2 production occurred after 2 days and dramatically dropped on day 5 and was zero on day 6. The total amount of CO_2 was about 22 mmol. The acid treated experiments (Fig. 28) showed very little production of CO_2 in total and CO_2 production stopped after 5 days. The acid treated food waste produced a total volume of CO_2 of about 6.5 mmol/day. The results of cumulative CO_2 production obtained from each experiment were combined and are shown in Fig. 29. Carbon dioxide gas production increased from zero at time 0 until about day 4 and remained constant thereafter in all experiment. The results shown in Fig. 29 implies that the autoclaved food waste had the most available metabolisable sugar, the 80 ^{o}C treated waste had about half as much available sugar and the acid treated food waste has the least amount.

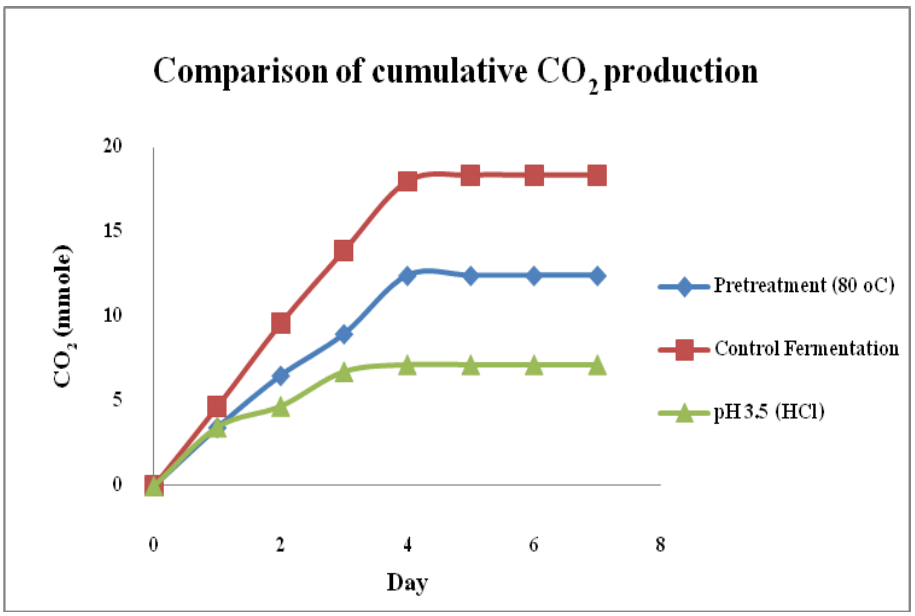


Fig. 29 Comparison of cumulative production of CO_2 gas using by *S. cerevisiae* grown on autoclave, pasteurized and acid treated food waste

4.10 Ethanol production during the fermentation of autoclaved, pasteurized and acid treated food waste

Ethanol productions by the three different cultures were measured using gas chromatography. See calibration curve in Appendix (Fig. 36).

Time(day)	Ethanol (%V/V) (Autoclaved FW)	Ethanol (%V/V) (pasteurize FW)	Ethanol (%V/V) (Acid treated FW)
0	0.55	0.34	0.36
1	0.48	0.36	0.43
2	0.54	0.46	0.45
3	1.39	0.74	0.48
4	0.45	0.42	0.39
5	0.48	0.26	0.28
6	0.33	0.12	0.16
7	0.29	0.11	0.15

Table4. Concentration of ethanol from fermentation by *S. cerevisiae* on various pretreated food waste. The volume of the inoculums and concentration of the food waste was 10 ml and 175 gFW respectively

Table 4 shows that the maximum alcohol production (1.39% V/V) obtained from autoclaved feedstock was after only 3 day incubation period. The production was less for 80 ^{o}C treated food waste and least for acid treated waste. As can be seen in the table, the maximum ethanol concentrations in all three experiments were low. After reaching the highest concentration the ethanol content decreased with time probably as a result of evaporation. Pretreatment at 80 ^{O}C led to satisfactory fermentation (0.74%V/V of ethanol). Acid treated food waste gave the worst fermentation results. These results indicate that autoclaving the food waste did increase the amount of fermentable carbohydrate while HCl treatment was an unsatisfactory pretreatment for the waste. The pH of anaerobic digestion in the autoclaved control dropped from 5.3 to 4.2 at day 12 and to pH 3.5 at day 30. According to Lee et al. (2004) if pH falls below 4 uptake of sugars by yeast was significantly reduced. The results demonstrate that pasteurization (80 ^{o}C) was a satisfactory pretreatment for food waste before fermentation using yeast. It was also confirmed that autoclaving was making a large proportion of the carbohydrate and protein available to the yeast by hydrolysis at high temperature and pressure.

CHAPTER 5

Conclusions

Phuket Province, Thailand is a major tourist area. Restaurants, hospitals, hotels, residences, cafeterias and other industrial sites produce large volumes of food waste. The tourist industry in Phuket continues to expand resulting in the continued increases in the number of restaurants and street food vendors in response to demand. Fermentative breakdown of food waste seems a viable alternative to feeding to pigs or garbage disposal. Food waste is difficult to dispose of by incineration because it has a high moisture content and very high amount of organic matter, such as carbohydrate, protein, lipid (fats and oils), cellulose etc. Household garbage has unacceptably high levels of toxins and heavy metals and so it is difficult to make use of garbage that has been digested using fermentation processes. Food waste has an inherently low level of heavy metals and so digested waste is more likely to be recyclable. In this study we used yeast (*Saccharomyces cerevisiae*) for anaerobic fermentation of food waste. In the experiments, autoclaved food waste was mainly used as the substrate and so some of the recalcitrant starches were hydrolysed by heating. Experiments in which a milder 80 ^{o}C heat treatment was used gave similar results to the autoclaved food waste, though breakdown of carbohydrates was less complete. Like in the case of Korean food waste, the main components of Thai food waste are partially hydrolyzed rice, vegetables and fish, pork or chicken and unlike European food has low levels of potato starch. It has a high level of lipids derived from cooking oil. Long fermentation times of 20, 25, 30 days were tested for the most effective incubation time, the most effective combination of food waste and water volume and the optimum temperature. It was found that yeast readily ferments food waste producing CO_2 and small amount of alcohol and reduces COD and BOD_5 (COD, 89.7%; BOD_5, 85.6%) under anaerobic conditions. Yeast very effectively digested carbohydrate (measured as reducing sugar) and total sugar (>80%). It was also able to slowly breakdown the partially hydrolyzed rice starch if being incubated long enough (about 75% after 30 day incubation). As expected, yeast metabolized protein (68% removal) and reducing sugars (87.4%) in food waste but a surprising result was that yeast was much better at breaking

down cooking oil and lipids than expected (> 85%). With a 25 day incubation there is about 85% breakdown of total sugar, reducing sugar, lipid and protein. COD decreased by about 90% and BOD_5 decreased by about 86%. The optimum temperature for breaking down the waste was 40 ^{o}C, the optimum combination of food waste with water was 175 gFW/l and the optimum inoculation rate for starting the fermentation was 5×10^6 cells.

From the results when the experiments were started with 10 ml yeast inoculum about 95% of total sugar and 70% protein were reduced. Yeast produced large amounts of CO_2 only during the first few days of the incubation. CO_2 gas production was high after 2 or 3 days but then rapidly declined to zero due to rapid digestion of easily metabolized organic compounds. Digestion of organic compounds (lipid, total sugar, reducing sugar and starch) was most completed at 40 ^{o}C. This is consistent with previous studies on yeast digestion (Masaaki et al., 2008). The effect of temperature on digestion of lipids and starch was more pronounced than in the case of total sugar and reducing sugar or protein. Thus higher temperature improves digestion of those wastes that would be expected to be less digestible than sugars and amino acids and proteins.

Maximum CO_2 production of 43 mmol of CO_2 was obtained when the food waste was fermented for 25 days at 40 ^{o}C using 10 ml inoculums and 175 gFW/250ml. CO_2 gas would have been produced mainly from the sugars present in the feedstock during the process of EtOH synthesis. It seemed that most CO_2 was produced in the first few days of the incubation from readily available sugars. After the first week of the incubation there were considerable amounts of starches and lipids still present and these were broken down in the period from 7 to 25 days. Yeast needed time for fermentation and digestion of starch and lipids. Results obtained after for incubation for 20 days showed that much of the starch was still present and digestion of starch was only apparent after 25 or 30 days of incubation. Starch was not digested well because it has long and branching chain which yeast cannot digest very well. From the literature (Shigeki et al., 1997; Barriga et al., 1999; Arsova, 2010; Cho et al., 2010) it was thought that yeast would not be able to break down lipids very well but it was quickly found in the project that this was not correct (Suwannarat and Ritchie 2013a, b). Lipolytic bacteria might not be necessary for efficient digestion of lipids (Cirne et al., 2006). *S.cerevisiae* could digest about 80% of lipids or even more after 25 day incubation and digestion of lipids appears to be greatly accelerated at higher

temperatures. The bioemulsifier produced by yeast greatly helps in the digestion of lipids (Barriga et al., 1999).

Fermentation of autoclaved food waste was shown to produce ethanol by Gas Chromatography, however the maximum yield was rather low (1.39%) but this was more than found in the case of pasteurized food waste (80 ^{o}C). Acid pretreatment gave very poor digestion of food waste and very low production of ethanol. Protein was reduced by about 70% when autoclaved feedstock was used. Yeast cultures were very efficient in removing ammonia (85–90%, Fig. 24) and also for CO_2 production, ethanol production and digestion of food waste the autoclaved food waste proved to be the best growth medium for the yeast.

In conclusion, the optimal fermentation conditions were 10 ml yeast inoculum, 175 gFW/250 ml of autoclaved food waste, 40 ^{o}C temperature and 25 day incubation time. Pasteurised food waste was almost as good a feedstock as autoclaved food waste for yeast fermentation but acid-treated food waste was not even though other studies have used acid treated waste material successfully (Han and Shin, 2002; Akpan et al., 2005; Akpan et al., 2008; Asada et al., 2011).

The spent food waste obtained after the fermentation had a relatively low BOD_5 and COD and low sugars, protein, lipids and starches. It was also shown that the ammonia content of the spent food waste would also be very low. The maximum ethanol production found in the present study was low (1.39% V/V) and it might not be economically viable to try and recover it. The spent food waste does not appear to be a significant environmental hazard and could be used as a fertilizer on soils. The study has shown that it is practical to use yeast to digest food waste to produce an environmentally harmless product.

5.1 Suggestions for future work

5.1.1 Alcohol production can also be measured by using standard biochemical assays based on alcohol dehydrogenase and NAD/NADH measurement by spectrophotometry (Vallee and Hoch, 1955).

5.1.2 Aerobic digestion should be investigated to improve the mobilization of organic materials in the food waste in a two-stage process and determine if a two-stage process improves alcohol production in the fermentation step.

5.1.3 This study did not include attempts to digest the cellulose of food waste. The results of the experiments on acid treatment of food waste were disappointing and so using steam hydrolysis to digest food waste might be unsatisfactory because yeast might not grow on the acid + heat hydrolysed food waste (Shigeki et al., 1997; Han and Shin 2002; Akpan et al., 2005; Akpan et al., 2008; Bonciu et al., 2010). An enzymatic digestion process to break down cellulose to increase the amount of available sugar in the food waste and maximum conversion of organic material into inorganic material appears to be more feasible (Wataru et al., 2004; Moon et al., 2009; Watanabe et al., 2009; Arsova, 2010; Yan et al., 2011) as is the use of genetically modified yeast that can digest cellulose (Liang et al., 2010).

5.1.4 Drying and dry grinding food waste before fermentation may greatly improve the breakdown of food waste by yeast.

References

Adler, L., Falk, K-E., Norkrans, B., Ångström, J. (1981). Polyol and water in the salt-tolerant yeast *Debaryomyces hansenii* as studied by 1H nuclear magnetic resonance. FEMS Microbiology Letters **11:** 269-271.

Adler, D., Santschi, P.H., Amdurer, M., Li, Y-H., Bell, J.J. (1981). Thorium isotopes as analogues for "particle-reactive" pollutants in coastal marine environments. Earth and Planetary Science Letters **47:** 327-335.

APHA. Standard methods for the examination of water and wastewater. 20th Edition. Eds: LS Cleseri, AE Greenberg, AD Eaton. American Public health Association, Washington, 1998.

Akande, K.B., Azeez, J.O., Adekunle, I.O., Atiku, O.O., Jamiu-Azeez, S.O. (2009). Effect of nine years of animal waste deposition on profile distribution of heavy metals in Abeokuta, south-western Nigeria and its implication for environmental quality. Waste Management **29**: 2582-2586.

Akpan, U.G., Alhakim, A.A., Ijah, U.J.J. (2008). Production of Ethanol Fuel from Organic and Food Wastes. Leonardo Electronic Journal of Practices and Technologies **13:** 1-11.

Akpan, U.G., Kovo, A.S., Abdullahi, M., Ijah, J.J. (2005). The Production of ethanol from maize cobs and groundnut Shells. Assumption University Journal of Technology **9:** 106-110.

Amonsit, M., Petsom, A. (1995). Principles and technical analysis tools. Chuan Printing Publ. Bangkok.

Arsova, L. (2010). Anaerobic digestion of food waste: current status, problems and an alternative product. Engineering and Applied Science Columbia University **1**:1-77.

Asada, C., Kita, A., Sasaki, C., Nakamura, Y. (2011). Ethanol production from disposable aspen chopsticks using delignification pretreatments. Carbohydrate Polymers **85**:196-200.

Barriga J.A.T, Cooper, D.G., Idziak, E.S., Cameron, D.R. (1999). Components of the bioemulsifier from *S. cerevisiae*. Enzyme and Microbial Technology **25:** 96-102.

Barry, K., Hanagan, A-M., Coughlan, B., Ryan, E., Cooney, R., Crowe, J. (2000). Analysis of sibling pairs presenting with hereditary haemochromatosis". Gastroenterology **118**: 4, A997.

Bodík, I., Blštáková, A., Sedlácek, S., Hutnan, M. (2009). Biodiesel waste as source of organic carbon for municipal WWTP denitrification. Bioresource Technology **100**: 2452-2456.

Bonciu, C., Tabacaru, C., Bahrim, G. (2010). Yeast isolation and selection for bioethanol production from inulin hydrolysates. Innovative Romanian Food Biotechnology **6:** 29-34.

Bouquin, N., Barral, Y., Courbeyrette, R., Blondel, M., Snyder, M., Mann, C. (2000). Regulation of cytokinesis by the Elm 1 protein kinase in *Saccharomyces cerevisiae*. Journal of Cell Science **113**:1435-2777.

Carneiro, J., Milutinovic, D., Athans, M., Lima, P. (2007). Modeling dynamics of cell population molecule expression distribution. Nonlinear Analysis: Hybrid Systems **1**: 81–94.

Carneiro, P., Duarte, M., Videira, A. (2007). The external alternative NAD(P)H dehydrogenase NDE3 is localized both in the mitochondria and in the cytoplasm of *Neurospora crassa*. Journal of Molecular Biology **368**: 1114-1121.

Chen, Y., Cheng, J.J., Creamer, K.S. (2008). Inhibition of anaerobic digestion process: A review. Bioresource Technology **99:** 4044-4064.

Cheng, S-W., Junhu, J-Z., Su, X-B., Kefa, H-C. (2010). Cogeneration of hydrogen and methane from protein-mixed food waste by two-phase anaerobic process. International Journal of Hydrogen Energy **35:** 3141-3146.

Cho, J-K., Park, S-C., Chang, H-N. (2010). Biochemical methane potential and solid state anaerobic digestion of Korean food wastes. Bioresource Technology **52**: 245-253.

Cirne, D.G., Bjornsson, L., Alves, M., Mattiasson, B. (2006). Effects of bioaugmentation by an anaerobic lipolytic bacterium on anaerobic digestion of lipid-rich waste. Journal of Chemical Technology and Biotechnology **81:**1745–1752.

Coenen-Stass, D., Lamprecht, I., Motzkus, Ch., Schaarschmidt, B. (1990). Pentachlorophenol an environmental pollutant: microcalorimetric investigations of an ecological model system. Thermochimica Acta **172**: 87-94.

Davis, R.A. (2008). Parameter estimation for simultaneous saccharification and fermentation of food waste into ethanol using Matlab Simulink. Applied Biochemistry and Biotechnology **147:**11–21.

Donaldson, A.D., Blow, J.J. (1999). The regulation of replication origin activation. Current Opinion in Genetics and Development **9:** 62-68.

Ergun, M., Mutlu, S.F (2000). Application of a statistical technique to the production of ethanol from sugar beet molasses by *Saccharomyces cerevisiae*. Bioresource Technology **73**: 251-255.

Fleet, G.H., Taniwaki, M.H., Hocking, A.D., Pitt, J.I. (2009). Growth and mycotoxin production by food spoilage fungi under high carbon dioxide and low oxygen atmospheres. International Journal of Food Microbiology **132**: 100-108.

Forster-Carneiro, T., Perez, M., Romero, L.I., Sales, D. (2007). Dry-thermophilic anaerobic digestion of organic fraction of the municipal solid waste: Focusing on the inoculum sources. Bioresource Technology **98:** 3195–3203.

Gene, P.F., Speece, R.E., Gallagher, D. (1983). Nickel stimulation of anaerobic digestion. Water Research **17:** 677-683.

Gonzales, H.B., Takyu, K., Sakashita, H., Nakano, Y., Nishijima, W., Okada, M. (2005). Biological solubilization and mineralization as novel approach for the pretreatment of food waste. Chemosphere **58**: 57-63.

Han, S-K., Shin, H-S. (2002). Enhanced acidogenic fermentation of food waste in a continuous-flow reactor. Waste Management Research **20:** 110-118.

Haug, J-P., Spichiger, U., Simon, W., Bakker, E., Lerchi, M., Bühlmann, P., Kuratli, M., Ozawa, S., West, S. (1993). Optical sensors based on neutral carriers. Sensors and Actuators B: Chemical **11**: 1-8.

Hertzel, A.V., Bernlohr, D.A. (2000). The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. Trends in Endocrinology and Metabolism **11:** 175-180.

Hwang, E-Y., Namkoong, W., Park, J-S., Choi, J-Y. (2002). Bioremediation of diesel-contaminated soil with composting. Environmental Pollution **119**: 23-31.

Kaeberlein, M., Powers, R.W. 3rd, Steffen, K.K., Westman, E.A., Hu, D., Dang, N., Kerr, E.O., Kirkland, K.T., Fields, S., Kennedy, B.K. (2005). Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science **310:** 1193-1196.

KEGG (Kyoto Encyclopedia of Genes and Genomes) (2013). http://www.genome.jp.Kegg/Kegg2/html [accessed 14 Dec 2013]

Kim, J-H., Lee, J-C., Pak, D. (2011). Feasibility of producing ethanol from food waste. Waste Management **31:** 2121-2125.

Kim, J.K., Oh, B.R., Shin, H.J., Eom, C.Y., Kim, S.W (2008). Statistical optimization of enzymatic saccharification and ethanol fermentation using food waste. Process Biochemistry
43: 1308–1312.

Kim, S-H., Cheon, H-C., Lee, C-Y. (2000). Enhancement of hydrogen production by recycling of methanogenic effluent in two-phase fermentation of food waste. International Journal of Hydrogen Energy **37:** 13777-13782.

Kim, S-H., Han, S-K., Shin, H-S. (2004). Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge. International Journal of Hydrogen Energy **29:**1607-1616.

Kreith, F. (1992). Solid waste management in the U.S. and 1989-1991 state legislation. Energy **17**:427-476.

Liang, X-X., Yoshida, T., Uryu, T. (2010). Direct saccharification and ethanol fermentation of cello-oligosaccharides with recombinant yeast. Carbohydrate Polymers **91:** 157-161.

Lowry, O.H., Rosebrough, N.J., Farr, L., Randall, R.J. (1951). Folin phenol reagent protein measurement with Folin phenol reagent. Journal of Biological Chemistry **193:** 265 – 275.

Madigan, T.M., Martinko, M.J., Parker, J. (1997) Brock: Biology of Microorganisms. Prentice Hall Publ., Upper Saddle River, New Jersey, USA.

Manning, L.R., Dumoulin, A., Jenkins, W.T., Winslow, R.M., Manning, J.M. (1999). Determining subunit dissociation constants in natural and recombinant protein. Methods in Enzymology **306:** 113-129.

Masaaki, T.K., Hitoshi, O., Akira, A., Shin, J.K., Hiroshi, M.I. (2008). Basic characteristics of food waste and food ash on steam gasification. Chemistry Research **47:** 2414-2419.

Moon, H.C., Song, S., Kim, J.C., Shirai, Y., Lee, D.H., Kim, J.K., et al. (2009). Enzymatic hydrolysis of food waste and ethanol fermentation. Energy Research **33:** 164-72.

Qian, C, Zhang, L., Zhao, H., Gan, M-Z, Jin, Y-L, Gao, X-F, Guan, J., Wang, Z. (2008). Application of simultaneous saccharification and fermentation (SSF) from viscosity reducing of raw sweet potato for bioethanol production at laboratory, pilot and industrial scales. Bioresource Technology **102**: 4573-4579.

Ritchie, R.J., Raghupathi, S.S. (2008). Al-toxicity studies in yeast using gallium as an aluminum analogue. Biometals **21:** 379-393.

Shigeki, S., Inoue, S., Minowa, T., Tsukahara, K., Ogi, T. (1997). Thermochemical liquidization and anaerobic treatment of kitchen garbage. Journal of Fermentation and Bioengineering **83**: 451-455.

Shimoda, C. (2004). Forespore membrane assembly in yeast: coordinating SPBs and membrane trafficking. Journal of Cell Science **17:** 389-395.

Solorzano, L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography **14:** 799-801.

Song, Y-E., Kim, M-H., Song, H-B., Kim, J-W., Hwang, S-J. (2010). Evaluation of food waste disposal options by LCC analysis from the perspective of global warming: Jungnang case, South Korea". Waste Management **31:** 2112-2120.

Suwannarat, J., Ritchie, R.J. (2013a). Anaerobic fermentation of restaurant and street food using yeast. 5th International Conference on Fermentation Technology for Value Added Agricultural Products FerVAAP 2013, 21-23 August 2013, Centara Hotel and Convention Centre, Khon Kaen, Thailand, Abstract Fer 1-02-p 8, paper 4-8.

Suwannarat, J., Ritchie, R.J. (2013b). Anaerobic fermentation of food waste using yeast. The International Conference on Waste Management and Environment (ICWME) 2013, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia. 26 – 27th August 2013, University of Malaya, Kuala Lumpur, p 55.

Watanabe, T., Masaki, K., Iwashita, K., Fujii, T., Iefuji, H. (2009). Treatment and phosphorus removal from high-concentration organic wastewater by the yeast *Hansenula anomala* J224 PAWA. Bioresource Technology **100**: 1781-1785

Watanabe, M., Takahashi, M., Sasano, K., Kashiwamura, T., Ozaki, Y., Tsuiki, T., Hidaka, H., Kanemoto, S. (2009). Bioethanol production from rice washing drainage and rice bran. Journal of Bioscience and Bioengineering **108:** 524–526.

Watanabe, H., Yoshino, H. (2009). Biohydrogen using leachate from an industrial waste landfill as inoculums. Renewable Energy **35:** 921-924.

Wataru. N., Hazel, B.G., Hideki, S., Yoichi, N., Mitsumasa, O. (2004). Improvement of biological solubilization and mineralization process for food waste. Water Environment Technology **2:** 57-64.

Westendorf, M.L., Dong, Z.C., Schoknecht, P.A. (1998). Recycled cafeteria food waste as a feed for swine: nutrient content digestibility. Journal of Animal Science **76:** 2976-2983.

Westendorf, M.L., Wohlt, J.E. (2002). Brewing by-products: their use as animal feeds. Veterinary Clinics of North America: Food Animal Practice **18**: 233-252.

Yan, S-B. , Chen, L-J., Jingyong, X-W., Ye, W-P., Jianming, J-Y. (2011). Enzymatical hydrolysis of food waste and ethanol production from the hydrolysate. Renewable Energy **36:** 1259-1265.

Yang, S.Y., Ji, K.S., Baik, Y.H., Kwak, W.S., McCaskey, T.A. (2006). Lactic acid fermentation of food waste for swine feed. Bioresource Technology **97:** 1858-1864.

Yixiang Xu, Loren Isom, Milford A. Hanna. (2010). Adding value to carbon dioxide from ethanol fermentations. Bioresource Technology **101**: 3311-3319

Zhang, Q-H, Chen, S., Ma, J., Qu, Y-Y. (2007). Solutions of linear ordinary differential equations with non-singular varying coefficients by using the corrected Fourier series. Applied Mathematics and Computation **187**: 765-776.

Appendix

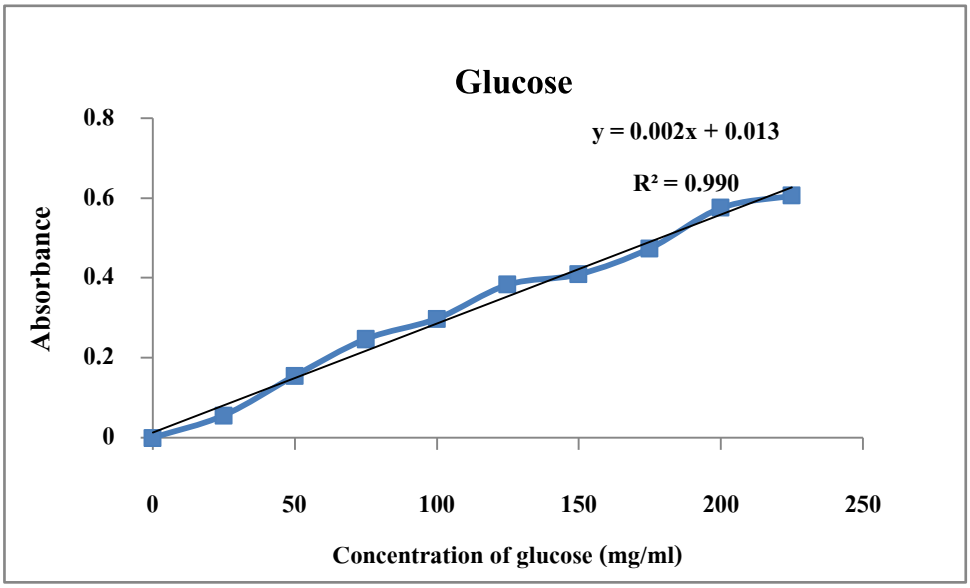


Fig. 30 Example of Standard curve of Glucose and is also used for the Carbohydrate Assays because carbohydrate was measured as equivalent glucose.

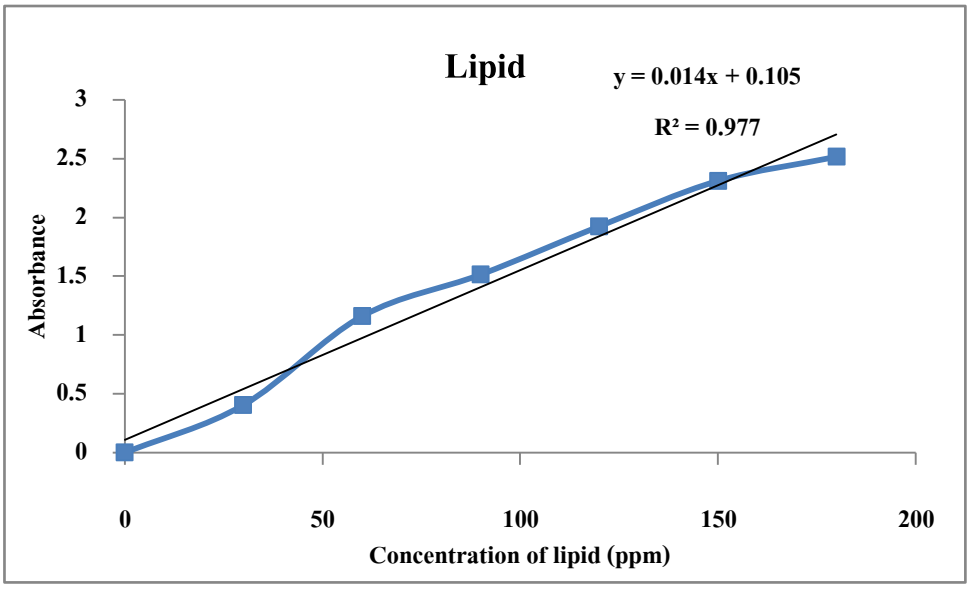


Fig. 31 Example of Lipid Assay Standard Curve

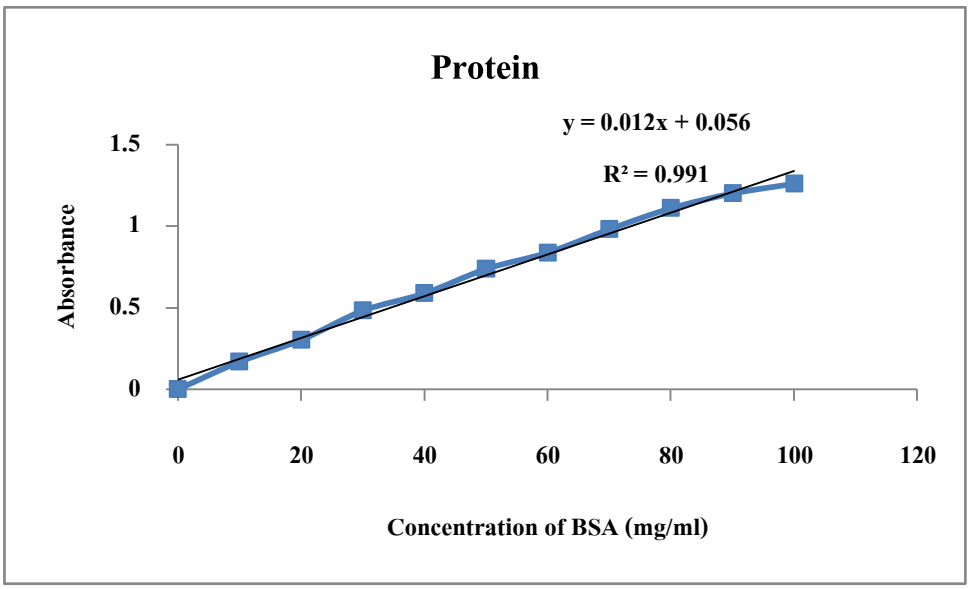


Fig. 32 Example of Standard curve for Protein

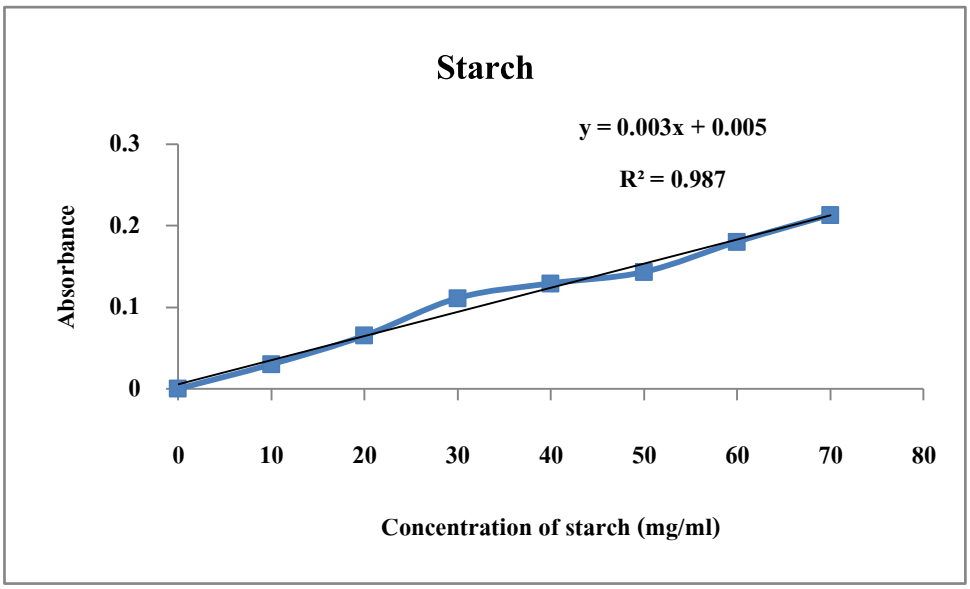


Fig. 33 Example of Standard curve of Starch

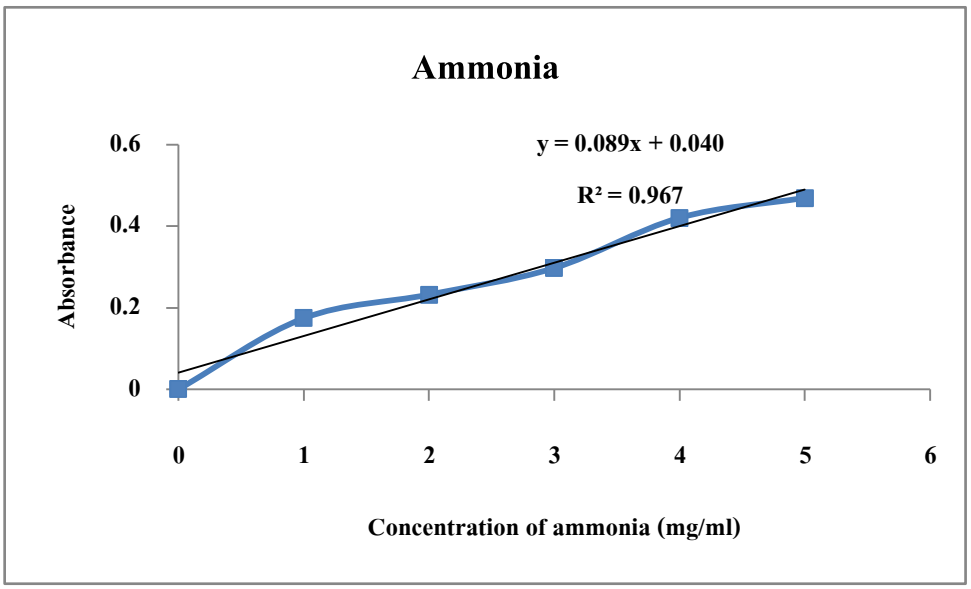


Fig. 34 Example of Ammonia Standard Curve

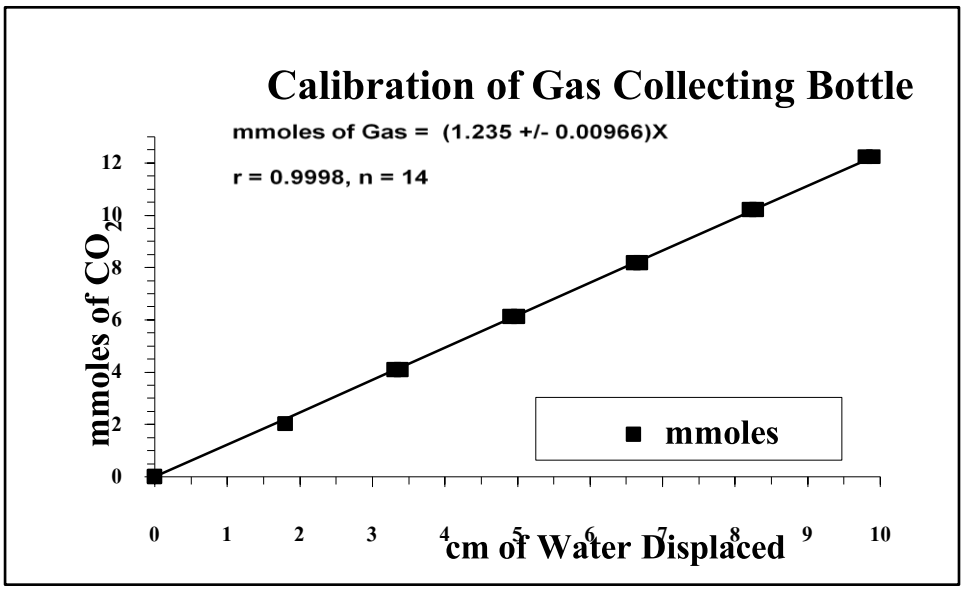


Fig. 35 Calibration of Collecting Bottle used for Collecting Gas in Fermentation Experiments.

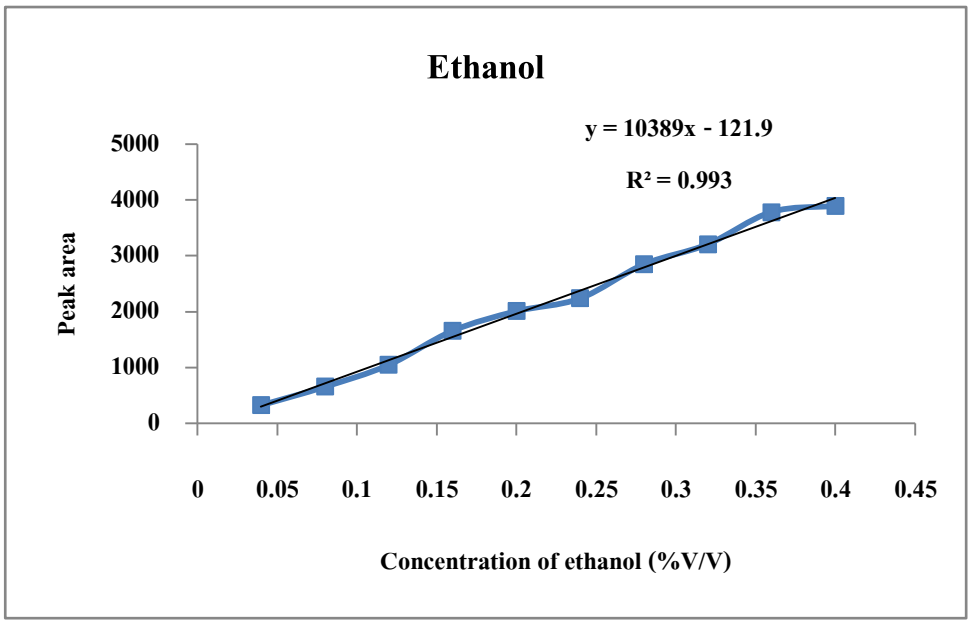


Fig. 36 Example of Ethanol Standard Curve on Gas Chromatograph

Analysis Protocols for the components of food waste:

- **Protein analysis using BSA standard (Lowry et al., 1951).**

Reagent C (Lowry et al., 1951)

- Solution A: dissolve 1 g of $NaCO_3$ into 50 ml of 0.4% NaOH (NaOH 0.2 g into 50 ml of distilled water)
- Solution B_1: 1% $CuSO_4 \cdot 5H_2O$ (0.02 g of $CuSO_4 \cdot 5H_2O$ in 2 ml of distilled water)
- Solution B_2: 2% $Na_3C_6H_5O_7 \cdot 2H_2O$ (0.04 g of $Na_3C_6H_5O_7 \cdot 2H_2O$ in 2 ml of distilled water)
- Reagent C = solution A: solution B_1: solution B_2 in ratios of 50:0.5:0.5. Reagent C must be prepared fresh prior to use.

Folin–Ciocalteus phenol reagent

Yellow stock solution 2 N: needs to be diluted 1:1 before use.

- 1 N concentration (1:1) kept in brown bottle at room temperature. It must be used fresh every time.
- Substrate solutions was prepared using bovine serum albumin (BSA) at a conc. of 1 mg/ml (0.1 g BSA in 100 ml DW).
- Preparation of bovine serum albumin Standard curve. 0, 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, 0.21, 0.24, 0.27, and 0.30 ml of BSA standard (1 mg/ml) were pipetted into test tubes, into each tube, DW was added to adjust the volume to 0.3 ml.
- 3 ml of reagent C was added to each tube at room temperature for 20 min after mixing.
- 0.3 ml of Folin–Ciocalteus phenol reagent was added and mixed immediately then kept at room temperature for 30 min.
- Absorbance was measured using a Shimadzu UV-1601 spectrophotometer (*Shimadzu*, Kyoto, Japan) at 750 nm.

Analysis of protein content

- 5 g of food waste was mixed in 100 ml of distilled water and 0.3g of sample into a test tube.
- 3 ml of reagent C was added to the test tube, mixed and left for 20 min at room temperature.
- 0.3 ml of Folin–Ciocalteus phenol reagent was added and mixed immediately then left for 30 min.
- Absorbance was measured at 750 nm (A_{750}) using distilled water as blank.
- The standard curve of A_{750} vs. BSA (mg) was used to calculate the protein content of the unknown sample using a curve fitted by linear regression (Fig. 32).

● **Carbohydrates analysis (APHA, 1998)**

Preparation of Reagents for Reducing sugar and Total Sugar Assay

-Standard glucose 10 mg/ml: dissolve 1 g of glucose in 100 ml distilled water.
- 1.5 M of sulphuric acid: mix 20 ml of concentrated sulphuric acid (18M) with about 200 ml of distilled water then adjust the volume to 250 ml.
- 10% of NaOH (2.5 M): 25 g of NaOH 250 ml of DW.
- DNS reagent: 10 g of DNS was first dissolved in 200 ml of 2 M NaOH. Sodium potassium tartrate 300 g was dissolved in 500 ml DW the two solutions were then mixed together and the volume was adjusted to 1 liter. The DNS reagent was kept in a brown bottle.

Preparation of glucose standards for a standard curve

- A range of 1 ml standard glucose solutions with concentrations of 0, 0.2, 0.4, 0.6, 0.8 and 10 mg/ml glucose were prepared using the Glucose stock solution.
- 1 ml of DNS reagent and 2 ml of distilled water mixing were added to each glucose standard.
- Each test tube was incubated in a water bath at 100 ^{o}C for 5 min and then cooled down to room temperature,

- Absorbance of each known glucose solution was measured at 540 nm and used to make a standard curve [A_{540} vs. Glucose (mg)] using the zero as a blank (Fig. 30).

Analysis of reducing sugar content

- 5 g of food waste was added to 50 ml of distilled water. The suspension was heated to 50 ^{o}C for 10 min to ensure dissolution of the reducing sugars present.
- The extract was then filtered with Whatman No.1 filter paper and the volume was adjusted with distilled water to 100 ml.
- Dilution step. 10 ml of the extracted sample was then placed in a flask and the volume was made up to 100 ml with distilled water.
- 1 ml of diluted sample was added to 1 ml of DNS reagent and 2 ml of distilled water and then mixing.
- The test tube was then incubated in a hot water bath at 100 ^{o}C 5 min and then cooled.
- Absorbance was measured at 540 nm and the standard curve used to calculate glucose equivalents in mg.

Analysis of Total sugar by Acid Hydrolysis

- 10 ml of 1.5 M H_2SO_4 was added to 2.5 g of food and heated in a water bath at 100 ^{o}C for 20 min and then cooled.
- The sample was then filtered through Whatman No.1 filter paper and the filtrate volume was made up to 100 ml with distilled water.
- 10 ml of the filtered sample was then diluted 10X by taking 10 ml and making it up to 100 ml using distilled water.
- 1 ml of DNS reagent was added to 1 ml of the diluted sample and 2 ml of distilled water added and mixed.
- The reaction was completed by incubating in a hot water bath at 100 ^{o}C for 5 min and then cooled.
- Absorbance was measured at 540 nm and the glucose standard curve (Fig. 30) was used to calculate total glucose equivalent in the hydrolysed food waste.

● **Lipid analysis**

Lipid analysis by UV- Spectrophotometer (APHA, 1998).

Total lipid analysis using standard graph comparison of standard solution of lipid dissolved in various solvents.

Preparation of standard curve

Using Standard Oil 10 mg dissolved in the solvent used in the analysis of the petroleum ether solution volume is 50 ml equal to 0.2 mg / ml or 200 ppm. Prepare various solvents. In a test tube in the volumes are shown in Table 1 to determine the absorbance wavelength at 210 nm. A calibration curve was prepared by plotting absorbance vs. concentration of standard solution of lipid (in ppm).

Tube no.	Stock standard (ml)	Solvent (ml)	Concentration of oil (ppm)
1	0.00	10.00	0
2	1.50	8.50	30
3	3.00	7.00	60
4	4.50	5.50	90
5	6.00	4.00	120
6	7.50	2.50	150
7	10.00	0	200

Table5. Preparation of lipid stock standard at different concentrations.

Analytical method

- suspend 5 g of food waste in 50 ml petroleum ether vigorously shake the suspension to dissolve the lipid in the organic solvent.
- Particulates were allowed to settle and the absorbance of the supernatant was measured at 210 nm.
- calculate the amount of lipids using the standard curve shown in Fig. 31.

● **Starch Analysis (APHA, 1998)**

Reagents

- Ethyl alcohol: C_2H_5OH 95%
- Sodium hydroxide: NaOH
- Glacial acetic acid: CH_3COOH
- Potassium iodide: KI
- Iodine: I_2
- Standard Starch solution: soluble starch laboratory grade 20 g dissolved in 1000 ml of hot distilled water.

Reagent solutions

- Sodium hydroxide 2 N: 80 g of sodium hydroxide was dissolved in 800 ml of distilled water and the volume was adjusted to 1000 ml after cooling.
- Glacial acetic acid 1 N: 60 ml of glacial acetic acid was mixed with 800 ml of distilled water then the volume was adjusted to 1000 ml.
- Iodine solution: 0.2 g of Iodine and 2 g of potassium iodide were dissolved in 80 ml of distilled water and the volume was adjusted to 100 ml. This solution is not stable and needs to be replaced periodically.

Standard curve preparation

- one ml of ethyl alcohol and 9 ml of sodium hydroxide was added to 0.04 g of amylose (soluble starch) and the volume was made up to 100ml with distilled water and stirred for 10 min.
- Prepare 5 x 100 ml of volumetric flasks and into each flask, 70 ml DW was added.

- To the first bottle 0.4 ml of glacial acetic acid was added. The 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} flask was added with 0.8, 1.2, 1.6 and 2 ml acetic acid. Two ml of iodine solution was added into each flask.

- 1, 2, 3, 4, 5 ml of the standard starch solution was then pipette into the five respective volumetric flasks and the volume in each flask was adjusted to 100 ml.

- Blank was prepared by adding 2 ml of glacial acetic acid, 2 ml of Iodine and adjusting the volume to 100 ml using distilled water.

- Absorbance was measured at 620 nm using the prepared blank and a standard curve of A_{620} vs. starch (mg) was prepared.

Analytical method

- one ml of Ethyl alcohol and 9 ml of Sodium hydroxide was added to 5 g of food waste and the volume was made up to 100 ml with distilled water and stirred for 10 min.

- Prepare 100 ml of beaker and add 70 ml of distilled water, 2 ml of glacial acetic acid, 2 ml of iodine solution and 5 ml of food waste. The solution was kept at room temperature for 10 min.

- Measure absorbance at 620 nm. The standard curve was used to calculate starch content (Fig. 33).

● **Ammonia analysis (Solorzano, 1969)**

Preparation of reagents

- Phenol solution : dissolve 5 g of phenol into 50 ml ethyl alcohol 95% (v/v) - Sodium nitroprusside solution [$Na_2Fe(CN)_5NO.2H_2O$] : 0.5 g of sodium nitroprusside solution [$Na_2Fe(CN)_5NO.2H_2O$] was mixed in 100 ml of distilled water and kept in a brown bottle.

- Oxidizing solution: 1 g of sodium dichloroisocyanurate, $C_3Cl_2N_3NaO_3$ or potassium dichloroisocyanurate, $C_3Cl_2N_3KO_3$ was dissolved in DW containing 5 g NaOH, 50 g of tri-sodium citrate dehydrate ($Na_3C_6H_5O_7 \bullet 2H_2O$). The volume was adjusted to 250 ml.

- Ammonia standard solution: 0.165 g of ammonium sulphate [$(NH_4)_2 SO_4$] (analytical grade) was dissolved in distilled water and the volume was adjusted to 1000 ml. The stock standard solution was kept in a brown bottle.

Preparation of standard curve

- five ml of stock ammonia standard solution was diluted to 500 ml with distilled water.
- 0, 5, 10, 20 and 40 ml of stock solution was added into 50 ml volume metric flask and adjust the volume to 50 ml with distilled water. The concentration of ammonia in each flask was 0, 0.035, 0.070, 0140 and 0.280 mg-N/L respectively.
- Two and a half ml of phenol solution, 5 ml of oxidizing solution and 2.5 ml of sodium nitroprusside was added to each 50 ml standard. After a 1 hr incubation absorbances were read at 640 nm and a standard curve of A_{640} vs. Ammonia (mg N) was prepared.

Analytical method

- Samples were assayed routinely using 10ml rather than 50 ml volumes. Ten ml of sample was placed in a screw cap tube to avoid loss of ammonia as gas.
- 0.5 ml of phenol solution, 1 ml of oxidizing solution, and 0.5 ml of sodium nitroprusside were added to a 10 ml sample volume and the solution was mixed. After incubation for 1 hr the absorbance was read at 640 nm and the standard curve used to calculate ammonia as mg N (Fig. 34).

- **BOD (Biological Oxygen Demand) method (APHA, 1998)**

Materials and reagents

- Standard BOD bottles with the capacity of 300 ml
- Diluted H_2SO_4: 2.8 ml of concentrated H_2SO_4 was added slowly to distilled water and the volume was adjusted to 100 ml with distilled water.
- Standard Starch solution: soluble starch laboratory grade 20 g were dissolved in 1000 ml of hot distilled water.

- Manganese sulphate solution: 480 g of manganese sulphate tetrahydrate ($MnSO_4.4H_2O$) or 400 g manganese sulphate dihydrate ($MnSO_4.2H_2O$) or 364 g manganese sulphate monohydrate ($MnSO_4.H_2O$) dissolved in distilled water and the volume was made up to 1000 ml. manganese sulphate solution should show no reaction when added to the potassium iodide solution.

- Alkali – Iodide – Azide solution: 500 g sodium hydroxide (NaOH) or 700 g potassium hydroxide (KOH), 135 g sodium iodide (NaI) or 150 g potassium iodide (KI) are first dissolved in about 800 ml distilled water and then made up to 1000 ml. ten g of sodium azide (NaN_3) is dissolved in 40 ml of distilled water and then added to the Alkali – Iodide solution and the total volume was adjusted to 1000 ml with distilled water.

- 0.025 N standard sodium thiosulphate titrant: 6.205 g of sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$) and 0.4 g of Sodium hydroxide (NaOH) were dissolved in distilled water and the volume was adjusted to 1000 ml.

- 0.025 N standard potassium bi-iodate solution: 0.8124 g of potassium bi-iodate [$KH(IO_3)_2$] dissolved in distilled water and the volume was adjusted to 1000 ml.

- Magnesium sulphate solution: 22.5 g of magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) dissolved in distilled water and the volume was adjusted to 1000 ml.

- Calcium chloride solution: 27.5 g of calcium chloride ($CaCl_2$) were dissolved in distilled water and the volume was adjusted to 1000 ml.

- Ferric chloride solution: 0.25 g of ferric chloride hexahydrate ($FeCl_3.6H_2O$) was dissolved in 1000 ml of distilled water.

- Phosphate buffer solution: 8.5 g of potassium dihydrogen phosphate (KH_2PO_4), 21.75 g of dipotassium hydrogen phosphate (K_2HPO_4), 33.4 g disodium hydrogen phosphate heptahydrate ($Na_2HPO_4.7H_2O$), and 1.7 g of ammonium chloride (NH_4Cl) were dissolved in 500 ml of distilled water and the volume was adjusted to 1000 ml.

- 1 N Sodium hydroxide (NaOH): 40 g of NaOH were dissolved in distilled water and the volume was adjusted to 1000 ml.

- 1 N Sulphuric acid solution: 28 ml of concentrated. H_2SO_4 (18 M, 36N) was added slowly to 600 ml of distilled water and the volume was adjusted to 1000 ml. The solution was kept in brown bottle at room temperature.

Analytical method for saturated O_2

- One ml of magnesium sulphate solution, calcium chloride solution, ferric chloride solution, and phosphate buffer solution were added to 1 liter of water.

- Equilibrate the solution to the atmosphere by aeration for about 1 – 2 hrs.)

- The water is then poured into BOD bottles and capped using the special BOD bottle gas plugs. Dissolved oxygen was measured after preparation at the Day (0) DO_0 the other bottles were incubated at $20 \pm 3\ ^{O}C$ for 5 days. Dissolved oxygen was determined on the maturity date (DO_5, dissolved oxygen after 5 day standard incubation) and compared to the dissolved O_2 recorded for the DO_0 bottles.

Determination of dissolved oxygen (DO)

- One ml of Manganese sulphate Solution and 1 ml of Alkali - Iodide-Azide solution were added to the BOD bottles, care was taken not to add bubbles of air to the water in the BOD bottle. The BOD bottle cap was then carefully replaced and the contents mixed carefully 15 times.

- The brown/white precipitate was allowed to settle.

- 1 M H_2SO_4 was then added avoiding any loss of the oxidised floc. The cap was then replaced and the bottle was shaken back and forth until the floc redissolved. Iodine is released from the floc in the presence of H_2SO_4 in equimolar amounts for the amount of oxygen dissolved in the water.

- Pour 99 ml of water from the BOD bottle into a flask.

- The iodine present was titrated with 0.025 N sodium thiosulphate standard solutions until the solution was light yellow. Starch solution was then added and the titration was continued until the dark color disappeared. The volume of

sodium thiosulphate titrant was recorded. One mole of thiosulphate used is equivalent to iodine.

Calculation of BOD_5 value:

$$BOD_5 \text{ (mg/l)} = DO_0 - DO_5$$

DO_0 = The DO of the sample at day zero.

DO_5 = Average DO of the sample at day 5.

- **COD (Chemical Oxygen Demand) analysis (APHA 1998)**

Reagents and equipment

- Borosilicate Winkler bottles size 25 ×150 mm screw Type.
- Hot air oven.
- 294.185 g/mole of potassium dichromate ($K_2Cr_2O_7$) was heated in a standard hot air oven 103 ^{O}C for 2 hours then left to cool in a desiccator.
- Concentrated H_2SO_4
- Ag_2SO_4, technical grade.
- $HgSO_4$
- Ferrous ammonium sulphate.
- 0.0167 M $K_2Cr_2O_7$ solution 0.4913 g $K_2Cr_2O_7$ was dissolved in 50 ml of distilled water, 16.7 ml of concentrated sulphuric acid was used added carefully to dissolve the chromate, and then carefully diluted to 100 ml.
- Ferroin indicator dissolved 1.485 g of 1, 10-phenanthroline monohydrate and 695 mg $FeSO_4.7H_2O$ were dissolved in distilled water, and then diluted to 100 ml.

Analysis of COD in a water sample

- Five ml of sample and 3 ml of potassium dichromate/H_2SO_4 digest solution were pipetted into a test tube.
- 7 ml of concentrated sulphuric acid were added gradually by flowing down the side of the tube.
- The tube was then closed tightly and shaken without inversion to complete solution.

- A blank was prepared using 5 ml of distilled water and prepared as above.
- The sample and blank were put in an oven at 150±2 ^{O}C for 2 h to remove the water.
- The sample and blank tubes were taken out of the oven and set to cool at room temperature, and then 1-2 drops of ferroin indicator were added to the solution.
- The solution was titrated with a standard solution of Ferrous Ammonium sulphate.