YEAST FROM PAPAYA PROCESSING WASTES AS AQUACULTURE FEED SUPPLEMENT

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BIOENGINEERING

AUGUST 2007

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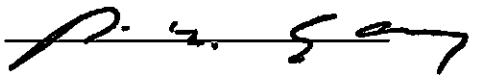

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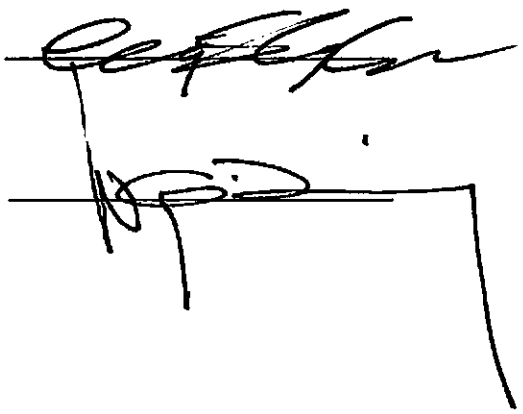

ACKNOWLEDGMENTS

This work was supported by College of Tropical Agriculture and Human Resources (CTAHR, University of Hawaii at Manoa) with the funding provided by Center for Tropical and Subtropical Aquaculture (CTSA, USDA) and co-investigation with Oceanic Institute (Waimanalo, HI). Additionally, this research gratefully acknowledges Super Food Ind. (Honolulu, HI) for providing the PPW.

I wish to express my thanks to the committee members for their guidance throughout the work. I am grateful to Dr. P.Y. Yang for offering this research opportunity and giving me the constant encouragement and advice on this work, Dr. W. G. Dominy for the valuable guidance on shrimp feed processing and feeding trial and Dr. C. S. Lee for the valuable suggestions on the revision. Also, I wish to thank Mr. Charles Nelson and Daniel Paquin for equipment set-up, Ward, Gavin and Masaya at Oceanic Institute for shrimp feed processing and feeding trial, Dr. C. C. Chien and Ms. He Xu for the further investigation, and Kuei Lin for the help of operation.

This work is dedicated to my parents for their support.

ABSTRACT

The Pacific Island is facing the challenge of short in cost-effective aquafeed. The protein source is the most costly ingredient in feed supplements. Yeast is enriched with protein and has been reported to be able to enhance shrimp's immune system, survival rate and average body weight. Hawaii produces million tons of fruit and food by-products each year which may have the potential to be upgraded into protein enriched value-added products. This study plans to develop a bioprocess procedure to convert fruit processing wastes into yeast biomass, to establish design and operational criteria for yeast production in batch and/or continuous/semi-continuous flow system, to evaluate the nutrient potential of the bioprocessed product as shrimp feed and the cost of the proposed production system.

Papaya processing waste collected from a food company in Honolulu was used for yeast growth (*S. cerevisiae*) in a 14-L fermenter mixing at 200 rpm under room temperature (22±2°C). PH, oxidation reduction potential (ORP) and dissolved oxygen (DO) were monitored with the change of soluble chemical oxygen demand (SCOD) removal and suspended solids increase in the growth medium to determine the required reaction time for maximum desirable product formation. An initial SCOD concentration ranged from 12,000 to 25,000 mg/l with 8-12h aeration was found for optimal and economical operation in the batch production system. It was able to remove more than 70% SCOD and produce 40-45% crude protein in suspended solid. Preliminary work of continuous/semi-continuous flow operation, shrimp (*L. vannamei*) feed trail and economic analysis on the batch production system indicated the successful development of the bioprocess system would be a mutual beneficial solution for local aqua-industries and environmental pollution control. In addition, the bioprocess could be applied widely to other agri-food by-products to produce the value-added products for the sustainability of agriculture production and environmental protection.

Keywords Fruit processing waste, value added product, yeast production, aquaculture feed, batch/semi-continuous or continuous flow operation, economic/sensitivity analysis, sustainability in fruit production, environmental protection.

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Chapter 1. Introduction

1.1 Background

The Pacific Island community is challenged by the high feed cost to maintain local aquaculture productions. Aquacultural feeds account more than 50% of variable operating costs and protein represents the most costly feed ingredient (Bassompierre et al. 1997). As global seafood demand increases, it is necessary to develop sustainable feed to meet the increasing industrial competition (Catacutan and Pagador 2004).

Fruit and food processing industries usually generates large amount of organic wastes that may cause environmental pollution problems. The island of Oahu produced approximately 1.76 million tons of total waste in 2005. Among these, 134,503 tons was food waste in which 76% entered the landfill or incineration and only 24% recycled (ENV 2005). Although agricultural industrial residues are rich in carbohydrates, their utilization is limited due to low protein content and poor digestibility (Pandy et al. 2001). These organic processing wastes contains soluble sugars, organic acids, nitrogen, minerals, sulfur, vitamins, and trace elements sources that may be biologically conversed into value-added products such as ethanol, protein enrichment or other valuable products.

Yeast is enriched with protein and has been reported to enhance the immune system of shrimp and fish by simulating their resistance to bacterial disease (Burgents et al. 2004; Raa 1990). Such effects may be due to the glucan content in yeast (Campa-Cordova et al. 2002; Chang et al. 2000). Yeast is able to utilize the soluble sugars as energy source for

cell growth. Yeast species such as *Candida* and *Saccharomyces spp* have been used for the bioconversion of organic wastes into protein enrichment such as vegetable processing waste (Stabnikova et al. 2005), potato starch waste (Rusendi and Sheppard 1995), pineapple cannery effluent (Nigam 1998), corn silage juice (Hang et al. 2003), Chinese cabbage juice (Choi et al. 2002), or effluents from paper mill and olive mill (Ejiofor et al. 1996; Gharsallah 1993; Nigam 1998). These bioprocessed products contain varied protein and carbohydrate that are suitable for animal/aquacultural feed supplement. These studies suggest that yeast is effective to treat these organic wastes for pollution control and production of protein enrichment products. The bioprocessed yeast from spent fruit flies medium was reported to increase the growth and survival rate of shrimp by incorporating it in commercial feed (Yang and Lin 1981).

From the environmental point of view, the use of yeast on bioprocessing organic wastes may be more attractive than the conventional treatments and disposal methods to provide value-added product. It is promising to develop an economic engineering process for the bioconversion of island agricultural fruit or food processing wastes into useful high nutrient products as feed ingredients. The success development of processing the island agriculture by-products into protein enrichment value-added products is beneficial to maintain local aquaculture productions with lower feed cost and the sustainability in fruit or agriculture production.

Papaya is one of the major agriculture products in Hawaii. The processing of papaya puree produces more than 50% of total weight as waste (Yang et al. 1984). The

investigation of bioprocessing papaya processing wastes into protein enrichment value-added products may provide a promising solution for sustainability of agriculture production, aquaculture industries and environmental pollution control.

1.2 Objectives

The overall goal of this study is to develop the bioconversion process of fruit/food processing waste into protein enrichment value added products for potential aquatic feed supplement. The island papaya processing waste was used for the investigation. The objectives are listed as follows:

1. To investigate various pretreatment methods of papaya processing wastes for yeast growth including liquefaction, blending, and nutrient requirements.
2. To develop operational criteria for the bioconversion of papaya processing wastes into high protein content products in batch and/or continuous flow system.
3. To evaluate the nutritional value of the bioprocessed products as shrimp feed supplement.
4. To assess the cost-effectiveness of the bioprocessed products.

Chapter 2. Literature Review

2.1 Treatments of fruit/food processing wastes

The wastes of fruit or food processing manufactories are required further treatment to reduce the organic load before disposal. These wastes usually contain soluble sugars, organic acids and various nutrients that can be utilized by microbes for the production of protein enrichment (Stabnikova et al. 2005), ethanol (Nigam 1999) or other value-added products. Banana fruit stalk waste has been used as solid substrate for commercial production of cellulase employing *Bacillus subtilis* (Krishna 1999).

The pretreatment methods of food wastes include extraction, anaerobic liquefaction, solid and slurry-phase decomposition. The extraction of dissolved organic matter from fruit and food processing wastes was usually applied as a pretreatment (Stabnikova et al. 2005). The extraction of solid food waste residue can be converted by aerobic treatment or composting into fertilizer or by anaerobic digestion into biogas (Srilatha et al. 1995; Wang et al. 2003; Wang et al. 2002; Yun et al. 2000).An anaerobic liquefaction of papaya waste has been reported to release 80% of COD within 10h of reaction (Yang and Chou 1986). Slurry-phase decomposition of food wastes showed approximately 82% of carbonaceous compounds was decomposed after 5-day reaction (Chang et al. 2000). The change in pH and DO (dissolved oxygen) level were found closely related to the decomposition process.

2.2 Bioconversion of fruit/food processing wastes for protein enrichment products as animal feed

A variety of fruit and food processing wastes have been investigated for protein enrichment such as Chinese cabbage juice (Choi et al. 2002), waste brine generated from kimchi production (Choi and Park 1999), deproteinized leaf juices (Chanda and Chakrabarti 1996), corn silage juice (Hang et al. 2003), or effluents from paper mill and olive mill (Ejiofor et al. 1996; Gharsallah 1993; Nigam 1998).

Apple pomace was used for yeast production after solid-state fermentation providing the yields varied from 54% to 65%. The product produced three times more crude protein than unfermented apple pomace powder which could be used profitably as animal feed (Joshi and Sandhu 1996). Vegetable and fruit processing waste was blended with water and shake for one day to take out the extract juice as substrate for yeast growth and produced 40-45% crude protein in the product (Stabnikova et al. 2005). Chinese cabbage waste was blended and centrifuged before using for yeast growth (Choi and Park 2003). The results showed that different species of yeast (*Candida utilis, Pichia stipitis, Kluyveromces marxianus and Saccharomyces cerevisiae)* produced similar protein content (35-44%) using cabbage juice as substrate compared to glucose 20g/l as substrate. Leaves juice collected from turnip, mustard, radish and cauliflower were used for yeast growth (Chanda and Chakrabarti 1996). The species of *Saccharomyces* was found to provide better soluble proteins than *Candida* and *Torula*. The bioconverted product contains 45% of protein and resulting in 46-60% of COD (chemical oxygen demand)

removal and 74-98% of BOD (biological oxygen demand) removal. Waste of banana skin was used for yeast growth (*Saccharomyces unarum*) and the cell protein content was increased with the addition of ammonia sulfate (Enwefa 1991).

The hydrolysis of rayon grade pulp mill effluent was used for yeast growth (Bhattacharya et al. 2005). With 1:1 dilution ratio of the effluent liquor, the process was effective to remove at least 50% of dissolved sugar and COD from the substrate after 5 day operation. Corn silage juice containing lactic acid, acetic acid and ethanol was used for yeast growth (Hang et al. 2003). The process was effective to remove the organic pollutants from corn silage and produced over 13 g/l of biomass (dry weight) from 21 mg/l of settled yeast volume index. Waste brine enriched with organic materials from kimchi production factory was used for the growth of osmotolerant yeast (Choi and Park 1999). Around 90% of BOD was removed after 24h operation and provided maximum cell yield of 0.69 g/l by dry cell and 40% protein in the product. The biomass produced was found to increase proportionally with the amount of waste brine cabbage juice added.

Different yeast species have been investigated under different growth environment for cell protein production and pollution control. For example, raw citrus waste usually contains predominantly mesophilic yeasts and low numbers of thermophilous bacteria and filamentous fungi (van Heerden et al. 2002). *Candida utilis* was selected for cultivation in concentrated food industry effluents after its anaerobic acidogenic treatment (Elmaleh et al. 1999). The yeast isolates, *C. halophila* and *Rhotorula glutinis*, could effectively remove above 85% of organic pollutants from wastewater containing

19 g/L of ammonium–nitrogen (Yang et al. 2003). The yeast *C. langeronii* was able to utilize L-arabinose above 42 °C in the absence of vitamins (Nigam 2000). The yeast *Galactomyces geotrichum* T2B provided consistently higher biomass yields and excellent nutrient removal from silage effluent (Arnold et al. 2000). Cultivation of yeast or filamentous fungi has been proposed for decreasing the content of organic acids in orange processing waste before its anaerobic treatment (Suhajda et al. 2000).

Several examples of the bioconversion process of yeast from fruit or food processing wastes for protein enrichment are presented as follows:

2.2.1 Vegetable processing wastes for cell protein conversion

Organic matter was easily extracted by water from vegetable and fruit processing wastes. The water extracts of cabbage, watermelon, a mixture of residual biomass of green salads and tropical fruits contained 1,420 to 8,900 mg/L of dissolved organic matter, 600 to 1,800 mg/l of nitrogen and with pH range from 4.1 to 6.4. Approximately 65–70% of TOC (total organic carbon) can be removed by extraction after 24 h operation (Stabnikova et al. 2005). The sterilization or pasteurization of extract is necessary to avoid bacterial species contaminations such as *Acinetobacter baumannii–A. calcoaceticus*. The yeast *Saccharomyces cerevisiae* CEE 12 was selected for growth at 30°C for 96h in the sterilized extracts without any nutrient supplements and pH control (Stabnikova et al. 2005).. The substrate concentration ranged from 6.4 to 8.2 g/l. The yield of the bioprocess was comparable with the yield of yeast biomass grown in potato

dextrose broth. The bioconverted product contains 40-45% of protein content in dry biomass.

2.2.2 Potato starch waste for cell protein conversion

Amylolytic yeast strains have been successfully used for protein enrichment on starch media (Bhalla and Joshi 1994; Rahmat et al. 1995; Touzi et al. 1982). The raw potato starch contains high water absorption capacity and is difficult to be used in solid state fermentation. The preliminary analysis found that potato starch caused major packing problems when solids content was higher than 25%. The potato slurry was then pretreated with barley malt flour that mainly produced maltose to reduce the solids from potato processing waste to 16.7% for further use. The fed-batch process is a cheaper alternative (protein yield 19.4%) than the batch process (protein yield 14.9%) to provide higher protein yield (Rusendi and Sheppard 1995).

Potato starch obtained from waste waters of chips manufacturing was used for yeast growth. The dewatered waste of potato starch cake containing 60% solids was diluted to 25% solids and treated with malt flour for starch hydrolysis. The batch fermentation was aerated at 26°C for 20h. Several yeast strains were investigated for the growth in the media. *Candida utilis* ATCC 9256 was found the most efficient protein-forming strain among 18 yeast strains tested (Gelinas and Barrette 2007). The scaled-up 100-L batch process was improved with molasses supplementation under fed-batch operation. After drying, the fermented starch contained 11-12% protein including 7- 8% yeast protein that

can be dehydrated for feeds. The results indicated that it would be difficult to get protein levels higher than 7-8% yeast protein after solid-state fermentation of potato starch.

2.2.3 Sugar cane hydrolysate for cell protein conversion

The hydrolysate of sugar cane bagasse hemicellulosic fraction was pretreated with 70 mg of sulphuric acid per gram of dry mass at 125 °C for 2 h and then used as substrate for the growth of yeast *Candida langeronii RLJ Y-019* at 42 °C, stirring at 700 rpm, aeration at 1.0 and 2.0 v/v/min with an initial pH of 6.0 (Nigam 2000). The D-glucose was firstly utilized before other carbon sources were utilized such as D-xylose, L-arabinose, and acetic acid. The kinetic parameters for growth at 1.0 and 2.0 v/v/min were as follows: maximum specific growth rate (μ_{max}) were 0.29 ± 0.01 h^{-1} and 0.43 ± 0.016 h^{-1}, yields ($Y_{x/s}$) were 0.36 ± 0.012 and 0.40 ± 0.012 g_x/g_s and productivity (Q_x) were 0.81 ± 0.016 and 0.97 ± 0.012 $g_x/l/h$, respectively. The results were favorably comparable with published results obtained from *Candida utilis* and *Geotrichum candidum*. *Candida langeronii* appeared superior to *C. utilis* for the growth on hemicellulose hydrolysate because it utilized L-arabinose and was capable to grow at higher temperatures. The biomass contained 48.2 % of protein, 23.4% of carbohydrate and essential amino acids for animal feed.

2.2.4 Salad oil manufacturing wastewater for cell protein conversion

Salad oil manufacturing wastewater (SOMW) is high-strength effluent from the water washing process separating the high amount of fatty acid from glyceride in raw vegetable

oil (Zheng et al. 2001). The initial N/C ratio drastically influenced the oil reduction efficiency, cell yield and protein content (Zheng et al. 2005). The N/C ratio range between 1/6 and 1/8 were recommended. *C. utilis* was chosen from five species (*Rhodotorula rubra, Candida tropicalis, C. utilis, C. boidinii, Trichosporon cutaneum*) as biomass producer due to its highest oil uptake rate (0.96 kg oil kg^{-1} biomass d^{-1}) and highest specific growth rate (0.25 h^{-1}). The bioconverted product contained 26% protein, 9% crude lipid, 55% carbohydrate and balanced amino acid compositions.

2.2.5 Silage waste for cell protein conversion

Silage effluent was treated by yeast strains *Candida utilis* -strain T2B and *Galactomyces geotrichum* (Arnold et al. 2000). The mean generation times varied from 4.5 to 26 h. The process was able to achieve 91-95% COD removal for dilute effluent and 74-79% COD removal for concentrated, very high removal of phosphate and some removal of ammonia. The largest removal in COD was from *ca.* 40,500 to 8,500 mg/l. The pH increased from 3.6-5.8 to 8.5-9.0 during the process. The Strain T2B gave consistently higher cell yields than *C. utilis*, producing a maximum yield of 8.6 g/l on dry weight from the 50% dilution of effluent.

2.2.6 Fish cannery processing wastewater for cell protein conversion

The yeasts isolated from a drainage canal in a fish food processing factory were investigated for their growth in the wastewater (Naoto et al. 2002). The decomposition abilities in organic matters such as proteins, sugars and total organic carbon (TOC), and

the taxonomy were examined. Three strains dominated the fish cannery effluent included Debaryomyces occidentalis (P1), Trichosporon ovoides (P19), and an unidentified strain (S27). Strain P19 had the highest TOC removal activity and was immobilized onto chitosan beads in the fish cannery wastewater.

2.2.7 Pineapple cannery effluent for cell protein conversion

The pineapple effluent rich in microbial utilizable sugars such as glucose, fructose and other nutrient sucrose was used for the growth of *Candida utilis* by supplement of diammonium hydrogen phosphate as nitrogen source. (Nigam 1998). The specific growth rate and yields varied with carbohydrate concentration. Higher growth rate and yields were obtained with lower concentrations of carbohydrate providing an appropriate growth condition for cell protein production. The maximum specific growth rate (μ_m = $0.46h^{-1}$) and cell yield coefficient (Yc/s = 0.30) were obtained at 23.2 g/l carbohydrate in the growth medium and achieved 90-95% COD removal. Freeze-dried yeast contained 55.3% crude protein, 51.2% true protein, and 27.4% carbohydrate. The yeast had a balanced amino acid profile, except for sulphur-containing amino acids. The results suggested that yeast could be effectively used to treat pineapple cannery waste for protein enrichment product.

2.2.8 Chinese cabbage juice from kimchi for cell protein conversion

During kimchi production, approximately 30% of Chinese cabbage (*Brassica campestris*) was discarded as waste that contained more than 90% water and reducing sugars (Choi et

al. 2002). Four species of yeast, *Candida utilis*, *Pichia stipitis*, *Kluyveromyces marxianus*, and *Saccharomyces cerevisiae* were cultured in cabbage waste juice extract. All of them except *Candida utilis* were able to provide higher protein yield compared to the growth on yeast extract malt (YM) broth of same sugar concentration.

An osmotolerant yeast *Pichia guilliermondii* A9 selected from 70 isolates of yeast was used to treat the waste brine from a kimchi factory for cell protein production (Choi and Park 1999). Supplement of nutrients in the waste growth media was found to effectively increase the biomass production. The growth of *P. guilliermondii* A9 in kimchi waste brine was drastically inhibited when NaCl concentration was above 12% (w/v) yet not inhibited when the concentration was 10% (w/v). Approximately 90% BOD was removed after 24h cultivation. The maximum cell yield was 0.69 g/l dry cells, containing 40% protein. The biomass increased proportionally with the amount of added organic material. The results suggested large amounts of waste brine generated from kimchi production could be used directly for the culture of the osmotolerant yeast *P. guilliermondii* A9.

2.3 Solid-state fermentation of fruit/food processing waste for cell protein production

2.3.1 Engineering aspects of solid state fermentation

Various studies have investigated on yeast growth for protein enrichment in liquid media (refer to section 2.2) (Ghaly et al. 2005; Lotz et al. 1991; Stabnikova et al. 2005). The liquid state fermentation (LSF) usually involved extensive use of water that had to be

discharged after the process. In liquid state fermentation, the potato starch containing 2-3% solids pretreated with amylolytic yeasts could provide a 50% biomass yield based on starch dry matter and 25% protein enrichment of the basic substrate after 24h of fermentation (Skogman 1976). The semi-solid state fermentation of beet sugar residues (25% solids) by yeast could provide about 10% of protein as feed ingredient (Durand and Chereau 1988).

Solid state fermentation (SSF) has been considered as an alternative for the fermentation process to minimize the excess waste discharge. Solid-state fermentation is normally performed at 25-70% solids without free water and very limited oxygen transfer (Lonsane et al. 1985; Pandey 1991). Traditional fermentation tanks originally designed for liquids could be used for solid-state fermentation (Almanza et al. 1995; Chamielec et al. 1994). The operation design in solid state fermentation include substrate uptake, oxygen transfer, growth characteristics, growth estimation, control systems for maintaining parameters, mathematical models, design of fermenters and automation of fermentations (Lonsane et al. 1985). It is important to develop mathematical models of heat and mass transfer for scale-up solid state fermentation processes (Mitchell et al. 2000).

Several investigations regarding solid state fermentation for protein enrichment are presented as follows:

2.3.2 Solid state fermentation of apple pomance

Solid state fermentation and liquid state fermentation of apple pomace in the co-culture of cellulolytic moulds and yeasts were found to increase the protein yield (Bhalla and Joshi 1994). The co-culture of *Candida utilis* and *Aspergillus niger* was found to be the best combination that produced 20% protein content in dried product under SSF (Bhalla and Joshi 1994). *Kloeckera apiculata* or *Candida utilis* Y15 was used for SSF of apple pomace and could produce 7.5% (w/w) crude protein after 72 h of fermentation with more than 2-fole increase in the concentration of essential amino acids and enhanced nutritional value that could be serve as a pig-feed supplement (Rahmat et al. 1995).

2.3.3 Solid state fermentation of sweet potato residue

Starchy agricultural waste from sweet potato containing 20-35% solids, 65-69 % moisture, and 0.98-0.99 water activity was inoculated with amylolytic yeasts under solid state fermentation at 30°C for 2-3 days to produce an average of 10-12% protein product as feed supplement (Yang 1988). The optimal operational condition was performed at an initial moisture content of 65%, pH of 4.5, and a 1:1 mixture of ammonium sulfate and urea which added incrementally into the medium with 1% added at zero time, 1% added at 24 h, and 0.5% added at 48 h to produce 16.11-20.82% protein.

2.3.4 Laboratory and pilot-scale solid-state fermentation of sugar beet pulp

A lab-scale reactor (1-L) was designed for aseptic solid state cultivations on sugar beet pulp medium (Almanza et al. 1995). A pilot scale reactor (50-L) was used for solid-state cultivations in sterile conditions with aeration system and planetary agitation device. The medium containing wheat bran wetted with a glucose solution was kept sterile for 8 days at 32°C (Chamielec et al. 1994). A pilot plant with a maximum working capacity of one ton (ca. 200 kg dry matter) has been investigated for solid-state fermentation for cell protein production from raw sugar beet pulp (Durand and Chereau 1988). The material and heat balance were monitored in relation with temperature and moisture level regulation. The protein content increased from 9% to 20-21% on dry basis after 48h fermentation.

2.4 Fruit/food processing wastes for ethanol production

The production of ethanol from fruit and food processing waste could be an attractive economic energy. The pineapple cannery waste is a good nutrient source of sugars, protein, vitamins for microbial growth that could be recovered as ethanol for waste pollution control (Alain et al. 1987; Chye and Meng 1975; Prior et al. 1980).

Pineapple cannery waste was mechanically chopped and pressed to obtain a turbid juice (450-500 L of juice produced from one ton of waste) and then pasteurized (80°C, 15 min), cooled, and centrifuged (6000g, 15 min) to obtain clear liquid with mean composition of

the effluent containing 82 g/l total sugar providing a suitable substrate for ethanol production (Nigam 1999). The heating process could lower undesired microbial and reduce total solids in the growth medium.

The continuous ethanol production from pineapple cannery waste by the respiration deficient strain *Saccharomyces cerevisiae* ATCC 24553 has been studied at 30°C with pH of 4.5 (Nigam 1999). Maximum ethanol yield (92.5% of the theoretical) was obtained at a dilution rate of 0.05 h^{-1}. The maximum values for volumetric ethanol and biomass productivities were 3.75 $g_p\ l^{-1}\ h^{-1}$ and 0.63 $g_x\ l^{-1}\ h^{-1}$, respectively, at a dilution rate of 0.15 h^{-1}. The maintenance energy coefficient was 1.12 $g_s\ g_x^{-1}\ h^{-1}$. The maximum specific ethanol productivity and specific sugar uptake rate were 0.98 $g_p\ g_x^{-1}\ h^{-1}$ and 2.54 $g_s\ g_x^{-1}\ h^{-1}$, respectively. The ethanol production under fed-batch operation was simulated with experimental results and sensitivity analyses by using a constant feeding rate to simulate a limited oxygen transfer rate and to allow the uptake of residual sugar uptake (Ejiofor et al. 1994a).

Additional carbohydrate may be required to be added into the pineapple cannery effluent to obtain higher ethanol concentrations in the reactor for economic distillation (Nigam 1999). The economics of such fermentation process depends on several factors such as the stability and availability of the effluent throughout the year so that the process can run continuously and efficiently. The ethanol yield from pineapple cannery waste appears to be quite attractive which offered excellent possibilities for the economic production of fuel ethanol.

2.5 Application of yeast as animal feed

2.5.1 Nutrient composition of yeast

The general composition of yeast *Saccharomyces cerevisiae* is shown in Table 1. The protein content in the yeast depended on the growth medium and growth condition (Reed and Nagodawithana 1991). The crude protein was usually determined by the Kjeldahl nitrogen multiplied by a factor of 6.25. The crude protein includes 8-15% of nitrogen from nucleic acids. The true protein content is more appropriate to be estimated by a factor of 5.5 (Reed and Nagodawithana 1991).

Table 1 Gross nutritional composition of S. cerevisiae

Moisture	2-5%	Solids	27%
Protein, crude (N*6.25)	50-52%	C	12.3%
protein	42-46%	N	2.3%
RNA and DNA	6-8%	P	0.28%
Minerals	7-8%	K	0.54%
Total lipids	4-5%	Mg	0.03%
Carbohydrates	30-37%	Ca	0.01%
Dietary fiber	17%		

Source: Reed and Nagodawithana (1991).

2.5.2 Benefits of yeast supplement on shrimp's immune system

Yeast supplement has been reported to increase shrimp's immune system (Burgents et al. 2004). Such effects may be due to the glucan content in yeast biomass which can enhance immune response and increase disease resistance (Campa-Cordova et al. 2002; Chant et al. 2000). Spent fruit fly media has been investigated for protein enrichment by yeast as shrimp feed (Yang and Lin 1981). These studies showed that the commercial feed combined with the bioconversed yeast supplement resulted in higher shrimp survival rate and growth than the original commercial feed.

2.5.3 Application of yeast as feed with selenium enrichment

Selenium (Se) is an important antioxidant in the diet and an essential nutrient for human and animal organisms. Organic selenium-containing compounds, mainly selenomethionine, is the best source of selenium for organisms (Demirci et al. 1999; Suhajda et al. 2000). Yeast enriched with selenium has been proved to be an appropriate supplement for humans (Alfthan et al. 2000; Schurauzer 2001) and animals (Pehrson et al. 1999).

Under appropriate conditions yeasts are capable of accumulating trace elements into cell mass. In conventional batch process, addition of water-soluble selenium salt in yeast growth medium results in a substantial amount of selenium absorbed by the yeast cell. Using a culture medium supplemented with 30 μg/ml sodium-selenite during the

exponential growth phase resulted in selenium accumulation of 1,200-1,400 μg/g in dried baker's yeast (*Saccharomyces cerevisiae*) (Suhajda et al. 2000).

The factors influence the accumulation of selenium in biomass including pH, dissolved oxygen, and yeast strains. A 0.40-0.50 mg/g h^{-1} specific selenium consumption rate was found to be appropriate to obtain selenium-enriched bakers' yeast with high quality (Suhajda et al. 2000). Under suitable conditions the undesirable inorganic selenium content of the yeast could be suppressed in the final biomass.

The transformation of extracts of vegetable and fruit processing wastes into selenium enriched yeast biomass has been investigated (Stabnikova et al. 2005). The nutrient of yeast *S. cerevisiae* CEE12 could be enhanced by adding 5 μg/ml of selenium in the medium for biomass to contain 150 μg Se/g of dry matter in product. It was possible to produce 10 kg of dry yeast biomass with high protein and selenium content using the extract from 1 tonne of fresh vegetable and fruit processing wastes (Stabnikova et al. 2005).

2.5.4 Economic evaluation for yeast production

The processed yeast biomass can be obtained by centrifuging, washing and drying. Several fruit and food processing wastes have been used for yeast production. From one tonne of sterilized extract from vegetable and fruit processing wastes, 10 kg of dry yeast could be produced with protein content ranged from 37- 49% (Stabnikova et al. 2005). Corn silage juice (containing 2.06 g/l of glucose) could produce 4.71 or 12.63 g/l of yeast

S. cerevisiae in 24h or 48h reaction time, respectively (Hang et al. 2003). The fermentation of deproteinized leaf juice (1 ml/25 ml of the substrate) for 96h could provide 0.61-1.00 g/100 ml of the yeast *S. cerevisiae* (Chanda and Chakrabarti 1996). The average yield of yeast (*Candida utilis*) from 100kg rice polishings is 90 kg. It is used as an ingredient in poultry feed and the feed cost was reduced by $ 0.33-0.51 per100 kg bag (Rajoka et al. 2006).

2.6 Yeast fermentation

The commercial production of Baker's yeast *S. cerevisiae* usually uses cane or beet molasses as substrate with additional nitrogen and phosphate and some mineral, vitamins or trace elements source (Reed and Nagodawithana 1991). The fermentation is under strictly aerobic condition with growth rate restricted to less than 0.25 h^{-1} to obtain optimal growth and produced 4-6% yeast solids. Parameters regarding yeast fermentation are presented as follows:

2.6.1 PH and temperature condition for yeast growth

Yeast can grow well between pH 3.6 to 6. The optimum pH for growth is between 4.5 and 5 (Reed and Nagodawithana 1991). Commercial baker's yeast fermentation is usually controlled at temperature 30°C with PH starting at 4.5 and end at 6 or above (Reed and Nagodawithana 1991).

2.6.2 Carbon sources for yeast growth

Carbon source serves as energy source for yeast growth. For commercial production of baker's yeast (*S. cerevisiae*), beet and cane molasses are used as substrate due to its less cost. However, the price fluctuates and affects the manufacturing cost accordingly (Reed and Nagodawithana 1991). Alternatively, potential sugar sources from starches, cheese whey or other food and fruit processing wastes have been investigated. Corn syrup consisting almost entirely of glucose and fructose has been reported to provide suitable yeast growth rates and yields (Reed and Nagodawithana 1991). Starch or lactose can not be assimilated by *S. cerevisiae*. It requires hydrolysis into glucose and galactose to be utilized by yeast (Moulin and Galzy 1984). Ethanol can be utilized directly by baker's yeast and results in the same yields as the yield from sugar on equivalent carbon basis.

2.6.3 Yeast growth from lactose source

The aerobic conversion of yeast growth from lactose source to energy and new cells is a combination of respiration and growth. This can be described by the following equation (Ghaly and Ben-Hassan 1994):

Respiration

$$C_{12}H_{22}O_{11} + 12\ O_2 \rightarrow 12\ CO_2 + 11\ H_2O + \text{Energy}$$

Growth

$13\ C_{12}H_{22}O_{11} + 24\ NH_4 \rightarrow 12\ C_{13}H_{20}N_2O_7 + 59H_2O + 24\ H^+$

Growth of yeast from lactose

$14\ C_{12}H_{22}O_{11} + 12\ O_2 + 24\ NH_4 \rightarrow 12\ C_{13}H_{20}N_2O_7 + 70H_2O + 12\ CO_2 + 24\ H^+ + \text{Energy}$

The utilization of 1 g of lactose requires 0.08 g of O_2 and 0.09 g of NH_4 and results in 0.79 g of cell biomass, 0.11 g of CO_2, 0.26 g of H_2O and 0.005 g of H^+ and release 82.5 J of energy (Ghaly et al. 2005).

2.6.4 Effect of sugar concentration

Yeast (*S. cerevisiae*) growth is sensitive to glucose concentrations (Reed and Nagodawithana 1991). When concentration of carbon source, mainly glucose is above a critical value of 0.2%, the respiratory of yeast growth is switched to another metabolism and results in the formation of metabolism intermediary products such as ethanol. This is known as catabolite repression or the Crabtree effect. Such metabolism occurs whether the dissolved oxygen in the medium is sufficient or not. In order to obtain maximum biomass growth, the sugar concentration in the growth medium needs to be maintained under the critical concentration (Reed and Nagodawithana 1991).

2.6.5 Effect of acetic acid

Baker's yeast can produce trace amount of acetic acids under certain condition and use acetic acid as carbon source when pH is above 5 (Casal et al. 1996). Acetic acid is

inhibitor to yeast growth at low pH or when concentration is above 0.5 g/l (Maiorella et al. 1983). Acetic acid can affect yeast growth and metabolism and is one cause of stuck and sluggish fermentation (Edwards et al. 1999; Huang et al. 1996; Rasmussen et al. 1995).

2.6.6 Oxygen requirement for growth

Oxygen is essential for yeast growth. The composition of yeast is $C_6H_{10}NO_3$ (Harrison 1967) or 45% as C, 6.8% as H, 9.0% as N, and 30.6% as O (Wang et al. 1977). One gram of oxygen is required for 1 gram of dry yeast cell formation (Mateles 1971). In strict aerobic fermentation, about one-half of oxygen is converted into yeast cell, and the other half converted into CO_2 (Reed and Nagodawithana 1991). Generally, oxygen is difficult to supply due to its low solubility in growth medium. Additional aeration system and mixing devices are required to provide sufficient oxygen for growth.

As yeast cell grows, it consumes oxygen in the medium and the dissolved oxygen (DO) level decreases accordingly. The DO in growth medium drops lower than 0.4 ppm or 5% saturation at 30°C of fermentation (Strohm and Dale 1961). The critical oxygen concentration for limited yeast growth is 0.059 ppm at 20°C and 0.073 ppm at 35°C (Finn 1967). For yeast growth in the waste of spent fruit fly media, an airflow rate of 0.8 l/l/min supplied with mechanical mixing was suggested (Yang and Lin 1981).

2.6.7 Effect of specific growth rate

The specific growth rate (μ) of yeast in an exponential growth phase is defined by the equation (Reed and Nagodawithana 1991):

$$dP/dt = \mu \times P$$

where P is mass of yeast (or biomass) and t is time.

The specific growth rate can be determined by the slope in the graph of increase in biomass versus reaction time in the exponential growth phase. The generation time is defined by the equation $t = ln\ (P_t/P_0)/\mu$ when $P_t/P_0 = 2$.

Yeast can grow under aerobic and anaerobic conditions. The best yield (Y_S) of yeast under aerobic condition is 0.54 (54 g dry yeast cell/100 g of glucose). Under anaerobic condition the yield is usually less than 0.075 (Reed and Nagodawithana 1991). Under anaerobic condition, alcohol (ethanol) is produced and the growth of yeast biomass is restricted. Sufficient aerobic environment and optimal substrate conditions are required to maximize cell yield. The substrate sugar concentration also requires to be maintained under a critical limit to avoid catabolite repression.

The yeast growth kinetics was inhibited by the substrate when its concentration was higher than a critical value (Elmaleh et al. 1999). Higher initial substrate concentration results in high osmotic pressure and low water activity and therefore decreases the growth which can be observed during ethanol fermentation (D'Amore and Stewart 1987;

Ghaly and El-Taweel 1995) or lactic acid fermentation (Tango and Ghaly 1999). For optimal growth of baker's yeast, the specific growth rate was suggested to be under $0.2h^{-1}$ (Reed and Nagodawithana 1991). At steady-state continuous fermentation, when the growth rate was higher than 0.18, the cell yield would decrease because the fermentation was shifted to anaerobic growth and ethanol was formed (Dellweg et al. 1977). Similar effect was reported when a critical specific growth rate was above 0.23 h^{-1} (Meyenburg 1969).

2.6.8 Kinetics of cell growth

The growth of the microbial cell is first increasing in size and then cell division occurs. Cell growth is measured in the increase of cell number rather than cell size. The time required for cell to grow and divide into two cells is the generation time. The growth depends on cell species and the characteristics of growth medium, such as maintenance and transport of nutrients. The growth of microorganisms usually undergoes four phases in batch system: lag phase, exponential growth phase, stationary phase and death phase. The exponential growth phase can be described by the following equation (Aiba et al. 1973):

$$dX/dt = \mu X$$

where dX/dt is the growth rate (g/L/h); X is the concentration of viable cells (g/L); and μ is the specific cell growth rate (h^{-1}).

Under optimal conditions, the increase rate of biomass is proportional to the quantity of viable cell in the medium (Aiba et al. 1973). The limited nutrients in the growth medium will first depleted and then the growth of cell will start to decline and then cease. The specific growth rate is proportional to substrate concentration and approaches a maximum value at the critical substrate concentration (Mulchandani and Luong 1989). The relationship between specific growth rate and limiting substrate concentration can be described by the equation (Aiba et al. 1973):

$$\mu = \mu_m S / (K_S + S)$$

Where μ_m is the maximum specific growth rate (h^{-1}); S is the concentration of the limiting substrate (g/l); K_s is the saturation constant or the substrate concentration (g/l) at one-half the maximum specific growth rate (g/l).

2.7 Batch, fed-batch and continuous flow process for yeast fermentation

2.7.1 Bioprocess control strategies for yeast fermentation

The control strategies of yeast fermentation include carbon source addition and nutrient supply for the control of growth phase and metabolic conditions (O'Connor et al. 1992). It concluded that single control strategy for yeast growth bioprocess was not appropriate under all possible operating conditions. An oxygen uptake rate-based control strategy performs better when a mean respiratory quotient (RQ) value was less than 1.1 during an

oxygen limitation condition than an ethanol control strategy with a mean RQ of 14. Under different operational conditions, the designed strategies were able to consistently provide the achievement of high cell densities 78.7g/l and yields 0.50 g/g glucose in the optimal fermentation conditions (O'Connor et al. 1992).

Several investigations of batch, fed-batch and continuous operations for yeast production are presented as follows:

Definition of batch, semi-batch, fed-batch, and continuous process (Doran 1995):

- A batch process operates in a closed system. All materials are added to the system at the start of the process; the system is then closed and products removed only when the process is complete.
- A semi-batch process allows either input or output of mass, but not both.
- A fed-batch process allows input of material to the system but not output.
- A continuous process allows matter to flow in and out of the system. If rates of mass input and output are equal, continuous processes can be operated indefinitely.

2.7.2 Fed-batch fermentation of *S. cerevisiae* on glucose

The respiro-fermentative growth of yeast *Saccharomyces cerevisiae*, DSM 2155 on glucose in fed-batch fermentation was performed in a simulated 5-phase feeding strategy on the Universal BIoprocess CONtrol (UBICON) system (Ejiofor et al. 1994b). The

growth rate was designed between 0.20-0.23 h^{-1}. The estimated and simulated values of specific growth rates showed good agreements.

The biomass yield, specific substrate uptake and O_2 consumption rates were analyzed for the consistency of the data using carbon and electron balances (Ejiofor et al. 1994b). It showed there was little possibility for ethanol formation due to the high average value of true biomass energetic yield of 0.707, and a low value of maintenance coefficient m_e of 0.0114 h^{-1} at high density fermentation. After 24h of fermentation, the yeast concentration reached 54 g/l. The yeast produced had also good dough-leavening characteristics. It is possible to operate a yeast plant without using respiratory quotient as the controlling parameter (Ejiofor et al. 1994b).

2.7.3 Batch and continuous fermentation of bakers' yeast on potato and wheat starch processing wastes

Wastes of potato and wheat starch were processed in batch and continuous cultures for baker's yeast production (Lotz et al. 1991). The growth media was cultivated in a 10-L laboratory stirred tank, an 80-L laboratory airlift tower loop and a 4 m^3 pilot-plant airlift tower loop reactor. Bioconversed glucose source from potato protein liquor, potato liquor retentate, potato liquor residue, and wheat process wastewater were used as substrate. The performance of the cultivation (cell concentrations, specific growth rates in the first (glucose) and second (ethanol) growth phases, productivities, and yield coefficients), and the qualities of the effluents (concentrations of phosphate, dissolved organic carbon,

dissolved organic nitrogen, and COD) were determined in different reactors. The optimal conditions were evaluated with yield performance and effluent quality. The performances do not vary with the scale of the reactors. The performance in continuous cultures is considerably better than that in batch cultures.

2.7.4 Fed-batch operation of *Saccharomyces cerevisiae* on Cassava starch waste

The cassava starch hydrolysate containing about 80.7% of glucose was used for baker's yeast fermentation under fed-batch operation (Ejiofor et al. 1996). The fermentation was controlled at a specific growth rate of 0.18-0.23 h^{-1}, a biomass yield coefficient of 0.5 g/g, and a feed substrate concentration of 200 g/L. The on-line off-gas analysis by mass spectrometry for the calculation of the oxygen uptake rate, carbon dioxide evolution rate, and respiratory quotient and the off-line determinations of biomass, ethanol, and glucose by dry weight, gas chromatography, and spectrophotometry were used as control parameters. Cell mass concentrations of 50-58 g/l were achieved in all experiments within 28 h with the last 15h in the fed-batch mode. The average biomass yields for the cassava and glucose media were identical at 0.49 g/g and no significant differences between leavening activities of the products. Waste cassava starch hydrolysate provides a suitable low cost replacement for glucose in the production of baking-quality yeast.

2.7.5 Continuous fermentation of *Kluyveromyces fragilis* on lactose

Cheese whey is the liquid waste generated from cheese making process. It contains about 92% water, 5% lactose, 1.9% protein and fats, 0.9% salt and a small amount of vitamins (Ghaly and Ben-Hassan 1995). The continuous flow operation has been successfully used for the production of cell protein from cheese whey using the yeast *Kluyveromyces fragilis* by the utilization of lactose (Ghaly and Ben-Hassan 1994; Ghaly and Ben-Hassan 1995; Ghaly et al. 1992; Mickle et al. 1974; Moresi et al. 1990; Reisman et al. 1968).

The fermentation of *Kluyveromyces fragilis* from cheese whey has been investigated for modeling (Ghaly et al. 2005). The kinetic model was developed from principles of mass balance and the combined effects of substrate utilization, maintenance, substrate concentration, and cell death rate during the fermentation process to describe the continuous production of cell protein. This model was tested with experimental data obtained from a continuous system operated at various retention times (12, 18 and 24 h), mixing speeds (200, 400 and 600 rpm) and air flow rates (1 and 3 vvm) and was capable of predicting the effluent cell and substrate concentrations with R^2 ranging from 0.95 to 0.99. A lactose concentration below 1.91 g/l limited yeast growth whereas the concentration above 75 g/l inhibited yeast growth. The viable cell mass and lactose consumption ranged from 1.3 to 34.3 g/l and from 74.31% to 99.02%, respectively. A cell yield of 0.74 g cell/g lactose (close to the stoichiometric value of 0.79 g cell/g lactose) was achieved at the 12 h retention time (3 vvm air flow rate, and 600 rpm mixing speed) and produced 37g/l of biomass output (viable and dead cells).

2.7.6 Continuous fermentation of *Candida utilis* on food industry waste

The concentrated food industry effluent was treated with an anaerobic acidogenic reactor followed by a yeast reactor (Elmaleh et al. 1999). The main carbon source includes acetic acid, propionic acid and butyric acid. *Candida utilis* was selected and operated in batch and in a continuous stirred tank reactor. The pH was maintained at 3.5 to minimize bacterial contamination. The yeast growth was found inhibited when substrate concentration was higher than a critical value. The growth and substrate utilization were significant different in batch reactor and the transient state in the completely mixed stirred reactor. The conveniently operated continuous flow system provided 97% TOC removal with the loading rate 30 kg TOC m^{-3} day. The TOC removal in continuous flow operation was independent of inlet concentration and space time provided the space time higher than the washout value. The TOC removal depends on the conversion of the initial compounds in acids. The organic acids were completely oxidized. The sludge production was of the same order of magnitude as in a conventional activated sludge reactor. The solids were easily flocculated and settled after neutralization.

2.8 Summary

Fruit and food processing wastes can be utilized by microbial cells for the removal of organic compounds to minimize environmental pollution and to produce value added products. The bioprocessed products from various organic wastes by yeast for protein

enrichment have been widely researched (Stabnikova et al. 2005; Rusendi and Sheppard 1995; Arnold et al. 2000; Zheng et al. 2005; Nigam 1998) as feed supplement. The protein content in the product is varied with different growth medium. The survival rate and growth of shrimp were increased by adding the bioprocessed yeast converted from spent fruit fly media into commercial feed (Yang and Lin 1981). The immune response of shrimp was found to be increased by incorporating yeast into aqua-feed (Burgents et al. 2004).

Several pretreatment methods for the fruit and food wastes have been applied such as mechanical chopping and pressing, extraction, or partially anaerobic digestion. The heating process reduced solids content and lowered undesired bacteria contamination (Nigam 1999). Sterilization or pasteurization was necessary to avoid bacteria contamination in the growth medium (Stabnikova et al. 2005). The heating temperature was suggested to be kept low to minimize protein loss during the heating process if the raw organic waste contained any protein (Choi et al. 2002).

Several operational parameters are considered during the yeast fermentation such as pH, DO, temperature, nutrients requirement, substrate concentration and utilization for optimal cell growth. For the better growth of yeast *S. cerevisiae*, pH was suggested to be between 3 and 6. The specific growth rate should not exceed 0.2 h^{-1} and substrate concentration was not higher than 0.2% (Reed and Nagodawithana 1991). Sufficient oxygen supply is necessary for yeast biomass growth. Additional mechanical aeration and

mixing system may be required to provide sufficient oxygen transfer (O'Connor et al. 1992).

The fed-batch, continuous flow system and scaled-up pilot plant have been investigated for mass production of yeast from fruit or food processing waste (Ejiofor et al. 1996; Ejiofor et al. 1994b; Elmaleh et al. 1999; Ghaly et al. 2005; Lotz et al. 1991). The scaled-up operation systems were found to provide similar performance as laboratory system (Elmaleh et al. 1999; Lotz et al. 1991). The scaled-up batch process was improved with the supplement of molasses under fed-batch process (Gelinas and Barrette 2007). The continuous culture was considerably better than batch culture to provide higher performance in effluent quality (concentrations of dissolved nitrogen, phosphate, and COD) and cell growth (cell concentrations, yield coefficients) (Lotz et al. 1991).

Solid state fermentation (SSF) can minimize excess water requirement and discharge during liquid state fermentation (LSF) process. Solid state fermentation usually performs at 25-70% of solids without free water (Lonsane et al. 1985; Pandey 1991; Skogman 1976). Traditional fermentation tanks originally designed for liquids can be used for solid state fermentation (Almanza et al. 1995; Chamielec et al. 1994) supplied with aeration and agitation system for sufficient oxygen and nutrient transfer (Chamielec et al. 1994). The operational parameters in solid state fermentation include substrate uptake, oxygen transfer, growth characteristics, growth estimation, control systems for maintaining parameters, mathematical models, design of fermenters and automation of fermentations (Lonsane et al. 1985). Several fruit and food processing wastes have been investigated

under solid state fermentation for protein enrichment such as apple pomance (Bhalla and Joshi 1994; Rahmat et al. 1995), sweet potato residue (Yang 1988), and sugar beet pulp (Almanza et al. 1995; Chamielec et al. 1994; Durand and Chereau 1988). The bioprocessed product contains around 20% of protein content in optimum performance for 2-3 days of fermentation (Bhalla and Joshi 1994; Durand and Chereau 1988; Yang 1988). Although the fermentation performance varies with microbial cell, substrate characteristics and growth environment, protein yield in SSF may not be as good as that in LSF which was able to provide 30-50% of protein after 24 h of fermentation (Choi and Park 1999; Nigam 1998; Nigam 2000). SSF of the organic wastes for nutrition enhancement as feed supplement (Bhalla and Joshi 1994; Rahmat et al. 1995; Yang 1988) and scaled-up or pilot-scale process for potential use (Almanza et al. 1995; Chamielec et al. 1994; Durand and Chereau 1988) appears a potential alternative from LSF.

The utilization of fruit and food processing waste by yeasts for ethanol production is another treatment for pollution control and value-added product production. Pineapple cannery (Nigam 1999) has been investigated for ethanol production. The economics of such bioprocess depends on several factors such as the ethanol yield or the stability and availability of substrate from organic wastes to achieve attractive ethanol yield and economic production (Nigam 1999).

It is, therefore, that the area of studies needed further investigations for the study using papaya processing waste for protein enrichment can be included in the following:

- Pretreatment method initiation

- Evaluation of optimal growth condition for yeast growth
- Evaluation of batch, semi-batch, fed-batch, or continuous fermentation
- Evaluation of scaled-up mass production
- Economic evaluation
- Potential application of solid state fermentation

Chapter 3. Materials and Methods

Papaya processing wastes were investigated for yeast growth in laboratory scale to determine the appropriate pretreatment methods, nutrient requirements and kinetic constants for optimal production in batch operation. Preliminary scaled-up batch operation and continuous/semi-continuous flow operations were also investigated. The bioprocessed product was tested as the potential ingredient in shrimp feed at Oceanic Institute (OI), Waimanalo, Hawaii.

3.1 Composition of papaya processing waste

Papaya processing waste (PPW) collected from Super Food Inc. (Honolulu, HI) was kept in freezer for future use (Figure 1). The composition of raw papaya and blended papaya waste slurries excluded seeds (30±5% of total weight) are shown in Table 2 and Table 3, respectively.

Figure 1 Raw papaya processing waste

Table 2 Composition of raw papaya

Nutrient*	(g/100g)	Minerals	(mg/100g)
Water	88.83	Calcium	24
Protein	0.61	Iron	0.1
Total lipid	0.14	Magnesium	10
Ash	0.61	Phosphorus	5
Carbohydrate	9.81	Potassium	257
Fiber	1.8	Sodium	3
Sugars	5.9	Zinc	0.07
		Copper	0.016
		Manganese	0.011
		Selenium	0.006

* Exclude seeds and skin (about 33% of total waste)

Source: National nutrient database for standard reference (USDA 2007)

Table 3 Composition of papaya processing waste

(in the form of blended slurry, exclude seeds ~30±5% of total weight)

(unit: mg/l)	mean	SD
PH	6.0	0.3
TS (total solids)	50,299	12,237
SS (suspended solids)	20,649	7,379
TCOD (total chemical oxygen demand)	126,288	6,945
SCOD (soluble chemical oxygen demand)	92,088	11,454
TN (total nitrogen)	1,620	241
STN (soluble total nitrogen)	358	166
NH_3-N (soluble ammonia nitrogen)	145	21
TCOD/TN	85	
SCOD/STN	257	
SCOD/TCOD	0.73	

3.2 Pretreatment methods of papaya processing waste

Several pretreatment methods of PPW were investigated for the performance of yeast growth. These included anaerobic liquefaction, blending method, and nutrients requirement.

3.2.1 Anaerobic liquefaction of PPW for yeast growth

Liquefaction pretreatments of PPW were carried out anaerobically at 30°C to determine the required reaction time for TCOD (total chemical oxygen demand) to be released into soluble form for further utilization by yeast. Chopped PPW with an average size of $10cm^3$ and blended PPW with an average size less than 0.5 cm^3 were used as liquefaction medium. The liquefaction seed was obtained from former anaerobic digestion liquor of papaya wastes at 30°C. The ratio of PPW to water to liquefaction seed was 1:1:0.1. After liquefaction, the filtered liquor and the mixed slurry were separately used as substrate for yeast growth.

3.2.2 Blending pretreatment of PPW for yeast growth

PPW was blended with tap water to an average solid size less than 0.5 cm^3. The blended PPW slurry was then centrifuged to obtain clear liquid using continuous flow centrifuge (CEPA Model LE, Germany) (Figure 2) by continuous feeding at 30 l/hr with motor speed at 1800 rpm and cylinder speed at 16,000 rpm. The PPW centrifuged juice and mixed slurry were separately used for yeast growth.

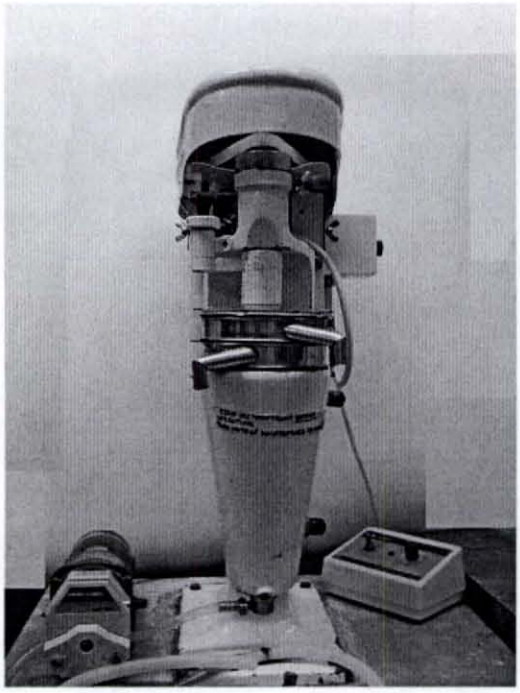


Figure 2 Continuous flow centrifuge (CEPA Model LE, Germany)

3.2.3 Nutrient requirements for yeast growth

Ammonium sulfate and potassium phosphate were used as nutrient source to investigate the effect on yeast growth. Ammonium sulfate and potassium phosphate were added at a ratio of SCOD: N: P = 100: 5: 1 in the growth medium at the beginning of fermentation. The centrifuged PPW juice obtained from blending method with initial SCOD ranged from 5000 to 6000 mg/l was used as substrate for the investigation.

3.3 Equipment set-up and operation

The liquefaction of PPW was carried out anaerobically at a ratio of PPW to water to seed 1:1:0.1 at 5-L working volume under 30°C. After one day of the reaction, the filtered liquefied liquor and the mixed liquefied slurry were separately inoculated with 2% baking yeast (*S. cerevisiae*) (Red Star, USA) and aerated at room temperature (22±2°C) at 2-L working volume in a 10-L reactor. The experiments of pretreatment methods of blending PPW for yeast growth were carried out at 2 to 4L of working volume in a 10-L reactor using air diffusers for aeration.

The batch operations were performed in a 14-L fermentor (New Brunswick Scientific Company, NJ) with working volume ranged from 4 to 8 L. The substrate of PPW was treated with HTST (high temperature short time) pasteurization at 72°C for at least 15 seconds (Houska et al. 2006). The growth medium was inoculated with 2% (w/v, g of yeast/100ml of growth medium) of baking yeast (*S. cerevisiae*, Red Star) stirred at 200 rpm under room temperature (22±2°C). Ammonium sulfate and potassium phosphate were used as nitrogen and phosphate source at a ratio of SCOD: N: P = 100: 5: 1, unless indicated otherwise. Initial substrate concentrations ranged from 5,000 to 30,000 mg/l and air flow rates ranged from 2 to 12 v/v/m were carried out to investigate the effects of substrate concentration and air supply on cell growth. The scaled-up batch operations were carried out with 100-L working volume in a 165-L container provided with air diffusers under room temperature (22±4°C).

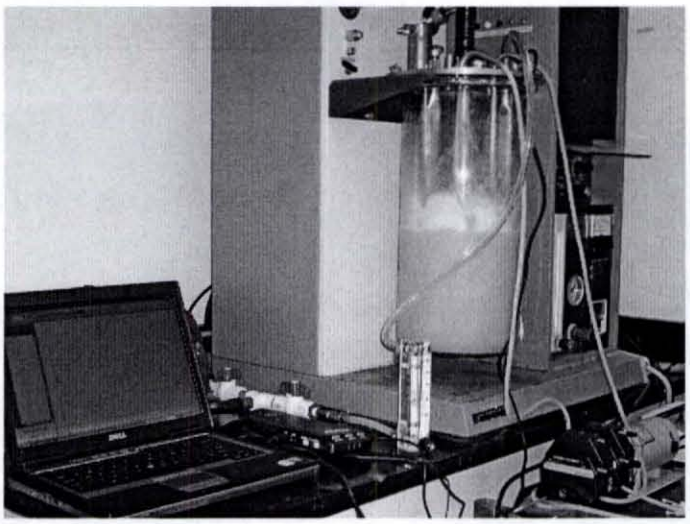


Figure 3 Lab-scale fermentation equipment setup

The continuous and semi-continuous flow operations were carried out in the 14-L fermentor with working volume ranged from 3 to 8-L, aerated at 7.5 v/v/m and stirred at 200 rpm under room temperature (22±2°C). Blended PPW slurry with SCOD ranged from 12,000 to 18,000 mg/l were used as substrate. Hydraulic retention time (HRT) ranged from 4 to 12h were investigated in the continuous flow operation and 2.74 to 8.2h HRT were investigated in the semi-continuous flow operation. The lab scale equipment set-up is shown in Figure 3.

3.3 Analytical methods

The performance of cell growth was examined by pH and ORP (Oakton 510 meter), dissolved oxygen (DO) (YSI model 85 meter, YSI, USA), suspended solids (SS), soluble chemical oxygen demand (SCOD), soluble total nitrogen (STN), soluble ammonia-

nitrogen, soluble phosphate and protein content. SS and SCOD were determined according to Standard Methods (APHA 1998). Total nitrogen, ammonia nitrogen, nitrate nitrogen, and phosphate were determined by persulfate digestion method, Nessler method, cadmium reduction method, and ascorbic acid method using the reagents provided from Hach Company (USA), respectively. Protein content was determined by Biuret method (Ramanathan 1968) or by the conversion of solid nitrogen multiplied a factor of 6.25. A mass balance of STN converted into cell protein was calculated by the amount of decrease in mixed liquor STN multiplied a factor of 6.25. The estimated protein content in increased SS was expressed as 6.25*ΔSTN/ΔSS.

3.4 Determination of kinetic parameters

Kinetic parameters were determined according to Aiba et al. (1973) with modification. Volumetric rate of substrate consumption was determined from a plot of SCOD (mg/l) versus reaction time (h). Suspended solids mass productivity (g/l/h by dry weight) was determined from a plot of dry solids (g/l) versus reaction time. Yield (Y) of suspended solids was determined from $dSS/dSCOD$. Specific growth rate of suspended solids was determined from the relationship of $dX/dt = f(X, S)$, and $\mu t = ln(X/X_0)$, where X = mass of suspended solids per unit volume (mg/L), S = concentration of growth-limiting substrate in SCOD (mg/l). Dependency of specific suspended solids growth rate on limiting substrate concentration was determined from the relationship $\mu = \mu_{max}\ (S/(Ks + S))$, where μ_{max} = maximum suspended solids growth rate when the substrate is unlimited, Ks= substrate concentration in SCOD (mg/l) at which the specific growth rate

is one-half of the maximum value. The yield of suspended solids (SS) growth to SCOD removal ($Y_{SS/SCOD}$) was determined from ΔSS/ΔSCOD. The yield of STN removal to SCOD removal ($Y_{STN/SCOD}$) was determined from ΔSTN/ΔSCOD. The estimated yield of protein formation ($Y_{STN*6.25/SCOD}$) was calculated by assuming the conversion of STN into cell protein and multiplied $Y_{STN/SCOD}$ by a factor of 6.25. The estimated yield of SS formation was calculated by assuming 40% of protein content in product solids and divided $Y_{STN*6.25/SCOD}$ by a factor of 0.4.

3.5 Nutrient evaluation of bioprocessed product as potential shrimp feed

Samples of mixed liquor and dried solids of the bioprocessed products from batch operation on blended PPW slurry with an initial SCOD of 15,000 mg/l were analyzed by the Agricultural Diagnostic Service Center (ADSC), University of Hawaii at Manoa, to determine the potential as feed ingredients. The samples of bioprocessed products from batch, preliminary scaled-up batch and continuous-flow operations were stored in a -80°C freezer and then freeze-dried for nutrient analysis by Oceanic Institute (OI), Waimanalo, Hawaii.

3.6 Shrimp feeding trial for the bioprocessed PPW product

The shrimp feeding trial was started in May, 2007 for 8 weeks at Oceanic Institute (OI, Waimanalo, HI, USA) to ascertain the protein replacement effects of varying inclusion levels of bioprocessed PPW product on the growth of *L. vannamei* juveniles. The PPW diets were controlled at 35% crude protein formulated according to Akiyama, Dominy, & Lawrence (1992). Three test diets were formulated with 0%, 50% and 100% replacement of bioprocessed PPW product. The freeze-dried bioprocessed PPW product from scaled-up batch operation was pulverized using Jacobson pulverizer (serial no. 47077, Model 16H, MI), mixed with formulated nutrient in a Hobart mixer (Model A120, OH) and then made for PPW diet pellet using pellet mill (Serial no. 384324, Series CL, California laboratory pellet mill, CA).

An initial of 12 shrimp was stocked in each rectangular glass aquaria with four aquaria per treatment. The growth trial was conducted at an indoor controlled-environment laboratory at OI. The shrimp was counted and weighed at two-week intervals (weeks 0, 2, 4, 6, and 8). The FCR is calculated by the weight of feed fed divided by the biomass gain of shrimp over the trail. The detailed information of the shrimp feeding protocol is shown in Appendix A. The results of PPW diets were compared with commercial shrimp feed 35/2.5 (purchased from Land O'Lake, Kapolei, HI).

3.7 Economic analysis

Economic analysis of the proposed bioprocess system included the estimation of material cost, capital cost such as facilities investment, and operational cost such as labor, maintenance and utilities. The cost of bioprocess per kg of PPW or per kg of protein were analyzed by net present worth and annual worth methods on a life span of 10 years and an annual interest rate of 5.75% (Kongsil, 2006). Sensitivity analysis was used for analyzing the impact of different factors on the production cost. The factors considered included capital cost, operational cost, material cost, life expectancy and annual interest rate. The sensitivity of annual worth to percent deviation changes of the most likely estimates of the concerned factor over a range of ±50% was analyzed by assuming the other factors remaining at their most likely estimates. The most likely estimate values were based on bioprocessing 100kg of PPW per day. The production cost of per kg PPW is calculated by dividing annual worth of the project cost to annual amount of PPW processed. The production cost of per kg protein is calculated by assuming the yield of 0.15 g protein/g PPW.

Chapter 4. Results and Discussion

4.1 Liquefaction pretreatment for yeast growth

4.1.1 Required reaction time for liquefaction of PPW

The chopped PPW and blended PPW were used as substrate for anaerobic liquefaction respectively. The SCOD is the indicator of carbohydrate concentration that can be utilized easily by yeast. It required one day for chopped PPW to release 75% TCOD into soluble form (Figure 4a). The blended PPW released about 72% TCOD immediately after blending without any liquefied treatment (Figure 4b). The blending method of PPW without liquefaction process was similar to chopped PPW with one day liquefaction to release similar portion of SCOD.

The anaerobic liquefaction of papaya processing waste showed about 80% COD was released within 10 h of reaction when using anaerobically digested effluent in the hybrid system as seed in the ratio of 1:1 with raw papaya processing waste (Yang and Chou 1986). Such short reaction time may be due to the specific hybrid operation process and high digested seed ratio.

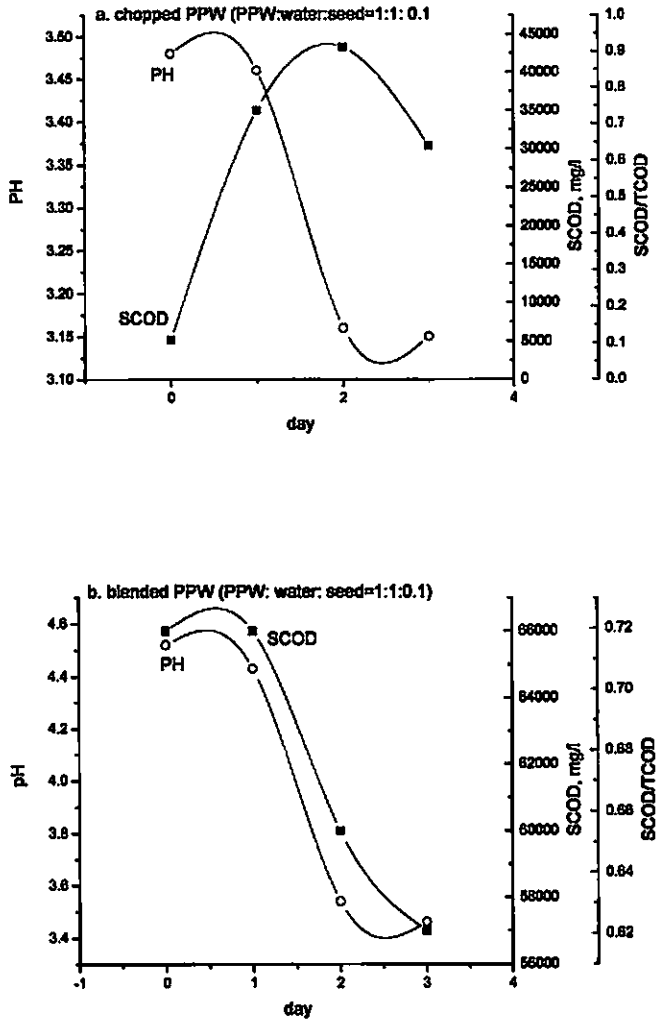


Figure 4 Liquefaction performance of PPW

4.1.2 Performance of liquefied PWW for yeast growth

Liquefied PPW in the form of filtered liquor and mixed slurry were separately used as substrate for yeast growth (Figure 5). The results showed that for one day reaction, about 93% and 84% SCOD were removed and the products contained 35% and 30% biuret protein in suspended solids for filtered liquefied liquor and mixed liquefied slurry, respectively (Table 4). The anaerobic liquefaction process may produce some intermediate products or contain certain bacteria which may require further product analysis and study on the potential or modification of the anaerobic liquefaction process.

Table 4 Performance of liquefied PPW for yeast growth

(unit in mg/l)	Filtered liquefied liquor	Mixed liquefied slurry
Reaction time (d)	1	1
Initial condition (unit in mg/l)		
S_0, SCOD	61000	64000
TCOD	75000	110000
SS	9600	37700
SCOD/TCOD	0.81	0.58
SCOD/SS	6.35	1.70
STN	550	560
End of operation		
SCOD	4000	10000
removal %	93%	84%
SS	12000	10800
Protein % in SS		
Biuret protein	35%	30%

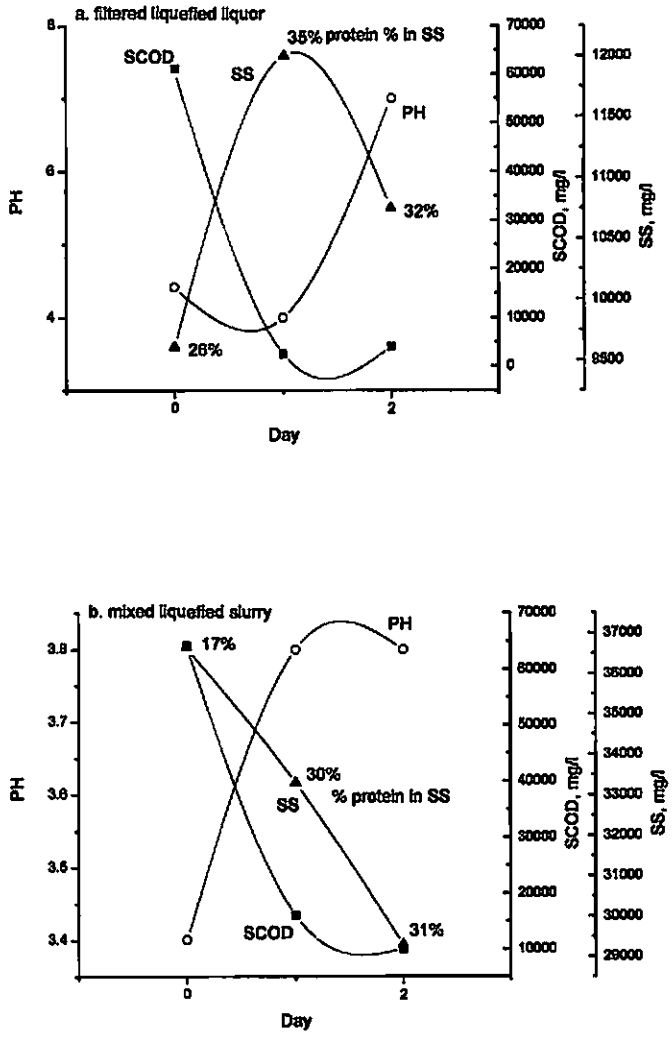


Figure 5 Performance of liquefied PPW for yeast growth

a. filtered liquefied PPW. b. mixed slurry liquefied PPW

4.2 Blending pretreatment for yeast growth

4.2.1 Effect of nitrogen and phosphate on yeast growth

The centrifuged PPW juice with initial SCOD ranged from 5000 to 6000 mg/l was used as substrate to investigate the effect of nitrogen and phosphate on yeast growth. Ammonium sulfate and potassium phosphate were added at a ratio of SCOD: N: P = 100: 5: 1. As shown in Table 5, the results show that with and without N and P addition, the required reaction time for yeast growth is 8 h and 20 h, providing the specific growth rate of 0.0135 h^{-1} and 0.00698 h^{-1}, yield $Y_{SS/SCOD}$ of 0.5057 and 0.0859, estimated yield of protein formation $Y_{STN*6.25/SCOD}$ 0.1391 and 0.07244, and biuret protein of 46% and 39% in the products, respectively. Without N and P addition, the required reaction time was much longer and the yield values and protein content in the product were much less. Therefore, the addition of nitrogen and phosphate is required for yeast growth using PPW substrate.

Table 5 Effect of additional nitrogen and phosphate source for yeast growth

Additional N and P source	SCOD:N:P=100:5:1	None
Reaction time (h)	8	20
Initial condition (unit in mg/l)		
S_0, SCOD	5880	5505
SS	6190	6500
SCOD/SS	0.95	0.85
STN	252	55
End of reaction		
SCOD	2860	1557
removal %	51%	72%
SS	6920	7150
STN	200	8.4
removal %	21%	85%
Product protein % in SS		
Biuret protein	46%	39%
6.25*ΔSTN/ΔSS	36%	45%
Specific growth rate, v (h^{-1})	0.0135	0.00698
SD	7.32E-04	0.00103
R^2	0.99	0.97
Yield, $Y_{SS/SCOD}$ (ΔSS/ΔSCOD)	0.5057	0.0859
SD	0.03699	0.02144
R^2	0.92	0.99
Yield, $Y_{STN/SCOD}$ (ΔSTN/ΔSCOD)	0.02225	0.01159
SD	0.00105	1.012E-4
R^2	0.96	0.99
Estimated yield of protein formation		
$Y_{STN*6.25/SCOD}$	0.1391	0.07244
Estimated yield of SS formation		
$Y_{STN*6.25/0.4/SCOD}$	0.3477	0.1811

4.2.2 Performance of PPW centrifuged juice and mixed slurry for yeast growth

The centrifuged juice and the mixed slurry of blended PPW with initial SCOD of 5600-5900mg/l and nutrient addition (N and P) were separately investigated for yeast growth. As shown in Table 6, the reaction provides 51-73% SCOD removal after 8 h of aeration for centrifuged juice and mixed slurry with the specific growth rate 0.0135 h^{-1} and 0.0130 h^{-1}, and similar yields values of suspended solids yields ($Y_{SS/SCOD}$) 0.5057 and 0.5175, estimated yield of protein formation ($Y_{STN*6.25/SCOD}$) 0.1391 and 0.1391, and estimated yield of SS formation ($Y_{STN*6.25/SCOD/0.4}$) 0.3477 and 0.3477, respectively. The results show that the juice and the slurry provide similar yield values for yeast growth from PPW substrate. The operation of blended slurry is simpler than that of centrifuged juice. It is, therefore, blended PPW slurry was used as the substrate for the yeast growth in this study.

Table 6 Performance of centrifuged juice and mixed slurry PPW for yeast growth

	centrifuged PPW juice	mixed PPW slurry
Aeration time (h)	8	8
Initial condition (unit in mg/l)		
S_0, SCOD	5880	5600
SS	6190	7840
SCOD/SS	0.95	0.71
STN	252	248
P	226	141
End of reaction		
SCOD	2860	1490
removal %	51%	73%
SS	6920	9610
STN	200	164
removal %	21%	34%
P	179	77
removal %	21%	45%
Product protein % in SS		
Biuret protein	46%	36%
6.25*ΔSTN/ΔSS	36%	30%
Specific growth rate, v (h^{-1})	0.0135	0.0130
SD	7.32E-04	0.00197
R^2	0.99	0.94
Yield, $Y_{SS/SCOD}$ (ΔSS/ΔSCOD)	0.5057	0.5175
SD	0.03699	0.00315
R^2	0.92	0.92
Yield, $Y_{STN/SCOD}$ (ΔSTN/ΔSCOD)	0.02225	0.02225
SD	0.00105	0.00105
R^2	0.96	0.99
Estimated yield of protein formation $Y_{STN*6.25/SCOD}$	0.1391	0.1391
Estimated yield of SS formation $Y_{STN*6.25/0.4/SCOD}$	0.3477	0.3477

4.2.3 The relationship of required aeration time to pH, DO and ORP

In order to determine the required aeration time for yeast growth, pH, DO and ORP were monitored during yeast growth. As shown in Figure 6, the results show that during yeast growth, pH decreases, ORP increases accordingly and the DO level is decreasing from 5 mg/l to 3.5 mg/l. When SCOD removal is up to 70% in the growth medium, the DO concentration increases sharply. Also, the increase of SS is following the decrease of SCOD.

The result is similar to the commercial Baker's yeast (*S. cerevisiae*) growth using molasses as substrate (Reed and Nagodawithana 1991). As yeast cell grows, the DO in growth medium drops lower than 0.4 ppm (1 ppm = 1 mg/kg or 1 mg/l when density is 1 kg/l) or 5% saturation at 30°C of reaction (Strohm and Dale 1961). The critical oxygen concentration for limited yeast growth is 0.059 ppm at 20°C and 0.073 ppm at 35°C (Finn 1967). At the end of yeast growth, the pH increases from 4 to 6 and DO concentration increases sharply (Reed and Nagodawithana 1991).

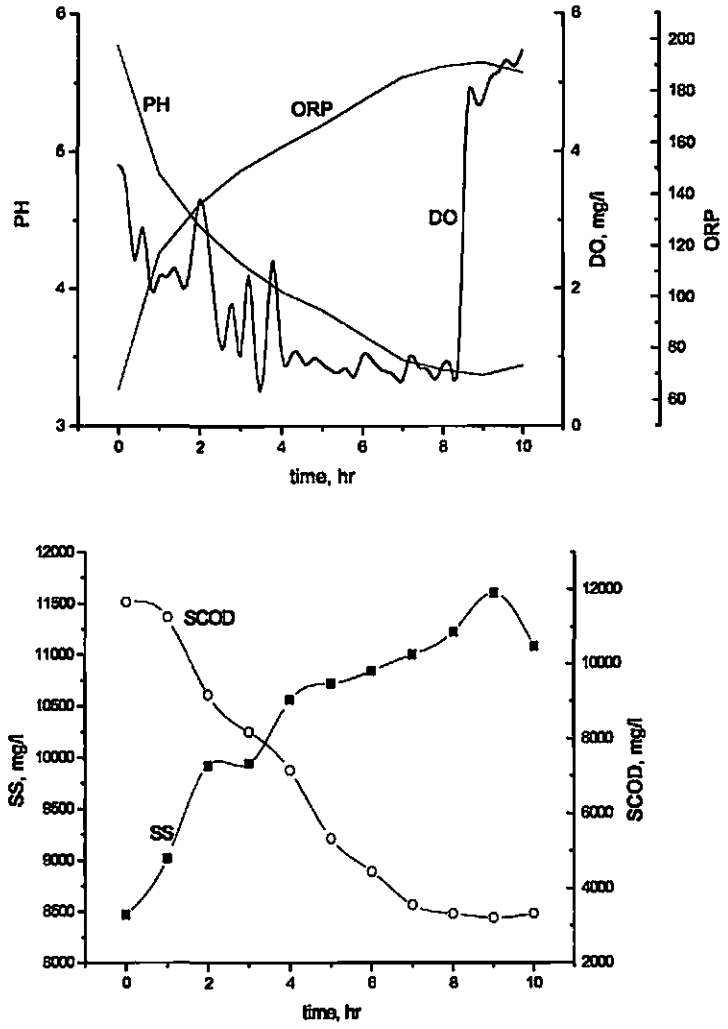


Figure 6 Relation of pH, DO and ORP curve with SCOD and SS during yeast growth

4.2.4 Buffer effect (acetic acid) for yeast growth

The pH in the growth medium of blended PPW slurry usually automatically maintained between 3 and 6 and sometimes below 3.5 without any additional adjustment (Figure 7a, 7b). Yeast can grow well between pH 3.6 to 6 and the optimum pH for growth is between 4.5 and 5 (Reed and Nagodawithana 1991). To maintain an optimum pH for yeast growth, the addition of acetic acid as buffer solution to maintain pH around 4.5 was investigated. With the addition of acetic acid, the pH was maintained between 4 and 5 during yeast growth (Figure 7c). However, acetic acid contributes to SCOD and can be utilized as carbon source for growth by baker's yeast *S. cerevisiae* (Casal et al. 1996). In this study, the acetic acid contributed about 30% (7400 mg/l) of total initial SCOD (25240 mg/l) in the buffered PPW substrate. The required reaction time to achieve similar SCOD removal (around 70%) in the acetic acid buffered substrate (25240mg/l of total SOCD with 17850 mg/l of PPW SCOD, 30h) was much longer compared to that in the substrate without acetic acid addition of similar initial total SCOD (25440 mg/l, 12h) or initial PPW slurry SCOD (18480 mg/l, 12h) (Table 7).

The yeast growth is inhibited when the concentration of acetic acid is higher than 0.5 g/l at low pH (Maiorella et al. 1983). Acetic acid can affect yeast growth and metabolism and may cause stuck and sluggish fermentation (Edwards et al. 1999; Huang et al. 1996; Rasmussen et al. 1995). The feed analysis of bioprocessed PPW product without pH adjustment indicated that the product has appropriate nutrition with high protein content (presented in section 4.5). Therefore, the addition of acetic acid as buffer is not suggested

for the use of pH adjustment in the PPW substrate. It may be replaced by using sodium bicarbonate as buffer or using pH control system which requires further study.

Table 7 Effect of buffer addition (acetic acid) on blended PPW slurry for yeast growth

Substrate (unit in mg/l)	PPW slurry without buffer addition		with buffer addition
Reaction time (h)	12	12	30
Initial condition			
S_0, SCOD	18480	25440	25240
			PPW SOCD 70%, 17850
			buffer SCOD 30%, 7400
TCOD	33500	42200	26800
SS	10440	15900	10360
SCOD/TCOD	0.55	0.60	0.94
SCOD/SS	1.77	1.60	2.44
STN	770	1020	690
P	600	1000	490
End of operation			
SCOD	4650	5260	8370
removal %	75%	79%	67%
SS	13130	10660	13930
STN	380	700	490
removal%	51%	31%	29%
P	113	560	390
removal%	81%	44%	20%
Product protein % in SS			
Biuret protein	32%	24%	26%
6.25*ΔSTN/ΔSS	43%	-2.89	44%

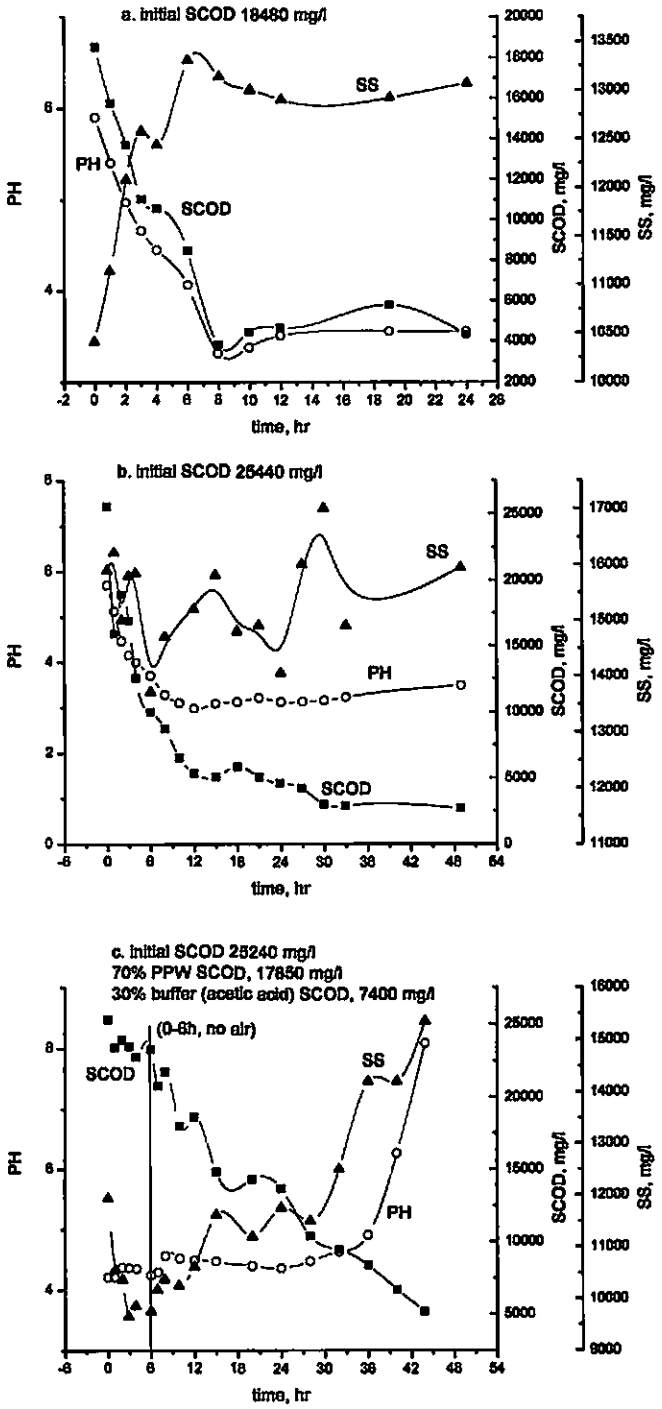


Figure 7 Buffer effect (acetic acid) on blended PPW substrate for yeast growth

a. S_0 18480 mg/l. b. S_0 25440 mg/l. c. S_0 25240 mg/l, PPW substrate 70% (17850mg/l), buffer 30% (7400 mg/l).

4.3 PPW for yeast growth in batch operation

4.3.1 Effect of initial substrate concentration on yeast growth

The effect of different initial substrate concentration of blended PPW slurry on yeast growth was investigated. As shown in Table 8 and Figure 8, the results show that substrates with initial SCOD ranged from 5600 mg/l to 18500 mg/l provide about 70% of SCOD removal and 30-40 % of protein content in suspended solids within 8 to 12 h of reaction time. For initial substrate concentrations ranged from 5600 to 18500 mg/l of SCOD, the resulting specific growth rate ranged from 0.013 to 0.04, the yield $Y_{SS/SCOD}$ ($\Delta SS/\Delta SCOD$) ranged from 0.52-0.21, the yield $Y_{STN/SCOD}$ ($\Delta STN/\Delta SCOD$) ranged from 0.022 to 0.026, the estimated yield of protein formation ($Y_{STN*6.25/SCOD}$) ranged from 0.139 to 0.166, and the estimated yield of SS formation ranged from 0.348 to 0.412, respectively.

As the substrate concentration increases from SCOD of 5600mg/l to 18500 mg/l, the specific growth rate increases accordingly, providing 72-75% of SCOD removal. The biuret protein content slightly decreases from 36% to 32% which may be due to the higher portion of PPW solids content in the higher substrate slurry. The estimated protein content $6.25*\Delta STN/\Delta SCOD$ increases as substrate concentration increases which may be due to higher initial STN concentration in the higher initial substrate slurry. The estimated protein of $6.25*\Delta STN/\Delta SCOD$ may be a better estimate for the increase in protein in the PPW slurry substrate. The yield values of $Y_{SS/SCOD}$ ($\Delta SS/\Delta SCOD$) decrease

as the PPW substrate concentration increases which may be due to higher portion of PPW solids decays in higher initial substrate concentration slurry. The growth of SS is the combined effect of growth in yeast biomass and decay in PPW solids. The estimated yield values of SS formation increase accordingly as the slurry substrate concentration increases may be a better estimate for biomass growth in the PPW slurry. The yield values of $Y_{STN/SCOD}$ (ΔSTN/ΔSCOD) increase as substrate concentrate increases which may be due to higher initial STN content in the higher initial substrate slurry similar to the situations in the estimation of protein content 6.25*ΔSTN/ΔSS.

Table 8 Effect of initial substrate concentration of blended PPW slurry on yeast growth in batch operation

Aeration time (h)	8	8	8	10	12
Initial condition					
S_0, SCOD	5600	11820	12110	15130	18480
TCOD	11200	23750	19520	30520	33500
SS	7840	7880	8850	9040	10440
SCOD/TCOD	0.50	0.50	0.62	0.50	0.55
SCOD/SS	0.71	1.50	1.37	1.67	1.77
STN	250	390	550	540	770
P	141	269	443	434	600
End of reaction					
SCOD	1490	2960	3390	3800	4650
removal %	73%	73%	72%	75%	75%
SS	9610	10660	11240	12600	13130
STN	160	250	410	290	380
removal%	36%	36%	25%	46%	51%
P	77	90	297	171	113
removal%	45%	67%	33%	61%	81%
Product protein % in SS					
Biuret protein	36%	33%	32%	30%	32%
6.25*ΔSTN/ΔSS	30%	30%	36%	41%	43%
Specific growth rate (h^{-1})	0.01302	0.02203	0.02468	0.02969	0.04038
SD	0.00197	0.00510	0.00443	0.00424	0.00824
R^2	0.94	0.93	0.90	0.94	0.94
Yield, $Y_{SS/SCOD}$ (ΔSS/ΔSCOD)	0.5175	0.3269	0.2716	0.3313	0.2135
SD	0.00315	0.01208	0.01673	0.01092	0.01695
R^2	0.92	0.97	0.94	0.98	0.91
Yield, $Y_{STN/SCOD}$ (ΔSTN/ΔSCOD)	0.02225	0.02651	0.01768	0.02346	0.02651
SD	0.00105	0.00118	0.00136	0.00141	0.00118
R^2	0.96	0.98	0.93	0.94	0.98
Estimated yield of protein formation $Y_{STN*6.25/SCOD}$	0.1391	0.1657	0.1105	0.1466	0.1657
Estimated yield of SS formation $Y_{STN*6.25/0.4/SCOD}$	0.3477	0.4142	0.2763	0.3666	0.4142

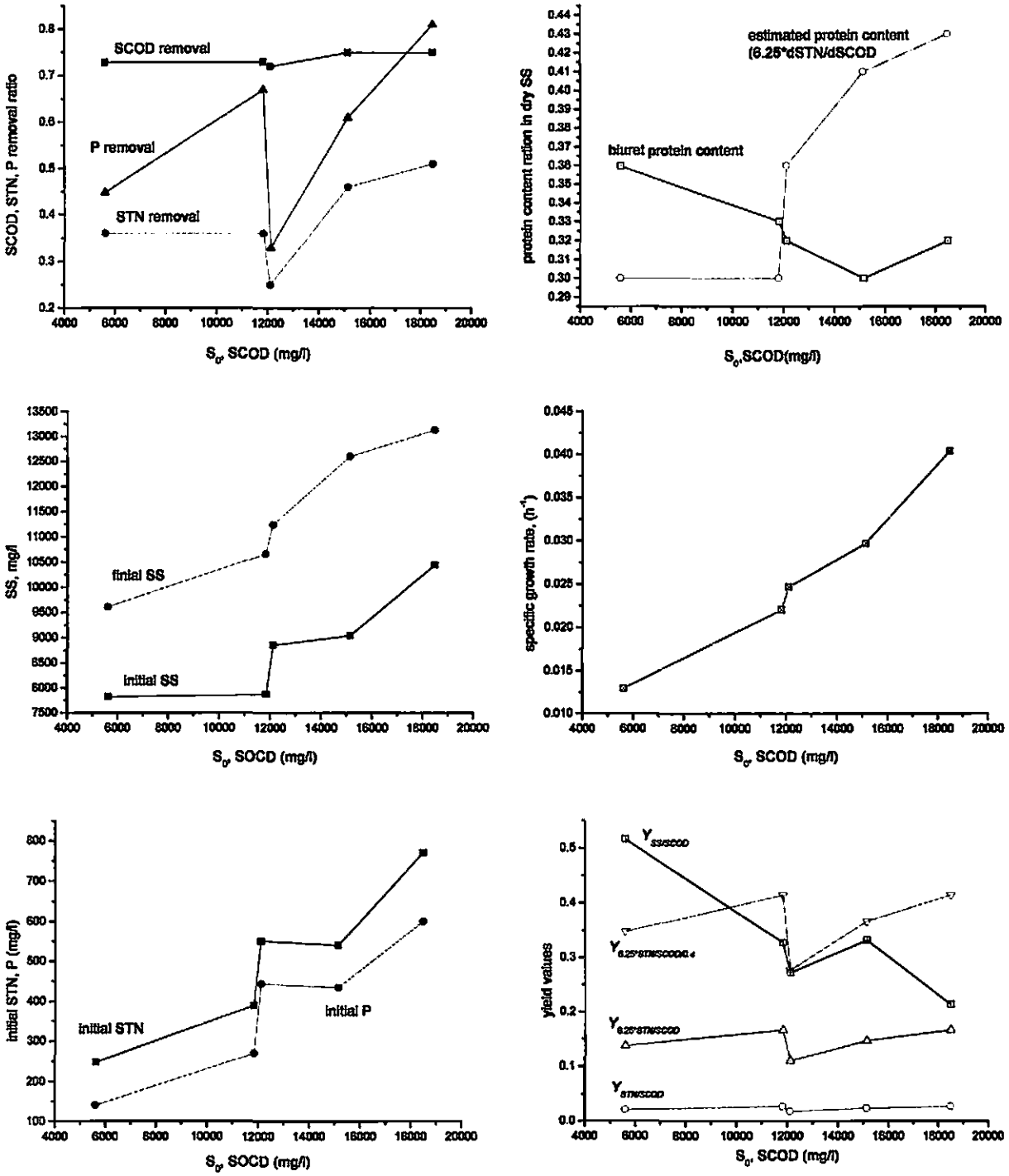
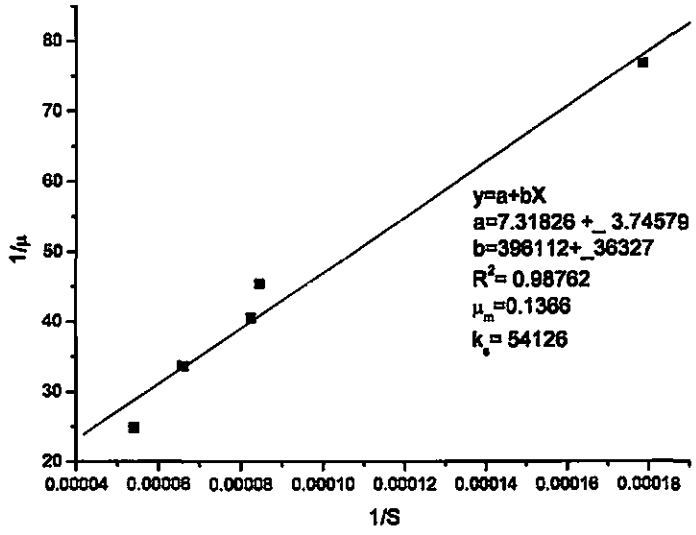


Figure 8 Performance of different initial substrate concentration of blended PPW slurry on yeast growth in batch operation

4.3.2 Kinetic parameters for optimal yeast growth in batch operation

Kinetic parameters for yeast growth in batch operation using blended PPW slurry as substrate were determined. As shown in Figure 9, mean values of maximum specific growth rate (μ_{max}) and saturation constant (k_S) are 0.1366 h^{-1} (ranged from 0.0904-0.2798) and 54,130 mg/l of SCOD (ranged from 100,654-59,090), respectively. To achieve the mean μ_{max} and k_S, assumed at 0.1h^{-1} specific growth rate, the suggested S_0 concentration is 145,440 mg/l of SCOD. However, such high concentration is difficult to achieve in the form of PPW slurry solely. The blended PPW slurry without any water addition is able to provide around 100,000 mg/l of SCOD. If using blended PPW slurry as sole substrate, the high slurry phase in the growth medium is not suitable for cell growth due to less oxygen transfer and high osmotic pressure. Therefore, based on the experimental results, it is suggested that it is appropriate to provide blended PPW slurry with initial SCOD concentration ranged from 12,000 to 25,000 mg/l for sufficient oxygen and nutrient transfers in the growth medium. This requires 8 to 12h of reaction which is much applicable for the yeast growth in batch operation.



S_0 (mg /l)	μ (h^{-1})	SD	R^2
5600	0.01302	0.00197	0.94
11820	0.02203	0.00510	0.93
12110	0.02468	0.00443	0.90
15130	0.02969	0.00424	0.94
18480	0.04038	0.00824	0.94

	μ_m (h^{-1})	k_S(mg SCOD/l)
mean	0.1366	54126
range	0.0904~0.2798	100654~59090

Figure 9 Determination of specific growth rate on blended PPW slurry

4.3.3 Effect of aeration on yeast growth

Blended PPW slurries were used as substrate to investigate the effect of aeration on yeast growth in batch operation. The medium of initial concentration 15000-16500 mg/l of SCOD were supplied with different air flow rate (2.5, 5, 6.25, 7, 9 and 11.7 l/l/m) and

stirred at 200 rpm in a 14-L fermentor at room temperature (22±2°C). As shown in Table 9 and Figure 10, the required reaction time for yeast growth decreases as air flow rate increases while providing similar SCOD removal (70-75%) and protein content (26-30%) in the product. As air flow rate increases, the specific growth rate increases. The cell yield values ($Y_{SS/SCOD}$, $Y_{6.25*STN/SCOD}$, $Y_{6.25*STN/SCOD/0.4}$) also increases accordingly. For aeration between 7 to 9 l/l/m, the required reaction time is 6-10h, providing 71-75% of SCOD removal, and 30% biuret protein content in SS.

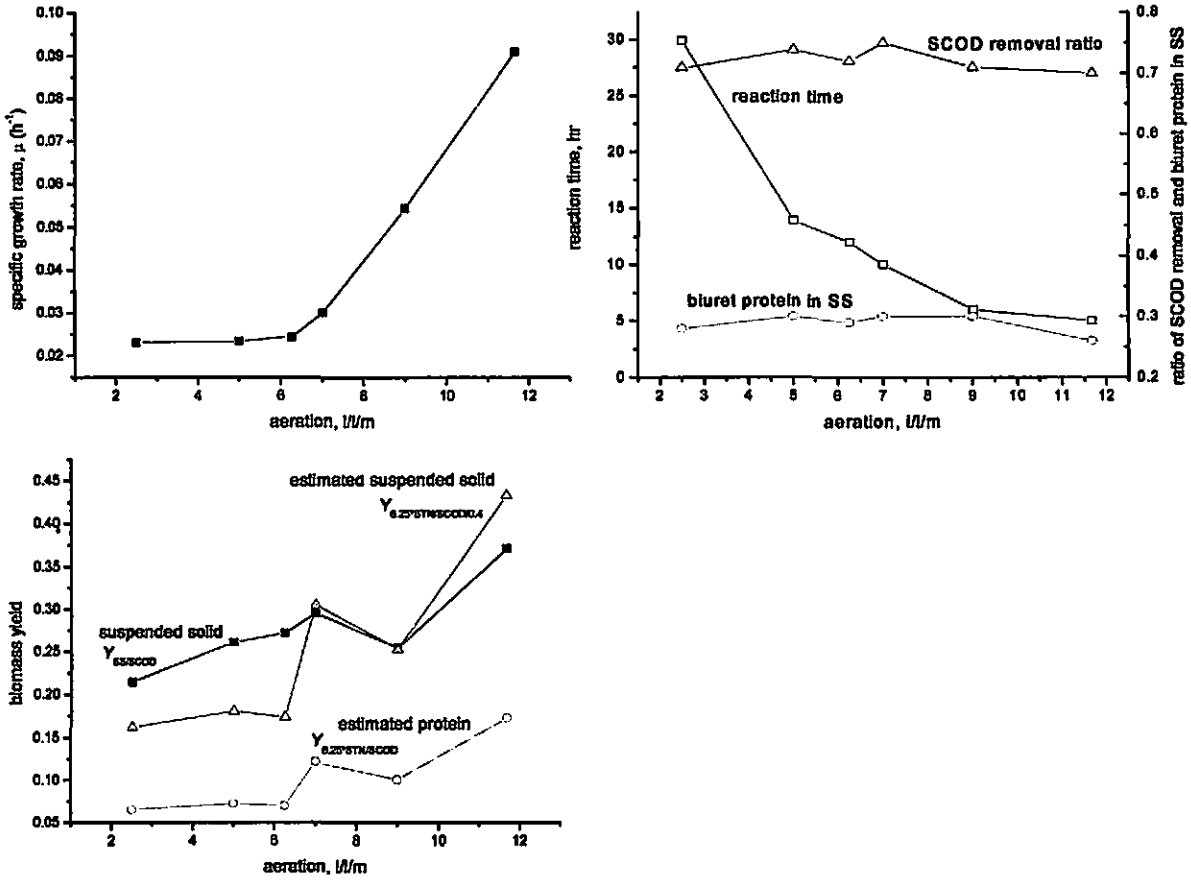


Figure 10 Effect of aeration on blended PPW slurry for yeast growth in batch operation

Table 9 Effect of aeration on blended PPW slurry for yeast growth in batch operation

aeration (l/l/m)	11.7	9	7	6.25	5	2.5
Reaction time (h)	5	6	10	12	14	30
Initial condition (mg/l)						
S_0, SCOD	16500	16270	15640	15320	15030	15700
TCOD	24200	20490	30750	27540	23210	21820
SS	8840	8040	9020	8740	9120	9530
SCOD/TCOD	0.68	0.79	0.51	0.56	0.65	0.72
SCOD/SS	1.87	2.02	1.73	1.75	1.65	1.65
STN	812	600	540	460	570	600
P	557	450	430	440	530	490
End of operation						
SCOD	4955	4680	3900	4260	3910	4560
removal %	70%	71%	75%	72%	74%	71%
SS	13080	10960	12860	11800	12600	11240
STN	328	330	290	270	370	380
removal%	60%	45%	46%	41%	35%	37%
P	160	150	170	230	300	290
removal%	71%	67%	60%	48%	43%	41%
Product protein % in SS						
Biuret protein	26%	30%	30%	29%	30%	28%
6.25ΔSTN/ΔSS	71%	50%	41%	40%	40%	51%
Specific growth rate (h^{-1})	0.09093	0.05427	0.03013	0.02448	0.02336	0.02307
SD	0.01485	0.03233	0.03963	0.00279	0.00109	8.58E-04
R^2	0.95	0.97	0.94	0.96	0.99	0.99
$Y_{SS/SCOD}$ (ΔSS/ΔSCOD)	0.03697	0.2532	0.2948	0.2718	0.2609	0.2146
SD	0.03546	0.02601	0.03377	0.03229	0.03045	0.01791
R^2	0.98	0.98	0.96	0.96	0.97	0.99
$Y_{STN/SCOD}$ (ΔSTN/ΔSCOD)	0.02764	0.0161	0.0195	0.01113	0.01155	0.01038
SD	0.00329	0.00181	0.00308	7.81E-04	9.48E-04	0.00168
R^2	0.97	0.98	0.96	0.99	0.99	0.97
Estimated yield of protein formation $Y_{STN*6.25/SCOD}$	0.1728	0.1006	0.1219	0.0696	0.0722	0.0649
Estimated yield of SS formation $Y_{STN*6.25/0.4/SCOD}$	0.4319	0.2516	0.3047	0.1739	0.1805	0.1622

4.3.4 Scaled-up batch operation

The batch operation in a 14-L fermentor was scaled up to a 165-L reactor with working volume of 100-L to investigate the reaction performance under room temperature (22±2°C). After 16h of reaction, the DO starts to increase. After 20h of reaction, the SCOD removal is up to 78% providing 35% of buiret protein content in suspended solid (Figure 11). The cell yield value of $Y_{SS/SCOD}$ is 0.2998, $Y_{STN/SCOD}$ is 0.01657, estimated yield of protein formation $Y_{6.25*STN/SCOD}$ is 0.1036, and estimated yield of SS formation $Y_{6.25*STN/0.4/SCOD}$ is 0.2589 (Table 10). The SCOD removal and protein content is similar to that obtained from 14-L fermentor scale. The longer reaction time may be due to the less oxygen transfer because of the different aeration and agitation system in scaled-up reactor. The yield value of $Y_{6.25*STN/SCOD}$ (0.1036) is also slightly less than that in 14-L reactor ($Y_{6.25*STN/SCOD}$ 0.139-0.166) which may also due to less oxygen transfer.

Providing similar condition, the scaled-up operation systems has been found to provide similar performance as laboratory system (Lotz et al. 1991). The cultivation of Baker's yeast from starch wastes indicate that the performances do not vary significantly with the scale of reactors (10-L laboratory stirred tank, 80-L laboratory airlift tower loop reactor and $4m^3$ airlift tower loop pilot-plant reactor) (Lotz et al. 1991). The scale-up batch fermentation process of *C. utilits* from 5-L to 13-L or 100-L did not change protein content in the fermented product from potato processing waste (Gelinas and Barrette 2007).

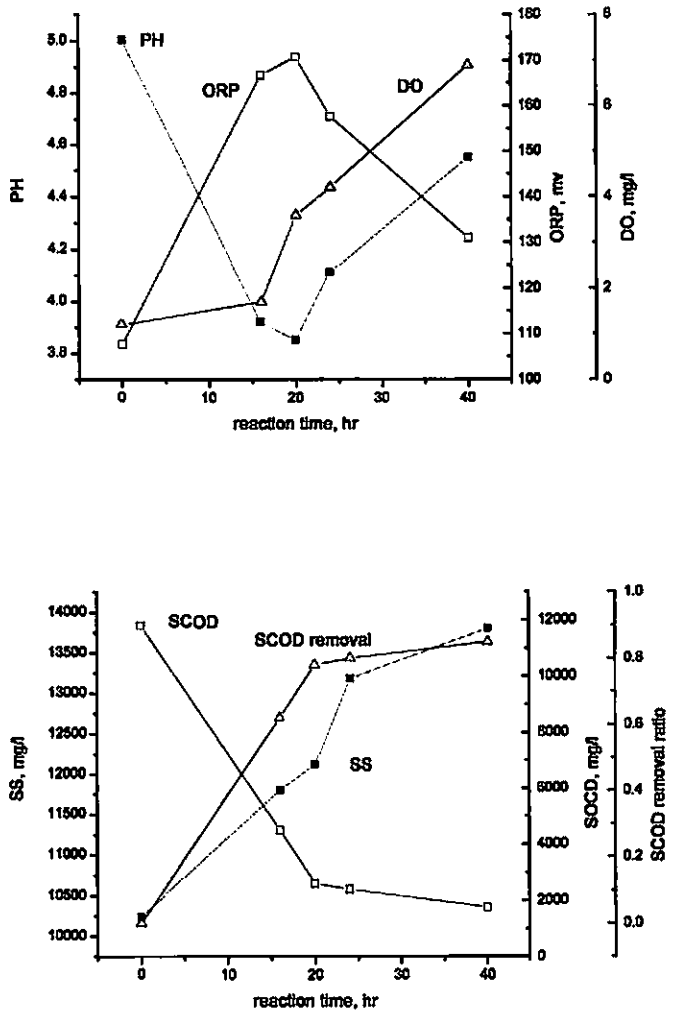


Figure 11 Performance of scaled-up batch operation on blended PPW slurry in 100-L working volume

Table 10 Yields of scaled-up batch operation on blended PPW slurry in 100-L working volume

	yield	SD	R^2
$Y_{SS/SCOD}$ (ΔSS/ΔSCOD)	0.2998	0.08073	0.91
$Y_{STN/SCOD}$ (ΔSTN/ΔSCOD)	0.0167	0.01013	0.85
Estimated yield of protein formation			
$Y_{STN*6.25/SCOD}$	0.1036		
Estimated yield of SS formation			
$Y_{STN*6.25/0.4/SCOD}$	0.2589		

4.4 Preliminary investigation of yeast growth on PPW in continuous and semi-continuous flow operation

4.4.1 Preliminary performance of continuous flow operation

Blended PPW slurries were used as substrate to investigate the performance of yeast growth in continuous operation. As shown in Table 11 and Figure 12, influent substrate concentrations were ranged from 15000 to 17500 mg/l SCOD with several different HRT ranged from 4-12 h. At the steady state, the effluent PH ranged from 2.24 to 3.89, ORP ranged from 168 to 260mv, the SCOD removal ranged from 38-76%, and biuret protein ranged from 16-25%. The reaction with HRT ranged from 6 to 12h, provides 64-76% of SCOD removal. The reaction with 10h HRT provides higher protein content (25%) and SCOD removal (75%). Based on batch experiments, the reaction time for initial SCOD ranged from 5000-18000 mg/l is 8-12 h (Table 7). The HRT for continuous flow operation with similar substrate concentrations is therefore suggested to be 8-12 h or

shorter. However, further study is required to determine the appropriate HRT for optimal performance regarding SCOD removal and protein formation. The stability of continuous flow operation and performance of scale-up system also require further investigations.

The main problem encountered in continuous flow operation is that the slurry phase PPW substrates unpredictably clog the pipeline that shut down the continuous flow operation. To solve the problem, the pipeline may be changed to larger size to provide stable continuous flow operation. Other problems are that the biuret protein content in continuous flow products is less than that in batch products and the pH decreases below 3 during reaction. The nutrient analysis (presented in Section 4.5) shows that the protein content is more 40% in the continuous-flow PPW products. This shows the potential to refine the continuous-flow operation in the problems in pipeline clogging, pH decrease, oxygen transfer and HRTs for optimal production. A pH and DO monitoring/control system may be applied for the operation.

The performance of protein content in continuous flow operation with HRT ranged from 6-12hr seems lower than that in batch operation in this preliminary study. The continuous culture of Baker's yeast is found considerably better than batch culture to provide higher performance in effluent quality (concentrations of dissolved nitrogen, phosphate, and COD) and biomass cultivation (cell concentrations, specific growth rates, productivities, and yield coefficients) using wastes of potato and wheat starch as substrate (Lotz et al. 1991). Further investigations of continuous flow operation needs to be carried out for the

process improvements of optimal yeast growth, stability of long-term operation and mass production.

Table 11 Preliminary performance of continuous flow operation for yeast growth

HRT	4.33	6.25	8	9.07	10	10.8	12
Influent (mg/l)							
PH	6.56	6.5	6.43	6.49	6.5	6.45	6.42
ORP	20	23.3	26.6	23.6	26	25.6	28.1
SS	7520	7670	7760	7080	7520	7090	7700
SCOD	15550	16000	17240	15700	16980	15240	17240
TCOD	20560	23020	21520	20800	20500	19640	20260
SCOD/TCOD	0.76	0.70	0.80	0.75	0.83	0.78	0.85
SCOD/SS	2.07	2.09	2.22	2.22	2.26	2.15	2.24
STN	592	556	432	580	552	576	536
P	442	434	412	460	297	456	437
Effluent							
PH	3.89	2.58	2.54	2.73	2.24	2.53	2.27
ORP	168	242	244	228	260	241	258
SS	3900	4340	6880	6320	8320	8460	6180
SCOD	9650	5690	5340	4600	4300	3630	5340
removal %	38%	64%	69%	71%	75%	76%	69%
STN	356	248	164	220	161	216	202
removal %	40%	55%	62%	62%	67%	63%	62%
P	265	193	135	185	197	208	183
removal %	37%	56%	67%	60%	63%	54%	58%
Product protein % in SS							
Biuret protein	19%	20%	18%	18%	25%	20%	16%
6.25ΔSTN/ΔSS	38%	44%	24%	36%	28%	27%	34%

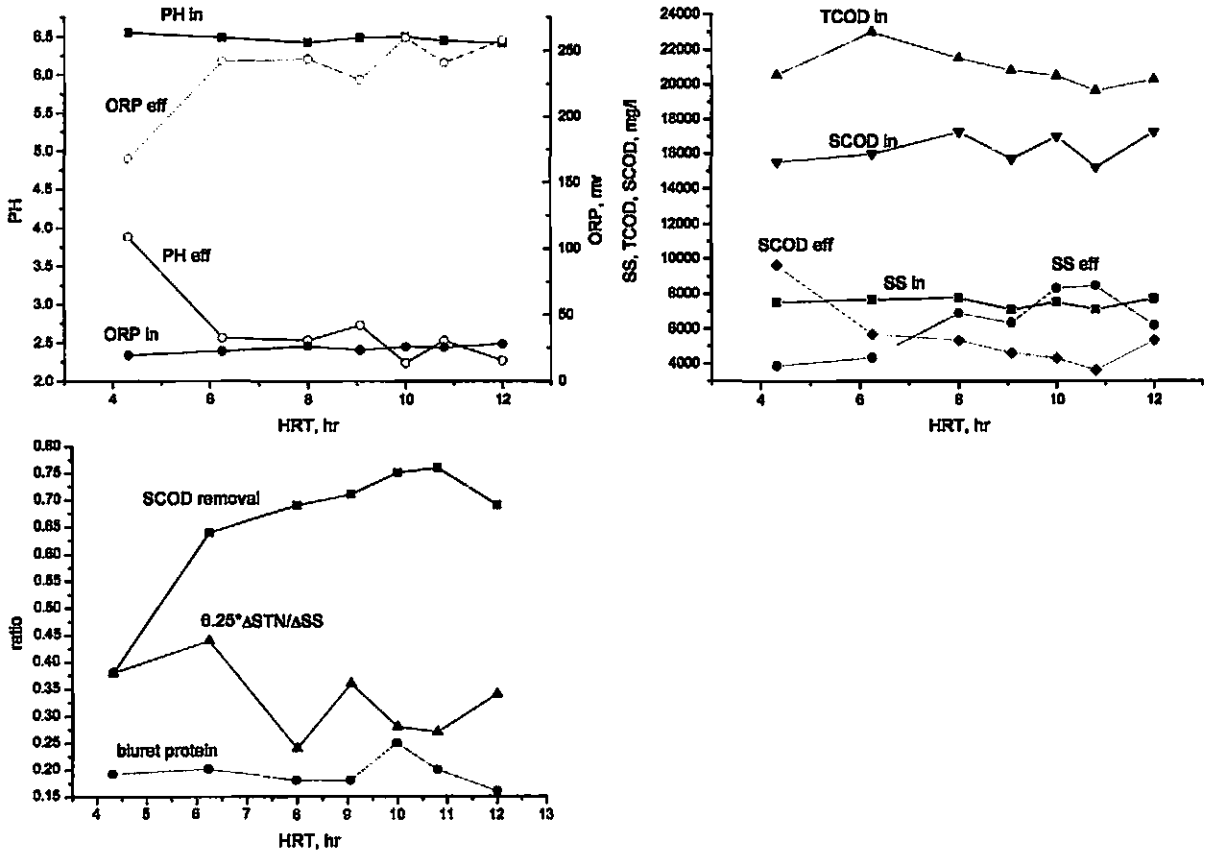


Figure 12 Preliminary performance of continuous flow operation for yeast growth

4.4.2 Preliminary performance of semi-continuous flow operation

Blended PPW slurries were used as substrate to investigate the performance of yeast growth in semi-continuous flow operation by intermittent pumping influent substrate and effluent products. As shown in Table 12 and Figure 13, influent substrate concentrations were ranged from 12,400 to 16,200 mg/l SCOD with several different HRT ranged from 2.74 to 8.2 h. At steady state, the effluent PH ranged from 2.47 to 4.58, ORP ranged from 132 to 247mv, the SCOD removal ranged from 26%-75%, and biuret protein ranged from 9-28%. The reaction with 4-8h HRT provides higher protein content (26-28%) and SCOD removal (60-63%).

The overall performance regarding SCOD removal and protein content of continuous flow operation seems lower than that in batch operation in this study. The cultivation of Baker's yeast from starch wastes indicate that higher performance can be attained in continuous cultures than in batch cultures (Lotz et al. 1991). The fed-batch process has found to provide higher protein yield than the batch process for yeast cultivation from potato starch waste. It was possible to increase the performance of yeast protein production through fed-batch process, yielding 19.4% of protein compared to 14.9% in batch process (Rusendi and Sheppard 1995).

Table 12 Performance of semi-continuous operation for yeast growth

HRT	2.74	2.96	3.52	4.11	6.17	8.2
Influent (mg/l)						
PH	6.34	6.31	6.3	6.35	6.31	6.46
ORP	32.9	34	37.5	22.4	34	25.6
SS	7240	9440	9380	7480	9440	9760
SCOD	12400	15820	14780	14790	15700	16160
TCOD	18960	18720	18700	19020	18740	21940
SCOD/TCOD	0.65	0.85	0.79	0.78	0.84	0.74
SCOD/SS	1.71	1.68	1.58	1.98	1.67	1.66
STN	664	584	548	560	584	608
P	573	475	462	450	275	467
effluent						
PH	4.58	4.1	3.95	2.82	2.58	2.47
ORP	132	157.8	166.6	231	223	247
SS	7840	7720	9960	8040	8620	8240
SCOD	9160	9760	3680	5950	5840	6290
removal %	26%	38%	75%	60%	63%	61%
STN	460	368	316	332	314	248
removal %	31%	37%	42%	41%	46%	59%
P	529	282	251	200	286	259
Removal %	8%	41%	46%	56%	40%	45%
end product protein % in SS						
biuret protein	9%	17%	15%	27%	26%	28%
6.25ΔSTN/ΔSS	16%	17%	15%	18%	17%	27%

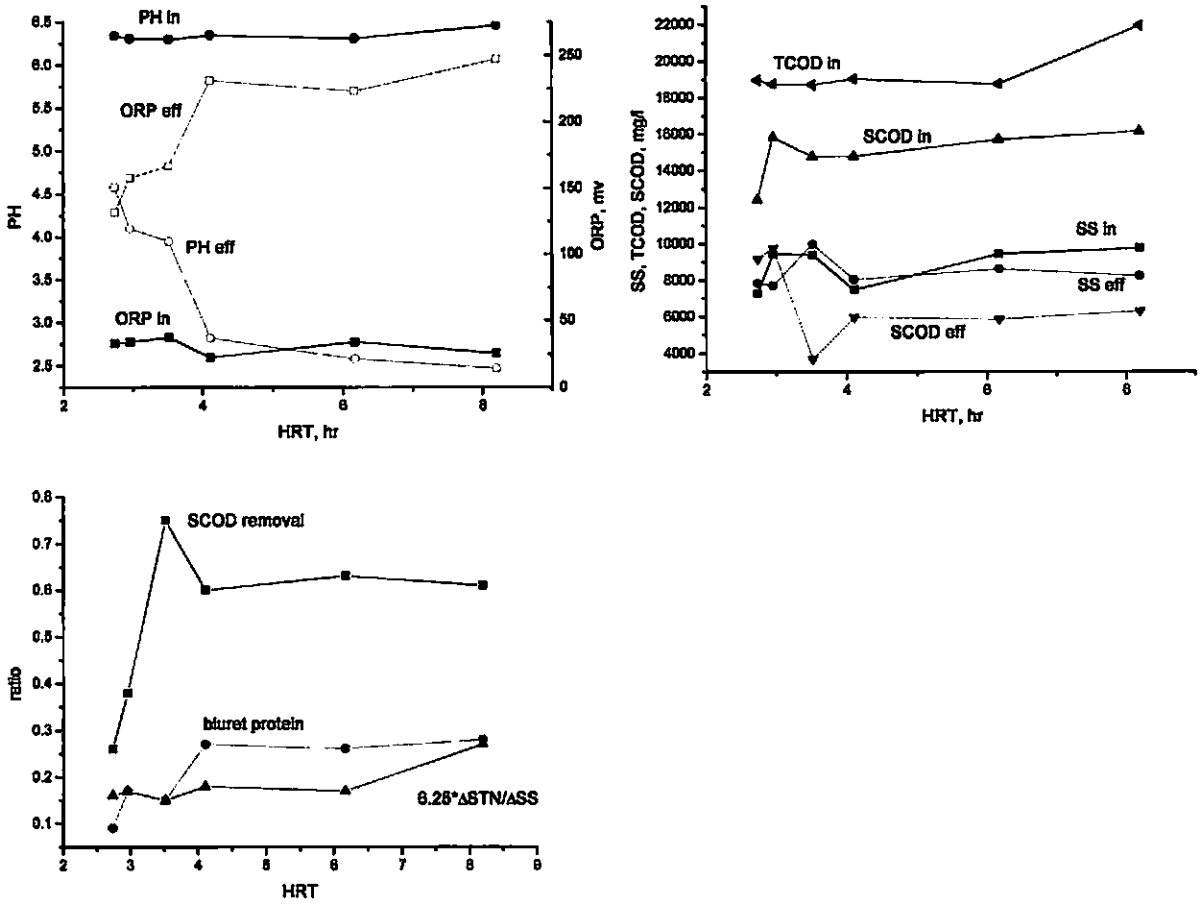


Figure 13 Preliminary performance of semi-continuous flow operation for yeast growth

4.4.3 Preliminary performance of pH control during continuous flow operation

In order to control the pH decrease during continuous and semi-continuous flow operation, sodium bicarbonate ($NaHCO_3$) is added into the fermentor intermittently when pH drops below 3. A preliminary result of 12 h HRT continuous flow operation with pH control was compared to that without pH control and the results of batch operation. As shown in Table 13, the continuous flow operation with pH controlled around 4 provides the highest SCOD removal (92%) and highest biuret protein (42%) compared to the operations without pH control when 12 HRT is applied. The result shows that pH control is necessary for yeast growth in optimal condition. The long-term operation of continuous and/or semi-continuous flow with pH control is therefore needed further investigations for process refinement.

Table 13 Preliminary performance of 12 h HRT continuous flow operation with pH control

	Continuous flow operation[a]		Batch operation[b]
	No pH control	With pH control	No pH control
pH	2.27	3.97	3.50
ORP	258	259	197.7
SS	6180	11580	13130
SCOD	5340	1360	4650
SCOD removal %	69%	92%	75%
Biuret protein %	16%	42%	32%

[a] 12 h HRT continuous flow operation with influent SCOD ranged from 16000-18000 mg/l
[b] 12 h batch operation with initial SCOD 18480 mg/l

The control of pH, oxygen supply and sugar concentration during yeast fermentation is necessary to obtain high biomass yield. The optimum PH for Baker's yeast growth is between 4.5 to 5 (Reed and Nagodawithana 1991). The control of pH is needed to increase protein formation. In continuous fermentation, the oxygen supply with respect to dilution rate also affects the biomass yield. As dilution rate increases, the ethanol production increases which indicates the insufficient oxygen supply under higher dilution rate conditions for the cultivation of *C. utilis* (Lawford et al. 1979; Lee and Kim 2001). In aerobic Beker's yeast fermentation, high biomass yields are obtained under lower sugar concentration, less than 2%, while under higher sugar concentration, sugar is metabolized into ethanol and CO_2 that decreases the biomass yield, which is known as Crabtree effect (Reed and Nagodawithana 1991). Fed-batch operation is applied to controll the sugar concentration by incremental substrate feeding to achieve the maximum cell growth rate (Aiba et al. 1976). The fed-batch cultivation of *S. cerevisiae* from waste cassava starch is suggested a suitable low cost replacement for glucose in the production of baking quality yeast with the fermentation process controlled under a specific growth rate of 0.18-0.23 h^{-1}, a biomass yield coefficient of 0.5 g/g, and a feed substrate concentration of 200 g/l (Ejiofor et al. 1996).

Further study is therefore required for process refinement in continuous/semi-continuous flow operation to solve the problems in pipeline clogging, pH decrease, and appropriate dilution rate for stable performance and higher biomass yield. A pH and DO monitoring/control system may be applied for the control of operation. Fed-batch process may also be applied in the PPW bioprocess to achieve a higher biomass yield.

4. 5 Feasibility of bioprocessed product as feed ingredients

4.5.1 Preliminary feed analyses of bioprocessed PPW product

The bioprocessed product from blended PPW slurry with initial SCOD 15,000 mg/l was oven-dried for feed analysis. The protein content is 4.31% and 45% in mixed liquor and the dried suspended solids, respectively (Table 14). The protein content is 4.31% and 45% in the mixed liquor and the filtered suspended solids, respectively. The bioprocessed protein content is slightly less than that in the Baker's yeast *S. cerevisiae* which contains about 50-52% of crude protein (Reed and Nagodawithana 1991).

The bioprocessed yeast product from leaf juices by *S. cerevisiae* contains 10% moisture, 45.6% of crude protein, 8% lipids and 10.3% ash on dry matter (Chanda and Chakrabarti 1996). The yeast powder product processed from solid state fermentation of apple pomance by *Saccharomyces* contains 4.48 % moisture, 16.80% crude protein, 8% crude fat, and 18.30% crude fiber (Joshi and Sandhu 1996). The product processed from fruit and vegetable processing waste by *S. cerevesiae* CEE12 contains 37.5-48.7% of protein in total solids (Stabnikova et al. 2005). The product from Chinese cabbage juice by *S. cerevisiae* contains 35% protein (Choi et al. 2002). The product from virgin grape marc by *S. cerevisiae* contains 9-12% moisture, 50-54% crude protein, 5-8% crude fiber, and 4-6% ash on dry matter (Lo Curto and Tripodo 2001). The yeast product processed from spent fruit flies medium contains 21-35.6% protein in mixed liquor suspended solids (Yang and Lin 1981). When using as feed for Malaysian prawns, the result shows that the

mixed feed of processed yeast product with regular feed provides a higher survival rate and a more homogeneous growth in body weight compared to regular feed (Yang and Lin 1981). The yeast *S. cerevisiae* is commonly used as nutritional supplement as animal feed (Demirci et al. 1999). The addition of *S. cerevisiae* to common fish diet can activate the innate immune system (Ortuno et al. 2002; Siwicki et al. 1994). Compared to those results, it is therefore, that the yeast product from papaya processing waste shows a high potential as feed supplement.

4.5.2 Preliminary nutrient profile of frozen bioprocessed PPW product

The frozen samples of centrifuged bioprocessed PPW products were stored in a -80°C freezer and then freeze-dried for nutrient analysis. About 20% of product by weight was remained after freeze-dry. The nutrient profile of freeze-dried bioprocessed products from batch, continuous flow, and scaled-up batch operation is analyzed by Oceanic Institute (OI) as shown in Table 15. The batch product is from the process with initial SCOD of 12000-15000mg/l for10-12 hr of reaction. The continuous flow product is from the preliminary process of influent SCOD ranged from 12000-17000 mg/l and a HRT of 6-12 hr. The scaled-up batch product is from initial SCOD ranged from 12000-20000 mg/l for 24-48 hr of reaction at 100L and 150L working volume. The result of mytoxin screening for the bioprocessed PPW products shows no mycotoxin in the sample (Table 16).

Table 14 Feed analyses of bioprocessed PPW products

	Oven dried sample*	
Poduct	Mixed liquor	Filtered solid
DM %: Dry Matter	2.05	82.22
Ash %	0.3	0.87
CP %: crude protein	4.3125	45.62
EE %: crude fat	0.027	0.054
NDF %: neutral detergent fiber	0.45	9.14
ADF %: acid detergent fiber	0.21	6.78
PMF %: lignin	0.05	2.67
Cellulose %:	0.11	4.01
Minerals		
%P	0.03	0.191
%K	0.094	0.218
%Ca	0.006	0.058
%Mg	0.007	0.034
%Na	0.013	0.015
B ppm	0.03	1.02
Cu ppm	0.47	3.29
Fe ppm	2.96	14.31
Mn ppm	0.12	0.87
Mo ppm	0.02	0.06
Zn ppm	1.89	19.06

*Smple of batch product of PPW with initial SCOD 15000mg/l after 12hr of reaction, analyzed by Agricultural Diagnostic Service Center (ADSC), University of Hawaii at Manoa

Table 15 Nutrient profile of freeze-fried bioprocessed PPW product

Product	Batch[a]	Continuous flow[b]	Scaled-up batch[c]
DM %: Dry Matter	89.22	97.33	98.36
Ash %	12	5.89	6.29
CP %: crude protein	40.06	43.67	49.82
EE %: crude fat	0.77	1.02	1.61
NDF %: neutral detergent fiber	22.41	44.34	21.89
ADF %: acid detergent fiber	13.84	32.40	32.72
PMF %: lignin	5.85	27.99	30.58
Cellulose %:	7.74	4.36	2.13
Minerals			
%P	1.32	1.36	1.38
%K	4.86	1.69	2.02
%Ca	0.29	0.23	0.11
%Mg	0.33	0.22	0.2
%Na	0.34	0.04	0.02
B ppm	18	9	4
Cu ppm	25	28	16
Fe ppm	92	85	95
Mn ppm	10	8	10
Mo ppm	0	0	0
Zn ppm	78	194	372
Essential Amino Acids (g/100g)			
Arginine	1.54	2.41	2.31
Histidine	0.66	0.98	1.00
Isoleusine	1.8	2.82	2.86
Leusine	2.05	3.43	2.98
Lysine	2.44	3.76	3.20
Methionine	0.45	0.63	0.53
Phenylalanine	1.47	2.16	1.82
Threonine	1.92	2.49	2.33
Tryptophan	NA*	NA	NA
Valine	1.78	2.97	2.93

Non Essential AA (g/100g)			
Alanine	1.57	2.44	2.28
Aspartic + Asparagine	1.79	3.13	2.59
Cystine	0.37	0.35	0.18
Glutamine + Glutamine	2.35	4.12	4.54
Glycine	1.27	1.85	1.64
Proline	1.87	1.51	1.54
Serine	1.41	2.14	1.83
Tyrosine	1.33	1.84	1.69
Essential AA Subtotal	14.11	21.65	19.96
Non Essential AA Subtotal	11.96	17.38	16.28
Total AA	26.07	39.03	36.25
EE% OI analysis	0.55	0.96	2.07
Essential Fatty Acids (% of total FA)			
Linoleic (18:2w6)	9.85	5.59	16.36
Linolenic (18:3w3)	6.92	14.00	4.56
Arachidonic (20:5w6)	N.D.**	N.D.	N.D.
EPA (20:5w3)	N.D.	N.D.	N.D.
DHA (22:6w3)	N.D.	N.D.	N.D.
Non Essential Fatty Acids	83.23	80.41	79.08
Cholesterol	N.D.	N.D.	N.D.

[a]Batch product of PPW with initial SCOD 12000-15000mg/l, 10-12hr of reaction, analyzed by OI
[b]Continuous flow product of PPW with influent substrate SCOD ranged from 12000-17000 mg/l with 6-12 hr of HRT, analyzed by OI
[c]Sacled-up batch product of PPW with initial SCOD ranged from 12000-20000 mg/l, 24-48hr of reaction at 100 and 150 L working volume, analyzed by OI
* N.A. = Not Available
** N.D. = Not Dectable

Table 16 Mytoxin screening for the dried ground bioprocessed PPW slurry

Toxin*	Detection limit		
Aflatoxin B1	1.0 ppb	HPLC	ND
Aflatoxin B2	1.0 ppb	HPLC	ND
Aflatoxin G1	1.0 ppb	HPLC	ND
Aflatoxin G2	1.0 ppb	HPLC	ND
Ochratoxin A	2 ppb	HPLC	ND [2]
T-2 Toxin	0.1 ppm	TLC	ND
HT-2 Toxin	0.1 ppm	TLC	ND
Diacetoxyscirpenol	0.3 ppm	TLC	ND
Neosolaniol	0.1 ppm	TLC	ND
Fusarenon X	0.5 ppm	TLC	ND
Deoxynivalenol	0.1 ppm	TLC	ND
15 Acetyl-DON	0.1 ppm	TLC	ND
3 Acetyl-DON	0.1 ppm	TLC	ND
Nivalenol	0.5 ppm	TLC	ND
Zearalenone	100 ppb	HPLC	ND
Fumonisin B1	0.2 ppm	HPLC	ND
Fumonisin B2	0.2 ppm	HPLC	ND
Fumonisin B3	0.2 ppm	HPLC	[1]
Citrinin	267 ppb	TLC	ND

*Sacled-up batch product of PPW with initial SCOD ranged from 12000-20000 mg/l, 24-48hr of reaction at 100 and 150 L working volume, analyzed at Romer labs, Union, MO.
[1] Immunoaffinity column used, can not detect Fumonisin B3.
[2] Detection limit raised to 5 ppb. Matrix spike recovery of 60.4%. This does not meet Romer quality criteria.

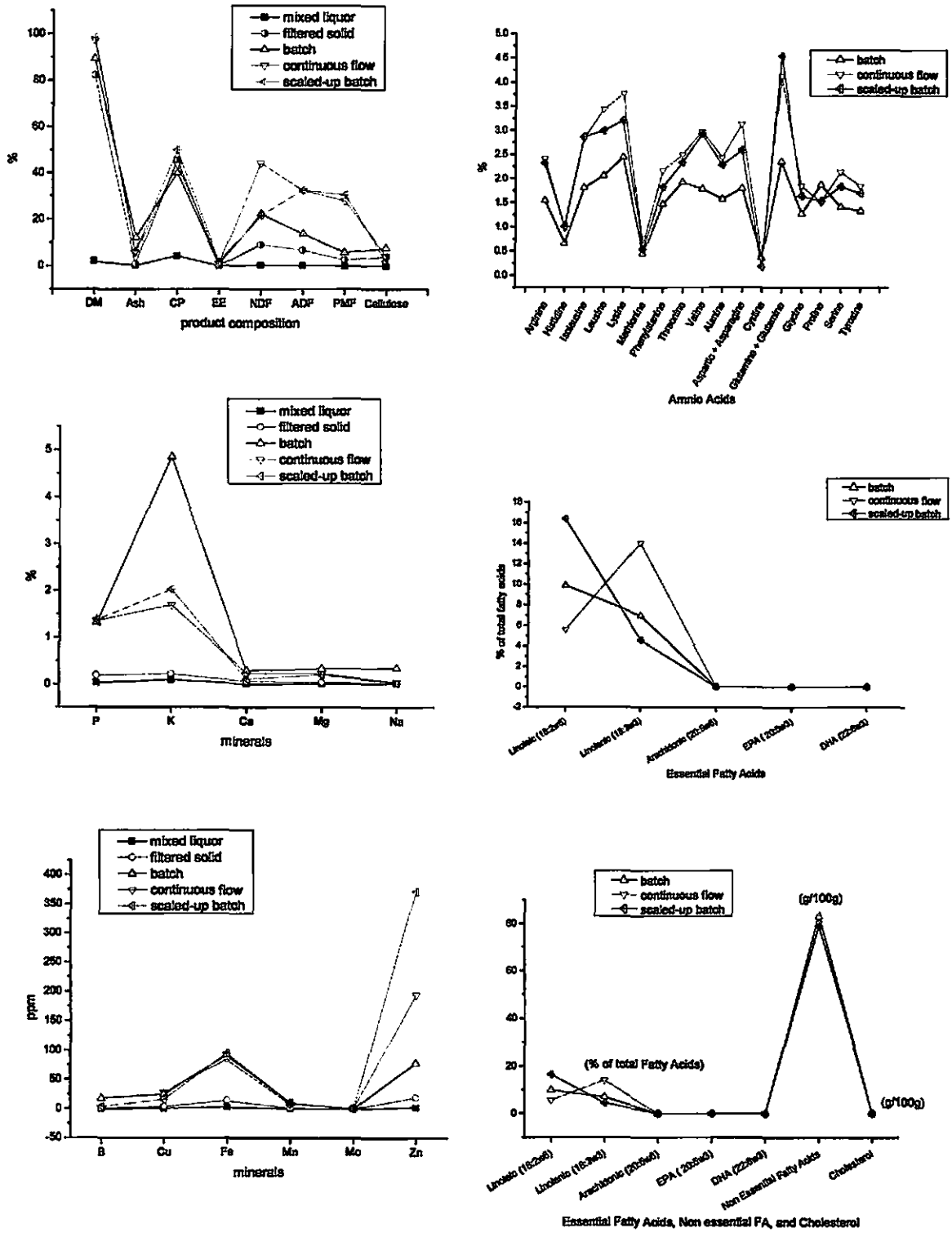


Figure 14 Nutrient profile of the bioprocessed products

The comparison of the bioprocessed PPW products from different operation process of the oven-dried samples of batch operation and freeze-dried samples of batch, continuous flow and scaled-up batch operation is shown in Figure 14. The level of nutrient concentration depends on the production process and the moisture content in the products. The moisture content in the mixed liquor and filtered samples are 98% and 18%, respectively. Therefore, the levels of nutrient concentration in the mixed liquor sample are much less than those in the filtered sample. The freeze-dried samples contain slightly less moisture than the oven-dried samples and therefore, the nutrient concentration levels are slightly higher than those in the oven-dried samples.

As shown in Figure 14, the freeze-dried samples from different operation process (i.e., batch, continuous flow and scaled-up batch) show similar nutrient composition except in a few nutrients such as the concentrations of NDF, ADF and PMF in product composition, and the concentrations of K and Zn in minerals composition. The amino acids profile of batch product is less than that in the continuous-flow and scaled-up batch operation. This may be due to different production process. The preliminary product of continuous-flow operation provides similar nutrient profile as the products from batch and scaled-up batch operation. Generally, the products contain 40-50% of crude protein for batch, scaled-up batch and continuous operation. The protein content is only slightly high or low to the nutrient of *S. cerevisiae* which contains about 50-52% of crude protein (Reed and Nagodawithana 1991). The results show that the bioprocessed product from papaya processing waste has the potential for feed supplement.

4. 6 Results of shrimp feeding trial

4.6.1 Formulation of PPW diets

The PPW diets for shrimp feeding trial were formulated according to Akiyama et al. (1992). Three diets with 0%, 50% and 100% PPW replacement were formulated to target 35% crude protein (CP) as shown in Table 17. The composition of formulated ingredients is shown in Table 18. The chemical composition of these three diets and commercial shrimp feed 35/2.5 (purchased from Land O'Lake, Kapolei, HI) are shown in Table 19. The crude protein content is 37.45%, 36.50% and 35.14% for 0%, 50% and 100% PPW control diet, respectively. The amnio acid profile and fatty acids profile are shown in Table 20 and 21, respectively.

Table 17 Formulation of PPW diets

Product*	Target		PPW 0%	PPW 50%	PPW 100%
Crude protein		35	35.20	35.02	34.81
Ether Extract		8	7.44	7.37	7.40
Calcium		>2.4	1.67	1.56	1.41
Growth Phosphorus		0	2.24	2.26	2.33
Potassium		1	1.92	1.66	1.51
AA	% CP	AA/protein			
Arginine	5.8	2.03	2.27	1.94	1.74
Methionine	2.4	0.84	78	59	42
Cystine	1.2	0.42	49	35	20
Met + Cys	3.6	1.26	1.27	94	62
Lysine	5.3	1.86	2.33	2.23	2.32
Threonine	3.6	1.26	1.61	1.58	1.69

*Formulated according to Akiyama et al. (1992) by Oceanic Institute (OI, Waimanalo, HI).

Table 18 CP 35% formulation of PPW diets

35% CP (crude protein) formulation* (wt%)	PPW 0%	PPW 50%	PPW 100%
PPW	0	30.50	68.00
Menhaden fish meal (select)	24.00	12.00	0
Squid meal	2.50	1.25	0
Poultry by-product meal	10.00	5.00	0
Soybean meal	12.00	6.00	0
Menhaden fish solubles	5.00	5.00	5.00
Whole Hard Red Winter wheat	35.55	29.30	16.05
Menhaden fish oil	1.10	2.30	3.60
Soy Lecithin	2.00	2.00	2.00
Trace min mix	0.50	0.50	0.50
Vitamin premix	0.35	0.35	0.35
Potassium phosphate monobasic	3.00	2.00	0.00
Diatomaceous earth	4.00	1.50	0.00
Calcium phosphate dibasic	0.00	2.30	4.50
Total	100.00	100.00	100.00

*Formulated according to Akiyama et al. (1992) by Oceanic Institute (OI, Waimanalo, HI).

Table 19 Chemical composition of the formulated diets and commercial shrimp feed

Product*	PPW 0%	PPW 50%	PPW 100%	Commercial feed 35/2.5
DM %: Dry Matter	94.31	93.63	93.14	91.61
Ash %	15.21	12.20	8.70	9.91
CP %: crude protein	37.48	36.50	35.14	40.90
EE %: crude fat	8.03	7.80	7.87	9.12
NDF %: neutral detergent fiber	48.42	45.26	23.59	49.48
ADF %: acid detergent fiber	12.93	21.00	14.44	13.29
PMF %: lignin	8.01	16.22	12.40	9.23
Cellulose %:	2.56	3.60	2.01	3.71
Minerals				
%P	2.13	2.25	2.01	1.57
%K	1.58	1.75	1.50	0.82
%Ca	1.80	1.82	1.29	2.45
%Mg	0.19	0.19	0.18	0.25
%Na	0.26	0.27	0.11	0.60
B ppm	n.d.	n.d.	n.d.	n.d.
Cu ppm	6	6	4	12
Fe ppm	181	494	292	36
Mn ppm	235	276	414	121
Mo ppm	298	276	119	121
Zn ppm	n.d.	n.d.	n.d.	n.d.

analyzed at Agricultural Diagnostic Service Center (ADSC), University of Hawaii at Manoa

Table 20 Amnio acid report of the formulated diets and commercial shrimp feed

Amino Acid* (unit: g/100g)	0% PPW	50% PPW	100% PPW
Non-essential AA			
Ala	1.96	2.13	2.10
Asp+ASN	2.68	1.98	1.86
Cys	0.00	0.00	0.00
Glu+Gln	4.66	4.37	4.03
Gly	2.58	2.23	1.82
Pro	2.68	2.72	2.72
Ser	1.35	1.34	1.44
Tyr	1.28	1.58	1.53
Taurine	0.60	0.38	0.12
Essential AA			
Arg	3.72	3.82	3.55
His	1.17	1.18	1.08
Ile	1.77	1.80	1.90
Leu	2.78	2.84	2.81
Lys	2.78	2.54	2.53
Met	0.83	0.72	0.58
Phe	1.74	1.73	1.78
Thr	1.45	1.45	1.23
Trp	0.00	0.00	0.00
Val	2.04	2.16	2.29
Subtotal of Non Essential AA	17.19	16.35	15.49
Subtotal of Essential AA	18.29	18.24	17.75
Total	35.48	34.59	33.24

* Analyzed at Oceanic Institute (OI), Waimanalo, HI

Table 21 Fatty acid profile of the formulated diets and commercial shrimp feed

		% of Total Fatty Acids				% of Sample			
		PPW 0%	PPW 50%	PPW 100%	Commercial 35/2.5	PPW 0%	PPW 50%	PPW 100%	Commercial 35/2.5
	%EE	7.48	7.63	8.35	9.00				
		%	%	%	%	%	%	%	%
Dodecanoic (Lauric)	C12:0	0.10	0.16	0.16	0.10	0.001	0.003	0.003	0.002
Tetradecanoic (Myristic)	C14:0	6.74	7.70	7.48	4.58	0.096	0.154	0.163	0.072
Pentadecanoate	C15:0	0.57	0.63	0.62	0.34	0.008	0.013	0.014	0.005
Hexadecanoic (Palmitic)	C16:0	17.65	16.40	14.31	17.14	0.250	0.328	0.311	0.271
Heptadecanoate	C17:0	0.23	0.25	0.25	0.17	0.003	0.005	0.005	0.003
Octadecanoic (Stearic)	C18:0	3.95	3.50	3.23	4.24	0.056	0.070	0.070	0.067
Ecosanoic (Arachidic)	C20:0	0.79	0.64	0.62	1.36	0.011	0.013	0.013	0.021
Hexadecenoic (Palmitoleic)	C16:1n-7	6.45	10.27	13.23	5.31	0.091	0.205	0.288	0.084
Octadecenoic (Oleic)	C18:1n-9	14.33	14.49	15.98	21.13	0.203	0.290	0.348	0.334
Octadecenoic (Oleic)*	C18:1n-7	2.34	2.16	2.14	2.74	0.033	0.043	0.047	0.043
Ecosenoic (Gadoleic)	C20:1n-9	0.00	0.00	0.00	0.25	0.000	0.000	0.000	0.004
Hexadecadienoic*	C16:2n-4	0.53	0.60	0.61	0.35	0.008	0.012	0.013	0.005
Octadecadienoic (Linoleic)	C18:2n-6	19.68	16.39	13.15	17.74	0.279	0.328	0.286	0.280
Octadecatrienoic (Linolenic)	C18:3n-3	2.45	2.76	2.92	2.18	0.035	0.055	0.063	0.034
Hexadecatrienoic*	C16:3n-4	0.61	0.63	0.58	0.40	0.009	0.012	0.013	0.006
Octadecatrienoic*	C18:3n-4	0.17	0.17	0.16	0.11	0.002	0.003	0.004	0.002
Octadecatetraenoic (Parinaric)*	C18:4n-3	1.39	1.67	1.89	0.78	0.020	0.033	0.041	0.012
Ecosatetraenoic	C20:4n-3	0.82	0.94	1.10	0.53	0.012	0.019	0.024	0.008
Ecosatetraenoic (Arachidonic)	C20:4n-6	1.01	0.81	0.59	0.84	0.014	0.016	0.013	0.013
Eicosapentanoic (EPA)	C20:5n-3	9.22	9.12	9.63	9.60	0.131	0.182	0.209	0.152
Decosapentanoic*	C22:5n-3	2.00	1.90	1.94	2.52	0.028	0.038	0.042	0.040
Docosahexanoic (DHA)	C22:6n-3	8.98	8.79	9.41	7.59	0.127	0.176	0.205	0.120
unidentified peaks		3.66	2.04	1.82	3.91				

* Fatty acids are quantified using individual quantitative standards from Sigma, with the exception of those marked with an asterisk *, which are estimated from known similar peaks. Analyzed by Oceanic Institute (OI), Waimanalo, HI

4.6.2 Results of shrimp feeding trial

The results of eight week shrimp feeding trial on PPW were compared with commercial 35/2.5 feed. As shown in Table 22 and Figure 15, the 50% PPW diet shows similar cumulative mean growth as commercial 35/2.5 feed and similar cumulative FCR as controlled 0% PPW diet. The survival rate is 100% for PPW 50% diet during the 8-week trial. Based on the severe growth condition in controlled indoor laboratory environment, the 50% PPW diets indicates high potential for shrimp feed supplement. Generally, the growth per week is able to be doubled when out-door growth environment is applied. The eight week feeding study showed that shrimp could grow on 100% PPW diet and 50% PPW or under diets had high potential to be a functional shrimp feed.

Table 22 Results of 8-week shrimp feeding trial on formulated PPW diets

Average weight of shrimp, g/shrimp								
week	PPW 0%	Std	PPW 50%	std	PPW 100%	std	Commercial 35/2.5	Std
0	1.67	0.05	1.64	0.14	1.72	0.07	1.71	0.03
2	3.08	0.06	2.72	0.26	2.45	0.24	3.13	0.22
4	4.78	0.23	4.03	0.34	3.07	0.26	4.36	0.35
6	6.39	0.68	5.21	0.4	3.62	0.43	5.57	0.82
8	8.11	1.46	6.61	0.56	4.15	0.42	6.94	1.45

Total feed amount per 2 week interval, g feed/2 week								
week	PPW 0%	Std	PPW 50%	std	PPW 100%	std	Commercial 35/2.5	Std
2	34.75	1.15	34.38	1.28	35.13	1.29	29.23	3.2
4	43.92	11.02	39.9	2.27	43.49	2.83	23.32	6.13
6	49.13	28.53	41.56	3.61	45.02	10.48	21.63	12.39
8	49.86	45.62	47.08	15.16	36.56	20.11	21.63	14.21
Cumulative fed	177.65	85.22	162.92	18.14	160.20	31.99	95.81	35.78

Growth per week, g shrimp/week								
week	PPW 0%	Std	PPW 50%	std	PPW 100%	std	Commercial 35/2.5	Std
2	0.7	0.05	0.54	0.08	0.37	0.08	0.71	0.1
4	0.85	0.09	0.66	0.05	0.31	0.07	0.62	0.09
6	0.80	0.28	0.59	0.07	0.27	0.09	0.60	0.26
8	0.86	0.45	0.70	0.17	0.27	0.06	0.78	0.46
Cumulative mean growth	6.44	1.41	4.97	0.48	2.43	0.35	5.24	1.43

Feed conversion ratio (FCR), g shrimp/g feed								
week	PPW 0%	std	PPW 50%	std	PPW 100%	std	Commercial 35/2.5	std
2	2.07	0.17	2.69	0.32	4.15	0.95	1.72	0.1
4	2.32	0.75	2.54	0.14	6.09	1.64	1.57	0.28
6	2.51	0.64	2.97	0.40	7.04	1.17	1.86	0.34
8	2.75	1.67	2.77	0.51	9.50	5.31	1.19	0.15
Cumulative FCR	2.39	0.86	2.73	0.11	6.05	1.29	1.56	0.11

Survival rate								
week	PPW 0%	std	PPW 50%	std	PPW 100%	std	Commercial 35/2.5	std
2	100%	0.00%	100%	0.00%	100%	0.00%	100%	0.00%
4	97.92%	4.17%	100%	0.00%	100%	0.00%	100%	0.00%
6	97.92%	4.17%	100%	0.00%	100%	0.00%	94.44%	4.81%
8	93.75%	7.89%	100%	0.00%	93.75%	4.17%	97.22%	4.81%

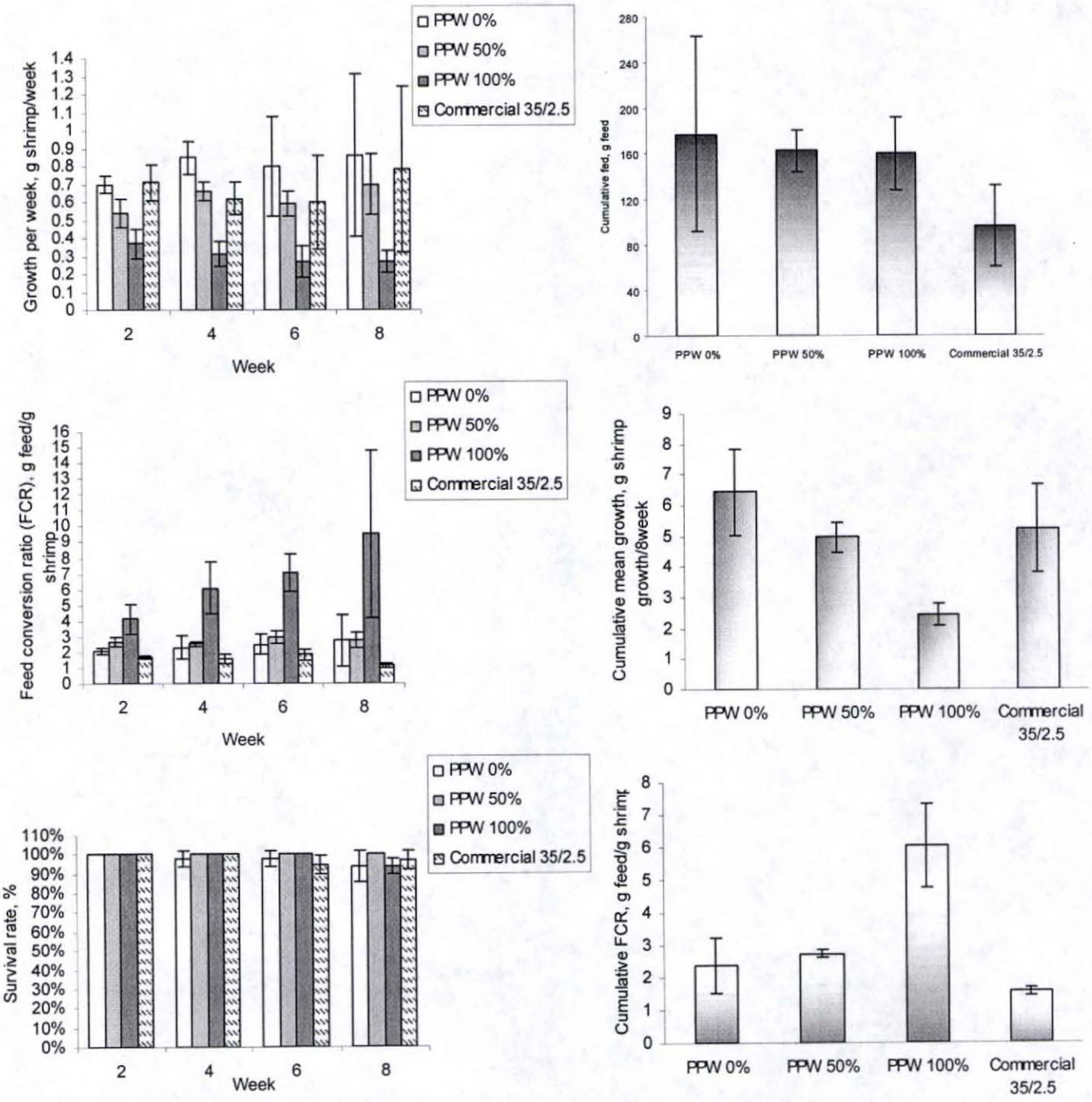


Figure 15 Results of 8-week shrimp feeding trial on formulated PPW diets

The yeast processed from spent fruit flies medium (SFFM) mixed with commercial feed shows a higher survival rate and more homogeneous body weight growth in Malaysian prawns compared to commercial feed (Yang and Lin 1981). The SFFM contains various different nutrients from PPW. When fed with shrimp, the product processed from SFFM and PPW may have different results, which requires further analyses after the 8-week shrimp feeding trial is complete.

The yeast cell wall reduces digestibility and represents up to 50% of cell weight with low protein. After removal of cell wall produces the yeast extracts *(S. cerevisiae)* NuPro®, a functional food produced by Alltech Inc. (Bangkok, Thailand). NuPro® is highly digestible and contains 47-50% crude protein and 5% nucleotide content that improves immunity and gut health. The shrimp fed with NuPro® for 15 weeks had higher body weight and better immune system response such as significantly higher blood cell counts, more granular hemocytes, and cleared bacteria from the blood faster and longer than the control group (Sritunyalucksana et al. 2005). The extraction of PPW yeast by removal of cell wall to produce higher digestible and consistent protein source may be applied for further study in aquafeed.

4.7 Economic Evaluation

4.7.1 Economic analysis for batch bioprocess system

Operational assumptions

According to Super Food Inc. (Honolulu, HI), the amount of average organic waste generated per day was estimated to be 400-500 lb or 181-226 kg with seasonal fluctuation. Based on the operational condition in batch fermentation, the PPW substrate was suggested to be ranged from 12,000-25,000mg/l of SCOD supplied with nitrogen and phosphate, aerated for 8h under room temperature (22±2°C). The estimated SS yield is 0.3 gSS/gPPW, and the estimated protein yield is 0.15 g protein/gPPW. The estimated costs of material, capital, operation & maintenance were based on bioprocessing 100kg PPW per day.

Material cost

The required nutrients, ammonium sulfate and potassium phosphate, were added at the ratio of SCOD: N: P at 100:5:1. Raw papaya processing waste was estimated to provide 100,000 mg/l SCOD/kg PPW. The operational substrate SCOD was estimated to be 20,000 mg/l. For every kg of PPW, the operational volume after shredding, blending and dilution to meet substrate SCOD requirement was 5L. For every 100 kg of PPW, the estimated cost for nutrients was $2.4576/100kg PPW as listed in Table 23 (based on the price of chemicals for whole sale provided by Brewer Environmental Industries (BEI, Honolulu, HI).

Table 23 Estimation of material cost for bioprocessing 100 kg of PPW

Material	amount	unit price	cost
PPW	1000 kg	$0	$0.00
Ammonium Sulfate*	23.5 kg	$0.5057/kg	$11.8847
Potassium Phosphate*	5.6 kg	$2.2663/kg	$12.6914
Total material cost for bioprocessing 1000 kg of PPW			$24.5761
Total material cost for bioprocessing 100 kg of PPW			$2.4576

*Based on whole sale price provided by Brewer Environmental Industries (BEI), Honolulu, Hi. USA.

Capital cost

Capital cost including juice press, grinder for fruit shredding and filter press for dewatering, fermenter, storage tank, air providing system, associated pumping, electrical and infrastructural system was estimated based on the pilot plant scale for wastewater treatment (Kongsil, 2006). The total estimated capital cost was $40,366 (Table 24).

Table 24 Estimation of capital cost for the proposed bioprocess system

Item	Description	material	labor	Total cost
Fermentation tank*	Tank	$ 1,111	$ -	$ 1,111
Aeration unit*	air distributor	$ 150	$ 75	$ 3,340
	air blower	$ 2,136	$ 600	
	Ametex Rotron Inc., Model DR523K58/Grainger, Catalog No. 396			
	air flow meter	$ 263	$ -	
	standard media filter	$ 116	$ -	
Feeding unit*	feeding pump	$ 350	$ -	$ 1,000
	Grainger Catalog No. 396			

	feeding flow-rate control unit	$	150	$	150		
	piping system and tank fitting	$	200	$	150		
Influent storage tank*	tank	$	425	$	-	$	925
	piping system and tank fitting	$	200	$	300		
Effluent storage tank*	tank	$	425	$	-	$	925
	piping system and tank fitting	$	200	$	300		
Infrastructure systems*	high voltage electrical system	$	-	$	3,500	$	8,200
	electrical system	$	500	$	900		
	piping system	$	750	$	450		
	site preparation	$	1,200	$	900		
Juice Press	X6 Commerical Juice Press Capable of outputs of 40 - 60 U.S. gallons (150 - 225 liters) per hour. Capacity: 480 lbs Output: 40-60 GPH Goodnature Products Inc., NY	$	8,600			$	8,600
Grinder	All Purpose Shredder EG400/50 Specifications Capacity: Up to 6 tons (5,443 kg) per hour, HP: 5 HP Standard Power Requirements: 230/460V, 3 Phase, 15/7.5 Amps - 60 HZ Screens: Two screens are included. Hole sizes available from 1/4" to 1 1/8" (6.35 mm to 28.57 mm) Goodnature Products Inc., NY	$	4,000			$	4,000
Filter Press	Lanco, manual, 2.5 cu. ft., 470MM filter press, 13 gasketed polypro plates, center feed, 4 corner return, manual hydraulic closure with distance piece to expand to 3.5 cu. ft. (used price) Met-Chem Inc. Cleveland, OH	$	7,000			$	7,000
subtotal							$35,101
Sundries	(15% of total main item cost)					$	5,265
Total cost							$40,366

*Based on the pilot plant for wastewater treatment (Kongsil 2006)

Operation and maintenance costs

Operation and maintenance costs included labor cost, electricity and water utilities. The labor cost was $15/hr/person for regular checking, operation and maintenance. The electricity cost was based on the power requirement of main equipment such as pump and air blower based on the pilot plant scale for wastewater treatment (Kongsil, 2006). The water cost was based on the price provided by Board of Water Supply, Honolulu (2007). For processing 100kg of PPW per day, 365 day per year, the annual operational cost was estimated to be $34,893 as shown in Table 25.

Table 25 Estimation of operation and maintenance cost for processing 100kg of PPW

	Description	annual cost	
Labor	$15/hr*2.5hr/day*365day/year	$	13,688
Electricity[a]	main pump: 3.30kW*12hr/day, air blower: 22.08kW*12hr/day, $0.19/kW	$	21,121
Water[b]	105.67gallon/100kgPPW/day*365 day/year: 38,569 gallon/year $5.15 two month billing charge, $2.24/1000gallons for first 13,000 gallons or $ 0.95/1000 gallons for any part thereof	$	84
Total	For per 100kg PPW/day*365day/year	$	34,893

[a] Based on the pilot plant for wastewater treatment (Kongsil 2006)
[b] Based on price provided by Board of Water Supply, Honolulu, USA (2007)

Disposal cost avoided from landfill

The disposal cost of organic waste avoided from the reuse of PPW was estimated from tipping fee. The tipping fee for MSW landfill was estimated at $33 per ton in 2000 (USEPA, 2000). The annual cost avoided from landfill disposal was $1205 estimated based on dispose 100kg of PPW per day, 365 day per year as shown in Table 26.

Table 26 Estimation of cost avoided from landfill disposal for per 100kg of PPW

	Description	annual benefit	
Tipping fee for landfill	$33/ton [a] 100kg/day*365day/year	$	1,205

[a] Based on the tipping fee in 2000 (USEPA 2000)

Annual production cost

The net present worth of the project and annual production cost were calculated based on an annual interest rate of 5.75% (Kongsil, 2006) and a life span of 10 year. The annual production cost were calculated by sum of annual operational and material cost less sum of annual disposal cost avoided from landfill and annual equivalent of capital recovery. As shown in Table 27, the net present worth of the project is $297,961 and annual production cost is $40,005 to treat 100 kg of PPW per day, 365 day per year. The cost of treating 1 kg of PPW was $1.096 (calculated by: $40,005/36500 kg PPW per year). Considering the yield (Y_{SS}=0.3 and $Y_{protein}$=0.15), the cost of production per kg of SS was $3.653 and per kg of protein was $7.307, respectively.

Table 27 Cost analysis for processing 100kg PPW per day

Capital investment		$ 40,366	
Annual operational cost		$ 34,893	
Annual material cost		$ 897	
Annual disposal cost avoided from landfill		$ 1,205	
Annual expenses		$ 34,586	
Annual interest rate		5.75%	
Life expectancy		10 years	
End of period	Capital cost	Annual expenses	Net cash flow
0	$ 40,366		$ 40,366
1		$ 34,586	$ 34,586
2		$ 34,586	$ 34,586
3		$ 34,586	$ 34,586
4		$ 34,586	$ 34,586
5		$ 34,586	$ 34,586
6		$ 34,586	$ 34,586
7		$ 34,586	$ 34,586
8		$ 34,586	$ 34,586
9		$ 34,586	$ 34,586
10		$ 34,586	$ 34,586
Net present worth of the project			$ 297,961
Annual production cost			$ 40,005
Bioprocess cost of per kg of PPW			$ 1.096
Production cost of per kg of SS (yield 0.3)			$ 3.653
Production cost of per kg of protein (yield 0.15)			$ 7.307

4.7.2 Sensitivity Analysis for the bioprocess system

Sensitivity analysis is used to make explicit the impact of uncertainty in the estimates of each factor of concern on the economic measure of merit (Sullivan, Wicks, & Luxhoj, 2003). Several factors including amount of PPW processed per day, capital cost, annual operational cost, annual material cost, life expectancy and annual interest rate were considered for their impact on the annual production cost for the batch system.

Change in amount of PPW processed

To evaluate the production cost regarding change in amount of PPW processed per day, the cost of annual material, disposal cost avoided and utilities cost were estimated with proportional to the amount of PPW processed and water required, respectively. The capital cost, annual interest rate and life expectancy were the same as shown in Table 27. The resulting data is shown in Table 28.

As shown in Figure 16, the annual worth decreases as amount of PPW processed per day increases. The production cost reduces from \$1.096 to \$0.213 for per kg of PPW, \$3.653 to \$0.177 for per kg of suspended solids and \$7.307 to \$1.422 for per kg of protein, respectively, when amount of PPW processed per day increases from 100 to 500 kg. As the amount of PPW processed per day increases, the proposed system provides an attractive cost-effective protein source for feed supplement.

Table 28 Estimation of production cost based on bioprocessing 100kg PPW per day

Capital investment $ 40,366

Annual interest rate 5.75%

Life expectancy 10 years

PPW, kg/day	Annual Operational cost	Annual Material cost	Annual disposal cost avoided	Annual expenses	Annual production cost	Processing cost, $/kg PPW	Production cost, $/kg SS	Production cost, $/kg protein
100	$ 34,893	$ 897	$ 1,204.5	$ 34,586	$ 40,005	$ 1.096	$3.653	$ 7.307
200	$ 34,930	$ 1,794	$ 2,409.0	$ 34,315	$ 39,734	$ 0.544	$1.814	$ 3.629
300	$ 34,966	$ 2,691	$ 3,613.5	$ 34,044	$ 39,464	$ 0.360	$1.201	$ 2.403
400	$ 35,003	$ 3,588	$ 4,818.0	$ 33,773	$ 39,193	$ 0.268	$0.895	$ 1.790
500	$ 35,040	$ 4,485	$ 6,022.5	$ 33,502	$ 38,922	$ 0.213	$0.711	$ 1.422
600	$ 35,076	$ 5,382	$ 7,227.0	$ 33,231	$ 38,651	$ 0.176	$0.588	$ 1.177
700	$ 35,113	$ 6,279	$ 8,431.5	$ 32,961	$ 38,380	$ 0.150	$0.501	$ 1.001
800	$ 35,150	$ 7,176	$ 9,636.0	$ 32,690	$ 38,109	$ 0.131	$0.435	$ 0.870
900	$ 35,186	$ 8,073	$ 10,840.5	$ 32,419	$ 37,839	$ 0.115	$0.384	$ 0.768
1000	$ 35,223	$ 8,970	$ 12,045.0	$ 32,148	$ 37,568	$ 0.103	$0.343	$ 0.686
1100	$ 35,259	$ 9,867	$ 13,249.5	$ 31,877	$ 37,297	$ 0.093	$0.310	$ 0.619
1200	$ 35,296	$10,764	$ 14,454.0	$ 31,606	$ 37,026	$ 0.085	$0.282	$ 0.564
1300	$ 35,333	$11,661	$ 15,658.5	$ 31,336	$ 36,755	$ 0.077	$0.258	$ 0.516
1400	$ 35,369	$12,558	$ 16,863.0	$ 31,065	$ 36,484	$ 0.071	$0.238	$ 0.476
1500	$ 35,406	$13,455	$ 18,067.5	$ 30,794	$ 36,214	$ 0.066	$0.220	$ 0.441
1600	$ 35,443	$14,352	$ 19,272.0	$ 30,523	$ 35,943	$ 0.062	$0.205	$ 0.410
1700	$ 35,479	$15,249	$ 20,476.5	$ 30,252	$ 35,672	$ 0.057	$0.192	$ 0.383
1800	$ 35,516	$16,146	$ 21,681.0	$ 29,981	$ 35,401	$ 0.054	$0.180	$ 0.359
1900	$ 35,553	$17,043	$ 22,885.5	$ 29,711	$ 35,130	$ 0.051	$0.169	$ 0.338
2000	$ 35,589	$17,940	$ 24,090.0	$ 29,440	$ 34,859	$ 0.048	$0.159	$ 0.318

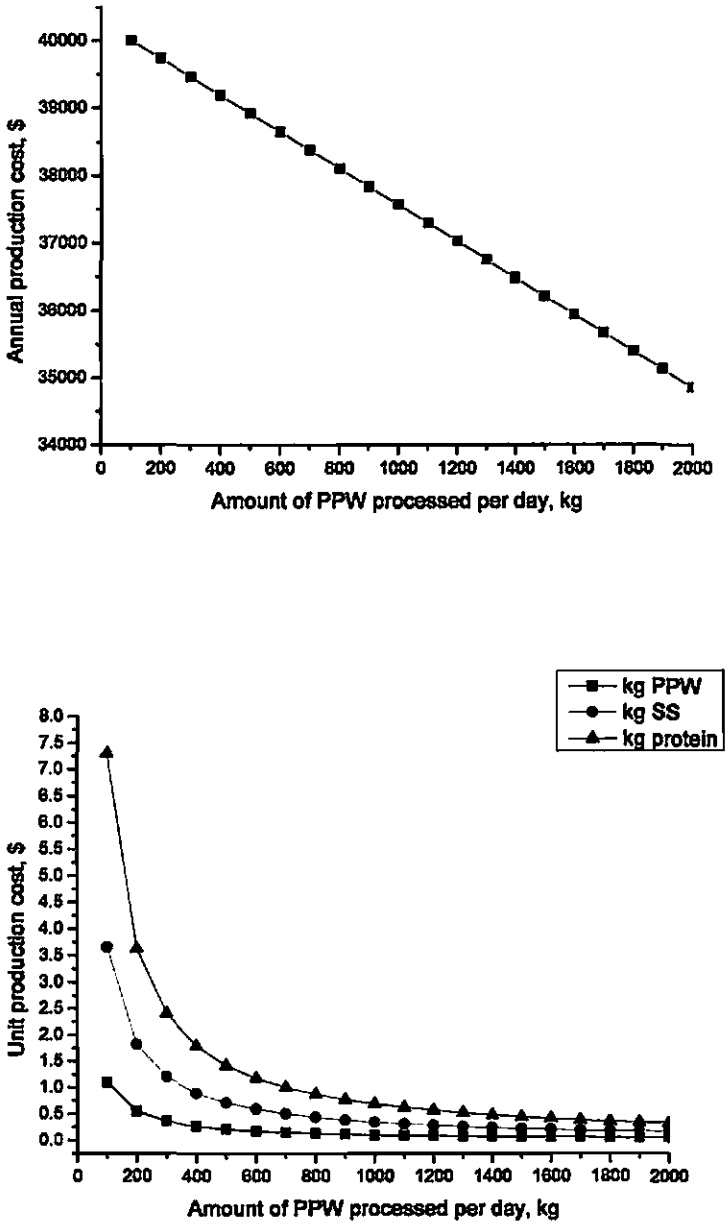


Figure 16 Sensitivity analysis of the amount of PPW processed per day on annual production cost

Sensitivity of capital, O&M, material cost, life expectancy and annual interest rate on the proposed system

Five factors including capital cost, annual operational cost, annual material cost, life expectancy and annual interest rate were considered for their impact on annual production cost for the proposed bioprocess system. The most likely estimate values are based on the analysis presented in Section 4.7.1 or Table 27 with 100 kg of PPW processed per day. The sensitivity of annual worth and production cost with respect to percent deviation changes of one factor over a range of ±50% from most like value is analyzed by assuming the other factors remaining at their most likely values. The resulting data is shown in Table 29.

The relative degree of sensitivity of the annual worth or production cost is indicated by the slope of the curves. As shown in Figure 17, the sensitivity of annual project cost regarding capital, operational and material cost showed a linear relationship with percent change from most like estimates while life expectancy and annual interest rate showed a nonlinear relationship. Among these factors, the annual operational cost appears to be the most sensitive factor for the cost of the proposed system. The next sensitive factors are life expectancy and capital cost, and the least sensitive factors are annual interest rate and material cost. The life expectancy is more influential when it is less than 7 year. The annual worth/production cost decreases as life expectancy increases. As annual interest rate increases, the annual worth/production cost slightly increases.

Table 29 Data of the sensitivity analysis of five factors for the proposed bioprocess system

Most likely estimate values					
Capital investment	$ 40,366				
Annual Operational cost	$ 34,893	For 100kg PPW/day*365day/year			
Annual material cost	$ 897	For 100kg PPW/day*365day/year			
Annual tipping fee for landfill avoided	$ 1,205	For 100kg PPW/day*365day/year			
Annual expenses	$ 34,586				
Annual interest rate	5.75%				
Life expectancy	10	years			
			Bioprocess cost	Production cost	
% change	Capital cost	Annual production cost	kg PPW	kg SS	kg Protein
-50%	$ 20,183	$ 37,295	$ 1.022	$ 3.406	$ 6.812
-40%	$ 24,220	$ 37,837	$ 1.037	$ 3.455	$ 6.911
-30%	$ 28,256	$ 38,379	$ 1.051	$ 3.505	$ 7.010
-20%	$ 32,293	$ 38,921	$ 1.066	$ 3.554	$ 7.109
-10%	$ 36,330	$ 39,463	$ 1.081	$ 3.604	$ 7.208
0%	$ 40,366	$ 40,005	$ 1.096	$ 3.653	$ 7.307
10%	$ 44,403	$ 40,547	$ 1.111	$ 3.703	$ 7.406
20%	$ 48,439	$ 41,089	$ 1.126	$ 3.752	$ 7.505
30%	$ 52,476	$ 41,631	$ 1.141	$ 3.802	$ 7.604
40%	$ 56,513	$ 42,173	$ 1.155	$ 3.851	$ 7.703
50%	$ 60,549	$ 42,715	$ 1.170	$ 3.901	$ 7.802
% change	Operational cost	Annual production cost	kg PPW	kg SS	kg Protein
-50%	$ 17,447	$ 22,559	$ 0.618	$ 2.060	$ 4.120
-40%	$ 20,936	$ 26,048	$ 0.714	$ 2.379	$ 4.758
-30%	$ 24,425	$ 29,537	$ 0.809	$ 2.697	$ 5.395
-20%	$ 27,914	$ 33,027	$ 0.905	$ 3.016	$ 6.032
-10%	$ 31,404	$ 36,516	$ 1.000	$ 3.335	$ 6.670
0%	$ 34,893	$ 40,005	$ 1.096	$ 3.653	$ 7.307
10%	$ 38,382	$ 43,495	$ 1.192	$ 3.972	$ 7.944
20%	$ 41,872	$ 46,984	$ 1.287	$ 4.291	$ 8.582
30%	$ 45,361	$ 50,473	$ 1.383	$ 4.609	$ 9.219
40%	$ 48,850	$ 53,962	$ 1.478	$ 4.928	$ 9.856
50%	$ 52,340	$ 57,452	$ 1.574	$ 5.247	$ 10.493
% change	Material cost	Annual production cost	kg PPW	kg SS	kg Protein
-50%	$ 449	$ 39,557	$ 1.084	$ 3.612	$ 7.225
-40%	$ 538	$ 39,646	$ 1.086	$ 3.621	$ 7.241
-30%	$ 628	$ 39,736	$ 1.089	$ 3.629	$ 7.258
-20%	$ 718	$ 39,826	$ 1.091	$ 3.637	$ 7.274
-10%	$ 807	$ 39,916	$ 1.094	$ 3.645	$ 7.291
0%	$ 897	$ 40,005	$ 1.096	$ 3.653	$ 7.307
10%	$ 987	$ 40,095	$ 1.098	$ 3.662	$ 7.323

20%	$ 1,076	$ 40,185	$ 1.101	$ 3.670	$ 7.340
30%	$ 1,166	$ 40,274	$ 1.103	$ 3.678	$ 7.356
40%	$ 1,256	$ 40,364	$ 1.106	$ 3.686	$ 7.372
50%	$ 1,346	$ 40,454	$ 1.108	$ 3.694	$ 7.389
% change	Life expectancy	Annual production cost	kg PPW	kg SS	kg Protein
-50%	5	$ 44,103	$ 1.208	$ 4.028	$ 8.055
-40%	6	$ 42,730	$ 1.171	$ 3.902	$ 7.805
-30%	7	$ 41,752	$ 1.144	$ 3.813	$ 7.626
-20%	8	$ 41,022	$ 1.124	$ 3.746	$ 7.493
-10%	9	$ 40,456	$ 1.108	$ 3.695	$ 7.389
0%	10	$ 40,005	$ 1.096	$ 3.653	$ 7.307
10%	11	$ 39,638	$ 1.086	$ 3.620	$ 7.240
20%	12	$ 39,335	$ 1.078	$ 3.592	$ 7.184
30%	13	$ 39,079	$ 1.071	$ 3.569	$ 7.138
40%	14	$ 38,861	$ 1.065	$ 3.549	$ 7.098
50%	15	$ 38,674	$ 1.060	$ 3.532	$ 7.064
% change	Interest rate	Annual production cost	kg PPW	kg SS	kg Protein
-50%	2.88%	$ 39,288	$ 1.076	$ 3.588	$ 7.176
-40%	3.45%	$ 39,427	$ 1.080	$ 3.601	$ 7.201
-30%	4.03%	$ 39,569	$ 1.084	$ 3.614	$ 7.227
-20%	4.60%	$ 39,712	$ 1.088	$ 3.627	$ 7.253
-10%	5.18%	$ 39,858	$ 1.092	$ 3.640	$ 7.280
0%	5.75%	$ 40,005	$ 1.096	$ 3.653	$ 7.307
10%	6.33%	$ 40,155	$ 1.100	$ 3.667	$ 7.334
20%	6.90%	$ 40,306	$ 1.104	$ 3.681	$ 7.362
30%	7.48%	$ 40,460	$ 1.108	$ 3.695	$ 7.390
40%	8.05%	$ 40,615	$ 1.113	$ 3.709	$ 7.418
50%	8.63%	$ 40,772	$ 1.117	$ 3.723	$ 7.447

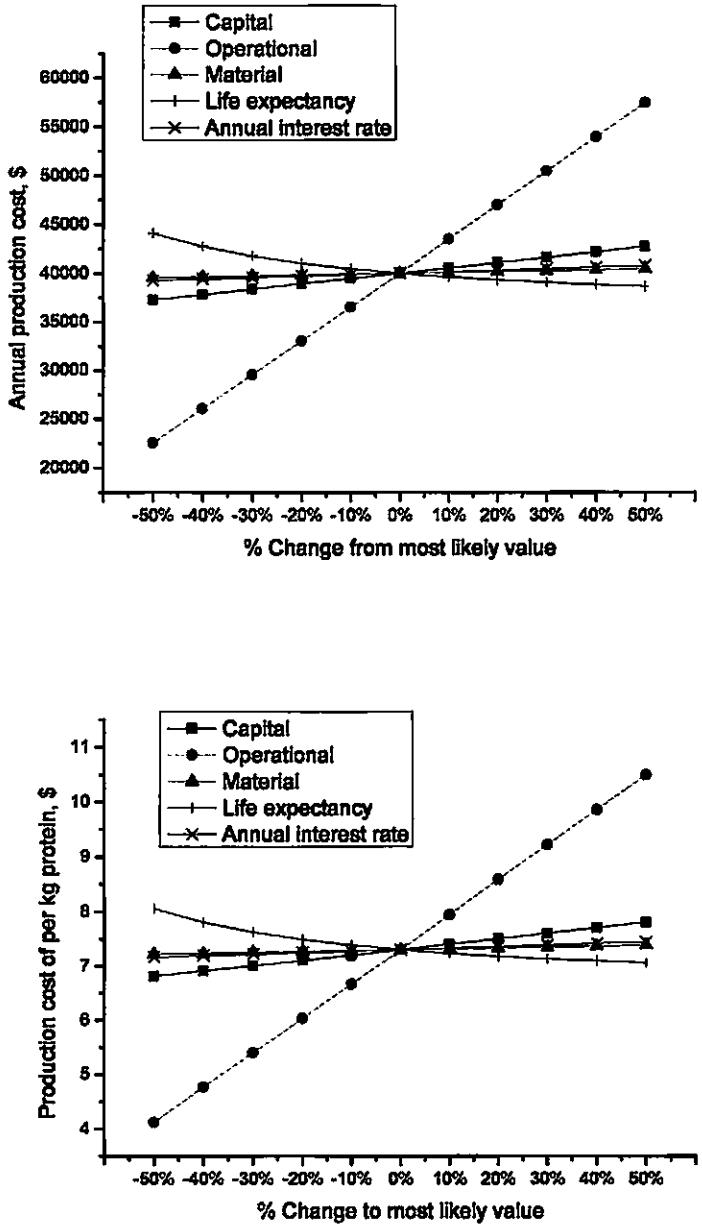


Figure 17 Sensitivity analyses of five factors for the proposed bioprocess system

The cost of the infrastructure, sterilization process and overhead charges considerably increase the production cost of single cell protein (Joshi & Sandhu, 1996). The cost of molasses, the most common source for Baker's yeast fermentation, is about $120-150 per metric tonne excluded transportation fee (Sasson, 1990). In this study, the raw material cost containing only the supply of nutrient cost is much less than molasses and appears the least influential factor on the sensitivity of production cost. The cost of annual operational as the most influential factor on production cost, is therefore suggested to be minimized by reducing cost of labor, electricity or water utilities.

4.7.3 Selling price and expected benefits

When considering the expected benefits from the sell of bioprocessed PPW product, the annual worth of the project can be modified by including annual revenue which is the amount of product multiplied unit selling price. The selling price of bioprocessed product is estimated by the cost of commercial shrimp feed from Commercial feed 35/2.5 (40.9% crude protein) which is $29.99 per 20kg bag or $1.4995 per kg purchased from Land O'Lakes (Kapolei, HI). Assuming the cost of protein is proportional to its composition in the feed, the protein cost is $0.6133 per kg. The selling price of the bioprocessed PPW is therefore estimated at $0.5 per kg of protein. By including selling of the bioprocessed product, the change of annual profit with respect to amount of PPW processed per day was evaluated.

By including selling of the bioprocessed product, the estimation of annual profit with change on amount of PPW processed per day is shown in Table 30. The cost of capital

investment and annual expenses was estimated as presented in section 4.7.1. The annual expenses with respect to change of amount of PPW processed were the same as presented in Table 28. The annual revenue from selling was calculated by amount of PPW processed per day *365day/year *0.15 yield of protein/PPW *$0.5/kg of protein.

As shown in Figure 18, when the amount of PPW processed is increased, the annual profit is increased from negative to positive values. When amount of PPW processed is more than 1400 kg per day or 511,000 kg per year, the annual profit is positive which indicates the minimum operational scale for possible net profit based on the selling price of $0.5 per kg of protein for the proposed bioprocess system.

Table 30 Data of annual profit with respect to change on amount of PPW processed per day when considering selling benefit

Capital investment $ 40,366

Annual interest rate 5.75%

Life expectancy 10 years

Selling price of per kg protein $0.5

PPW, kg/day	Annual expenses and recovery of capital cost	Annual revenue	Annual profit
100	$ 40,005	$ 2,738	$ -37,268
200	$ 39,734	$ 5,475	$ -34,259
300	$ 39,464	$ 8,213	$ -31,251
400	$ 39,193	$ 10,950	$ -28,243
500	$ 38,922	$ 13,688	$ -25,234
600	$ 38,651	$ 16,425	$ -22,226
700	$ 38,380	$ 19,163	$ -19,218
800	$ 38,109	$ 21,900	$ -16,209
900	$ 37,839	$ 24,638	$ -13,201
1000	$ 37,568	$ 27,375	$ -10,193
1100	$ 37,297	$ 30,113	$ -7,184
1200	$ 37,026	$ 32,850	$ -4,176
1300	$ 36,755	$ 35,588	$ -1,168
1400	$ 36,484	$ 38,325	$ 1,841
1500	$ 36,214	$ 41,063	$ 4,849
1600	$ 35,943	$ 43,800	$ 7,857
1700	$ 35,672	$ 46,538	$ 10,866
1800	$ 35,401	$ 49,275	$ 13,874
1900	$ 35,130	$ 52,013	$ 16,882
2000	$ 34,859	$ 54,750	$ 19,891
2100	$ 34,589	$ 57,488	$ 22,899
2200	$ 34,318	$ 60,225	$ 25,907
2300	$ 34,047	$ 62,963	$ 28,916
2400	$ 33,776	$ 65,700	$ 31,924
2500	$ 33,505	$ 68,438	$ 34,932
2600	$ 33,234	$ 71,175	$ 37,941
2700	$ 32,964	$ 73,913	$ 40,949
2800	$ 32,693	$ 76,650	$ 43,957
2900	$ 32,422	$ 79,388	$ 46,966
3000	$ 32,151	$ 82,125	$ 49,974

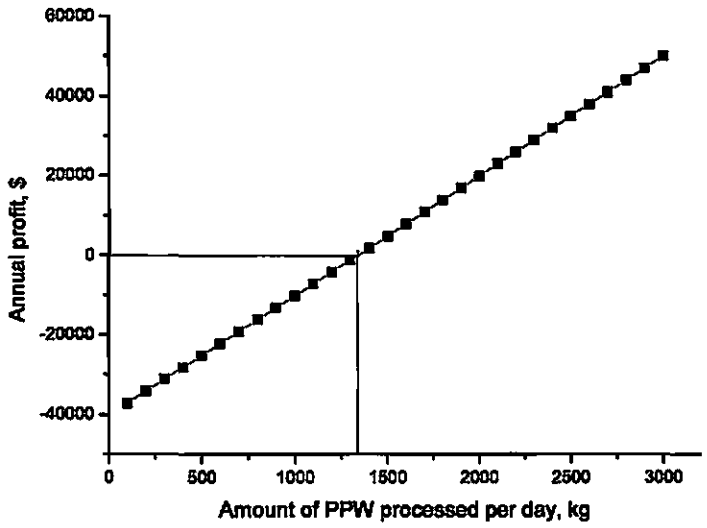


Figure 18 Relationship of annual profit and the amount of PPW processed

Sensitivity of annual worth on selling price and other factors

The sensitivity of annual profit on selling price and other factors including capital cost, annual operational and material cost, interest rate and life expectancy is evaluated when the amount of PPW processed is 1400 kg per day or 511 metric ton per year. The most likely values of capital cost, annual interest rate, life expectancy, annual expenses, and selling price were based on Table 30. The calculations of annual profit with change on these factors were as presented at previous section. The resulting data is shown in Table 31.

As shown in Figure 19, the linear relationship is found between annual profit with respect to change in selling price, capital cost, annual operational and material cost, while the nonlinear relationship is found in annual interest rate and life expectancy. The selling price of per kg protein and annual operational cost appeared to be the most influential factors. The next influential factors were annual material cost and capital cost and the least influential factors were annual interest rate and life expectancy. The life expectancy appeared to be more influential when it is less than 8 year. The cost of material appears to be more influential than the capital cost when the operational capacity is larger, i.e., 1400kg PPW per day, which is different from the results when operational capacity is 100kg PPW per day.

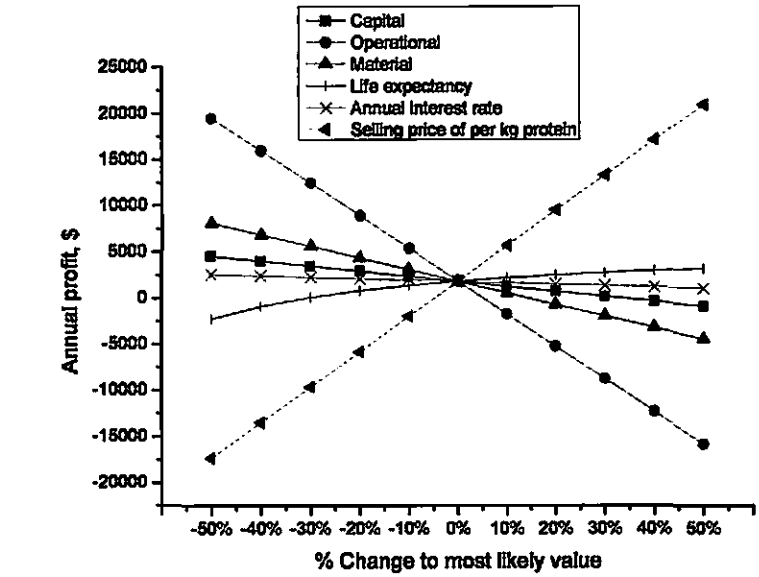
The annual profit increases as selling price increases. When the selling price is more than $0.476 per kg of protein, the annual worth is positive which indicates the possible net profit. With the cost estimation of other factors remains unchanged, the selling price for net profit can be reflected from the production cost based on amount of PPW processed as shown in Table 28 and Figure 16. When the selling price is more than the production cost, it is possible to gain net profit.

Table 31 Sensitivity analysis of annual profit based on 1400 kg PPW processed per day

Most likely estimate values	1400 kg	PPW processed per day
Annual interest rate	5.75%	
Life expectancy	10	years
Capital investment	$ 40,366	
Annual Operational cost	$ 35,369	
Annual material cost	$ 12,558	
Annual disposal cost avoided	$ 16,863	
Selling price	$ 0.5	kg/protein
Annual revenue	$ 38,325	

% change	Capital cost	Annual profit	Operational cost	Annual profit	Material cost	Annual profit
-50%	$ 20,183	$ 4,550	$ 17,685	$ 19,525	$ 6,279	$ 8,120
-40%	$ 24,220	$ 4,008	$ 21,222	$ 15,988	$ 7,535	$ 6,864
-30%	$ 28,256	$ 3,467	$ 24,759	$ 12,451	$ 8,791	$ 5,608
-20%	$ 32,293	$ 2,925	$ 28,295	$ 8,914	$ 10,047	$ 4,352
-10%	$ 36,330	$ 2,383	$ 31,832	$ 5,378	$ 11,303	$ 3,096
0%	$ 40,366	$ 1,841	$ 35,369	$ 1,841	$ 12,558	$ 1,841
10%	$ 44,403	$ 1,299	$ 38,906	$ -1,696	$ 13,814	$ 585
20%	$ 48,439	$ 757	$ 42,443	$ -5,233	$ 15,070	$ -671
30%	$ 52,476	$ 215	$ 45,980	$ -8,770	$ 16,326	$ -1,927
40%	$ 56,513	$ -327	$ 49,517	$ -12,307	$ 17,582	$ -3,183
50%	$ 60,549	$ -869	$ 53,054	$ -15,844	$ 18,838	$ -4,439

% change	Life expectancy	Annual profit	Interest rate	Annual profit	Selling price	Annual profit
-50%	5	$ -2,257	2.88%	$ 2,558	$ 0.25	$ -17,322
-40%	6	$ -884	3.45%	$ 2,419	$ 0.30	$ -13,489
-30%	7	$ 93	4.03%	$ 2,277	$ 0.35	$ -9,657
-20%	8	$ 824	4.60%	$ 2,134	$ 0.40	$ -5,824
-10%	9	$ 1,390	5.18%	$ 1,988	$ 0.45	$ -1,992
0%	10	$ 1,841	5.75%	$ 1,841	$ 0.50	$ 1,841
10%	11	$ 2,207	6.33%	$ 1,691	$ 0.55	$ 5,673
20%	12	$ 2,511	6.90%	$ 1,540	$ 0.60	$ 9,506
30%	13	$ 2,767	7.48%	$ 1,386	$ 0.65	$ 13,338
40%	14	$ 2,984	8.05%	$ 1,231	$ 0.70	$ 17,171
50%	15	$ 3,172	8.63%	$ 1,074	$ 0.75	$ 21,003



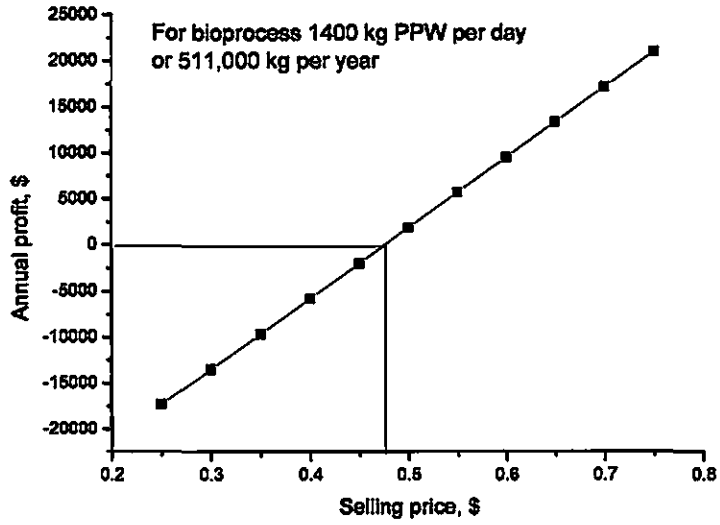


Figure 19 Sensitivity analysis of annual profit based on 1400 kg PPW processed per day

Estimation of year of return

The payback period method was applied to estimate the year of return which indicates how fast an investment can be recovered by calculating the number of years required for cash inflows to just equal cash outflows or for cumulative present worth to turn positive (Sullivan et al., 2003). The evaluation of year of return is based on the related capital cost, annual expenses, annual revenue and selling price as presented in Table 31. An example of how to determine the year of return or payback period based on processed 1400 kg PPW per day is shown in Table 32. The year of return is 6 year for processing 1400kg PPW per day because the cumulative present worth turns positive at the end of year 6. The result of year of return with respect to amount of PPW processed is presented in Table 33.

As shown in Figure 20, the year of return decreases as amount of PPW processed increases. The year of return is 6 year for processing 1400kg PPW per day. When the amount of PPW processed per day is more than 1500 kg, the year of return is 4 year. When it is more than 1700 kg, the year of return is 3 year. When it is more than 1900 kg, the year of return is 2 year. When it is more than 2600 kg, the year of return is 1 year.

Based on the economic analysis, it can be concluded that when the selling price of $0.5 per kg protein is considered, it requires an operational capacity of at least 1400 kg PPW per day or 551 metric ton per year to gain net profit and the year of return is 6 year. When the operational capacity is more than 551 metric ton, the year of return will be less than 6 year with more profits depends on the amount of PPW processed.

Table 32 Example of payback period calculation

Capital investment $ 40,366

Annual interest rate 5.75%

Selling price $0.5 per kg protein

When amount of PPW processed per day is 1400 kg

End of year	Capital investment	Annual expenses	Annual revenue	Net cash flow	Cumulative PW
0	$ -40,366			$ -40,366	$ -40,366
1		$ 31,065	$ 38,325	$ 7,260	$ -33,106
2		$ 31,065	$ 38,325	$ 7,260	$ -25,846
3		$ 31,065	$ 38,325	$ 7,260	$ -18,585
4		$ 31,065	$ 38,325	$ 7,260	$ -11,325
5		$ 31,065	$ 38,325	$ 7,260	$ -4,065
6		$ 31,065	$ 38,325	$ 7,260	$ 3,196 *
7		$ 31,065	$ 38,325	$ 7,260	$ 10,456
8		$ 31,065	$ 38,325	$ 7,260	$ 17,716
9		$ 31,065	$ 38,325	$ 7,260	$ 24,976
10		$ 31,065	$ 38,325	$ 7,260	$ 32,237

* The payback period is 6 year because the cumulative discounted balance turns positive at end of year 6.

Table 33 Year of return based on amount of PPW processed

Amount of PPW processed per day, kg	Amount of PPW processed per year, kg	Year of return
1,300	474,500	10
1,400	511,000	6
1,500	547,500	4
1,600	584,000	4
1,700	620,500	3
1,800	657,000	3
1,900	693,500	2
2,000	730,000	2
2,100	766,500	2
2,200	803,000	2
2,300	839,500	2
2,400	876,000	2
2,500	912,500	2
2,600	949,000	1
2,700	985,500	1
2,800	1,022,000	1
2,900	1,058,500	1
3,000	1,095,000	1

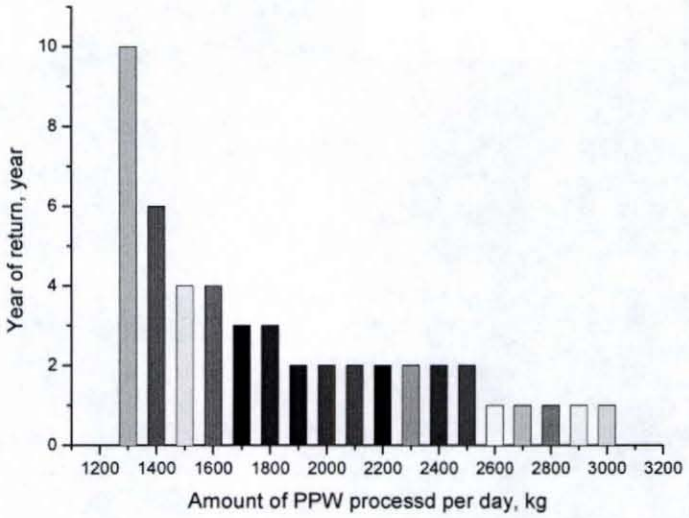


Figure 20 Relationship of year of return and amount of PPW processed per day

The selling price of commercial Baker's yeast is $2.20 per kg yeast or $14.68 per kg protein (based on the estimation of 50% of protein content and 30% of dry matter, Red Star Baker's yeast, provided by HFM, Honolulu, HI). The functional yeast extract for aquafeed (Alltech Inc., Bangkok, Thailand) is about $2 per kg and the protein from soybean is $0.44-0.66 per kg. The production cost of feed protein from defatted rice polishings by *Candida utilis* including over-head charges of labor, electricity and processing cost was $2.58 per100 kg of rice polishings or $0.121 per kg of protein (based on an average yield of 90kg yeast biomass/100kg rice polishings and 23.6% of true protein in biomass). It could reduce the poultry feed cost by $0.35 and $0.65 per 100kg bag by replacing 25% or 50% with the processed product, respectively, in Pakistan (Rajoka et al. 2006). The production cost of *C. utilis* estimated from substrate prices was $1.08 and $143.22 per kg dry mass for the growth in molasses medium and complex

media containing what in batch fermentation, respectively (Lee and Kim 2001). Under fed-batch fermentation in what complex media, the cost was $1.71 per kg. With the estimation of 62% raw material cost in total production cost including utilities, labor and supervision, fixed charges, maintenance, etc, in single cell protein production (Stanbury et al. 1995), the production cost of *C. utilis* in what complex media was estimated to be $ 2.76 per kg in fed-batch process, which appears much cost-effective than that in batch process (Lee & Kim, 2001). The fed-batch process was suggested as a cheaper alternative than the batch process to provide higher protein yield for yeast cultivation from potato starch waste (Rusendi and Sheppard 1995). The continuous-flow operation is also considered to provide higher cell yield than batch operation (Lotz et al. 1991). It is suggested that the bioprocessing of PPW in feb-batch or continuous process may provide higher biomass/protein yield with lower production cost than the batch process.

Due to the treatment cost involved, agricultural effluents are often not subject to treatment prior to disposal. However, as the regulation is getting more rigid, the consideration of economical treatment process is needed (Arnold et al. 2000). The economic analyses of the utilization of organic wastes often show marginal or sometimes negative results that government subsidies may be needed initially to help the cost of further processing (Westerman and Bicudo 2005). The economic analysis of processing apple pomance under solid state fermentation for protein enrichment after dried and sold as animal feed did not justify its production commercially in New Zealand (Kennedy 1994). Such process requires a large-size of fermenter, provision for aeration and costly separation of products from the fermented substrate (Kennedy 1994). The economic

analysis of papaya processing waste for methane production is able to obtain net profits when treating more than 3000 metric tons of waste per year. With smaller waste treated, the methane produced is insufficient to cover the investment cost (Chou and Yang 1986).

For reference, the cost of direct use of crop residue as livestock feed are $41.90/ton for corn, $42.51/ton for sorghum, $21.21/ton for wheat, $32.09/ton for barley, $34.25/ton for oats, $6.31/ton for sugarcane, and $25.10/ton for rice in 1997. Crop residues is also available to processing plants in the $14/ton to $30/ton range for all major field crops and regions (Gallagher et al. 2003).

The costs and benefits of environmental alternative waste management policies is not easy to determine (Westerman and Bicudo 2005). For example, the average cost for MSW composting is $59 per ton (USEPA 1995), while the average market value of compost is $10-35 per ton (USEPA 2000). The composting facility for the biological stabilization of waste organic material is $8,409 per facility source (GAO 1999). The national savings over conventional disposal from composting vary from $9 to $37 per cubic yard depending on the method of composting selected and the type of waste (USEPA 2000). Economic analysis indicates that vary few facilities receive any revenues from the sale of MSW compost to offset operating costs and the financial grounds cannot be justified in most parts of the US at present, although it may be competitive with land disposal where the cost of landfill is high (Renkow and Rubin 1998). Negative public reactions to MSW composting include odor problems and cost overruns (Goldstein and Steutville 1996). Compared to those organic waste treatment and disposal methods, the

bioprocess of PPW appears a promising solution for organic waste treatment that avoids the odor and land-requirement problems while providing a competitive cost-effective feed when higher capacity of PPW is treated or lower operational cost is applied.

Chapter 5. Conclusions and Recommendations

5.1 Conclusions

The operational conditions of bioconverting papaya processing waste into protein enrichment have been achieved in batch system. Blended PPW slurry with initial SCOD ranged from 15000-25000 mg/l and the addition of nutrient was able to achieve more than 70% of SCOD removal with 40-45% crude protein in the product after 8-12 h of reaction. The estimated yield of protein formation ranged from 0.11-0.17. The estimated yield of suspended solids formation ranged from 0.14-0.28. The maximum specific growth rate and saturation constant were 0.1366 h^{-1} and 54130 mg SCOD/l, respectively. The biokinetic constants are able to provide some guidance for the operational criteria for the continuous flow system. In batch operation, the continuous monitoring of pH, ORP and DO was able to determine the required reaction time when pH starts to increase, ORP starts to decrease and DO starts to increase to more than 5 mg/l. The adjusting of pH was not needed in batch operation which automatically maintained around 3-4 during reaction. The scaled-up batch operation with 100-L working volume was able to provide similar SCOD removal and protein content as lab batch scale fermentor.

The preliminary continuous flow operation with HRT ranged from 6-12 h was able to provide 60-78% SCOD removal and 16-25% biuret protein without pH control. The semi-continuous flow operation with HRT ranged from 4-8 h was able to provide 60-75% SCOD removal and 15-28% biuret protein without pH control. The preliminary investigations of continuous and semi-continuous flow operation showed that the pH

control around 4-5 for optimal growth condition was necessary in order to obtain higher SCOD removal about 90% and biuret protein content about 42% in product. Further investigations of the HRT's with more than 7.3 hours or maximum cell growth rate of less than 0.1366 h^{-1} in continuous/semi-continuous flow operation with pH control are required for the long-term observation of the process stability.

The nutrient analysis of the bioprocessed product showed 40-50% crude protein with various nutrients. The results of shrimp feeding trial indicated that the bioprocessed PPW product had favorable potential as aquafeed supplement.

Economic analysis of the batch operation shows that the amount of PPW processed and annual operational cost were influential factors to reduce the production cost. The production cost could be reduced from \$7.307 to \$1.422 for per kg of protein when amount of PPW processed per day increased from 100 to 500 kg. When the selling price \$0.5 per kg protein was considered, it required an operational capacity of at least 1400 kg PPW per day or 551 metric ton per year to gain net profit and the year of return was 6 year. As the production cost reduces, the proposed system provides an attractive cost-effective protein source for feed supplement.

5.2 Recommendations

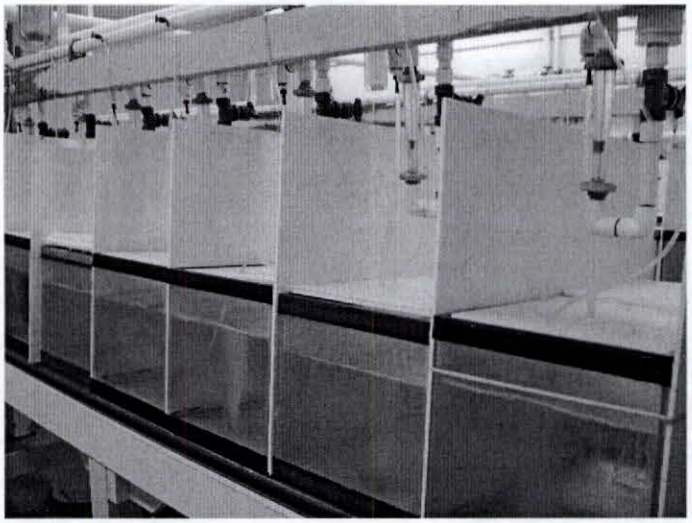
Based on the results in this study, the following are recommended for further investigation. Further studies in continuous/semi-continuous flow or fed-batch operation for higher protein yield and long-term performance stability are required for mass

production. Furthermore, in order to minimize the extensive water use and discharge after liquid state fermentation, solid state fermentation may be applied for the PPW bioprocess. With further investigations on shrimp feeding trial, the PPW bioprocess may be refined to produce more effective feed supplement. In addition, the developed bioprocess may be applied to various other island agriculture by-products or food processing waste for the sustainability of Hawaii's local aquacultural production and environmental protection.

Appendix A

Protocol of shrimp feeding trial

HYPOTHESIS:	
OBJECTIVE:	To ascertain the protein replacement effects of varying inclusion levels of papaya processing waste (PPW) on the growth of *L. vannamei* juveniles.
TREATMENTS:	
1	0% Papaya Processing Waste Control
2	50% Papaya Processing Waste
3	100% Papaya Processing Waste
# REPLICATES:	4
HUSBANDRY	Shrimp stocked in rectangular glass aquaria (76 x 31 x 31-cm dimensions and 52-L water volume) with four aquaria per treatment. Initial stocking density of 12 shrimp (50-shrimp/m^2 and ca. 1.0 g individual body weight) per aquarium. Seawater flow-through system at 1L/min (water exchange rate of about 100% /hour). Air flow at 250 to 300 ml/5 sec. Aquaria cleaned every morning before first feeding by siphoning out uneaten feed, feces, molts, and dead shrimp. Remove dead shrimp from aquaria as soon as possible to prevent cannibalism and record mortality/number of shrimp left in the tank. Shrimp group weighed by tank at the start and end of the trial and at two week intervals (weeks 0, 2, 4, 6 and 8) in all treatments. Lighting will be reduced in the room using red filters, doors kept closed. Morts will be replaced for a period of 72 hours after stocking.
FEEDING:	All treatments will be fed by hand three times daily at 0830, 1130, and 1530. Ration amount to be initially determined by logarithmic equation, then assessed and adjusted by tank according to consumption patterns (± 5-7%). Feed amount is recorded daily.
START OF TRIAL:	May 2007
TRIAL DURATION:	56 days (8 weeks)
OUTPUTS:	Water – Daily: Water flow rate, Airflow rate, Temperature; Dissolved oxygen Weekly: Salinity & pH in selected tanks per row. NH_3 (HACH) week 0, 4, & 8. Feeds – UH F11 (proximate, crude fiber, minerals), water stability, biochemical composition (e.g. amino acids, fatty acids, vitamins, carotenoid pigments, phospholipids) Shrimp - survival, final body weight, weight gain, FCR, proximate composition, initial and final biochemical composition
TECHNICAL STAFF:	AFN technical staff



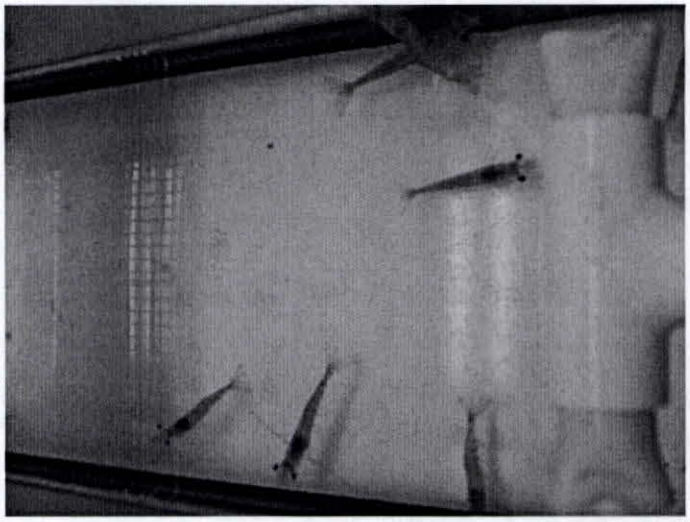


Figure A.1.1 Shrimp feeding trail

Nomenclature

μ_{max}	Maximum specific growth rate (h^{-1})
k_S	Saturation constant, substrate concentration when specific growth rate is at one-half of its maximum value (SCOD mg/l)
6.25ΔSTN/ΔSS	Estimate protein content in suspended solids formation (%, $g_{protein}/g_{SS}$)
$Y_{SS/SCOD}$	Yield of suspended solids coefficient, ΔSS/ΔSCOD (g_{SS}/g_{SCOD})
$Y_{STN/SCOD}$	Yield of biomass total nitrogen coefficient, ΔSTN/ΔSCOD (g_{STN}/g_{SCOD})
$Y_{6.25*STN/SCOD}$	Yield estimate of protein formation coefficient, ΔSTN*6.25/ΔSCOD ($g_{protein}/g_{SCOD}$)
$Y_{6.25*STN/SCOD/0.4}$	Yield estimate of suspended solids formation coefficient, ΔSTN*6.25/ΔSCOD/0.4 (g_{SS}/g_{SCOD})

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