POLYHYDROXYALKANOATES PRODUCTION FROM
HIGH ORGANIC CONTENT WASTEWATER
BY MIXED MICROBIAL CULTURES AND
ITS TECHNO-ECONOMIC ANALYSIS

การผลิต POLYHYDROXYALKANOATES จากน้ำเสียที่มีสารอินทรีย์สูง
โดยจุลินทรีย์แบบผสมและการวิเคราะห์ทางเทคนิคและเศรษฐศาสตร์

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Thesis Title	Polyhydroxyalkanoates Production from High Organic Content Wastewater by Mixed Microbial Cultures and Its Techno-economic Analysis
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ABSTRACT

Nowadays, almost 4.8 to 12.7 million-ton plastics made by fossil fuel are entering the ocean from land. One of the potential bio-degradable plastics is Polyhydroxyalkanoates (PHAs). The high organic wastewater as a subtract was studied for reducing the production price of PHAs from Mixed Microbial Consortia. PHAs is mainly composed of PHB and PHV. Pyruvate is one of the precursors to produce Polyhydroxyalkanoates (PHAs) especially for PHV production. In this study, the pyruvate concentrations ranging from 0, 0.25, 0.5 to 1.0 g Pyruvate/L were conducted in Sequencing Batch Reactor (SBR) to enhance the productivities of PHV and PHAs. It was found that the peak production was of 53.6 g PHAs/g VSS (%) and 3,350 mg VSS/L while the pyruvate concentration was 1 g /L, initial COD was 57,440 mg/L, pH was 4.5 and DO was 0.47 mg/L. PHA production system is 994,143 USD for 20 years, and lifetime. The operation cost is 159,711 USD every year. The payback period is 6.21 yesrs, and the internal rate of return (IRR) is 16 %, respectively.

Keywords : Polyhydroxyalkanoates, Mixed Microbial Consortia, Pyruvate, High Organic Wastewater

หัวข้อวิทยานิพนธ์	:การผลิต Polyhydroxyalkanoates จากน้ำเสียที่มีสารอินทรีย์สูง โดยจุลินทรีย์แบบผสมและการวิเคราะห์ทางเทคนิค และเศรษฐศาสตร์
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บทคัดย่อ

ในปัจจุบันพลาสติกเกือบ 4.8 ถึง 12.7 ล้านตันที่ผลิตจากเชื้อเพลิงฟอสซิลกำลังถูกย่อยสลายคืนสู่มหาสมุทร หนึ่งในพลาสติกที่สามารถย่อยสลายทางชีวภาพคือ Polyhydroxyalkanoates (PHAs) โดยการศึกษานี้ได้ทำการวิจัยน้ำเสียอินทรีย์เพื่อลดค่าใช้จ่ายในการผลิต PHAs จากกลุ่มจุลินทรีย์ Consortia แบบผสม ซึ่งสาร PHAs ส่วนใหญ่ประกอบด้วย PHB และ PHV โดย Pyruvate ที่เป็นหนึ่งในสารตั้งต้นในการผลิต Polyhydroxyalkanoates (PHAs) ที่สำคัญสำหรับการผลิต PHV ในการศึกษานี้ได้ทำการศึกษาปฏิกิริยาความเข้มข้นของ Pyruvate ตั้งแต่ 0, 0.25, 0.5 ถึง 1.0 กรัม การศึกษาการใช้ Pyruvate ต่อลิตร ใน Sequencing Batch Reactor (SBR) เพื่อเพิ่มประสิทธิภาพของ PHV และ PHAs พบว่าการผลิตสูงสุดที่ 53.6 กรัม PHAs ต่อกรัม VSS (%) และ 3,350 มิลลิกรัม VSS / L ในขณะที่ความเข้มข้นของ Pyruvate 1 กรัม ต่อ ลิตรค่าซีโอดีเริ่มต้น 57,440 มิลลิกรัม ต่อลิตร ค่า pH เท่ากับ 4.5 และ DO เท่ากับ 0.47 มิลลิกรัม ต่อ ลิตร ท้ายที่สุดแล้วกระบวนการระบบการผลิต PHA มีค่าใช้จ่ายประมาณ 994,143 เหรียญสหรัฐ สำหรับการใช้งานระบบในระยะเวลา 20 ปี ต้นทุนการดำเนินการอยู่ที่ 159,711 เหรียญสหรัฐทุกปี โดยคำนวณระยะเวลาคืนทุน 6.21 ในอัตราผลตอบแทนภายใน (IRR) คือ 16% ตามลำดับ

คำสำคัญ : โพลีไฮดรอกซีอัลคาโนเอต, กลุ่มจุลินทรีย์ผสม, สารไพรูเวท, น้ำเสียอินทรีย์สูง

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CHAPTER 1

INTRODUCTION

Background and Rationale

Nowadays, almost 4.8 to 12.7 million-ton plastics made of fossil fuel are entering the ocean form land. How to decrease the plastics is a big issue in the world. It will increase to twice amount in 2025 compared to year 2015 (Jambeck et al., 2015). Therefore. To find environmentally friendly products to replace petrochemical plastic is play an important role to the community people. One of the potential bio-degradable plastics is Polyhydroxyalkanoates (PHAs). PHAs are synthesized by microorganisms intracellularly. Characteristics of PHAs are similar as fossil fuel-based plastics. But PHAs are more eco-friendly, due to the product made by PHAs can be biodegraded (Serafim et al., 2008). There are many kinds of organic compounds can be the substrate to constituent PHAs. Especially, these organic compounds we can find industrial, agricultural, and even municipal wastewaters (Davidso & Brande, 1981). The process to make PHAs. Not only decrease the Non-biodegradable plastics we need but also fix the waste we must deal with. There are many ways can enhance accumulation of PHAs. Such as control phosphorus, nitrogen, and carbon concentration. Dissolved oxygen (Serafim et al., 2008) and repetitive feast/famine phases in sequencing batch operation (SBR) are also very significant conditions that we can control. PHA is kinds of biodegradable plastic monomer that can be stored in many microorganisms. Some of bacteria can convert the carbon sources into PHAs in the bioreactor. When energy is needed, the ATP obtained by decomposing PHAs. Therefore, PHAs are biodegradable and biocompatible. Compared with PHAs, petrochemical plastics and PLA, PHAs can be completely decomposed without burning and burying. PHAs have been pointed out many advantages in recent years. PHAs are simple processing. PHAs can resistant UV. PHAs will not be dissolved in Water immediately (Gholami et al., 2016, Mannina et al., 2019). PHAs show thermoplastic properties that is up to the substrate and the ways to ferment. PHA are ideal bioplastic to replace some of oil-based plastics such as polyethylene (PE), polyethylene terephthalate (PET) or polypropylene (PP) (Bugnicourt et al., 2014). PHA monomer is composed by (R)-Hydroxy fatty acid. Figure 1.1 (Sharma et al., 2021) is the basic structure of PHA. The units are connected by ester bonds, and each monomer has a side chain R group. Namely saturated alkyl or unsaturated alkyl substituted alkyl and branched alkyl. In this study, most of PHA is consisted by PHB and PHV. HB is hard and Heat intolerant, because don't have enough long chain. The High molecular polymer of PHBV is Polymerized by 3-HB and 3-HV. PHBV is an ideal bioplastic material. It has not only Sufficient mechanical strength, but also resilient (Gahlawa & Soni, 2017).

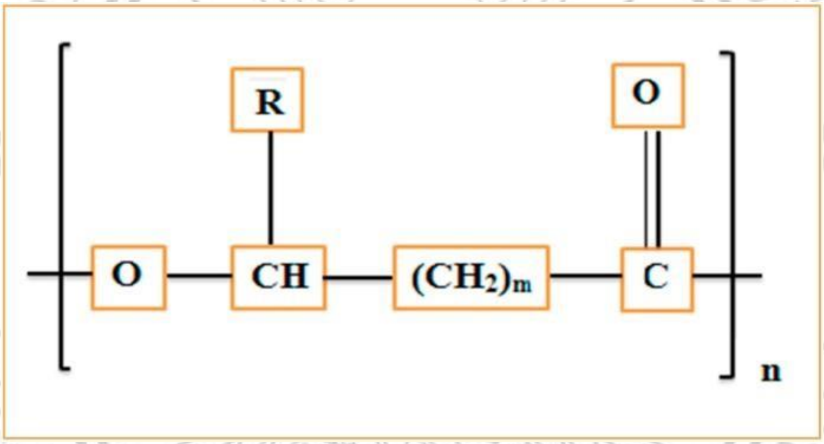

Figure 1.1 Structure of PHAs

So far, most studies force on how to use pure cultures to accumulation PHAs or use pure substrates as acetate and glucose to produces PHA (Serafim et al., 2008). Currently, the products from the big scale are still too expensive. In fact, the prize of PHAs polymer around 2.2 to 5.0 €/kg. But the prices of conventional oil-based plastic are always lower than 1 €/kg. The prize of PHA is more than three times higher than the polymer from petrochemical resources (Gholam et al., 2016, Mannin et al., 2019). Many studies show one of the reasons is downstream process (DSP). Most of the DSP cost higher 50% of the PHA production (Perez-Rivero et al., 2019). These ways need very methods cannot be commercialized and requires very high costs to maintain operation. Using mixed microbial cultures (MMCs) to produce PHAs is one way to reduce the cost from the process. MMCs not only don't need aseptic operation but also have bigger range to metabolic different substrates. This characteristic can allow them to deal with cheaper feedstocks (Carvalho et al., 2014).

In this research, batch experiments are conducted under different ratio of the mix substrate. Using the waste of acid production reactor with waste of hydrogen production reactor from our lab. There are two different sludges. One of them is the aerobic MMCs from waste treatment of pig from the south of Taiwan. One of them is the aerobic MMCs of Municipal wastewater from Feng Chia. Comparing these two different sludges which one can produce PHAs more efficient in batch. Feast and famine (F/F) is a very significant condition. Use Sequencing batch reactor to accumulate PHAs in different periods of F/F.

Research Objectives

1. To compare the three different sludges from Taiwan with high organic content wastewater to produce polyhydroxyalkanoates (PHA).

2. To compare the ratios of two different substrates to produce polyhydroxyalkanoates (PHA).

3. To add pyruvate in the substrate to optimize the production of PHA

4. To perform techno-economic analysis which includes the net present value (NPV), benefit cost ratio (BCR) payback period and the internal rate of return of polyhydroxyalkanoates (PHA) Production technology.

Research Hypotheses

1. The inoculum source influences anaerobic digestion process.

2. The type of substrate has an effect on PHA production via anaerobic digestion.

3. Pyruvate can enhance PHA yield during anaerobic digestion.

4. The PHA production has both technical and economic possibilities to be scaled up.

Research Scope

To compare the PHA production of three different sludges in Taiwan. To find the best ratios of substrate to produce PHA by two kinds of acidification efferent. To find the best amount of additional pyruvate to upgrade PHA production. To calculate techno-economic of PHA production.

Scope of Content

For Polyhydroxyalkanoates (PHA) Production by Mixed Microbial Cultures from High Organic Content Wastewater will run at Feng Chia University. This research is divided into three parts to carry out the experiment. The first part is the experiment of PHAs production test by three different aerobic sludges from Feng Chia University Wastewater treatment system, Tainan pig farm, Chiayi pig farm. The substrate was obtained from Effluent from sucrose acidification fermenter and Effluent from Molasses waste acidification fermenter. The ratios of these two effluents were 1:1. The second part is to use the sludge from the aerobic pool of the Tainan pig farm as the seed sludge, and the effluent of sucrose acid fermenter and molasses wastewater acid fermenter at different ratios 0:1, 1:1, 3:1, 1:3, To carry out the PHAs production experiment. The third part is to use the sludge of aerobic pool of the Tainan pig farm as the seed sludge. The substrate was obtained from Effluent of acidification fermenter. Adding 0g, 0.25g, 0.5g, and 1g Pyruvate in each reactor when each cycle being. To conduct PHA production experiments.

Definitions of terms

This study focuses to optimize polyhydroxyalkanoates (PHA) production by mixed Microbial cultures from high organic content wastewater, and to perform Techno-economic Analysis of polyhydroxyalkanoates (PHA) Production technology. The author design three parts of experiment and Techno-economic Analysis of PHA to prove that PHA is a feasible solution.

Conceptual Framework

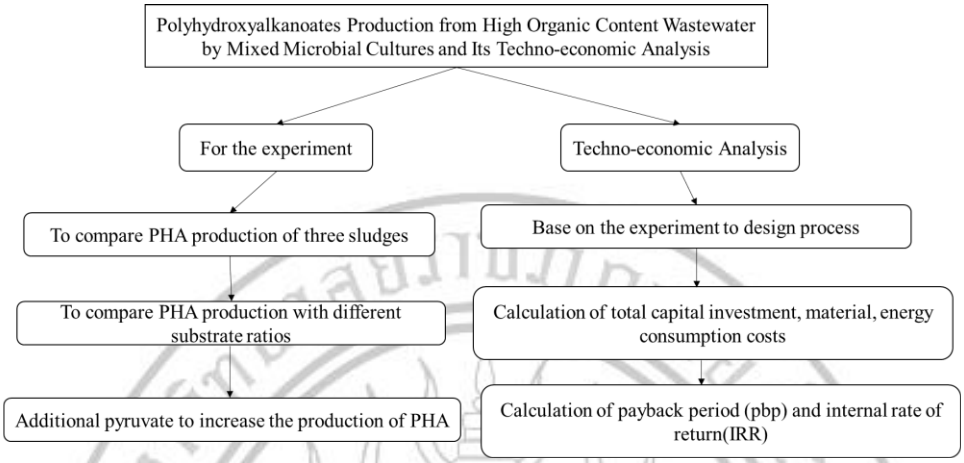

Figure 1.2 Conceptual Framework

CHAPTER 2

LITERATURE REVIEW

This chapter is about four main conditions (bacteria source, substrate, dissolved oxygen, pH) which can affect PHA production.

Bacteria Source

PHAs are polymers produced by organisms, which are aggregated by a variety of organisms. When carbon sources are lacking, such as some archaea and certain bacterial groups, gram-positive bacteria, gram Pha-negative bacteria, photosynthetic bacteria and a mixture of different microorganisms can accumulate PHA under aerobic and anaerobic conditions. It has now been found that more than 150 species of bacteria have the ability to produce PHAs (Mitra et al., 2020). When PHAs are produced in MMC, they do not need to be produced in a sterile environment. Compared with the single metabolic path of pure bacteria, the diversified metabolic pathways can reduce the production cost of PHAs and can use diversified metabolic pathways. Substrate source, such as industrial or agricultural wastewater (Carvalho et al., 2014). When cultivating mixed bacteria to produce PHAs, the repeated feast and famine are often used to stimulate the ability of the MMC to produce PHAs (Sabapathy et al., 2020).

In recent years halophiles have attracted the interest of many scientists. This group of bacteria is a unique and diverse group, capable of living in high-salt environments, such as salt lakes, salt pans, and salt marshes. They are roughly distributed in three different strains, Bacteria, Archaea, Eukarya (Edbeib et al., 2016). Therefore, under the characteristics of halophilic bacteria, They can adapt to the environment of high concentration of potassium and high concentration of organic matters (Youssef et al., 2014). In addition, the hydrolytic enzymes (including amylase, cellulase, protease and xylanase) secreted by halophilic bacteria are also very useful for environments with high concentrations of organic matters. The most important thing is that almost all halophilic bacteria have the ability to accumulate PHAs in the cell(Mitra et al., 2020). Yeasts and fungi in sludge have the ability to produce and synthesize biopolymers (triacylglycerol, TAG), wax esters and PHAs, especially under conditions that limit nitrogen or phosphorus (Kumar et al., 2018). PHAs production process, through the MMC that produces PHAs in the reactor, use a matrix source of high content of organic acids to convert them into PHAs (Ghosh & Chakraborty, 2020) (Valentino et al., 2020). Figure 2 shows that Metabolic pathway of PHAs production by microorganisms. When organic wastewater is used as a substrate and co-fermented with MMC to produce PHAs, it can be roughly divided into three stages: (1) Hydrolyze and acidify the substrate to obtain the carbon source and volatile organic acid that can be used by MMC (2) Find its rapid production PHAs flora (3), extract and purify PHAs at the PHAs concentration that bioaccumulates the most. In the experiment of producing PHAs, the main challenge is how to choose MMC that can store high concentration of PHAs (Argiz et al.,2020, Albuquerque et al., 2010). Using MMC to produce PHA has a number of advantages. MMC can produce PHA from a variety of wastewater substrates (Lorini et al., 2021). Conditions are easier to manage when MMC is used to produce PHA (Valentino et al., 2016). MMC is a less costly way to produce PHA (Wang et al., 2020).

In order for MMC to effectively produce PHAs, many scientists will use continuous batch reactor (SBR) to train MMC to produce more PHA (Albuquerque et al.,2010, Third et al.,2003, Beccari et al., 2009).

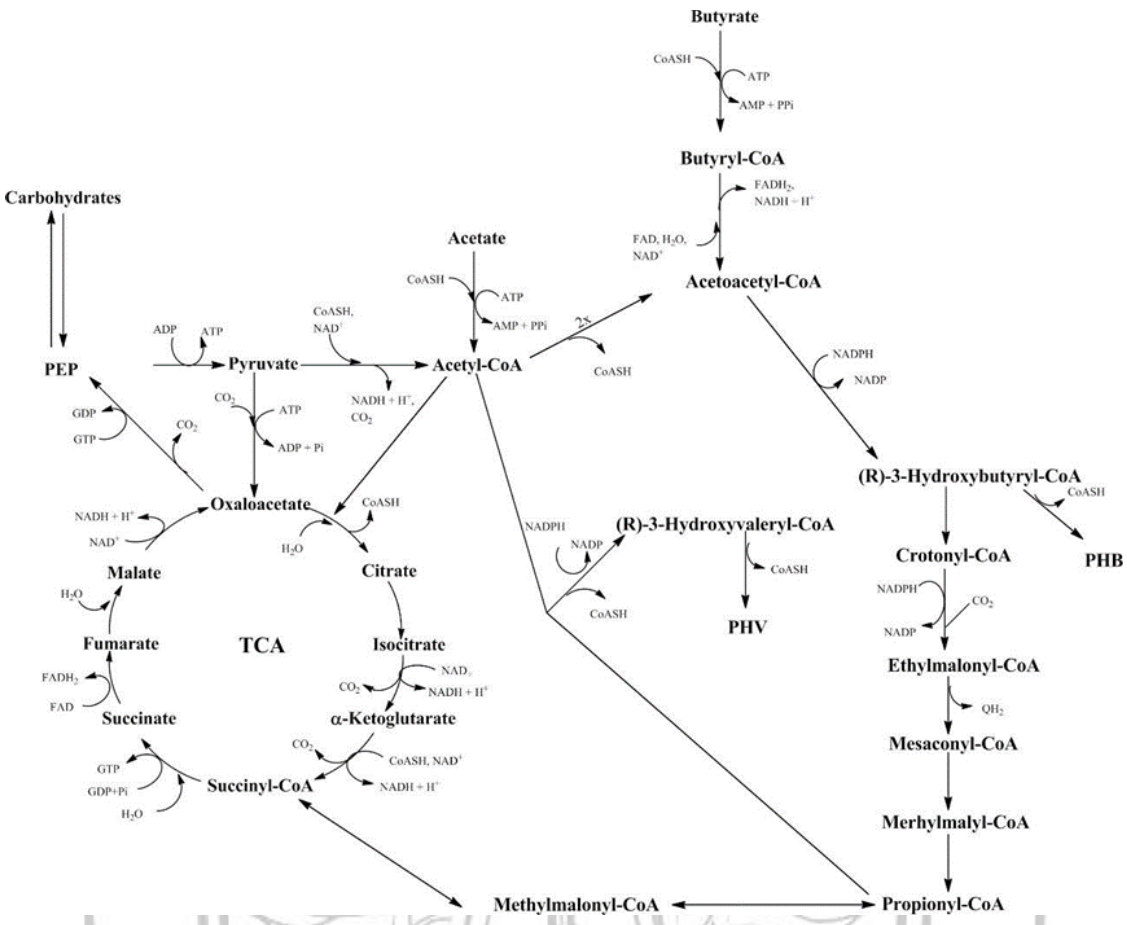

Figure 2.1 Metabolic pathway of PHAs production by microorganisms

Substrate

In recent years, most research are using MMC to produce PHAs. The content of substrates are mostly organic acid. The by-products of many organic waste water are rich organic acid and carbon source (Coats et al., 2007), olive oil factory wastewater (Beccari et al., 2009), molasses wastewater (Carvalho et al., 2014), pulp wastewater (Bengtsson et al., 2008) that can be used by MMC. The use of these wastewater as a substrate is to effectively reduce the production cost of PHAs. However, in order to make these wastewater sources can be used effectively, it is necessary to decompose the larger carbon source into useful organic acids after acidification pre-treatment (Albuquerque et al., 2010).

The common substrates for PHAs are volatile organic acids (Wang et al., 2017) and crude glycerin. Among them, acetic acid and butyric acid are mostly used to produce PHB, while propionic acid and valeric acid can be produced as PHV (Tu, Zhang, &Wang, 2019, Wang et al., 2018), When propionic acid is used to produce PHAs, it will inhibit the growth of MMC, so this is why the concentration of PHV in PHAs is lower than that of PHB.

Pyruvate is an important reactant found at the crossroads of glycolysis and oxidative phosphorylation. Pyruvate can be converted to acetyl-CoA by pyruvate dehydrogenase (PDH), which then enters the citric acid (TCA) cycle. Pyruvate is converted to lactate by cytoplasmic lactate dehydrogenase during glucose fermentation (anaerobic glycolysis) (LDH) (Rao et al., 2021). Acetyl-CoA and lactate can be the substrates to produce PHA.

One of the pyruvate metabolic pathways suggests PHAs production directly proportional to the pyruvate addition. The pathway which carries out the generation of PHB appears to be directly proportional to the amount of pyruvate being added. (Guerra-Blanco et al., 2018). in particular, Pyruvic acid can boost PHV synthesis. The structure of PHV differs from that of PHB; thus, a higher PHV concentration allows PHA to have reduced crystallinity and more flexibility (Siracusa & Blanco, 2020).

Dissolved Oxygen

Although the production cost increases in an aerobic environment, there are many points that indicate that the degree of dissolved oxygen is related to the production of PHAs. When using SBR to produce PHAs, dissolved oxygen is a very important indicator. In many studies, they showed different DO levels during feast and famine (Third et al., 2003). According to the change of its dissolved oxygen, its replacement cycle of supernatant (Wang et al., 2017).Table 1 shows using VFA to produce PHA (Tu et al., 2019). When the oxygen supply is less, cell growth needs to use more ATP to accumulate PHA. So the efficiency way to accumulate PHA is to use Butyrate and Valerate. On the other hands, when the oxygen supply is more, PHA be accumulated which don’t need so much ATP. Acetate and propionate can be started to use (Wang et al., 2017). Therefore, Therefore, dissolved oxygen is an extremely important indicator in the period of F/F.

Table 2.1 Using VFA to produce PHAs chemical

Reaction	Stoichiometry
Acetate → Acetyl-CoA	CH_2O + ATP → $CHO_{0.5}$ + 0.5H_2O
Propionate → Propionyl-CoA	$CH_2O_{2/3}$ + 0.67ATP → $CH_{4/3}O_{1/3}$ + 0.33H_2O
Butyrate → Acetyl-CoA	$CH_2O_{0.5}$ + 0.5ATP → $CHO_{0.5}$ + 0.5NADH
Valerate → Acetyl-CoA	$CH_2O_{0.4}$ + 0.4ATP → 0.4$CHO_{0.5}$ + 0.6$CH_{4/3}$
+ Propionyl-CoA	$O_{1/3}$ + 0.4NADH

Source: Tu et al., 2019

pH

pH is one of the important conditions for biological activity. Regarding the research of microbial production. pH will lead the products to be produced by organisms. In the process of producing PHAs, pH plays a very important role. Many sciences have studied the production of PHAs from MMC by pH (Chua et al., 2003, Villano et al., 2010), and pointed out that when the pH is 8.0-9.0, the accumulation of PHAs in the organism can be the best, and when the pH is 6.0-7.0, The bioaccumulation of PHAs activity decreases (Kourmentza & Kornaros, 2016). It is also observed that the production efficiency of HV is the best when the pH is 7.5-9.0.

Techno-Economic Analysis

In the PHA production system, there three processes (acidification, PHA accumulation and downstream process) must be included. The first of PHA production is acidification process (Salehizadeh & VanLoosdrecht, 2004). In this process, the big carbon sources will be digest to the VFA or small carbon sources. Acidification process fermented under the anaerobic condition. The effluent with high VFA can be substrate of PHA accumulation process.

After acidification process, MMC can use the high VFA reactant to accumulate PHA. PHA accumulation is under aerobic digestion in SBR. After the PHA accumulation, PHA-rich biomass is obtained. But the PHA cannot be used when it is in the cell.

The downstream process (DSP) is the way to extract the PHA out. The efficient DSP play an important role. PHA DSP is include five steps as Fig 2.2 (del Oso et al., 2021). It started biomass separation with physical ways. And then extraction, there are two ways: (1) solubilizing the non-cellular PHA mass though chemical digestion or mechanical disruption. (2) solubilizing the PHA through solvent extraction. After the step of extraction. The PHA will be precipitation, filtration, or sedimentation, or more alternatives for higher-value products such as liquid-liquid extraction or air classification. The last step, PHA need to be purified by redissolution with water or ethanol. High quality PHA can be produced (Koller et al., 2013, Saavedra et al., 2020)

There are many different DSP, alkali-surfactant (Jiang et al., 2015), surfactant-hypochlorite (Jacquel et ai., 2008), dichloromethane solvent (Gurieff & Lant, 2007). Dichloromethane (DCM) solvent DSP is a very efficient way to extract intracellular PHA. The DCM can get not only 99.9 wt% purity PHA but also the boiling point is 40 degrees. The low boiling point is easy to recycle the solvent and no need too much energy to maintain all process. The extraction rate of PHA from the biomass is around 82.2% (Fernández-Dacosta et al., 2015).

After understanding the production process of PHA, techno-economic analysis is an important indicator of commercialization. In the General, the analysis of economic feasible ability of system. The cost will be divided in two parts: Capital costs and Operation costs. Capital costs include land, buildings, construction, and equipment used in the production of goods or in the rendering of services. Operation costs include which have immediate effect on the production rate, raw materials, operating labor and utilities costs, maintenance of the operations. The general costs include overheads for (Shahzad et al., 2017).

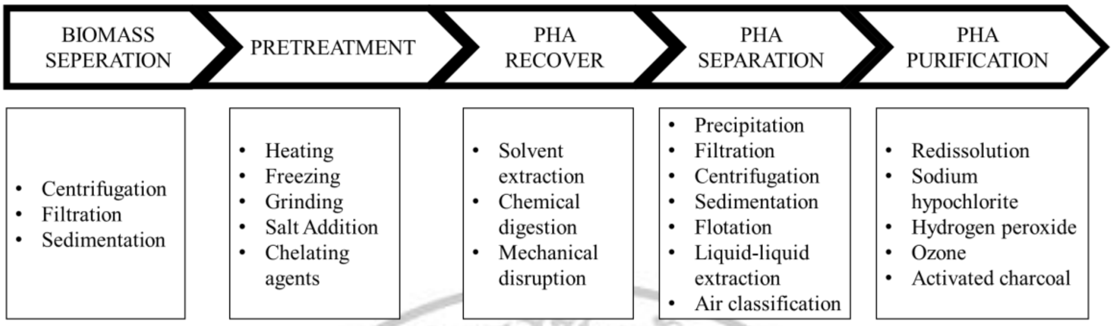

Figure 2.2 Conventional steps in the PHA downstream processing

CHAPTER 3

RESEARCH METHODOLOGY

Research Design

This research is divided into three parts to carry out the experiment. The first part is the experiment of PHAs production test by three different aerobic sludges from Feng Chia University Wastewater treatment system, Tainan pig farm, Chiayi pig farm. The substrate was obtained from effluent from sucrose acidification fermenter and effluent from Molasses waste acidification fermenter. The ratio of these two effluents were 1:1. To carry out the PHA production experiment.

The second part is to use the sludge from the aerobic pool of the Tainan pig farm as the seed sludge, and the effluent of sucrose acid fermenter and molasses wastewater acid fermenter at different ratios control set, 1:1, 3:1, 1:3. To carry out the PHAs production experiment.

The third stage is to adopt the best seed from second part as the seed sludge. The substrate was obtained from Effluent of acidification fermenter. Control set, adding 0.25g, 0.5g, 1g Pyruvate in each reactor when each cycle being. To conduct PHA production experiments.

These three experiments all used reactors with a volume of 250mL. The working volume was 100 mL, and the temperature was controlled at 30°C. Fermentation under aerobic conditions is used in this experiment. When the pH is less than 6, or when the PHA concentration has stabilized. The experiments were stopped. Each cycle time was 24 hours. After 22.5 hours, the reaction is stopped for half an hour to precipitate, and 50 ml is extracted in 15 minutes. The supernatant is analysis including pH, DO, and VFA. Bottom solution was taken for another 15 minutes. Bottom solution was analyzed the PHAs content and VSS. Fresh substrate was added in the last half an hour. This cycle is continuous for one week. the hydraulic retention time (HRT) is 2 days, and the sludge retention time (SRT) is 4 days. Observe the changes in water quality and the changes in the content of PHAs in the biomass. The hole experiment process is Figure 3.1.

For the techno economic analysis, first step is to check the bioconversion process from the input wastewater to PHAs product. The process of PHA production in this study consists of three parts including acidification, PHA accumulation, and downstream process as presented in figure 3.2. The lifetime of the equipment is assumed to be 20 years of PHA production system as recommended by (Fernández-Dacosta et al., 2015) (Shahzad et al., 2017).

The economic feasibility of the two process options was analyzed by considering capital costs, and operation costs. The capital costs included land, buildings, construction, and equipment used in the production of PHAs. The operation costs include maintenance, labor, utilities and material every year (Fernández-Dacosta et al., 2015).

In this study, there were four different indicators applied to determine the economic feasibility of the project. First, Net present value (NPV) is the difference between the net present benefit (NPB) and the net present cost (NPC) presented in equation (1). When NPV is positive, it proves that the project can be invested (Gunjal & Amankwah, 1999).

$$NPV=NPB-NPC \tag{1}$$

Second, Benefit Cost Ratio (BCR) is calculated by using equation (2) the ratio of the two present values. Thus, When the BCR is bigger than one, the proposed project is considered financially feasible (Gunjal &Amankwah, 1999).

$$BCR=NPV/NPC \tag{2}$$

Third, the payback period (PBP) of the PHAs production system will be determined. The payback period refers to the amount of time it takes to recover the cost of an investment (Shahzad et al., 2017).

PBP= Initial Investment/ Net Cash Flow per Period (3)

Fourth, the Internal rate of return (IRR) which is the discount rate that makes the net present value (NPV) of zero. The calculation was performed using Microsoft Excel. When determined, the rate is compared with the interest rate in Taiwan. If IRR is bigger than this global interest rate then the investment is feasible (Gunjal & Amankwah, 1999).

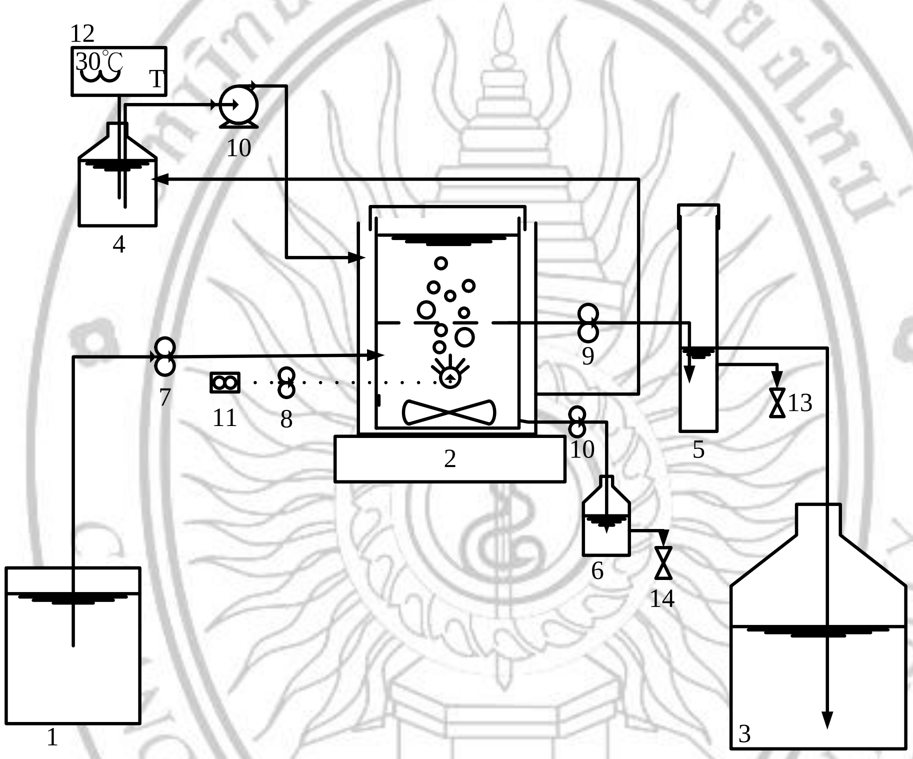

Figure 3.1 Schematic diagram of PHAs production process.

Table 3.1 Components of Schematic diagram of PHAs production process.

Contents	Number
Substrate Container	1
Sequencing batch reactor	2
Effluent container	3
Constant temperature water bath	4
Sample storage tank	5, 6
Pumps	7, 8, 9, 10
Gas pump	11
Thermostat	12
Sample point	13, 14

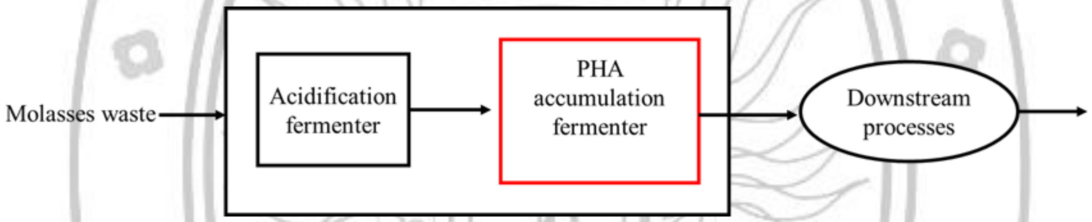

Figure 3.2 Schematic diagram of PHA polymer production process.

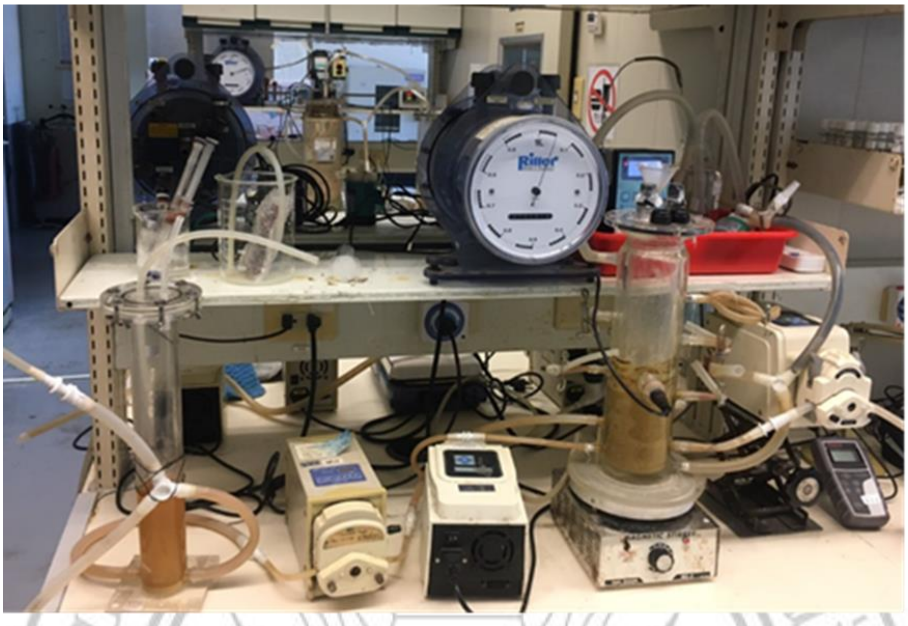

Figure 3.3 Photo of PHAs production process in the laboratory

Population and Sample Group

The substrate will be obtained from the effluent of sucrose acidification fermenter and effluent from molasses waste acidification fermenter. The substrate quality analysis for pH, COD, TS, VS, VSS and VFA was shown in Table 1. The ratio of effluent from sucrose acidification fermenter mixed with efferent from molasses waste acidification fermenter was also studied. The VFA analysis of different ratio for effluent from sucrose acidification fermenter and effluent from molasses waste acidification fermenter was shown in Table 3.2.

Three kind of seed sludges were selected from Tainan pig farm, Chiayi pig farm and wastewater treatment system of Feng Chia University. The initial values of COD, pH, TS and VS were analyzed before experiments as Table 3.3.

Table 3.2 Water quality analysis for substrate

Substrate	Efferent from sucrose acidification fermenter	Efferent from Molasses waste acidification fermenter
pH	5.63	6.02
COD (mg/L)	26,600	25,600
TS (mg/L)	211,960	227,260
VS (mg/L)	208,100	217,480
VSS (mg/L)	10,000	11,200
Acetic acid (mg/L)	1,112	4,087
Propionic acid (mg/L)	4,789	2,430
Butyric acid (mg/L)	3,465	8,151

Table 3.3 Water quality analysis for seed sludge

Seed Sludge	Wastewater treatment system, Feng Chia University	Tainan pig farm	Chiayi pig farm
pH	7.04	7.49	7.21
COD (mg/L)	17,500	19,200	18,500
TS (mg/L)	9,730	12,790	4,430
VS (mg/L)	8,580	7,310	2,290
VSS (mg/L)	10500	6000	4500

Research Instruments

pH Electrode (BROADLEY-JAMES CORP), syringe and circulating pump, Gas Chromatography with flame ionization detector (GC-FID), electronic balance (BP221s, Startorius), Centrifuge (CN-1050, HSIANGTAI), High temperature oven, DR1900 Portable Spectrophotometer. These equipments will used in this study. The photos of the equipment were in the appendix. The chemicals used in this study was listed in the Table 3.4.

Table 3.4 The list of chemicals

Chemicals	Chemical's source
NaOH, Ag_2SO_4, H_2SO_4	UNION CHEMICAL WORKS LTD.
$CHCl_3$,CH_3OH_2, $CH_3COCOOH$, PHA	Uni-onward

Data Collection

In this study, Total solids and total volatile solids (TS & VS) were analyzed based on (Kopp et al., 1979). Total Suspended Solids (TSS), Volatile Suspended Solids (VSS) were determinded base on (Kopp et al., 1979), Chemical Oxygen Demand (COD) was quantified based on (Geerdink et al.,). Finally, VFA and PHA analysis will be based on (Tu et al., 2019).

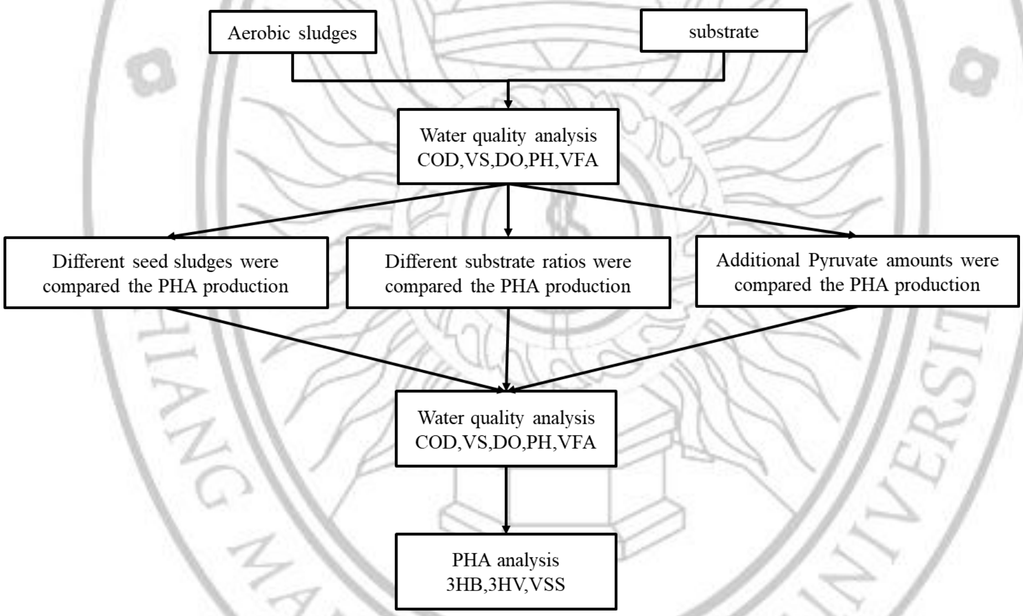

Figure 3.4 Experimental procedure of the PHAs production.

CHAPTER 4

RESULTS AND DATA ANALYSIS

Seed Sludge

In this study, three kinds of seed sludges were selected from Tainan pig farm, Chiayi pig farm and wastewater treatment system of Feng Chia University. These sludges were fed in the different SBR to compared production of PHA. The substrate was proportioned 1:1 from the effluent of sucrose acidification fermenter and effluent from Molasses waste acidification fermenter. Temperature was controlled around 30 degrees Celsius. HRT was 2 days. SRT was 4 days.

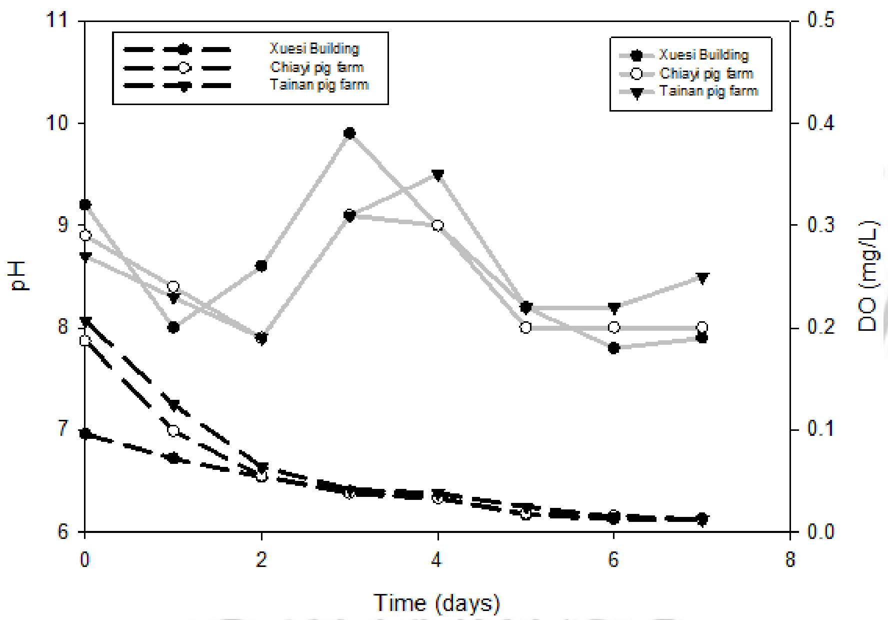

Figure 4.1 Changes of dissolved oxygen and pH of different sludge in continuous batch reactor with time

According to figure 4.1, when experiment started, DO of three different reactors were around 0.3 mg/L. After 3 days, the effluent of three reactors Significant raised. This reaction showed the bacteria in the reactors started to storage PHA in the cell. In this study, aerobic sludge was the seed sludge. When DO concentration rise, the reactions were in femine stage (Wang et al., 2017). After six days, DO concentration dropped. Because pH of three reactors dropped, PHA storage of bacteria decreased activity. As Yi Zhanga (Zhang et al., 2018) study shows, pH was a significant conditions of PHA storage. When the pH was 5, the PHAs-producing bacteria were inactive. When the pH was above 6.5, it can maintain its ability to produce PHA. As figure 4.1, pH dropped to the fifth day. Below 6.5, this was one of the main reasons for the decrease in the amount of bioaccumulated PHAs.

The temperature was kept at a constant 30°C. Because the sludge came from a wastewater treatment plant. The majority of these sludge microorganisms were mesophilic. 30-35°C was ideal for mesophilic microorganisms (Lin et al., 2018). The average temperature in Taiwan was around 30°C. Under 30°C, PHA accumulation was easily controlled. Also, according to (Tu et al., 2019), maintained a temperature of 30°C.

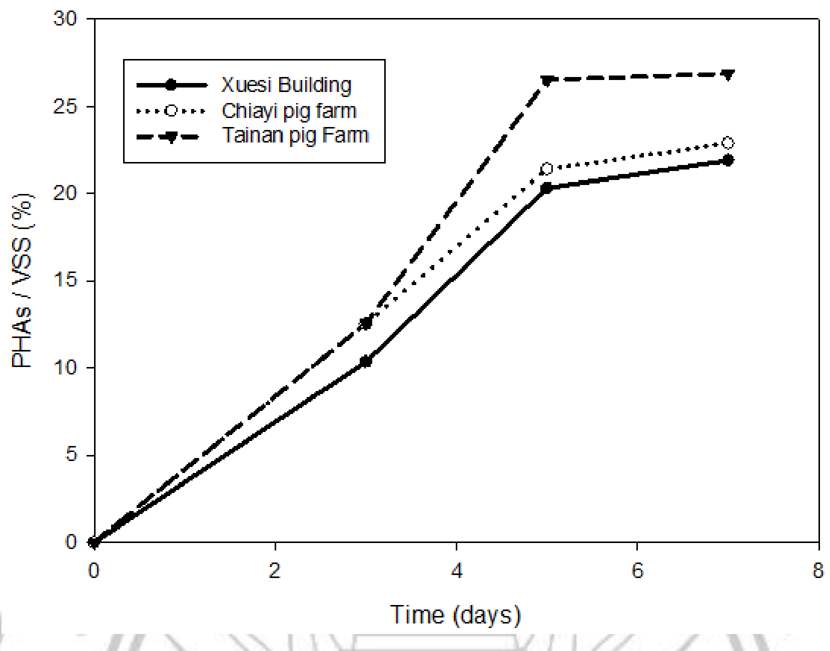

Figure 4.2 Changes of PHAs concentration of different sludge in SBR with time

From Figure 4.2, PHA concentration of three reactor had a linear increase trend in the first five days. Sixth day and seventh day, PHA concentration of three reactors increased tends to be stable. Each of three seed sludge of the highest PHA concentration from Tainan pig farm, Chiayi pig farm and wastewater treatment system of Feng Chia University were 26.88%, 22.88%, 21.90%. Tainan pig farm sludge was the best sludge to accumulate PHA. In this study, the aeration tank sludge of the Tainan pig farm was used as seed sludge for follow-up research.

Effect of Substrate Ratio

In this study, the ratios of the two substrate from the efflent of sucose acidfidication fermenter and the efflent from molasses waste acidfidication fermenter were control set, 1:1, 3:1, 1:3. This experiment was run in the SBR to accumulate PHA. Seed sludge were obtained from Tainan pig farm. Temperature was controlled around 30 degrees Celsius. HRT was 2 days. SRT was 4 days.

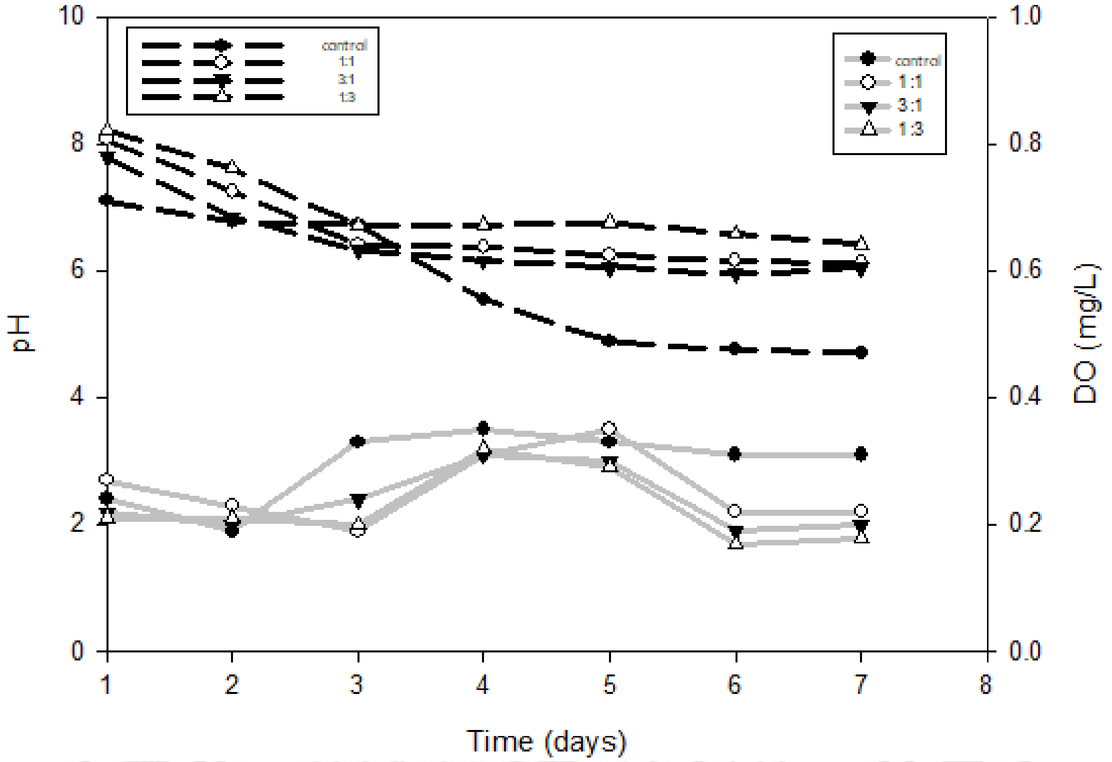

Figure 4.3 Changes of dissolved oxygen and pH with time in a continuous batch reactor with different substrate ratios

According to table 4.3, pH dropped and DO raised during second day to fourth day. This phenomenon showed the bacteria in these four reactors storage to produce PHA (Wang et al., 2017).

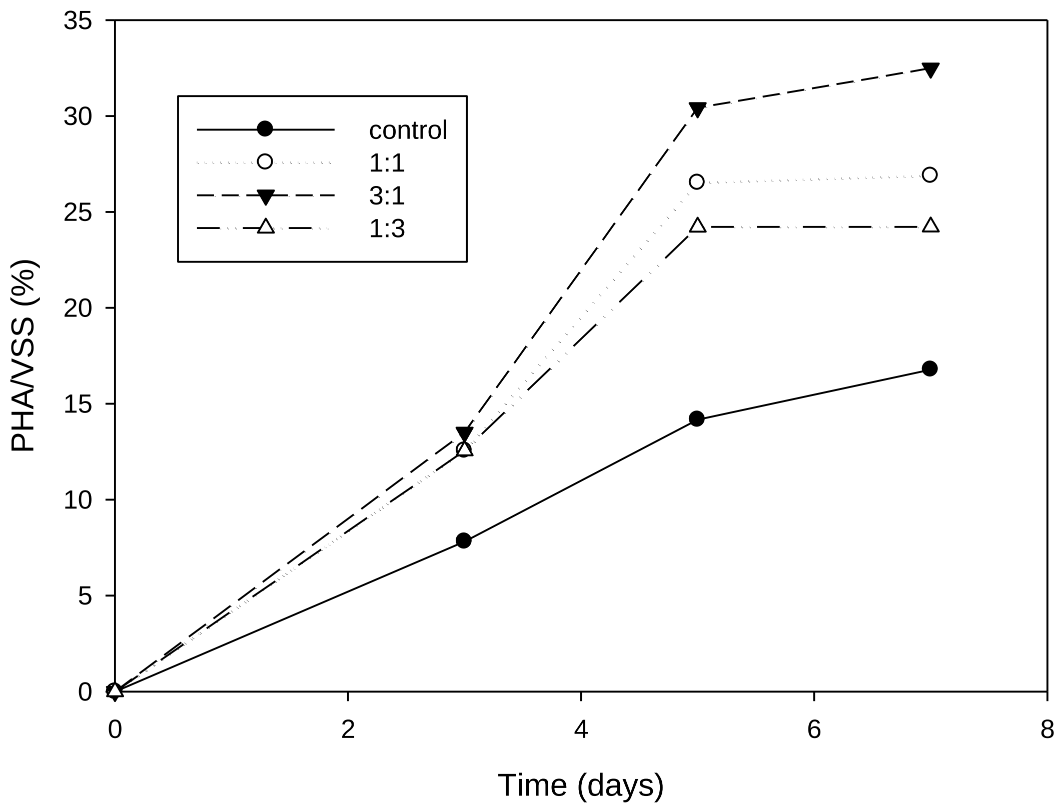

Figure 4.4 Changes of dissolved oxygen and pH with time in a continuous batch reactor with different substrate ratios

Study of Weiming Tu (Tu et al., 2019) maintained acetic acid and butyric acid can increase the content of PHB. Propionic acid inhibits the activity of PHAs flora. So The substrate of high content acetic acid and butyric acid can increase PHAs content. In Figure 4.4, the substrate contained more Efferent from Molasses waste acidification fermenter can accumulate higher PHA concentration. In table 4.4, the efluent from Molasses waste acidfidication fermente had more acetic acid and butyric acid than the eflurent of sucose from acidfidication fermenter. The efluent from Molasses waste acidfidication fermente had less propionic acid than the eflurent of sucose from acidfidication fermenter. These two reasons can explain the substrate contained had more fluent from Molasses waste acidfidication fermenter had ability to produce higher content of PHA. On the 7th day, the ratios of the efluent of sucose acidfidication fermenter and the eflurent from molasses waste acidfidication fermenter were control set, 1:1, 3:1, 1:3. the PHA concentrations were 18.33% and 24.21%, 26.88%, 32.48%.

Metabolites

The substrate was proportioned 1:1 from the effluent of sucrose acidification fermenter and effluent from Molasses waste acidification fermenter. Seed sludge was selected from Tainan pig farm to accumulate PHA. After feeding substrate, experiment was analyzed VFA metabolism at eighth day.

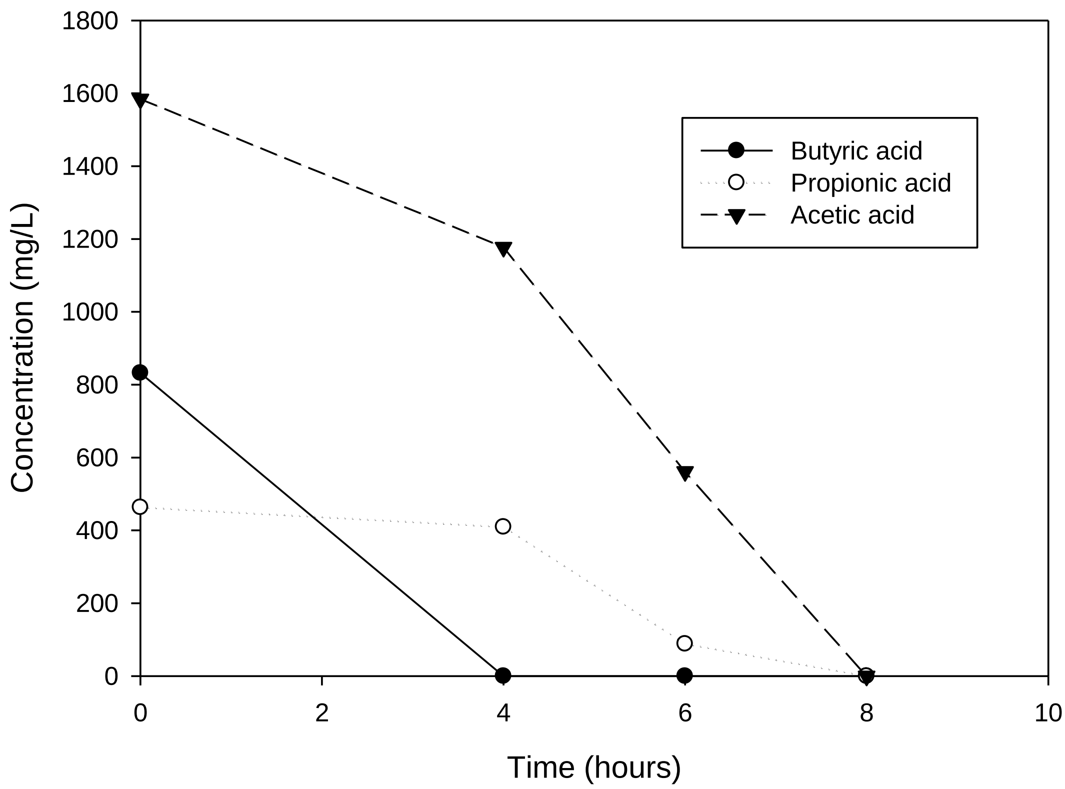

Figure 4.5 The concentration of acetic acid, propionic acid, and butyric acid changed with time when the substrate ratio is 1:1

As Figure 4.5, acetic acid, propionic acid, and butyric acid were Completely metabolized at eighth hour. Metabolic pathway of acetic acid and butyric acid is PHB. Metabolic pathway of propionic acid PHV. But propionic acid leads to reduce the production efficiency of PHA (Tu et al., 2019). The substrate was used in this experiment with high concentration of acetic acid and butyric acid. Therefore, PHA can be increased quickly.

Pyruvate Effect

The effluent of sucrose acidification fermenter was used as substrate in this research. To Observed Metabolism of pyruvate to produce PHA. Control set, 0.25g, 0.5g and 1g of Pyruvate was added in different SBR after adding the substrate. Seed sludge were obtained from Tainan pig farm. Temperature was controlled around 30 degrees Celsius. HRT was 2 days. SRT was 4 days.

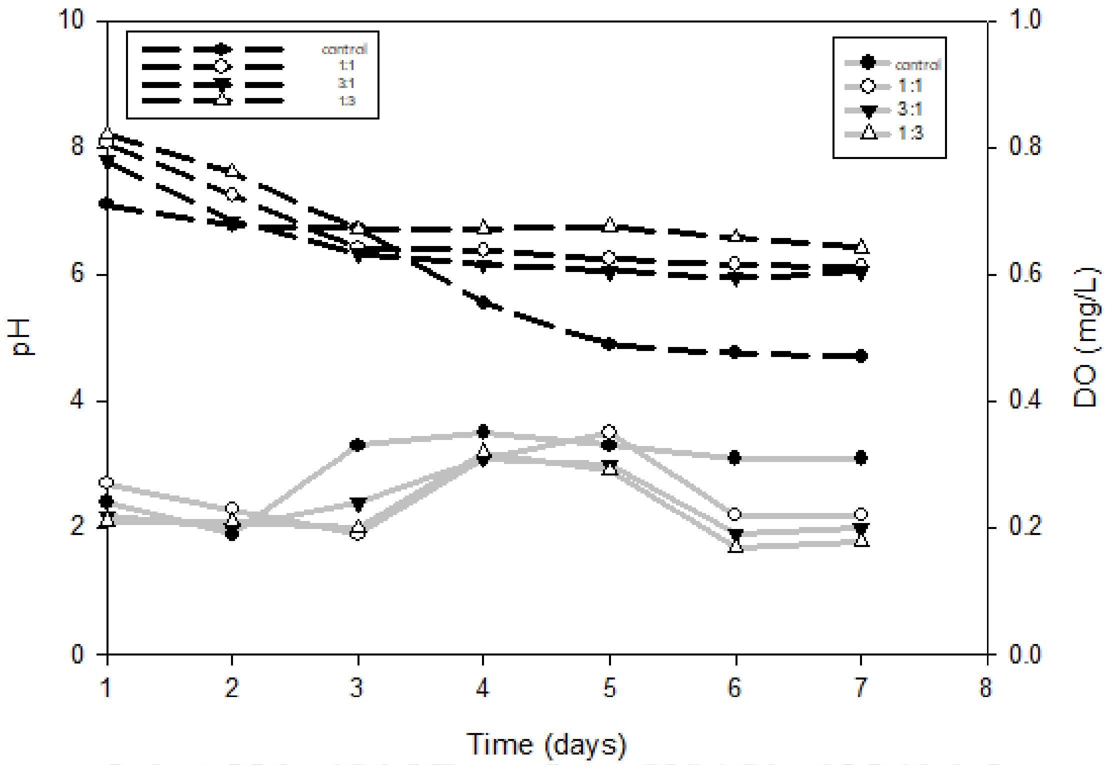

Figure 4.6 Changes in dissolved oxygen and pH with time in continuous batch reactors with different amounts of pyruvate

As figure 4.6, pH dropped and DO rised druning second day to fourth day. This phenomenon showed the bateria in these four reactors storaged to produce PHA (Wang et al., 2017, Albuquerque et al., 2010). As figure 4.7d, PHA accumilation was getting slow after three days that showed the same result.

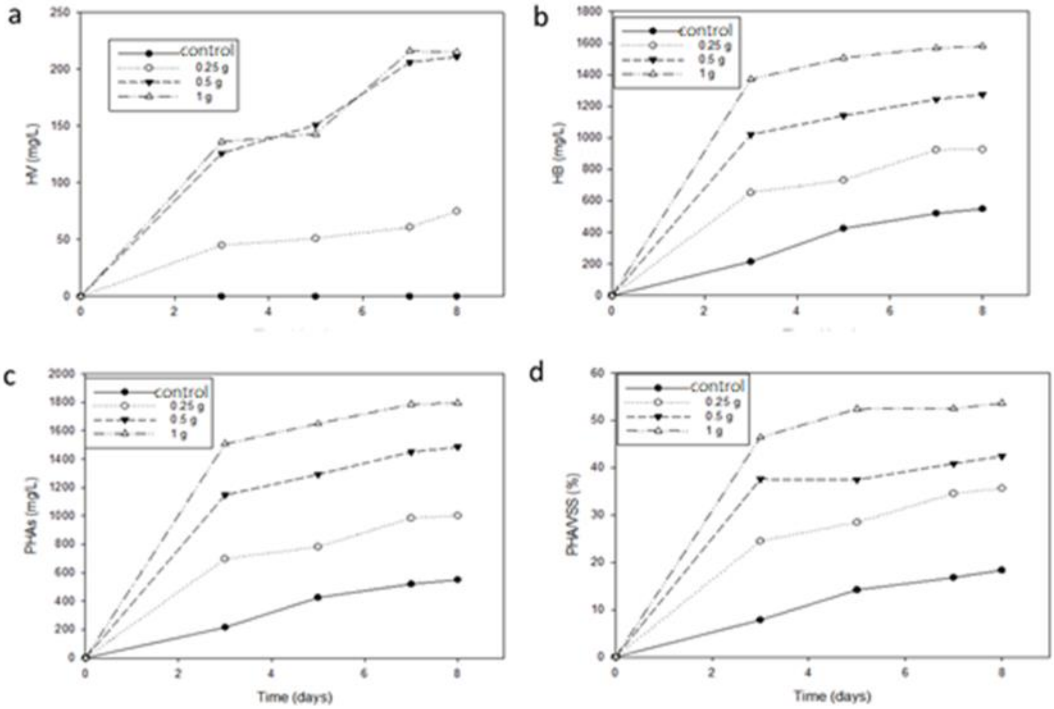

Figure 4.7 The change of PHB, PHV, PHAs, PHAs/VSS in continuous batch reactor by adding different amounts of pyruvate with time

As figure 4.7a, when the added amount of pyruvate was control 0.25 g, 0.5 g, and 1 g, the PHV concentration on the eighth day was control, 75 mg/L, 221 mg/L, and 215 mg/L. There was no PHV production when we added 0g pyruvate. 0.25g, 0.5g, 1g of pyruvate were added in SBR. PHV was found in each reactor. One of the Pyruvate Metabolic pathway was PHV (Guerra-Blanco et al.,2018). Additional pyruvate indeed increased production of PHV. However, Additional 0.5g and 1g pyruvate almost produced same amount of PHV. This result can prove Additional 0.5g pyruvate was the most value amount in 100mL working volume. As figure 4.7b, when the added amount of pyruvate was control set, 0.25 g, 0.5 g, and 1 g, the PHB concentration on the eighth day was control, 926 mg/ L, 1274mg/L, 1580 mg/L. Pyruvate was added more in the SBR, PHB was higher. Because Pyruvate was an important reactant found at the crossroads of glycolysis and oxidative phosphorylation. Pyruvate can be converted to acetyl-CoA by pyruvate dehydrogenase (PDH) (Rao et al., 2021). acetyl-CoA was one of the substrate can produce PHA (Tu et al., 2019). The more pyruvate added, the more

PHB produced. Because one of the Pyruvate Metabolic pathway was PHB (Guerra-Blanco et al., 2018). As figure 4.7c, the total amount of PHB and PHV was PHA in this study. Pyruvate was added more in the SBR, PHB was higher. When the added amount of pyruvate was control, 0.25 g, 0.5 g, and 1 g, PHA concentration on the eighth day was 550 mg/L, 1001 mg/ L, 1485 mg/L, 1795 mg/L. The main product PHA was PHB. As figure 4.7d, When the added amount of pyruvate was control, 0.25 g, 0.5 g, and 1 g, PHAs/VSS% on the eighth day was 18.33 %, 35.63%, 42.42%, 53.58%. Each cycle added more pyruvate, the PHAs concentration in the biomass increases significantly. Acid production effluent of sucrose was used as the substrate. Sludge from the aeration tank of the Tainan pig farm was added with an additional amount of 1 g of pyruvate at the same time. It can achieve the best state of producing PHA.

Techno-economic analysis of PHA

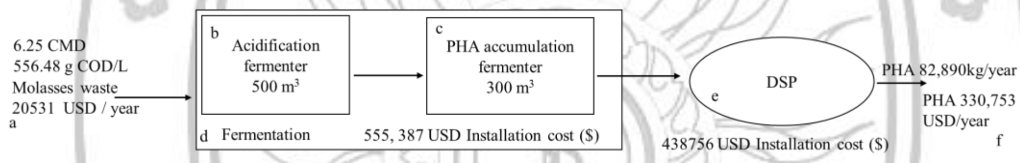

Figure 4.8 The diagram of PHA production with its size and cost

Figure 4.8 has 6 processes of analysis including with a. substrate (Arshad et al., 2019), b. Acidification fermenter, c. PHA accumulation fermenter, d. Fermentation (b+c) (Li et al., 2015) e. Downstream processes (Fernández-Dacosta et al., 2015) f. PHA product price (Gholami et al., 2016) (Mannina et al., 2019)

As presented in the figure 4.8. There were three main steps in the PHA production process. In this study, the size of PHAs production system was assumed that 6.25 cubic meter per day (CMD) of the molasses waste was fed in. The concentration of molasses waste was 556 g COD/L, but it needed to be diluted 11 times to 25 g COD/L. The cost of molasses per year was 20,531 USD (Arshad et al., 2019). The size of acidification and PHA accumulation systems were assumed to be 500m^2 and 300m^2, respectively. Because the acidification and PHA accumulation system were similar to the biogas plant, the installation price of these two systems were around 555,387 USD

(Li et al., 2015). This experiment uses DSP with dichloromethane (DCM) as the solven (Gurieff &Lant, 2007). There were three reasons to justify that DMC was the most suitable DSP in this study. First, the boiling point of DCM was 40°C that was easy to recycle the solvent, and it also don't need significant amount of energy to maintain all process. Second the extraction rate of PHA from the biomass by DCM was around 82.2% (Fernández-Dacosta et al., 2015). Third DCM was the solvent like chloroform. According to (Fernández-Dacosta et al., 2015) the cost of DSP were larger than those for the fermentation stage. The cost contribution of DCM was79 % of all the PHA production processes. The price of DSP in this study was 438,756 USD. The capital cost of all PHA production process was 994,143 USD.

Table 4.1 Total cost of PHA production process

Items		**PHA production process (USD)**	**References**
Capital costs			
Capital investment	B	994,143	(Arshad et al., 2019) (Fernández-Dacosta et al., 2015)
Operation costs (1+2+3+4)	G		
1. Maintenance (3% of capital costs)	C	29,824	
2. Labor (10% of capital costs)	D	99,414	(Fernández-Dacosta et al., 2015)
3. Utilities (1% of capital costs)	E	9,941	
4. Material	F	20,531	(Arshad et al., 2019)
Total		**159,711**	
Revenue			(Gholami et al., 2016)
PHA (4 USD/kg)	H	330,753	(Mannina et al., 2019)

As presented in table 4.1, the cash flow was showed by following assumptions: the capital cost of PHA production which was include acidification, PHA accumulation, and downstream process was 994,143USD (Arshad et al., 2019, Fernández-Dacosta et al., 2015). The maintenance cost was 3% of the capital costs. Labor cost was 10% of the capital costs. Utilities was 1% of the capital costs. Thes operation cost was 159,711USD that was including maintenance, labor, utilities, and materials. The revenue comes from the produced PHAs (Fernández-Dacosta et al., 2015). In this study PHAs yield was 53.58% PHA/VSS. The DSP in this study can extract up to 82.2% from the biomass (Fernández-Dacosta et al., 2015). Thus, 227 kg PHA can be produced per day or 82,890kg PHA can be produced per year. The unit selling price of 1 kg PHA was 4 USD (Gholami et al., 2016, Mannina et al., 2019). So, the total revenue of this process was 330,753 USD per year.

Table 4.2 Financial analysis for of PHA production

Project	**NPB***	**NPC***	**NPV***	**BCR**	**PBP (years)**	**IRR (%)**
PHAs production process	$2,948,300	$1,616,567	$1,331,733	1.82	6.21	16.31

*Discount rate of 8% (Gunjal &Amankwah, 1999)
NPB -Net Present Benefit
NPC -Net Present Cost
NPV -Net Present Value
BCR -Benefit Cost Ration
PBP -Payback Period
IRR -Internal Rate of Return

As showed in table 4.2, the life time in this study was assumed to be 20 years (Fernández-Dacosta et al., 2015) (Shahzad et al., 2017). First, the results of the study showed that the NPV for the PHAs production system was positive and the BCR was greater than one, thus, the proposed project was considered financially feasible (Gunjal

& Amankwah, 1999). Second, Taiwan Bank averaging loan interest rate was 8 % from July 1961 to March 2021 (Zhang et al., 2017), and the IRR results indicated that annual rates of return 16.3% with 20 years period. The IRR of PHAs production system in this study was higher 8.4% higher compared to the loan interest rate in Taiwan. And based on 105 countries, the average of global loan interest rate for 2019 was 11.57% (Shaddady et al., 2019). The IRR of PHAs production system in this study was also higher than 11.57%. This proved that the project can be invested. The payback period in this study was 6.21 years. Compared to the study of Khurram Shahzad (Shahzad et al., 2017) payback period that was 3.25- 4.5 years. There were many revenues which included meat and bone meal (MBM), Biodiesel, Biogas (heat and electricity) and PHA. But this study just focusses on PHA production, so the PHA was only one revenue. So this system was worth the investment.

CHAPTER 5

CONCLUSION, DISCUSSION, LIMITATIONS AND RECOMMENDATIONS

Conclusion

In this study, sequency batch reactor was used to successfully use aerobic fermentation to produce PHA. The comparison of three different seed sludge with high-organic wastewater to produce PHA, the comparison of two different high-concentration organic wastewater ratios with the sludge of Tainan pig farm to produce PHA and its VFA metabolism process, the comparison of the additional pyruvate to improve PHA production capacity. According to the estimated cost of the 6.25 CMD PHA production system showed an investment in acidification, PHA accumulation and downstream process about 994,143 USD for 20 years lifetime. the operation costs were 159,711 USD every year.

Discussion

In the sludge comparison experiment of Tainan pig farm, Chiayi pig farm and wastewater treatment system of Feng Chia University. The dissolved oxygen level increased significantly on the third day, and the PHA concentration was also obvious on the third day. When the dissolved oxygen level increases, the PHA-producing bacteria will enter the state of storing PHA.

Compared with other sludge, Tainan Pig Farm has the best PHA production capacity.

In ratios of the two substrates from the effluent of sucrose acidification fermenter and the effluent from molasses waste acidification fermenter were 0:1, 1:1, 3:1, 1:3. More the effluent from molasses waste acidification fermenter was contained, more PHA was produced. Because the effluent from molasses waste acidification fermenter contains more volatile organic acids than effluent of sucrose acidification fermenter, it can produce more PHA. The substrate ratio of the effluent of sucrose acidification fermenter and the effluent from molasses waste acidification fermenter was 1:1 on the eighth day of the experiment. The organic acid was run out in the eighth hour after adding substrate.

The additional Pyruvate can produce more PHA, and change the ratio of PHB and PHV in PHA

The results indicated that 6.25 CMD PHA production system financially feasible under the base run scenario. The net present value of PHA production system was bigger than 0 and benefit cost ration of PHA production system was bigger than 1.0. The payback period was 6.21 years and the internal rate of return (IRR) was 16 % was bigger than the global interest rate and the interest rate of Taiwan Bank. This was a worthwhile investment.

The use of high-organic wastewater with MMC was the way of the future and adding pyruvate can improve PHA output production while also changing the PHV ratio in PHA. Systems that employ wastewater to make PHA were commercially viable for medium- to large-scale locations, and PHA was the future trend.

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APPENDICES

Appendix A

Experimental equipments

Table A 1. Specification of measurement equipment

No.	Name	Description	Figure
1.	pH Electrode	BROADLEY-JAMES CORPC-MAG HST, IKA	
2.	Gas Chromatography Detectors-flame ionization detector	Thermo Scientific FOCUS 12550050 GC with Flame Ionization Detector 115VAC ; Column : Stabilwax-DA, 30m, 0.32mm ID, 0.25µm (cat. #11024)	
3.	Electronic balance	BP221s, S Tartorius	

No.	Name	Description	Figure
4.	Centrifuge	CN-1050, HSIANGTAI	
5.	Oven	JA72, PRECISION	

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