“การใช้กากเนื้อลำไยอบแห้งปราศจากน้ำตาลโมเลกุลสายสั้นเป็นแหล่งอาหารคาร์บอนในการผลิตเอนไซม์ไพรูเวตดีคาร์บอกซิเลส และ *R* - phenylacetylcarbinol”

“The Application of Oligosaccharides Depleted Dried Longan Flesh Meal as a Carbon Source in the Production of Pyruvate Decarboxylase and *R* - phenylacetylcarbinol”

Research Team

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Sponsored by

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Final Report

13 September 2010

Preface

The final report of the project entitled “The Application of Oligosaccharides Depleted Dried Longan Flesh Meal as a Carbon Source in the Production of Pyruvate Decarboxylase and R - phenylacetylcarbinol” is intended for submission to National Research Council of Thailand The research team is grateful for NRCT which provided financial support that lead to the formation of research team which consisted of lecturers and students within the Faculty of Agro-Industry, Chiang Mai University.

Signed _____

(Asst.Prof. Dr. Noppol Leksawasdi)

Project Team Leader

13 September 2010

Abstract

Longan is a crucial exported economic crop of Thailand and ranked at the fourth position after pineapple, durian, and young corn. Each year, the majority of longan produced in the Northern region is amount to about 500,000 tons with the exportation values of no less than 5 billion baht. The processing of fresh longan to dried longan for added value purpose had encountered a deadstock problem which prevented 67,000 tons of the processed longans from being transferred aboard during 2003 – 2004. It took more than 6 years to reach the final resolution to rectify the problem. One alternative strategy is to retrieve dried longan with high level of sugars and utilize it for ethanol production as biofuels. The attained biomass can also be used as biocatalyst for the production of a precursor for medicines, namely, ephedrine and pseudoephedrine with the properties of relieving the allergic and nasal congestion symptoms. The previous research report suggested that a microbial strain which belonged to the group of *Candida utilis* was the best biocatalyst. This study was therefore focused on the cultivation of six *C. utilis* strains which included TISTR 5001, 5032, 5043, 5046, 5198, and 5352 to evaluate the growth and ethanol production kinetics in detail. The cultivation level was 150 ml with dried longan extract as a carbon source under static condition for 192 h at 25.6^{o}C and 4 replicates. The concentrations of sugars such as sucrose, glucose, and fructose as well as ethanol were determined by HPLC. The strain which was able to generate the highest level of ethanol and biomass was *C. utilis* TISTR 5198 at 0.46 ± 0.14 g l^{-1} and 6.47 ± 0.12 g l^{-1}, respectively. The highest specific growth rate was 0.044 ± 0.011 h^{-1} which corresponded to the doubling time of 15.7 ± 3.8 h. Growth kinetic of *C.utilis* TISTR 5352 for 1,500 ml scale was level of ethanol and biomass at 0.100 ± 0.011 g l^{-1} and 0.115 ± 0.005 g l^{-1}, respectively. The highest specific growth rate was 0.008 ± 0.000 h^{-1} which corresponded to the doubling time of 83.0 ± 4.1 h. The cultivation of *C. utilis* TISTR 5198 and TISTR 5352 in DDLFH medium at TSS levels of 20 and 40^{o}Brix indicated the growth inhibition. The two-phase PAC biotransformation of *C. utilis* TISTR 5198 using whole cells harvested at 192 h in DDLFH medium with 6.12 g l^{-1} of dried biomass equivalent resulted in the overall PAC production level of 1.76 ± 0.06 mM.

The implementation of microorganism such as *Saccharomyces cerevisiae* TISTR 5606, which was capable of converting sugars in the overproduced fresh longan or deadstock dried longan to fermented broth with high ethanol concentration level, was an example of biochemical process application in order to replace fossil energy with a new energy source. The development of this biofuel would be beneficial in assisting to rectify not only global warming but also devalued longan problems. In current study, the

research team investigated the effect of varied inoculum concentration levels (1, 5, and 10% (v/v)) to elucidate a cost reduction strategy for the microbial cultivation in dried longan extract from the conventional level of 10% (v/v). In addition, the detailed growth and ethanol production kinetics were also evaluated simultaneously. The cultivation level was 1,500 ml in static condition for 36 h at 25.6^{o}C. The results suggested that inoculum level of 1% (v/v) was able to produce the highest levels of ethanol concentration and yield of 53.8 ± 0.5 g l^{-1} and 0.49 ± 0.01 g ethanol per g of consumed sugars. Moreover, the achieved dried biomass concentration was also the highest at 7.47 ± 0.08 g l^{-1} which was not significantly different ($p > 0.05$) in comparison to the cultivation with inoculum level of 5% (v/v) (7.39 ± 0.10 g l^{-1}). In fact, the inoculum level of 10% (v/v) yielded the lower level of dried biomass concentration (6.31 ± 0.11 g l^{-1}). The method of score weighing was introduced later by considering three factors which included costing (20%), microbial growth (30%), and substrates as well as product (50%). The most appropriate yeast inoculum level was 1% (v/v) followed by 5 and 10% (v/v) with the corresponding weighing scores of 91.2, 82.3, and 79.5, respectively. DLE medium was the most suitable carbon source for batch cultivation in 5,000 ml scale with an initial aeration period of 12 h from the overall 36 h cultivation period at 25.6^{o}C. The ethanol production of *S. cerevisiae* TISTR 5606 in DLE was the highest in comparison with DDLFH medium with corresponding ethanol concentration and yield of 73.77 ± 0.48 g l^{-1} and 0.53 ± 0.01 g ethanol per g of consumed sugars, respectively. The level of dried biomass concentration obtained was also the highest at 10.81 ± 0.08 g l^{-1} which was significantly different ($p \leq 0.05$) from that of DDLFH medium (0.17 ± 0.03 g l^{-1}). The toxicity of DDLFH medium in comparison to DLE medium was elucidated in the fed batch experiment. The ethanol production from the system with DLE medium feeding was 24.93 ± 1.13 g l^{-1}, which was significantly different ($p \leq 0.05$) from DDLFH medium feeding at 8.61 ± 0.56 g l^{-1}. Dried biomass concentration obtained from DLE medium feeding was also higher at 5.72 ± 0.13 g l^{-1} which was significantly different ($p \leq 0.05$) from DDLFH medium feeding at 3.00 ± 0.17g l^{-1}. The two-phase separated PAC biotransformation using whole cells cultivated in 5,000 ml scale with DLE and DDLFH media did not result in PAC production.

The problems of longan overproduction and dried longan deadstock that spanned over 6 Northern provincial area of Thailand gave rise to the research endeavour which concentrated on finding the solution for these issues. Previous research results suggested that *Saccharomyces cerevisiae* TISTR 5606 was the microbial strain with the highest capability in producing ethanol from three types of sugars commonly found in dried longan extract, namely, glucose, fructose and sucrose. The development of mathematical model for ethanol production kinetics in batch system for *S. cerevisiae* TISTR 5606 was essential in the further optimization of final ethanol concentration level and productivity based on fed batch and

continuous production systems. The research team carried out the detailed investigation of growth and ethanol production kinetics in batch system for this microbial strain in 1,500 ml scale. The utilized carbon source was pure analytical grade sugar with an initial concentration of 40 g l^{-1} under static condition for 36 h, 25.6^{o}C, and five replicates. The analyses of sugars and ethanol concentrations were done with HPLC. The developed mathematical model included the constants such as ethanol concentration threshold (P_{ip}) and maximum ethanol concentration (P_{mp}). The numerical integration was applied in the simulation of microbial cultivation kinetics with an individual pure sugar. The simulated curves predicted the experimental profiles relatively well (average RSS of 58.9, $R^2 > 0.98$). The parameter values such as maximum specific growth rate constant (μ_{max}), maximum specific substrate consumption rate constant ($q_{s,max}$), and maximum specific ethanol production rate constant ($q_{p,max}$) for each sugar were obtained. The subsequent mathematical model development and simulation for static batch cultivation using triple pure sugars (glucose/fructose/sucrose at 20/20/20, 30/30/30, 40/40/40, and 60/60/60) and dried longan extract (60, 120, and 180 g l^{-1}) resulted in the good agreement of model fitting to experimental data with the corresponding total RSS and average R^2 of (1,033, 0.97) and (1,894, 0.96), respectively.

Pyruvate decarboxylase1 (PDC1) converts benzaldehyde and pyruvate into (*R*)-phenylacetylcarbinol (*R* - PAC) in enzymatic biotransformation. *R*-PAC is the precursor for the production of ephedrine and pseudoephedrine, used as anti-asthmatic and nasal decongestants, respectively. In this study the PDC1 gene was amplified from *Saccharomyces cerevisiae* 5606. The amplicon was ligated into pPICZA. The resulting pPICZA-PDC1 was transformed into *Pichia pastoris* X-33. Three clones of recombinant *P. pastoris* found on selective media containing 500 ug ml^{-1} zeocin were cultured and induced with methanol. The activities of PDC1 expressed from *P. pastoris* were similar to *S. cerevisiae* TISTR5606 and *C. utilis* TISTR5198. Further studies are needed in order to optimize the PDC1 expression.

Contents

Contents of Figures

Contents of Figures (Continuous)

Contents of Figures (Continuous)

Contents of Figures (Continuous)

Contents of Figures (Continuous)

Figure Number **Pages**

Contents of Tables

Table Number **Pages**

Contents of Tables (Continuous)

Contents of Tables (Continuous)

Contents of Tables (Continuous)

Contents of Tables (Continuous)

Contents of Tables (Continuous)

Contents of Tables (Continuous)

Contents of Tables (Continuous)

Table Number **Pages**

Contents of Tables (Continuous)

Contents of Tables (Continuous)

Contents of Tables (Continuous)

Table Number **Pages**

Contents of Tables (Continuous)

Chapter 1

Introduction and objective

Longan is one of the important economic crops of Thailand, forth only to pineapple (exporting values of $US361 millions), durians ($US87 millions), and young corns ($US58 millions) as shown in Fig. 1.1. In each year, Thailand exported fresh longan to various countries with the total values of $US57.8 millions (Agriculture Economics Office, 2007). The longan products which were consumed domestically and passed through processing steps were 30 and 40% of the overall produce. The leftover products were exported (DOA, 2004). Besides, the longan also possessed medicinal properties and used in the treatment of mental illness, insomnia, and stomachache (Choo, 2000).

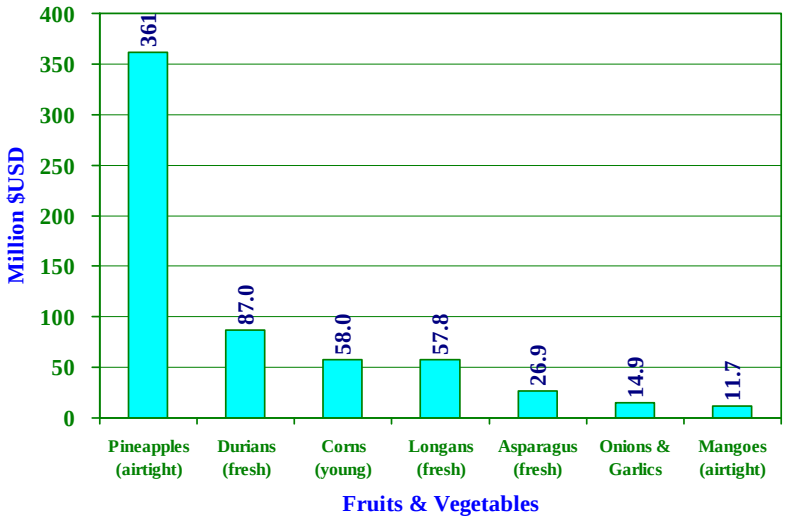


Figure 1.1: The exporting values of vegetables and fruits in Thailand, year 2006 (Agriculture Economics Office, 2007).

The increase values of longan has led to the processing of fresh longan into various products such as crunchy longan, canned longan, and dried longan with an immense popularity from the consumers. The majority of consumers preferred dried longan with the complete removal of peels and seeds (Boonmak *et al*., 2005). In 2006, the overall exporting value of longan in the Northern part of Thailand was up to $US112 million which was higher than lychee and orange by 5.92 and 13.4 times, respectively as given in Fig. 1.2.

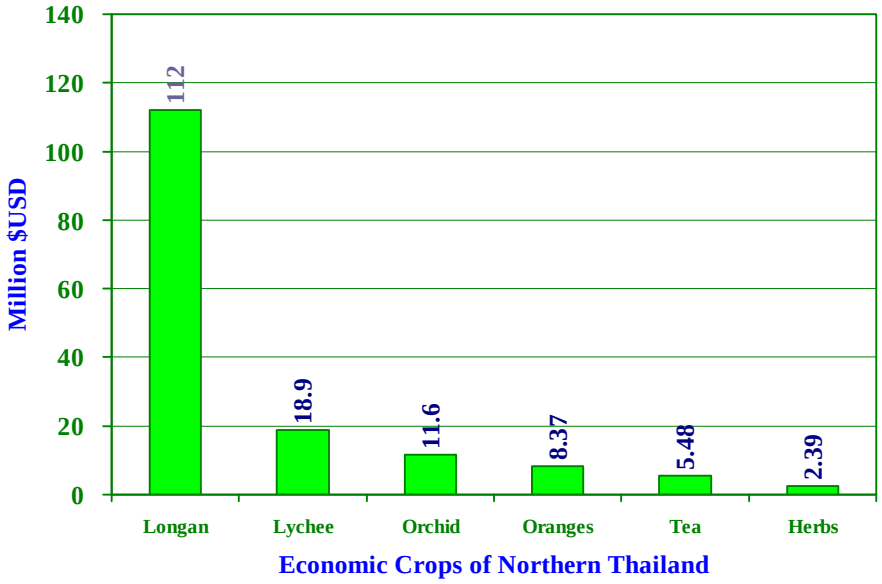


Figure 1.2: The exporting values of Northern economic crops, year 2006 (Agriculture Economics Office, 2007).

The overall plantation area of longan in Thailand during 2001 was 600,000 Rai (in comparison to 636,000 Rai at the present, according to The Committee of Economics and Social Development Office, 2005). This was compared to China whose plantation area was about 3 millions Rai (Prachachat Business, 2005). Two-third of longan cultivation area were located in Chiang Mai and Lumphun with the total exportation volume of 5,050 million Bahts in 2000 (in 204, this figure had increased to 5,142 million Bahts) which were divided into 2,414 million Bahts for dried longan, 2,040 million Bahts for fresh longan, and the rest was in the form of canned longan and frozen longan (Horticulture Promotion Department, 2001). Poapongsakorn (2002) had elaborated in the website of FAO that Thailand was one of the major dried longan exporter (Fig. 1.3). The reason of processing the fresh longan was due to its relatively short life after harvesting (Choo, 2000).

However, the substandard dried longan which was prohibited from export and overproduction (up to 30,000 tonnes which covered the area of 8 Northern provinces) were problems that the Royal Thai Government had attempted to set up the relief strategy (TISC, 2005). The disposal methods by burning or burying were considered less useful than ethanol production which could be mixed with gasoline to generate gasohol. This strategy played the contributing part to the relief effort of rising petrol price. The world's crude oil price had risen from $US25 per barrel in 2002 (BBC, 2008) to the unprecedented levels

of $US105 and $US119 on 28 March and 26 April 2009, respectively (Bloomberg, 2008). These were the results of unrest situation in Nigeria and Pakistan as well as the weakening US currency and rapid economical growth in China and India.

The ratio was calculated based on fresh longan (year 2004) whose production level was 565,062 tonnes.

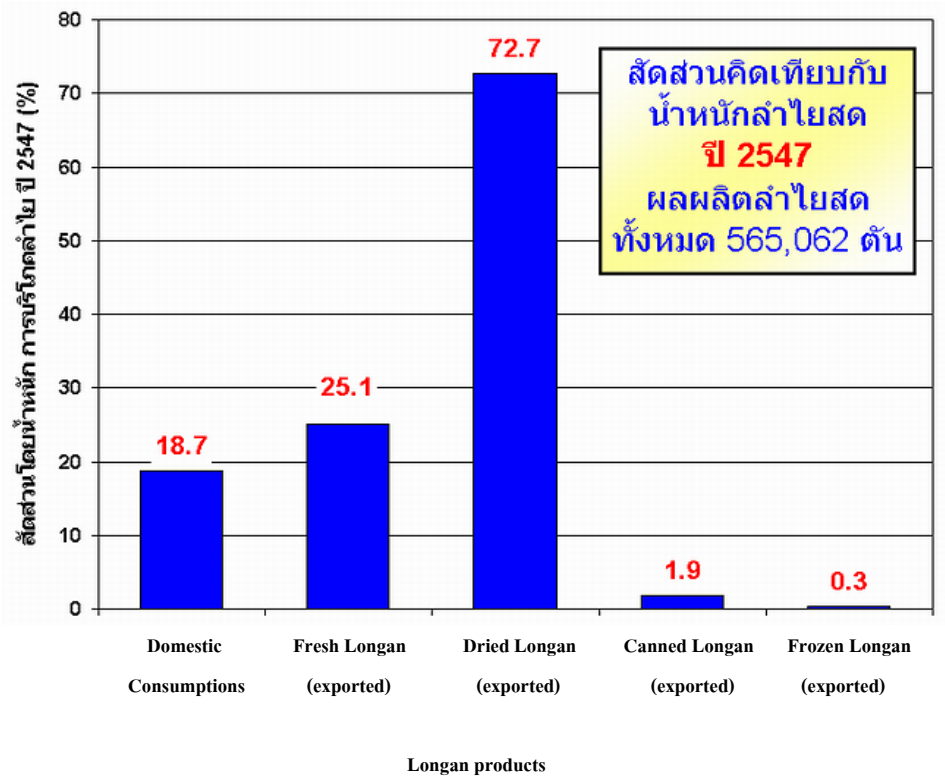


Figure 1.3: Weight ratio (%) of longan consumption in 2004 (The Committee of Economics and Social Development Office, 2005).

The experiment conducted in the current study was one of the steps in establishing biotransformation research based in Thailand by commencing with the improvement of *R*-phenylacetylcarbinol (PAC) production from raw materials level. The expired dried longan was used instead of glucose and the alteration of buffer species in the biotransformation process from MOPS (88,400 Bahts/kg) to the much cheaper phosphate buffer (840 Bahts/kg) by 100 times (OV Chemicals, 2007). Leksawasdi *et al.* (2005) illustrated that the implementation of digital pH control to the two-phase system between 20 mM MOPS buffer solution and 2.5 M dipropylene glycol in octanol. This was

accompanied by overhead stirrer to mix both phases well. The enzymatic extract from *C. utilis* was used as biocatalyst and led to the PAC production of 151 g/l and 17.2 g/l in the organic and aqueous phases, respectively within 48 h which was equivalent to the more expensive system that utilized 2.5 M MOPS buffer.

Objectives

1. For screening of whole cells from six *C. utilis* strains to select the best ethanol producers in 150 ml batch system with dried longan extract (DLE) as a carbon source by examining the detailed growth kinetics.
2. For producing ethanol in; (a) DLE using 1,500 ml batch system with *C. utilis* TISTR 5352, and (b) digested dried longan flesh hydrolysate (DDLFH) with initial total soluble solid levels of 20 and 40^{o}Brix in 150 ml scale.
3. For using the whole cells of *C. utilis* TISTR 5198 and TISTR 5352 harvested from the system with DLE and DDLFH at 20^{o}Brix as carbon sources were used in the two-phase biotransformation system to elucidate the level of PAC production.
4. The comparison of growth kinetics from *S. cerevisiae* TISTR 5606 in 5,000 ml batch system with an initial aeration period of 12 h from the overall 36 h with dried longan extract (DLE) and digested dried longan fresh hydrolysate (DDLFH) as carbon sources.
5. The examination of growth and ethanol production kinetics in fed batch systems with the addition of; (a) 200 ml DLE medium, and (b) 200 ml DDLFH medium.
6. For using the whole cells of *S. cerevisiae* TISTR 5606 harvested from the system which utilized DLE and DDLFH medium as carbon sources were later used in the two-phase separated biotransformation system to elucidate the level of PAC production.
7. For constructing a mathematical model describing the kinetic profiles involving the utilization of triple sugar substrates (glucose, fructose, and sucrose) as well as production of ethanol and dried biomass in the cultivation system which utilizes dried longan extract as a carbon source.
8. The developed mathematical model could be used later in the subsequent prediction of ethanol production, the design of concentrated substrates feeding profile in fed batch system, as well as the prediction of ethanol concentration at steady state in the continuous culture system. This would, in turn, lead to the development of a process that resulted in optimal ethanol concentration and yield.

Chapter 2

Materials and Methods

2.1 The Production of *R*-phenylacetylcarbinol Using Whole Cells of *Candida utilis* in Biphasic Biotransformation System with Concentrated Phosphate Solution as Buffer Species

2.1.1 Microorganisms

Six wild type strains of *Candida utilis* were purchased from Thailand Institute of Scientific and Technological Research (TISTR), namely, TISTR 5001, 5032, 5043, 5046, 5198, and 5352. These strains were propagated in Erlenmeyer flasks and kept at -20^{o}C in yeast-malt extract medium which contained 20% v/v glycerol as stock culture in 1 ml volume.

2.1.2 Cultivation media preparation

Three types of cultivation media were applied. Yeast-malt (YM) seed medium in 15 ml scale: glucose (10 g l^{-1}); yeast extract (3 g l^{-1}); malt extract (5 g l^{-1}); peptone (5 g l^{-1}). Dried longan extract (DLE) medium in 150 ml scale was prepared by adding dried longan flesh aged 2 yrs old (Sanpathong District, Chiang Mai, Thailand) into boiling water (30% w/v) for 30 min (Agustina *et al*. 2009) prior to separation of extract from insoluble solids by filtration technique. Digested dried longan flesh hydrolysate (DDLFH) medium in 150 ml scale was obtained in a stepwise manner as follows; (1) insoluble solids, which were separated from DLE medium preparation, was mixed in 1:10 ratio with a predigesting solution that contained 1% w/v NaOH and 2% w/v glacial acetic acid of equal volume (adapted from Yoon *et al.* 2005) pH 5, (2) the freshly prepared dried longan flesh/acetate buffer mixture was then immediately subjected to heat treatment in the pressurized sterilizer (All Americans, Model No. 1925x) at 121^{o}C for 30 min before cooling down to room temperature, (3) three enzyme mixtures which included α-amylase (Ronozyme A), carbohydrase (Ronozyme VP), and endo-xylanase (Ronozyme WX) were subsequently added (1% w/v of each) to the pretreated dried longan pomace which was followed by static incubation at 30^{o}C for another 24 h, (4) the filtration was carried out later to remove the remnant solids and the collected slurry was concentrated on the stove with natural gas heating in order to obtain two types of final hydrolysate whose total soluble solids levels were 20 and 40^{o}Brix, respectively. All media were sterilized by claving at 121^{o}C for 15 min.

2.1.3 Kinetics Studies

The inocula in all cultivations were propagated statically in 15 ml aliquot at 25.6^{o}C which was the average climatic temperature of Chiang Mai province during 17 yrs period from 1988 – 2005 (Poodtatep *et al.,* 2008). The batch cultivation of each *C. utilis* strain was carried out in a 150 ml jam jar under the similar constant conditions at 25.6^{o}C and initial pH of 6.5 with four replicates and 10% v/v inoculum size. The cultivation in DLE medium were conducted to obtain kinetics data of all six *C. utilis* strains and to select two appropriate strains for latter experiments in DDLFH medium with initial total soluble solids levels of 20 and 40^{o}Brix. The sampling was scheduled on a regular interval of 12 h for 192 h cultivation period for both DLE and DDLFH media. The samples were maintained at -20^{o}C for further analysis.

2.1.4 Biotransformation Studies

The whole cells of two *C. utilis* strains (TISTR 5198 and 5352) based on the highest level of produced ethanol which indirectly reflects the level of PDC production after 192 h cultivation period from previous kinetics studies were selected for the subsequent PAC biotransformation. The dried biomass equivalent concentration in 1.2 M phosphate buffer with 300 mM pyruvate was 6.12 g l^{-1}. Two types of cofactors were also added, namely, 1.0 mM TPP (thiamine pyrophosphate) and 1.0 mM $MgSO_4.7H_2O$. The initial pH level was adjusted to 6.0 prior to whole cells addition. Five ml octanol was used as an organic phase with 1.75 M benzaldehyde (Leksawasdi *et al*., 2005). After addition of whole cells, the biotransformation bottle (dia. 2.6 cm × height 5.9 cm) was placed in a shaking incubator at 250 rpm, 8^{o}C for 72 h.

2.1.5 Analytical methods

2.1.5.1 Ethanol Production

The sugars (fructose, glucose, and sucrose) and ethanol concentrations in the sample supernatants were determined utilizing HPLC (1200 series, Agilent Technologies, USA) with an Aminex HPX-87H (BioRad) column at 40^{o}C and RI detector. The mobile phase was 5 mM H_2SO_4 with flow rate of 0.75 ml min^{-1}. The sample injection volume was 20 µl. The biomass concentrations (X) were correlated to optical density (OD) measurements at 600 nm (Perkin Elmers, Waltham, USA, Model No. Lambda 25).

The conversion of OD readings to dried biomass was based on the following polynomial equation; $X = -1.5861 \times 10^{-5} \times OD^3 + 2.5423 \times 10^{-3} \times OD^2 + 1.4118 \times 10^{-1} \times OD$ with correlation coefficient (R^2) of 0.9942. The determination of pH and total soluble solid (TSS) for the sample supernatants were conducted using a pH meter (Eutech, Model pH 510, Japan) and a refractometer (Atago, Model No. N-1α, Japan). All analyses were carried out in five replicates. The computation of statistical mean, standard error, as well as hypothesis testing of experimental mean comparison were based on the techniques described by Skoog *et al*. (1996) with NLST_Diff.xls Version 1.0 (W1.3281). The separation of protein for subsequent analysis was based on the method described in Rosche *et al*. (2001). Bradford assay (Bradford, 1976) was used for the determination of total protein concentration. PDC activity was quantified based on carboligase assay as described previously (Rosche *et al*. 2002). All analyses were done in four replicates.

2.1.5.2 PAC Production

The analyses of pyruvate and acetaldehyde concentrations were performed using the method of Czok and Lamprecht (1974) as well as Bernt and Bergmeyer (1974), respectively. The determination of benzyl alcohol, PAC, benzoic acid, and benzaldehyde were carried out using Alltima™C8 5 µm as described by Rosche *et al*. (2002). All analyses were done in five replicates.

The computation of statistical mean, standard error, as well as hypothesis testing of experimental mean comparison were based on the techniques described by Skoog *et al*. (1996) with NLST_Diff.xls Version 1.0 (W1.3281).

2.2 The Kinetics of Ethanol and PAC Biotransformation Production from Dried Longan Extract

2.2.1 Microorganisms

The freeze dried ampoule of *Saccharomyces cerevisiae* TISTR 5606 was obtained from Thailand Institute of Scientific and Technological Research (TISTR). The primary stock culture of this yeast strain was maintained in 1 ml aliquot at -20^{o}C in yeast-malt extract medium which contained 20% v/v glycerol.

2.2.2 Cultivation media preparation

Preseed yeast-malt (YM) medium consisted of (per litre): 10.0 g Glucose, 3.0 g yeast extract, 5.0 g malt extract, and 5.0 g peptone. Dried longan extract (DLE) medium which was also used as a seed inoculum contained (per litre): 300 g dried longan flesh aged 2 yrs old (Sanpathong District, Chiang Mai, Thailand), 4.5 g yeast extract, 7.5 g malt extract, and 7.5 g peptone for additional nitrogen sources. The extraction was done in boiling water with mass to volume extraction ratio of 30.0 g dried longan per 100 ml distilled water for 30 min (Agustina *et al*. 2009) prior to removal of insoluble solids by filtration. Both media were sterilized by claving at 121^{o}C for 15 min. Digested dried longan flesh hydrolysate (DDLFH) medium was obtained in a stepwise manner as follows; (1) insoluble solids, which were separated from DLE medium preparation, was mixed in 1:10 ratio with a predigesting solution that contained 1% w/v NaOH and 2% w/v glacial acetic acid of equal volume (adapted from Yoon *et al.* 2005) pH 5, (2) the freshly prepared dried longan flesh/acetate buffer mixture was then immediately subjected to heat treatment in the pressurized sterilizer at 121^{o}C for 30 min before cooling down to room temperature, (3) three enzyme mixtures which included α-amylase (Ronozyme A), carbohydrase (Ronozyme VP), and endo-xylanase (Ronozyme WX) were subsequently added (1% w/v of each) to the pretreated dried longan pomace which was followed by static incubation at 30^{o}C for another 24 h, (4) the filtration was carried out later to remove the remnant solids and the collected slurry was concentrated on the stove with natural gas heating in order to obtain the final hydrolysate with total soluble solid level of approximately 20^{o}Brix.

2.2.3 Kinetic studies: Effect of inoculum size

S. cerevisiae was cultivated statically in a 2,000 ml sterilized bottle (Isolab) with working volume of 1,650 ml. The propagation of preseed and various percentages of seed inocula (1, 5, and 10% v/v) were carried out sequentially in 15 and 150 ml sterile stationary jam jars with cultivation period of 48 h (exponential phase) for each inoculum type. All cultivations were done at 25.6^{o}C which was the average climatic temperature of Chiang Mai province during 17 yrs period from 1988 – 2005 (Poodtatep *et al*. 2008) with an initial pH of 6.5. The variation of seed inocula percentages at 1 and 5% v/v were achieved by diluting 10% v/v inocula with corresponding volumes of DLE medium until the final volume of seed inocula reached the similar level of 150 ml as their 10% v/v counterpart. The sampling was scheduled on a regular interval of 3 h for the first 24 h which was later extended to 6 h until the termination of cultivation period at 36 h. Five replicates were collected at each time point and kept frozen at -20^{o}C pending subsequent analyses.

2.2.4 Kinetics Studies: Batch cultivations with aeration in 5,000 ml scale

The batch fermentation of DLE and DDLFH media in 5,000 ml scale were carried out in 20 L high density polyethylene drums (height × diameter = 45 × 27 cm^{2}) for 36 h at 25.6^{o}C. Each drum was washed thoroughly with hot water for 5 min prior to soaking for 24 h with 200 ppm potassium metabisulphite (KMS, Wechavit), which was filled to the fullest level of the drum. The replacement of KMS solution in the drum with 4.95 L DLE medium and 0.001% v/v antifoam (propylene glycol, Fluka, Steinheim, Germany, Cat. No. 43560) was performed aseptically. Similar preparation strategies of preseed and seed inocula were carried out as described previously with addition of 1% v/v seed inoculum to initiate the fermentation. The aeration was achieved by an aquarium pump which pushed the air through delivery tube system with connection to a 0.2 µm sterile air filtration unit (Sartorius, USA, Midisart 2000). The air bubbles were released at the bottom of the fermentation drum with an aeration period of 12 h after which the pump was turned off. Five sample replicates were withdrawn from the drum every 3 h during the first 24 h of cultivation period and every 6 h for the next 12 h. All samples were maintained at -20^{o}C for further analyses.

2.2.5 Kinetics Studies: Fed batch cultivations in 600 ml scale

The fed batch cultivation of *S. cerevisiae* TISTR 5606 was initiated as 1,500 ml batch cultivation in DLE medium as a carbon source with addition of 1% v/v seed inoculum. Five replicates were collected at each time point (0, 3, 6, 9, 12, 15, 18, 21, 24, 30, and 36 h) at 25.6^{o}C. The culture was then separated into two portions of 400 ml. The first bottle was fed with 200 ml DLE medium while the other was fed with 200 ml of DDLFH medium. The cultivations in both bottles were continued until 60 h and the sampling was scheduled on a regular interval of 3 h starting from 36 h for the period of 24 h (36, 39, 42, 45, 48, 51, 54, 57, and 60 h) at 25.6^{o}C. Five replicates were collected at each time point and kept frozen at -20^{o}C pending subsequent analyses.

2.2.6 Two-phase Separated Biotransformation Studies

The experiment of two-phase PAC biotransformation was conducted by adopting whole cells *S. cerevisiae* TISTR 5606 cultivated in DLE and DDLFH media. Firstly, whole cells from DLE medium was adjusted to dried biomass equivalent level of 3.06 g l^{-1}, 6.12 g l^{-1}, and 12.24 g l^{-1}, respectively. Secondly, whole cells from DDLFH medium was adjusted to dried biomass equivalent level of 3.06 g l^{-1}. The organic phase contained 1.75 M benzaldehyde in octanol with the total volume of 5 ml (Leksawasdi *et al*., 2005). The aqueous phase of equal volume consisted of 300 mM sodium pyruvate, 1 mM thiamine pyrophosphate (TPP), and 1 mM $MgSO_4 \cdot 7H_2O$ in 1.2 M phosphate buffer. After addition of whole cells, the biotransformation bottle (dia. 2.6 cm × height 5.9 cm) was placed in a shaking incubator at 250 rpm, 8^{o}C for 72 h.

2.2.5 Analytical methods

2.2.5.1 Ethanol Production

The sugars (fructose, glucose, and sucrose) and ethanol concentrations in the sample supernatants were determined utilizing HPLC (1200 series, Agilent Technologies, USA) with an Aminex HPX-87H (BioRad) column at 40^{o}C and RI detector. The mobile phase was 5 mM H_2SO_4 with flow rate of 0.75 ml min^{-1}. The sample injection volume was 20 µl. The biomass concentrations (X) were correlated to optical density (OD) measurements at 600 nm (Perkin Elmers, Waltham, USA, Model No. Lambda 25). The conversion of OD readings to dried biomass was based on the following polynomial equation; X =

- $1.5861 \times 10^{-5} \times OD^3 + 2.5423 \times 10^{-3} \times OD^2 + 1.4118 \times 10^{-1} \times OD$ with correlation coefficient (R^2) of 0.9942. The determination of pH and total soluble solid (TSS) for the sample supernatants were conducted using a pH meter (Eutech, Model pH 510, Japan) and a refractometer (Atago, Model No. N-1α, Japan). All analyses were carried out in five replicates. The computation of statistical mean, standard error, as well as hypothesis testing of experimental mean comparison were based on the techniques described by Skoog *et al*. (1996) with NLST_Diff.xls Version 1.0 (W1.3281). The separation of protein for subsequent analysis was based on the method described in Rosche *et al*. (2001). Bradford assay (Bradford, 1976) was used for the determination of total protein concentration. PDC activity was quantified based on carboligase assay as described previously (Rosche *et al*. 2002). All analyses were done in four replicates.

2.2.5.2 PAC Production

The analyses of pyruvate and acetaldehyde concentrations were performed using the method of Czok and Lamprecht (1974) as well as Bernt and Bergmeyer (1974), respectively. The determination of benzyl alcohol, PAC, benzoic acid, and benzaldehyde were carried out using Alltima™C8 5 µm as described by Rosche *et al*. (2002). All analyses were done in five replicates.

The computation of statistical mean, standard error, as well as hypothesis testing of experimental mean comparison were based on the techniques described by Skoog *et al*. (1996) with NLST_Diff.xls Version 1.0 (W1.3281).

2.3 The Development of Mathematical Model for Ethanol Production from Dried Longan Extract in a Static Condition of *Saccharomyces cerevisiae* TISTR 5606

2.3.1 Microorganisms

The freeze dried ampoule of *Saccharomyces cerevisiae* TISTR 5606 was obtained from Thailand Institute of Scientific and Technological Research (TISTR). The primary stock culture of this yeast strain was maintained in 1 ml aliquot at -20^{o}C in yeast-malt extract medium which contained 20% v/v glycerol.

2.3.2 Cultivation media composition and preparation

Preseed yeast-malt (YM) medium in 15 ml scale consisted of (per litre): 10.0 g glucose, 3.0 g yeast extract, 5.0 g malt extract, and 5.0 g peptone. In the first experiment of kinetic determination based on single pure sugar, seed inocula and cultivation media in 150 and 1,500 ml scales contained (per litre): 40.0 g of a suitable sugar type (glucose, fructose, or sucrose) with 1.5 times the corresponding amount of nitrogen sources in preseed YM medium as described previously for 150 and 1,500 ml scales. The procedures were carried out similarly in the second and third experiments where the mixture of pure sugars and longan extract were used. The yeast cultivation in the second experiment was done with similar media with the glucose/fructose/sucrose concentration ratio of (g l^{-1}); 20/20/20, 30/30/30, 40/40/40, and 60/60/60 as carbon sources. Three overall sugar concentration levels of 60, 120, and 180 g l^{-1} of dried longan extract were employed in the third experiment. All media were sterilized by claving at 121^{o}C for 15 min

2.3.3 Fermentation Studies

The microbial propagation was initiated by transferring two primary stock cultures to preseed YM medium which was followed by incubating statically at 25.6^{o}C for 48 h. The readied preseed was added to seed medium (10% v/v inoculum) and cultivated in the same condition and incubation period. The exponential stage seed culture of *S. cerevisiae* TISTR 5606 was immediately combined with cultivation media (10% v/v inoculum) to begin the fermentation. The cultivation period was 36 h with the regular sampling interval of 3 h for the first 24 h and 6 h afterwards. Each withdrawn sample from the specified collection time point was maintained at - 20^{o}C and subsequently analyzed in five replicates.

2.3.4 Analytical methods

The sugars (fructose, glucose, and sucrose) and ethanol concentrations in the sample supernatants were determined utilizing HPLC (1200 series, Agilent Technologies, USA) with an Aminex HPX-87H (BioRad) column at 40^{o}C and RI detector. The mobile phase was 5 mM H_2SO_4 with flow rate of 0.75 ml min^{-1}. The sample injection volume was 20 µl. The calibration accuracy was regularly verified with corresponding sugars and ethanol standards with 2% w/v glacial acetic acid as an internal standard. The biomass concentrations (X) were correlated to optical density (OD) measurements at 600 nm (Perkin Elmers, Waltham, USA, Model No. Lambda 25). The conversion of OD readings to dried biomass was based on the following polynomial equation; $X = - 1.5861 \times 10^{-5} \times OD^3 + 2.5423 \times 10^{-3} \times OD^2 + 1.4118 \times 10^{-1} \times OD$ with correlation coefficient (R^2) of 0.9942. The determination of pH and total soluble solid (TSS) for the sample supernatants were conducted using a pH meter (Eutech, Model pH 510, Japan) and a refractometer (Atago, Model No. N-1α, Japan). All analyses were carried out in five replicates. The statistical mean, standard error, as well as hypothesis testing of experimental mean comparison were based on the techniques described by Skoog *et al*. (1996).

2.3.5 Mathematical model and simulation methods

The implemented modeling and simulation strategies were based on the previously published (Leksawasdi *et al*. 2001) Euler's method of numerical integration (Kreyszig, 1993) and systematic grid-search of parameter values using VBA (Visual Basic for Applications) 6.3 in Microsoft EXCEL 2003.

2.3.6 Triple Substrate Model Development

Three sets of differential equations describing rates of microbial growth, substrates utilization, and ethanol production in the static condition for *S. cerevisiae* TISTR 5606 were modified from the previously established mathematical models of ethanol production from *Zymomonas mobilis* ZM4 using glucose (Lee & Rogers, 1983) and *Z. mobilis* ZM4(pZB5) using glucose and xylose (Leksawasdi *et al*., 2001), as well as lactic acid production from *Lactococcus lactis* NZ133 using lactose (Boonmee *et al*., 2003). These rate equations consist of common parameter types which are defined fully in the Nomenclature section. The equation of microbial growth rate is represented by Equation (1).

$$\frac{dx}{dt} = \left[\alpha r_{x,1} + \beta r_{x,2} + (1-\alpha-\beta) r_{x,3}\right] x \qquad (1)$$

For glucose:

$$r_{x,1} = \mu_{\max,1}\left(\frac{s_1}{K_{sx,1}+s_1}\right)\left(1-\frac{p-P_{ix,1}}{P_{mx,1}-P_{ix,1}}\right)\left(\frac{K_{ix,1}}{K_{ix,1}+s_1}\right) \quad (2)$$

For fructose:

$$r_{x,2} = \mu_{\max,2}\left(\frac{s_2}{K_{sx,2}+s_2}\right)\left(1-\frac{p-P_{ix,2}}{P_{mx,2}-P_{ix,2}}\right)\left(\frac{K_{ix,2}}{K_{ix,2}+s_2}\right) \quad (3)$$

For sucrose:

$$r_{x,3} = \mu_{\max,3}\left(\frac{s_3}{K_{sx,3}+s_3}\right)\left(1-\frac{p-P_{ix,3}}{P_{mx,3}-P_{ix,3}}\right)\left(\frac{K_{ix,3}}{K_{ix,3}+s_3}\right) \quad (4)$$

For sugar utilization, the rate of each pure sugar is considered in separate rate equation. The glucose, fructose, and sucrose consumption rates as shown in Equation (5), (6) and (7), respectively,

$$\frac{ds_1}{dt} = -\alpha q_{s\max 1}\left(\frac{s_1}{K_{ss,1}+s_1}\right)\left(1-\frac{p-P_{is,1}}{P_{ms,1}-P_{is,1}}\right)\left(\frac{K_{is,1}}{K_{is,1}+s_1}\right)x + ks_3 x \quad (5)$$

$$\frac{ds_2}{dt} = -\beta q_{s\max 2}\left(\frac{s_2}{K_{ss,2}+s_2}\right)\left(1-\frac{p-P_{is,2}}{P_{ms,2}-P_{is,2}}\right)\left(\frac{K_{is,2}}{K_{is,2}+s_2}\right)x + ks_3 x \quad (6)$$

$$\frac{ds_3}{dt} = -(1-\alpha-\beta)q_{s\max 3}\left(\frac{s_3}{K_{ss,3}+s_3}\right)\left(1-\frac{p-P_{is,3}}{P_{ms,3}-P_{is,3}}\right)\left(\frac{K_{is,3}}{K_{is,3}+s_3}\right)x - ks_3 x \quad (7)$$

For ethanol production, the rate is interpreted by Equation (8). The rate of ethanol production is dependent on the relative sugar uptake rate of glucose, fructose, and sucrose by the following equations.

$$\frac{dp}{dt} = \left[\alpha r_{p,1} + \beta r_{p,2} + (1-\alpha-\beta)r_{p,3}\right]x \quad (8)$$

For glucose:

$$r_{p,1} = q_{p,\max,1}\left(\frac{s_1}{K_{sp,1}+s_1}\right)\left(1-\frac{p-P_{ip,1}}{P_{mp,1}-P_{ip,1}}\right)\left(\frac{K_{ip,1}}{K_{ip,1}+s_1}\right) \quad (9)$$

For fructose:

$$r_{p,2} = q_{p,\max,2}\left(\frac{s_2}{K_{sp,2} + s_2}\right)\left(1 - \frac{p - P_{ip,2}}{P_{mp,2} - P_{ip,2}}\right)\left(\frac{K_{ip,2}}{K_{ip,2} + s_2}\right) \quad (10)$$

For sucrose:

$$r_{p,3} = q_{p,\max,3}\left(\frac{s_3}{K_{sp,3} + s_3}\right)\left(1 - \frac{p - P_{ip,3}}{P_{mp,3} - P_{ip,3}}\right)\left(\frac{K_{ip,3}}{K_{ip,3} + s_3}\right) \quad (11)$$

The ultimate purpose of a mathematical model developed in this study was to construct simulation curves for the cultivation kinetics of *S. cerevisiae* TISTR 5606 in static condition which utilized dried longan extract as a carbon source. The best way to achieve an acceptable parameter set in the mathematical model was through a step by step calculation which started from the cultivation in three individual pure sugars which were commonly found in dried longan, namely, glucose fructose and sucrose at 40 g l^{-1}. This was followed by subsequent cultivation in the fermentation media with triple sugars and dried longan extract, respectively. At the beginning, initial guess parameters of the model were determined from the cultivation kinetics with individual pure sugar substrate and previously published values (Leksawasdi *et al*. 2001). These guessed parameters were then applied to triple substrate kinetics data. The important microbial growth parameters such as μ_{max}, $q_{s,max}$ and $q_{p,max}$ were allowed to "float" within 20% of the originally guessed value using the minimization process of total RSS. The searched parameters from triple substrate system were later used for the cultivation kinetics data from dried longan extract during the overall sugar concentration range of 60 - 180 g l^{-1}. The complete mathematical model with all kinetic parameters was then formulated during the final parameter search.

2.4 Cloning and Expression of *Saccharomyces cerevisiae* Pyruvate Decarboxylase in *Pichia pastoris*

2.4.1 Genomic DNA extraction

Genomic DNA was extracted from *Saccharomyces cerevisiae* TISTR5606 using modified method from Sambrook *et al.* (2001) and Invitrogen Corp (1999).

2.4.2 Test of amplification of *pdc*1 gene

The condition to amplify *pdc*1 gene was carried out using *Taq* DNA polymerase enzyme (Vivantis, Poland), specific primer (F_PDC1_SC; 5'-ATGTCTGAAATTACTTTGGGTAAATATTTG-3', R_PDC1_SC; 5'-TTATTGCTTAGCGTTGGTAGCAGCAGTCAA-3').

2.4.3 Amplification of *pdc*1 gene using hi-fidelity DNA polymerase

*pdc*1 gene was amplified from *S. cerevisiae* TISTR5606 genomic DNA using hi-fidelity Phusion® DNA polymerase (Finnzyme, Finland) and specific hanging primer (added restriction site *Xho* I and *Not* I at 5' and 3', respectively) (F_PDC1_SC_X; 5'-AGTCGTCCTCGAGAAAAGAGAG GCTGAAGCTAT G TCTGAAATT-3', R_PDC1_SC_N 5'-AATATGCGGCCGCTTATTGCTTAGCGT TGGTAGCAGC-3'). After that the *pdc*1 amplicon was cut with *Eco* RI enzyme for restriction analysis. The best condition to amplify was pre-denaturing 95 ^{o}C 10 minutes followed by 35 cycles of denaturating 95 ^{o}C for 50 seconds, annealing 58 ^{o}C for 50 seconds, and elongation 72 ^{o}C for 1 minute.

2.4.4 Transformation of pPICZA-PDC1 into *E. coli*

Amplified *pdc*1 gene and pPICZA vector (Invitrogen, USA) were cut with restriction enzyme *Xho* I and *Not* I. After that, they were ligated with T4 ligase (fermentas, USA). The recombinant vector, pPICZA-PDC1, was transformed into competent *E. coli* XL-1 blue using heat shock method. (Sambrook *et al*., 2001). The transformed cells were selected on LB low salt plates containing 25 ug/ml zeocin and incubated at 37 ^{o}C for overnight. All clones were analyzed for the present of large recombinant vector using size screening technique. The clones containing larger plasmid were confirmed by colony PCR technique using primers specific to *pdc*1 gene.

2.4.5 Transformation of pPICZA-PDC1 into *P. pastoris*

pPICZA-*pdc*1 vector was purified from positive transformant using geneJET^{TM} plasmid miniprep kit. Vector was the linearlized with *Sac* I. Linear recombinant plasmid was transformed into *P. pastoris* X-33 using electroporation method. The transformed cells were selected on YPDS agar containing 100 200 and 500 μg ml^{-1} zeocin. The clones were confirmed by colony PCR technique using primers specific to *pdc*1 gene.

2.4.6 Analysis of PDC1 enzyme expression

Positive transformant *P. pastoris* and *P. pastoris* containing pPICZA vector (negative control) were inoculated into 25 ml of BMGY broth in baffles flask and incubated overnight at 30 °C, 250 rpm. Induction was carried out by addition of methanol to final concentration of 0.5% (v/v) at 12 and 24 hour and final concentration of 1% (v/v) at 36, 48 and 60 hour. *S. cerevisiae T*ISTR 5606 (wild type) and *C. utillis* TISTR 5198 (control) were incubated in YM broth and incubated for 24 hours, 250 rpm. Then the cells were harvested by centrifugation and broken using freeze-thawing with liquid nitrogen. The PDC1 activity was analyzed using carboligase activity, which is corresponding to PDC1 activity, using carboligase assay (Forlani, 1999, Roche *et al.*, 2001; Leksawasdi, 2003; 2004). R-PAC produced in the reaction was detected by High Performance Liquid Chromatography (HPLC) (Agilent technologies, Germany) using C8 column (Bio-Rad, USA).

Chapter 3

Results and Discussion

3.1 The Production of *R*-phenylacetylcarbinol Using Whole Cells of *Candida utilis* in Biphasic Biotransformation System with Concentrated Phosphate Solution as Buffer Species

3.1.1 Kinetics Studies of Six *C. utilis* strains in 150 ml Dried Longan Extract (DLE)

This experiment utilized dried longan aged two years which still possessed high level of sugar concentrations in order to obtain dried longan extract with supplementation of extra nitrogen sources such as yeast extract, malt extract, and peptone. All cultivations were performed at 150 ml scale for 192 h in the static condition at 25.6^{o}C. The cultivation kinetic profiles of six *C. utilis* strains in dried longan extract are shown in Fig. 3.1 for strain TISTR5001, Fig. 3.2 for TISTR 5032, Fig. 3.3 for TISTR 5043, Fig. 3.4 for TISTR 5046, Fig. 3.5 for TISTR 5198, and Fig. 3.6 for TISTR 5352. Each figure is divided into two parts, namely; part (a) describes the kinetic profiles of TSS, pH level, OD600, and dried biomass concentration which could be related to OD600 by third order polynomial equations as previously mentioned in Table 3.1; part (b) portrays the kinetic profiles of substrates such as sucrose, glucose, and fructose concentrations, as well as the product or ethanol concentration. These profiles were further analyzed to obtain ethanol yield ($Y_{P/S}$) which described the ratio of the produced ethanol concentration over the consumed sucrose, glucose and fructose concentrations as shown in Table 3.5.

Table 3.1: List of β values in the third order polynomial equations that correlated OD and X for six *C. utilis* strains.

Strain (TISTR)	β_1	β_2	β_3	R^2
5001	4.290×10^{-1}	-1.343×10^{-2}	5.297×10^{-4}	0.999
5032	4.583×10^{-1}	-9.683×10^{-3}	1.396×10^{-3}	1.000
5043	4.410×10^{-1}	-1.262×10^{-2}	6.291×10^{-4}	1.000
5046	5.021×10^{-1}	-1.897×10^{-2}	1.060×10^{-3}	1.000
5198	1.999×10^{-1}	9.397×10^{-3}	8.040×10^{-5}	0.998
5352	2.868×10^{-1}	1.322×10^{-3}	2.482×10^{-4}	0.999

The detailed analysis of each cultivation profile with hypothesis testing across six *C. utilis* strains is tabulated in Table 3.2 – 3.7. The first three tables (Table 3.2 – 3.4) portray the statistical comparison of TSS, pH level, OD600, and dried biomass concentration data extracted from Fig. 3.1(a) – 3.6(a) which include the analyses of differences between the final and initial levels, average, and maximum rates. Similar analyses and comparisons were also carried out for sugars and ethanol concentrations. Table 3.5 – 3.7 presents these information in terms of differences, average as well as maximum rates.

The kinetic profiles describing the microbial growth of six *C.utilis* strains had similar trends as shown in Fig. 3.1(a) – 3.6(a). In term of pH level and TSS decreasing, there was negligible change with a slight continuous decreasing trend with cultivation period. The profiles of dried biomass concentration and OD600 for the cultivation of all six *C. utilis* strains were similar in shape and trend.

From Table 3.2, the highest decreasing trend of TSS was 6.39 ± 0.35 ^{o}Brix for *C. utilis* TISTR 5352 which was significantly different statistically ($p \leq 0.05$) from TISTR 5198 with the second highest TSS decreasing trend of 3.61 ± 0.07^{o}Brix. The average and maximum TSS decreasing rates of TISTR 5352 were the highest with the corresponding values of 0.033 ± 0.009^{o}Brix h^{-1} and 0.078 ± 0.016^{o}Brix h^{-1}, respectively. These were different statistically ($p \leq 0.05$) from TISTR 5032 and 5046 whose TSS level remained constant throughout 192 h cultivation period (Table 3.3 and 3.4).

This was compared to the highest increasing trend of 15.48 ± 0.58 in OD600 unit for the cultivation of TISTR 5198 which was not different statistically ($p > 0.05$) than TISTR 5352 (13.61 ± 0.90 ODU) (Table 3.2). The average OD600 increasing rate of TISTR 5198 was the highest at 0.081 ± 0.056 ODU h^{-1} which was followed by TISTR 5352 at 0.076 ± 0.034 ODU h^{-1}, both values were not different statistically ($p > 0.05$) (Table 3.3). The maximum OD600 increasing rate of TISTR 5198 at 0.262 ± 0.081 ODU h^{-1} was also at the highest level and did not differ statistically ($p > 0.05$) from TISTR 5001, whose OD600 increasing rate was 0.204 ± 0.030 ODU h^{-1} (Table 3.4).

The first two *C. utilis* strains that could generate the highest level of dried biomass concentration were TISTR 5198 (6.43 ± 0.12 g l^{-1}) and TISTR 5352 (4.98 ± 0.26 g l^{-1}), respectively (Table 3.2) which were also different statistically ($p \leq 0.05$). This was in contrary to average dried biomass production rate of both strains at 0.034 ± 0.022 g l^{-1} h^{-1} and 0.028 ± 0.013 g l^{-1} h^{-1} in Table 3.3 which appeared to be insignificantly different ($p > 0.05$). Similar trend was also spotted for the maximum rate of dried biomass production rates (Table 3.4) of both strains at 0.107 ± 0.029 g l^{-1} h^{-1} and 0.063 ± 0.001 g l^{-1} h^{-1}, respectively. It was evident from the trends of OD600 and dried biomass concentration of all six

strains in Fig. 3.1(a) – 3.6(a) that the growth profiles could be divided into two stages. After the first stage of uninterrupted growth for the duration of 96 h, the trends of OD600 or dried biomass concentration began to drop thereafter prior to the second stage of growth. It was possible that the culture of *C. utilis* was subjected to the osmotic dehydration from the initial TSS level of 20^{o}Brix in the cultivation media and exhibited two growth stages.

Dried longan extract was able to resist pH change during 192 h cultivation relatively well as evident in Table 3.3 where the average pH level was maintained between 4.723 ± 0.093 for TISTR 5046 and 5.415 ± 0.040 for TISTR 5043. However, both values were statistically different ($p \leq 0.05$) from one another. The presence of buffer species in dried longan extract might played parts in resisting pH change in the similar ways as molasses (Cazetta *et al*., 2007). In addition, *C. utilis* also did not generate organic acids at a relatively high level during cultivation in static condition (Poodtatep *et al*., 2008).

The average specific growth rate was analyzed as shown in Table 3.3 with the highest value belonged to *C. utilis* TISTR 5352 at 0.015 ± 0.005 h^{-1} which was closely followed by TISTR 5198 at 0.014 ± 0.008 h^{-1}. Both values were not significantly different ($p > 0.05$). In term of maximum specific growth rate as illustrated in Table 3.4, *C. utilis* TISTR 5198 possessed the highest level at 0.044 ± 0.011 h^{-1} which was not different statistically ($p > 0.05$) than the lowest value from TISTR 5046 at 0.024 ± 0.003 h^{-1}.

The average doubling time computed directly from the average specific growth rate revealed the relatively broad range between 46.4 ± 16.8 h for TISTR 5352 to 119.1 ± 160.7 h for TISTR 5032. Due to the relatively large error, the statistically different in average doubling time was not observed ($p > 0.05$). This was compared to TISTR 5198 whose average doubling time was 50.2 ± 30.4 h. Furthermore, the analysis of minimum doubling time in Table 3.4 suggested that TISTR 5198 was the *C. utilis* strain that was able to undergo cells budding process with the shortest time of 15.7 ± 3.8 h which was immediately followed by TISTR 5352 with the corresponding minimum doubling time of 19.8 ± 2.0 h which was not different statistically from its predecessor ($p > 0.05$).

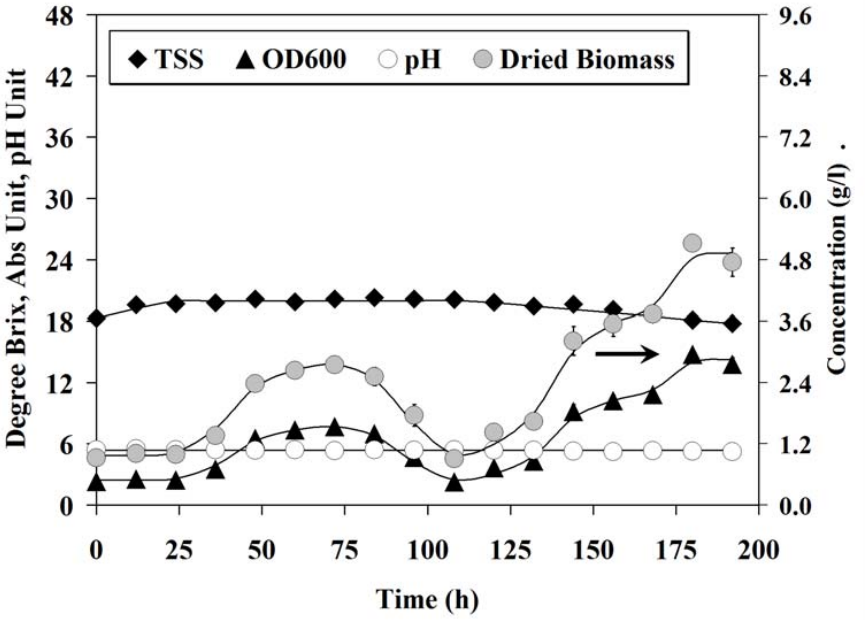
From Fig. 3.1(b) – 3.6(b) and Table 3.5, the sucrose concentration level of TISTR 5043 dropped most drastically at 71.69 ± 1.96 g l^{-1} which could be significantly compared ($p \leq 0.05$) to TISTR 5198 with the least decreasing level of only 6.31 ± 0.99 g l^{-1}. In term of an average sucrose decreasing rate (Table 3.6), TISTR 5043 decreased at the highest rate of 0.384 ± 0.166 g $l^{-1}h^{-1}$ and was followed by TISTR 5001 (0.383 ± 0.202 g $l^{-1}h^{-1}$) which was not significantly different from each other ($p > 0.05$).

Further comparison on the maximum sucrose decreasing rate (Table 3.7) resulted in TISTR 5001 whose rate was at the highest level of 1.450 ± 0.551 g $l^{-1}h^{-1}$. This was immediately followed by TISTR 5043 at the decreasing rate of 1.347 ± 0.341 g $l^{-1}h^{-1}$ which was also not significantly different ($p > 0.05$) from the previous rate from TISTR 5001.

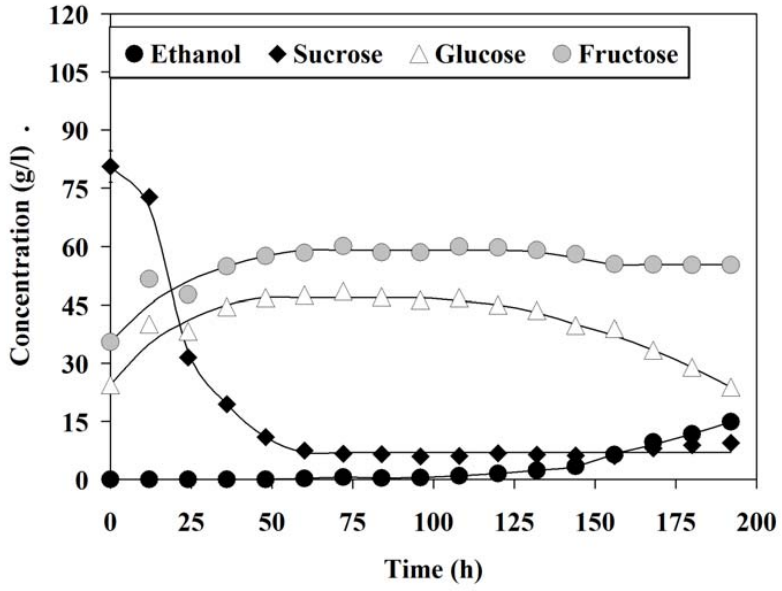
In term of glucose consumption (Table 3.5), TISTR 5352 was able to uptake this sugar at the highest level of 20.2 ± 0.17 g l^{-1} while the increases in glucose concentration were observed for TISTR 5032, TISTR 5043, and TISTR 5046. The average glucose decreasing rates from the cultivation of all six *C. utilis* strains were compared in Table 6 with TISTR 5352 as the best consumer with corresponding consumption rate of 0.105 ± 0.067 g $l^{-1}h^{-1}$ which was followed by TISTR 5198 (0.095 ± 0.040 g $l^{-1}h^{-1}$) whose rate was not significantly different from the former ($p > 0.05$). The maximum glucose decreasing rate in Table 3.7 suggested that TISTR 5198 had the highest rate of 0.363 ± 0.026 g $l^{-1}h^{-1}$ while TISTR 5001 was the second runner up with consumption rate of 0.337 ± 0.038 g $l^{-1}h^{-1}$. Both values were not significantly different from one another ($p > 0.05$).

C. utilis TISTR 5198 was able to consume the maximum level of fructose at 30.14 ± 0.32 g l^{-1} which could be significantly compared ($p \leq 0.05$) to TISTR 5043 whose fructose level had increased by 20.18 ± 1.55 g l^{-1}. The average fructose decreasing rate of TISTR 5198 in Table 6 was the highest at 0.143 ± 0.089 g $l^{-1}h^{-1}$, which was followed by the increasing rate at 0.109 ± 0.059 g $l^{-1}h^{-1}$ for TISTR 5043.

However, both values were not significantly different ($p > 0.05$) from one another. The maximum fructose decreasing rate could be obtained from Table 3.7. *C. utilis* TISTR 5198 was able to consume this sugar at the maximum level of 0.549 ± 0.292 g $l^{-1}h^{-1}$ while TISTR 5352 was the second candidate that was able to consume fructose at the slower rate of 0.391 ± 0.043 g $l^{-1}h^{-1}$. The maximum fructose consumption rates from both strains were not significantly different ($p > 0.05$). The elevation of glucose and fructose concentrations in comparison to the initial level throughout 192 h cultivation profiles of certain *C. utilis* strains as evident from Fig. 3.1(b) – 3.6(b) might be explained by the invertase activity that converted sucrose to glucose and fructose (Takeshige *et al.*, 1995).

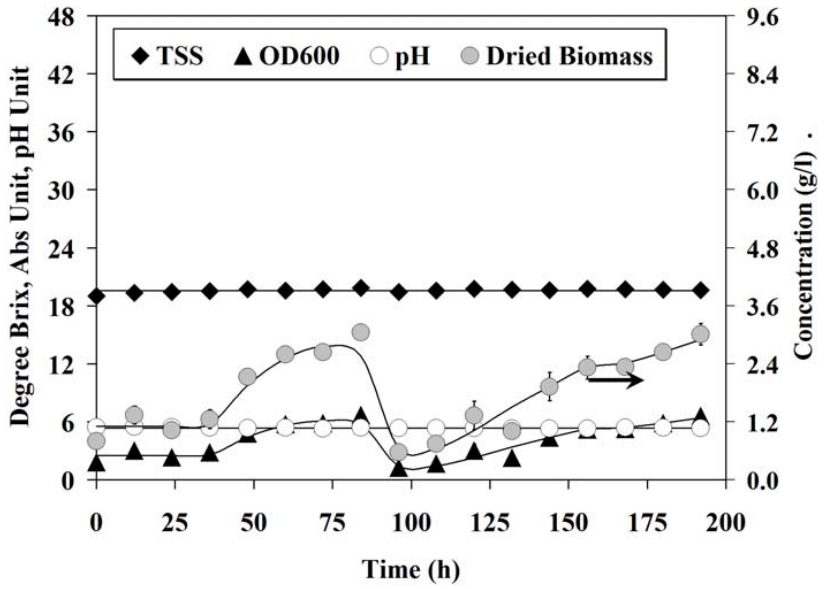


(a)

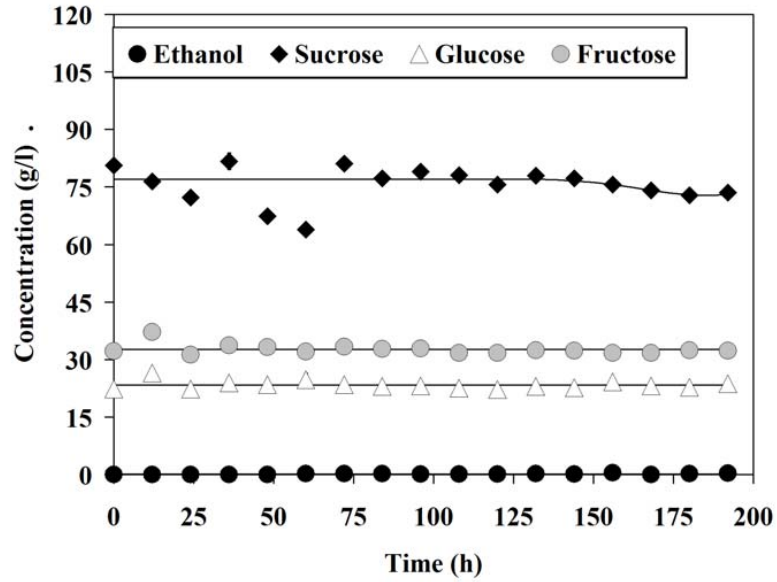


(b)

Figure 3.1: Growth kinetics of *C. utilis* TISTR 5001 during 192 h cultivation period in a static condition with DLE at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

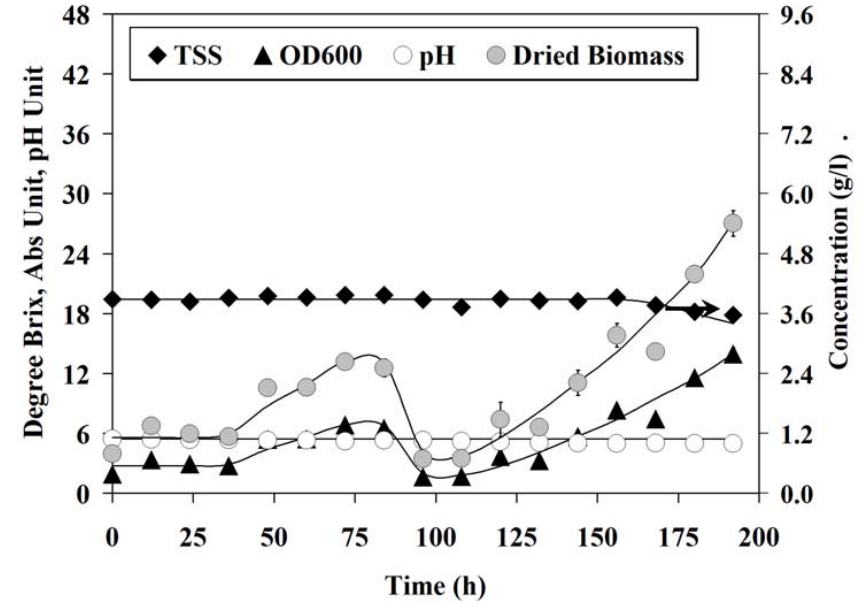


(a)

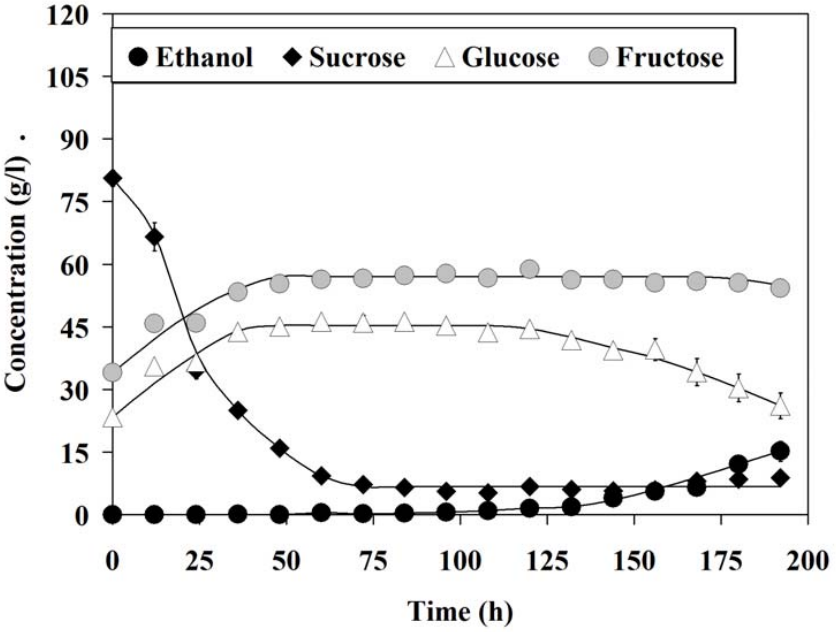


(b)

Figure 3.2: Growth kinetics of *C. utilis* TISTR 5032 during 192 h cultivation period in a static condition with DLE at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

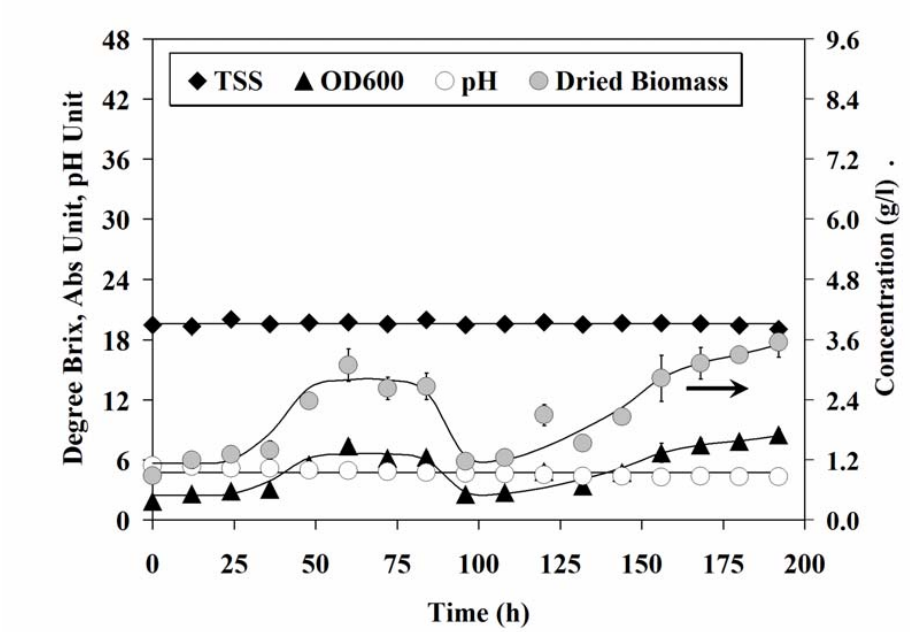


(a)

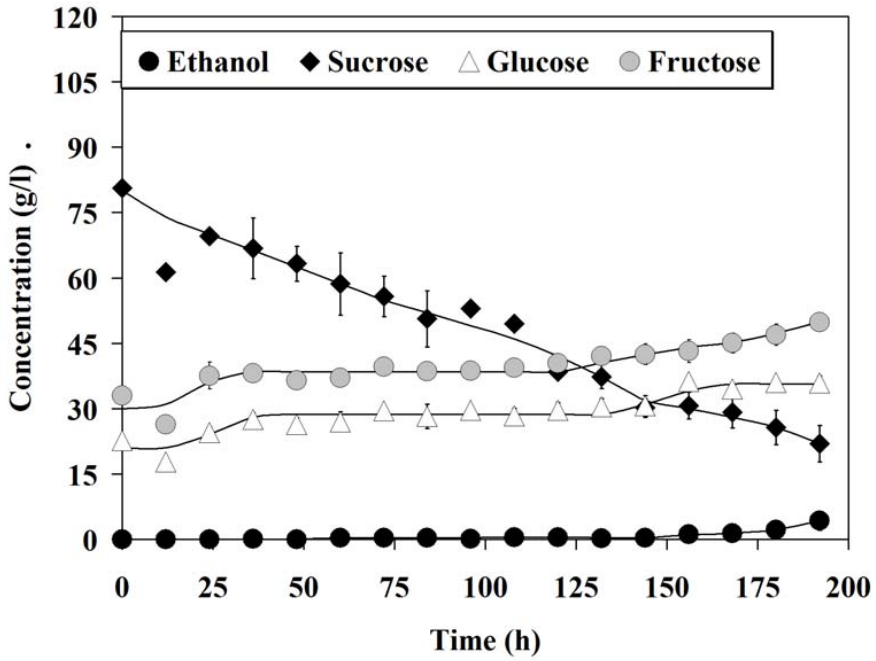


(b)

Figure 3.3: Growth kinetics of *C. utilis* TISTR 5043 during 192 h cultivation period in a static condition with DLE at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

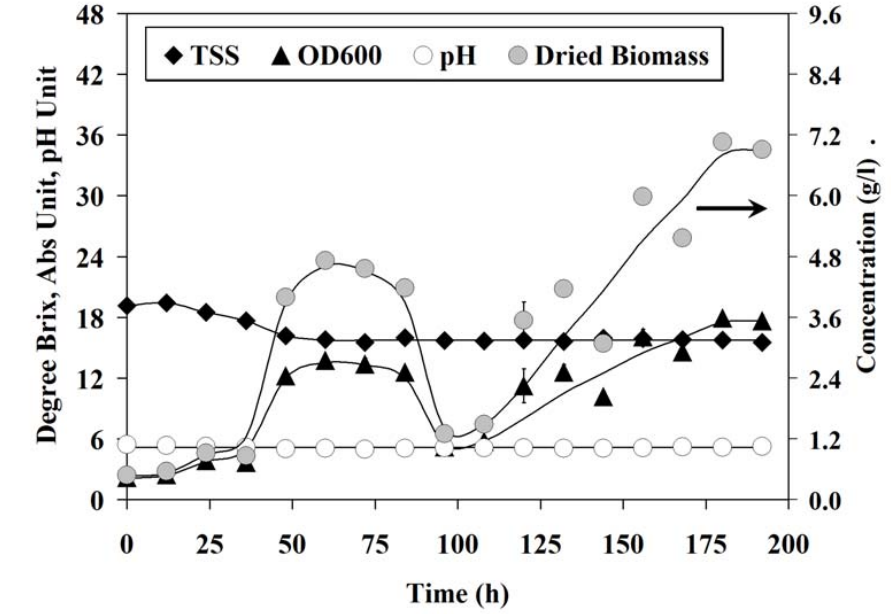


(a)

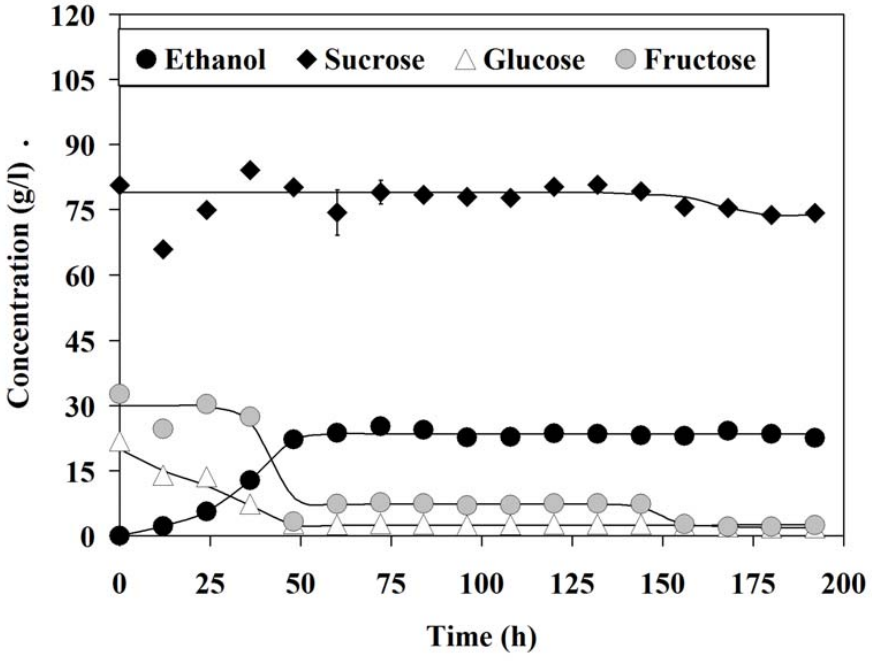


(b)

Figure 3.4: Growth kinetics of *C. utilis* TISTR 5046 during 192 h cultivation period in a static condition with DLE at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

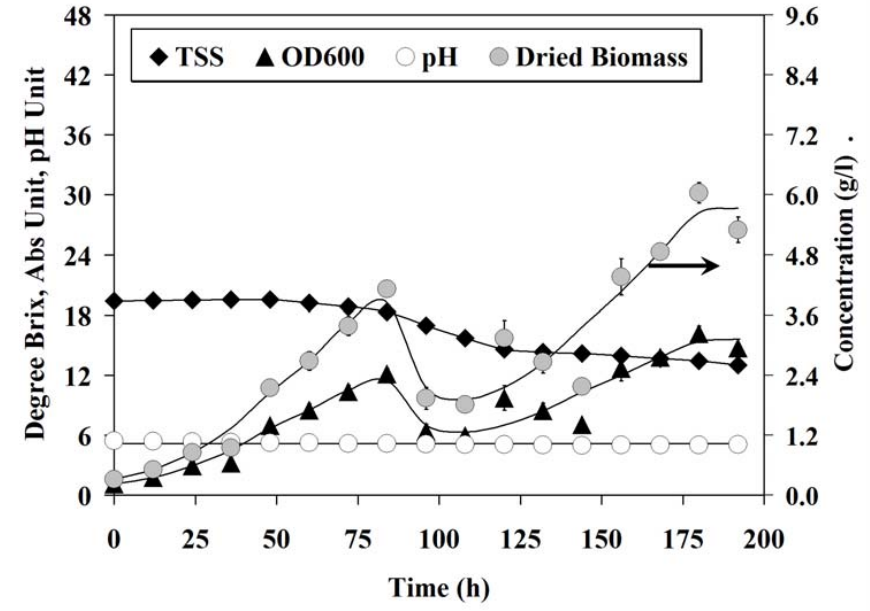


(a)

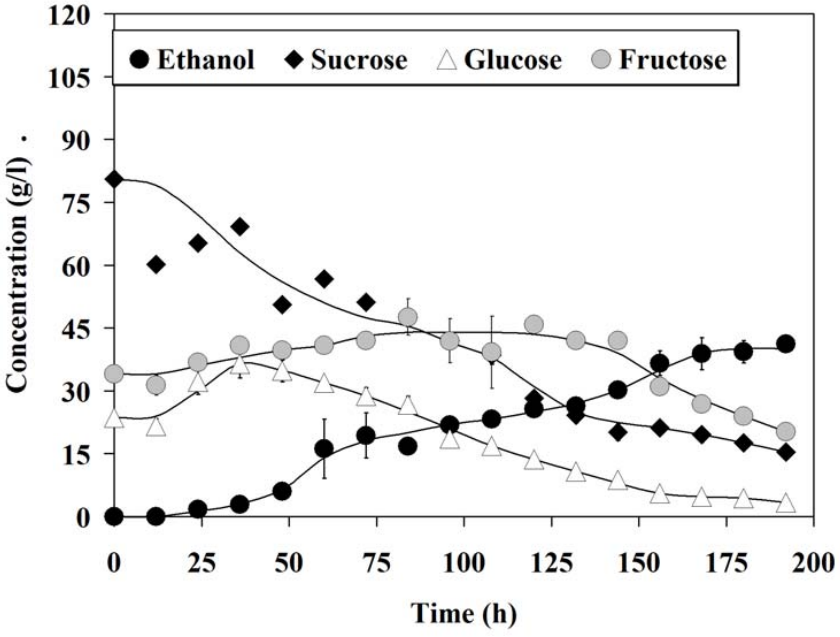


(b)

Figure 3.5: Growth kinetics of *C. utilis* TISTR 5198 during 192 h cultivation period in a static condition with DLE at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.



(a)



(b)

Figure 3.6: Growth kinetics of *C. utilis* TISTR 5352 during 192 h cultivation period in a static condition with DLE at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

Table 3.2: The differences in TSS, OD600, and dried biomass (X) concentration (g l^{-1}) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 150 ml static cultivation in dried longan extract using six *C. utilis* strains at 25.6°C.

Investigated parameters	TISTR 5001		TISTR 5032		TISTR 5043		TISTR 5046		TISTR 5198		TISTR 5352	
TSS decreasing level	0.50 ± 0.35	I, III, IV	-0.57 ± 0.20	II	1.54 ± 0.30	III	0.41 ± 0.28	IV	3.61 ± 0.07	V	6.39 ± 0.35	VI
OD600 increasing level	11.47 ± 0.67	I	4.81 ± 0.56	II, III	12.07 ± 0.60	I	6.63 ± 0.62	III	15.48 ± 0.58	IV	13.61 ± 0.90	I, IV
X production level	3.82 ± 0.28	I, III	2.21 ± 0.25	II, IV	4.61 ± 0.26	III, VI	2.67 ± 0.30	IV	6.43 ± 0.12	V	4.98 ± 0.26	VI

The numbers with the same Roman numeral (I-VI) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.3: The average pH level, average TSS decreasing rate (^{o}Brix h^{-1}), average OD600 increasing rate (ODU h^{-1}), average dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), average specific growth rate (h^{-1}), and average doubling time (h) during 192 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 150 ml static cultivation in dried longan extract using six *C. utilis* strains at 25.6^{o}C.

Investigated parameters	TISTR 5001		TISTR 5032		TISTR 5043		TISTR 5046		TISTR 5198		TISTR 5352	
pH level	5.361 ± 0.019	I	5.367 ± 0.013	I	5.415 ± 0.040	I	4.723 ± 0.093	II	5.143 ± 0.029	III	5.157 ± 0.037	III
TSS decreasing rate	-0.003 ± 0.008	I, II	0.000 ± 0.000	I	-0.013 ± 0.007	I, II, III	0.000 ± 0.000	II	-0.018 ± 0.010	I, II, III	-0.033 ± 0.009	III
OD600 increasing rate	0.062 ± 0.034	I	0.020 ± 0.028	I	0.059 ± 0.035	I	0.031 ± 0.025	I	0.081 ± 0.056	I	0.076 ± 0.034	I
X increasing rate	0.021 ± 0.011	I	0.009 ± 0.012	I	0.022 ± 0.013	I	0.012 ± 0.010	I	0.034 ± 0.022	I	0.028 ± 0.013	I
Specific growth rate	0.008 ± 0.006	I	0.006 ± 0.008	I	0.009 ± 0.007	I	0.006 ± 0.005	I	0.014 ± 0.008	I	0.015 ± 0.005	I
Doubling time	82.8 ± 56.1	I	119.1± 160.7	I	79.9 ± 64.8	I	116.5 ± 98.9	I	50.2 ± 30.4	I	46.4 ± 16.8	I

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.4: The maximum TSS decreasing rate (^{o}Brix h^{-1}), maximum OD600 increasing rate (ODU h^{-1}), maximum dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), maximum specific growth rate (h^{-1}), and minimum doubling time (h) during 192 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 150 ml static cultivation in dried longan extract using six *C. utilis* strains at 25.6^{o}C.

Investigated parameters	TISTR 5001		TISTR 5032		TISTR 5043		TISTR 5046		TISTR 5198		TISTR 5352	
TSS decreasing rate	-0.027 ± 0.000	I	0.000 ± 0.000	II	-0.041 ± 0.019	I, II, III	0.000 ± 0.000	II	-0.061 ± 0.021	I, III	-0.078 ± 0.016	III
OD600 increasing rate	0.204 ± 0.030	I	0.098 ± 0.014	II	0.165 ± 0.013	I, III	0.118 ± 0.020	II, III, IV	0.262 ± 0.081	I, II	0.162 ± 0.006	I, IV
X increasing rate	0.068 ± 0.010	I, II	0.044 ± 0.006	I	0.063 ± 0.007	I, II	0.047 ± 0.008	I, II	0.107 ± 0.029	I, II	0.063 ± 0.001	II
Specific growth rate	0.032 ± 0.004	I	0.031 ± 0.004	I	0.028 ± 0.002	I	0.024 ± 0.003	I	0.044 ± 0.011	I	0.035 ± 0.003	I
Doubling time	21.8± 2.7	I	22.7 ± 3.2	I	24.6 ± 2.1	I	28.7± 4.1	I	15.7 ± 3.8	I	19.8 ± 2.0	I

The numbers with the same Roman numeral (I-IV) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.5: The differences in sugars (sucrose, glucose, and fructose) concentration levels (g l^{-1}), ethanol concentration levels (g l^{-1}), lag time (sucrose, glucose, fructose, and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 150 ml static cultivation in dried longan extract using six *C. utilis* strains at 25.6°C.

Investigated parameters	TISTR 5001		TISTR 5032		TISTR 5043		TISTR 5046		TISTR 5198		TISTR 5352	
Sucrose decreasing level	71.15 ± 4.06	I, IV	7.02 ± 0.59	II	71.69 ± 1.96	III, IV	58.62 ± 4.38	I	6.31 ± 0.99	II	65.2 ± 2.20	I, III
Glucose decreasing level	0.55 ± 0.47	I	-1.37 ± 0.16	II	-2.74 ± 3.10	I, II	-13.31 ± 1.64	III	19.97 ± 0.22	IV	20.2 ± 0.17	IV
Fructose decreasing level	-19.80 ± 0.53	I	-0.18 ± 0.62	II	-20.18 ± 1.55	I	-16.90 ± 2.05	I	30.14 ± 0.32	III	13.8 ± 0.33	IV
Ethanol producing level	14.83 ± 1.31	I	0.32 ± 0.05	II	15.17 ± 2.33	I	4.26 ± 2.15	II	22.55 ± 0.36	III	41.2 ± 1.61	IV
Sucrose lag time	0.00 ± 0.00	I	132.0 ± 13.20	II	0.00 ± 0.00	I	0.00 ± 0.00	I	132.0 ± 13.20	II	0.0 ± 0.00	I
Glucose lag time	0.00 ± 0.00	I	192.0 ± 19.20	II	0.00 ± 0.00	I	12.00 ± 1.20	III	0.00 ± 0.00	I	0.0 ± 0.00	I
Fructose lag time	0.00 ± 0.00	I	192.0 ± 19.20	II	0.00 ± 0.00	I	0.00 ± 0.00	I	12.0 ± 1.20	III	12.0 ± 1.20	III
Ethanol lag time	48.00 ± 4.80	I	192.0 ± 19.20	II	48.00 ± 4.80	I	48.00 ± 4.80	I	0.00 ± 0.00	III	12.0 ± 1.20	IV
$Y_{P/S}$	0.29 ± 0.03	I	0.06 ± 0.01	II	0.31 ± 0.05	I, III	0.15 ± 0.08	I, II	0.40 ± 0.01	III	0.42 ± 0.02	III

The numbers with the same Roman numeral (I-IV) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.6: The average sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), average ethanol production rate (g l^{-1} h^{-1}), average specific rate of sugars consumption (Avg Q_s, g g^{-1} h^{-1}), and average specific rate of ethanol production (Avg Q_p, g g^{-1} h^{-1}) during 192 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 150 ml static cultivation in dried longan extract using six *C. utilis* strains at 25.6°C.

Investigated parameters	TISTR 5001		TISTR 5032		TISTR 5043		TISTR 5046		TISTR 5198		TISTR 5352	
Sucrose consumption rate	-0.383 ± 0.202	I, II	-0.021 ± 0.010	II	-0.384 ± 0.166	I, II	-0.302 ± 0.023	I	-0.027 ± 0.015	II	-0.339 ± 0.051	I
Glucose consumption rate	-0.003 ± 0.085	I, II	0.000 ± 0.000	I	0.014 ± 0.077	I, II	0.076 ± 0.029	II	-0.095 ± 0.040	I	-0.105 ± 0.067	I
Fructose consumption rate	0.104 ± 0.062	I, II	0.000 ± 0.000	I	0.109 ± 0.059	I, II	0.104 ± 0.030	II	-0.143 ± 0.089	I	-0.072 ± 0.056	I
Ethanol production rate	0.077 ± 0.025	I	0.000 ± 0.000	II	0.079 ± 0.026	I	0.022 ± 0.011	I, II	0.122 ± 0.059	I, II, III	0.208 ± 0.040	III
Avg Q_s of sucrose	-0.335 ± 0.198	I, II	-0.009 ± 0.004	II	-0.312 ± 0.147	I, II	-0.170 ±0.026	I	-0.005 ± 0.003	II	-0.216 ± 0.060	I
Avg Q_s of glucose	0.067 ± 0.069	I, II	0.000 ± 0.000	I, II	0.061 ± 0.058	I, II	0.044 ± 0.019	II, III	-0.110 ± 0.059	I	0.017 ± 0.061	I, III
Avg Q_s of fructose	0.101 ± 0.059	I, II, III	0.000 ± 0.000	I, IV	0.100 ± 0.051	I, III	0.060 ± 0.023	III	-0.062 ± 0.037	II, IV	0.006 ± 0.024	I, II, III
Avg Q_p of ethanol	0.026 ± 0.007	I, II	0.000 ± 0.000	III	0.030 ± 0.008	I	0.007 ± 0.003	II, III	0.104 ± 0.047	I, II, III, IV	0.088 ± 0.017	IV

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.7: The maximum sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), maximum ethanol production rate (g l^{-1} h^{-1}), maximum specific rate of sugars consumption (Max Q_s, g g^{-1} h^{-1}), and maximum specific rate of ethanol production (Max Q_p, g g^{-1} h^{-1}) during 192 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 150 ml static cultivation in dried longan extract using six *C. utilis* strains at 25.6°C.

Investigated parameters	TISTR 5001		TISTR 5032		TISTR 5043		TISTR 5046		TISTR 5198		TISTR 5352	
Sucrose consumption rate	-1.450 ± 0.551	I, II, III	-0.083 ± 0.019	IV	-1.347 ± 0.341	I	-0.417 ±0.036	II	-0.109 ± 0.042	III, IV	-0.625 ± 0.042	I
Glucose consumption rate	-0.337 ± 0.038	I	0.000 ± 0.000	II	-0.290 ± 0.031	I	0.000 ± 0.000	II	-0.363 ± 0.026	I	-0.314 ± 0.016	I
Fructose consumption rate	-0.077 ± 0.034	I, II	0.000 ± 0.000	I	-0.042 ± 0.029	I, II	0.000 ± 0.000	I, II	-0.549 ± 0.292	I, II, III	-0.391 ± 0.043	III
Ethanol production rate	0.240 ± 0.013	I	0.000 ± 0.000	II	0.243 ± 0.012	I	0.081 ± 0.030	III	0.458 ± 0.136	I, IV	0.423 ± 0.069	IV
Max Q_s of sucrose	-1.309 ± 0.600	I, II, III	-0.037 ± 0.007	I	-1.155 ± 0.331	II	-0.304 ±0.047	III	-0.020 ± 0.007	I	-0.549 ± 0.131	II, III
Max Q_s of glucose	-0.097 ± 0.006	I	0.000 ± 0.000	II	-0.100 ± 0.009	I	0.000 ± 0.000	II	-0.438 ± 0.148	I	-0.127 ± 0.015	I
Max Q_s of fructose	-0.031 ± 0.011	I	0.000 ± 0.000	II	-0.009 ± 0.006	I, II	0.000 ± 0.000	II	-0.237 ± 0.119	I, II, III	-0.094 ± 0.015	III
Max Q_p of ethanol	0.060 ± 0.007	I	0.000 ± 0.000	II	0.074 ± 0.004	I	0.026 ± 0.008	III	0.408 ± 0.055	IV	0.181 ± 0.029	V

The numbers with the same Roman numeral (I-V) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Two strains of *C. utilis*, namely TISTR 5032 and TISTR 5198, were not able to consume sucrose as evident from the maximum lag time period of 132 ± 13.2 h in Table 3.5. This was significantly compared ($p \leq 0.05$) to TISTR 5001, 5043, 5046, and 5352 without the presence of any lag period for this sugar. In fact, TISTR 5032 was also unable to consume either glucose or fructose under static condition. *C. utilis* TISTR 5043 was able to consume glucose without any lag period. The lag periods of TISTR 5198 and TISTR 5352 in consuming fructose were 12 ± 1.2 h.

From Table 3.6, the highest average specific rate of sucrose consumption at 0.335 ± 0.198 g $g^{-1}h^{-1}$ belonged to TISTR 5001 which did not differ significantly ($p > 0.05$) to TISTR 5198 with the lowest average specific rate of 0.005 ± 0.003 g $g^{-1}h^{-1}$. In term of average specific rate for glucose consumption, TISTR 5198 was able to consume this sugar at the fastest specific rate of 0.110 ± 0.059 g $g^{-1}h^{-1}$ which was not significantly different ($p > 0.05$) from the apparent production rate of TISTR 5001 (0.067 ± 0.069 g $g^{-1}h^{-1}$). In the situation of average specific rate for fructose consumption, the highest rate belonged to TISTR 5198 (0.062 ± 0.037 g $g^{-1}h^{-1}$) which was not significantly different ($p > 0.05$) from the apparent production rate of TISTR 5001 (0.101 ± 0.059 g $g^{-1}h^{-1}$).

The maximum specific rate of sucrose consumption for TISTR 5001 was at the highest level of 1.309 ± 0.600 g $g^{-1}h^{-1}$ as evident from Table 3.7 which was not significantly different ($p > 0.05$) from TISTR 5198 with the lowest maximum specific rate of only 0.020 ± 0.007 g $g^{-1}h^{-1}$. This was compared to the maximum specific rate of glucose consumption, in which TISTR 5198 yielded the highest value of 0.438 ± 0.148 g $g^{-1}h^{-1}$ and was followed by TISTR 5352 with the corresponding value of 0.127 ± 0.015 g $g^{-1}h^{-1}$. Both maximum specific rates were not significantly different ($p > 0.05$). Similar trend and insignificant difference ($p > 0.05$) were also observed with the maximum specific rate of fructose consumption in which TISTR 5198 had the highest value of 0.237 ± 0.119 g $g^{-1}h^{-1}$ and was again followed by TISTR 5352 (0.094 ± 0.015g $g^{-1}h^{-1}$)

The analysis of ethanol production level in Table 3.5 resulted in the best three ethanol producers which included TISTR 5352 (41.2 ± 1.6 g l^{-1}), TISTR 5198 (22.6 ± 0.4 g l^{-1}), and TISTR 5043 (15.2 ± 2.3 g l^{-1}), respectively. These were significantly different ($p \leq 0.05$) to TISTR 5032 whose ethanol production level was only 0.32 ± 0.05 g l^{-1}. The average ethanol production rate of TISTR 5352 as shown in Table 3.6 was the highest with the corresponding value of 0.208 ± 0.040 g $l^{-1}h^{-1}$. The comparison to TISTR 5198 at 0.122 ± 0.059 g $l^{-1}h^{-1}$ suggested that both figures were not significantly different ($p > 0.05$). The comparison of maximum ethanol production rates are given in Table 3.7. The highest ethanol

producer was TISTR 5198 with the maximum rate of 0.458 ± 0.136 g $l^{-1}h^{-1}$ which was followed by TISTR 5352 at 0.423 ± 0.069 g $l^{-1}h^{-1}$. Both values were not significantly different ($p > 0.05$).

The average specific rate of ethanol production for TISTR 5198 (Table 3.6) was at the highest level of 0.104 ± 0.047 g $g^{-1}h^{-1}$ which did not differ statistically ($p > 0.05$) from TISTR 5032 in the absence of ethanol production. In term of maximum specific rate of ethanol production (Table 3.7), TISTR 5198 gained the highest value of 0.408 ± 0.055 g $g^{-1}h^{-1}$ which was significantly different ($p \leq 0.05$) from the second runner up, namely TISTR 5352 (0.181 ± 0.029 g $g^{-1}h^{-1}$).

The ethanol yields of *C. utilis* TISTR 5352 and TISTR 5198 were at the highest level (Table 3.5) at 0.42 ± 0.02 and 0.40 ± 0.01 g ethanol g^{-1} sugars consumed, respectively which were not significantly different ($p > 0.05$).

3.1.2 Kinetics Studies of *C. utilis* TISTR 5352 in 1,500 ml Dried Longan Extract (DLE)

This experiment utilized dried longan aged two years which still possessed high level of sugar concentrations in order to obtain dried longan extract with supplementation of extra nitrogen sources such as yeast extract, malt extract, and peptone. All cultivations were performed at 1,500 ml scale for 192 h in the static condition at 25.6°C, to compare with the cultivation in 150 ml scale from section 3.1.1. The cultivation kinetic profiles of *C. utilis* TISTR 5352 in dried longan extract are shown in Fig. 3.7. The figure is divided into two parts, namely; part (a) describes the kinetic profiles of TSS, pH level, OD600, and dried biomass concentration which was related to OD600 by third order polynomial equations as previously mentioned in Table 3.7; part (b) portrays the kinetic profiles of substrates such as sucrose, glucose, and fructose concentrations, as well as the product or ethanol concentration. These profiles were further analyzed to obtain ethanol yield ($Y_{P/S}$) which described the ratio of the produced ethanol concentration over the consumed sucrose, glucose and fructose concentrations as shown in Table 3.11.

The detailed analysis of each cultivation profile with hypothesis testing for *C. utilis* TISTR 5352 in 1,500 ml and 150 ml static cultivation are tabulated in Table 3.8 – 3.13. The first three tables (Table 3.8 – 3.10) describe the statistical comparison of TSS, pH level, OD600, and dried biomass concentration data extracted from Fig. 3.6(a) for 150 ml cultivation and Fig. 3.7(a) for 1,500 ml cultivation which include the analyses of differences between the final and initial levels, average, and maximum rates. Similar analyses and comparisons were also carried out for sugars and ethanol concentrations. Table 3.11 – 3.13 presents these information in terms of differences, average as well as maximum rates.

The kinetic profiles describing the microbial growth of *C.utilis* TISTR 5352 for 1,500 ml and 150 ml static cultivation had similar trends as shown in Fig. 3.6(a) – 3.7(a). In term of pH level and TSS decreasing, there was negligible change with a slight continuous decreasing trend with cultivation period. The profiles of dried biomass concentration and OD600 for the cultivation of *C. utilis* TISTR 5352 for 1,500 ml and 150 ml static cultivation were similar in shape and trend.

From Table 3.8, the highest decreasing trend of TSS was 6.39 ± 0.35^{o}Brix for *C. utilis* TISTR 5352 for 150 ml static cultivation was significantly different statistically ($p \leq 0.05$) from 1,500 ml static cultivation with the TSS increasing trend of 1.26 ± 0.23^{o}Brix. The average and maximum TSS decreasing rates for the cultivation in 150 ml scale were the highest with the corresponding values of 0.033 ± 0.009^{o}Brix h^{-1} and 0.078 ± 0.016 ^{o}Brix h^{-1}, respectively. These were different statistically ($p \leq 0.05$) from 1,500 ml scale whose TSS level remained constant throughout 192 h cultivation period (Table 3.9 and 3.10).

The increasing trend of OD600 at 34.26 ± 1.30 in OD600 unit for the cultivation in 1,500 ml scale differed statistically ($p \leq 0.05$) from 150 ml scale at 13.61 ± 0.90 ODU as indicated in Table 3.8. The average OD600 increasing rate for 1,500 ml scale was the highest at 0.182 ± 0.075 ODU h^{-1} which was followed by 150 ml scale at 0.076 ± 0.034 ODU h^{-1}, however both values were not different statistically ($p > 0.05$) (Table 3.9). The maximum OD600 increasing rate for the cultivation in 1,500 ml scale at 0.400 ± 0.017 ODU h^{-1} was also at the highest level and did not differ statistically ($p > 0.05$) from 150 ml scale, whose OD600 increasing rate was only 0.162 ± 0.006 ODU h^{-1} (Table 3.10).

The cultivation of *C. utilis* TISTR 5352 in 1,500 ml scale could generate a significantly ($p \leq 0.05$) higher level of dried biomass concentration (9.87 ± 0.37 g l^{-1}) than that of 150 ml scale (4.98 ± 0.26 g l^{-1}) as indicated in Table 3.8. This was in contrary to the average rate of dried biomass production where both scales did not appear to be significantly different ($p > 0.05$) (Table 3.9). The opposite trend was observed for the maximum rate of dried biomass production rates (Table 3.10) at 0.115 ± 0.005 g l^{-1} h^{-1} and 0.063 ± 0.001 g l^{-1} h^{-1} for 1,500 and 150 ml scales, respectively. It was evident from the trends of OD600 and dried biomass concentration in Fig. 3.6(a) – 3.7(a) that the growth profiles of 1,500 and 150 ml scales could be divided into two stages. After the first stage of uninterrupted growth for the duration of 108 h, the trends of OD600 or dried biomass concentration began to decrease prior to the second stage of growth.

Dried longan extract was able to resist pH change during 192 h cultivation relatively well as evident in Table 3.9 where the average pH level was maintained between 4.601 ± 0.024 for 1,500 ml

scale and 5.157 ± 0.037 for 150 ml scale. However, both values were statistically different ($p \leq 0.05$) from one another. The presence of buffer species in dried longan extract might play parts in resisting pH change in the similar ways as molasses (Cazetta *et al*., 2007). In addition, *C. utilis* also did not generate organic acids at a relatively high level during cultivation in static condition (Poodtatep *et al*., 2008).

The average specific growth rate was analyzed as shown in Table 3.9 with the highest value belonged to 150 ml scale at 0.015 ± 0.005 h^{-1} which was followed by 1,500 ml scale at 0.004 ± 0.002 h^{-1}. Both values were not significantly different ($p > 0.05$). In term of maximum specific growth rate as illustrated in Table 3.10, cultivation in 150 ml scale resulted in the highest rate at 0.035 ± 0.003 h^{-1} which was significantly different statistically ($p \leq 0.05$) from 1,500 ml scale at 0.008 ± 0.000 h^{-1}.

The average doubling time calculated directly from the average specific growth rate were 46.4 ± 16.8 h for 150 ml scale and 155.6 ± 60.2 h for 1,500 ml scale. Due to the relatively large error, the statistically different in average doubling time was not observed ($p > 0.05$). Furthermore, the analysis of minimum doubling time in Table 3.10 suggested that the cultivation in 150 ml scale was able to undergo cells division process with the shortest time of 19.8 ± 2.0 h which was different statistically ($p \leq 0.05$) from the cultivation in 1,500 ml scale (83.0 ± 4.1 h).

From Fig. 3.6(b) – 3.7(b) and Table 3.11, the sucrose concentration level of the cultivation in 150 ml scale was decreased by 65.2 ± 2.20 g l^{-1} after 192 h which was not different significantly ($p > 0.05$) from 1,500 ml scale (65.0 ± 0.31 g l^{-1}). In term of an average sucrose decreasing rate (Table 3.12), both 1,500 and 150 ml scale had the similar value of 0.339 g l^{-1} h^{-1}. Further comparison of the maximum sucrose decreasing rate (Table 3.13) indicated the highest rate of 0.625 ± 0.042 g l^{-1} h^{-1} for 150 ml scale. While the rate for 1,500 ml scale was significantly lower ($p \leq 0.05$) at 0.500 ± 0.024 g l^{-1} h^{-1}.

In term of glucose consumption (Table 3.11), 150 ml scale was able to uptake this sugar at the highest level of 20.2 ± 0.17 g l^{-1} while the increase in glucose concentration was observed for 1,500 ml scale. The average glucose decreasing rate for 150 ml scale of 0.105 ± 0.067 g l^{-1} h^{-1} (Table 3.12) was significantly ($p \leq 0.05$) higher than 1,500 ml scale whose rate was, in fact, increasing. The maximum glucose decreasing rate in Table 3.13 suggested that 150 ml scale had the highest rate of 0.314 ± 0.016 g $l^{-1}h^{-1}$ while the rate for 1,500 ml scale was lower with the consumption rate of 0.104 ± 0.021 g l^{-1} h^{-1}. Both values were significantly different from one another ($p \leq 0.05$).

The cultivation of *C. utilis* TISTR 5352 in 150 ml scale was able to consume the maximum level of fructose at 13.8 ± 0.33 g l^{-1} which could be significantly compared ($p \leq 0.05$) to 1,500

ml scale whose fructose level had actually increased by 24.8 ± 0.6 g l^{-1}. The average fructose decreasing rate of 150 ml scale in Table 3.12 was the highest at 0.072 ± 0.056 g l^{-1} h^{-1}, where the increasing trend in rate at 0.128 ± 0.019 g $l^{-1}h^{-1}$ was observed stead for 1,500 ml scale. Both values were significantly different ($p \leq 0.05$) from one another. The maximum fructose decreasing rate could be obtained from Table 3.13. The cultivation in 150 ml scale was able to consume this sugar at the maximum level of 0.391 ± 0.043 g $l^{-1}h^{-1}$ which could be significantly compared ($p \leq 0.05$) to 1,500 ml scale whose fructose concentration level had increased by 0.031 ± 0.010 g l^{-1} h^{-1}. The elevation of glucose and fructose concentrations in comparison to the initial level throughout 192 h cultivation profile at 1,500 ml scale as evident from Fig. 3.6(b) – 3.7(b) might be explained by the invertase activity that converted sucrose to glucose and fructose (Takeshige *et al.*, 1995).

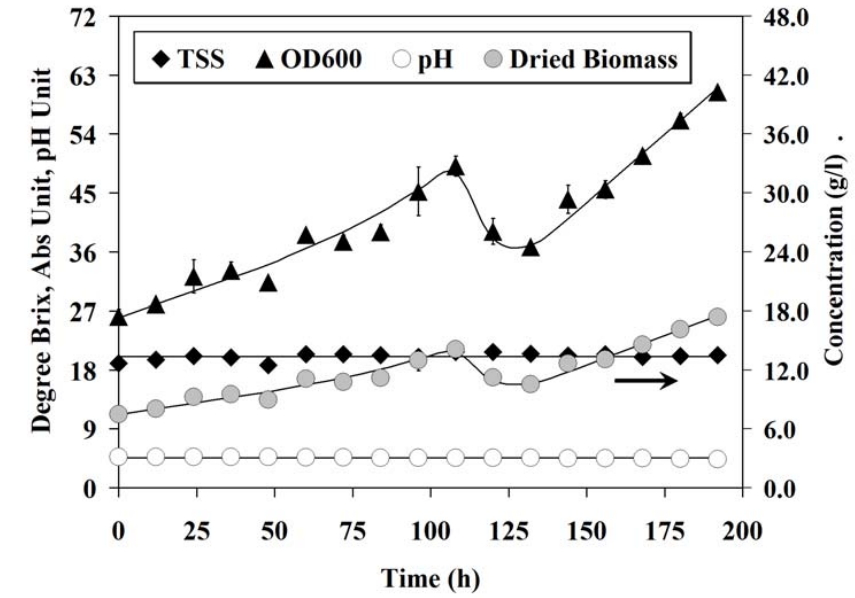
The cultivation of *C. utilis* TISTR 5352 in 150 and 1,500 ml scales resulted in the absence of lag period for sucrose and glucose consumption (Table 11). In fact, the lag period only existed for the utilization of fructose during the cultivation in 150 ml scale at 12.0 ± 1.20 h. The lag period for fructose consumption for 1,500 ml scale was also absence as there concentration of this sugar appeared to elevate throughout the time course.

From Table 3.12, the highest average specific rate of sucrose consumption at 0.216 ± 0.060 g g^{-1} h^{-1} belonged to 150 ml scale which differ significantly ($p \leq 0.05$) from 1,500 ml scale with the lowest average specific rate of 0.033 ± 0.005 g g^{-1} h^{-1}. In term of average specific rate for glucose consumption, 150 ml scale was able to consume this sugar at the fastest specific rate of 0.017 ± 0.061 g g^{-1} h^{-1} which was significantly different ($p \leq 0.05$) from the specific consumption rate of 1,500 ml scale (0.008 ± 0.003 g g^{-1} h^{-1}). In the situation of average specific rate for fructose consumption, the highest rate belonged to 150 ml scale (0.006 ± 0.024 g g^{-1} h^{-1}) which was not significantly different ($p > 0.05$) from the 1,500 ml scale at 0.012 ± 0.002 g $g^{-1}h^{-1}$.

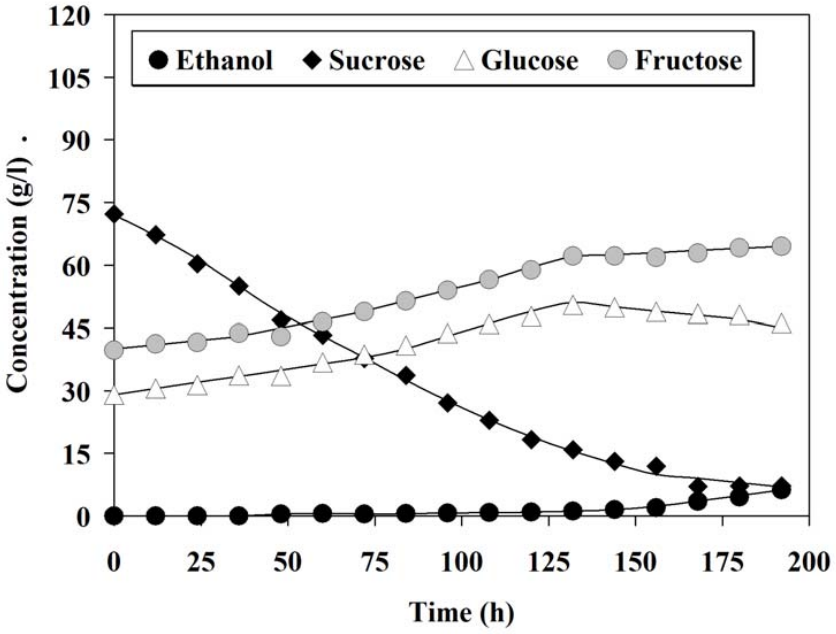
The maximum specific rate of sucrose consumption for 150 ml scale was at the highest level of 0.549 ± 0.131 g $g^{-1}h^{-1}$ as evident from Table 13 which was significantly different ($p \leq 0.05$) from 1,500 ml scale with the lowest maximum specific rate of only 0.057 ± 0.002 g $g^{-1}h^{-1}$. This was compared to the maximum specific rate of glucose consumption, in which 150 ml scale yielded the highest value of 0.127 ± 0.015 g $g^{-1}h^{-1}$ and was followed by 1,500 ml scale with the corresponding value of 0.007 ± 0.001 g $g^{-1}h^{-1}$. Both maximum specific rates were significantly different ($p \leq 0.05$). Similar trend and significant difference ($p \leq 0.05$) were also observed with the maximum specific rate of fructose consumption in

which 150 ml scale had the highest value of 0.094 ± 0.015 g $g^{-1}h^{-1}$ and was followed by 1,500 ml scale (0.002 ± 0.001 g $g^{-1}h^{-1}$).

The analysis of ethanol production level in Table 3.11 indicated that the cultivation in 150 ml scale (41.24 ± 1.61 g l^{-1}) was significantly different ($p \leq 0.05$) from 1,500 ml scale whose ethanol production level was only 6.28 ± 0.06 g l^{-1}. The average ethanol production rate in 150 ml scale as shown in Table 3.12 was the highest with the corresponding value of 0.208 ± 0.040 g $l^{-1}h^{-1}$. The comparison with 1,500 ml scale at 0.033 ± 0.011 g $l^{-1}h^{-1}$ suggested that both figures were significantly different ($p \leq 0.05$). The comparison of maximum ethanol production rates are given in Table 3.13.



(a)



(b)

Figure 3.7: Growth kinetics of *C. utilis* TISTR 5352 for 1,500 ml during 192 h cultivation period in a static condition with DLE at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

Table 3.8: The differences in TSS, OD600, and dried biomass (X) concentration (g l^{-1}) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml and 150 ml static cultivation in DLE using *C. utilis* TISTR 5352 at 25.6°C.

Investigated parameters	1,500 ml		150 ml	
TSS decreasing level	-1.26 ± 0.23	I	6.39 ± 0.35	II
OD600 increasing level	34.26 ± 1.30	I	13.61 ± 0.90	II
X production level	9.87 ± 0.37	I	4.98 ± 0.26	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.9: The average pH level, average TSS decreasing rate (°Brix h^{-1}), average OD600 increasing rate (ODU h^{-1}), average dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), average specific growth rate (h^{-1}), and average doubling time (h) during 192 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml and 150 ml static cultivation in DLE using *C. utilis* TISTR 5352 at 25.6°C.

Investigated parameters	1,500 ml		150 ml	
pH level	4.601 ± 0.024	I	5.157 ± 0.037	II
TSS decreasing rate	0.000 ± 0.000	I	-0.033 ± 0.009	II
OD600 increasing rate	0.182 ± 0.075	I	0.076 ± 0.034	I
X increasing rate	0.053 ± 0.022	I	0.028 ± 0.013	I
Specific growth rate	0.004 ± 0.002	I	0.015 ± 0.005	I
Doubling time	155.6 ± 60.2	I	46.4 ± 16.8	I

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.10: The maximum TSS decreasing rate (^{o}Brix h^{-1}), maximum OD600 increasing rate (ODU h^{-1}), maximum dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), maximum specific growth rate (h^{-1}), and minimum doubling time (h) during 192 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml and 150 ml static cultivation in DLE using *C. utilis* TISTR 5352 at 25.6^{o}C.

Investigated parameters	1,500 ml		150 ml	
TSS decreasing rate	0.000 ± 0.000	I	-0.078 ± 0.016	II
OD600 increasing rate	0.400 ± 0.017	I	0.162 ± 0.006	II
X increasing rate	0.115 ± 0.005	I	0.063 ± 0.001	II
Specific growth rate	0.008 ± 0.000	I	0.035 ± 0.003	II
Doubling time	83.0± 4.1	I	19.8 ± 2.0	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.11: The differences in sugars (sucrose, glucose, and fructose) concentration levels (g l^{-1}), ethanol concentration levels (g l^{-1}), lag time (sucrose, glucose, fructose, and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 1,500 ml and 150 ml static cultivation in DLE using *C. utilis* TISTR 5352 at 25.6°C.

Investigated parameters	1,500 ml		150 ml	
Sucrose decreasing level	65.01 ± 0.31	I	65.16 ± 2.20	I
Glucose decreasing level	-17.12 ± 0.42	I	20.21 ± 0.17	II
Fructose decreasing level	-24.75 ± 0.59	I	13.76 ± 0.33	II
Ethanol producing level	6.28 ± 0.06	I	41.24 ± 1.61	II
Sucrose lag time	0.00 ± 0.00	I	0.00 ± 0.00	I
Glucose lag time	0.00 ± 0.00	I	0.00 ± 0.00	I
Fructose lag time	0.00 ± 0.00	I	12.00 ± 1.20	II
Ethanol lag time	36.00 ± 3.60	I	12.00 ± 1.20	II
$\mathbf{Y_{P/S}}$	0.27 ± 0.01	I	0.42 ± 0.02	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.12: The average sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), average ethanol production rate (g l^{-1} h^{-1}), average specific rate of sugars consumption (Avg Q_s, g g^{-1} h^{-1}), and average specific rate of ethanol production (Avg Q_p, g g^{-1} h^{-1}) during 192 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml and 150 ml static cultivation in DLE using *C. utilis* TISTR 5352 at 25.6°C.

Investigated parameters	1,500 ml		150 ml	
Sucrose consumption rate	-0.339 ± 0.039	I	-0.339 ± 0.051	I
Glucose consumption rate	0.083 ± 0.034	I	-0.105 ± 0.067	II
Fructose consumption rate	0.128 ± 0.019	I	-0.072 ± 0.056	II
Ethanol production rate	0.033 ± 0.011	I	0.208 ± 0.040	II
Avg Q_s of sucrose	-0.033 ± 0.005	I	-0.216 ± 0.060	II
Avg Q_s of glucose	0.008 ± 0.003	I	0.017 ± 0.061	I
Avg Q_s of fructose	0.012 ± 0.002	I	0.006 ± 0.024	I
Avg Q_p of ethanol	0.002 ± 0.001	I	0.088 ± 0.017	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.13: The maximum sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), maximum ethanol production rate (g l^{-1} h^{-1}), maximum specific rate of sugars consumption (Max Q_s, g g^{-1} h^{-1}), and maximum specific rate of ethanol production (Max Q_p, g g^{-1} h^{-1}) during 192 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml and 150 ml static cultivation in DLE using *C. utilis* TISTR 5352 at 25.6°C.

Investigated parameters	1,500 ml		150 ml	
Sucrose consumption rate	-0.500 ± 0.024	I	-0.625 ± 0.042	II
Glucose consumption rate	-0.104 ± 0.021	I	-0.314 ± 0.016	II
Fructose consumption rate	0.031 ± 0.010	I	-0.391 ± 0.043	II
Ethanol production rate	0.100 ± 0.011	I	0.423 ± 0.069	II
Max Q_s of sucrose	-0.057 ± 0.002	I	-0.549 ± 0.131	II
Max Q_s of glucose	-0.007 ± 0.001	I	-0.127 ± 0.015	II
Max Q_s of fructose	0.002 ± 0.001	I	-0.094 ± 0.015	II
Max Q_p of ethanol	0.007 ± 0.001	I	0.181 ± 0.029	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

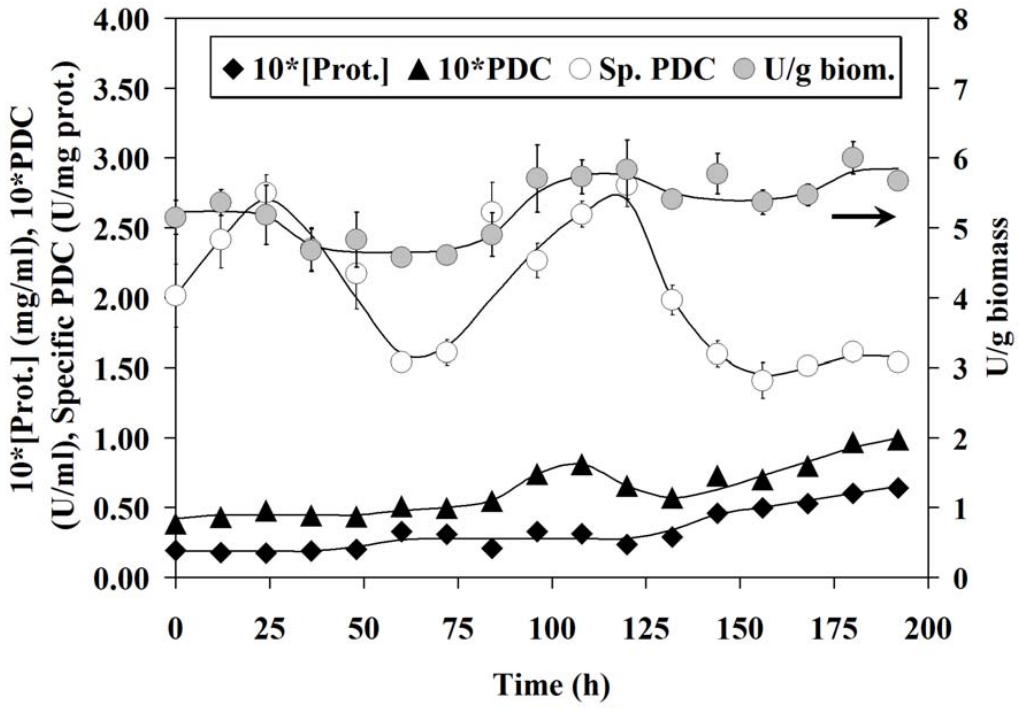


Figure 3.8: Kinetics of protein production (mg ml^{-1}), PDC activity (U ml^{-1}), specific PDC activity (both U mg^{-1} protein and U g^{-1} biomass) of *C. utilis* TISTR 5352 during 192 h cultivation period in a static condition at 25.6°C with DLE in 1,500 ml scale.

The highest ethanol production was achieved at 150 ml scale with the corresponding rate of 0.423 ± 0.069 g $l^{-1}h^{-1}$ which was significantly different ($p \leq 0.05$) from 1,500 ml scale at 0.100 ± 0.011 g $l^{-1}h^{-1}$. The average specific rate of ethanol production for 150 ml scale (Table 3.12) was at the highest level of 0.088 ± 0.017 g $g^{-1}h^{-1}$ which differed statistically ($p \leq 0.05$) from 1,500 ml scale (0.002 ± 0.001 g $g^{-1}h^{-1}$). In term of maximum specific rate of ethanol production (Table 3.13), 150 ml scale gained the highest value of 0.181 ± 0.029 g g^{-1} h^{-1} which was significantly different ($p \leq 0.05$) from 1,500 ml scale (0.007 ± 0.001 g $g^{-1}h^{-1}$).

The ethanol yields of 150 ml and 1,500 ml scales were 0.42 ± 0.02 and 0.27 ± 0.01 g ethanol g^{-1} sugars consumed (Table 3.11), respectively which were significantly different ($p \leq 0.05$) from one another.

The kinetics of protein production, PDC activity, and specific PDC activity of *C. utilis* TISTR 5352 during 192 h cultivation period in a static condition at 25.6°C with dried longan extract in

1,500 ml scale is illustrated in Fig. 3.8. The initial protein concentration of 0.019 ± 0.002 mg ml^{-1} was increased to 0.064 ± 0.001 mg ml^{-1} after 192 h cultivation period. The PDC activity increased from 0.039 ± 0.001 to 0.099 ± 0.001 U ml^{-1} between 0 and 192 h. This was compared to the decrease in specific PDC activity from 2.01 ± 0.23 to 1.54 ± 0.03 U mg^{-1} proteins. The elevation of biomass specific PDC activity from 5.15 ± 0.25 to 5.67 ± 0.09 U g^{-1} biomass after 192 h cultivation periods were observed.

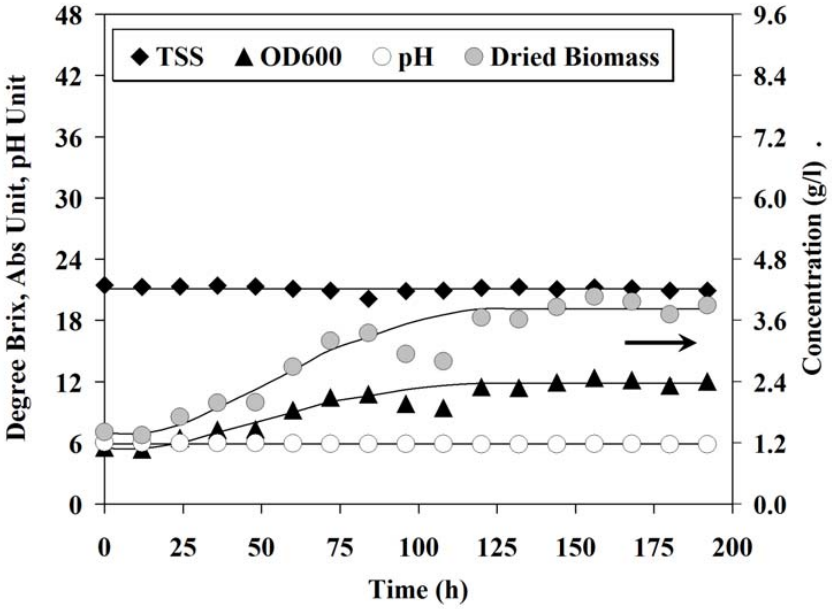
3.1.3 Kinetics Studies in Digested Dried Longan Flesh Hydrolysate (DDLFH)

From the previous experiment, *C.utilis* TISTR 5198 and TISTR 5352 were suitable microbes for ethanol production in 150 ml scale which utilized DLE as carbon source. These yeasts were later cultivated in DDLFH medium with the initial TSS values of 20 and 40^{o}Brix for 192 h in a static condition at 25.6^{o}C to investigate the growth kinetics. The kinetic profiles for the initial TSS value of 20^{o}Brix are shown in Fig. 3.9 and 10 for TISTR 5198 and TISTR 5352, respectively. This was compared to Fig. 3.11 and 3.12 for TISTR 5198 and TISTR 5352 for initial TSS value of 40^{o}Brix. Each figure is divided into two parts, namely; part (a) describes the kinetic profiles of TSS, pH level, OD600, and dried biomass concentration; part (b) portrays the kinetic profiles of substrates such as sucrose, glucose, and fructose concentrations, as well as the product or ethanol concentration.

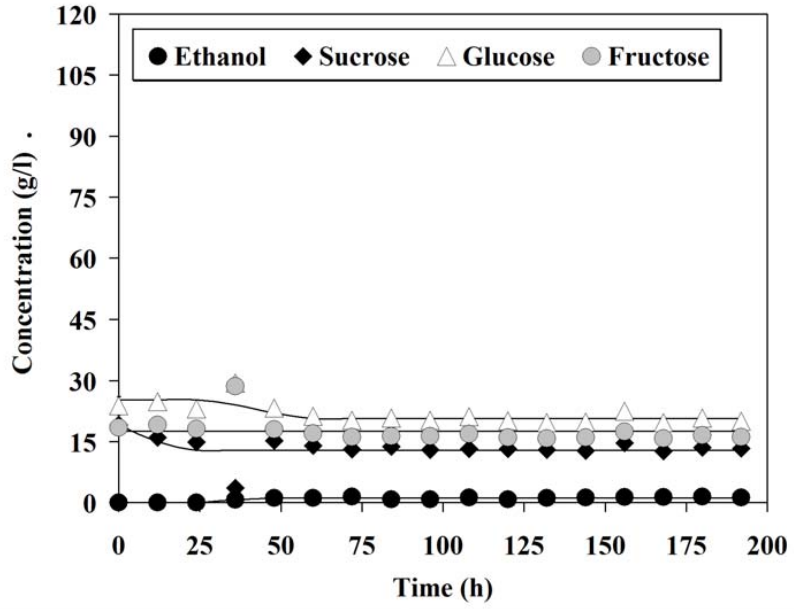
From Fig. 3.9(a) and 3.10(a) in term of pH and TSS level increasing when the cultivation was carried out with DDLFH medium with the initial TSS level of 20^{o}Brix, there was negligible change with a slight continuous decreasing trend with cultivation period. The OD600 increasing for TISTR 5198 was 6.46 ± 0.64 ODU which could be not significantly compared ($p > 0.05$) to TISTR 5352 was 5.59 ± 0.33 ODU. The dried biomass increasing for TISTR 5198 was 2.48 ± 0.13 g l^{-1} which could be significantly compared ($p \leq 0.05$) to TISTR 5352 whose dried biomass level had increased to 1.86 ± 0.10 g l^{-1}. The cultivation of *C. utilis* TISTR 5198 and TISTR 5352 in DDLFH media at TSS levels of 20 and 40^{o}Brix clearly indicated the growth inhibition as the process involved in the production of hydrolysate with a number of heat treatment steps might generate the toxic compounds, namely;furans,furfural, and hydroxymethylfurfural (HMF) (Pienkos *et al*., 2009). Similar trends were also observed in Fig. 3.11(a) and 3.12(a) for pH and TSS level in which the negligible change with a slight continuous decreasing trend were observed throughout the cultivation period for 40^{o}Brix. The decreasing trend of OD600 was observed for TISTR 5198 at 2.44 ± 1.07 ODU which could be significantly compared ($p \leq 0.05$) to TISTR 5352 whose OD600 increased by 1.21 ± 0.88 ODU after 192 h. The decreasing trend of dried biomass

concentration for TISTR 5198 was also observed at 1.57 ± 0.22 g l^{-1} which could be significantly compared ($p \leq 0.05$) to TISTR 5352 whose value increased by 0.61 ± 0.25 g l^{-1}.

From Fig. 3.9(b) and 3.10(b), there was a slight change in sugars concentrations throughout the time course for 20°Brix. For *C. utilis* TISTR 5198, the sucrose concentration decreased by 5.82 ± 0.78 g l^{-1}. This was compared with TISTR 5352 at 4.58 ± 0.56 g l^{-1}. However, both values were not significantly different ($p > 0.05$). The glucose decreasing level of *C. utilis* TISTR 5198 was 3.85 ± 2.25 g l^{-1} which did not differ statistically ($p > 0.05$) from TISTR 5352 at 0.37 ± 0.73 g l^{-1}.

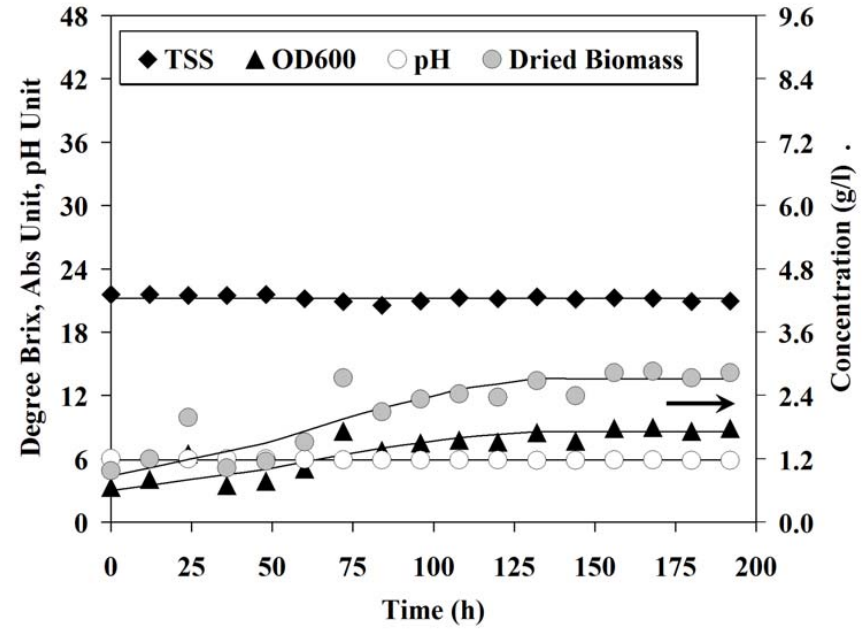


(a)



(b)

Figure 3.9: Growth kinetics of *C. utilis* TISTR 5198 during 192 h cultivation period in a static condition at 25.6^{o}C with digested dried longan flesh hydrolysate at 20^{o}Brix; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.



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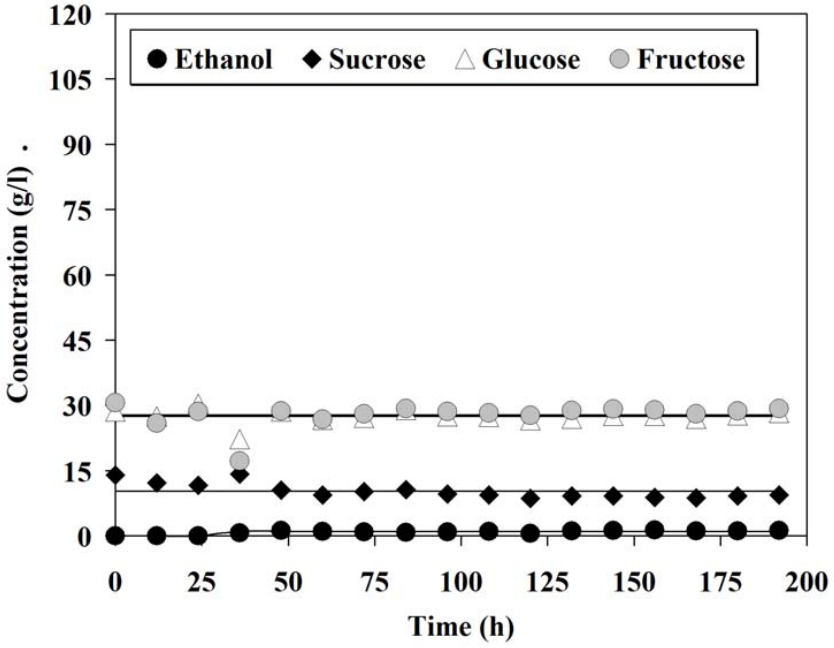
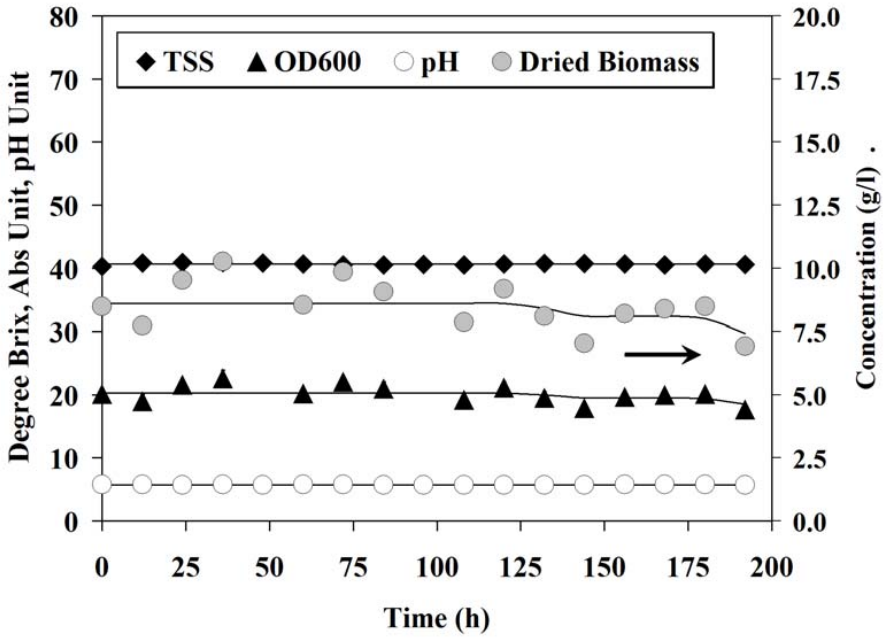
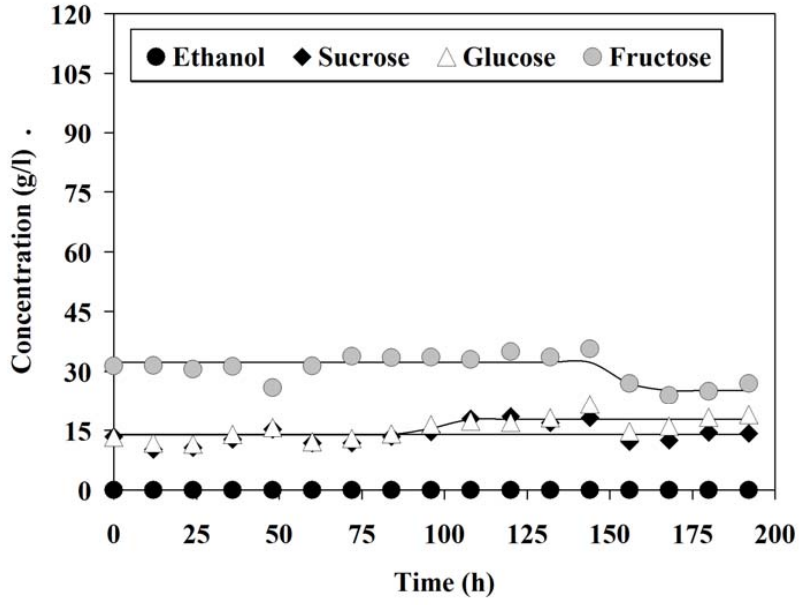


(b)

Figure 3.10: Growth kinetics of *C. utilis* TISTR 5352 during 192 h cultivation period in a static condition at 25.6°C with digested dried longan flesh hydrolysate at 20°Brix; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

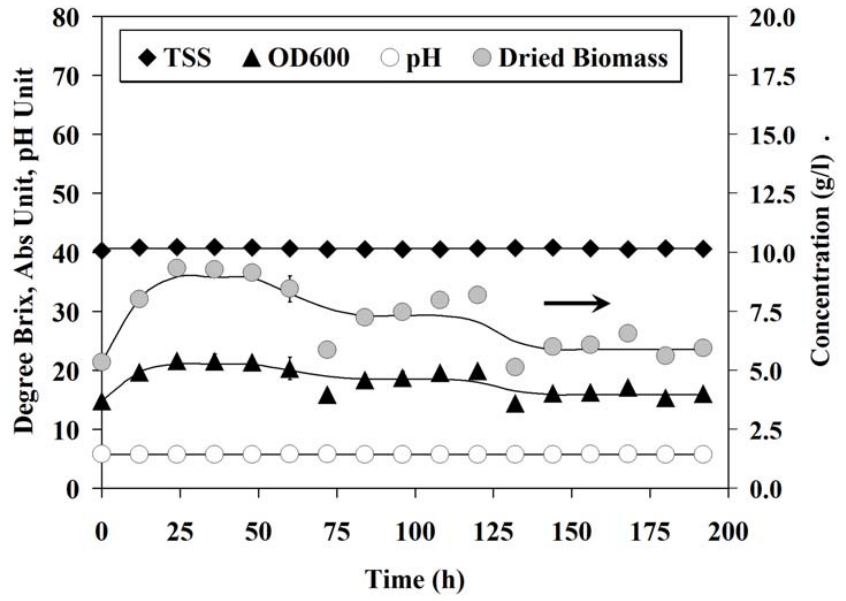


(a)

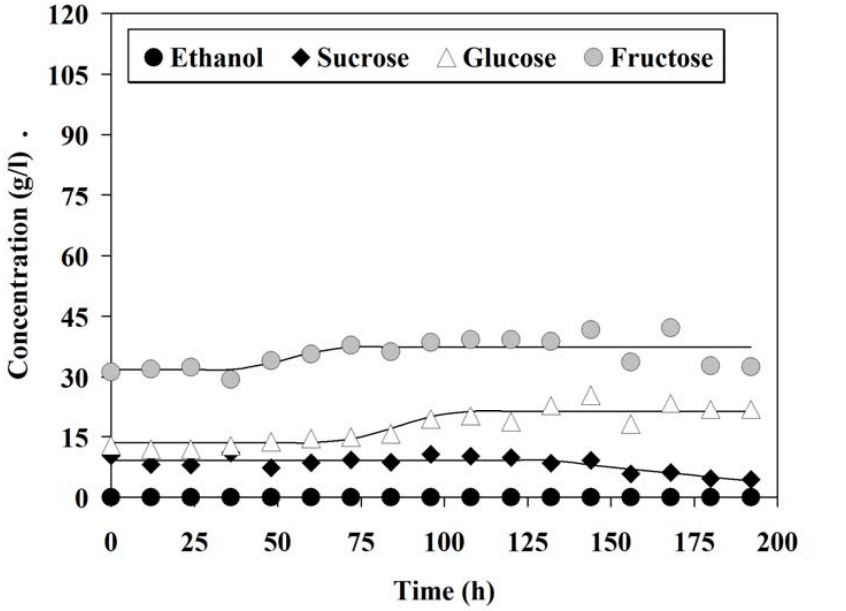


(b)

Figure 3.11: Growth kinetics of *C. utilis* TISTR 5198 during 192 h cultivation period in a static condition at 25.6^{o}C with digested dried longan flesh hydrolysate at 40^{o}Brix; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.



(a)



(b)

Figure 3.12: Growth kinetics of *C. utilis* TISTR 5352 during 192 h cultivation period in a static condition at 25.6°C with digested dried longan flesh hydrolysate at 40°Brix; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

The fructose decreasing level of *C. utilis* TISTR 5198 at 2.31 ± 1.56 g l^{-1} was not significantly different ($p > 0.05$) to TISTR 5352 at 1.38 ± 0.45 g l^{-1}. The ethanol production level of TISTR 5198 was 1.26 ± 0.04 g l^{-1} which was statistically similar ($p > 0.05$) to TISTR 5352 at 1.22 ± 0.06 g l^{-1}. For 40°Brix, the sucrose decreasing trends as indicated in Fig. 3.11(b) and 3.12(b) for TISTR 5352 and TISTR 5198 were 5.89 ± 0.31 g l^{-1} and 0.81 ± 0.59 g l^{-1} which were statistically different ($p \leq 0.05$). The glucose concentration was observed to increase throughout the time course for *C. utilis* TISTR 5198 at 5.67 ± 0.25 g l^{-1} which significantly differ ($p \leq 0.05$) from the increasing glucose level of TISTR 5352 at 9.02 ± 1.00 g l^{-1}. The fructose decreasing level of TISTR 5198 at 4.37 ± 0.16 g l^{-1} was significantly different ($p \leq 0.05$) from TISTR 5352 which increased by 1.21 ± 1.84 g l^{-1}.

It should be noted that both strains of *C. utilis* neither produce ethanol in DDLFH media at initial TSS levels of 20 nor 40°Brix.

3.1.4 Two-phase Separated Biotransformation Studies

The two-phase PAC biotransformation of *C. utilis* TISTR 5198 and TISTR 5352 using whole cells harvested at 192 h in DLE and DDLFH media with 6.12 g l^{-1} of dried biomass equivalent are shown in Fig. 3.13 and Table 3.14 – 3.17.

The volume ratio of buffer and organic phase was used to calculate the overall PAC production level in two-phase PAC biotransformation system. The results of non-unity volume ratio in all experiments might stem from 1) the absorption of organic phase to the whole cells and/or 2) the emulsifier properties of whole cells that facilitate the dissolution of organic into the buffer phase. The range of volume ratio was between 0.81 ± 0.01 to 0.87 ± 0.02. This may suggest that the whole cells of *C. utilis* TISTR 5352 from different type of media did not affect the volume ratio significantly ($p > 0.05$).

The concentration of produced PAC in aqueous phase of the two-phase separated biotransformation in an orbital shaker incubator at 250 rpm at 8°C for 72 h is shown in Table 3.15. Whole cells of *C. utilis* TISTR 5352 from DLE medium could produce 0.20 ± 0.02 mM PAC. This was compared to the situation of TISTR 5198 in which the PAC production was absent. The PAC production from whole cells of *C. utilis* TISTR 5198 from DDLFH medium was 0.45 ± 0.02 mM which was statistically different ($p \leq 0.05$) from TISTR 5352 whose PAC production was not occurred.

Table 3.16 illustrated the production of PAC in organic phase, whole cells of *C. utilis* TISTR 5352 from DLE medium could produce up to 1.39 ± 0.03 mM which was significantly different (p

≤ 0.05) from *C. utilis* TISTR 5198 whose PAC production level was only 0.42 ± 0.02 mM. In fact, the PAC production level from whole cells of TISTR 5198 cultivated in DDLFH medium was 3.35 ± 0.13 mM which was statistically different ($p \leq 0.05$) from TISTR 5352 that failed to produce PAC.

The overall PAC concentration produced in both phases could be calculated from the volume ratio and generated PAC value in each phase as indicated in Fig. 3.13 and Table 3.17. The overall PAC level produced from whole cells *C. utilis* TISTR 5352 using DLE medium was 0.75 ± 0.02 mM which was statistically different ($p \leq 0.05$) from TISTR 5198 whose overall PAC production level was only 0.19 ± 0.01 mM. In fact, whole cells of *C. utilis* TISTR 5352 cultivated in DDLFH medium did not produce PAC. However, the overall PAC production level from whole cells of TISTR 5198 was the highest at 1.76 ± 0.06 mM. The overall PAC production in this study was significantly lower than Agustina *et al.* (2009) (51.9 ± 4.2 mM) who employed *C. utilis* TISTR 5198 in the same biotransformation condition but in the relatively well-mixed phases.

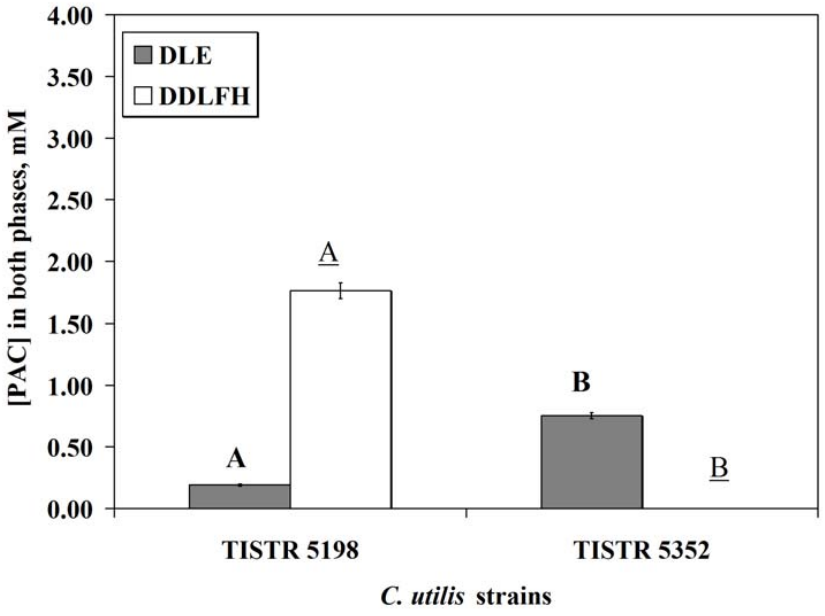


Figure 3.13: The overall PAC (mM) production level using whole cells of *C. utilis* TISTR 5198 and 5352 from DLE and DDLFH medium at 8°C for 72 h in two-phase separated conditions.

Table 3.14: The statistical comparison of organic to aqueous phase volume ratio in two-phase separated biotransformation using whole cells of *C. utilis* TISTR 5198 and 5352 at 250 rpm and 8°C.

Investigated parameters	Volume ratio (no unit, v/v)					
	DLE			DDLFH		
TISTR 5198	0.83 ± 0.02	A	I	0.83 ± 0.01	A	I
TISTR 5352	0.87 ± 0.02	A	I	0.81 ± 0.01	A	I

The numbers with the same alphabet (A), for comparison between each row of the same column, or Roman numeral (I), for comparison between each column of the same row, indicated no significant difference ($p > 0.05$).

Table 3.15: The statistical comparison of the PAC level in aqueous phase of two-phase separated PAC biotransformation using whole cells of *C. utilis* TISTR 5198 and 5352 at 250 rpm and 8°C.

Investigated parameters	[PAC] (mM)					
	DLE			DDLFH		
TISTR 5198	0.00 ± 0.00	A	I	0.45 ± 0.02	A	II
TISTR 5352	0.20 ± 0.02	B	I	0.00 ± 0.00	B	II

The numbers with the same alphabet (A-B), for comparison between each row of the same column, or Roman numeral (I-II), for comparison between each column of the same row, indicated no significant difference ($p > 0.05$).

Table 3.16: The statistical comparison the PAC level in organic phase of two-phase separated PAC biotransformation using whole cells of *C. utilis* TISTR 5198 and 5352 at 250 rpm and 8°C.

Investigated parameters	[PAC] (mM)					
	DLE			DDLFH		
TISTR 5198	0.42 ± 0.02	A	I	3.35 ± 0.13	A	II
TISTR 5352	1.39 ± 0.03	B	I	0.00 ± 0.00	B	II

The numbers with the same alphabet (A-B), for comparison between each row of the same column, or Roman numeral (I-II), for comparison between each column of the same row, indicated no significant difference ($p > 0.05$).

Table 3.17: The statistical comparison the overall PAC production level in both phases of two-phase separated PAC biotransformation using whole cells of *C. utilis* TISTR 5198 and 5352 at 250 rpm and 8°C.

Investigated parameters	[PAC] (mM)					
	DLE			DDLFH		
TISTR 5198	0.19 ± 0.01	A	I	1.76 ± 0.06	A	II
TISTR 5352	0.75 ± 0.02	B	I	0.00 ± 0.00	B	II

The numbers with the same alphabet (A-B), for comparison between each row of the same column, or Roman numeral (I-II), for comparison between each column of the same row, indicated no significant difference ($p > 0.05$).

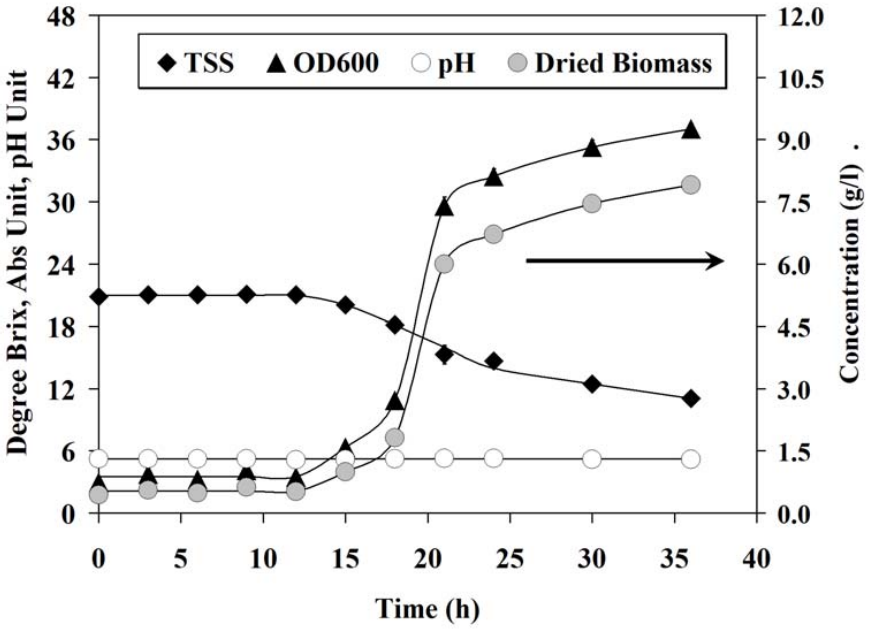
3.2 The Kinetics of Ethanol and PAC Biotransformation Production from Dried Longan Extract

3.2.1 Kinetics Studies: Effects of inoculum size

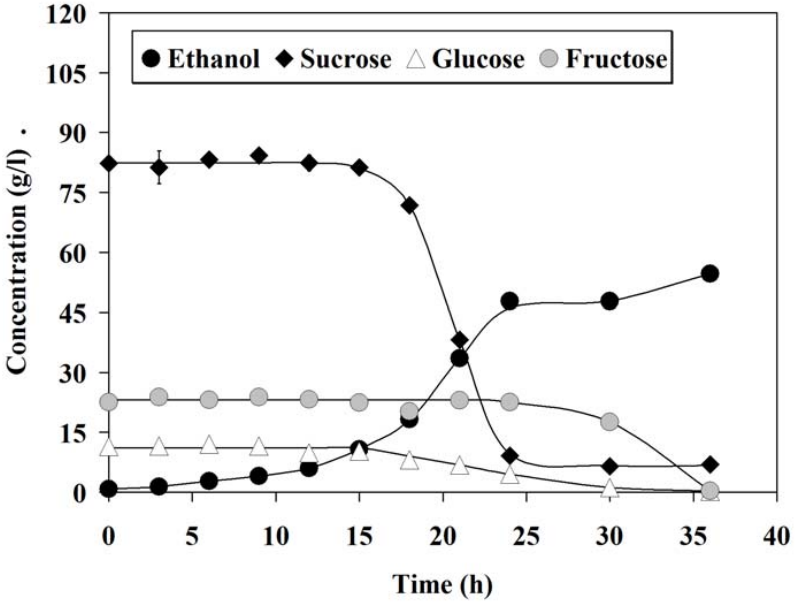
This experiment investigated the effect of three *S. cerevisiae* TISTR 5606 inoculum levels (1, 5, and 10% (v/v)) on growth kinetic profiles of 1,500 ml batch cultivation using dried longan extract aged two years with supplementation of extra nitrogen sources such as yeast extract, malt extract, and peptone. All cultivations were carried out for 36 h in the static condition at 25.6^{o}C. The cultivation kinetic profiles of three inoculum levels for *S. cerevisiae* TISTR 5606 in dried longan extract are shown in Fig. 3.14 for 1% (v/v), Fig. 3.15 for 5% (v/v), and Fig. 3.16 for 10% (v/v). Each figure is divided into two parts, namely; part (a) describes the kinetic profiles of TSS, pH level, OD600, and dried biomass concentration which could be related to OD600 by third order polynomial equations as previously mentioned in previous section; part (b) portrays the kinetic profiles of substrates such as sucrose, glucose, and fructose concentrations, as well as the product or ethanol concentration.

The detailed analysis of each cultivation profile with hypothesis testing across three inoculum levels is tabulated in Table 3.18 – 3.23. The first three tables (Table 3.18 – 3.20) portray the statistical comparison of TSS, pH level, OD600, and dried biomass concentration data extracted from Fig. 3.14(a) – 3.16(a) which include the analyses of differences between the final and initial levels, average, and maximum rates. Similar analyses and comparisons were also carried out for sugars and ethanol concentrations. Table 3.21 – 3.23 presents these information in terms of differences, average as well as maximum rates.

As indicated in Fig. 3.14(a), 3.15(a), and 3.16(a), the maximum change in TSS level was observed with 5% (v/v) inoculum level at 10.08 ± 0.14°Brix. This was significantly different ($p \leq 0.05$) from 9.34 ± 0.09°Brix (Table 3.18) obtained with 10% (v/v) inoculum level. The average rate of TSS decreasing (Table 3.19) and maximum rate of TSS decreasing (Table 3.20) for all three inoculum levels were not different statistically ($p > 0.05$) with the corresponding range of rates between 0.282 – 0.301 and 0.496 – 0.582^{o}Brix h^{-1}, respectively.

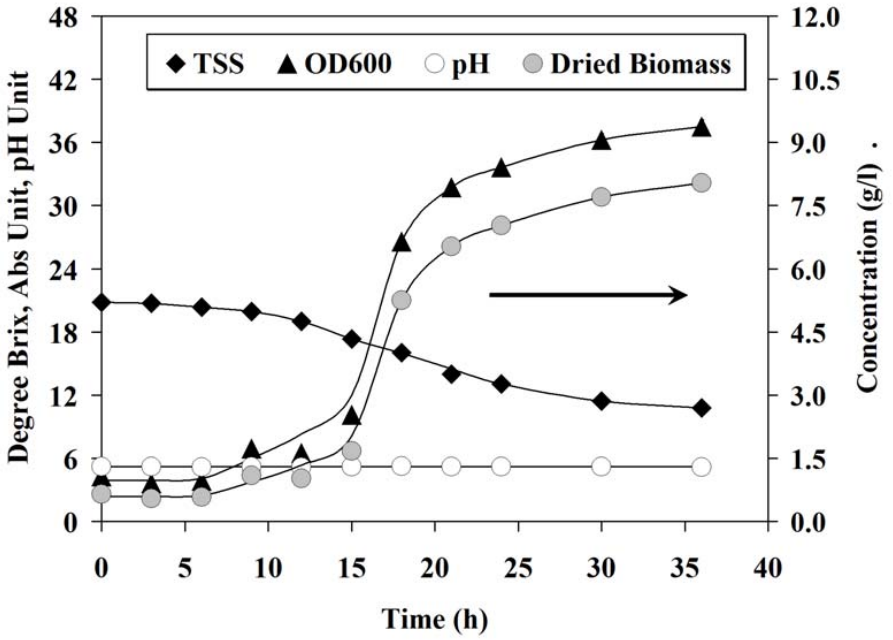


(a)

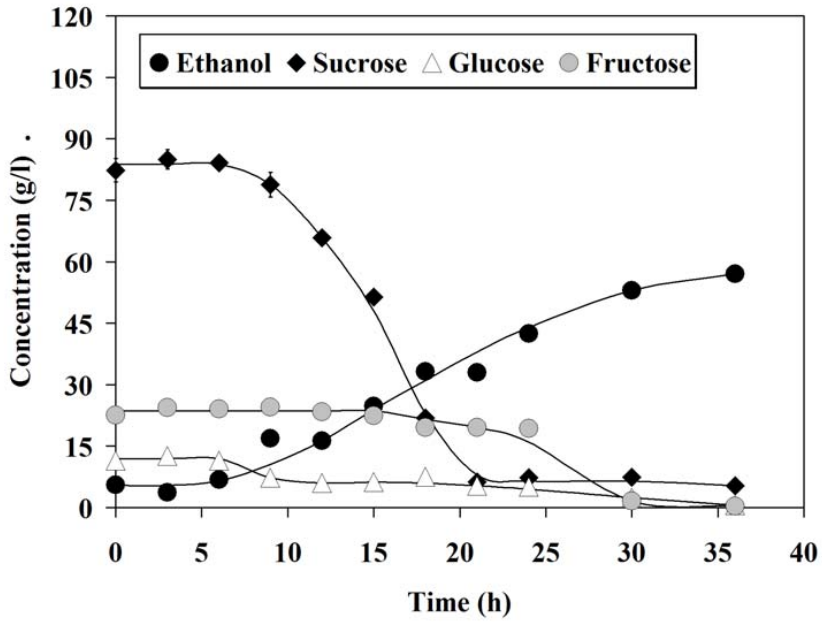


(b)

Figure 3.14: Growth kinetics of *S. cerevisiae* TISTR 5606 using 1% (v/v) inoculum during 36 h cultivation period in a static condition with dried longan extract at 25.6°C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

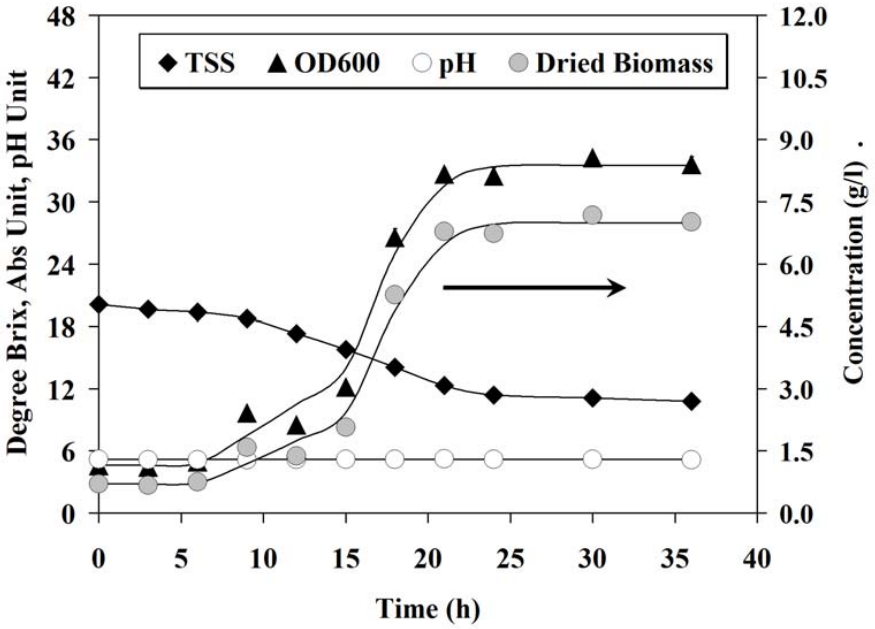


(a)

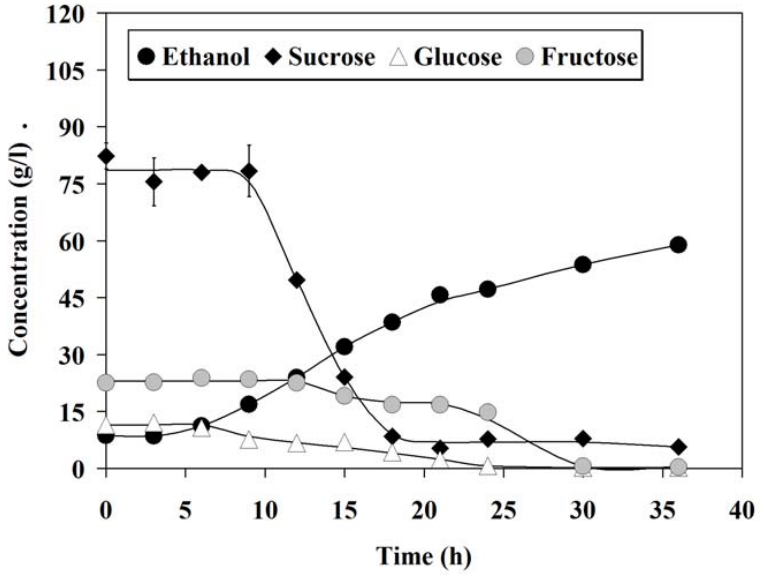


(b)

Figure 3.15:Growth kinetics of *S. cerevisiae* TISTR 5606 using 5% (v/v) inoculum during 36 h cultivation period in a static condition with dried longan extract at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.



(a)



(b)

Figure 3.16:Growth kinetics of *S. cerevisiae* TISTR 5606 using 10% (v/v) inoculum during 36 h cultivation period in a static condition with dried longan extract at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

Table 3.18: The differences in TSS, OD600, and dried biomass (X) concentration (g l^{-1}) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three inoculum levels.

Investigated parameters	Inoculum levels					
	1% (v/v)		5% (v/v)		10% (v/v)	
TSS decreasing level	9.74 ± 0.27	I, II	10.08 ± 0.14	I	9.34 ± 0.09	II
OD600 increasing level	34.05 ± 0.53	I	33.23 ± 0.69	I	29.01 ± 0.76	II
X production level	7.47 ± 0.08	I	7.39 ± 0.10	I	6.31 ± 0.11	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.19: The average pH level, average TSS decreasing rate (^{o}Brix h^{-1}), average OD600 increasing rate (ODU h^{-1}), average dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), average specific growth rate (h^{-1}), and average doubling time (h) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for three inoculum levels.

Investigated parameters	Inoculum levels					
	1% (v/v)		5% (v/v)		10% (v/v)	
pH level	5.216 ± 0.010	I	5.196 ± 0.009	I, II	5.169 ± 0.011	II
TSS decreasing rate	-0.282 ± 0.093	I	-0.298 ± 0.060	I	-0.301 ± 0.069	I
OD600 increasing rate	1.040 ± 0.601	I	1.056 ± 0.454	I	0.959 ± 0.373	I
X increasing rate	0.226 ± 0.134	I	0.231 ± 0.101	I	0.209 ± 0.085	I
Specific growth rate	0.082 ± 0.040	I	0.082 ± 0.029	I	0.075 ± 0.025	I
Doubling time	8.440 ± 4.075	I	8.448 ± 3.010	I	9.288 ± 3.116	I

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.20: The maximum TSS decreasing rate (^{o}Brix h^{-1}), maximum OD600 increasing rate (ODU h^{-1}), maximum dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), maximum specific growth rate (h^{-1}), and minimum doubling time (h) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for three inoculum levels.

Investigated parameters	Inoculum levels					
	1% (v/v)		5% (v/v)		10% (v/v)	
TSS decreasing rate	-0.582 ± 0.093	I	-0.496 ± 0.024	I	-0.535 ± 0.026	I
OD600 increasing rate	2.410 ± 1.283	I	2.143 ± 0.927	I	2.000 ± 0.612	I
X increasing rate	0.515 ± 0.293	I	0.474 ± 0.207	I	0.440 ± 0.147	I
Specific growth rate	0.198 ± 0.065	I	0.171 ± 0.041	I	0.154 ± 0.025	I
Doubling time	3.492 ± 1.146	I	4.046 ± 0.976	I	4.499 ± 0.734	I

The numbers with the same Roman numeral (I) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.21: The differences in sugars (sucrose, glucose, and fructose) concentration levels (g l^{-1}), ethanol concentration levels (g l^{-1}), lag time (sucrose, glucose, fructose, and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three inoculum levels.

Investigated parameters	Inoculum levels					
	1% (v/v)		5% (v/v)		10% (v/v)	
Sucrose decreasing level	75.38 ± 0.63	I	77.04 ± 2.80	I	76.66 ± 3.38	I
Glucose decreasing level	11.26 ± 0.06	I	10.90 ± 0.40	I	11.19 ± 0.39	I
Fructose decreasing level	22.18 ± 0.07	I	22.22 ± 0.76	I	22.12 ± 0.59	I
Ethanol production level	53.85 ± 0.50	I	51.52 ± 0.25	II	50.24 ± 0.46	III
Sucrose lag time	12.00 ± 1.20	I	6.00 ± 0.60	II	6.00 ± 0.60	II
Glucose lag time	15.00 ± 1.50	I	6.00 ± 0.60	II	6.00 ± 0.60	II
Fructose lag time	21.00 ± 2.10	I	15.00 ± 1.50	I, II	12.00 ± 1.20	II
Ethanol lag time	12.00 ± 1.20	I	6.00 ± 0.60	II	6.00 ± 0.60	II
$Y_{P/S}$	0.49 ± 0.01	I	0.47 ± 0.01	I	0.46 ± 0.01	I

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.22: The average sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), average ethanol production rate (g l^{-1} h^{-1}), average specific rate of sugars consumption (Avg Q_s, g g^{-1} h^{-1}), and average specific rate of ethanol production (Avg Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three inoculum levels.

Investigated parameters	**Inoculum levels**					
	1% (v/v)		**5% (v/v)**		**10% (v/v)**	
Sucrose consumption rate	-2.476 ± 1.351	I	-2.597 ± 0.967	I	-2.407 ± 1.135	I
Glucose consumption rate	-0.292 ± 0.111	I	-0.311 ± 0.144	I	-0.365 ± 0.105	I
Fructose consumption rate	-0.390 ± 0.288	I	-0.515 ± 0.247	I	-0.544 ± 0.239	I
Ethanol production rate	1.651 ± 0.552	I	1.505 ± 0.282	I	1.478 ± 0.266	I
Avg Q_s of sucrose	-0.727 ± 0.340	I	-1.269 ± 0.486	I	-1.253 ± 0.653	I
Avg Q_s of glucose	-0.091 ± 0.050	I	-0.247 ± 0.192	I	-0.193 ± 0.099	I
Avg Q_s of fructose	-0.052 ± 0.038	I	-0.083 ± 0.036	I	-0.130 ± 0.064	I
Avg Q_p of ethanol	0.927 ± 0.212	I	0.718 ± 0.213	I	0.737 ± 0.229	I

The numbers with the same Roman numeral (I) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.23: The maximum sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), maximum ethanol production rate (g l^{-1} h^{-1}), maximum specific rate of sugars consumption (Max Q_s, g g^{-1} h^{-1}), and maximum specific rate of ethanol production (Max Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three inoculum levels.

Investigated parameters	Inoculum levels					
	1% (v/v)		5% (v/v)		10% (v/v)	
Sucrose consumption rate	-6.071 ± 2.548	I	-5.902 ± 0.878	I	-5.796 ± 1.802	I
Glucose consumption rate	-0.689 ± 0.048	I	-0.654 ± 0.287	I	-0.660 ± 0.107	I
Fructose consumption rate	-0.974 ± 0.658	I	-1.233 ± 0.407	I	-1.311 ± 0.314	I
Ethanol production rate	3.335 ± 0.814	I	2.303 ± 0.113	I	2.271 ± 0.172	I
Max Q_s of sucrose	-1.801 ± 0.478	I	-2.936 ± 0.423	I	-3.094 ± 1.146	I
Max Q_s of glucose	-0.222 ± 0.097	I	-0.607 ± 0.453	I	-0.436 ± 0.200	I
Max Q_s of fructose	-0.130 ± 0.085	I	-0.201 ± 0.045	I	-0.317 ± 0.108	I
Max Q_p of ethanol	1.600 ± 0.198	I	1.395 ± 0.256	I	1.524 ± 0.167	I

The numbers with the same Roman numeral (I) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.24: Marking of each inoculum level based on cost factor

Cost (20%)	Inoculum levels (% (v/v))			Weighting
Data	**1**	**5**	**10**	**(%)**
Media volume (ml)	30[@]	90[#]	165[$]	20.00
Ratio media volume	1.0	3.0	5.5	N/a
100 × Inverse ratio	100.00	33.33	18.18	N/a
Sub total	**20.00**	**6.67**	**3.64**	**20.00**

[@] 15 ml preseed volume + seed volume at 1% × 1,500 ml or 15 ml; 15 + 15 = 30 ml

[#] 15 ml preseed volume + seed volume at 5% × 1,500 ml or 75 ml; 15 + 75 = 90 ml

[$] 15 ml preseed volume + seed volume at 10% × 1,500 ml or 150 ml; 15 + 150 = 165 ml

Table 3.25: Marking of each inoculum level based on growth factor

Growth (30%)	Inoculum levels (% (v/v))			Weighting
Marking	**1**	**5**	**10**	**(%)**
TSS change (initial level - final level)	0.97	1.00	0.93	1.00
OD600 change (final level - initial level)	9.00	9.00	7.67	9.00
X change (final level - initial level)	9.00	9.00	7.60	9.00
pH level	0.99	0.99	1.00	1.00
Average rate of TSS decreasing (degree Brix h^{-1})	1.00	1.00	1.00	1.00
Average rate of OD600 increasing (ODU h^{-1})	1.00	1.00	1.00	1.00
Average rate of X increasing (g l^{-1} h^{-1}	1.00	1.00	1.00	1.00
Average specific growth rate (h^{-1})	1.00	1.00	1.00	1.00
Average doubling time (h)	1.00	1.00	1.00	1.00
Maximum rate of TSS decreasing (degree Brix h^{-1})	1.00	1.00	1.00	1.00
Maximum rate of OD600 increasing (ODU h^{-1})	1.00	1.00	1.00	1.00
Maximum rate of X increasing (g l^{-1} h^{-1})	1.00	1.00	1.00	1.00
Maximum specific growth rate (h^{-1})	1.00	1.00	1.00	1.00
Maximum doubling time (h)	1.00	1.00	1.00	1.00
Sub total	**29.96**	**29.99**	**27.20**	**30.00**

Table 3.26: Marking of each inoculum level based on substrates & product factor

Substrates & Product (50%)	Inoculum levels (% (v/v))			Weighting
Marking	**1**	**5**	**10**	
Sucrose decreasing (g l^{-1})	0.50	0.50	0.50	0.50
Glucose decreasing (g l^{-1})	0.50	0.50	0.50	0.50
Fructose decreasing (g l^{-1})	0.50	0.50	0.50	0.50
Ethanol production (g l^{-1})	9.05	8.66	8.44	9.05
Lag Time sucrose (h)	2.80	5.60	5.60	5.60
Lag Time glucose (h)	2.10	5.60	5.60	5.60
Lag Time fructose (h)	3.15	3.85	5.60	5.60
Lag Time ethanol (h)	5.60	3.92	5.60	5.60
Yield (g ethanol/g sugars consumed), 36 h (no unit)	9.05	8.55	8.36	9.05
Average rate of sucrose decreasing (g l^{-1} h^{-1})	0.50	0.50	0.50	0.50
Average rate of glucose decreasing (g l^{-1} h^{-1})	0.50	0.50	0.50	0.50
Average rate of fructose decreasing (g l^{-1} h^{-1})	0.50	0.50	0.50	0.50
Average rate of ethanol producing (g l^{-1} h^{-1})	0.50	0.50	0.50	0.50
Average specific rate of sucrose decreasing (g g^{-1} h^{-1})	0.50	0.50	0.50	0.50
Average specific rate of glucose decreasing (g g^{-1} h^{-1})	0.50	0.50	0.50	0.50
Average specific rate of fructose decreasing (g g^{-1} h^{-1})	0.50	0.50	0.50	0.50
Average specific rate of ethanol production (g g^{-1} h^{-1})	0.50	0.50	0.50	0.50
Maximum rate of sucrose decreasing (g l^{-1} h^{-1})	0.50	0.50	0.50	0.50
Maximum rate of glucose decreasing (g l^{-1} h^{-1})	0.50	0.50	0.50	0.50
Maximum rate of fructose decreasing (g l^{-1} h^{-1})	0.50	0.50	0.50	0.50
Maximum rate of ethanol production (g l^{-1} h^{-1})	0.50	0.50	0.50	0.50
Maximum specific rate of sucrose decreasing (g g^{-1} h^{-1})	0.50	0.50	0.50	0.50
Maximum specific rate of glucose decreasing (g g^{-1} h^{-1})	0.50	0.50	0.50	0.50
Maximum specific rate of fructose decreasing (g g^{-1} h^{-1})	0.50	0.50	0.50	0.50
Maximum specific rate of ethanol production (g g^{-1} h^{-1})	0.50	0.50	0.50	0.50
Sub total	**41.25**	**45.68**	**48.70**	**50.00**

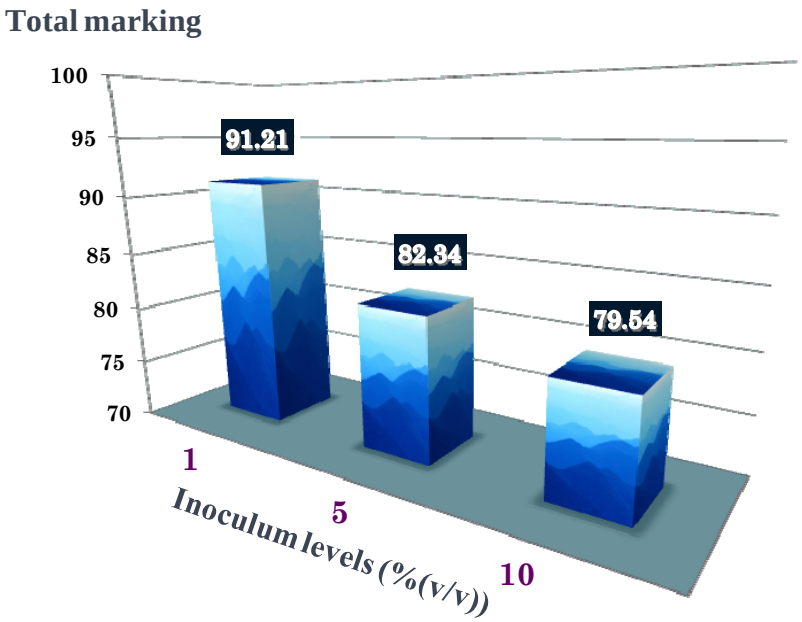


Figure 3.17: Total marking for selection of the most suitable inoculum level.

As indicated in Fig. 3.14(a), 3.15(a), and 3.16(a), the maximum change in TSS level was observed with 5% (v/v) inoculum level at 10.08 ± 0.14°Brix. This was significantly different ($p \leq 0.05$) from 9.34 ± 0.09°Brix (Table 3.18) obtained with 10% (v/v) inoculum level. The average rate of TSS decreasing (Table 3.19) and maximum rate of TSS decreasing (Table 3.20) for all three inoculum levels were not different statistically ($p > 0.05$) with the corresponding range of rates between 0.282 – 0.301 and 0.496 – 0.582°Brix h^{-1}, respectively.

The maximum change in OD600 level of 34.05 ± 0.53 ODU was observed with 1% (v/v) inoculum level as indicated in Table 18 which was not different statistically from 5% (v/v) inoculum level at 33.23 ± 0.69 ODU. This was compared to 10% (v/v) inoculum level with corresponding ODU change of 29.01 ± 0.76 ODU which differed statistically ($p \leq 0.05$) from its 1% counterpart. The comparison of OD600 increasing rate suggested that the application of 5% (v/v) inoculum level resulted in the highest OD600 elevation rate of 1.056 ± 0.454 ODU h^{-1} , which was not found to be significantly different ($p > 0.05$) from that of 10% (v/v) inoculum level with the corresponding rate of 0.959 ± 0.373 ODU h^{-1} (Table 3.19). Furthermore, the comparison of maximum OD600 increasing rate in Table 3.20 suggested that all three inoculum levels yielded the same level of rate ($p > 0.05$) within the range of 2.00 – 2.41 ODU h^{-1}.

The increasing trend of dried biomass concentration which corresponded directly to OD600 level suggested that 1% (v/v) inoculum level could generate the highest dried biomass

concentration of 7.47 ± 0.08 g l^{-1}. This differed significantly ($p \leq 0.05$) from the minimum figure of 6.31 ± 0.11 g l^{-1} which was obtained after the cultivation with 10% (v/v) inoculum level (Table 3.18). The average increasing rate of dried biomass concentration in Table 3.19 and the maximum increasing rate of dried biomass concentration in Table 3.20 for all three inoculum levels were not significantly different from one another ($p > 0.05$) with the corresponding range of rates between 0.209 – 0.231 g l^{-1} h^{-1} and 0.440 – 0.515 g l^{-1} h^{-1}, respectively.

The resistance to pH change throughout the cultivation period was generally observed for all three cases of varied inoculum levels which utilized dried longan extract as cultivation medium with the pH range of 5.169 – 5.216 (Table 3.19). This might be due to the presence of buffer species existed previously in dried longan extract.

The average specific growth rate presented in Table 3.19 for both 1% and 5%(v/v) inoculum levels were peaked at 0.082 ± 0.040 h^{-1} and 0.082 ± 0.029 h^{-1}. However, both values were not different statistically ($p > 0.05$) from the average specific growth rate determined from 10% (v/v) inoculum level at 0.075 ± 0.025 h^{-1}. The shortest period of average doubling time in Table 3.19 was observed with 1% (v/v) inoculum level at 8.44 ± 4.08 h which could be compared to the longest period of doubling time for 10% (v/v) inoculum level at 9.29 ± 3.12 h. The statistical analyses of maximum specific growth rate (μmax) and corresponding minimum doubling time (td, min) in Table 3.20 revealed that inoculum levels of 1, 5, and 10% (v/v) did not significantly alter ($p > 0.05$) both values. In case of 1% (v/v) inoculum level, the observed μ_{max} and t_d, min was 0.198 ± 0.065 h^{-1} and 3.49 ± 1.15 h, respectively. This was subsequently compared to 10% (v/v) inoculum level with (μ_{max}, t_d, min) of 0.154 ± 0.025 h^{-1} and 4.50 ± 0.73 h.

The consideration of lag time period for each respective sugar in Table 3.21 using 1% (v/v) inoculum level revealed the longest lag period of 12.0 ± 1.2 h, 21.0 ± 2.1 h, and 15.0 ± 1.50 h for sucrose, fructose, and glucose consumption. The lag time for sucrose and glucose consumption at 1%(v/v) inoculum level were significantly different ($p \leq 0.05$) from the other two inoculum levels. This was compared to lag time for fructose consumption in which the significant difference was not observed between 1% and 5%n(v/v) inoculum levels ($p > 0.05$) but the significant difference occurred for the comparison between 1% and 10% (v/v) inoculum levels ($p \leq 0.05$). The lag periods for ethanol production for all three inoculum levels are also listed in Table 3.21. The highest lag period of 3.00 ± 0.30 h for 5% (v/v) inoculum level was evident which differed statistically from 1% and 10%.

The decreasing level of each sugar for all three inoculum levels being investigated after 36 h cultivation period was not differed statistically ($p > 0.05$) as illustrated in Table 3.21 with the decreasing

ranges of 75.4 – 77.0 g l^{-1}, 10.9 – 11.3 g l^{-1}, and 22.1 – 22.2 g l^{-1} for sucrose, glucose, and fructose, respectively. The similar trend of insignificant difference ($p > 0.05$) was also observed for average rate of sugars decreasing (Avg RS, Table 3.22), average specific rate of sugar decreasing (Avg Qs, Table 3.22), maximum rate of sugars decreasing (Max RS, Table 23), and maximum specific rate of sugars decreasing (Max Qs, Table 3.23). For sucrose, the corresponding ranges of these four rates were Avg RS of 2.407 – 2.597 g l^{-1} h^{-1}, Avg Qs of 0.727 – 1.269 g g^{-1} h^{-1}, Max RS of 5.796 – 6.071 g l^{-1} h^{-1}, and Max Qs of 1.801 – 3.094 g g^{-1} h^{-1}. For glucose, the corresponding ranges of these four rates were Avg RS of 0.292 – 0.365 g l^{-1} h^{-1}, Avg Qs of 0.091– 0.247 g g^{-1} h^{-1}, Max RS of 0.654 – 0.689 g l^{-1} h^{-1}, and Max Qs of 0.222 – 0.607 g g^{-1} h^{-1}. For fructose, the corresponding ranges of these four rates were Avg RS of 0.390 – 0.544 g l^{-1} h^{-1}, Avg Qs of 0.052 – 0.130 g g^{-1} h^{-1}, Max RS of 0.974 – 1.311 g l^{-1} h^{-1}, and Max Qs of 0.130 – 0.317 g g^{-1} h^{-1}.

Fig. 3.14(b), 3.15(b), and 3.16(b) portrayed the increasing trends of ethanol production for all three inoculum levels. The utilization of 1% (v/v) inoculum level (Fig. 3.14(b)) resulted in the stationary phase during the first 12 h prior to rapid increase in ethanol production until 24th h. From this time period, the increasing trend began to slow down up to the end of cultivation period at 36th h. The five folds increase in inoculum level to 5% (v/v) had shortened the lag period by half to 6 h as observed in Fig. 3.15(b). The inoculation of dried longan extract medium with 10% (v/v) inoculum also resulted in the similar lag period of 6 h (Fig. 3.16(b)). It was thus evident that the application of 5 and 10% (v/v) inoculum level could accelerate the production time of ethanol, however, the final ethanol concentration obtained after 36 h cultivation periods differed statistically ($p \leq 0.05$) as shown in Table 3.21. The highest ethanol production level was 53.8 ± 0.5 g l^{-1} for 1% (v/v) inoculum level. This was followed by 51.5 ± 0.2 g l^{-1} and 50.2 ± 0.5 g l^{-1} for 5 and 10% (v/v) inoculum levels, respectively.

The influence of varied inoculum levels on the average rate of ethanol production (Avg RP, Table 3.22), average specific rate of ethanol production (Avg QP, Table 3.22), maximum rate of ethanol production (Max RP, Table 3.23), and maximum specific rate of ethanol production (Max QP, Table 3.23) were elucidated in the insignificant difference ($p > 0.05$) with the corresponding range of these rates as following; Avg RP between 1.478 – 1.651 g l^{-1} h^{-1}, Avg QP between 0.718 – 0.927 g g^{-1} h^{-1}, Max RP between 2.271 – 3.335 g l^{-1} h^{-1}, and Max QP between 1.395 – 1.600 g g^{-1} h^{-1}, respectively.

The highest ethanol yield was observed with 1% (v/v) inoculum level with the corresponding value of 0.49 ± 0.01 g produced ethanol g-1 consumed sugars, however, this was not significantly different ($p > 0.05$) to ethanol yields from 5 and 10% (v/v) inoculum levels.

The evaluation of the most appropriate inoculum level is shown in Table 3.24 – 3.26 for the analyses based on; (1) cost factor (20%, Table 3.24); (2) growth factor (30%, Table 3.25); and (3)

substrate & product factor (50%, Table 3.26). The comparison of overall marking for each inoculum is presented in Figure 3.17. In term of costing factor (Table 3.24), the lower level of inoculum reflected the lower expense. Therefore, 1% (v/v) inoculum level was rated with full mark of 20.00 which was compared to 6.67 and 3.64 marks from 5% and 10% (v/v) inoculum level. From Table 3.25, the highest weighting factors on microbial growth of 0.09 were given to changes in OD600 and dried biomass concentration as these were considered more important than the other parameters with weighting factor of 0.01. As the higher level of biomass concentration cultivated in the same condition generally reflected the presence of a useful pyruvate decarboxylase enzyme which could be used later in the biotransformation system for PAC production. The marks summation in term of growth factor were thus 29.96, 29.99, and 27.20 for 1%, 5%, and 10% (v/v) inoculum levels, respectively. The last factor being considered was substrates & product as given in Table 3.26, the highest weight factor of 0.0905 was applied to ethanol production level (g l^{-1}) and ethanol yield (g g^{-1}) as ethanol were the desired principal product after cultivation. The obtained scores based on this factor were 48.70, 45.68, and 41.25 for 10%, 5%, and 1% (v/v) inoculum levels. The combined score from all three factors in Fig. 3.17 clearly suggested that 1% (v/v) inoculum level was the most suitable for *S. cerevisiae* TISTR 5606 cultivation in static condition with the highest score of 91.2 which was followed by 82.3 and 79.5 for 5% and 10% (v/v) inoculum levels, respectively.

3.2.2 Kinetics Studies: Batch cultivations with aeration in 5,000 ml scale

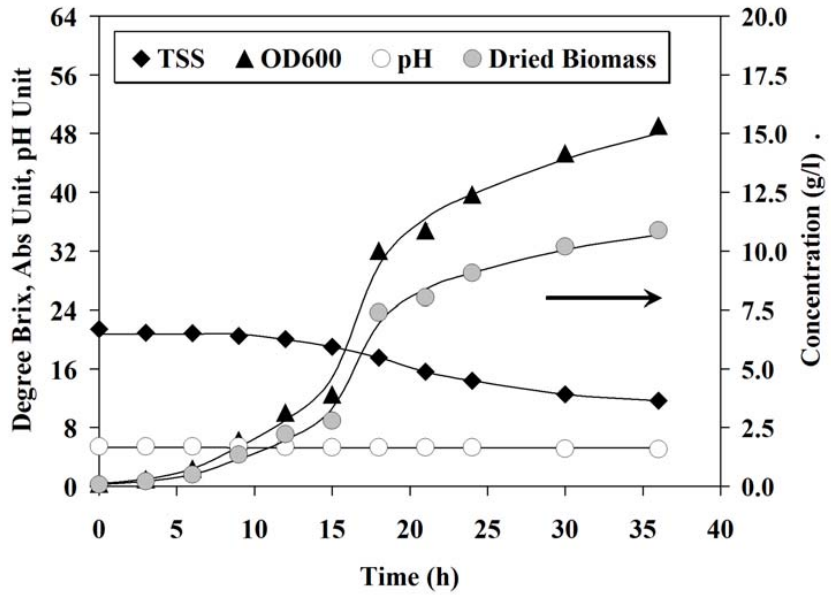
This study investigated the batch cultivation kinetics of *S. cerevisiae* TISTR 5606 in 5,000 ml scale for 36 h using DLE and DDLFH media as carbon sources. The supplementation of nitrogen sources such as yeast extract, malt extract, and peptone was also added. The cultivation was carried out for 36 h with aeration during the first 12 h in the static condition at 25.6^{o}C. The cultivation kinetic profiles of both carbon sources for *S. cerevisiae* TISTR 5606 are shown in Fig. 3.18 for DLE medium and Fig. 3.19 for DDLFH medium. Each figure is divided into two parts, namely; part (a) describes the kinetic profiles of TSS, pH level, OD600, and dried biomass concentration which could be related to OD600 by third order polynomial equations as previously mentioned in previous section; part (b) portrays the kinetic profiles of substrates such as sucrose, glucose, and fructose concentrations, as well as the product or ethanol concentration.

The detailed analysis of each cultivation profile with hypothesis testing for both carbon sources is tabulated in Table 3.27 – 3.32. The first three tables (Table 3.27 – 3.29) portray the statistical

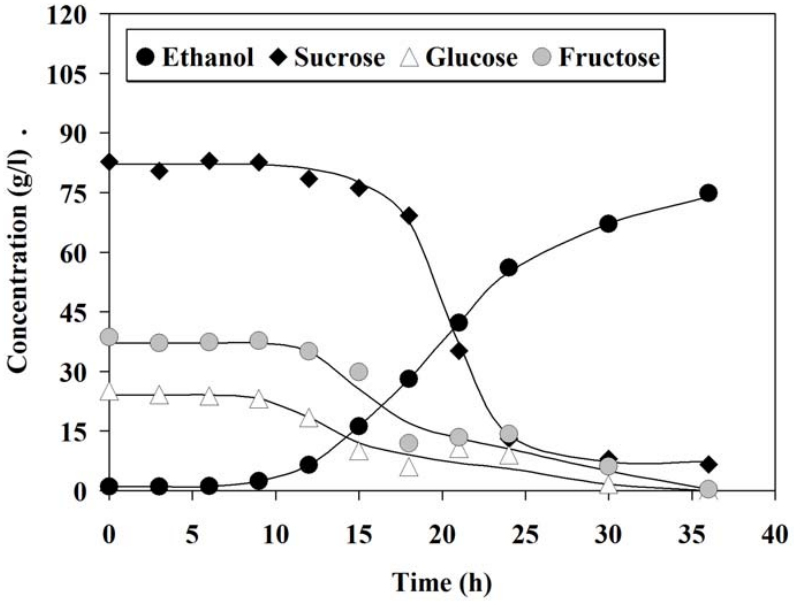
comparison of TSS, pH level, OD600, and dried biomass concentration data extracted from Fig. 3.18(a) – 3.19(a) which include the analyses of differences between the final and initial levels, average, and maximum rates. Similar analyses and comparisons were also carried out for sugars and ethanol concentrations. Table 3.30 – 3.32 presents these information in terms of differences, average as well as maximum rates.

As indicated in Fig. 3.20(a) and 3.21(a), the maximum change in TSS level was observed with DLE medium at 9.80 ± 0.13°Brix. This was significantly different ($p \leq 0.05$) from 0.96 ± 0.49°Brix (Table 3.23) obtained with DDLFH medium. The average rate of TSS decreasing (Table 3.24) and maximum rate of TSS decreasing (Table 3.25) for DLE medium resulted in the highest values of 0.258 ± 0.070 and 0.467 ± 0.067°Brix h^{-1}, respectively. These two rates differed statistically ($p \leq 0.05$) from one another.

The maximum change in OD600 level of 48.71 ± 0.38 ODU was observed with DLE medium as indicated in Table 3.27 which was different statistically ($p \leq 0.05$) from DDLFH medium at 0.81 ± 0.12 ODU. The comparison of OD600 increasing rate suggested that the application of DLE medium resulted in the highest OD600 elevation rate of 1.449 ± 0.445 ODU h^{-1} which was found to be significantly different ($p \leq 0.05$) from that of DDLFH medium (Table 3.28). Furthermore, the comparison of maximum OD600 increasing rate in Table 3.29 suggested that DLE medium yielded the highest level of rate at 2.583 ± 0.852 ODU h^{-1}.

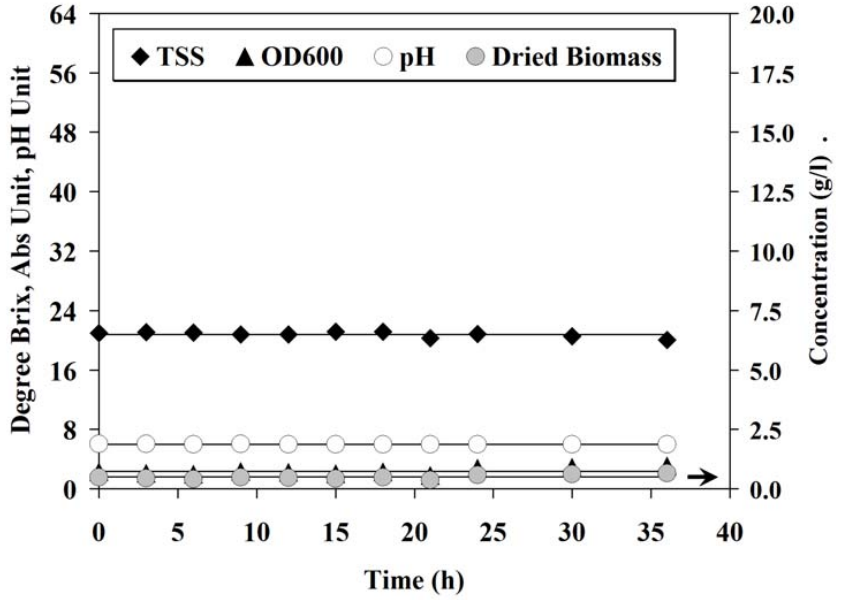


(a)

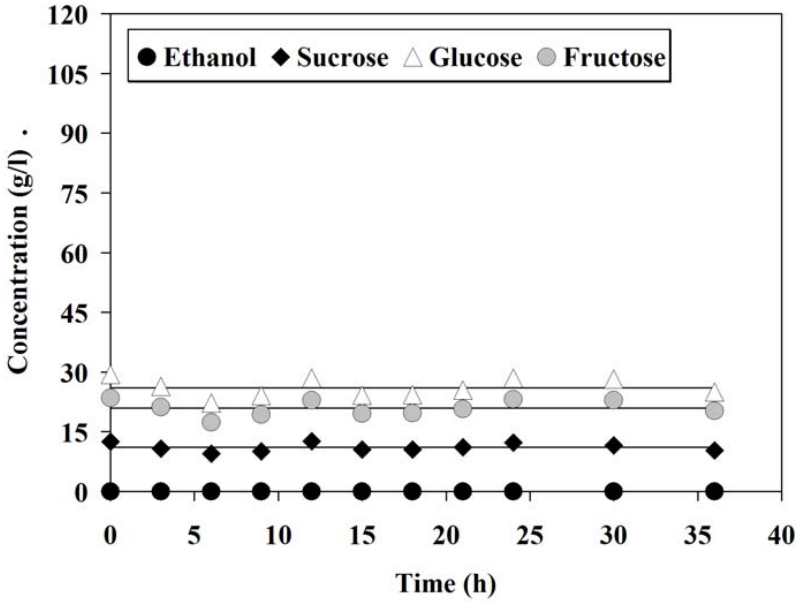


(b)

Figure 3.18: Growth kinetics of *S. cerevisiae* TISTR 5606 in 5,000 ml batch system using DLE medium as a carbon source during 36 h cultivation with an initial aeration period of 12 h from the overall 36 h at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.



(a)



(b)

Figure 3.19: Growth kinetics of *S. cerevisiae* TISTR 5606 in 5,000 ml batch system using DDLFH medium as a carbon source during 36 h cultivation period with an initial aeration period of 12 h from the overall 36 h at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

Table 3.27: The differences in TSS, OD600, and dried biomass (X) concentration (g l^{-1}) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for two carbon sources.

Investigated parameters	Carbon sources			
	DLE		DDLFH	
TSS decreasing level	9.80 ± 0.13	I	0.96 ± 0.49	II
OD600 increasing level	48.71 ± 0.38	I	0.81 ± 0.12	II
X production level	10.81 ± 0.08	I	0.17 ± 0.03	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.28: The average pH level, average TSS decreasing rate (^{o}Brix h^{-1}), average OD600 increasing rate (ODU h^{-1}), average dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), average specific growth rate (h^{-1}), and average doubling time (h) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for two carbon sources.

Investigated parameters	Carbon sources			
	DLE		DDLFH	
pH level	5.317 ± 0.036	I	5.999 ± 0.007	II
TSS decreasing rate	-0.258 ± 0.070	I	0.000 ± 0.000	II
OD600 increasing rate	1.449 ± 0.445	I	0.000 ± 0.000	II
X increasing rate	0.327 ± 0.107	I	0.000 ± 0.000	II
Specific growth rate	0.154 ± 0.037	I	0.000 ± 0.000	II
Doubling time	4.491 ± 1.070	I	N/a	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.29: The maximum TSS decreasing rate (^{o}Brix h^{-1}), maximum OD600 increasing rate (ODU h^{-1}), maximum dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), maximum specific growth rate (h^{-1}), and minimum doubling time (h) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for two carbon sources.

Investigated parameters	Carbon sources			
	DLE		DDLFH	
TSS decreasing rate	-0.467 ± 0.067	I	0.000 ± 0.000	II
OD600 increasing rate	2.583 ± 0.852	I	0.000 ± 0.000	II
X increasing rate	0.601 ± 0.205	I	0.000 ± 0.000	II
Specific growth rate	0.271 ± 0.015	I	0.000 ± 0.000	II
Doubling time	2.561 ± 0.144	I	N/a	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.30: The differences in sugars (sucrose, glucose, and fructose) concentration levels (g l^{-1}), ethanol concentration levels (g l^{-1}), lag time (sucrose, glucose, fructose, and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for two carbon sources.

Investigated parameters	Carbon sources			
	DLE		DDLFH	
Sucrose decreasing level	76.14 ± 1.59	I	2.29 ± 0.20	II
Glucose decreasing level	25.21 ± 0.62	I	4.30 ± 0.48	II
Fructose decreasing level	38.33 ± 0.95	I	3.23 ± 0.38	II
Ethanol production level	73.77 ± 0.48	I	0.00 ± 0.00	II
Sucrose lag time	6.00 ± 0.60	I	36.00 ± 3.60	II
Glucose lag time	6.00 ± 0.60	I	36.00 ± 3.60	II
Fructose lag time	9.00 ± 0.90	I	36.00 ± 3.60	II
Ethanol lag time	6.00 ± 0.60	I	36.00 ± 3.60	II
$\mathbf{Y_{P/S}}$	0.53 ± 0.01	I	0.00 ± 0.00	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.31: The average sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), average ethanol production rate (g l^{-1} h^{-1}), average specific rate of sugars consumption (Avg Q_s, g g^{-1} h^{-1}), and average specific rate of ethanol production (Avg Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for two carbon sources.

Investigated parameters	Carbon sources			
	DLE		DDLFH	
Sucrose consumption rate	-2.386 ± 1.155	I	0.000 ± 0.000	I
Glucose consumption rate	-0.709 ± 0.218	I	0.000 ± 0.000	II
Fructose consumption rate	-1.059 ± 0.353	I	0.000 ± 0.000	II
Ethanol production rate	2.113 ± 0.571	I	0.000 ± 0.000	II
Avg Q_s of sucrose	-0.371 ± 0.144	I	0.000 ± 0.000	II
Avg Q_s of glucose	-0.263 ± 0.114	I	0.000 ± 0.000	I
Avg Q_s of fructose	-0.265 ± 0.119	I	0.000 ± 0.000	I
Avg Q_p of ethanol	0.482 ± 0.128	I	0.000 ± 0.000	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.32: The maximum sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), maximum ethanol production rate (g l^{-1} h^{-1}), maximum specific rate of sugars consumption (Max Q_s, g g^{-1} h^{-1}), and maximum specific rate of ethanol production (Max Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 5,000 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for two carbon sources.

Investigated parameters	Carbon sources			
	DLE		DDLFH	
Sucrose consumption rate	-5.583 ± 2.058	I	0.000 ± 0.000	II
Glucose consumption rate	-1.343 ± 0.321	I	0.000 ± 0.000	II
Fructose consumption rate	-2.043 ± 0.559	I	0.000 ± 0.000	II
Ethanol production rate	4.048 ± 0.307	I	0.000 ± 0.000	II
Max Q_s of sucrose	-0.825 ± 0.193	I	0.000 ± 0.000	II
Max Q_s of glucose	-0.598 ± 0.188	I	0.000 ± 0.000	II
Max Q_s of fructose	-0.593 ± 0.214	I	0.000 ± 0.000	II
Max Q_p of ethanol	0.868 ± 0.129	I	0.000 ± 0.000	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

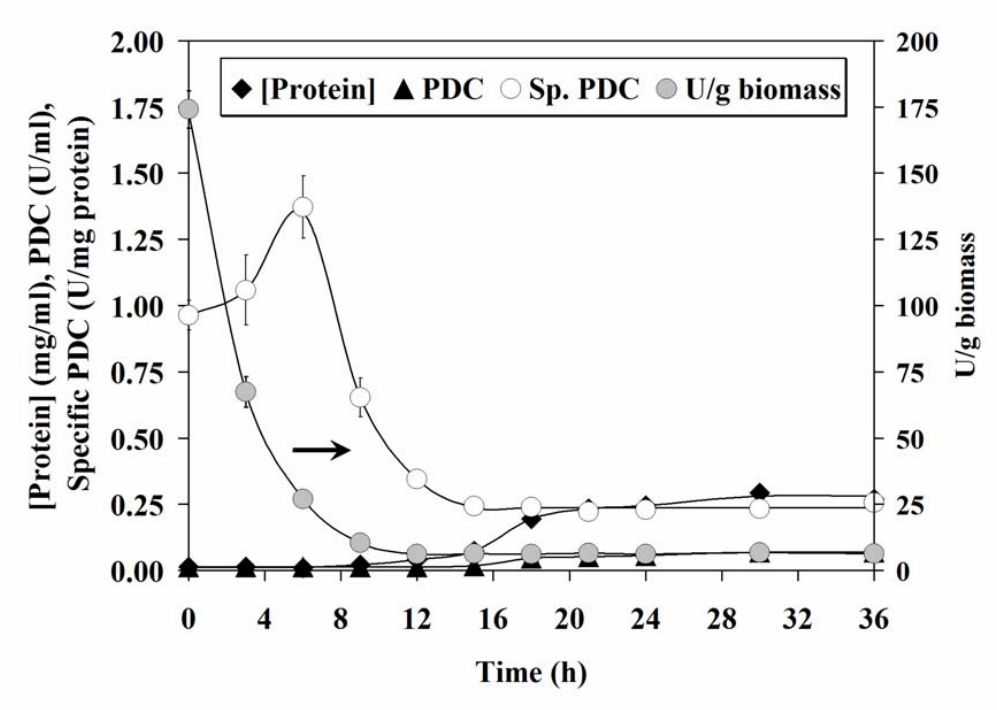


Figure 3.20: PDC activity of *S. cerevisiae* TISTR 5606 in batch system using DLE as a carbon source during 36 h cultivation period in a static condition with dried longan extract at 25.6°C. Profiles of protein concentration (mg ml^{-1}), PDC activity (U ml^{-1}), sp. PDC (U mg $protein^{-1}$), sp. PDC (U mg $biomass^{-1}$)

The increasing trend of dried biomass concentration which corresponded directly to OD600 level suggested that DLE medium could generate the highest dried biomass concentration of 10.81 ± 0.08 g l^{-1}. This differed significantly ($p \leq 0.05$) from 0.17 ± 0.03 g l^{-1} obtained after the yeast cultivation with DDLFH medium (Table 3.27). The average increasing rate of dried biomass concentration in Table 3.28 and the maximum increasing rate of dried biomass concentration in Table 3.29 for DLE medium were peaked at 0.327 ± 0.107 g l^{-1} h^{-1} and 0.601 ± 0.205 g l^{-1} h^{-1}, respectively. These two rates differed significantly ($p \leq 0.05$) from each other.

The average value of pH level throughout the cultivation period was generally observed with DLE medium at 5.317 ± 0.036 (Table 3.28). This was significantly different ($p \leq 0.05$) from 5.999 ± 0.007 obtained with DDLFH medium.

The average specific growth rate presented in Table 3.28 for DLE medium was peaked at 0.154 ± 0.037 h^{-1}, which was different statistically ($p \leq 0.05$) from DDLFH medium. The shortest period

of average doubling time in Table 3.28 was 4.491 ± 1.070 h with DLE medium. The maximum specific growth rate (μ_{max}) and corresponding minimum doubling time (t_d, min) in Table 3.12 for DLE medium was significantly different from DDLFH medium ($p \leq 0.05$). In case of DLE medium, the observed μ_{max} and t_d, min was 0.271 ± 0.015 h^{-1} and 2.561 ± 0.144 h, respectively.

The consideration of lag time period for each respective sugar in Table 3.30 using DLE medium revealed the shortest lag period of 6.00 ± 0.60 h, 6.00 ± 0.60 h, and 9.00 ± 0.90 h for sucrose, glucose, and fructose consumption. The lag time for sugars consumption for DLE medium were significantly different ($p \leq 0.05$) from DDLFH medium. The lag periods for ethanol production for two carbon sources are also listed in Table 3.30. The shortest lag period of 6.00 ± 0.60 h for DLE medium was evident which differed statistically ($p \leq 0.05$) from DDLFH medium.

The decreasing level of each sugar for two carbon sources being investigated after 36 h cultivation period differed statistically ($p \leq 0.05$) as illustrated in Table 3.30 with the decreasing level of 76.14 ± 1.59 g l^{-1}, 25.21 ± 0.62 g l^{-1}, and 38.33 ± 0.95 g l^{-1} for sucrose, glucose, and fructose, respectively. The highest average rate of sucrose decreasing (Avg RS, Table 3.31) for DLE was 2.386 ± 1.155 g l^{-1} h^{-1} which did not significantly differ ($p > 0.05$) from DDLFH medium.

The average specific rate of sucrose decreasing (Avg Qs of sucrose, Table 3.31), maximum rate of sucrose decreasing (Max RS, Table 3.32), and maximum specific rate of sucrose decreasing (Max Qs, Table 3.32) for DLE medium was significantly higher ($p \leq 0.05$) than DDLFH medium. For DLE medium, the corresponding value of these three rates were Avg Qs of 0.371 ± 0.144 g g^{-1} h^{-1}, Max RS of 5.583 ± 2.058 g l^{-1} h^{-1}, and Max Qs of 0.825 ± 0.193 g g^{-1} h^{-1}. These were compared to the average specific rates of glucose and fructose decreasing (Avg Qs, Table 3.31) which were not significantly different ($p > 0.05$) from DDLFH medium. For DLE medium, the corresponding values of Avg Qs for these two sugars were 0.263 ± 0.114 g g^{-1} h^{-1} and 0.265 ± 0.119 g g^{-1} h^{-1}, respectively.

For glucose, the average rate of sugar decreasing (Avg RS, Table 3.31), maximum rate of sugars decreasing (Max RS, Table 3.32), and maximum specific rate of sugars decreasing (Max Qs, Table 3.32) in DLE medium were Avg RS of 0.709 ± 0.218 g l^{-1} h^{-1}, Max RS of 1.343 ± 0.321 g l^{-1} h^{-1}, and Max Qs of 0.598 ± 0.188 g g^{-1} h^{-1}. For fructose, the corresponding ranges of these three rates were Avg RS of 1.059 ± 0.353 g l^{-1} h^{-1}, Max RS of 2.043 ± 0.559 g l^{-1} h^{-1}, and Max Qs of 0.593 ± 0.214 g g^{-1} h^{-1}. These three rates of DLE medium were significantly higher ($p \leq 0.05$) than DDLFH medium.

Fig. 3.18(b) and 3.19(b) portrayed the increasing trends of ethanol production for both carbon sources. The utilization of DLE medium (Fig. 3.18(b)) resulted in the stationary phase during the

first 6 h period which was followed by a rapid increase in ethanol production until 36th h. The cultivation of DDLFH medium did not produce ethanol as observed in Fig. 3.19(b). Lastly, the final ethanol concentration obtained after 36 h cultivation periods differed statistically ($p \leq 0.05$) as shown in Table 3.30. The highest ethanol production level was 73.77 ± 0.48 g l^{-1} for DLE medium while none was detected for DDLFH medium.

The influence of DLE medium on the average rate of ethanol production (Avg RP, Table 3.31), average specific rate of ethanol production (Avg QP, Table 3.31), maximum rate of ethanol production (Max RP, Table 3.32), and maximum specific rate of ethanol production (Max QP, Table 3.32) were elucidated in the significant different ($p \leq 0.05$) from DDLFH medium with the corresponding level of these rates as following; Avg RP of 2.113 ± 0.571 g l^{-1} h^{-1}, Avg QP of 0.482 ± 0.128 g g^{-1} h^{-1}, Max RP of 4.048 ± 0.307 g l^{-1} h^{-1}, and Max QP of 0.868 ± 0.129 g g^{-1} h^{-1}, respectively.

The highest ethanol yield was observed with DLE medium with the corresponding value of 0.53 ± 0.10 g produced ethanol (g consumed sugars)$^{-1}$.

The kinetics profiles of protein production, PDC activity, and specific PDC activity (Fig. 3.20) of *S. cerevisiae* TISTR 5606 during 36 h overall cultivation period with aeration during the first 12 h in a static condition at 25.6^{o}C with dried longan extract as carbon source in 5,000 ml scale are illustrated in Fig. 3.22. The initial protein concentration of 0.014 ± 0.001 mg ml^{-1} was increased to 0.270 ± 0.001 mg ml^{-1} after 36 h cultivation period. The PDC activity increased from the initial value of 0.013 ± 0.001 U ml^{-1} to 0.069 ± 0.001 U ml^{-1} after 36 h. This was compared to the decrease in specific PDC activity from 0.962 ± 0.056 to 0.257 ± 0.004 U mg^{-1} proteins during the same period. The elevation of biomass specific PDC activity from 174.1 ± 6.99 to 6.38 ± 0.10 U g^{-1} biomass after 36 h cultivation periods was also observed.

3.2.3 Kinetics Studies: Fed batch cultivation in 600 ml scale

This study investigated the fed batch cultivation of *S. cerevisiae* TISTR 5606 by starting with 1,500 ml batch cultivation for 36 h using DLE medium as a carbon source. The cultivation culture was then divided into two portions of 400 ml. The addition of 200 ml DLE medium was then followed to the first portion while the equivalent volume of DDLFH medium was then carried out to the latter. The cultivation was then allowed to proceed for the next 24 h at 25.6^{o}C. The detailed kinetic profiles of the

microbial growth with addition of DLE and DDLFH media are shown in Fig. 3.21 and 3.22, respectively. Each figure is divided into two parts, namely; part (a) describes the kinetic profiles of TSS, pH level, OD600, and dried biomass concentration which could be related to OD600 by third order polynomial equations as previously mentioned in previous section; part (b) portrays the kinetic profiles of substrates such as sucrose, glucose, and fructose concentrations, as well as the product or ethanol concentration.

The detailed analysis of each cultivation profile with hypothesis testing for DLE and DDLFH feeding is tabulated in Table 3.33 – 3.38. The first three tables (Table 3.33 – 3.35) portray the statistical comparison of TSS, pH level, OD600, and dried biomass concentration data extracted from Fig. 3.21(a) – 3.22(a) which include the analyses of differences between the final and initial levels, average, and maximum rates. Similar analyses and comparisons were also carried out for sugars and ethanol concentrations. Table 3.36 – 3.38 presents these information in terms of differences, average as well as maximum rates.

As indicated in Fig. 3.21(a) and 3.22(a), the maximum change in TSS level before feeding was 9.15 ± 0.12°Brix. This was significantly different ($p \leq 0.05$) from the addition of DLE medium during 36 – 60 h (DLE (36 - 60)) and DDLFH medium during the same period (DDLFH (36 – 60)) as indicated in Table 3.33. TSS change of DLE (36 – 60) was 3.82 ± 0.05°Brix which was also significantly different ($p \leq 0.05$) from 1.72 ± 0.21°Brix for DDLFH (36 – 60). The average rate of TSS decreasing (Table 3.34) and maximum rate of TSS decreasing (Table 3.35) for all three cases were not different statistically ($p > 0.05$) with the corresponding range of rates between 0.081 – 0.236^{o}Brix h^{-1} and 0.161 – 0.378^{o}Brix h^{-1}, respectively.

The maximum change in OD600 level of 30.51 ± 1.17 ODU before feeding as indicated in Table 3.33 was significantly higher ($p \leq 0.05$) than DLE (36 – 60) and DDLFH (36 – 60) with the corresponding values of 22.20 ± 0.91 ODU and 12.86 ± 1.20 ODU, respectively. The comparison of OD600 increasing rate suggested that all three conditions were not different statistically ($p > 0.05$) with the corresponding range of rates between 0.503 – 1.040 ODU h^{-1} (Table 3.34). In addition, the highest maximum OD600 increasing rate in Table 3.18 belonged to before feeding of 2.014 ± 0.954 ODU h^{-1}, but this was not different statistically ($p > 0.05$) from DLE (36 – 60) and DDLFH (36 – 60).

The increasing trend of dried biomass concentration which corresponded directly to OD600 level suggested that the highest dried biomass concentration was obtained before feeding at 6.58 ± 0.17 g l^{-1}, which was significantly higher ($p \leq 0.05$) than 5.72 ± 0.13 g l^{-1} and 3.00 ± 0.17 g l^{-1} (Table 3.33) obtained from DLE (36 – 60) and DDLFH (36 – 60), respectively. The average increasing rate of

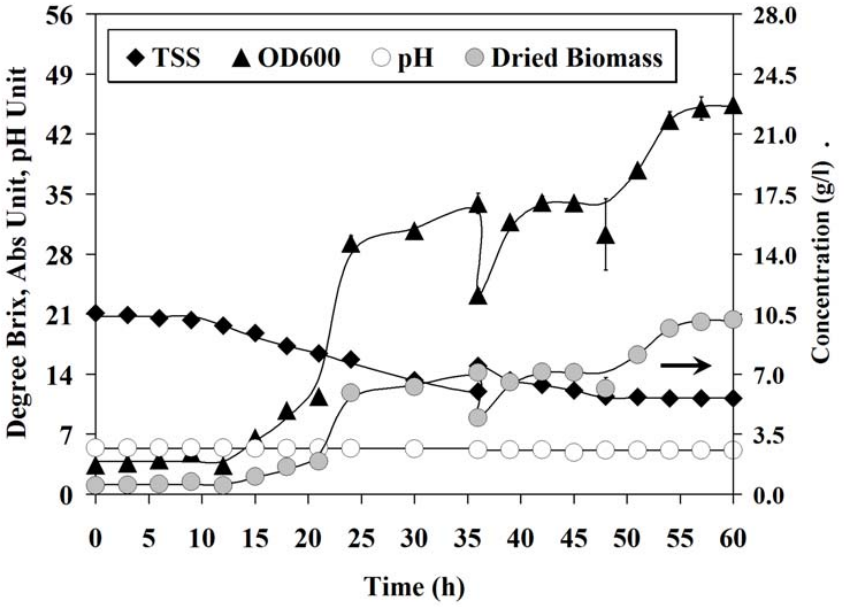
dried biomass concentration in Table 3.34 suggested that all three conditions were not different statistically ($p > 0.05$) with the corresponding range of rates between 0.117 – 0.889 g l^{-1} h^{-1}. The highest maximum increasing rate of dried biomass concentration in Table 3.35 for DLE (36 – 60) was 0.430 ± 0.088 g l^{-1} h^{-1}, which was not different statistically ($p > 0.05$) from before feeding of 0.418 ± 0.226 g l^{-1} h^{-1}. However, this value was higher than 0.178 ± 0.023 g l^{-1} h^{-1} which belonged to DDLFH (36 – 60).

The resistance to pH change throughout the cultivation period was generally observed for DLE (36 – 60) at 5.545 ± 0.004 (Table 3.34), which was significantly higher ($p \leq 0.05$) than before feeding and DDLFH (36 – 60) with the corresponding values of 5.349 ± 0.017 and 5.177 ± 0.035, respectively.

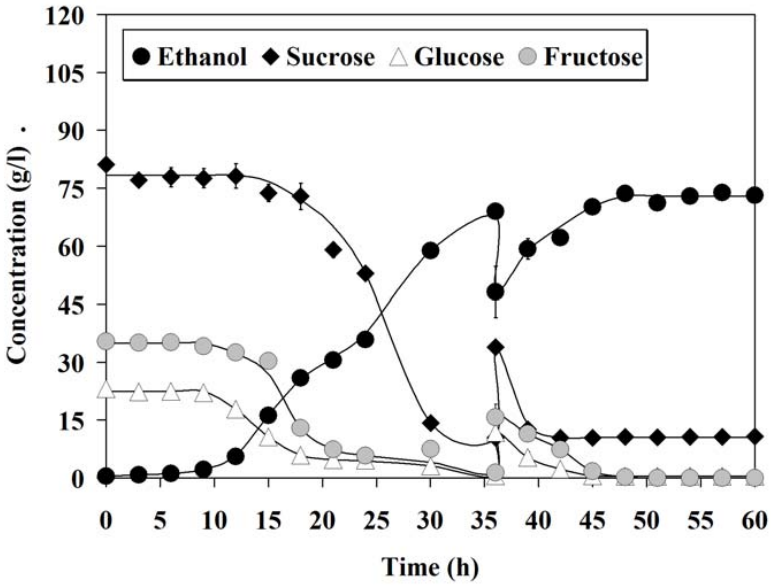
The average specific growth rate presented in Table 3.34 for all three conditions were not different statistically ($p > 0.05$) with the corresponding range of rates between 0.027 – 0.188 h^{-1}. The shortest period of average doubling time in Table 3.34 occurred with the kinetics profile before feeding at 9.06 ± 3.70 h which could be compared to the longest period of doubling time for DDLFH (36 – 60) at 25.60 ± 7.20 h. The statistical analyses of maximum specific growth rate (μmax) indicated that the value of before feeding was the highest at 0.183 ± 0.032 h^{-1}. This was significantly different ($p \leq 0.05$) from DLE (36 – 60) and DDLFH (36 – 60). In case of minimum doubling time (td, min), before feeding had the lowest value of 3.79 ± 0.66 h which was not different statistically ($p > 0.05$) from DLE (36 – 60). The highest minimum doubling time (td, min) belonged to DDLFH (36 – 60) at 16.30 ± 3.80 h.

The decreasing level of each sugar for all three conditions being investigated differed statistically ($p \leq 0.05$) as illustrated in Table 3.36. The highest level of sugars decreasing for before feeding were 70.67 ± 0.46 g l^{-1}, 22.48 ± 0.60 g l^{-1}, and 34.01 ± 0.91 g l^{-1} for sucrose, glucose, and fructose, respectively. The decreasing levels of each sugar for DLE (36 – 60) were 23.24 ± 1.07 g l^{-1}, 11.37 ± 0.17 g l^{-1}, and 15.69 ± 0.22 g l^{-1} for sucrose, glucose, and fructose, respectively. These were significantly higher ($p \leq 0.05$) than DDLFH (36 – 60). The average rate of sugars decreasing level (Avg RS, Table 3.37 was not differed statistically ($p > 0.05$) between all three conditions. For sucrose, the corresponding range of Avg RS was 0.000 – 1.577 g l^{-1} h^{-1}. For glucose, the corresponding ranges of Avg RS was 0.322 – 0.666 g l^{-1} h^{-1}. For fructose, the corresponding range of Avg RS was 0.367 – 1.043 g l^{-1} h^{-1}. In case of average specific rate for sucrose decreasing (Avg Qs, Table 3.37), before feeding had the highest value but was not significantly different ($p > 0.05$) from DLE (36 – 60) with the corresponding range of rate between 0.175 – 0.543 g g^{-1} h^{-1}. The average specific rates of glucose and fructose decreasing (Avg Qs, Table 3.37) were not significantly different ($p > 0.05$) for all three conditions. For glucose, the corresponding range of this

rate was 0.080 – 0.742 g g^{-1} h^{-1}. For fructose, the corresponding range of this rate was 0.093 – 0.889 g g^{-1} h^{-1}. The maximum rate of sucrose decreasing (Max RS, Table 3.38) was the highest for before feeding but was not significantly different ($p > 0.05$) from DLE (36 – 60) with corresponding range of rate between 1.948 – 3.598 g l^{-1} h^{-1}. The maximum rates of glucose and fructose decreasing (Max RS, Table 3.38) were not significantly different ($p > 0.05$) from one another. For glucose, the corresponding range of this rate was 0.643 – 1.486 g l^{-1} h^{-1}. For fructose, the corresponding range was 0.697 – 2.290 g l^{-1} h^{-1}. The maximum specific rates of sugars decreasing levels (Max Qs, Table 3.38) were the highest in case of before feeding at 1.158 ± 0.064 g g^{-1} h^{-1}, 1.822 ± 0.665 g g^{-1} h^{-1}, and 2.161 ± 0.627 g g^{-1} h^{-1} for sucrose, glucose, and fructose, respectively. These rates for before feeding differed significantly ($p \leq 0.05$) from DLE (36 – 60) and DDLFH (36 – 60). But the latter pair did not differ statistically ($p > 0.05$) from one another.

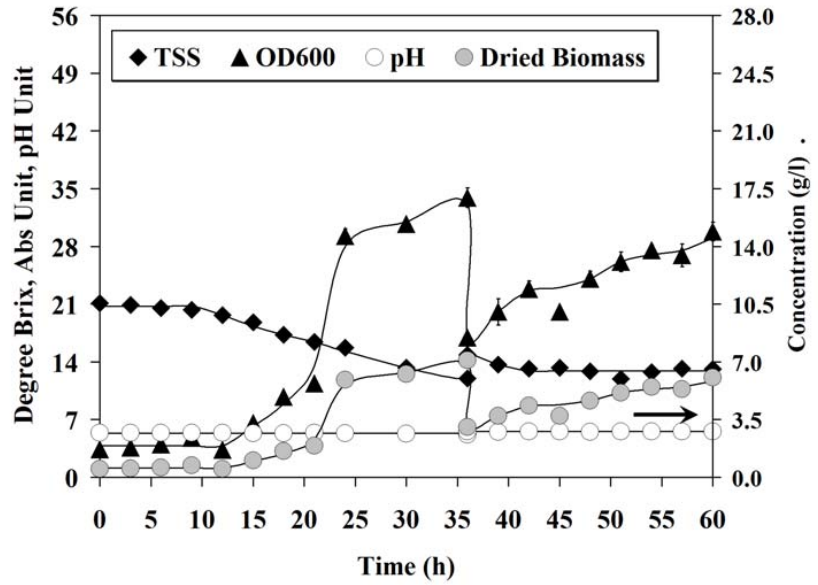


(a)

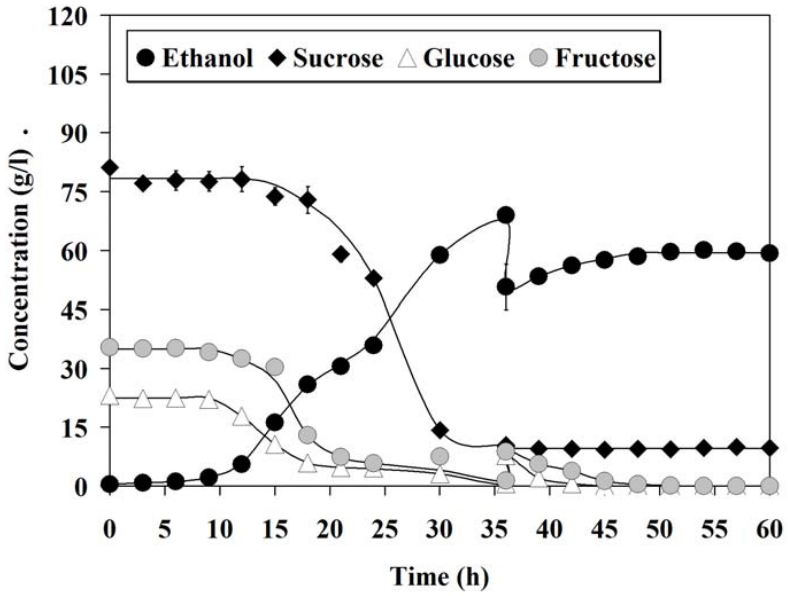


(b)

Figure 3.21: Growth kinetics of *S. cerevisiae* TISTR 5606 in fed batch system which utilized DLE medium in batch stage for 36 h before feeding of DLE medium for the next 24 h during 60 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.



(a)



(b)

Figure3.22: Growth kinetics of *S. cerevisiae* TISTR 5606 in fed batch system which utilized DLE medium in batch stage for 36 h before feeding of DDLFH medium for the next 24 h during 60 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

Table 3.33: The differences in TSS, OD600, and dried biomass (X) concentration (g l^{-1}) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three conditions.

Investigated parameters	**Conditions**					
	Before 36 h		**DLE (36 – 60 h)**		**DDLFH (36 – 60 h)**	
TSS decreasing level	9.15 ± 0.12	I	3.82 ± 0.05	II	1.72 ± 0.21	III
OD600 increasing level	30.51 ± 1.17	I	22.20 ± 0.91	II	12.86 ± 1.20	III
X production level	6.58 ± 0.17	I	5.72 ± 0.13	II	3.00 ± 0.17	III

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.34: The average pH level, average TSS decreasing rate (^{o}Brix h^{-1}), average OD600 increasing rate (ODU h^{-1}), average dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), average specific growth rate (h^{-1}), and average doubling time (h) during 60 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for three conditions.

Investigated parameters	Conditions					
	Before 36 h		DLE (36 – 60 h)		DDLFH (36 – 60 h)	
pH level	5.349 ± 0.017	I	5.177 ± 0.035	II	5.545 ± 0.004	III
TSS decreasing rate	-0.236 ± 0.055	I	-0.155 ± 0.072	I	-0.081 ± 0.054	I
OD600 increasing rate	1.040 ± 0.467	I	0.916 ± 0.340	I	0.503 ± 0.118	I
X increasing rate	0.889 ± 0.105	I	0.236 ± 0.086	I	0.117 ± 0.026	I
Specific growth rate	0.188 ± 0.031	I	0.034 ± 0.014	I	0.027 ± 0.008	I
Doubling time	9.06 ± 3.70	I	20.40 ± 8.50	I	25.60 ± 7.20	I

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.35: The maximum TSS decreasing rate (^{o}Brix h^{-1}), maximum OD600 increasing rate (ODU h^{-1}), maximum dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), maximum specific growth rate (h^{-1}), and minimum doubling time (h) during 60 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for three conditions.

Investigated parameters	Conditions					
	Before 36 h		DLE (36 – 60 h)		DDLFH (36 – 60 h)	
TSS decreasing rate	-0.378 ± 0.024	I	-0.311 ± 0.089	I	-0.161 ± 0.098	I
OD600 increasing rate	2.014 ± 0.954	I, II	1.679 ± 0.366	I	0.771 ± 0.117	II
X increasing rate	0.418 ± 0.226	I, II	0.430 ± 0.088	I	0.178 ± 0.023	II
Specific growth rate	0.183 ± 0.032	I	0.064 ± 0.018	II	0.043 ± 0.010	II
Doubling time	3.79 ± 0.66	I	10.90 ± 3.10	I, II	16.30 ± 3.80	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.36: The differences in sugars (sucrose, glucose, and fructose) concentration levels (g l^{-1}), ethanol concentration levels (g l^{-1}), lag time (sucrose, glucose, fructose, and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three conditions.

Investigated parameters	Conditions					
	Before 36 h		DLE (36 – 60 h)		DDLFH (36 – 60 h)	
Sucrose decreasing level	70.67 ± 0.46	I	23.24 ± 1.07	II	0.36 ± 0.25	III
Glucose decreasing level	22.48 ± 0.60	I	11.37 ± 0.17	II	7.72 ± 0.20	III
Fructose decreasing level	34.01 ± 0.91	I	15.69 ± 0.22	II	8.80 ± 0.18	III
Ethanol production level	68.42 ± 0.43	I	24.93 ± 1.13	II	8.61 ± 0.56	III
$Y_{P/S}$	0.54 ± 0.01	I	0.50 ± 0.02	I	0.51 ± 0.04	I

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.37: The average sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), average ethanol production rate (g l^{-1} h^{-1}), average specific rate of sugars consumption (Avg Q_s, g l^{-1} h^{-1}), and average specific rate of ethanol production (Avg Q_p, g l^{-1} h^{-1}) during 60 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three conditions.

Investigated parameters	Conditions					
	Before 36 h		DLE (36 – 60 h)		DDLFH (36 – 60 h)	
Sucrose consumption rate	-1.577 ± 0.685	I	-0.974 ± 0.823	I	0.000 ± 0.000	I
Glucose consumption rate	-0.666 ± 0.271	I	-0.473 ± 0.277	I	-0.322 ± 0.230	I
Fructose consumption rate	-1.043 ± 0.457	I	-0.654 ± 0.274	I	-0.367 ± 0.149	I
Ethanol production rate	1.735 ± 0.421	I	1.032 ± 0.464	I, II	0.363 ± 0.145	II
Avg Q_s of sucrose	-0.543 ± 0.182	I	-0.175 ± 0.152	I, II	0.000 ± 0.000	II
Avg Q_s of glucose	-0.742 ± 0.381	I	-0.080 ± 0.050	I	-0.090 ± 0.068	I
Avg Q_s of fructose	-0.889 ± 0.415	I	-0.101 ± 0.043	I	-0.093 ± 0.041	I
Avg Q_p of ethanol	1.247 ± 0.444	I	0.167 ± 0.082	II	0.092 ± 0.038	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.38: The maximum sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), maximum ethanol production rate (g l^{-1} h^{-1}), maximum specific rate of sugars consumption (Max Q_s, g l^{-1} h^{-1}), and maximum specific rate of ethanol production (Max Q_p, g l^{-1} h^{-1}) during 60 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation in dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three conditions.

Investigated parameters	Conditions					
	Before 36 h		DLE (36 – 60 h)		DDLFH (36 – 60 h)	
Sucrose consumption rate	-3.598 ± 1.080	I	-1.948 ± 1.590	I, II	0.000 ± 0.000	II
Glucose consumption rate	-1.486 ± 0.409	I	-0.947 ± 0.457	I	-0.643 ± 0.442	I
Fructose consumption rate	-2.290 ± 0.822	I	-1.289 ± 0.286	I	-0.697 ± 0.174	I
Ethanol production rate	3.052 ± 0.342	I	2.064 ± 0.541	I	0.727 ± 0.100	II
Max Q_s of sucrose	-1.158 ± 0.064	I	-0.350 ± 0.296	II	0.000 ± 0.000	II
Max Q_s of glucose	-1.822 ± 0.665	I	-0.160 ± 0.087	II	-0.181 ± 0.126	II
Max Q_s of fructose	-2.161 ± 0.627	I	-0.200 ± 0.045	II	-0.180 ± 0.053	II
Max Q_p of ethanol	2.545 ± 0.725	I	0.334 ± 0.112	II	0.184 ± 0.035	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Fig. 3.21(b) and 3.22(b) portrayed the increasing trends of ethanol production. The application of DLE and DDLFH media feeding in Fig. 3.21(b) and 3.22(b) resulted in the stationary phase during the first 9 h prior to rapid elevation in ethanol production until 36^{th} h. At the beginning of feeding step on the 36^{th} h, the rapid decrease in ethanol concentration was generally observed due to the dilution of the cultivation culture by the feeding media. This was followed by the increasing trend until the 60^{th} h. In case of DDLFH medium feeding, the slower increasing trend was observed before reaching the plateau at the 45^{th} h. Such phenomenon may illustrate the toxicity of DDLFH medium which slowed down the ethanol production rate. The final ethanol concentration differed statistically ($p \leq 0.05$) as shown in Table 3.36. The highest ethanol concentration level at 68.42 ± 0.43 g l^{-1} was obtained for before feeding. The ethanol concentration of DLE (36 – 60) was 24.93 ± 1.13 g l^{-1} which was significantly different ($p \leq 0.05$) from 8.61 ± 0.56 g l^{-1} of DDLFH (36 – 60).

The highest average rate of ethanol production (Avg RP, Table 3.37) for before feeding was 1.735 ± 0.421 g l^{-1} h^{-1}, which was not significantly different ($p > 0.05$) from DLE (36 – 60) at 1.032 ± 0.464 g l^{-1} h^{-1}. The highest average specific rate of ethanol production (Avg QP, Table 3.37) for before feeding was 1.247 ± 0.444 g g^{-1} h^{-1}, which was significantly different ($p \leq 0.05$) from DLE (36 – 60) and DDLFH (36 – 60) with the corresponding range of rate between 0.092 – 0.167 g g^{-1} h^{-1}. The highest maximum rate of ethanol production (Max RP, Table 3.38) was before feeding at 3.052 ± 0.342 g l^{-1} h^{-1}, which was not significantly different ($p > 0.05$) from DLE (36 – 60) at 2.064 ± 0.541 g l^{-1} h^{-1}. The highest maximum specific rate of ethanol production (Max QP, Table 3.38) belonged to before feeding at 2.545 ± 0.725 g g^{-1} h^{-1}. This value was significantly different ($p \leq 0.05$) from DLE (36 – 60) and DDLFH (36 – 60) with the corresponding range of rate between 0.184 – 0.334 g g^{-1} h^{-1}.

The highest ethanol yield was observed with before feeding in Table 3.36 with the corresponding value of 0.54 ± 0.01 (this was higher than the theoretical value of 0.538) g produced ethanol g^{-1} consumed sugars. Such yield was not significantly different ($p > 0.05$) to DLE (36 – 60) and DDLFH (36 – 60) with the corresponding yield values of 0.50 ± 0.02 and 0.51 ± 0.04, respectively.

3.2.4 Two-phase Separated Biotransformation Studies

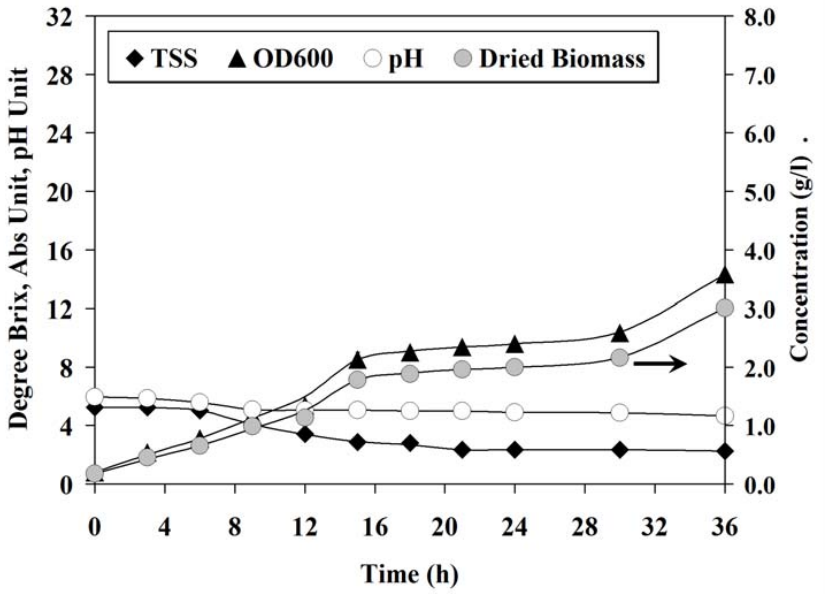
The experiment of two-phase PAC biotransformation was conducted by adopting whole cells *S. cerevisiae* TISTR 5606 cultivated in DLE and DDLFH media. Firstly, whole cells from DLE medium was adjusted to dried biomass equivalent level of 3.06 g l^{-1}, 6.12 g l^{-1}, and 12.24 g l^{-1}, respectively. Secondly, whole cells from DDLFH medium was adjusted to dried biomass equivalent level of 3.06 g l^{-1}. The absence of PAC production was evident which might be the result of separated organic/aqueous phase which minimized the exposure of whole cells to benzaldehyde substrate. This was in contrast to a well-mixed two-phase biotransformation system in which whole cells of *C. utilis* TISTR 5198 could produce a higher level of PAC concentration at 83.8 ± 6.8 mM (Agustina, 2009). Further comparison could be made to the studies of Tangsuntornkhan and Ktanyu (2010) in which *C. utilis* TISTR 5352 whole cells cultivated in the similar DLE medium and biotransformation conditions could generate the overall PAC concentration at the level up to 0.75 ± 0.02 mM. While *C. utilis* 5198 whole cells in DDLFH medium yielded the overall PAC concentration of 1.76 ± 0.06 mM.

3.3 The Development of Mathematical Model for Ethanol Production from Dried Longan Extract in a Static Condition of *Saccharomyces cerevisiae* TISTR 5606

3.3.1 Cultivation in Individual Pure Sugar Conditions

This experiment examined the cultivation kinetics of *S. cerevisiae* TISTR 5606 on the media containing a different type of single carbon source, namely, glucose only, fructose only, and sucrose only with the initial sugar concentration of 40 g l^{-1}. The batch cultivation was performed statically on a 1,500 ml scale with supplementation of extra nitrogen sources such as yeast extract, malt extract, and peptone. All cultivations were carried out for 36 h in the static condition at 25.6^{o}C. The microbial cultivation kinetic profiles of each pure sugar for *S. cerevisiae* TISTR 5606 are illustrated in Fig. 3.23 for glucose, Fig. 3.24 for fructose, and Fig. 3.25 for sucrose. Each figure is divided into two parts, for example; part (a) describes the kinetic profiles of TSS, pH level, OD600, and dried biomass concentration which could be related to OD600 by third order polynomial equations as previously mentioned in previous section; part (b) portrays the kinetic profiles of substrates such as glucose, fructose, and sucrose concentrations, as well as the product or ethanol concentration.

The detailed analyses of each cultivation profile with hypothesis testing across three types of carbon sources are tabulated in Table 3.39 – 3.44. The first three tables (Table 3.39 – 3.41) portray the statistical comparison of TSS, pH level, OD600, and dried biomass concentration data extracted from Fig. 3.23(a) – 3.25(a) which include the analyses of differences between the final and initial levels, average, and maximum rates. Similar analyses and comparisons were also carried out for sugars and ethanol concentrations.



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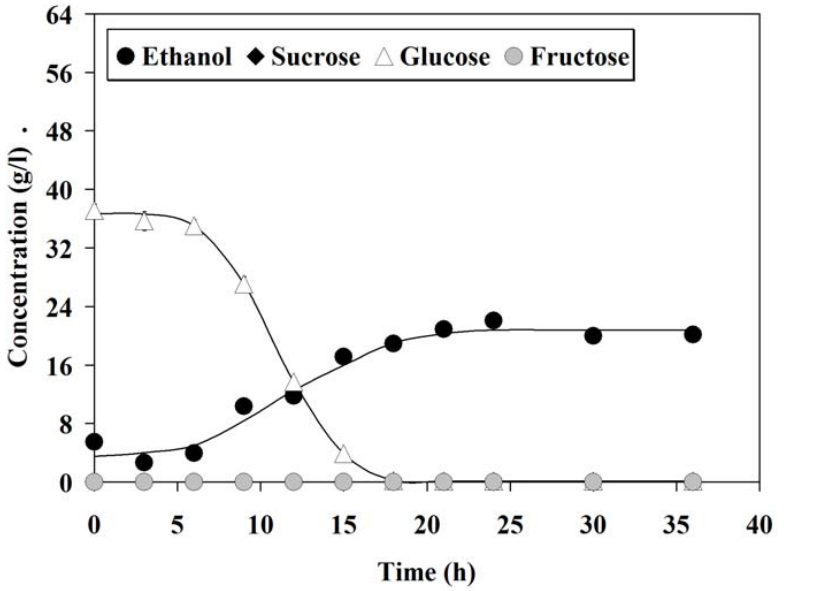
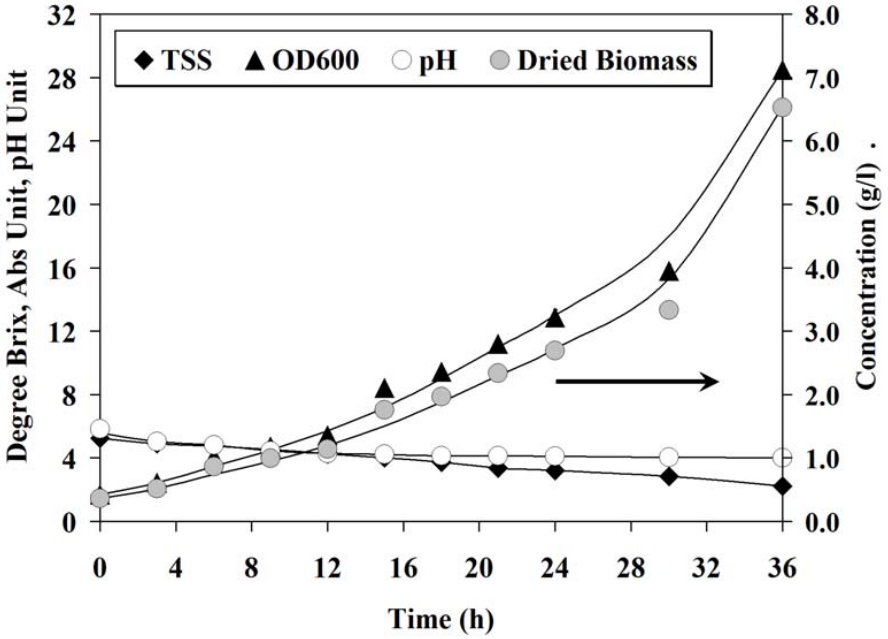
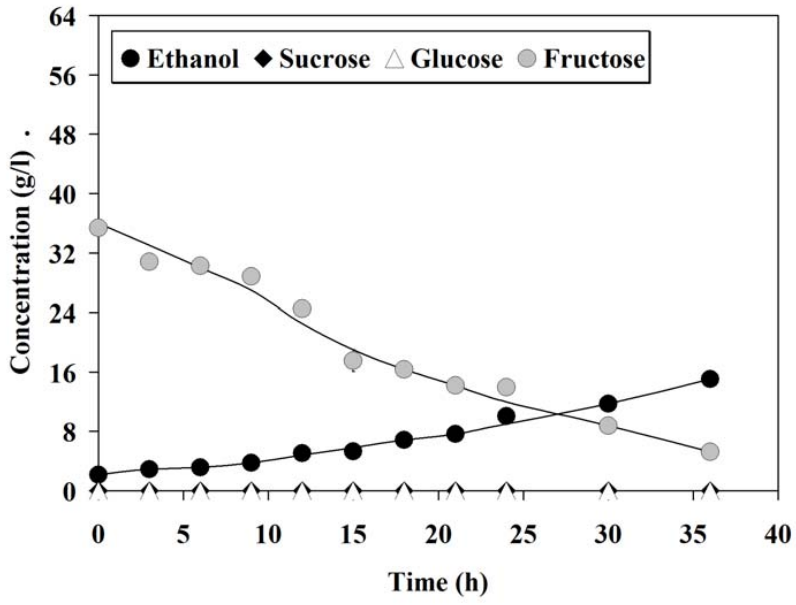


(b)

Figure 3.23: Growth kinetics of *S. cerevisiae* TISTR 5606 using glucose as a sole carbon source during 36 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

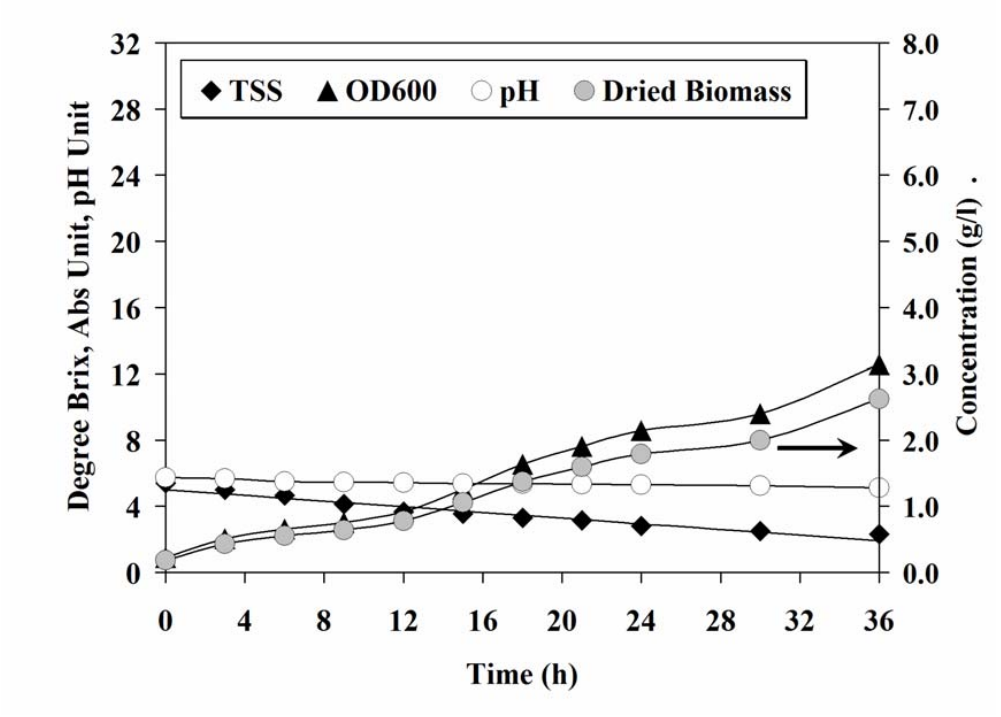


(a)

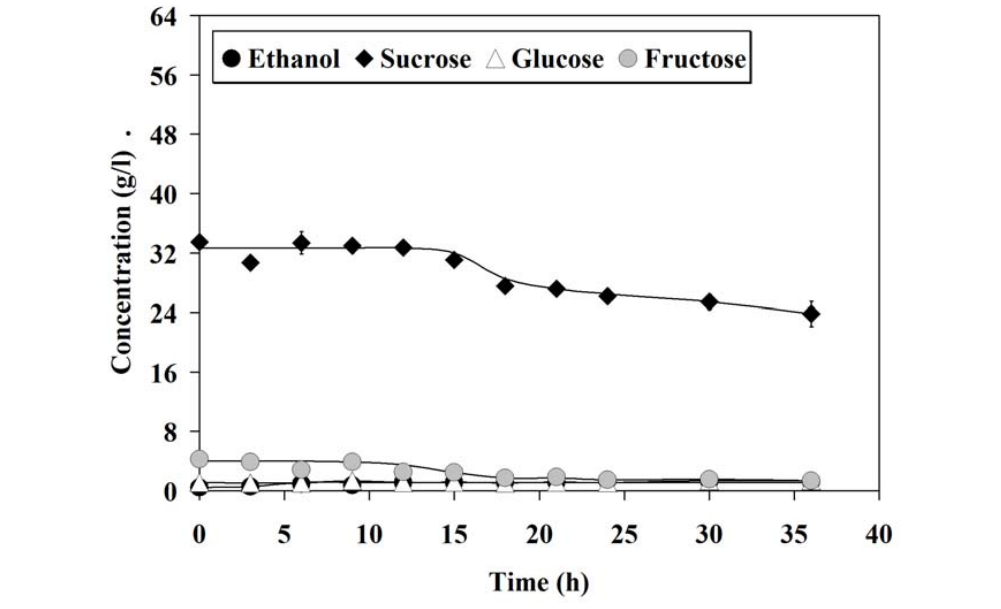


(b)

Figure 3.24: Growth kinetics of *S. cerevisiae* TISTR 5606 using fructose as a sole carbon source during 36 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.



(a)



(b)

Figure 3.25: Growth kinetics of *S. cerevisiae* TISTR 5606 using sucrose as a sole carbon source during 36 h cultivation period in a static condition at 25.6°C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

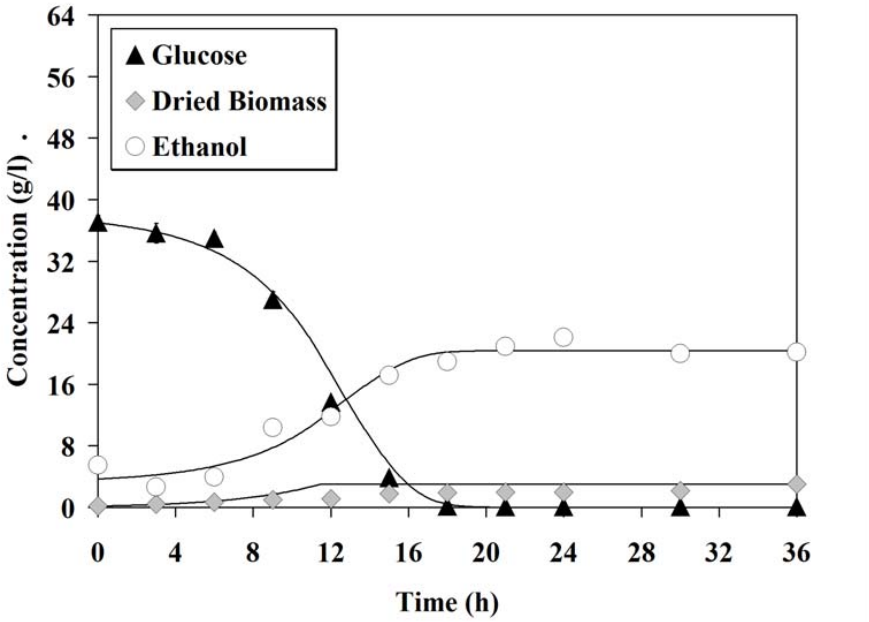


Figure 3.26: Simulated curves of the individual pure sugar and experiment data for *S. cerevisiae* using 40 g l^{-1} glucose in a static condition at 25.6°C. The R^2 and RSS value were 0.9954 and 26.0, respectively.

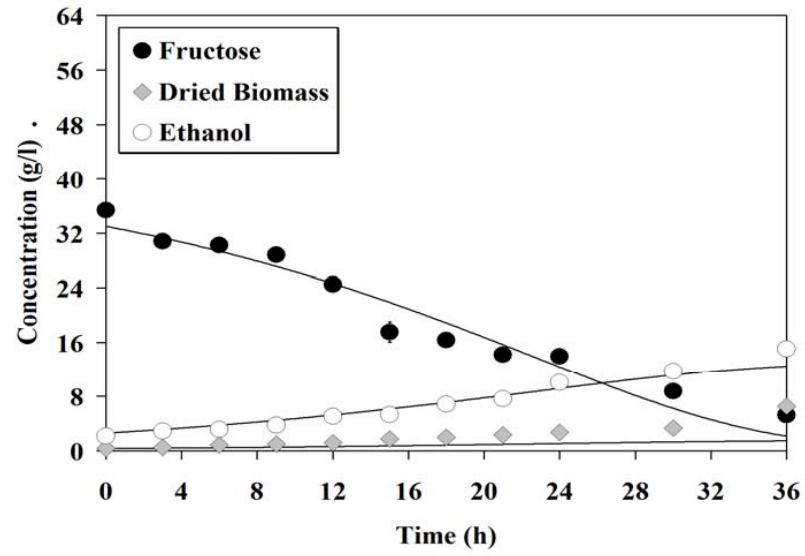


Figure 3.27: Simulated curves of the individual pure sugar and experiment data for *S. cerevisiae* using 40 g l^{-1} fructose in a static condition at 25.6°C. The R^2 and RSS value were 0.9849 and 67.7, respectively.

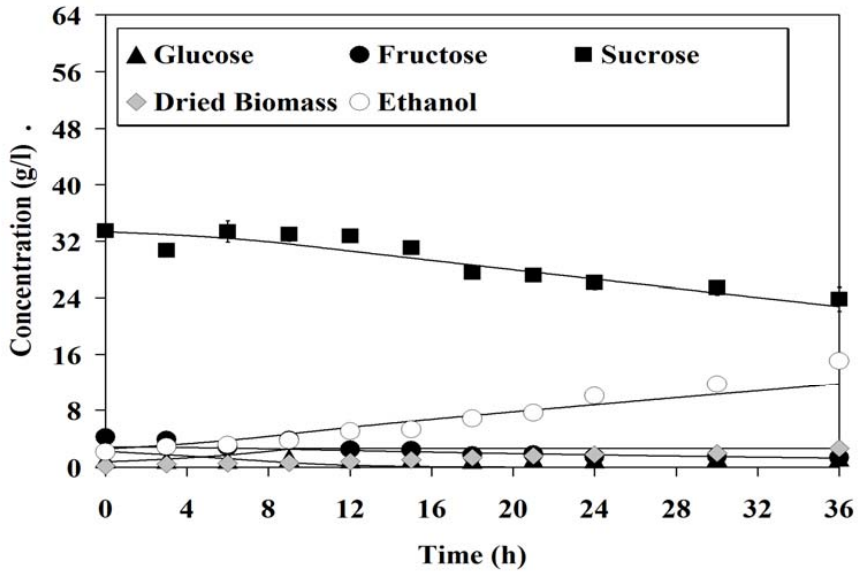


Figure 3.28: Simulated curves of the individual pure sugar and experiment data for *S. cerevisiae* using 40 g l^{-1} sucrose in a static condition at 25.6°C. The R^2 and RSS value were 0.9877 and 83.0, respectively.

Table 3.39: The differences in TSS, OD600, and dried biomass (X) concentration (g l^{-1}) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with each pure sugar using *S. cerevisiae* TISTR 5606 at 25.6°C for three types of sugars.

Investigated parameters	Sugar type					
	Glucose		Fructose		Sucrose	
TSS decreasing level	2.98 ± 0.05	I	3.01 ± 0.06	I	3.09 ± 0.03	I
OD600 increasing level	13.51 ± 0.16	III	26.78 ± 0.36	II	11.67 ± 0.26	I
X production level	2.83 ± 0.03	III	6.17 ± 0.08	II	2.44 ± 0.06	I

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.40: The average pH level, average TSS decreasing rate (^{o}Brix h^{-1}), average OD600 increasing rate (ODU h^{-1}), average dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), average specific growth rate (g g^{-1} h^{-1}), and average doubling time (h) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with each pure sugar using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for three types of sugars.

Investigated parameters	Sugar type					
	Glucose		Fructose		Sucrose	
pH level	5.96 ± 0.13	I	5.60 ± 0.17	I	5.74 ± 0.05	I
TSS decreasing rate	-0.10 ± 0.03	I	-0.08 ± 0.01	I	-0.09 ± 0.00	I
OD600 increasing rate	0.37 ± 0.08	I	0.63 ± 0.14	I	0.32 ± 0.04	I
X increasing rate	0.08 ± 0.02	I	0.14 ± 0.04	I	0.07 ± 0.01	I
Specific growth rate	0.09 ± 0.03	I	0.08 ± 0.01	I	0.08 ± 0.02	I
Doubling time	8.10 ± 2.50	I	8.50 ± 0.70	I	8.70 ± 2.40	I

The numbers with the same Roman numeral (I-I) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.41: The maximum TSS decreasing rate (^{o}Brix h^{-1}), maximum OD600 increasing rate (ODU h^{-1}), maximum dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), maximum specific growth rate (g g^{-1} h^{-1}), and minimum doubling time (h) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation with each pure sugar using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for three types of sugars.

Investigated parameters	Sugar type					
	Glucose		Fructose		Sucrose	
TSS decreasing rate	-0.21 ± 0.04	II	-0.11 ± 0.00	II	-0.09 ± 0.00	I
OD600 increasing rate	0.62 ± 0.09	I	0.98 ± 0.26	I	0.46 ± 0.03	I
X increasing rate	0.13 ± 0.02	I	0.23 ± 0.08	I	0.10 ± 0.01	I
Specific growth rate	0.16 ± 0.04	I	0.10 ± 0.01	I	0.13 ± 0.04	I
Doubling time	4.20 ± 1.00	I	6.80 ± 0.70	I	5.20 ± 1.60	I

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.42: The differences in sugars (sucrose, glucose, and fructose) concentration levels (g l^{-1}), ethanol concentration levels (g l^{-1}), lag time (sucrose, glucose, fructose, and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with each pure sugar using *S. cerevisiae* TISTR 5606 at 25.6°C for three types of sugars.

Investigated parameters	Sugar type					
	Glucose		Fructose		Sucrose	
Sucrose decreasing level	N/a	II	N/a	II	9.68 ± 1.72	I
Glucose decreasing level	36.97 ± 0.88	II	N/a	I	-0.28 ± 0.06	I
Fructose decreasing level	N/a	III	30.14 ± 0.70	II	2.93 ± 0.27	I
Ethanol producing level	14.70 ± 0.90	II	12.87 ± 0.19	II	0.84 ± 0.11	I
Sucrose lag time	N/a	II	N/a	II	12.00 ± 1.20	I
Glucose lag time	3.00 ± 0.30	II	N/a	I	N/a	I
Fructose lag time	N/a	II	N/a	II	6.00 ± 0.60	I
Ethanol lag time	0.00	I	0.00	I	0.00	I
$\mathbf{Y_{P/S}}$	0.40 ± 0.03	II	0.43 ± 0.01	II	0.07 ± 0.01	I

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.43: The average sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), average ethanol production rate (g l^{-1} h^{-1}), average specific rate of sugars consumption (Avg Q_s, g g^{-1} h^{-1}), and average specific rate of ethanol production (Avg Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with each pure sugar using *S. cerevisiae* TISTR 5606 at 25.6°C for three types of sugars.

Investigated parameters	**Sugar type**					
	Glucose		**Fructose**		**Sucrose**	
Sucrose consumption rate	N/a	II	N/a	II	-0.25 ± 0.11	I
Glucose consumption rate	-1.22 ± 0.52	II	N/a	I	0.00 ± 0.02	I
Fructose consumption rate	N/a	I	-0.91 ± 0.09	II	-0.09 ± 0.04	I
Ethanol production rate	0.58 ± 0.17	II	0.33 ± 0.04	II	0.02 ± 0.02	I
Avg Q_s of sucrose	N/a	II	N/a	II	-0.18 ± 0.09	I
Avg Q_s of glucose	-1.12± 0.48	II	N/a	I	0.00 ± 0.02	I
Avg Q_s of fructose	N/a	I	-0.87 ± 0.23	II	-0.09 ± 0.04	I
Avg Q_p of ethanol	0.54 ± 0.16	II	0.23 ± 0.04	II	0.05 ± 0.04	I

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.44: The maximum sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), maximum ethanol production rate (g l^{-1} h^{-1}), maximum specific rate of sugars consumption (Max Q_s, g g^{-1} h^{-1}), and maximum specific rate of ethanol production (Max Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation with each pure sugar using *S. cerevisiae* TISTR 5606 at 25.6°C for three types of sugars.

Investigated parameters	**Sugar type**					
	Glucose		**Fructose**		**Sucrose**	
Sucrose consumption rate	N/a	II	N/a	II	-0.53 ± 0.22	I
Glucose consumption rate	-2.90 ± 0.67	III	N/a	II	-0.04 ± 0.02	I
Fructose consumption rate	N/a	III	-1.17 ± 0.12	II	-0.20 ± 0.06	I
Ethanol production rate	1.17 ± 0.08	III	0.45 ± 0.04	II	0.06 ± 0.04	I
Max Q_s of sucrose	N/a	II	N/a	II	-0.40 ± 0.19	I
Max Q_s of glucose	-2.62 ± 0.66	III	N/a	II	-0.05 ± 0.02	I
Max Q_s of fructose	N/a	III	-1.61 ± 0.24	II	-0.21 ± 0.07	I
Max Q_p of ethanol	1.00 ± 0.19	II	0.34 ± 0.07	I	0.14 ± 0.09	I

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.45: The initial guess parameters which maximum specific growth rate (μ_{max}, h^{-1}), maximum specific substrate consumption rate ($q_{s,max}$, $g\ g^{-1}\ h^{-1}$), maximum specific ethanol production rate ($q_{p,max}$, $g\ g^{-1}\ h^{-1}$) for 1,500 ml static cultivation of *S. cerevisiae* TISTR 5606 with each pure sugar at 25.6°C .The values are expressed as the average of five consecutive maximum values ± standard error (S.E.).

Investigated parameters	Sugar type					
	Glucose		Fructose		Sucrose	
μ_{max}	0.16 ± 0.04	I	0.10 ± 0.01	I	0.13 ± 0.04	I
$q_{s,max}$	-2.62 ± 0.66	I	-1.61 ± 0.24	II	-0.40 ± 0.19	III
$q_{p,max}$	1.00 ± 0.19	I	0.34 ± 0.07	II	0.14 ± 0.09	III

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.46: Results of parameter search for mathematical model describing the growth kinetics of *S. cerevisiae* TISTR 5606 in the cultivation media containing only 40 g l^{-1} glucose, fructose, and sucrose.

(a) Initial values for the batch cultivation of TISTR 5606 on media containing a single carbon source

Concentration (g l^{-1})	Glucose only	Fructose only	Sucrose only
x_0	0.02	1.81	0.18
S_1	37.12	0.00	0.01
S_2	0.00	35.38	4.38
S_3	0.00	0.00	31.95
p_0	3.68	0.00	2.03

(b) Optimal kinetic parameters

Glucose only		Fructose only		Sucrose only	
Weighting factor for sugar consumption					
α	0.1598	β	0.3400	γ	0.5000
Biomass production model					
$\mu_{max,1}$	0.207	$\mu_{max,2}$	0.007	$\mu_{max,3}$	0.134
$K_{sx,1}$	20.41	$K_{sx,2}$	4.91	$K_{sx,3}$	5.01
$P_{mx,1}$	41.27	$P_{mx,2}$	56.30	$P_{mx,3}$	56.19
$K_{ix,1}$	600	$K_{ix,2}$	600	$K_{ix,3}$	596
$P_{ix,1}$	30.0	$P_{ix,2}$	26.6	$P_{ix,3}$	26.7
Sugar consumption model					
$q_{smax,1}$	2.721	$q_{smax,2}$	0.487	$q_{smax,3}$	0.305
$K_{ss,1}$	25.0	$K_{ss,2}$	10.9	$K_{ss,3}$	25.0
$P_{ms,1}$	36.29	$P_{ms,2}$	81.18	$P_{ms,3}$	35.00
$K_{is,1}$	600	$K_{is,2}$	600	$K_{is,3}$	600
$P_{is,1}$	29.8	$P_{is,2}$	30.0	$P_{is,3}$	26.0
Ethanol production model					
$q_{pmax,1}$	1.208	$q_{pmax,2}$	0.231	$q_{pmax,3}$	0.516
$K_{sp,1}$	25.0	$K_{sp,2}$	10.9	$K_{sp,3}$	25.0
$P_{mp,1}$	36.29	$P_{mp,2}$	81.18	$P_{mp,3}$	35.00
$K_{ip,1}$	600	$K_{ip,2}$	600	$K_{ip,3}$	600
$P_{ip,1}$	29.8	$P_{ip,2}$	30.0	$P_{ip,3}$	26.0

Table 3.42 – 3.44 presents these information in terms of differences, average as well as maximum rates.

The kinetic profiles describing the microbial growth using three pure sugars had similar trends as shown in Fig. 3.23(a) – 3.25(a). In term of pH level and TSS decreasing, there was negligible change with a slight continuous decreasing trend with cultivation period. The profiles of dried biomass concentration and OD600 for the cultivation using glucose and sucrose as carbon sources appeared to increase continuously during the first 12 h before the increasing rate was slowed down until the 24^{th} h period, during which a rapid increasing trend was immediately followed. In case of the cultivation using fructose, the initial increasing trend was extended from 12 to 24 h before the similar rapid increasing trend was observed.

From Table 3.39, the decreasing trend of TSS for all three cultivation conditions were not differed statistically ($p > 0.05$). This was compared to the highest increasing trend of 26.78 ± 0.36 in OD600 unit for the cultivation that used fructose as a sole carbon source which was found to be significantly difference ($p \leq 0.05$) than its counterparts that utilized other carbon sources. The comparison of dried biomass production in Table 3.39 also suggested that fructose could enhance its production significantly ($p \leq 0.05$) than the other sugars and reach the production level of 6.17 ± 0.08 g l^{-1}.

The average pH level (Table 3.40) in all three cultivation conditions did not differ significantly ($p > 0.05$) which was similar to the average TSS decreasing rate (0.08-0.10 ^{o}Brix h^{-1}), average OD600 elevation rate (0.32-0.63 ODU h^{-1}), as well as average specific growth rate (0.08-0.09 h^{-1}). Because of the insignificant difference ($p > 0.05$) from the average specific growth rates, the average doubling times of *S. cerevisiae* TISTR 5606 cultivated in the media containing these individual sugars were also found to be similar to one another (8.10-8.70 h).

The maximum decreasing rate of TSS in Table 3.41 for the cultivation using glucose (0.21 ± 0.04^{o}Brix h^{-1}) was the highest but was not found to be significantly different ($p > 0.05$) from fructose. However, the TSS decreasing rate of 0.09 ± 0.00^{o}Brix h^{-1} for sucrose was the lowest and significantly different ($p \leq 0.05$) from the other two sugars. This was in contrary to the increasing rate of OD600 at which the significant difference was not observed ($p > 0.05$). In fact, the same trend of insignificant difference ($p > 0.05$) was also spotted for maximum dried biomass increasing rate, maximum specific growth rate, and minimum doubling time.

Fig. 3.23(b) described the glucose consumption profile of *S. cerevisiae* TISTR 5606 with the overall consumption level of 36.97 ± 0.88 g l^{-1} and glucose depletion time of 18 h. Further comparison could be made to the fructose and sucrose consumption profiles in Fig. 3.24(b) and 3.24(c) with the

corresponding overall sugars consumption levels of 30.14 ± 0.70 and 9.68 ± 1.72 g l^{-1}, respectively (Table 3.42). This phenomenon clearly suggested the predominant role of glucose over the other two sugars. The production profile of ethanol exhibited the highest production level of 14.70 ± 0.90 g l^{-1} when glucose was used as a substrate. The lower levels of ethanol produced of 12.87 ± 0.19 and 0.84 ± 0.11 g l^{-1} were obtained when fructose and sucrose were utilized individually as substrates. The corresponding ethanol yields ($Y_{P/S}$) were 0.40, 0.43, and 0.07 for glucose, fructose, and sucrose which were slightly lower than the theoretical yield of 0.511 g ethanol g^{-1} consumed sugars.

Table 3.43 and 44 illustrated the analyses of average and maximum substrates consumption rates for all three sugars. The average and maximum glucose consumption rates of 1.22 ± 0.52 and 2.90 ± 0.67 g l^{-1} h^{-1} were significantly higher ($p \leq 0.05$) than fructose and sucrose, respectively. The corresponding ethanol production rates of three sugars were significantly different ($p \leq 0.05$) from one another. The highest ethanol production rate of 1.17 ± 0.08 g l^{-1} h^{-1} belonged to glucose which was followed by fructose (0.45 ± 0.04 g l^{-1} h^{-1}) and sucrose (0.06 ± 0.04 g l^{-1} h^{-1}), respectively. The specific ethanol production rate of glucose (1.00 ± 0.19 g g^{-1} h^{-1}) was also significantly higher ($p \leq 0.05$) than fructose and sucrose.

The obtained experimental kinetic parameter values such as μ_{max}, q_{smax}, and q_{pmax} from Table 3.41 and 3.44 are summarized in Table 3.45 and used as initial guesses for parameter search procedure in the RSS minimization program described previously. The predicted cultivation profiles for *S. cerevisiae* TISTR 5606 cultivation using an individual pure sugar are presented with accompanying R^2 and RSS values for fitting assessment in Fig. 3.26 for glucose, Fig. 3.27 for fructose, and Fig. 3.28 for sucrose. The optimal initial concentrations of substrates, product, and dried biomass, as well as corresponding kinetic parameters in the proposed model are tabulated in Table 3.46(a) and (b), respectively. The developed mathematical model could predict the experimental data relatively well with $R^2 > 0.98$ as well as relatively low level of RSS of 83.0 for the cultivation with sucrose only, 25.9 for glucose only, and 67.7 for fructose only.

3.3.2 Cultivation in Triple Pure Sugars Conditions

In case of the cultivation kinetics of *S. cerevisiae* TISTR 5606 on the media containing triple substrate which included (glucose/fructose/sucrose) 20/20/20, 30/30/30, 40/40/40 and 60/60/60 g l^{-1}. This cultivation was performed similarly to the individual pure sugar cultivation. The microbial cultivation kinetic profiles of triple sugar concentrations are illustrated in Fig. 3.29 for 20/20/20, Fig. 3.30 for 30/30/30, Fig. 3.31 for 40/40/40, and Fig. 3.32 for 60/60/60. Each figure is divided into two parts, for

example; part (a) describes the kinetic profiles of TSS, pH level, OD600, and dried biomass concentration which could be related to OD600 by third order polynomial equations as previously mentioned in previous section; part (b) portrays the kinetic profiles of substrates as well as the product or ethanol concentration.

The detailed analyses of each cultivation profile with hypothesis testing across three types of carbon sources are tabulated in Table 3.47 – 3.52. The first three tables (Table 3.47 – 3.49) portray the statistical comparison of TSS, pH level, OD600, and dried biomass concentration data extracted from Fig. 3.29(a) – 3.32(a) which include the analyses of differences between the final and initial levels, average, and maximum rates. Similar analyses and comparisons were also carried out for sugars and ethanol concentrations. Table 3.50 – 3.52 presents these information in terms of differences, average as well as maximum rates.

The kinetic profiles describing the microbial growth using four levels of triple sugar concentrations had similar trends as shown in Fig. 3.29(a) – 3.32(a). In term of pH level and TSS decreasing, there was negligible change with a slight continuous decreasing trend with cultivation period. The profiles of dried biomass concentration and OD600 for the cultivation using triple sugar concentrations at level of 20/20/20, 30/30/30, 40/40/40 and 60/60/60 as carbon sources appeared to increase continuously during the first 16 h before the increasing rate was slowed down until the 36 h period, during which a rapid increasing trend was immediately followed.

From Table 3.47, the decreasing trend of TSS and the increasing trend of dried biomass production for four tested levels of triple sugar concentrations were significantly different ($p \leq 0.05$). The highest TSS decreasing of 7.30 ± 0.10 belonged to 40/40/40 and dried biomass increasing of 2.85 ± 0.02 g l^{-1} belonged to 30/30/30. The comparison of OD600 decreasing revealed that the obtained values from 20/20/20 and 60/60/60 did not differ significantly ($p > 0.05$). In fact, the OD600 decreasing from 30/30/30 was significantly different ($p \leq 0.05$) from that of 40/40/40.

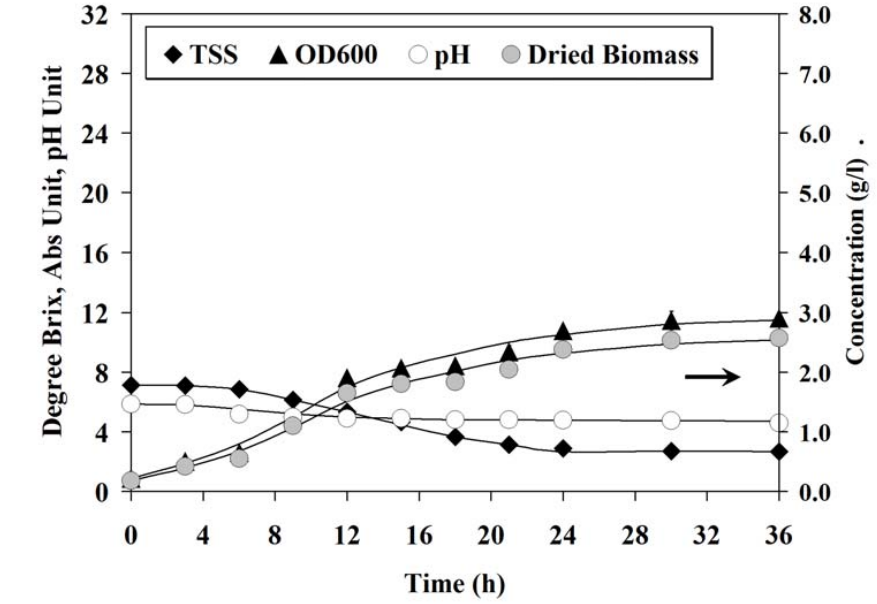
The average pH level (Table 3.48) in all four cultivation conditions did not differ significantly ($p > 0.05$). This trend of insignificant differences ($p > 0.05$) were also observed with average TSS decreasing rate (0.15-0.20 ^{o}Brix h^{-1}), average OD600 elevation rate (0.34-0.40 ODU h^{-1}), as well as average specific growth rate (0.08-0.09 h^{-1}). Because of the insignificant difference from the average specific growth rates, the average doubling times of *S. cerevisiae* TISTR 5606 cultivated in the media containing these triple sugar concentrations were also found to be similar to one another (7.40-8.20 h).

The maximum decreasing rate of TSS in Table 3.49 for the cultivation using concentration level at 30/30/30 was the highest (0.29 ± 0.03^{o}Brix h^{-1}) but was not found to be significantly different ($p > 0.05$) from three other concentrations. However, the TSS decreasing rate of 0.23 ± 0.01^{o}

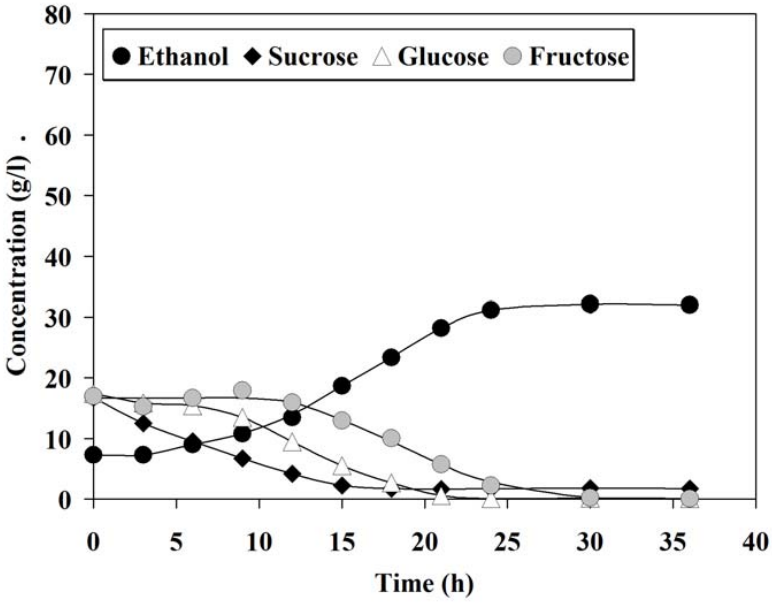
Brix h^{-1} for 60/60/60 was the lowest. This was similar to the increasing rate of OD600 at which the significant difference was not observed ($p > 0.05$). The trend of insignificant difference ($p > 0.05$) was also observed for maximum dried biomass increasing rate, maximum specific growth rate, and minimum doubling time.

Fig. 3.29(b) described the cultivation of 20/20/20 sugars consumption profile for *S. cerevisiae* TISTR 5606 with the sucrose consumption level of 15.04 ± 0.46 g l^{-1}, glucose consumption level of 17.30 ± 0.40 g l^{-1}, and fructose consumption level of 16.89 ± 0.45 g l^{-1}. The depletion of sugars occurred simultaneously during 24-26 h. Further comparison could be made to other triple sugars cultivation profiles in Fig. 3.30(b), 3.31(b) and 3.32(b) for 30/30/30, 40/40/40, and 60/60/60, respectively. The sucrose decreasing level in all four conditions differed statistically ($p \leq 0.05$) from one another. In all four cases, sugars were consumed completely after 36 h.

The ethanol production kinetics exhibited the highest level of ethanol concentration at 49.79 ± 0.49 g l^{-1} (Table 3.12) for 40/40/40. The lower levels of produced ethanol concentration were 24.64 ± 1.04, 31.21 ± 0.68, and 46.11 ± 1.03 g l^{-1} for 20/20/20, 30/30/30 and 60/60/60, respectively.

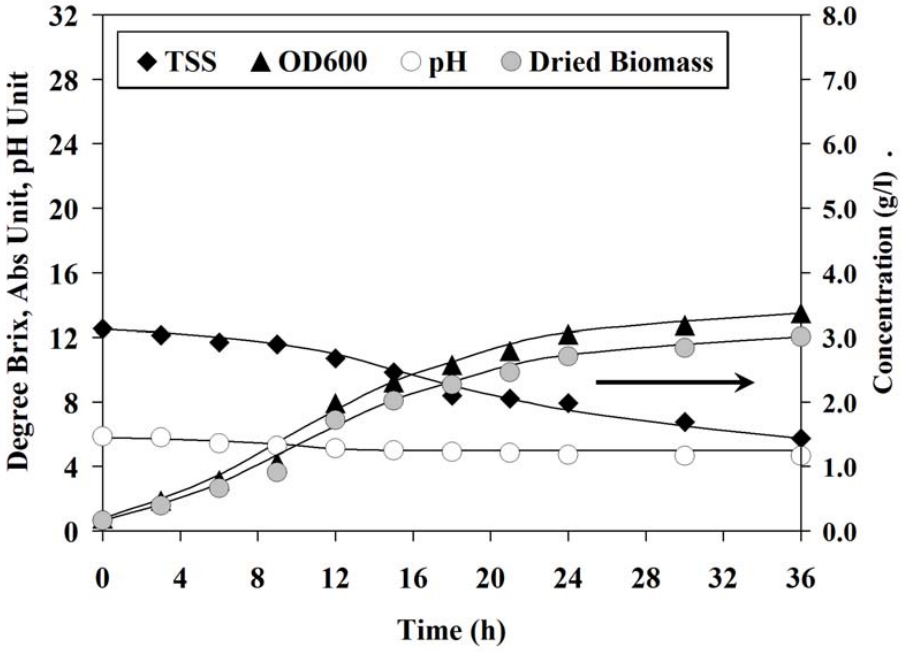


a)

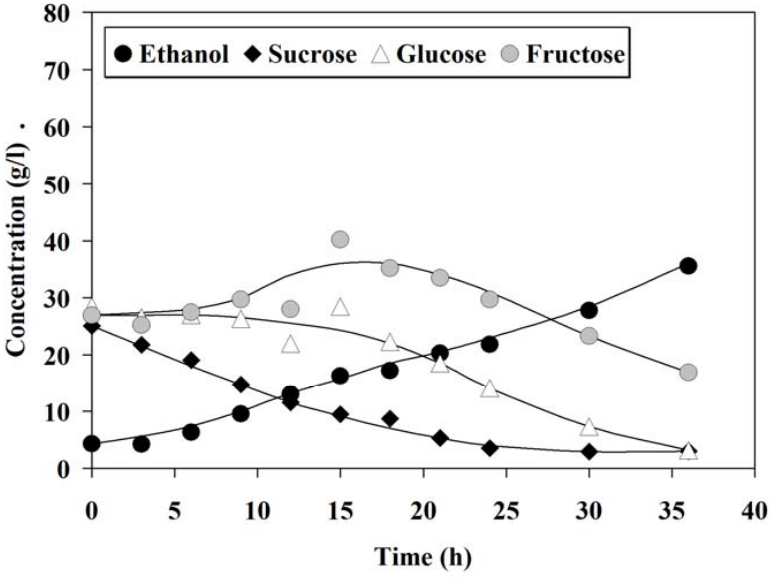


(b)

Figure 3.29: Growth kinetics of *S. cerevisiae* TISTR 5606 using triple sugars concentration ratio of 20/20/20 glucose/fructose/sucrose as carbon sources during 36 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

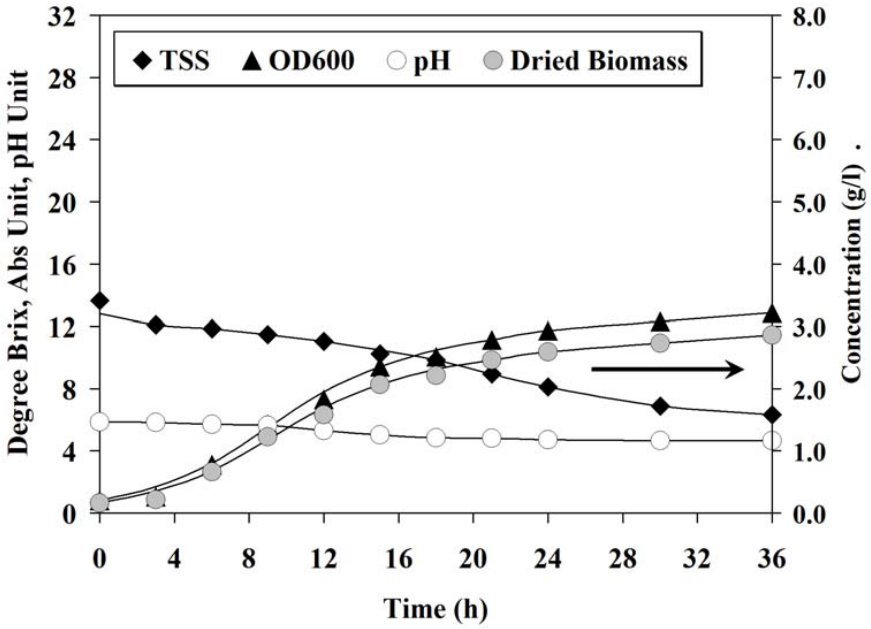


(a)

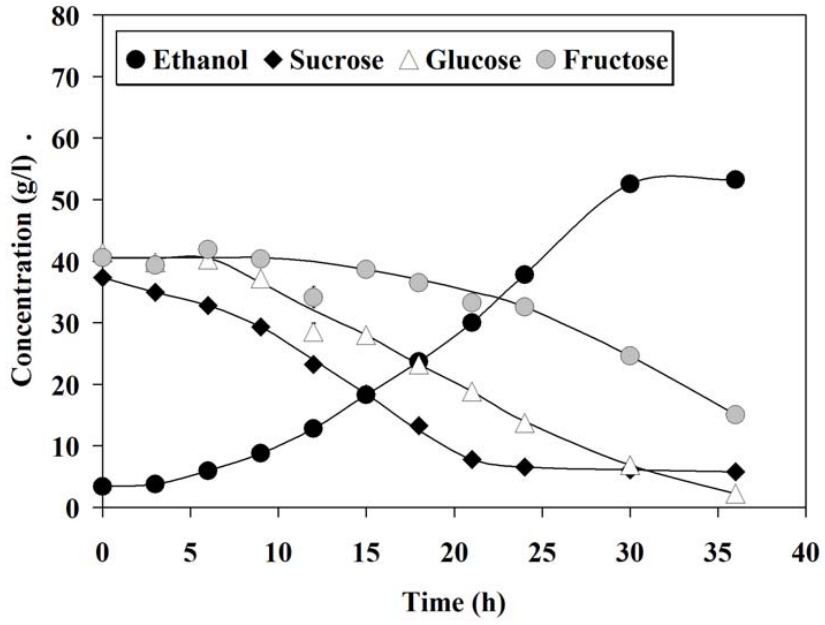


(b)

Figure 3.30: Growth kinetics of *S. cerevisiae* TISTR 5606 using triple sugars concentration ratio of 30/30/30 glucose/fructose/sucrose as carbon sources during 36 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

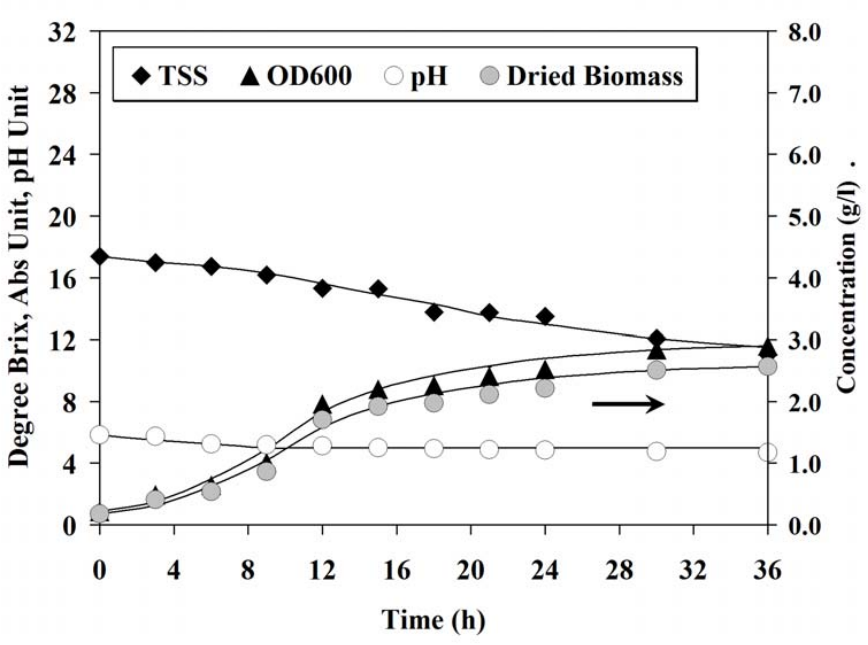


(a)

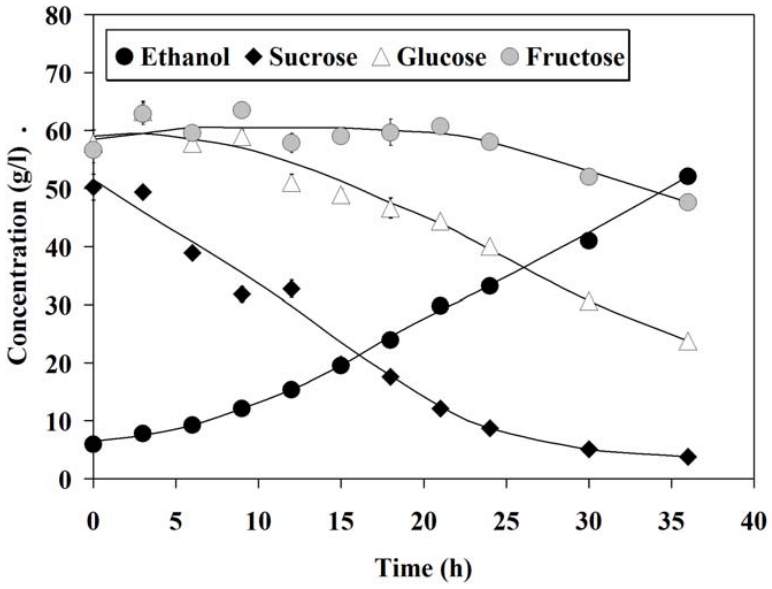


(b)

Figure 3.31: Growth kinetics of *S. cerevisiae* TISTR 5606 using triple sugars concentration ratio of 40/40/40 glucose/fructose/sucrose as carbon sources during 36 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.



(a)



(b)

Figure 3.32: Growth kinetics of *S. cerevisiae* TISTR 5606 using triple sugars concentration ratio of 60/60/60 glucose/fructose/sucrose as carbon sources during 36 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

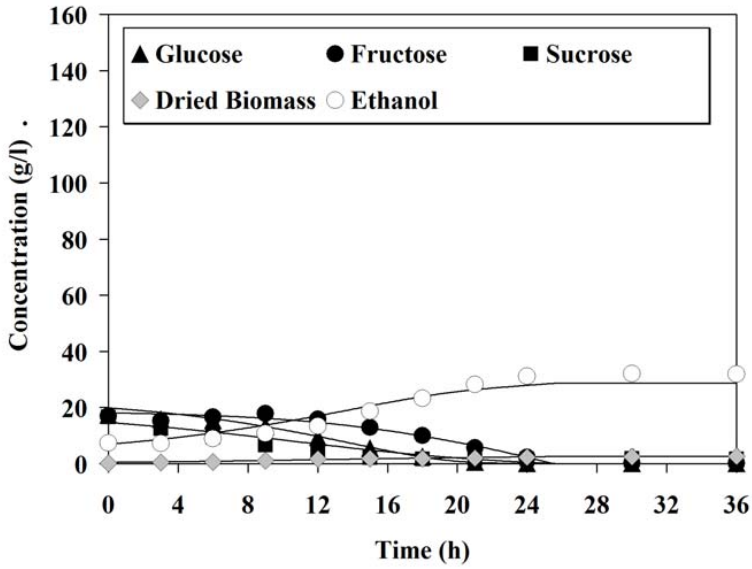


Figure 3.33: Simulated curves and experiment data for *S. cerevisiae* using triple sugars concentration ratio of 20/20/20 glucose/fructose/sucrose in a static condition at 25.6°C. The R^2 and RSS value were 0.9693 and 68.4, respectively.

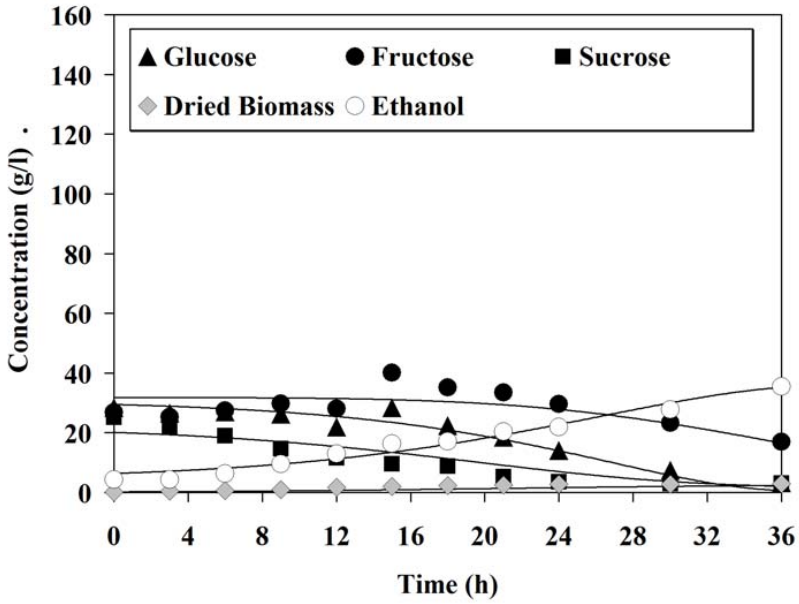


Figure 3.34: Simulated curves and experiment data for *S. cerevisiae* using triple sugars concentration ratio of 30/30/30 glucose/fructose/sucrose in a static condition at 25.6°C. The R^2 and RSS value were 0.9410 and 227, respectively.

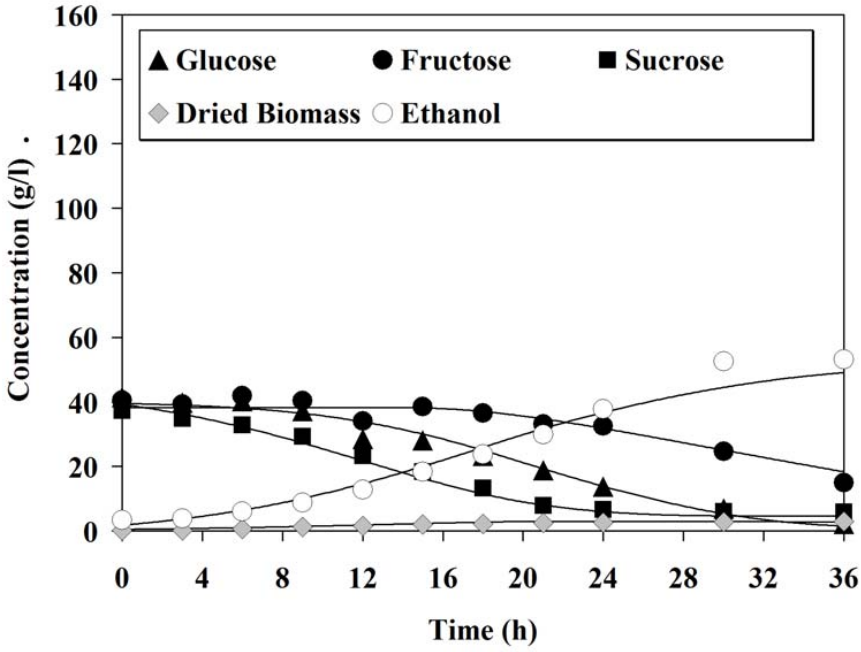


Figure 3.35: Simulated curves and experiment data for *S. cerevisiae* using triple sugars concentration ratio of 40/40/40 glucose/fructose/sucrose in a static condition at 25.6^{o}C. The R^2 and RSS value were 0.9834 and 101, respectively.

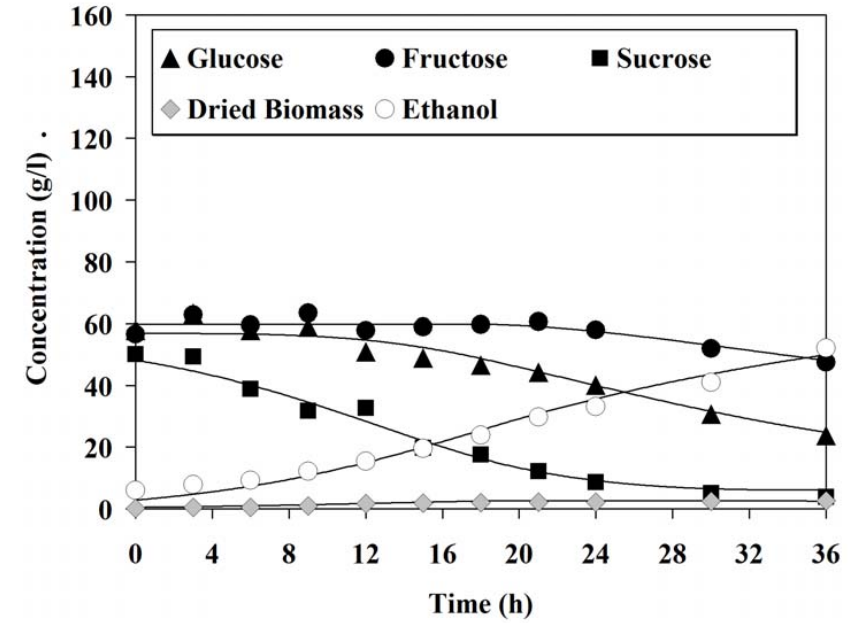


Figure 3.36: Simulated curves and experiment data for *S. cerevisiae* using triple sugars concentration ratio of 60/60/60 glucose/fructose/sucrose in a static condition at 25.6^{o}C. The R^2 and RSS value were 0.9915 and 83.8, respectively.

Table 3.47: The differences in TSS, OD600, and dried biomass (X) concentration (g l^{-1}) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with triple sugars using *S. cerevisiae* TISTR 5606 at 25.6°C for four concentration levels.

Investigated parameters	Conditions							
	202020		303030		404040		606060	
TSS decreasing level	4.46 ± 0.05	I	6.79 ± 0.05	II	7.30 ± 0.10	III	6.30 ± 0.04	IV
OD600 increasing level	10.75 ± 0.41	I	12.70 ± 0.12	II	12.06 ± 0.14	III	10.73 ± 0.26	I
X production level	2.39 ± 0.09	I	2.85 ± 0.02	II	2.70 ± 0.03	III	2.39 ± 0.05	IV

The numbers with the same Roman numeral (I-IV) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.48: The average pH level, average TSS decreasing rate (^{o}Brix h^{-1}), average OD600 increasing rate (ODU h^{-1}), average dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), average specific growth rate (g g^{-1} h^{-1}), and average doubling time (h) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with triple sugars using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for four concentrations.

Investigated parameters	Conditions							
	202020		303030		404040		606060	
pH level	5.86 ± 0.13	I	5.80 ± 0.13	I	5.87 ± 0.15	I	5.84 ± 0.11	I
TSS decreasing rate	-0.15 ± 0.04	I	-0.2 ± 0.03	I	-0.19 ± 0.02	I	-0.17 ± 0.02	I
OD600 increasing rate	0.34 ± 0.06	I	0.40 ± 0.07	I	0.38 ± 0.08	I	0.34 ± 0.08	I
X increasing Rate	0.08 ± 0.01	I	0.09 ± 0.02	I	0.09 ± 0.02	I	0.08 ± 0.02	I
Specific growth rate	0.08 ± 0.03	I	0.09 ± 0.03	I	0.09 ± 0.03	I	0.09 ± 0.03	I
Doubling time	8.20 ± 2.60	I	7.40 ± 2.40	I	7.60 ± 2.40	I	8.10 ± 2.50	I

The numbers with the same Roman numeral (I-I) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.49: The maximum TSS decreasing rate (^{0}Brix h^{-1}), maximum OD600 increasing rate (ODU h^{-1}), maximum dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), maximum specific growth rate (g g^{-1} h^{-1}), and minimum doubling time (h) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation with triple sugars using *S. cerevisiae* TISTR 5606 at 25.6^{0}C for four concentrations.

Investigated parameters	Conditions							
	202020		303030		404040		606060	
TSS decreasing rate	-0.27 ± 0.01	I	-0.29 ± 0.03	I	-0.26 ± 0.02	I	-0.23 ± 0.01	I
OD600 increasing rate	0.53 ± 0.06	I	0.61 ± 0.04	I	0.64 ± 0.07	I	0.61 ± 0.07	I
X increasing rate	0.12 ± 0.01	I	0.14 ± 0.01	I	0.14 ± 0.02	I	0.13 ± 0.02	I
Specific growth rate	0.17 ± 0.03	I	0.19 ± 0.04	I	0.19 ± 0.03	I	0.18 ± 0.02	I
Doubling time	4.00 ± 0.70	I	3.70 ± 0.80	I	3.70 ± 0.50	I	3.90 ± 0.40	I

The numbers with the same Roman numeral (I-I) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.50: The differences in sugars (sucrose, glucose, and fructose) concentration levels (g l^{-1}), ethanol concentration levels (g l^{-1}), lag time (sucrose, glucose, fructose, and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with triple sugars using *S. cerevisiae* TISTR 5606 at 25.6°C for four concentrations.

Investigated parameters	Conditions							
	202020		303030		404040		606060	
Sucrose decreasing level	15.04 ± 0.46	I	22.09 ± 0.37	II	31.53 ± 0.60	III	46.41 ± 2.22	IV
Glucose decreasing level	17.30 ± 0.40	I	25.29 ± 0.38	II	39.24 ± 0.45	III	34.01 ± 2.35	III
Fructose decreasing level	16.89 ± 0.45	I	9.98 ± 0.57	II	25.56 ± 0.44	III	9.04 ± 2.38	II
Ethanol producing level	24.64 ± 1.04	I	31.21 ± 0.68	II	49.79 ± 0.49	III	46.11 ± 1.03	IV
Sucrose lag time	0.00 ± 0.00	I	0.00 ± 0.00	II	0.00 ± 0.00	III	0.00 ± 0.00	I V
Glucose lag time	0.00 ± 0.00	I	3.00 ± 0.30	II	6.00 ± 0.60	III	0.00 ± 0.00	IV
Fructose lag time	0.00 ± 0.00	I	0.00 ± 0.00	II	9.00 ± 0.90	III	0.00 ± 0.00	IV
Ethanol lag time	0.00 ± 0.00	I	0.00 ± 0.00	I	0.00 ± 0.00	I	0.00 ± 0.00	I
$Y_{P/S}$	0.50 ± 0.02	I	0.54 ± 0.01	I	0.52 ± 0.01	I	0.52 ± 0.03	I

The numbers with the same Roman numeral (I-IV) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.51: The average sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), average ethanol production rate (g l^{-1} h^{-1}), average specific rate of sugars consumption (Avg Q_s, g g^{-1} h^{-1}), and average specific rate of ethanol production (Avg Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with triple sugars using *S. cerevisiae* TISTR 5606 at 25.6°C for four concentrations.

Investigated parameters	Conditions							
	202020		303030		404040		606060	
Sucrose consumption rate	-0.50 ± 0.17	I	-0.72 ± 0.13	I	-1.04 ± 0.23	I,II	-1.51 ± 0.20	II
Glucose consumption rate	-0.5 ± 0.16	I	-0.61 ± 0.17	I	-1.08 ± 0.20	I	-0.91 ± 0.17	I
Fructose consumption rate	-0.51 ± 0.17	I	-0.10 ± 0.28	I	-0.56 ± 0.18	I	-0.19 ± 0.14	I
Ethanol production rate	0.81 ± 0.20	I	0.84 ± 0.08	I	1.40 ± 0.29	I	1.21 ± 0.14	I
Avg Q_s of sucrose	-0.90 ± 0.48	I	-0.93 ± 0.41	I	-0.97 ± 0.29	I	-1.85 ± 0.70	I
Avg Q_s of glucose	-0.55 ± 0.18	I	-0.26 ± 0.06	I	-0.59 ± 0.14	I	-0.47 ± 0.13	I
Avg Q_s of fructose	-0.26 ± 0.09	I	-0.13 ± 0.16	I	-0.23 ± 0.06	I	-0.10 ± 0.17	I
Avg Q_p of ethanol	0.56 ± 0.13	I	0.64 ± 0.12	I	0.85 ± 0.11	I	0.87 ± 0.09	I

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.52: The maximum sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), maximum ethanol production rate (g l^{-1} h^{-1}), maximum specific rate of sugars consumption (Max Q_s, g g^{-1} h^{-1}), and maximum specific rate of ethanol production (Max Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation with triple sugars using *S. cerevisiae* TISTR 5606 at 25.6°C for four concentrations.

Investigated parameters	Conditions							
	202020		303030		404040		606060	
Sucrose consumption rate	-1.05 ± 0.13	I	-1.13 ± 0.03	I	-1.79 ± 0.09	II	-1.93 ± 0.06	II
Glucose consumption rate	-1.08 ± 0.16	I	-1.13 ± 0.14	I	-1.54 ± 0.04	II	-1.35 ± 0.08	I,II
Fructose consumption rate	-1.09 ± 0.08	I	-1.00 ± 0.13	I,II	-1.10 ± 0.22	I,II	-0.60 ± 0.17	II
Ethanol production rate	1.45 ± 0.13	I	1.04 ± 0.08	II	2.25 ± 0.18	III	1.57 ± 0.04	I
Max Q_s of sucrose	-2.12 ± 0.96	I	-2.02 ± 0.76	I	-1.74 ± 0.46	I	-3.67 ± 1.34	I
Max Q_s of glucose	-1.08 ± 0.24	I	-0.45 ± 0.04	II	-0.98 ± 0.17	I	-0.65 ± 0.02	I
Max Q_s of fructose	-0.56 ± 0.04	I	-0.37 ± 0.04	II	-0.42 ± 0.07	I,II	-0.25 ± 0.06	II
Max Q_p of ethanol	0.91 ± 0.05	I	1.03 ± 0.15	I	1.10 ± 0.09	I	1.14 ± 0.12	I

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.53: Results of parameter search for mathematical model describing the growth kinetics of *S. cerevisiae* TISTR 5606 in the cultivation media containing triple substrate which included (glucose/fructose/sucrose) 20/20/20, 30/30/30, 40/40/40 and 60/60/60 g l^{-1}.

(a) Initial values for the batch cultivation of TISTR 5606 on media containing triple sugars.

Concentration (g l^{-1})	**202020**	**303030**	**404040**	**606060**
x_0	0.51	0.21	0.50	0.50
S_1	19.90	29.47	39.56	56.87
S_2	18.11	31.78	38.27	59.94
S_3	14.68	20.14	39.30	48.22
p_0	6.91	6.36	1.76	2.82

(b) Optimal kinetic parameters

Glucose only		**Fructose only**		**Sucrose only**	
Weighting factor for sugar consumption					
α	0.4973	β	0.3400	γ	0.1627
Biomass production model					
$\mu_{max,1}$	0.128	$\mu_{max,2}$	0.094	$\mu_{max,3}$	0.156
$K_{sx,1}$	47.22	$K_{sx,2}$	0.0001	$K_{sx,3}$	14.06
$P_{mx,1}$	53.88	$P_{mx,2}$	47.16	$P_{mx,3}$	41.21
$K_{ix,1}$	50.00	$K_{ix,2}$	79.12	$K_{ix,3}$	600
$P_{ix,1}$	4.7	$P_{ix,2}$	27.4	$P_{ix,3}$	30.0
Sugar consumption model					
$q_{smax,1}$	2.262	$q_{smax,2}$	1.808	$q_{smax,3}$	0.480
$K_{ss,1}$	2.75	$K_{ss,2}$	0.0001	$K_{ss,3}$	0.0001
$P_{ms,1}$	79.79	$P_{ms,2}$	250	$P_{ms,3}$	35.00
$K_{is,1}$	600	$K_{is,2}$	600	$K_{is,3}$	600
$P_{is,1}$	30.0	$P_{is,2}$	250	$P_{is,3}$	30.0
Ethanol production model					
$q_{pmax,1}$	1.200	$q_{pmax,2}$	0.408	$q_{pmax,3}$	0.136
$K_{sp,1}$	2.75	$K_{sp,2}$	0.0001	$K_{sp,3}$	0.0001
$P_{mp,1}$	79.79	$P_{mp,2}$	250	$P_{mp,3}$	35.00
$K_{ip,1}$	600	$K_{ip,2}$	600	$K_{ip,3}$	600
$P_{ip,1}$	30.0	$P_{ip,2}$	30.0	$P_{ip,3}$	30.0

The corresponding ethanol yields ($Y_{P/S}$) were 0.50 ± 0.02, 0.54 ± 0.01, 0.52 ± 0.01 and 0.52 ± 0.03 for 20/20/20, 30/30/30, 40/40/40 and 60/60/60, respectively, which were close to the theoretical yield.

Table 3.51 shows the analyses of average substrates consumption rates for all four concentration levels of triple sugars. The average sucrose consumption rate at 1.51 ± 0.20 g l^{-1} for 60/60/60 was significantly higher ($p \leq 0.05$) than 20/20/20 and 30/30/30 g l^{-1}, respectively. However, this value did not differ significantly from 40/40/40 g l^{-1} ($p > 0.05$). The average glucose and fructose consumption rates for all four conditions did not differ significantly ($p > 0.05$). The corresponding ethanol production rates of four concentration levels were similar without obvious significant difference ($p > 0.05$). The highest ethanol production rate of 1.40 ± 0.29 g l^{-1} h^{-1} belonged to 40/40/40 which was followed by 1.21 ± 0.14 g l^{-1} h^{-1}, 0.84 ± 0.07 g l^{-1} h^{-1} and 0.81 ± 0.20 g l^{-1} h^{-1} for 60/60/60, 30/30/30 and 20/20/20, respectively.

The analyses of maximum substrate consumption rates for all four initial triple sugar concentrations are represented in Table 3.52. The maximum sucrose consumption rates of 1.93 ± 0.06 and 1.79 ± 0.09 g l^{-1} h^{-1} for 60/60/60 and 40/40/40 were significantly higher ($p \leq 0.05$) than 20/20/20 and 30/30/30, respectively. The maximum glucose consumption rate of 1.54 ± 0.04 g l^{-1} h^{-1} for 40/40/40 was significantly higher ($p \leq 0.05$) than 20/20/20 and 30/30/30, respectively. However, this value did not differ significantly from 60/60/60 ($p > 0.05$). The maximum fructose consumption rates for 20/20/20, 30/30/30 and 40/40/40 did not differ significantly ($p > 0.05$) from one another with the corresponding range of 1.00 – 1.10 g l^{-1} h^{-1}. The ethanol production rate of 20/20/20 and 60/60/60 did not differ significantly ($p > 0.05$) whereas 30/30/30 and 40/40/40 exhibited a significant difference ($p \leq 0.05$). The highest maximum ethanol production rate of 2.25 ± 0.18 g l^{-1} h^{-1} belonged to 40/40/40 which was followed by 1.57 ± 0.04 g l^{-1} h^{-1}, 1.45 ± 0.13 g l^{-1} h^{-1} and 1.04 ± 0.08 g l^{-1} h^{-1} for 60/60/60, 20/20/20 and 30/30/30 g l^{-1}, respectively.

The predicted cultivation profiles for *S. cerevisiae* TISTR 5606 cultivation using mixed triple sugars are presented with accompanying R^2 and RSS values for fitting assessment in Fig. 3.33 for 20/20/20, Fig. 3.34 for 30/30/30, Fig. 3.35 for 40/40/40, and Fig.3.36 for 60/60/60, respectively. The optimal initial concentrations of substrates, product, and dried biomass, as well as corresponding kinetic parameters in the proposed model are tabulated in the data from Table 3.53(a). The developed mathematical model could predict the experimental data relatively well

with $R^2 > 0.94$ as well as relatively low level of RSS of 68.4 for the cultivation with 20/20/20, 227 for 30/30/30, 101 for 40/40/40 and 83.8 for 60/60/60.

3.3.3 Cultivation in Dried Longan Extract Conditions

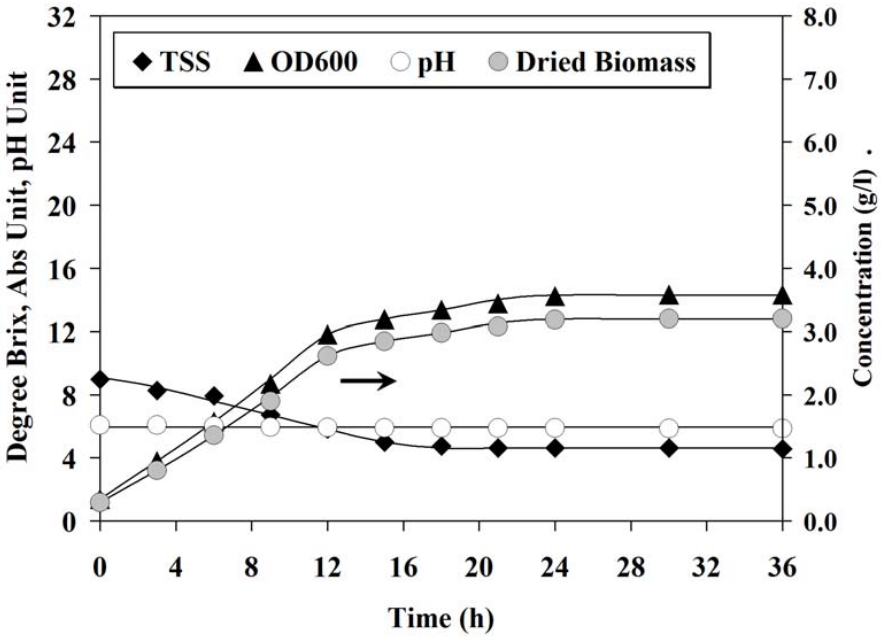
Finally, the third experiment examined the cultivation kinetics of *S. cerevisiae* TISTR 5606 on the media containing a different concentration of dried longan extract (LG) as carbon source at the level of 60, 120 and 180 g l^{-1}. This cultivation was performed similarly to the single pure and triple pure sugar cultivation. The microbial cultivation kinetic profiles of dried longan extract for *S. cerevisiae* TISTR 5606 are illustrated in Fig. 3.37 for LG60, Fig. 3.38 for LG 120 and Fig. 3.39 for LG180. Each figure is divided into two parts, for example; part (a) describes the kinetic profiles of TSS, pH level, OD600, and dried biomass concentration which could be related to OD600 by third order polynomial equations as previously mentioned in previous section; part (b) portrays the kinetic profiles of substrates such as LG60, LG120 and LG180 concentrations, as well as the product or ethanol concentration.

The detailed analyses of each cultivation profile with hypothesis testing across three conditions are tabulated in Table 3.54 – 3.59. The first three tables (Table 3.54 – 3.56) portray the statistical comparison of TSS, pH level, OD600, and dried biomass concentration data extracted from Fig. 3.37(a) – 3.39(a) which include the analyses of differences between the final and initial levels, average, and maximum rates. Similar analyses and comparisons were also carried out for sugars and ethanol concentrations. Table 3.57 –3.59 presents these information in terms of differences, average as well as maximum rates.

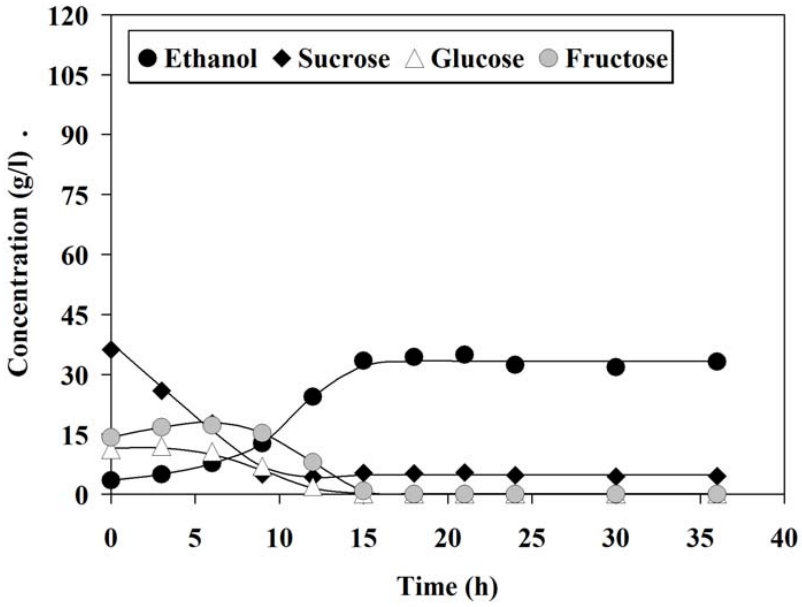
The kinetic profiles describing the microbial growth using three concentrations of dried longan extract had similar trends as shown in Fig. 3.37(a) – 3.39(a). In term of pH level and TSS decreasing, there was negligible change with a slight continuous decreasing trend with cultivation period. The profiles of dried biomass concentration and OD600 for the cultivation using LG60 as carbon sources appeared to increase continuously during the first 12 h before the increasing rate was slowed down until the 36 h period. In case of the cultivation using LG120, the initial increasing trend was increase continuously during the first 12 h from 12 to 24 h before the plateau was reached. During 24 to 30 h, the rapid increasing trend was observed before a steady state was followed until 36 h. In the cultivation condition with LG180 as carbon source, dried biomass concentration and OD600 appeared to increase continuously at a slow rate during the first 20 h which was followed by a faster rate until the 36^{th} h.

From Table 3.54, the decreasing trend of TSS for all three cultivation conditions was significantly difference ($p \leq 0.05$) and the highest increasing trend of 10.20 ± 0.11 was for LG 120. In term of OD600, the highest increasing trend of 23.57 ± 0.37 ODU for the cultivation that utilized LG180 as carbon source was found to be significantly difference ($p \leq 0.05$) from its counterparts that utilized other carbon sources. The production of dried biomass from LG180 was significantly higher ($p \leq 0.05$) than other sugar levels and achieved the production level of 5.49 ± 0.08 g l^{-1}.

The average pH level (Table 3.55) from LG180 was significantly difference ($p \leq 0.05$) from LG60 and LG180. The TSS decreasing rate in all three cultivation conditions did not differ significantly ($p > 0.05$) from one another within the range of 0.15 - 0.32 ^{o}Brix h^{-1}. The average OD600 elevation rate was between 0.43 to 0.72 ODU h^{-1}. The average specific growth rate and the average doubling times of *S. cerevisiae* TISTR 5606 cultivated in dried longan extract were relatively similar ($p > 0.05$) with the corresponding values within the range of 0.07 - 0.08 h^{-1} and 9.00 - 10.10 h, respectively.

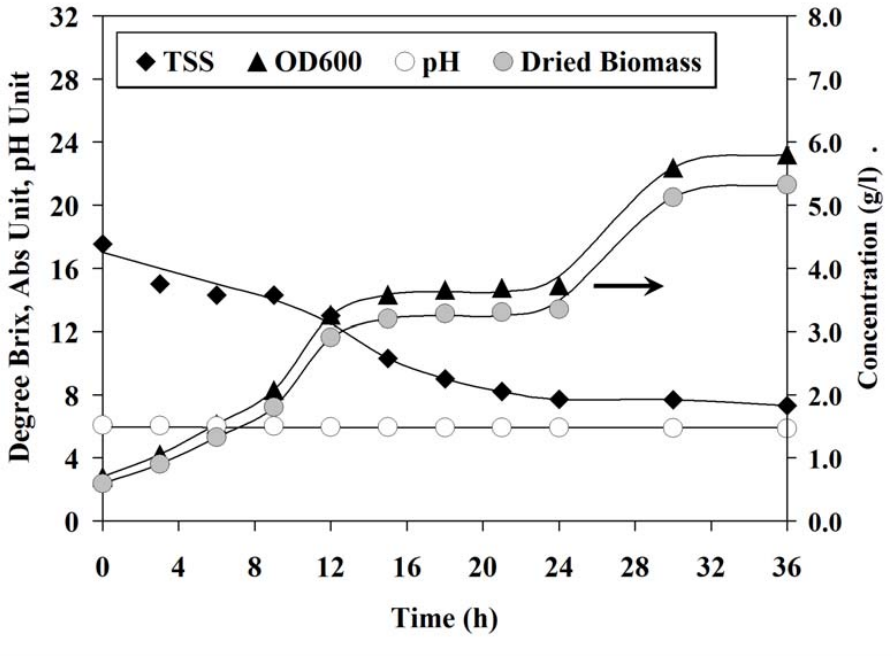


(a)

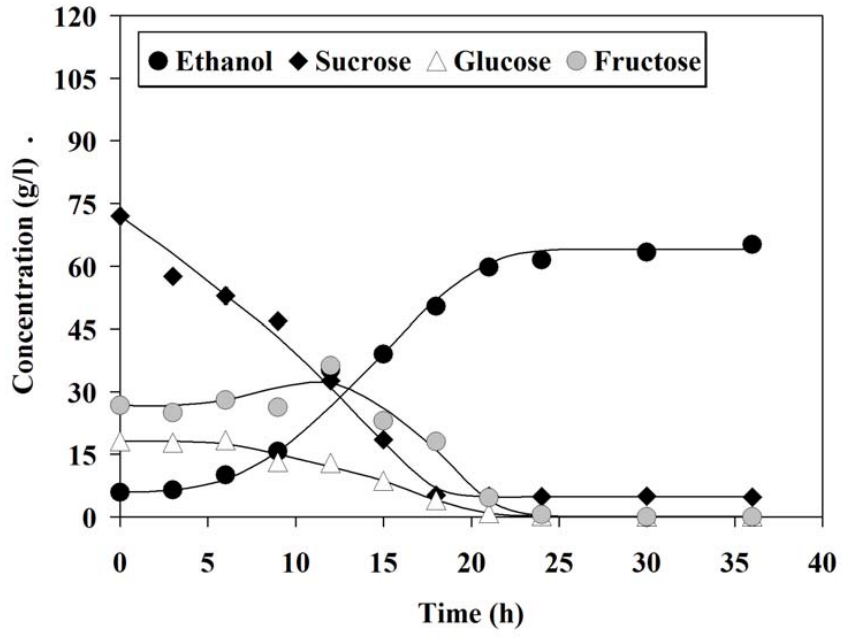


(b)

Figure 3.37: Growth kinetics of *S. cerevisiae* TISTR 5606 using 60 g l^{-1} dried longan extract as carbon sources during 36 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

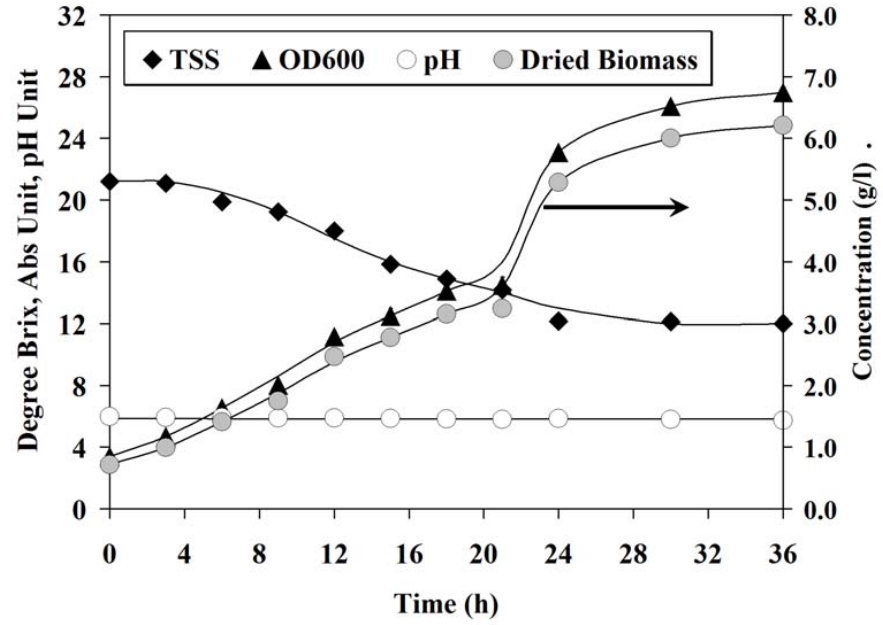


(a)

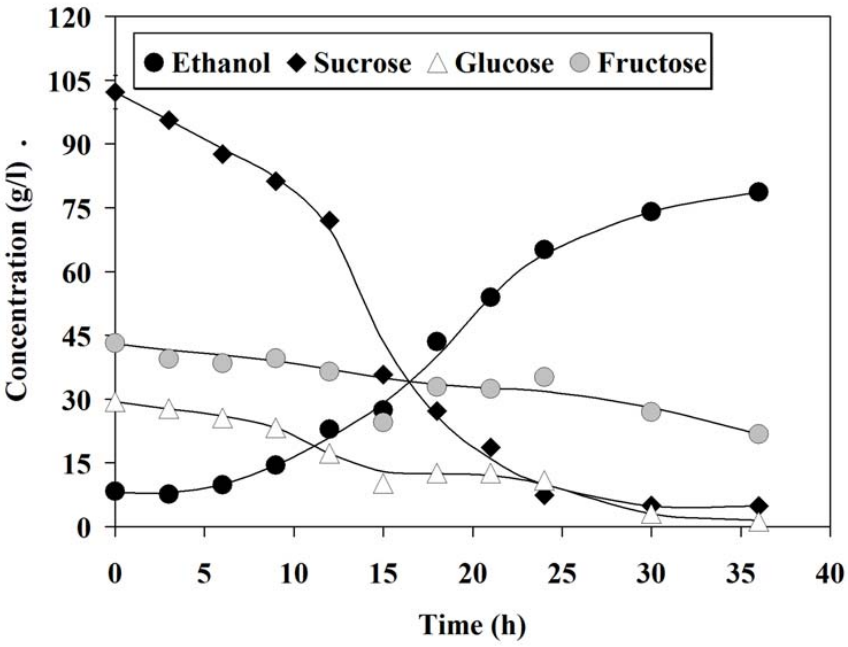


(b)

Figure 3.38: Growth kinetics of *S. cerevisiae* TISTR 5606 using 120 g l^{-1} dried longan extract as carbon sources during 36 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.



(a)



(b)

Figure 3.39: Growth kinetics of *S. cerevisiae* TISTR 5606 using 180 g l^{-1} dried longan extract as carbon sources during 36 h cultivation period in a static condition at 25.6^{o}C; (a) profiles of TSS, OD600, pH level, dried biomass concentration (g l^{-1}); (b) concentration profiles of ethanol, sucrose, glucose, and fructose concentrations.

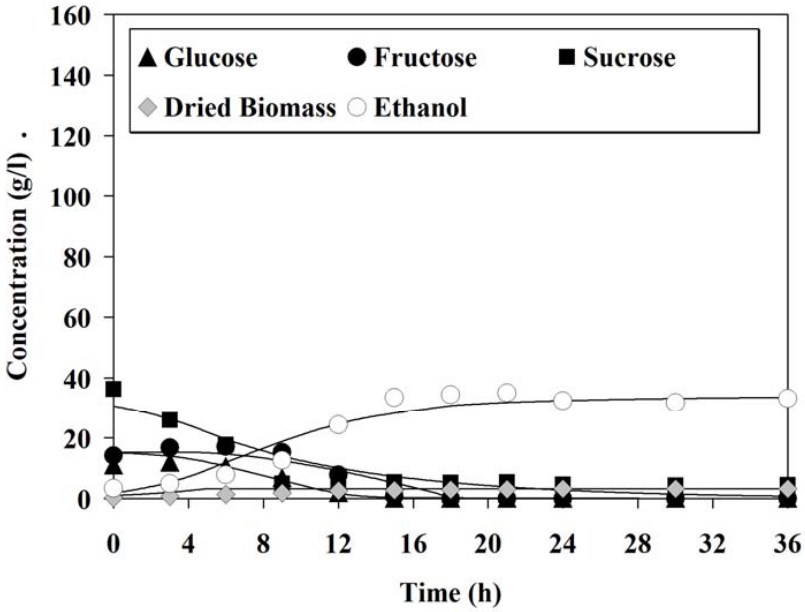


Figure 3.40: Simulated curves and experiment data for *S. cerevisiae* using dried longan extract concentration of 60 g l^{-1} in a static condition at 25.6°C. The R^2 and RSS value were 0.9471 and 183, respectively.

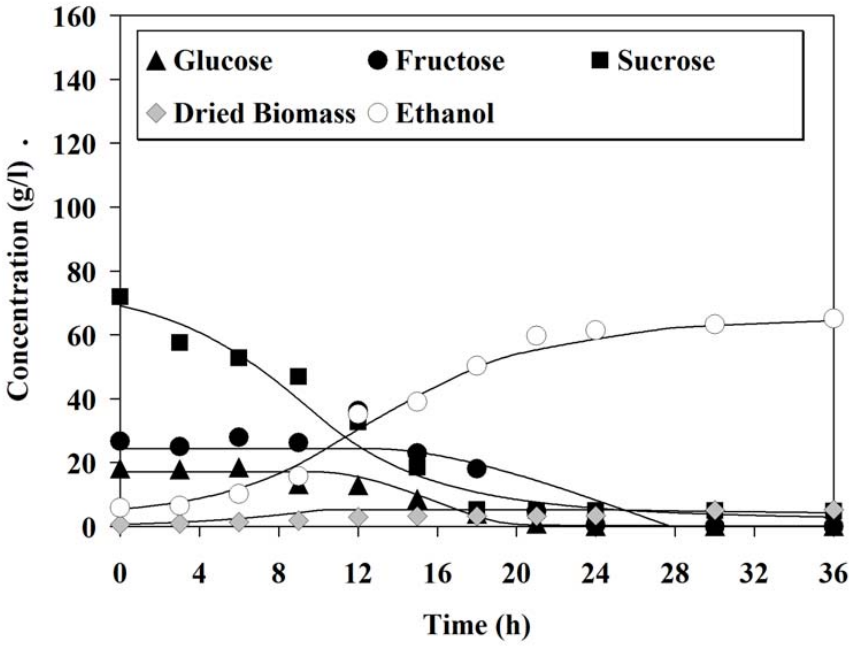


Figure 3.41: Simulated curves and experiment data for *S. cerevisiae* using dried longan extract concentration of 120 g l^{-1} in a static condition at 25.6°C. The R^2 and RSS value were 0.9723 and 320, respectively.

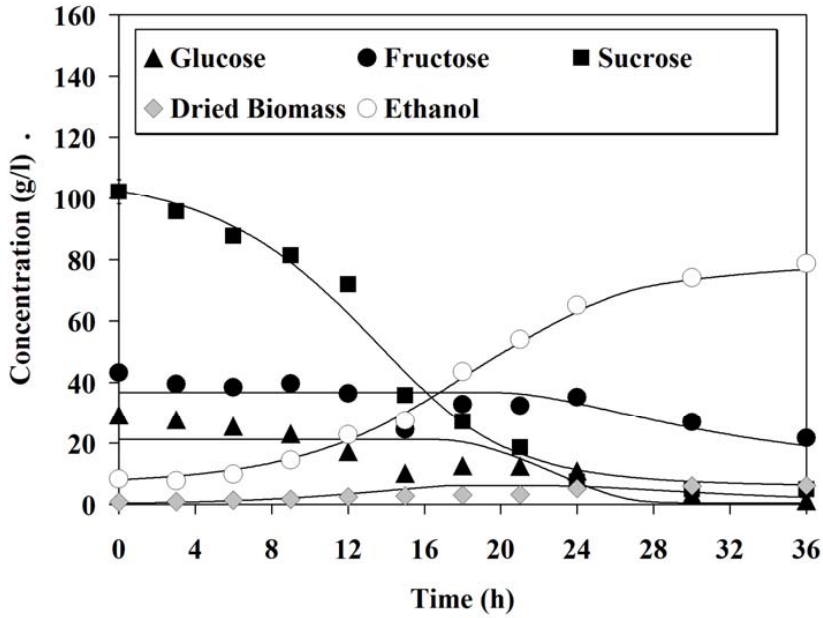


Figure 3.42: Simulated curves and experiment data for *S. cerevisiae* using dried longan extract concentration of 180 g l^{-1} in a static condition at 25.6°C in a static condition at 25.6°C. The R^2 and RSS value were 0.9771 and 359, respectively.

Table 3.54: The differences in TSS, OD600, and dried biomass (X) concentration (g l^{-1}) levels between the final and initial cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three concentrations.

Investigated parameters	Conditions					
	LG60		LG120		LG180	
TSS decreasing level	4.41 ± 0.02	I	10.20 ± 0.11	II	9.20 ± 0.09	III
OD600 increasing level	12.92 ± 0.08	I	20.39 ± 0.46	II	23.57 ± 0.37	III
X production level	2.91 ± 0.02	I	4.73 ± 0.10	II	5.49 ± 0.08	III

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.55: The average pH level, average TSS decreasing rate (^{o}Brix h^{-1}), average OD600 increasing rate (ODU h^{-1}), average dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), average specific growth rate (g g^{-1} h^{-1}), and average doubling time (h) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for three concentrations.

Investigated parameters	Conditions					
	LG60		LG120		LG180	
pH level	5.96 ± 0.03	I	5.96 ± 0.02	I	5.86 ± 0.02	II
TSS decreasing rate	-0.15 ± 0.04	I	-0.32 ± 0.07	I	-0.29 ± 0.06	I
OD600 increasing rate	0.43 ± 0.12	I	0.55 ± 0.16	I	0.72 ± 0.19	I
X increasing rate	0.10 ± 0.03	I	0.13 ± 0.04	I	0.17 ± 0.05	I
Specific growth rate	0.08 ± 0.03	I	0.07 ± 0.02	I	0.07 ± 0.01	I
Doubling time	9.00 ± 3.80	I	10.60 ± 3.10	I	10.10 ± 1.90	I

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.56: The maximum TSS decreasing rate (^{o}Brix h^{-1}), maximum OD600 increasing rate (ODU h^{-1}), maximum dried biomass (X) concentration increasing rate (g l^{-1} h^{-1}), maximum specific growth rate (g g^{-1} h^{-1}), and minimum doubling time (h) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation with dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6^{o}C for three concentrations.

Investigated parameters	Conditions					
	LG60		LG120		LG180	
TSS decreasing rate	-0.29 ± 0.00	I	-0.50 ± 0.08	II	-0.47 ± 0.05	II
OD600 increasing rate	0.87 ± 0.03	I	1.02 ± 0.22	I	1.10 ± 0.42	I
X increasing rate	0.19 ± 0.01	I	0.23 ± 0.05	I	0.26 ± 0.10	I
Specific growth rate	0.18 ± 0.05	I	0.13 ± 0.01	I	0.11 ± 0.01	I
Doubling time	4.00 ± 1.10	I	5.30 ± 0.40	I	6.20 ± 0.40	I

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.57: The differences in sugars (sucrose, glucose, and fructose) concentration levels (g l^{-1}), ethanol concentration levels (g l^{-1}), lag time (sucrose, glucose, fructose, and ethanol) (h) between the final and initial cultivation periods, as well as ethanol yield ($Y_{P/S}$; g ethanol produced over g of all three sugars consumed). The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three concentrations.

Investigated parameters	Conditions					
	LG60		LG120		LG180	
Sucrose decreasing level	31.77 ± 0.44	I	67.17 ± 1.91	II	97.25 ± 3.96	III
Glucose decreasing level	11.06 ± 0.06	I	18.05 ± 0.31	II	28.12 ± 1.01	III
Fructose decreasing level	14.21 ± 0.07	I	26.70 ± 0.72	II	21.37 ± 2.08	III
Ethanol producing level	29.79 ± 0.39	I	59.22 ± 0.42	II	70.31 ± 0.68	III
Sucrose lag time	0.00 ± 0.00	I	0.00 ± 0.00	II	0.00 ± 0.00	III
Glucose lag time	3.00 ± 0.30	I	0.00 ± 0.00	II	0.00 ± 0.00	III
Fructose lag time	0.00 ± 0.00	I	0.00 ± 0.00	II	0.00 ± 0.00	III
Ethanol lag time	0.00 ± 0.00	I	0.00 ± 0.00	I	3.00 ± 0.30	I
$Y_{P/S}$	0.52 ± 0.01	I	0.53 ± 0.01	I	0.48 ± 0.02	II

The numbers with the same Roman numeral (I-III) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.58: The average sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), average ethanol production rate (g l^{-1} h^{-1}), average specific rate of sugars consumption (Avg Q_s, g g^{-1} h^{-1}), and average specific rate of ethanol production (Avg Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as average ± standard error (S.E.) for 1,500 ml static cultivation with dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three concentrations.

Investigated parameters	Conditions					
	LG60		LG120		LG180	
Sucrose consumption rate	-1.10 ± 0.52	I	-2.24 ± 0.58	I	-3.16 ± 0.81	I
Glucose consumption rate	-0.38 ± 0.18	I	-0.60 ± 0.18	I	-0.79 ± 0.19	I
Fructose consumption rate	-0.47 ± 0.35	I	-0.88 ± 0.54	I	-0.54 ± 0.07	I
Ethanol production rate	1.00 ± 0.42	I	1.93 ± 0.54	I	2.11 ± 0.47	I
Avg Q_s of sucrose	-1.23 ± 0.72	I	-1.36 ± 0.44	I	-1.47 ± 0.35	I
Avg Q_s of glucose	-0.21 ± 0.10	I	-0.24 ± 0.07	I	-0.37 ± 0.10	I
Avg Q_s of fructose	-0.06 ± 0.23	I	-0.21 ± 0.19	I	-0.23 ± 0.05	I
Avg Q_p of ethanol	0.56 ± 0.19	I	0.79 ± 0.19	I	0.73 ± 0.15	I

The numbers with the same Roman numeral (I-I) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.59: The maximum sugars (sucrose, glucose, and fructose) consumption rate (g l^{-1} h^{-1}), maximum ethanol production rate (g l^{-1} h^{-1}), maximum specific rate of sugars consumption (Max Q_s, g g^{-1} h^{-1}), and maximum specific rate of ethanol production (Max Q_p, g g^{-1} h^{-1}) during 36 h cultivation periods. The values are expressed as the average of five consecutive maximum values ± standard error (S.E.) for 1,500 ml static cultivation with dried longan extract using *S. cerevisiae* TISTR 5606 at 25.6°C for three concentrations.

Investigated parameters	Conditions					
	LG60		LG120		LG180	
Sucrose consumption rate	-2.81 ± 0.66	I	-3.83 ± 0.22	I	-5.50 ± 1.23	I
Glucose consumption rate	-0.96 ± 0.27	I	-1.17 ± 0.12	I	-1.38 ± 0.23	I
Fructose consumption rate	-1.50 ± 0.54	I,II	-2.64 ± 0.63	I	-0.75 ± 0.10	II
Ethanol production rate	2.25 ± 0.64	I	3.73 ± 0.34	I	3.58 ± 0.41	I
Max Q_s of sucrose	-3.09 ± 1.39	I	-2.71 ± 0.51	I	-2.46 ± 0.36	I
Max Q_s of glucose	-0.53 ± 0.14	I,II	-0.45 ± 0.03	I	-0.67 ± 0.09	II
Max Q_s of fructose	-0.64 ± 0.21	I	-0.82 ± 0.19	I	-0.37 ± 0.07	I
Max Q_p of ethanol	1.14 ± 0.19	I,II	1.42 ± 0.04	I	1.17 ± 0.09	II

The numbers with the same Roman numeral (I-II) indicated no significant difference ($p > 0.05$) for comparison between each column of the same row.

Table 3.60: Results of parameter search for mathematical model describing the growth kinetics of *S. cerevisiae* TISTR 5606 in the cultivation media containing dried longan extract which included 60, 120, and 180 g l^{-1}.

(a) Initial values for the batch cultivation of TISTR 5606 on media containing dried longan extract

Concentration (g l^{-1})	**LG60**	**LG120**	**LG180**
x_0	1.00	0.67	0.36
S_{01}	15.17	17.11	21.23
S_{02}	15.35	24.36	36.71
S_{03}	30.60	69.13	102.3
p_0	1.86	5.39	8.07

(b) Optimal kinetic parameters

Glucose		**Fructose**		**Sucrose**	
Weighting factor for sugar consumption					
α	0.5040	β	0.3400	γ	0.1560
Biomass production model					
$\mu_{max,1}$	0.192	$\mu_{max,2}$	0.087	$\mu_{max,3}$	0.155
$K_{sx,1}$	0.0001	$K_{sx,2}$	0.01	$K_{sx,3}$	7.72
$P_{mx,1}$	62.45	$P_{mx,2}$	50.36	$P_{mx,3}$	41.80
$K_{ix,1}$	65.82	$K_{ix,2}$	93.67	$K_{ix,3}$	507
$P_{ix,1}$	30.0	$P_{ix,2}$	25.6	$P_{ix,3}$	29.2
Sugar consumption model					
$q_{smax,1}$	2.10	$q_{smax,2}$	1.93	$q_{smax,3}$	0.48
$K_{ss,1}$	1.46	$K_{ss,2}$	0.0001	$K_{ss,3}$	6.77
$P_{ms,1}$	250	$P_{ms,2}$	250	$P_{ms,3}$	39.9
$K_{is,1}$	600	$K_{is,2}$	600	$K_{is,3}$	272
$P_{is,1}$	30.0	$P_{is,2}$	30.0	$P_{is,3}$	30.0
Ethanol production model					
$q_{pmax,1}$	1.20	$q_{pmax,2}$	0.41	$q_{pmax,3}$	0.14
$K_{sp,1}$	1.46	$K_{sp,2}$	0.0001	$K_{sp,3}$	6.77
$P_{mp,1}$	250	$P_{mp,2}$	250	$P_{mp,3}$	39.9
$K_{ip,1}$	600	$K_{ip,2}$	600	$K_{ip,3}$	272
$P_{ip,1}$	30	$P_{ip,2}$	30	$P_{ip,3}$	30

The maximum decreasing rate of TSS is shown in Table 18 with the highest value of 0.50 ± 0.08 ^{o}Brix h^{-1} for LG120 which was not found to be significantly different ($p > 0.05$) from LG180. However, the TSS decreasing rate of 0.29 ± 0.00 ^{o}Brix h^{-1} for LG60 was the lowest and significantly different ($p \leq 0.05$) from the other. This was in contrary to the increasing rate of OD600 (0.87 – 1.10 ODU h^{-1}) in which the significant difference was not observed ($p > 0.05$) in all conditions. In fact, the same trend of insignificant difference ($p > 0.05$) was also spotted for maximum dried biomass increasing rate (0.19 – 0.29 g l^{-1} h^{-1}), maximum specific growth rate (0.11 – 0.18 h^{-1}), and minimum doubling time (4.0 – 6.2 h).

Fig. 3.37(b) described the sugars consumption profile of *S. cerevisiae* TISTR 5606 cultivated in LG60 with sucrose consumption level of 31.77 ± 0.44 g l^{-1}, glucose consumption level of 11.06 ± 0.06 g l^{-1}, and fructose consumption level of 14.21 ± 0.07 g l^{-1}. Both glucose and fructose were consumed completely during 16-18 h. This was in contrast to sucrose concentration in which less than 5 g l^{-1} was still remained after 36 h. The comparison could be made to the other cultivation kinetics in Fig. 3.38(b) and 3.39(b). The pattern of cultivation kinetics in Fig. 3.38(b) was similar to Fig. 3.37(b) with the extended period of glucose and fructose depletion time between 20 - 24 h. The sugar consumption profiles in Fig. 3.39(b) were slightly lower than the other with remnant sugars concentration after 36 h cultivation period. The highest production level of ethanol of 70.31 ± 0.68 g l^{-1} for LG180 was observed. The lower levels of ethanol production at 59.22 ± 0.42 and 29.79 ± 0.39 g l^{-1} were obtained for LG120 and LG60. The corresponding ethanol yields of 0.52 ± 0.01, 0.53 ± 0.01, and 0.48 ± 0.02 g ethanol produced g^{-1} sugars consumed were observed for LG60, LG120, and LG180, respectively. These yields were very similar to the theoretical yield.

Table 3.58 depicts the analyses of average substrates consumption rates for all three levels of dried longan extract. The average sugars consumption rates and ethanol production rates were not found to be significantly different ($p > 0.05$). The highest ethanol production rate of 2.11 ± 0.47 g l^{-1} h^{-1} belonged to LG180 which was followed by LG120 (1.93 ± 0.54 g l^{-1} h^{-1}) and LG60 (1.00 ± 0.42 g l^{-1} h^{-1}), respectively.

The analyses of maximum substrates consumption rates are clarified in Table 3.59. The maximum sucrose and glucose consumption rates were not significantly different ($p > 0.05$) from one another. The maximum fructose consumption rate of 2.64 ± 0.63 g l^{-1} h^{-1} for LG120 was the highest which was followed by LG60 (1.50 ± 0.54 g l^{-1} h^{-1}) and LG180 (0.75 ±

0.09 g l^{-1} h^{-1}). The highest ethanol production rate of 3.73 ± 0.34 g l^{-1} h^{-1} belonged to LG120 which was followed by LG180 (3.58 ± 0.41 g l^{-1} h^{-1}) and LG60 (2.25 ± 0.64 g l^{-1} h^{-1}), respectively.

The predicted cultivation profiles for *S. cerevisiae* TISTR 5606 cultivation using dried longan extract are presented with accompanying R^2 and RSS values for fitting assessment in Fig. 3.40 for LG60, Fig. 3.41 for LG120, and Fig. 3.42 for LG180. The optimal initial concentrations of substrates, product, and dried biomass, as well as corresponding kinetic parameters in the proposed model are tabulated in Table 3.44(a) and (b), respectively. The developed mathematical model could predict the experimental data relatively well with $R^2 > 0.94$ with RSS at 183 for the cultivation with LG60, 320 for LG120, and 359 for LG180.

3.4 Cloning and Expression of *Saccharomyces cerevisiae* Pyruvate Decarboxylase in *Pichia pastoris*

3.4.1 Genomic DNA extraction

Genomic DNA was successfully extracted from *Saccharomyces cerevisiae* TISTR 5606. The concentration of genomic DNA was 1250 ng μl^{-1} (estimated by Quantity One program).

3.4.2 Test of amplification of *pdc*1 gene

*pdc*1 gene was successfully amplified from genomic DNA of *S. cerevisiae* TISTR 5606 using specific primers (Figure 3.43a). The concentration of *pdc*1 gene was 15.14 ng μl^{-1} and the size of amplicon was approximate 1700 bp (estimated by Quantity One program) corresponded to the size of *pdc*1 (1692 bp) from NCBI database (Accession Number: NM_001181931).

3.4.3 Amplification of *pdc*1 gene using hi-fidelity DNA polymerase

*pdc*1 gene was successfully amplified by Hi-Fidelity PCR using hanging primer (Figure 3.43b) and restriction analysis of *pdc*1 with restriction enzyme *Eco* RI resulted in 2 fragments with approximate 800 and 900 bp as expected (Figure 3.43c).

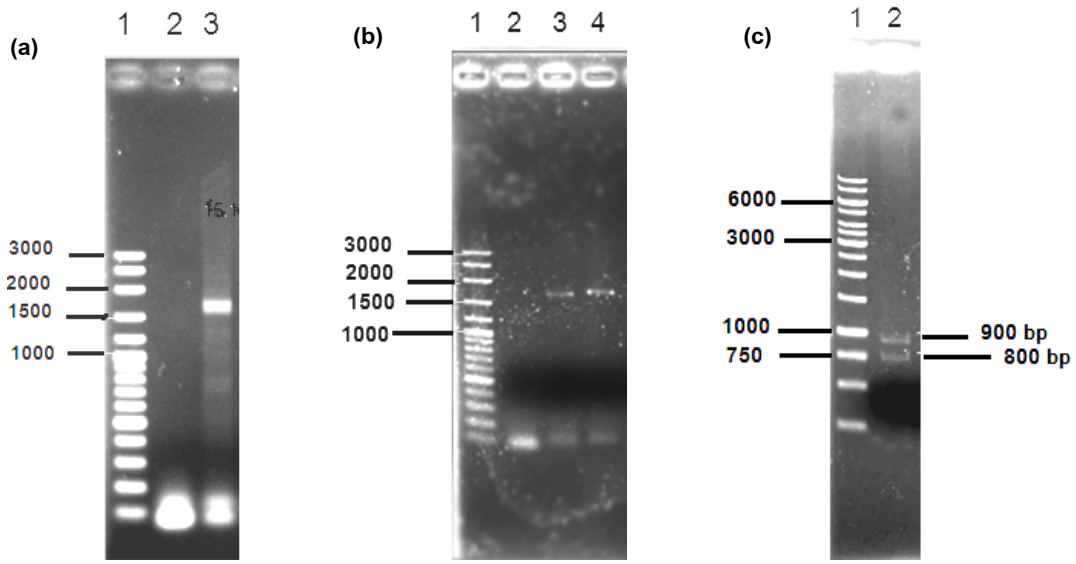


Figure 3.43 **(a)** PCR product of *pdc*1 gene amplified from yeast's genomic DNA (lane 1; 100bp ladder plus, lane 2; negative control, lane 3; PCR product from *S. cerecisiae* 5606 genomic DNA), **(b)** PCR product of PDC1 gene amplified with Phusion R DNA polymerase (lane 1; 100bp ladder plus, lane 2; negative control (dH_20), lane 3; PCR product amplified with specific primers, lane 4; PCR product amplified with hanging primers (add *Xho* I and *Not* I site), **(c)** The restriction analysis of *pdc*1 gene (lane 1; 1kb DNA ladder, lane 2; PDC1 gene cut with *Eco* RI).

3.4.4 Transformation of pPICZA-PDC1 into *E. coli*

28 colonies of transformants were found on LB low salt agar containing zeocin. All clones were analyzed for the present of recombinant vector using size screening technique. Only 1 colony (lane 4 Top, Figure 3.44a) was found to have plasmid with larger size than pPICZA. This clone was further tested by colony PCR technique. It was found that the size of PCR product (lane 4) was similar to positive control (Figure 3.44b). Sequencing technique reveal that this gene is similar to the sequence from PDC1 (Genbank Accession number NM_001181931) with approximate 99.9%. Our *pdc*1 gene differed from the sequence from Genbank at base position 1157 and 1521 (Figure 3.45) resulted in amino acid at the position 383 differed from the amino acid sequence from Genbank (Figure 3.46).

3.4.5 Transformation of pPICZA-PDC1 into *P. pastoris*

It was found that 54, 28 and 3 colonies were growth on YPDS containing zeocin 100, 200 and 500 μg ml^{-1}, respectively. 3 clones from YPDS containing zeocin 500 μg ml^{-1} were selected for present of *pdc*1 gene in *Pichia* genome using colony PCR technique. Because those colonies were growth on media containing high concentration of zeocin, so those clones have high copy number (Mansur, 2005). The resulting from colony PCR confirmed that all clones contain *pdc*1 gene (Figure 3.44c).

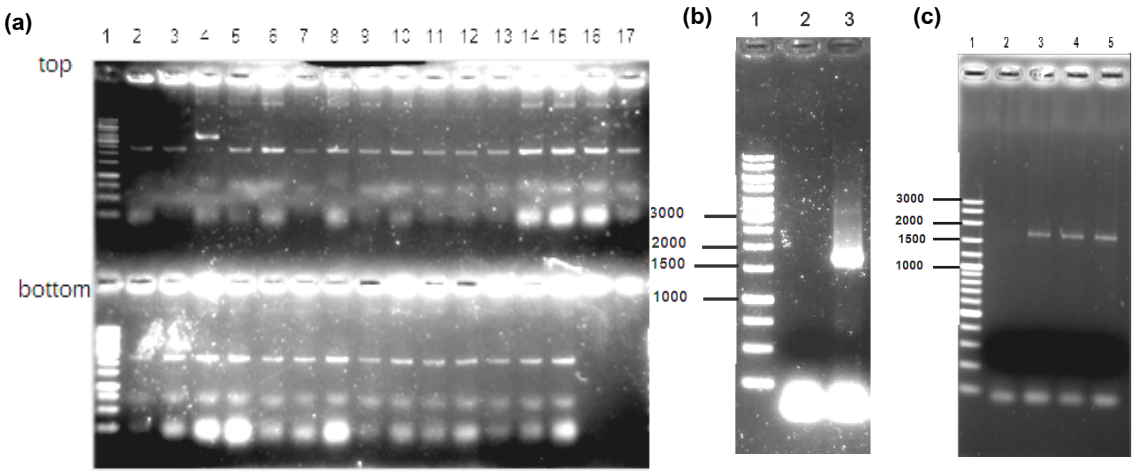


Figure 3.44 **(a)** The size screening analysis (Top; lane 1; 1kb DNA ladder, lane 2; pPICZ A (control), lane 3-17; transformant *E. coli* XL1-blue colony 1-15, bottom; lane 1; 1kb DNA ladder, lane 2; pPICZ A (control), lane 3-15; transformant *E. coli* XL1-blue colony 16-28**. (b)** Amplified PDC1 from transformant *E. coli* XL1-blue (lane 1; 1kb DNA ladder, lane 2; negative control (dH_2O), lane 3; PDC1 amplified from positive transformant)**. (c)** Amplified PDC1 from recombinant *P. pastoris* grew on LB low salt media containing zeocin 500 ug/ml (lane 1; 100bp ladder plus, lane 2; negative control (dH_2O), lane 3-5; PDC1 amplified from positive transformant colony 1-3, respectively).

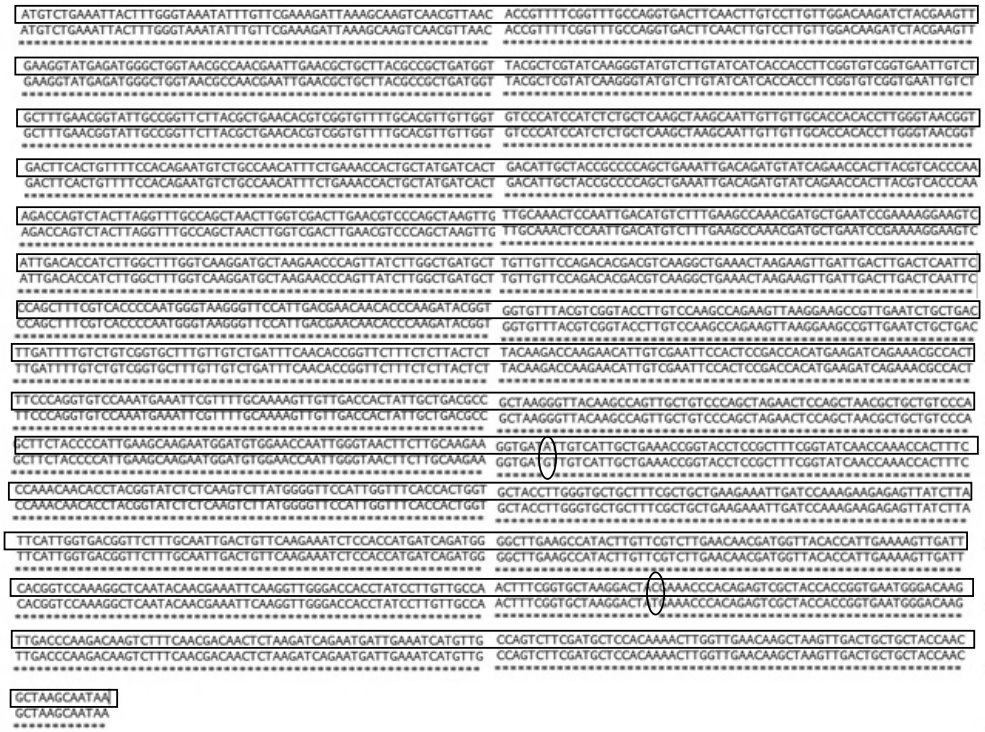


Figure 3.45 the sequence of pdc1 gene from Genbank (Accession number NM_001181931) (□) compare with our pdc1 gene (blank) (Analyzed by ClastalW program; http://clustalw.ddbj.nig.ac.jp/top-e.html).

3.4.6 Analysis of PDC1 enzyme expression

It was found that carboligase activities from recombinant *P. pastoris* clone 1-3 were 0.11, 0.16 and 0.12, respectively. The carboligase activities reported here were slightly lower than carboligase activity from *S. cerevisiae* (0.19 U/ml) and *C. utilis* (0.20 U/ml) (Table 3.61).

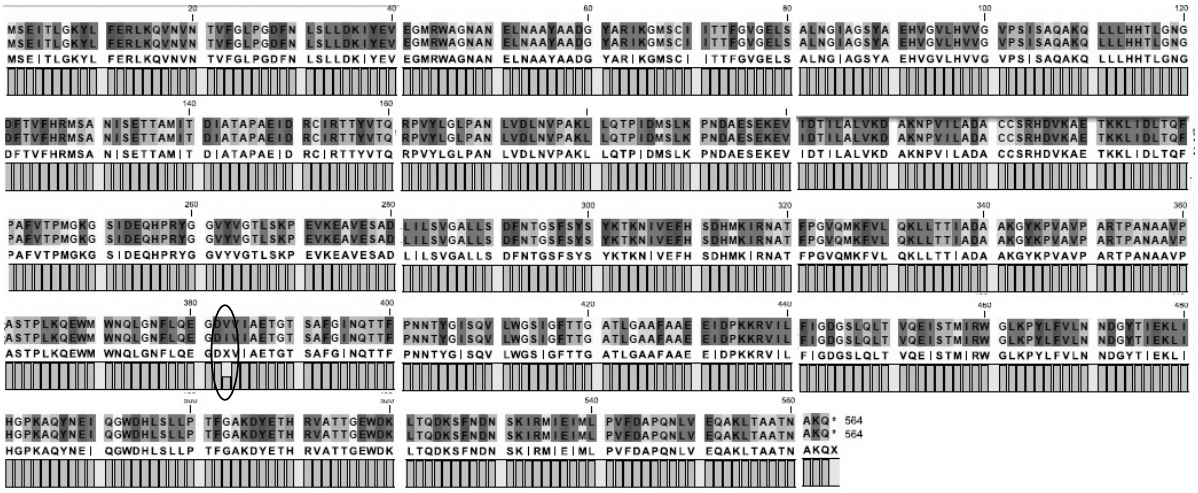


Figure 3.46 the PDC1 protein sequence from Genbank (Accession number: NM_001181931) (up) compare with our pdc1 gene (down) (Analyzed by CLC sequence Viewer 5.1.2 program).

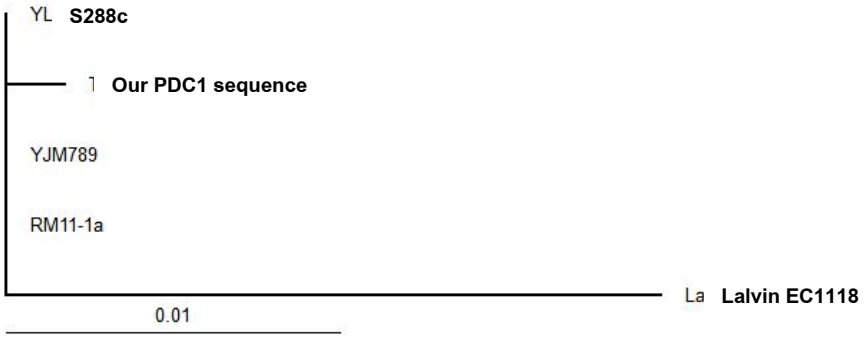


Figure 3.47 Relationship of the PDC1 protein sequences from *S. cerevisiae* strain S288c, our PDC1 sequence, YJM789, RM11-1a and Lavin EC1118 (Analyzed by ClastalW program; http://clustalw.ddbj.nig.ac.jp/top-e.html).

Table 3.61 Comparison of R-PAC production (mM) and PDC carboligase activity (U/ml) from positive transformant *P. pastoris* X-33 pPICZ A-PDC1 with other control and negative control.

Microbes	R-PAC production (mM)	PDC carboligase activity (U/ml)
P. pastoris X-33 pPICZ A-PDC1 clone 1	1.04	0.11
P. pastoris X-33 pPICZ A-PDC1 clone 2	1.46	0.16
P. pastoris X-33 pPICZ A-PDC1 clone 3	1.07	0.12
P. pastoris X-33 empty pPICZ A (negative control)	0	0
S. cerevisiae TISTR5606 (control)	1.73	0.19
C. utillis TISTR5339 (control)	1.82	0.20

The *pdc*1 gene from *S. cerevisiae* TISTR 5606 was successfully amplified and cloned into pPICZA vector. The size of *pdc*1 gene was 1700 bp and has *Eco* RI restriction site in the middle of sequence (Figure 3.45). The sequence of amplified *pdc*1 was verified and found that this gene is similar to the *pdc*1 sequence Genbank (accession number NM_001181931) with approximate 99.9%. Our *pdc*1 gene and PDC1 protein sequence differed from the sequence from Genbank 2 bases (Figure 3.45) and 1 amino acid (Figure 3.46) because the sequence from Genbank database was from another strain *S. cerevisiae* strain S288c. Although *pdc*1 gene from different organisms or strains could be various but the function of PDC1 enzymes were similarly. For instance, the PDC1 protein sequence from Genbank was similar to the sequence from *S. cerevisiae* strain YJM789 and RM11-1a but differ from *S. cerevisiae* strain Lavin EC1118 and our PDC1 sequence (The relationship of the PDC1 sequences show in figure 3.47) (Redzepovic *et al.*, 2003, Omura *et al.*, 2007, Wei *et al.,* 2007, Dimitrov *et al.*, 2009). Apart from *S. cerevisiae*, the PDC1 protein sequence from *Aspergillus nidulan* was similar to maize, *Zea may*, *Aspergillus oryzae* and *S. cerevisiae* (Kelley, 1989, Lockington, 1996). But Differ to *Rhizopus oryzae, Zymomonas mobilis, Arabidopsis and zygosaccharomyces bisporus* (Skory, 2002). The recombinant plasmid pPICZA-PDC1 was transformed into *P. pastoris.* The hyper-resistant clones on YPDS containing 500 mg μl^{-1} of zeocin may imply that *pdc*1 gene is present in multiple copies (Invitrogen Corp., 1998, Mansur, 2005). The carboligase activities from 3 recombinant clones were assessed. The carboligase activities were 0.11, 0.16 and 0.12 U ml^{-1}, respectively. The expression of PDC1 in *P. pastoris* was similar to wild type strain *S. cerevisiae*

(0.20 U ml^{-1}) and *C. utilis* (0.19 U ml^{-1}). The enzyme activity from this study was higher than previous study Augustina (2009). It was reported that carboligase activity from *Candida utillis* TISTR 5198 was highest among 15 wild type microbes, followed by carboligase from *S. cerevisiae* TISTR 5606 at 0.05, 0.01 U ml^{-1}, respectively. But the enzyme activity from this study was lower than Leksawasdi *et al.* (2004) study using different strain. It reported that carboligase activity from *C. utillis* strain 70940 was 5.0 U ml^{-1} in the same biotransformantion process. But PDC1 activity from *C. utillis* and *Rhizopus javanicus* was 8.4 U ml^{-1} in two-phase biotransformantion process at the best condition.

Chapter 4

Conclusion

C. utilis strain TISTR 5352 was able to produce ethanol at the highest level among six strains at 150 ml scale for 192 h cultivation period in a static condition using a carbon source from dried longan extract with other supplementary nitrogen sources at 25.6^{o}C. The second ethanol producer was *C. utilis* TISTR 5198. The ethanol yield obtained from the cultivation of *C. utilis* TISTR 5352 in 1,500 ml scale was 0.27 ± 0.01 g g^{-1}. The cultivation of *C. utilis* TISTR 5198 and TISTR 5352 in DDLFH medium at TSS levels of 20 and 40^{o}Brix indicated the growth inhibition. The two-phase PAC biotransformation of *C. utilis* TISTR 5198 using whole cells harvested at 192 h in DDLFH medium with 6.12 g l^{-1} of dried biomass equivalent resulted in the overall PAC production level of 1.76 ± 0.06 mM which was followed by *C. utilis* TISTR 5352 in DLE medium with PAC production level of 0.75 ± 0.02 mM.

Inoculum level at 1% (v/v) was the most suitable for a 1,500 ml scale batch cultivation of *S. cerevisiae* TISTR 5606 using dried longan extract as a carbon source in a static condition for 36 h and 25.6^{o}C. The consecutive runner ups were inoculum levels at 5 and 10% (v/v), respectively. The carbon source from DLE medium was the most suitable for batch cultivation in 5,000 ml scale with an initial aeration period of 12 h from the overall 36 h cultivation period at 25.6^{o}C. Fed batch system illustrated the toxicity of DDLFH medium in comparison to DLE medium. The two-phase separated PAC biotransformation using whole cells cultivated in 5,000 ml scale with DLE and DDLFH media did not result in PAC production.

The individual cultivation kinetics of *S. cerevisiae* TISTR 5606 on three types of carbon sources, namely, glucose, fructose, and sucrose at 40 g l^{-1} had been investigated as these sugars were commonly found in dried longan extract. The initial guesses of nine kinetics parameters necessary for carrying out the subsequent development of mathematical model was then obtained. The assessment of simulated curves for microbial cultivation kinetics with an individual pure sugar suggested good predictions with RSS levels between 26.0 – 83.0, and $R^2 > 0.98$. The triple substrate model for the cultivation media consisting of glucose/fructose/sucrose (in g l^{-1}) at 20/20/20, 30/30/30, 40/40/40, and 60/60/60 resulted in the good fitting with minimum total RSS value of 1,033. The proposed model of cultivation kinetics was then adapted to predict growth kinetics profile of *S. cerevisiae* TISTR 5606 on dried longan extract cultivation media which

contained the overall sugars concentrations of 60, 120, and 180 g l^{-1} with total RSS value of 1,894 and the following parameter values; α = 0.5040, β = 0.3400, γ = 0.1560, $\mu_{max,1}$ = 0.192 h^{-1}, $\mu_{max,2}$ = 0.087 h^{-1}, $\mu_{max,3}$ = 0.155 h^{-1}, $q_{smax,1}$ = 2.10 g g^{-1} h^{-1}, $q_{smax,2}$ = 1.93 g g^{-1} h^{-1}, $q_{smax,3}$ = 0.48 g g^{-1} h^{-1}, $q_{pmax,1}$ = 1.20 g g^{-1} h^{-1}, $q_{pmax,2}$ = 0.41 g g^{-1} h^{-1}, and $q_{pmax,3}$ = 0.14 g g^{-1} h^{-1}. The prediction agreed well the experimental profiles with $R^2 > 0.94$.

The *pdc*1 gene from *S. cerevisiae* TISTR 5606 was successfully amplified and cloned into *P. pastoris.* Carboligase activities from 3 recombinant clones were similar to carboligase activity from wild type *S. cerevisiae* (0.19 U ml^{-1}) and *C. utilis* (0.20 U ml^{-1}).

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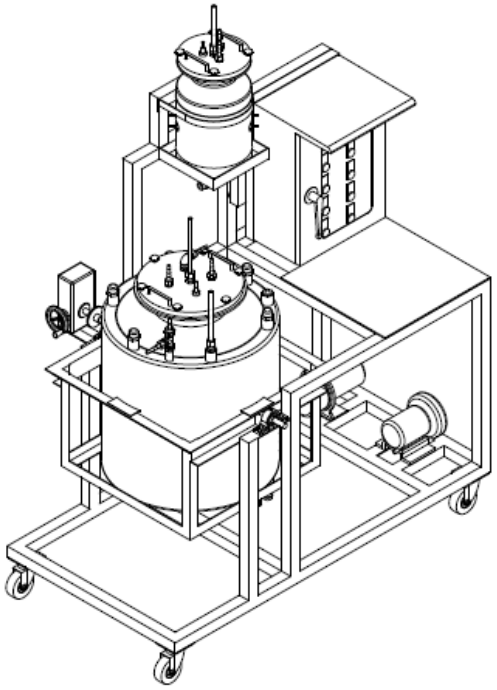
Appendix A

Designed Prototype of 100 L Scale Cultivation Tank



Isometric View

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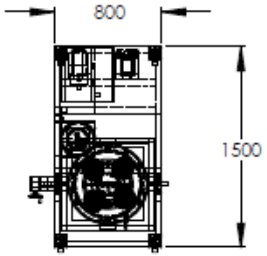

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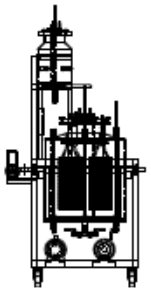
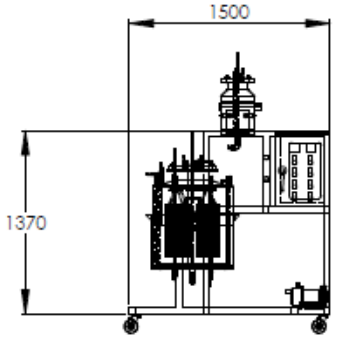


Front View



Right-Side View

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APP'VD						
MFG				MATERIAL:		DWG NO. FT - 02 A3
Q.A.						
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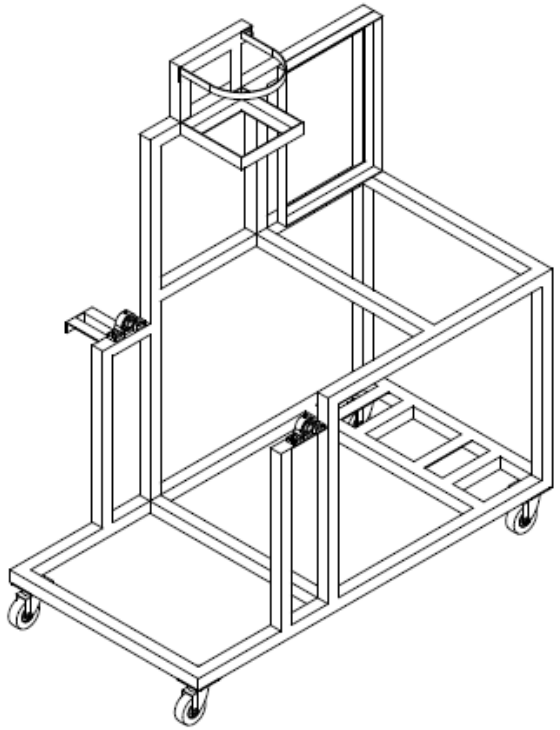

Isometric View

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					FEBIX INTERNATIONAL CO., LTD	
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APP'D						
MFG				MATERIAL:	DWG NO. FT - 03	A3
Q.A						
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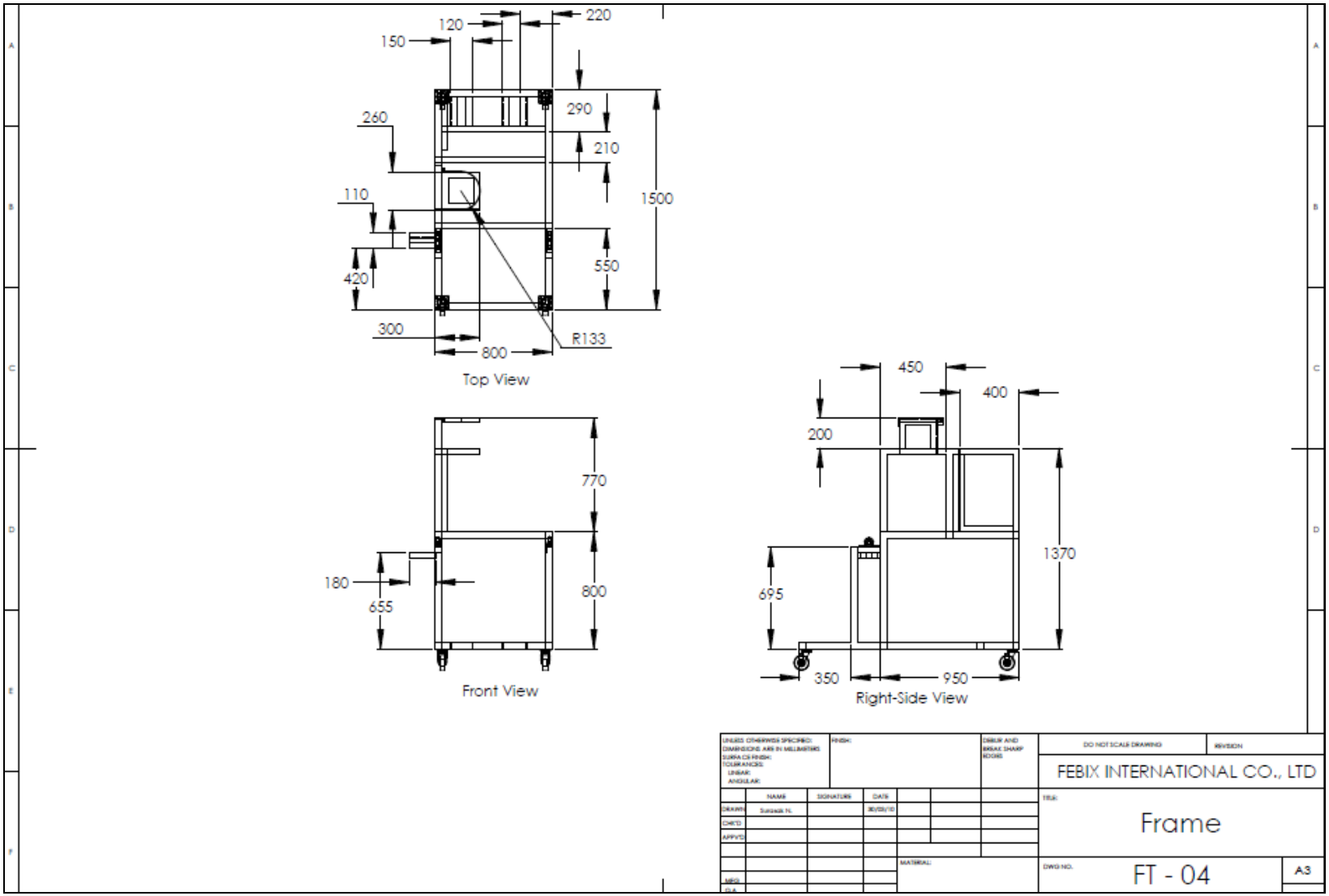
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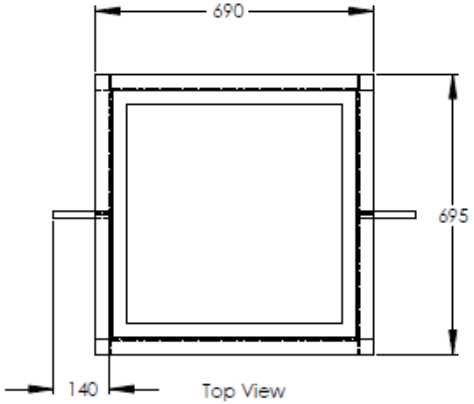
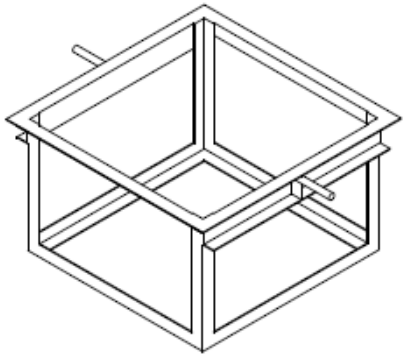
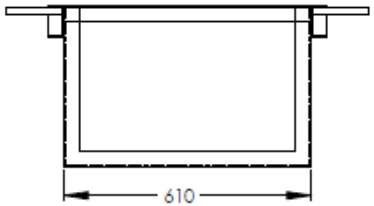
REVISION
FEBIX INTERNATIONAL CO., LTD
Frame
FT - 04
A3



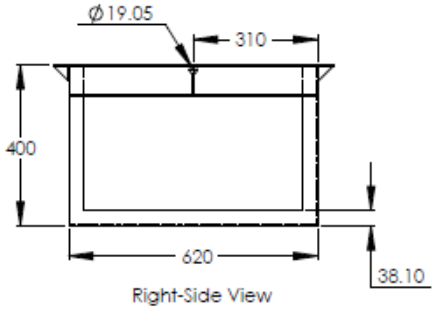
Top View



Isometric View



Front View



Right-Side View

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Q.A						
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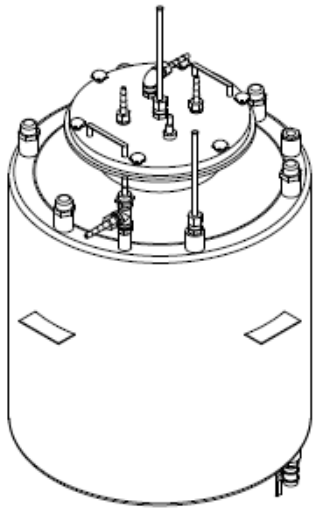

Isometric View

UNLESS OTHERWISE SPECIFIED: DIMENSIONS ARE IN MILLIMETERS SURFACE FINISH: TOLERANCES: LINEAR: ANGULAR:	FINISH:				DEBUR AND BREAK SHARP EDGES	DO NOT SCALE DRAWING
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APP'V'D						
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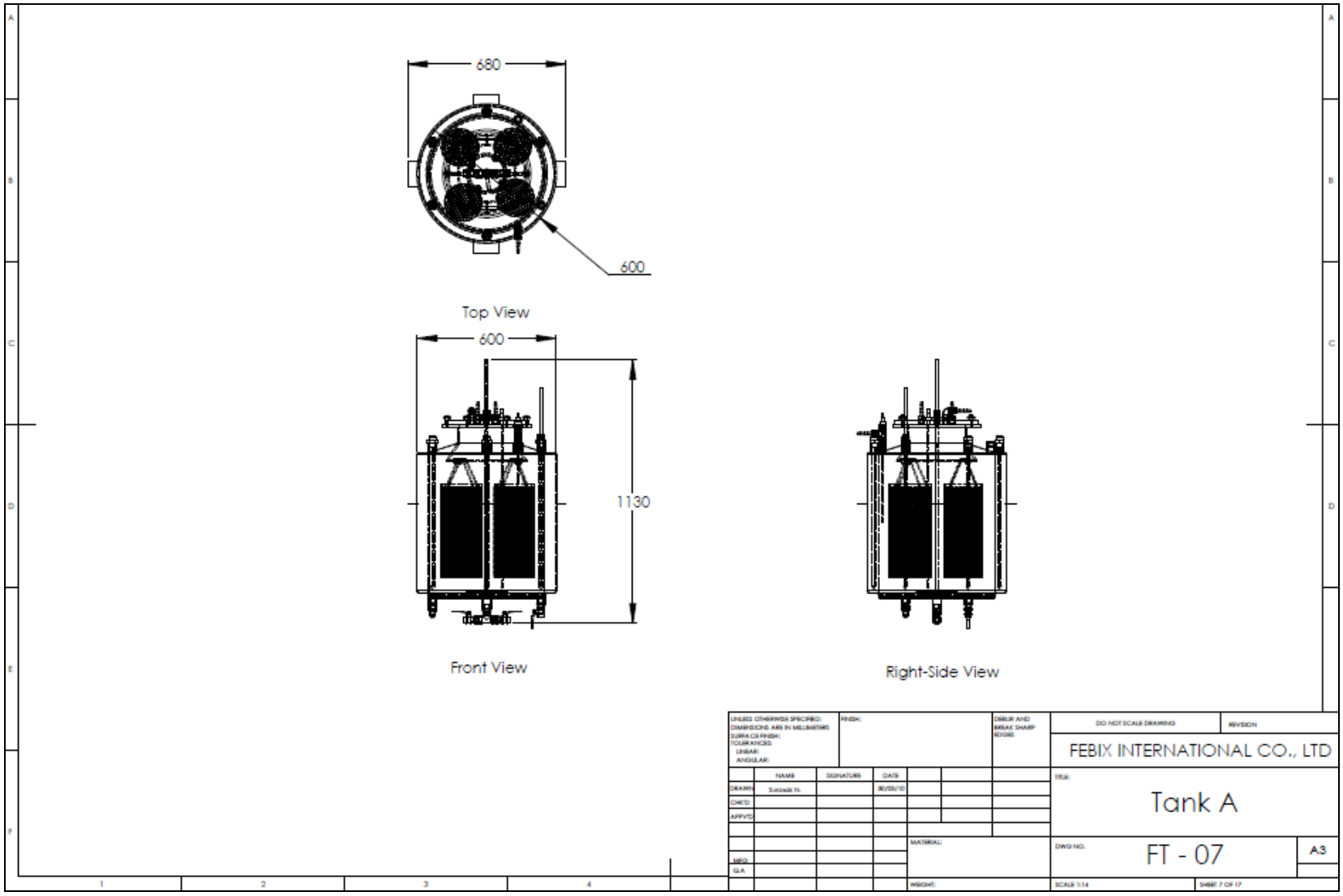
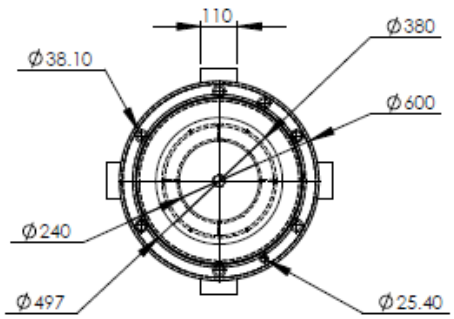
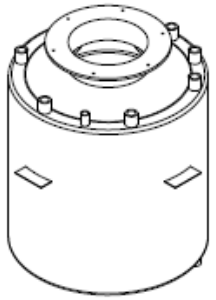
Q.A						A3
				WEIGHT:		SCALE: 1:3 SHEET 6 OF 17

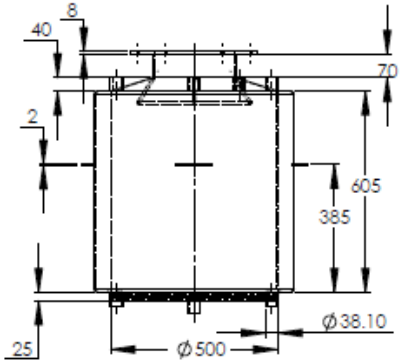

680
600
Top View
600
1130
Front View
Right-Side View
FEBIX INTERNATIONAL CO., LTD
Tank A
FT - 07
A3



Top View

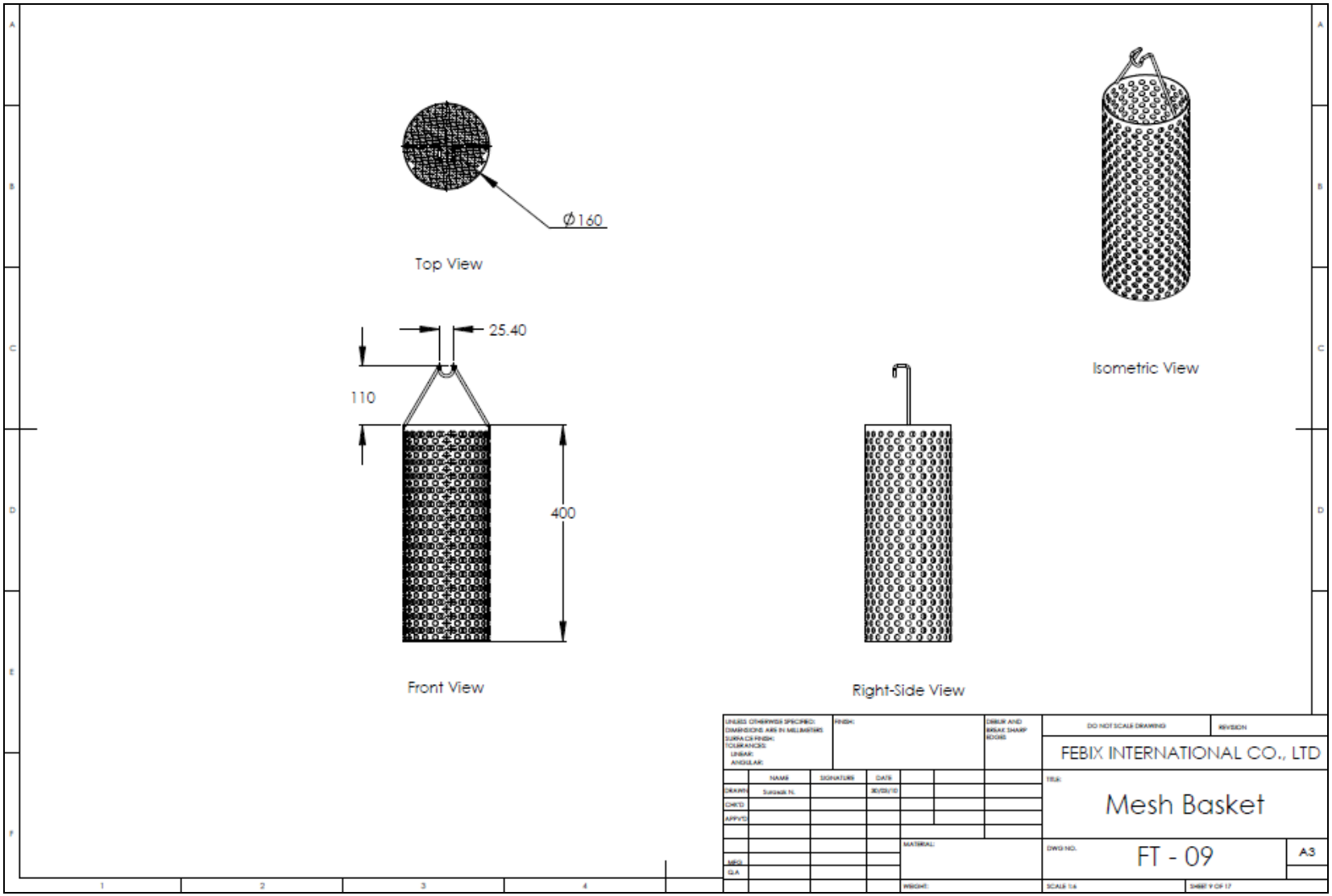


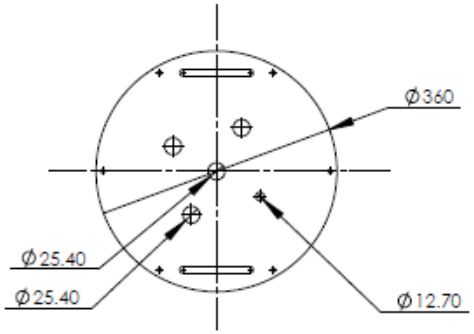
Isometric View



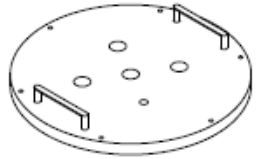
Front View

UNLESS OTHERWISE SPECIFIED: DIMENSIONS ARE IN MILLIMETERS SURFACE FINISH: TOLERANCES: LINEAR: ANGULAR:	FINISH:			DEBUR AND BREAK SHARP EDGES	DO NOT SCALE DRAWING	REVISION
					FEBIX INTERNATIONAL CO., LTD	
	NAME	SIGNATURE	DATE		TITLE: Tank A	
DRAWN	Sukasak N.		30/05/10			
CHK'D						
APPV'D						
MFG				MATERIAL:	DWG NO. FT - 08	A3
Q.A						
				WEIGHT:	SCALE 1:12	SHEET 8 OF 17

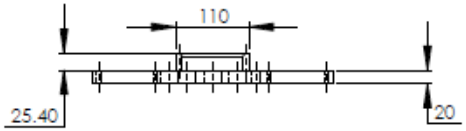

Ø160
Top View
25.40
110
400
Front View
Right-Side View
Isometric View
FEBIX INTERNATIONAL CO., LTD
Mesh Basket
FT - 09
A3



Top View



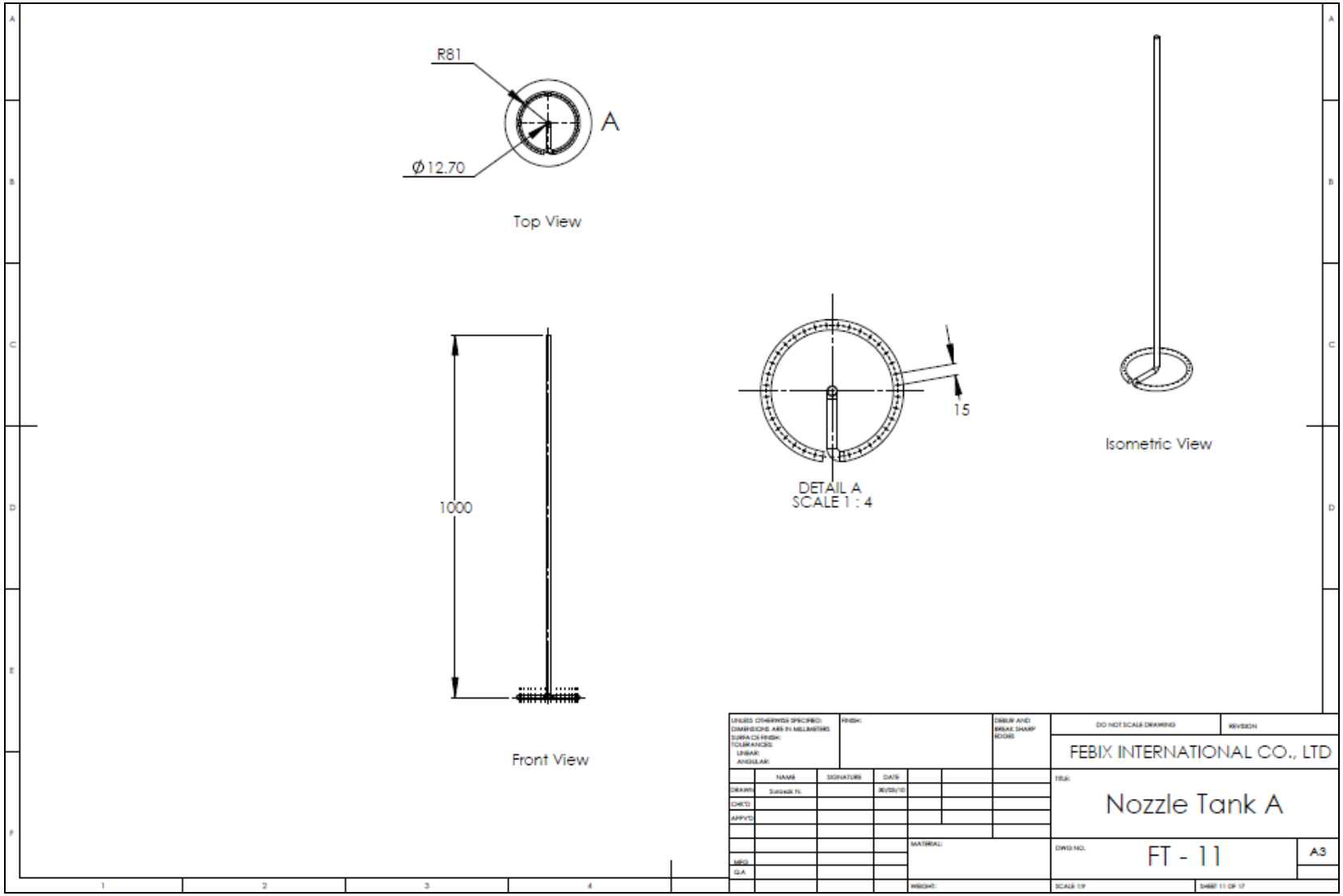
Isometric View

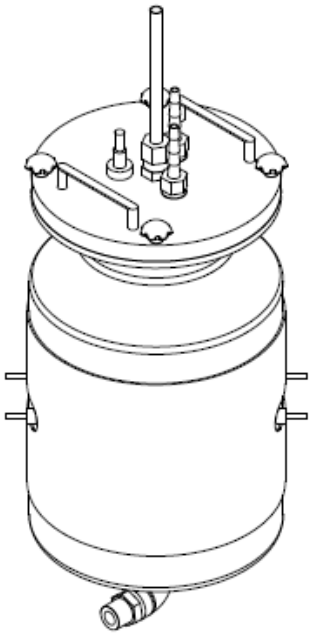


Front View

UNLESS OTHERWISE SPECIFIED: DIMENSIONS ARE IN MILLIMETERS SURFACE FINISH: TOLERANCES: LINEAR: ANGULAR:						FINISH:	DEBUR AND BREAK SHARP EDGES
	NAME	SIGNATURE	DATE				
DRAWN	Sutasak N.		30/03/10				
CHK'D							
APPV'D							
						MATERIAL:	
MFG							
Q.A							
						WEIGHT:	

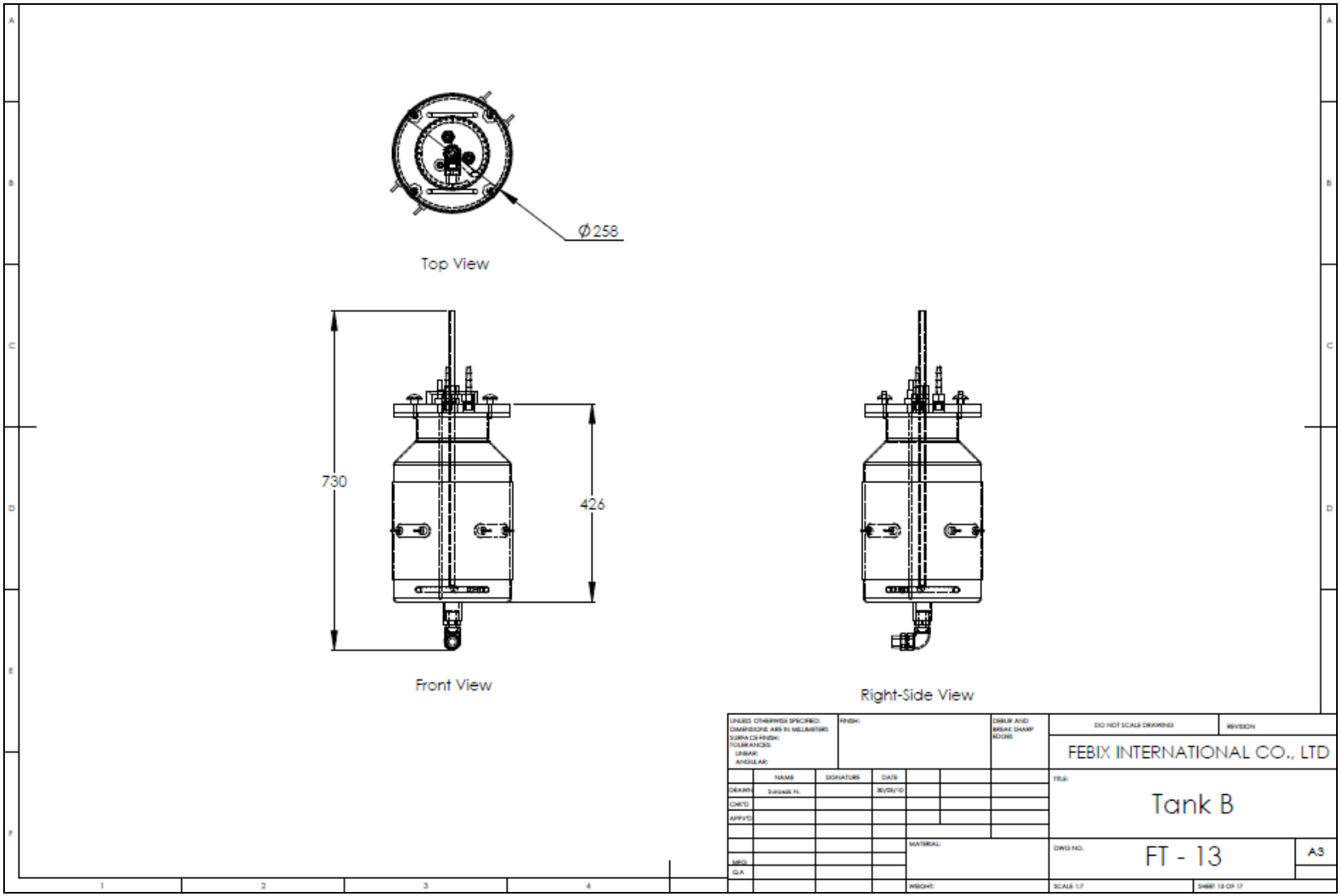
DO NOT SCALE DRAWING	REVISION
FEBIX INTERNATIONAL CO., LTD	
TITLE: Tank A Cap	
DWG NO. FT - 10	A3
SCALE 1:4	SHEET 10 OF 17

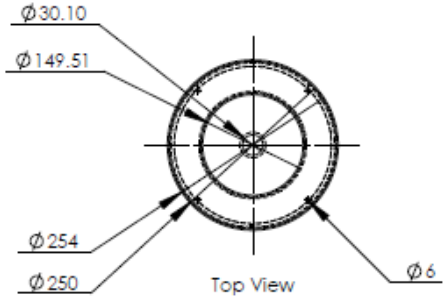

R81
Ø12.70
A
Top View
1000
Front View
15
DETAIL A
SCALE 1 : 4
Isometric View
FEBIX INTERNATIONAL CO., LTD
Nozzle Tank A
FT - 11
A3



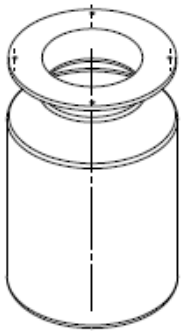
Isometric View

UNLESS OTHERWISE SPECIFIED: DIMENSIONS ARE IN MILLIMETERS SURFACE FINISH: TOLERANCES: LINEAR: ANGULAR:	FINISH:				DEBUR AND BREAK SHARP EDGES	DO NOT SCALE DRAWING
						FEBIX INTERNATIONAL CO., LTD
	NAME	SIGNATURE	DATE			TITLE: Tank B
DRAWN	Sukjeok N.		30/03/10			
CHK'D						
APP'VD						
MFG				MATERIAL:		DWG NO. FT - 12
Q.A						A3
				WEIGHT:		SCALE 1:4
						SHEET 12 OF 17

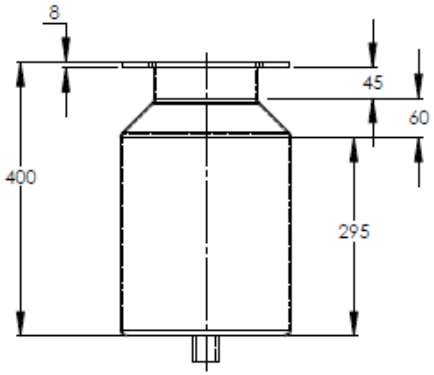

Ø258
Top View
730
426
Front View
Right-Side View
FEBIX INTERNATIONAL CO., LTD
Tank B
FT - 13
A3



Top View

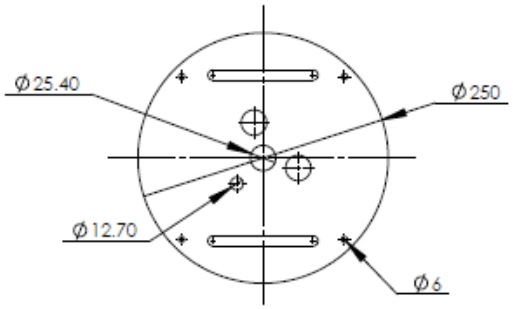


Isometric View

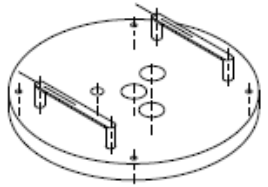


Front View

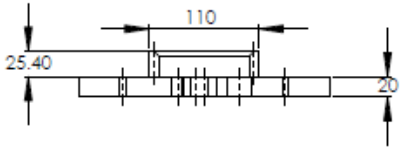
UNLESS OTHERWISE SPECIFIED: DIMENSIONS ARE IN MILLIMETERS SURFACE FINISH: TOLERANCES: LINEAR: ANGULAR:	FINISH:				DEBUR AND BREAK SHARP EDGES	DO NOT SCALE DRAWING
	NAME	SIGNATURE	DATE			FEBIX INTERNATIONAL CO., LTD
DRAWN	Sunpak N.		30/09/10			TITLE: Tank B
CHK'D						
APP'D						
MFG				MATERIAL:		DWG NO. FT - 14
Q.A						A3
				WEIGHT:		SCALE: 1:6 SHEET 14 OF 17



Top View

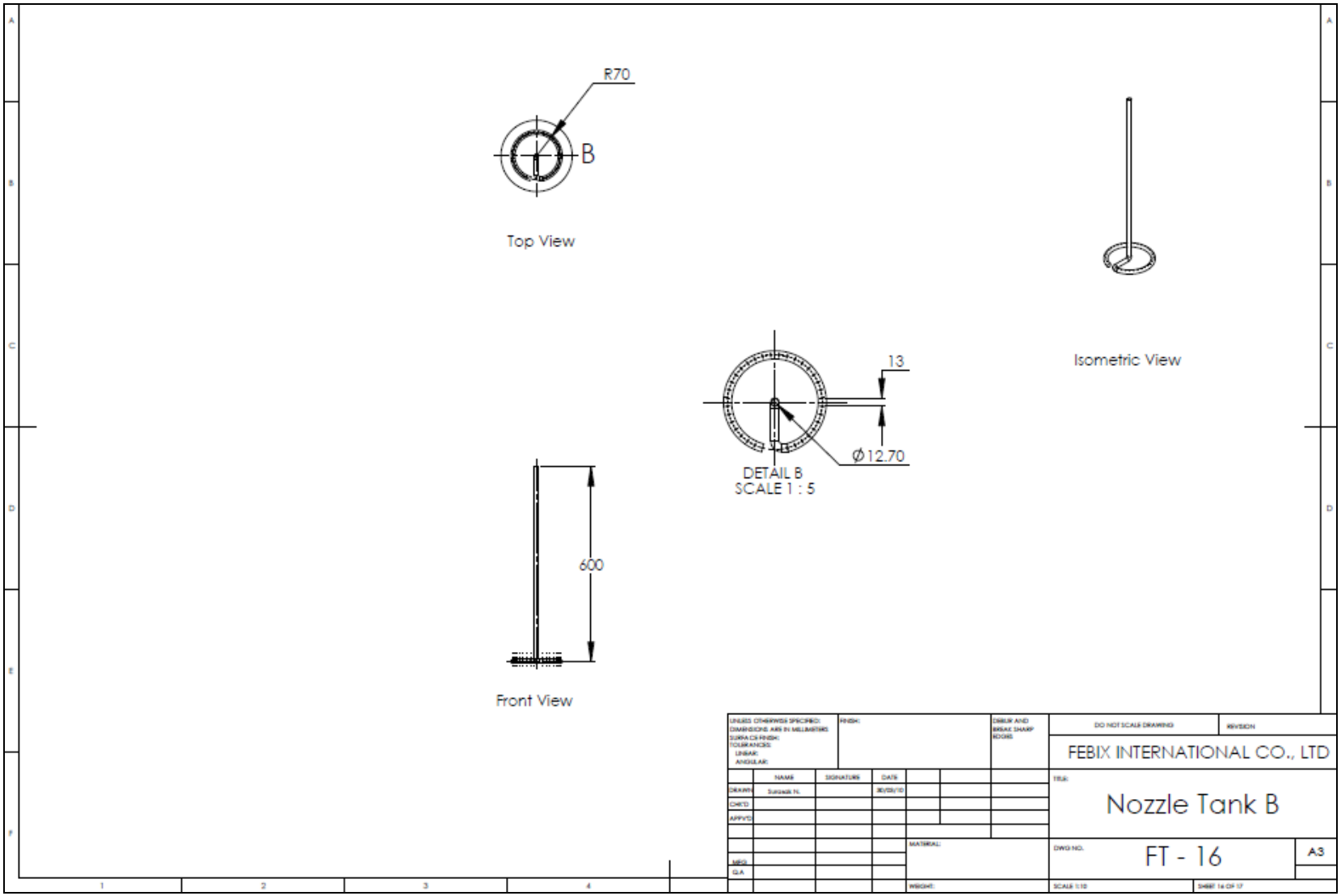


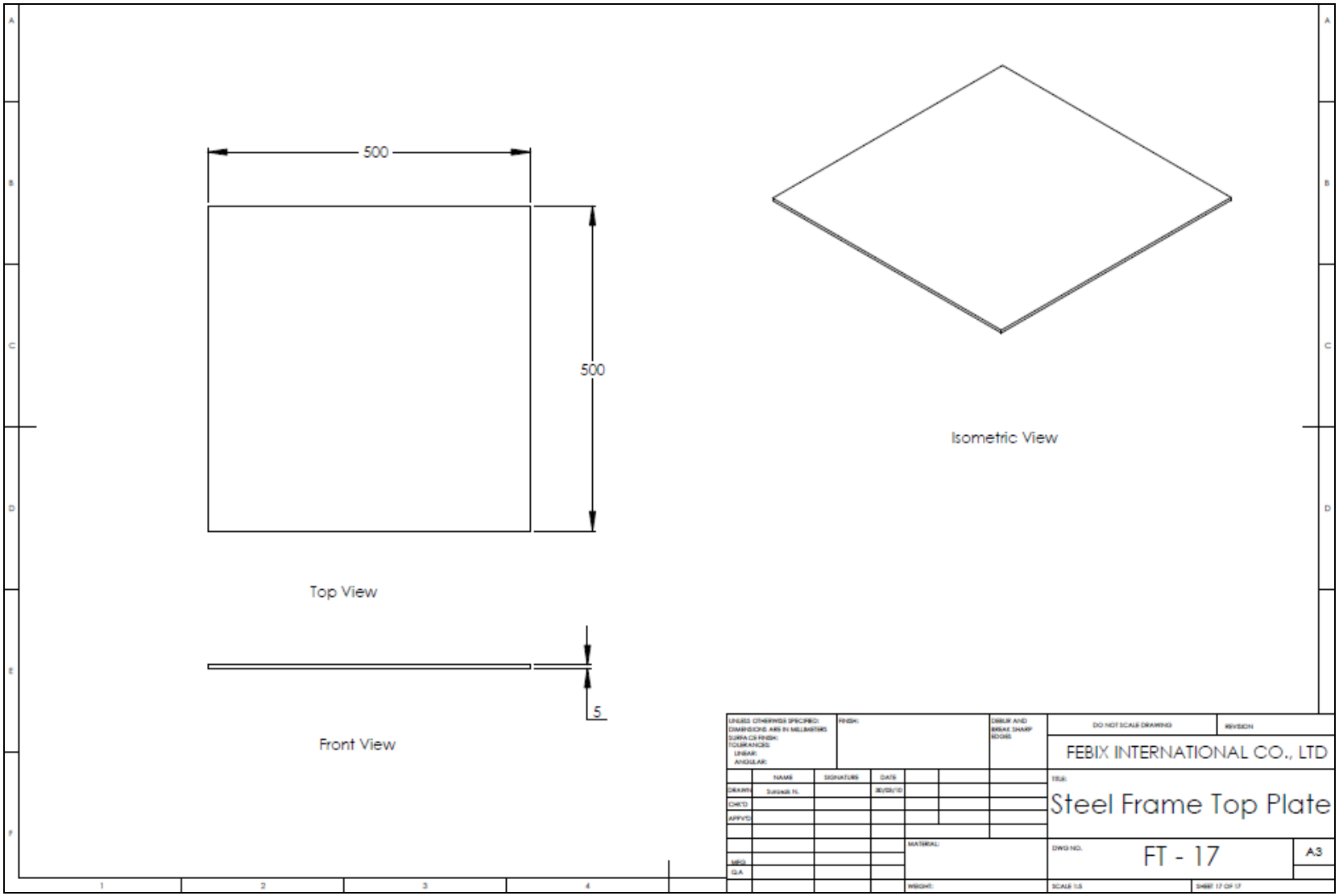
Isometric View



Front View

UNLESS OTHERWISE SPECIFIED: DIMENSIONS ARE IN MILLIMETERS SURFACE FINISH: TOLERANCES: LINEAR: ANGULAR:		FINISH:		DEBUR AND BREAK SHARP EDGES	DO NOT SCALE DRAWING	REVISION
					FEBIX INTERNATIONAL CO., LTD	
	NAME	SIGNATURE	DATE		TITLE: Tank B Cap	
DRAWN	Sunsuk N.		30/03/10			
CHK'D						
APP'VD						
MFG				MATERIAL:	DWG NO. FT - 15	A3
Q.A						
				WEIGHT:	SCALE 1:4	SHEET 15 OF 17


R70
B
Top View
Isometric View
13
Ø12.70
DETAIL B
SCALE 1 : 5
600
Front View
DO NOT SCALE DRAWING
REVISION
FEBIX INTERNATIONAL CO., LTD
Nozzle Tank B
FT - 16
A3


500
500
Top View
5
Front View
Isometric View
FEBIX INTERNATIONAL CO., LTD
Steel Frame Top Plate
FT - 17
A3