CHAPTER 2

LITERATURE REVIEW

2.1 Enzymatic digestion

2.1.1 α - amylase

α - amylase is an enzyme which aids the breakdown of α - 1,4 linkage in starch to maltose. α - amylase hydrolyzes bonds between glucose repeats as shown in Fig 2.1 (Toralballa and Eitingon, 1967).

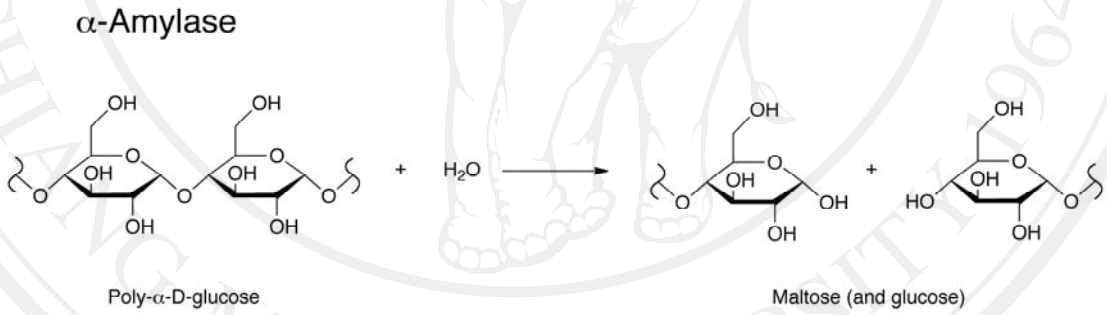

Figure 2.1 α - amylase activity

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2.1.2 Carbohydrase

Carbohydrase including endo - 1,3(4) - β - glucanase, is an enzyme that breaks down a glucan, a polysaccharide made of several glucose subunits, to glucose as shown in Fig 2.2 (Sigma, 2011).

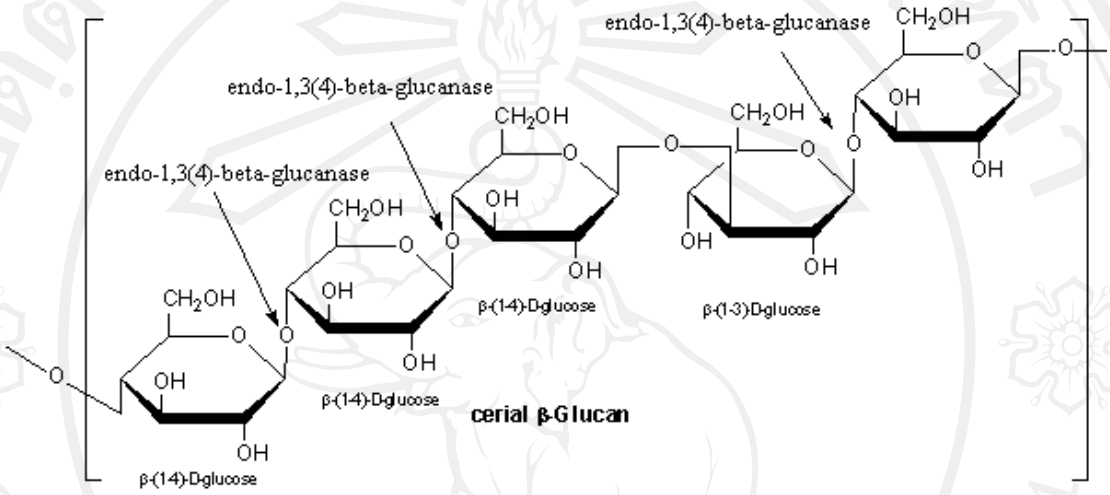

Figure 2.2 Carbohydrase activity

2.1.3 Endo – 1,4 – β – xylanase

Endo – 1,4 – β – xylanase is an enzyme that degrade the linear poly-saccharide β - 1,4 – xylan into xylose as shown in Fig 2.3 (Gulzar, 2004).

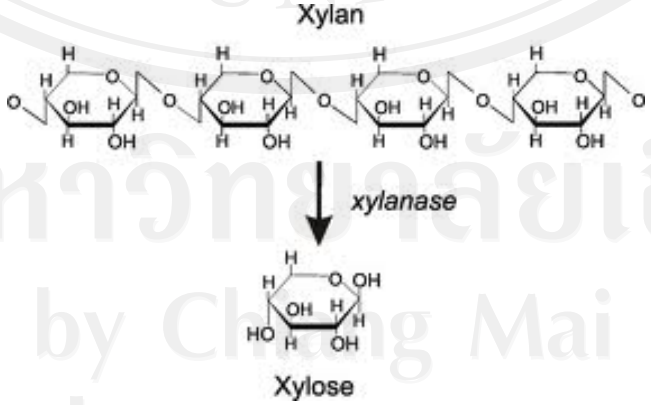

Figure 2.3 Endo – 1,4 – β – xylanase activity

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2.2 Present situation of ethanol production

The alleviation of global warming and climate changes effects was the urgent policy that Royal Thai government had stated to the parliament on 18 February 2008 with the alleviative measure and expedition of the project. Thai parliament was also hosting the 30th ASEAN Inter – Parliamentary Assembly (AIPA) between 2 – 8 August 2009 which focused on the discussion of food safety and global warming. The resolution from the eight member countries of AIPA indicated the joint cooperation to solve this problem (Thiaktong and Sripenprapa, 2009). This has indicated the concerns and willingness of the Royal Thai Government to take action on global warming and shortage of fossil fuels in the present situation. EPPO (2000) stated that at the present rate of fossil fuels consumption, crude oil, natural gas and coal would only be available for 30 – 40, 50 – 60 and 220 years, respectively. Forest destruction was the principal activity leading to the release of greenhouse gases such as carbon dioxide, methane and nitrous oxide to the atmosphere. The rectification measure are required to prevent negative impact to the coastal ecosystem, fishery, settlement of population and tourism industry of the country. The explosion of an underwater oil drilling platform in the Gulf of Mexico of British Petroleum (BP) company on 20th April 2010 resulted in the extensive damages on both coastal and marine food industry because the amount of sprilling crude oil to the sea was more than 7.2 million liters (Thansettakij, 2010). The United States Government prohibited the fishery activity within the area of 229,212 km^2 as a protective measure to ensure that the marine food product from the gulf of Mexico were safe for consumption which eventually led to the increase of seafood price (DITP, 2010). The limited option of an alternative energy has been narrowed down as evident from 2011 Tohoku earthquake and tsunami, which occurred on 11th March 2011 at the east coast of Japan, that resulted in a number of casualties and serious damages to several nuclear reactors at Fukushima Daiichi Nuclear Power Plant (Prakairangsee, 2011). The release of certain radioactive by – products (such as iodine – 131 and strontium – 90) from the fissile materials to the environment either by air or sea exceeding the stipulated amount (Hanlon, 2011) can cause harmful effects toone's health ranging from headache, fever, diarrhea, nausea, vomiting, occurrence of cancer and death

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(WNC NEWS, 2011). Such incident has significantly undermined the picture of clean and safe production process from nuclear energy in public view.

One strategy to mitigate the effect of global warming is to search for a new form of energy to replace the fossil energy. Biomass energy is one of the alternative energy which is renewable and can be found generally in nature with recyclability property. The collected energy in living organisms or products obtained from these organisms can be converted to other forms of energy for ease of use, for example, liquid alcohol fuels (ethanol and butanol) through the biochemical process and biodiesel through the chemical process. Thailand is the agricultural country with abundant biomass resource from the agriculture sector. The advantage in term of raw materials for biofuels production is thus clearly pronounced. Currently, the ethanol production has been carried out from several agricultural materials such as sugar cane, cassava and molasses which corresponds to the necessity in developing an alternative renewable eco – friendly energy that can be produced in Thailand. This has led to the expansion of ethanol production industry to produce gasohol which is the blend of ethanol with fossil fuels (KAPI, 2006). There exists many researches involving ethanol production from other agricultural materials such as Premjit *et al*. (2007) who utilized paper mulberry materials in the simultaneous saccharification and fermentation (SFF). In addition, Lueang – a papong (2005) performed the simple experiment by adding wine yeast into the mixture of dried longan and water and observed, however, it was possible that insufficient biomass and/or poor access to air might contribute to low level of ethanol production in absence of oxygen. The more paper systematic cultivation conditions for wine yeast or other ethanol producing microbes which included controls of temperature, pH level and oxygenation interval would result in the higher ethanol concentration level. Moreover, the efficiently of ethanol production should also be considered by comparing the ethanol yield (g ethanol produced/g consumed sugar) and ethanol productivity (g ethanol produced/L/h) with other production systems.

In June 2009, Thai Ethanol Manufacturing Association stated that there were 15 authorized ethanol factories with the overall production capacity of 2.272 million liters per day as shown in Table 2.1.

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Table 2.1 Thai ethanol production capacity in 2009 (Thai ethanol manufacturing association, 2009)

Factory	Location	Raw materials	Production capacity (Liters/day)
1. Pornwilai international company	Ayutthaya	Molasses	25,000
2. Thai alcohol public company limited	Nakornpathom	Molasses	200,000
3. Thai agro energy company limited	Suphanburi	Molasses	150,000
4. Thai yuan alcohol company limited	Khonkaen	Cassava	130,000
5. Khonkaen alcohol company limited	Khonkaen	Sugar cane/Molasses	150,000
6. Petrogreen company limited	Chaiyaphum	Sugar cane/Molasses	200,000
7. Thai sugar ethanol company	Kanchanaburi	Sugar cane/Molasses	100,000
8. K.I. ethanol company	Nakhonratchasima	Sugar cane/Molasses	100,000
9. Petrogreen company limited	Kalasin	Sugar cane/Molasses	200,000
10. Aekaratpatthana company limited	Nakhonsawan	Molasses	200,000
11. Thai rungruang power company	Saraburi	Molasses/Bagasse	120,000
12. E.S. power company limited	Sakaeo	Molasses/Cassava	150,000
13. Ratchaburi ethanol company limited	Ratchaburi	Molasses/Cassava	150,000
14. Maesod clean energy company	Tak	Sugar cane	200,000
15. Subtip company	Lopburi	Cassava	200,000
		TOTAL	**2,275,000**

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2.3 Growth phase of ethanol producing microbes

There exists two growth intervals of the ethanol producing microbes which include (1) aerobic cultivation and (2) ethanol production. In the first phase, the aeration will be supplied fully to maintain a respiration quotient (RQ or the mole fraction of carbon dioxide produced over consumed oxygen) of 1 – 4. This will be followed by the decrease in aeration during the second phase to induce ethanol production which will raise the RQ level to 12 – 25 (Shin and Roger, 1996).

Phase 1: Aerobic growth

In the situation where monosaccharides, such as glucose or fructose, are used as the substrates;

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \quad (1)$$

In the situation where disaccharide, such as sucrose, is used as the substrates;

$$C_{12}H_{22}O_{11} + 12O_2 \longrightarrow 12CO_2 + 11H_2O \quad (2)$$

Shuler and Kargi (1992) clearly portrayed the theoretical yield of dried biomass production per consumed glucose (Yx/s) was 0.4 which was compared to the experimental value of 0.38 – 0.51 g ethanol per g consumed sugars.

Phase 2: Ethanol production

In the situation where monosaccharides, such as glucose or fructose, are used as the substrates;

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \quad (3)$$

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The theoretical yield of ethanol production per consumed glucose/fructose (Yp/s) can be calculated as following;

(Yp/s) = $(2 \times 46)/180$

= 0.511 g ethanol/g glucose/fructose (theoretical Yp/s)

In the situation where disaccharide, such as sucrose, is used as the substrates;

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 4C_2H_5OH + 4CO_2 \quad (4)$$

The theoretical yield of ethanol production per consumed sucrose (Yp/s) can be calculated as following;

(Yp/s) = $(4 \times 46)/342$

= 0.538 g ethanol/g sucrose (theoretical Yp/s)

In fact, the experimental yield was approximately 90 – 95% of the theoretical yield because glucose was transformed to biomass and other by – products such as glycerol or acetate in metabolism process (Shuler and Kargi, 1992).

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2.4 Pyruvate decarboxylase

Biomass gained from ethanol production consisted of pyruvate decarboxylase which catalysed decarboxylation reaction of pyruvate which was the last product in the glycolysis pathway. Carbon dioxide was removed without oxidative reaction (Sergienko and Jordan, 2002; Iding *et al*., 1998) resulting in acetaldehyde which would be later transformed to ethanol (Leksawasdi, 2004) as indicated in chemical equation scheme shown inn Figure 2.4.

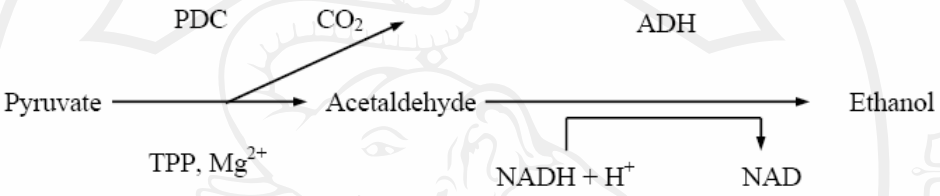

Figure 2.4 Ethanol production from pyruvate decarboxylase enzyme (Leksawasdi, 2004)

Leksawasdi (2004) stated that pyruvate decarboxylase enzyme extracted from yeast and bacteria consisted of four identical subunit with a molecular mass of 240 – 250 kDa. Two of these subunits were tightly, but noncovalently, bound at the interface with thiamine pyrophosphate (TPP) at pH values below 6.5. Ullrich and Donner (1970) found that pH level had an influence on the blinding strength of thiamine pyrophosphate to the binding sites of pyruvate decarboxylase. Each pyruvate decarboxylase subunit was a single polypeptide chain of 563 amino acids which was arranged into α, β and γ domains (Furey *et al*., 1998) as shown in Figure 2.5.

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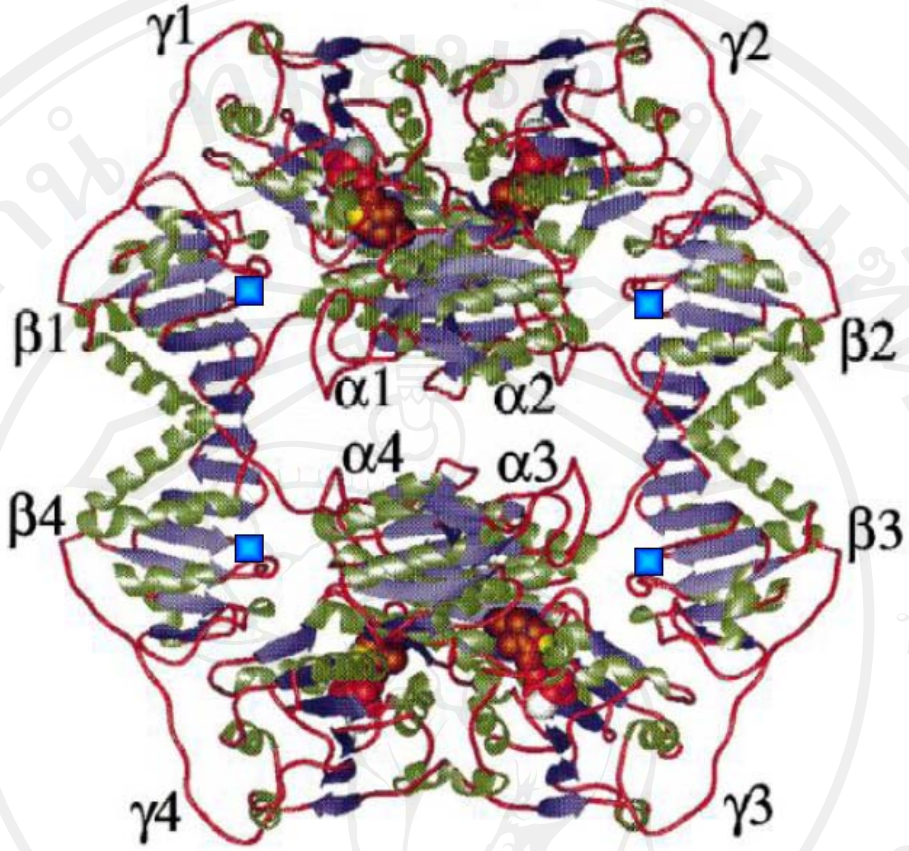

Figure 2.5 A complete ribbon drawing of PDC tetramer

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2.5 Phenylacetylcarbinol (PAC)

The biotransformation process is catalysed by enzyme pyruvate decarboxylase to produce PAC, a chiral precursor for the production of the pharmaceuticals ephedrine and pseudoephedrine with the properties of relieving the allergic and nasal congestion symptoms. It is produced from pyruvate and benzaldehyde substrates (as shown in Figure 2.6). Pyruvate is changed to by – products, such as acetaldehyde and acetoin (Rosche *et al*., 2002).

There are 3 methods of ephedrine production as following; (1) direct extraction from Ma Huang (*Ephedra* sp.) herb, (2) chemical synthesis alone and (3) combined biological and chemical process inn which PAC is produced from substrates pyruvate and benzaldehyde prior to chemical conversion into ephedrine (Leksawasdi, 2004).

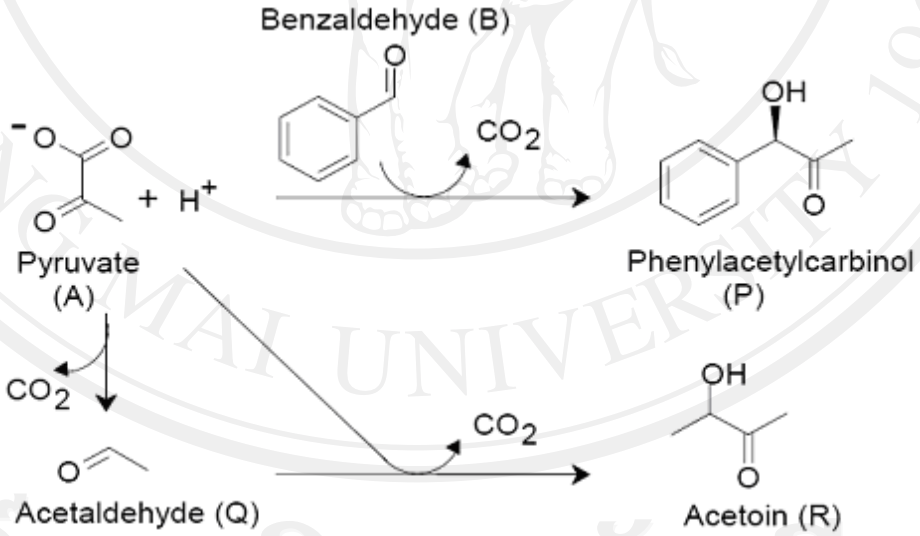

Figure 2.6 Production of PAC and by – products from pyruvate decarboxylase enzyme (Leksawasdi, 2004)

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2.6 Production of ethanol and PAC from dried longan extract

2.6.1 Selection of sugars and protein extraction methods from dried longan flesh aged 1.5 years

Achawasamit and Leksawasdi (2009) employed 5 extraction methods using 6 levels (10, 30, 50, 70, 100 and 130 g) of dried longan flesh aged 1.5 yrs. The specified mass of dried longan flesh was mixed with 100 ml distilled water in a 550 ml jam jar. The efficiency scores were determined, rated and ranked. The results showed that the best extraction method was 30 min boiling with the mass to volume ratio of dried longan flesh to distilled water at 30:100 as shown in Table 2.2.

Table 2.2 Selection of sugars and protein extraction methods from dried longan flesh aged 15 yrs

Researcher team	Extraction methods	Results
Achawasamit and Leksawasdi (2009)	1. Soaking in distilled water at room temperature for 24 h 2. Soaking in distilled water at room temperature for 24 h and followed by boiling for 30 min 3. Steaming for 30 min 4. Boiling for 30 min 5. Boiling for 30 min twice	Boiling strategy for 30 min with 30 g dried longan flesh per 100 ml distilled water. The highest extraction efficiency score of 74.1 ± 1.1 was obtained. There were 191 ± 3 g/l of total sugars with 127 ± 2 g/l sucrose, 23.4 ± 0.3 g/l glucose, 40.4 ± 0.6 g/l fructose and total protein concentration of 6.74 ± 0.26 g/l.

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2.6.2 Selection of the suitable time for seed cultivation

Kanchanwong *et al*. (2008), Poodthatep *et al*. (2008) and Phratong *et al*. (2008) investigated ethanolproduction using 15 microbial stains from *Candida utilis, Saccharomyces cerevisiae, Zymomonas mobilis, Escherichia coli* and *Klebsiella* sp. in a static system of 10 ml at 25.6°C with variation of seed cultivation time (Table 2.3). The cultivation medium with glucose as a sole carbon source with 1.5 folds of nitrogen source concentration used in the Yeast – Malt (YM) medium.

Table 2.3 Selection of the suitable time for seed cultivation

Researcher team	Seed cultivation time	Results
Kanchanwong *et al*. (2008)	24	*Z. mobilis* TISTR 405 produced the highest ethanol concentration of 9.47 ± 0.47 g/l with Yp/s = 0.48 g ethanol/g glucose
Poodthatep *et al*. (2008)	48	*Z. mobilis* TISTR 405 produced the highest ethanol concentration of 10.6 ± 0.53 g/l with Yp/s = 0.55 g ethanol/g glucose
Phratong *et al*. (2008)	72	*Z. mobilis* TISTR 405 produced the highest ethanol concentration of 10.6 ± 0.53 g/l with Yp/s = 0.54 g ethanol/g glucose

The results obtained from the experiment in selecting the suitable time for seed cultivation (Table 2.3) indicated that the cultivation time at 48 and 72 h resulted the highest level concentration. This was compared to the decreasing biomass production rates and increasing expenses for the extended cultivation time. The most suitable time for seed cultivation was 48 h based on the level of ethanol concentration being produced (Kanchanwong *et al*., 2008; Poodthatep *et al*., 2008; Phratong *et al*., 2008).

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2.6.3 Selection of the highest ethanol producing microbe using 6 mo old dried longan flesh

Kanchanwong *et al*. (2008), Poodthatep *et al*. (2008) and Phratong *et al*. (2008) performed the selection of microbial inocula by comparing the ethanol production from the inocula of 15 microbial strains mentioned previously in section 2.6.2 in a static system of 100 ml at 25.6°C for 48 h with variation of carbon source (Table 2.4). The other components in the cultivation medium were kept at the same level as in section 2.6.2. The results showed that *S. cerevisiae* TISTR 5606 and 5020 could produce the highest ethanol levels.

Table 2.4 Selection of the highest ethanol producing microbe using 6 mo old dried longan flesh

Researcher team	Carbon sources	Results
Kanchanwong *et al*. (2008)	Glucose only with concentration of 63.5 – 64.4 g/l	*S. cerevisiae* TISTR 5606 produced the highest ethanol concentration of 23.5 ± 2.6 g/l with Yp/s 0.37 ± 0.04 g ethanol/g glucose
Poodthatep *et al*. (2008)	Mixture of 6 mo old dried longan extract and molasses in 1:1 ratio with initial total sugar concentration of 74.4 ± 5.2 g/l	*S. cerevisiae* TISTR 5020 produced the highest ethanol concentration of 43.3 ± 4.0 g/l with Yp/s 0.56 g ethanol/g total sugar
Phratong *et al*. (2008)	Six mo old dried longan extract only with initial total sugar concentration of 74.4 ± 5.2 g/l	*S. cerevisiae* TISTR 5020 produced the highest ethanol concentration of 45.0 ± 6.1 g/l with Yp/s 0.50 g ethanol/g total sugar

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2.6.4 Selection of the highest ethanol producing microbe using 6 years old dried longan flesh

Kumtip *et al*. (2009), Tadkeaw *et al*. (2009) and Buakham *et al*. (2009) performed the ethanol production from the inocula of 15 microbial strains mentioned previously in section 2.6.2 in the static system of 100 ml at 25.6°C for 48 h with variation of carbon source (Table 2.5) without addition of extrageneous nitrogen sources. The results showed that *S. cerevisiae* TISTR 5606 and 5020 could produce the highest ethanol levels.

Table 2.5 Selection of the highest ethanol producing microbe using 6 years old dried longan flesh

Researcher team	Carbon sources	Results
Kumtip *et al*. (2009)	Molasses only with initial total sugar concentration of 74.4 – 5.2 g/l	*S. cerevisiae* TISTR 5606 produced the highest ethanol concentration of 50.5 ± 1.6 g/l with Yp/s 0.51 g ethanol/g total sugar
Tadkeaw *et al*. (2009)	Mixture of 6 years old dried longan extract and molasses in 1:1 ratio with initial total sugar concentration of 74.4 ± 5.2 g/l	*S. cerevisiae* TISTR 5606 produced the highest ethanol concentration of 38.4 ± 1.3 g/l with Yp/s 0.51 g ethanol/g total sugar
Buakham *et al*. (2009)	Six years old dried longan extract only with initial total sugar concentration of 74.4 ± 5.2 g/l	*S. cerevisiae* TISTR 5020 produced the highest ethanol concentration of 6.77 ± 2.2 g/l with Yp/s 0.26 ± 0.14 g ethanol/g total sugar

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2.6.5 Ethanol production of three *C. utilis* strain from dried longan extract and molasses in various ratio

Jaiwanglok *et al*. (2008) studied the ethanol production of *C. utilis* TISTR 5032, 5046 and 5352 using mixtures of 6 mo old dried longan extract and molasses in 5 proportions (0:100, 75:25, 50:50, 25:75 and 100:0) (Table 2.6) in static system for 48 h. The other components in the cultivation medium were kept at the same level as in section 2.6.2. The results showed that *C. utilis* TISTR 5352 which was cultivated in the medium with carbon sources ratio of 50:50 could produce the highest ethanol concentration.

Table 2.6 Ethanol production from three *C. utilis* strains using dried longan extract and molasses inn various ratio as substrates

Researcher team	Various ratio of mixture	Results
Jaiwanglok *et al*. (2008)	1. 0:100 2. 75:25 3. 50:50 4. 25:75 5. 100:0	*C. utilis* TISTR 5352 cultivated in the 50:50 mixture produced the highest ethanol concentration of 3.20 ± 0.41 g/l with Yp/s 0.38 g ethanol/g total sugar

The highest ethanol concentration obtained from this investigation was less than those of *S. cerevisiae* TISTR 5020 and 5606 (Poodthatep *et al*., 2008) which could produce ethanol at 43.4 ± 4.0 and 41.8 ± 1.2 g/l, respectively, by almost 26.6 times.

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2.6.6 Cultivation of ethanol producing microbes in the aerated condition

Agustina *et al*. (2009) investigated ethanol production from the inocula of 15 microbial strains mentioned previously in section 2.6.2. The cultivation media contained 10 mo and 6 years old dried longan extract only as sole carbon sources in 100 ml scale with aeration during the first 24 h by air pump which was connected to sterilized tubing and 0.2 μm pore diameter filter. The partially aerobic condition was simulated by turning off the air pump for the next 24 h (total cultivation time of 48 h). The other components in the cultivation medium were kept at the same level as in section 2.6.2. The utilization of 10 mo old dried longan extract as a carbon source resulted in the higher ethanol concentration than that of 6 years old dried longan (Table 2.7).

Table 2.7 Cultivation of the ethanol producing microbes in aerated condition

Researcher team	Age of dried longan extract	Results
Agustina *et al*. (2009)	10 mo old	*S. cerevisiae* TISTR 5606 produced the highest ethanol concentration of 24.7 ± 3.3 g/l with Yp/s 0.20 ± 0.09 g ethanol/g total sugar
Agustina *et al*. (2009)	6 yrs old	*C. utilis* TISTR 5001 produced the highest ethanol concentration of 1.26 ± 0.86 g/l with Yp/s 3.93 ± 10 g ethanol/g total sugar with was much higher than the theoretical yield and reflected the scarcity of initial sugar concentration in the extract

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2.6.7 Effect of inoculums concentration levels on growth and ethanol production kinetics of *S. cerevisiae* TISTR 5606

Chaweekunlayakun *et al*. (2010) investigated the effect of varied inoculums concentration levels (1, 5 and 10% (v/v)) to elucidate a cost reduction strategy for the microbial cultivation in 2 years old dried longan extract from the conventional level of 10% (v/v). The cultivation level was 1,500 ml in static condition for 36 h at 25.6°C. The method of score weighing was introduced later by considering three factors which included costing, microbial growth and substrates as well as product. The results suggested that inoculums level of 1% (v/v) was able to achieve the highest growth and ethanol production kinetics efficiency scores.

Table 2.8 Effect of inoculums concentration levels on growth and ethanol production kinetics of *S. cerevisiae* TISTR 5606

Researcher team	Inoculum concentration levels	Results
Chaweekunlayakun *et al*. (2010)	1. 1% 2. 5% 3. 10%	1% (v/v) inoculums level provided the highest efficiency score of 91.2 in relation to growth and ethanol production kinetics

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2.6.8 The growth and ethanol production kinetics of *S. cerevisiae* TISTR 5606 were studied in 1,000, 1,500 and 5,000 ml scale under static condition

2.6.8.1 *S. cerevisiae*

The growth and ethanol production kinetics of *S. cerevisiae* TISTR 5606 were studied in 1,000, 1,500 and 5,000 ml scale under static condition. Jaiwanglok *et al*. (2008) investigated the microbial cultivation in mixtures of 6 mo old dried longan extract and molasses in 1:1 ratio as carbon sources at room temperature for 72 h at 1,000 ml. Agustina *et al*. (2009) employed 10 mo old dried longan extract as a sole carbon source for cultivation at 25.6°C for 48 h at 1,500 ml. This was compared to Chaweekunlayakun *et al*. (2010) who investigated the microbial cultivation in two types of carbon sources, namely, two years old dried longan extract and digested dried longan flesh hydrolysate (DDLFH). The latter preparation was subjected to heat treatment in a pressurized sterilizer (at 121°C for 30 min) and enzymatic method (Ronozyme A, VP and WX were added at 1% (w/v) of each) with a static incubation at 30°C for 24 h. Both batch and fed batch cultivation were carried out at 25.6°C with an initial aeration period of 12 h from the overall 36 h cultivation period at 5,000 ml. The other components in the cultivation medium were kept at the same level as in section 2.6.2 (Table 2.9). The results showed that the cultivation at 5,000 ml scale in the fed batch experiment was able to generate the highest specific growth rate and ethanol production capability.

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Table 2.9 The growth and ethanol production kinetics of *S. cerevisiae* TISTR 5606

Researcher team	Levels of the microbial cultivations (ml)	Results
Jaiwanglok *et al*. (2008)	1,000	The highest ethanol concentration of 47.3 ± 1.5 g/l with Yp/s 0.32 ± 0.03 g ethanol/g total sugar were obtained. Maximum specific sucrose, glucose and fructose consumption rates were 3.31, 0.70 and 0.31 g sugar/g biomass/h with the corresponding maximum specific ethanol production rate, and maximum specific growth rate of 0.66 g ethanol/g biomass/h and 0.11 ± 0.03 per h, respectively.
Agustina *et al*. (2009)	1,500	The highest ethanol concentration was 75.5 ± 3.6 g/l with Yp/s 0.59 ± 0.04 g ethanol/g total sugar. The maximum specific sucrose, glucose and fructose consumption rates were 1.07, 0.099 and 0.161 g sugar/g biomass/h with the corresponding maximum specific ethanol production rate and maximum specific growth rate of 0.519 g ethanol/g biomass/h and 0.069 ± 0.003 per h, respectively.

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Table 2.9 (Cont.) The growth and ethanol production kinetics of *S. cerevisiae* TISTR 5606

Researcher team	**Levels of the microbial cultivations (ml)**	**Results**
Chaweekunlayakun *et al*. (2010)	5,000	The growth and ethanol production kinetics in dried longan extract were the highest in comparison with DDLFH medium. The additional ethanol production from the system with dried longan extract medium in a fed batch system was 24.9 ± 1.13 g/l (before feeding this was 68.4 g/l). The maximum specific sucrose, glucose and fructose consumption rates were 0.350, 0.160 and 0.200 g sugar/g biomass/h with the corresponding maximum specific ethanol production rate and maximum specific growth rate of 0.334 g ethanol/g biomass/h and 0.064 ± 0.018 per h, respectively.

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2.6.8.2 *C. utilis*

Tangsuntornkhan *et al*. (2010) investigated the growth and ethanol production kinetics of six *C. utilis* strains which included TISTR 5001, 5032, 5043, 5046, 5198 and 5352 at 150 and 1,500 ml scale. The cultivation level was 150 ml with (1) two years old dried longan extract, (2) 20°Brix DDLFH and (3) 40°Brix DDLFH as carbon sources. The cultivation level at 1,500 ml was carried out with two years old dried longan extract as a sole carbon source. The results showed that the cultivation in two years old dried longan extract was able to generate the better growth and ethanol production kinetics in comparison with DDLFH medium.

Table 2.10 The growth and ethanol production kinetics of six *C. utilis* strains

Researcher team	Levels of the microbial cultivations (ml)	Results
Tangsuntornkhan *et al*. (2010)	150	The highest ethanol concentration of 41.2 ± 1.6 g/l with Yp/s 0.42 ± 0.02 g ethanol/g total sugar were obtain from *C. utilis* TISTR 5352 in dried longan extract. Maximum specific sucrose, glucose and fructose consumption rate were 0.549, 0.127 and 0.094 g sugar/g biomass/h with the corresponding maximum specific ethanol production rate and maximum specific growth rate of 0.181 g ethanol/g biomass/h and 0.035 ± 0.003 per h, respectively.

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Table 2.10 (Cont.) The growth and ethanol production kinetics of six *C. utilis* strains

Researcher team	**Levels of the microbial cultivations (ml)**	**Results**
Tangsuntornkhan *et al*. (2010)	1,500	The highest ethanol concentration of 6.28 ± 0.06 g/l with Yp/s 0.27 ± 0.01 g ethanol/g total sugar were obtain from *C. utilis* TISTR 5352 in dried longan extract. Maximum specific sucrose, glucose and fructose consumption rate were 0.057, 0.007 and 0.002 g sugar/g biomass/h with the corresponding maximum specific ethanol production rate and maximum specific growth rate of 0.007 g ethanol/g biomass/h and 0.008 ± 0.000 per h, respectively.

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2.6.9 The development of mathematical model for ethanol production from three types of sugars commonly found in dried longan extract

Previous research results suggested that *S. 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, sucrose, glucose and fructose. The development of mathematical model for ethanol production kinetics in batch system for *S. cerevisiae* TISTR 5606 was essential for prediction of the kinetic profiles involving sugar utilization in fermentation system. This can be useful for the further optimization of final ethanol concentration level and productivity based on fed batch and continuous production systems.

Saikeaw *et al*. (2010) investigated growth and ethanol production kinetics of *S. cerevisiae* TISTR 5606 in 1,500 ml scale under static condition, 25.6°C for 36 h with various types of carbon source as following;

1. Pure analytical grade sugar (sucrose, glucose and sucrose) with an initial concentration of 40 g/l,
2. The mixture of sucrose, glucose and fructose (in g/l) at 20/20/20, 30/30/30, 40/40/40 and 60/60/60
3. Dried longan extract with the initial overall sugar concentration levels of 60, 120 and 180 g/l.

The analytical of RSS (Residual Sum of Square) value indicated good prediction of experimental data by the mathematical model with relatively low total RSS as shown in Table 2.11.

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Table 2.11 The development of mathematical model for ethanol production from three types of sugars commonly found in dried longan extract

Researcher team	Carbon sources	results
Saikeaw *et al*. (2010)	Pure analytical grade sugar with an initial concentration of 40 g/l	The simulated curves predicted the experimental profiles relatively well with RSS levels for sucrose, glucose and fructose of 83.0, 25.9 and 67.7, respectively.
	The mixture of sucrose, glucose and fructose (in g/l) at 20/20/20, 30/30/30, 40/40/40 and 60/60/60	The simulated curves predicted in experimental profiles relatively well. The triple substrate model for the cultivation media consisting of sucrose/glucose/fructose at 20/20/20, 30/30/30, 40/40/40 and 60/60/60 resulted in the good fitting with RSS values of 68.4, 227, 101 and 83.8, respectively.
	Dried longan extract with the initial overall sugar concentration levels of 60, 120 and 180 g/l	The simulated curves predicted the experimental profiles relatively well with total RSS value of 183, 320 and 359 for the overall sugars concentration of 60, 120 and 180 g/l, respectively.

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2.6.10 PAC production

2.6.10.1 PAC production in static system for a single solvent system

Kanchanwong *et al*. (2008) investigated the subsequent biotransformation experiment in a static system by utilizing the wet biomass of *S. cerevisiae* TISTR 5606 with 0.53 g dry biomass equivalent/l from section 2.6.3 at 4 and 20°C for 24 h. The buffer solution was replaced with four solvents as shown in Table 12. The production of PAC was not detected in any of the examined solvents.

Table 2.12 PAC production in a static condition for a single solvent system

Researcher team	Solvents	Results
Kanchanwong *et al*. (2008)	1. Distilled water 2. Octanol (C8) 3. Dipropylene Glycol (DPG) 4. Mixture of octanol and DPG in 1:1 ratio	The production of PAC was not detected in any of the examined solvents.

2.6.10.2 PAC production in static system for a two phase separated system

Poodtatep *et al*. (2008) and Phrathong *et al*. (2008) investigated the subsequent biotransformation experiment in a static system for a two – phase separated system in different condition by utilizing the selected microbes from Section 2.6.3 for PAC production as shown in Table 2.13. The results showed that the PAC production capability of *C. utilis* TISTR 5198 was better than *S. cerevisiae* TISTR 5606 at the same level of biomass concentration.

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Table 2.13 PAC production in static system for a two phases separated system

Researcher team	Experiment conditions	Results
Poodtatep *et al*. (2008)	The experiment utilized wet biomass of *S. cerevisiae* TISTR 5606 with 3.06 g/l dry biomass equivalent in a static system at 4°C and 20°C for 24 h. Octanol was used as a solvent.	*S. cerevisiae* TISTR 5606 generated overall PAC concentration of 3.97 and 3.72 mM at 4 and 20°C.
Phrathong *et al*. (2008)	The experiment utilized wet biomass of *C. utilis* TISTR 5198 and *S. cerevisiae* TISTR 5606 of 2.01 g/l at 4°C for 8 h by varying phosphate buffer concentration ranging from 0, 300, 600 and 900 mM.	The PAC production ability of *C. utilis* TISTR 5198 was diminished with elevating phosphate buffer concentration while the opposite was true for *S. cerevisiae* TISTR 5606 whose PAC production level of 1.19 ± 0.01 mM was observed at 900 mM phosephate concentration.

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2.6.10.3 PAC production in shaking system for a two phase separated system

Tangsuntornkhan *et al.* (2010) and Chaweekunlayakun *et al.* (2010) investigated the subsequent biotransformation experiment in a shaking system for PAC production by whole cells from two *C. utilis* strains (TISTR 5198 and 5352) and *S. cerevisiae* TISTR 5606. These microbes were cultivated in two years dried longan extract and DDLFH media with the following levels of dried biomass equivalent;

Microbes	**Carbon sources**	**Level of dried biomass equivalent (g/l)**
C. utilis TISTR 5198 and 5352	DDLFH	6.12
	Two years old dried longan extract	6.12
S. cerevisiae TISTR 5606	DDLFH	3.06
	Two years old dried longan extract	3.06, 6.12 and 12.24

The biotransformation bottles were placed in a shaking incubator at 250 rpm, 8°C for 72 h. The shaking speed utilized in this experiment could not thoroughly mixed the two liquid phases as evident from the clearly defined liquid layers. The absence or minimal level of PAC production was resulted which indicated the effect of separating organic/aqueous phase minimizing the exposure of whole cells to benzaldehyde substrate within the specified time period. The whole cells of *C. utilis* TISTR 5198 cultivated in a DDLFH medium with 6.12 g/l of dried biomass equivalent could produce the highest PAC production level of 1.76 ± 0.06 mM as shown in Table 2.14.

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Table 2.14 PAC production in shaking system for a two phases separated system

Researcher team	Microbes	Results
Tangsuntornkhan *et al*. (2010)	*C. utilis* TISTR 5198	The microbes which were cultivated in a DDLFH medium could produce the highest PAC concentration level of 1.76 ± 0.06 mM.
	C. utilis TISTR 5352	The microbes which were cultivated in two years dried longan extract medium could produce the highest PAC concentration level of 0.75 ± 0.02 mM.
Chaweekunlayakun *et al*. (2010)	*S. cerevisiae* TISTR 5606	The absence of the PAC production was detected.

2.6.10.4 PAC production in static system for a two phase emulsion system

Jaiwanglok *et al*. (2008) investigated the subsequent biotransformation experiment for PAC production by whole cells from *S. cerevisiae* TISTR 5606 with 3.06 g/l of dried biomass equivalent in a shaking incubator at 200 rpm, 6°C for 24 h. The organic phase contained various primary alcohol (C1 – C10) or DPG or mixture of various primary alcohol and DPG (C1 + DPG, C2 + DPG, C3 + DPG, C7 + DPG, C8 + DPG and C9 + DPG) in 1:1 ratio as shown in Table 2.15. The application of octanol phase yielded the highest PAC concentration.

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Table 2.15 PAC production in static system for a two phases emulsion system

Researcher team	Solvents in the organic phase	Results
Jaiwanglok *et al*. (2008)	1. C1 2. C2 3. C3 4. C4 5. C5 6. C6 7. C7 8. C8 9. C9 10. C10 11. DPG 12. C1 + DPG 13. C2 + DPG 14. C3 + DPG 15. C7 + DPG 16. C8 + DPG 17. C9 + DPG	The application of octanol as organic phase yielded the highest PAC concentration of 10.4 ± 0.2 mM.

Kumtip *et al*. (2009), Tadkeaw *et al*. (2009) and Buakham *et al*. (2009) investigated the subsequent biotransformation experiment of PAC in static condition for a two phases emulsion system. The whole cells from *S. cerevisiae* TISTR 5606, which was cultivated in variation of carbon source, were applied at 3.06 and 6.12 g/l of dried biomass equivalent. The organic phase contained various long chain alcohols (C7 – C9) or mixture of primary alcohol and DPG (C7 + DPG, C8 + DPG and C9 + DPG). The experiment was carried out in a shaking incubator at 250 rpm, 8°C for 72 h as shown in Table 2.16. The results showed that *S. cerevisiae* TISTR 5606, which was cultivated in 6 years old dried longan extract as a sole carbon source, could

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produce the highest average PAC concentration in both phases by using DPG and C8 as an organic solvent mixture.

Table 2.16 PAC production in static system for a two phase emulsion system with variation of carbon source

Researcher team	Carbon sources	Results
Tadkeaw *et al*. (2007)	Molasses only	The application of the whole cells with 6.12 g/l of dried biomass equivalent could produce the highest average PAC concentration in both phases of 29.3 ± 5.2 mM by using DPG and C8 as an organic solvent mixture.
Buakham *et al*. (2009)	Six years old dried longan extract only	The application of the whole cells with 6.12 g/l of dried biomass equivalent could produce the highest average PAC concentration in both phases of 34.2 ± 10.4 mM by using DPG and C8 as an organic solvent mixture.
Kumtip *et al*. (2009)	Mixture of 6 years old dried longan extract and molasses in 1:1 ratio	The application of the whole cells with 6.12 g/l of dried biomass equivalent could produce the highest average PAC concentration in both phases of 27.8 ± 4.4 mM by using DPG and C8 as an organic solvent mixture.

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Agustina *et al*. (2009) investigated the subsequent biotransformation experiment for a two phase emulsion system. The whole cells from *S. cerevisiae* TISTR 5606 and *C. utilis* TISTR, which were cultivated in 10 mo old dried longan extract as a sole carbon source, were applied at 6.12 and 12.24 g/l of dried biomass equivalent. The organic phase contained various primary alcohols (C1 – C10) or mixture of various primary alcohol and DPG (C1 + DPG, C2 + DPG, C3 + DPG, C4 + DPG, C5 + DPG, C6 + DPG, C7 + DPG, C8 + DPG, C9 + DPG and C10 + DPG). The experiment was carried out in a shaking incubator at 250 rpm, 8°C for 72 h as shown in Table 2.17. The results showed that whole cells from *C. utilis* TISTR 5198 could produce higher level of average PAC concentration than *S. cerevisiae* TISTR 5606.

Table 2.17 PAC production for a two phases emulsion system from whole cells of microbes with 10 mo old dried longan extract as a sole carbon source

Researcher team	**Whole cells from the microbes**	**Results**
Agustina *et al*. (2009)	*S. cerevisiae* TISTR 5606	The application of the whole cells with 12.24 g/l of dried biomass equivalent could produce the highest average PAC concentration in both phases of 37.4 ± 2.8 mM by using DPG and C8 as an organic solvent mixture.
	C. utilis TISTR 5198	The application of the whole cells with 12.24 g/l of dried biomass equivalent could produce the highest average PAC concentration in both phases of 83.8 ± 6.8 mM by using C9 as an organic solvent.

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Leksawasdi (2004) investigated the subsequent biotransformation experiment on a two phase emulsion system using partially purified PDC extracted from *C. utilis* UNSW 709400 with 1.55 M benzaldehyde and 1.83 M pyruvate as substrates. The aqueous phase contained various type of buffers (2.5 M 3-[N-morpholino] propanesulfonic acid (MOPS), 20 mM MOPS, and 20 mM MOPS + 2.5 DPG). The experiment was carried out at 4°C with pH control at 7.0 as shown in Table 2.18. The results showed that the application of partially purified PDC extracted from *C. utilis* UNSW 709400 in 20 mM MOPS and 2.5 M DPG could produce the similar level of average PAC concentration (1.01 M in organic phase and 115 mM in aqueous phase, the standard errors were not given) to the system with a more expensive 2.5 M MOPS buffer.

Table 2.18 PAC production for a two phases emulsion system from partially purified PDC extracted of *C. utilis* UNSW 709400 with the aqueous phase contained various type of buffers

Researcher team	Buffer	Results
Leksawasdi (2004)	1. 2.5 M MOPS 2. 20 mM MOPS 3. 20 mM MOPS + 2.5 DPG	The application of the partially purified PDC extracted from *C. utilis* UNSW 709400 with 20 mM MOPS and 2.5 M DPG could produce the similar level of average PAC concentration (1.01 M in organic phase and 115 mM in aqueous phase) to the system with a more expensive 2.5 M MOPS buffer.

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Rosche *et al*. (2005) performed the biotransformation study on a two phase emulsion system with 1.44 M benzaldehyde and 1.43 M pyruvate as substrates in 2.5 M MOPS buffer at 21°C using *C. utilis* UNSW 709400 PDC in the form of resting cells as summarised in Table 2.19. The results showed that the application of resting cells from *C. utilis* UNSW 709400 could produce the average PAC concentration level of 386 mM (standard error was not reported).

Table 2.19 PAC production for a two phases emulsion system from resting cells of *C. utilis* UNSW 709400

Researcher team	Type of PDC preparation	Results
Rosche *et al*. (2005)	resting cells	The application of the resting cells of *C. utilis* UNSW 709400 could produce the average PAC concentration level at 386 mM (standard error was not reported).

Gunawan (2006) investigated the two phase emulsion biotransformation system with 3.6 M benzaldehyde and 0.785 M pyruvate as substrates in a 20 mM MOPS buffer mixed with 2.5 M DPG at 20°C using pH control at 7.0. Two types of PDC preparation were employed as biocatalysts for PAC production, which were whole cells and partially purified PDC from *C. utilis* UNSW 709400 as shown in Table 2.20. The results showed that the application of whole cells from *C. utilis* UNSW 709400 could produce higher level of average PAC concentration than partially purified PDC.

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Table 2.20 PAC production for a two phases emulsion system from whole cells and partially purified PDC of *C. utilis* UNSW 709400

Researcher team	Type of PDC preparation	Results
Gunawan (2006)	resting cells	The application of the whole cells of *C. utilis* UNSW 709400 could produce the highest average PAC concentration in both phases of 172 mM (standard error was not reported).

Satianegara (2006) investigated the two phase emulsion biotransformation system using whole cells of *C. utilis* UNSW 709400 with 1.7 M benzaldehyde and 1.4 M pyruvate as substrates in 2.5 M MOPS buffer at 21°C with/without pH control at 6.5 as shown in Table 2.21. The results showed that the application of whole cells from *C. utilis* UNSW 709400 with and without pH control could produce the similar level of average PAC concentration of 400 mM (standard error was not reported).

Table 2.21 PAC production for a two phases emulsion system from whole cells of *C. utilis* UNSW 709400 with/without pH control

Researcher team	Conditons	Results
Satianegara (2006)	1. pH control 2. No pH control	The application of the whole cells of *C. utilis* UNSW 709400 with and without pH control could produce the average PAC concentration in both phases at the similar level of 400 mM (standard error was not reported).

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