CHAPTER 4

Optimization of fermentation conditions for soy isoflavone aglycones production by *Bacillus coagulans* PR03 in laboratory scale

4.1 Introduction

From the previous study, it was found that *Bacillus coagulans* PR03 was the suitable pure culture for soybean isoflavone aglycone production. Thus, in this experiment, the pure culture of *B. coagulans* PR03 was used to find optimal fermentation conditions for isoflavone aglycones production in a laboratory scale. The aim of experiment was to study solid state fermentation, which is the simplest technology with high productivity, good oxygen circulation, low energy requirement and resemble the natural habitat for several microorganisms (Couto and Sanromán, 2006; Pandey, 2003). For soybean fermentation, Lekhakula (2010) reported that optimum conditions for Thai fermented soybean were boiled soybean for 6 h and fermented at 30ºC for 3 days with mixed culture of selected *Bacillus* strains. While, Dajanta *et al.* (2009) reported optimum fermentation conditions for high aglycone fermented soybean was 42 ºC for 72 h. The distributions of individual isoflavones in soybean seeds differ in the hypocotyl or soygerm and cotyledon. Eldridge and Kwolek (1983) and Wang and Murphy (1994) reported that isoflavones content in the soygerm was higher than the content in cotyledon. So, there were several factors that need to be determined when using solid state fermentation to ensure an effective fermentation. For this chapter, fermentation condition was optimized including type of raw material, fermented condition, inoculum concentration and fermentation temperature.

Consequently, the first step in this study was designed to determine a suitable substrate for isoflavone glucoside production from various varieties and parts of soy beans. Next, the fermentation conditions including inoculum concentration and fermentation temperature was investigated. The optimum conditions were determined and used to

calculate the coefficient of the laboratory scale isoflavone aglycone production. Finally, kinetic study was used to predict a suitable time to harvest isoflavone aglycones.

4.2 Methods

4.2.1 Selection of suitable source of isoflavone glucoside production from various soy varieties

The seed of various soybean varieties cultivated in northern part of Thailand, such as Chiang Mai1, Chiang Mai 60 and SJ2, was purchased from the Chiang Mai field crop research center (Chiang Mai, Thailand). Each of soybean varieties was separated into germ, cotyledon and hull (Figure 4.1). All separated parts of soybean seed was analyzed for isoflavone glucoside content (see Appendix A). The part of soybean seed with highest isoflavone glucosides would be used as the source of isoflavone glucoside production

(A) **(B)** **(C)**

Figure 4.1 Different parts of soybean seed: (A) germ (B) cotyledon and (C) hull.

4.2.2 Optimum fermented condition for isoflavone aglycones production by *B. coagulans* PR03

The fermentation conditions were investigated to find the suitable condition including anaerobic, facultative anaerobic and aerobic fermentation conditions.

For the fermented soybean preparation, the suitable part and variety of soybean from section 4.2.1 were used. Initially, one hundred grams from a particular part of soybean were washed 2 times with clean water and drained for 30 min at ambient temperature (30-35°C). After draining, the part of soybean was transferred to 250 ml glass bottle (Duran®), filled with 200 ml of water, sealed with cotton wool and sterilized at 121ºC for 15 min. Then, the sterilized part of soybean was inoculated with 1% (v/w) of *B. coagulans* PR03. The inoculum of *B. coagulans* PR03 was cultured in nutrient broth (NB) and incubated at 37ºC and 120 rpm for 18 h before used.

The conditions for fermentation were divided into 3 treatments (Figure 4.2):

- Anaerobic fermentation: incubate in anaerobic jar
- Facultative anaerobic fermentation
- Aerobic fermentation: air flow rate 1 L/min

All treatments were incubated at 30ºC for 72 h. Finally, the fermented part of soybean was freeze dried and grinded into powder before isoflavones analysis (see Appendix A). The fermented condition with highest isoflavone aglycones was selected.

(A) (B) (C)

Figure 4.2 Fermentation conditions:

(A) anaerobic fermentation (B) facultative anaerobic fermentation and

(C) aerobic fermentation with air pump and 0.2 µm air filter

4.2.3 Optimum inoculum concentration of *B. coagulans* PR03 for isoflavone aglycones production

The suitable source of isoflavone glucosides and optimum condition for fermentation were used in this study. In this experiment, the concentration of *B. coagulans* PR03 was varied as followed; 1, 5, 10, 15 and 20 % (v/w). The fermented soybean preparation followed the process described in section 4.2.2. After the fermentation, fermented soybeans were freeze dried and grinded into powder before isoflavones analysis (see Appendix A). The optimum inoculum concentration with highest isoflavone aglycones was selected.

4.2.4 Optimum fermentation temperature for isoflavone aglycones production by *B. coagulans* PR03

The suitable source of isoflavone glucosides, fermentation condition and inoculum concentration were used in this study. The varied fermentation temperature were 30, 35, 40 and 45 ^{o}C. Soy samples were prepared according to the process in section 4.2.2. After the fermentation, fermented soybean samples were freeze dried and grinded into powder before isoflavones analysis (see Appendix A). The optimum fermentation temperature with highest isoflavone aglycones was selected.

4.2.5 Kinetic Study of isoflavone aglycones production by *B. coagulans* PR03

The optimum conditions from section 4.2.2-4.2.5 were used to produce isoflavone aglycones. The fermentation time was 10 days. During the fermentation period, fermented soybeans were daily analyzed for isoflavones content (see appendix A), β-glucosidase activity (see section 3.2.3) and total bacterial count (see section 3.2.3). The results were used to predict the suitable time of isoflavone aglycone harvesting. After the suitable harvesting time was determined, the results during that time were used to study the kinetic of laboratory scale isoflavone aglycones production including specific growth rate of *B. coagulans* PR03 (μ), product yield coefficient ($Y_{p/s}$), specific product usage rate (q_s) and specific product formation rate (q_p).

4.2.5.1 Specific growth rate of *B. coagulans* PR03 (μ)

Growth rate: $dX/dt = \mu X$ ---------- 1

When dX/dt = change of cell number (X) in time period (t)

$$dX/X = \mu \, . \, dt \quad \text{---------- 2}$$

Integrate Equation 2:

$$X_t = X_0 \, . \, e^{\mu t} \quad \text{---------- 3}$$

When X_0 = initial cell number of *B. coagulans* PR03

X_t = cell number of *B. coagulans* PR03 at t hr

Take natural logarithm in Equation 3:

$$\ln X_t = \ln X_0 + \mu t \quad \text{---------- 4}$$

Rearrange Equation 4 to equation of specific growth rate:

$$\mu = \ln (X_t/X_0) / t \quad \text{---------- 5}$$

4.2.5.2 Product yield coefficient

Product yield coefficient ($Y_{p/s}$) of isoflavone aglycones was calculated according to the following equation:

$$Y_{p/s} = \Delta P/ \Delta S \quad \text{---------- 6}$$

When $Y_{p/s}$ = product yield coefficient

ΔP = increase of product mass

ΔS = decrease of substrate mass

So: $$Y_{agy/glu} = (P_{agy}1- P_{agy}0)/(S_{glu}0- S_{glu}1) \quad \text{---------- 7}$$

When $Y_{agy/glu}$ = isoflavone aglycones yield coefficient

$P_{agy}1$ = final isoflavone aglycones content

$P_{agy}0$ = initial isoflavone aglycones content

$S_{glu}0$ = initial isoflavone glucosides content

$S_{glu}1$ = final isoflavone glucosides content

4.2.5.3 Kinetic of substrate usage

The isoflavone aglycones production is batch fermentation, which neither add nor reduce substrate during fermentation. So, the specific substrate usage rate (q_s) was calculated with this equation.

Substrate usage rate = growth rate – product formation – live energy

$-dS/dt = -\mu X/Y_{x/s} - q_p X/Y_{p/s} - mX$ ---------- 8

$-(1/X)dS/dt = -\mu/Y_{x/s} - q_p/Y_{p/s} - m = -q_s$ ---------- 9

$(1/X)dS/dt = \mu/Y_{x/s} + q_p/Y_{p/s} + m = q_s$ ---------- 10

So: $q_s = (1/X)dS/dt$ ---------- 11

When q_s = specific substrate usage rate

X = cell number

dS/dt = substrate usage rate

Divide Equation 11 with Equation 1:

$q_s/\mu = [(1/X)dS/dt] \times [X \bullet dt/dX]$ ---------- 12

$q_s/\mu = dS/dX$ ---------- 13

Specific substrate usage rate was found from Equation 14:

$q_s = (dS/dX)\,\mu$ ---------- 14

4.2.5.4 Kinetic of product formation

The isoflavone aglycones production is batch fermentation, which does not lose the product during fermentation. So, the specific product formation rate (q_p) was calculated with this equation.

$$dP/dt = (q_pX) \quad \text{---------- 15}$$

$$q_p = (1/X)dP/dt \quad \text{---------- 16}$$

When q_p = specific product formation rate

X = cell number

dP/dt = product formation rate

Divide Equation 16 with Equation 1:

$$q_p/\mu = [(1/X)dP/dt]\times[X\bullet dt/dX] \quad \text{---------- 17}$$

$$q_p/\mu = dP/dX \quad \text{---------- 18}$$

Specific substrate usage rate was determined from Equation 19:

$$q_p = (dP/dX)\,\mu \quad \text{---------- 19}$$

4.3 Results and discussion

4.3.1 Selection of suitable soybean varieties for source of isoflavone glucosides

The isoflavone glucoside contents in germ, cotyledon and hull from soybean seed (Chiang Mai 1, Chiang Mai 60 and SJ2 varieties) are shown in table 4.1. It was found that, isoflavone glucoside contents in each parts of soybean seed from different soybean varieties (Chiang Mai 1, Chiang Mai 60 and SJ2) were significantly different (P<0.05). Considering isoflavone glucoside contents in germ, it was found that SJ2 variety had the highest amount of glucosides (1,082.89 mg/100g), which included daidzin, genistin and glycitin in an amount of 570.98, 155.23 and 356.68 mg/100g, respectively. Whereas, Chiang Mai 60 and Chiang Mai 1 varieties had a similar total content of glucosides; 597.93 and 476.48 mg/100g, respectively. Glucosides from Chiang Mai 60 variety comprised of daidzin, genistin and glycitin in the number of 334.04, 75.76 and 118.12

mg/100g, respectively. On the other hand, glucosides from Chiang Mai 1 variety contained daidzin and genistin in the number of 404.90 and 71.59 mg/100g, respectively; while glycitin was not found. When examined the isoflavone glucoside contents in cotyledon and hull, it was found that they had a smaller amount of the compounds than germ. Cotyledon and hull had total glucosides contents between 21.26 – 37.59 mg/100g and 10.21 – 13.42 mg/100g, respectively. In addition, glycitin in cotyledon and hull were not detected. Thus, from the results, it indicated that isoflavone glucosides could be found in every part of soybean seed. However, it was mostly found in soygerm. This result was similar to the report from Wang and Murphy (1994) which showed that isoflavones content in the soygerm was higher than the content in cotyledon. On the other hand, Eldridge and Kwolek (1983) had studied isoflavones in two varieties of soybean (Amsoy and Tiger) and found that Amsoy variety has more isoflavone glucosides than Tiger variety. The content of isoflavone glucosides were highest in the soygerm (1,400-1,700 mg/100g) and lowest in the hull of the seed (10-20 mg/100g), with the cotyledons containing moderate amount between 150-320 mg/100g.

In conclusion, soygerm of SJ2 variety was suitable source of isoflavone glucosides for isoflavone aglycones production

Table 4.1 Isoflavone glucoside contents in different parts of soybean seed from Chiang Mai 1, Chiang Mai 60 and SJ2 varieties.

Part of soybean seed	Varieties of soybean	Isoflavone glucosides content (mg/100g dry weight)			
		Daidzin	Genistin	Glycitin	Total
Germ	Chiang Mai 1	404.90±1.66^{b}	71.59±0.80^{b}	ND	476.48±2.45^{c}
	Chiang Mai 60	334.04±4.38^{c}	75.76±0.38^{b}	188.12±2.02^{b}	597.93±6.78^{b}
	SJ2	570.98±13.76^{a}	155.23±2.26^{a}	356.68±10.01^{a}	1,082.89±26.03^{a}
Cotyledon	Chiang Mai 1	9.03±0.44^{b}	21.54±0.11^{b}	ND	30.57±0.56^{b}
	Chiang Mai 60	6.04±0.38^{c}	15.22±0.18^{c}	ND	21.26±0.55^{c}
	SJ2	12.38±0.26^{a}	25.21±0.21^{a}	ND	37.59±0.46^{a}
Hull	Chiang Mai 1	7.92±0.03	3.45±0.01^{b}	ND	11.37±0.03^{b}
	Chiang Mai 60	8.04±0.13	2.16±0.08^{c}	ND	10.20±0.31^{c}
	SJ2	7.98±0.16	5.43±0.06^{a}	ND	13.42±0.21^{a}

Note: ND = not detected. Means within same column with different superscripts in same part of soybean seed are significantly different (P<0.05).

4.3.2 Optimal fermentation condition for isoflavone aglycones production by *B. coagulans* PR03

The experiment in section 4.3.1 concluded that the soygerm of SJ2 variety was the suitable source of isoflavone glucosides. This experiment studied the fermentation condition using soygerm of SJ2 variety to produce isoflavone aglycones by *B. coagulans* PR03. The results are shown in Table 4.2. It was found that the fermentation conditions were significantly affected the quantities of isoflavone aglycones and isoflavones glucosides (P<0.05). The initial contents of daidzein, genistein, glycitein and total aglycones in non-fermented soygerm were 104.60, 29.52, 26.96 and 161.08 mg/ 100 g, respectively. After fermentation processes by *B. coagulans* PR03, it was found that aerobic fermentation was the most effective condition in increasing the isoflavone aglycone content. The contents of daidzein, genistein, glycitein and total aglycones were increased

to 278.42, 106.10, 163.97 and 548.57 mg/100 g, respectively. Facultative anaerobic fermentation was secondly affected the amount the compounds to 234.86, 74.39, 112.52 and 428.77 mg/100 g, respectively. While, the anaerobic fermentation showed the smallest effect with a small increase in the contents of daidzein, genistein, glycitein and total aglycones (117.09, 54.04, 40.01 and 211.14 mg/100 g, respectively). The increases of isoflavone aglycones were also reflected by the decreases of isoflavone glucosides. For instance, the values of daidzin, genistin, glycitin and total glucoside of aerobic fermentation were reduced to 255.14, 74.11, 175.22 and 504.47 mg/100 g, respectively. For facultative anaerobic fermentation, it had the values of isoflavone glucosides more than aerobic fermentation, which were 304.57, 101.32, 255.32 and 631.21 mg/100 g, respectively. Lastly, anaerobic fermentation had the highest values of isoflavone glucosides; 551.24, 145.24, 347.56 and 1,044.04 mg/100 g, respectively. The values were similar to the initial values of non-fermented soygerm, which were 570.98, 155.23, 356.68 and 1.082.89 mg/100 g, respectively.

The increasing amounts of isoflavone aglycones were resulted from hydrolysis of β-glycosidic linkage of isoflavone glucosides by β-glucosidase during fermentation (Kuo *et al.*, 2006; Toda *et al.*, 2001; Yin *et al.*, 2004). Therefore, aerobic fermentation condition might increase the production of β-glucosidase by *B. coagulans* PR03 more than anaerobic and facultative anaerobic fermentation could. As a result, the most suitable condition was aerobic fermentation and used in the next experiment.

4.3.3 Optimal inoculum concentration of *B. coagulans* PR03 for isoflavone aglycones production

The experiment in section 4.3.1 and 4.3.2 had found that the soygerm of SJ2 variety and aerobic fermentation were suitable for isoflavone aglycone production. This experiment aimed to study optimal inoculum concentration of *B. coagulans* PR03 for isoflavone aglycone production. The inoculum concentrations were changed from 1, 5, 10, 15 and 20% (v/w). The results are shown in Table 4.3. It was found that the inoculum concentrations of *B. coagulans* PR03 were significantly affected the quantities of isoflavone aglycones and isoflavone glucosides ($P<0.05$). The amounts of daidzein, genistein, glycitein and total aglycones in non-fermented soygerm started at 104.60, 29.52, 26.96 and 161.08 mg/ 100 g, respectively. After fermentation process, it was found that 15% (v/w) of inoculum

concentration was greatly increased total aglycones content to 943.61 mg/100 g which consisted of daidzein, genistein and glycitein in an amount of 493.35, 117.99 and 332.27 mg/100 g, respectively. 20% (v/w) of inoculum concentration was secondly affected the increasing of total aglycones to 938.17 mg/100 g which consisted of daidzein, genistein and glycitein at an amount of 494.72, 114.96 and 333.17 mg/100 g, respectively. For inoculum concentration of 10, 5 and 1% (v/w), isoflavone aglycones showed only a moderate increase with the least effect from 1% (v/w) of inoculum concentration, which had daidzein, genistein, glycitein and total aglycones at an amount of 312.67, 81.97, 144.59 and 539.23 mg/100 g, respectively. Furthermore, inoculum concentration of 15% (v/w) had also showed the highest decrease in isoflavone glucoside. It decreased the values of daidzin, genistin, glycitin and total glucoside to 150.40, 38.84, 116.79 and 302.03 mg/100 g, respectively. However, when considering the amounts of isoflavone aglycones at 15 and 20% (v/w) inoculum concentrations, the analysis showed that both conditions were not significantly difference (P>0.05). Therefore, the chosen suitable inoculum concentration of *B. coagulans* PR03 was 15 % (v/w) and was used in the next experiment.

Table 4.2 Isoflavone contents in fermented soygerm (SJ2 variety) at the different fermentation conditions

Fermentation condition	Aglycones (mg/100 g dry weight)				Glucosides(mg/100 g dry weight)			
	Daidzein	Genistein	Glycitein	Total aglycones	Daidzin	Genistin	Glycitin	Total glucosides
Anaerobic	117.09±0.53[a]	54.04±0.07[a]	40.01±0.07[a]	211.14±0.59[a]	551.24±3.11[a]	145.24±1.43[a]	347.56±4.54[a]	1,044.04±4.54[a]
Facultative anaerobic	234.86±1.02[b]	74.39±0.32[a]	119.52±0.39[a]	428.77±1.10[b]	304.57±0.44[b]	101.32±0.11[b]	225.32±4.54[a]	631.21±0.56[b]
Aerobic	278.42±0.50[b]	106.10±0.01[b]	163.97±0.06[b]	548.57±0.55[b]	255.14±0.03[c]	74.11±0.01[c]	175.22±0.01[c]	504.47±0.03[c]
Non fermented soygerm	104.60±3.08	29.52±0.72	26.96±0.97	161.08±4.76	570.98±13.76	155.23±2.26	356.68±10.01	1,082.89±26.03

Note: Means within same column with different superscripts are significantly different (P<0.05).

Table 4.3 Isoflavone contents in fermented soygerm (SJ2 variety) at the different inoculum concentrations of *Bacillus coagulans* PR03

Inoculum Concentration (%)	Aglycones content (mg/100 g dry weight)				Glucosides content (mg/100 g dry weight)			
	Daidzein	Genistein	Glycitein	Total Aglycones	Daidzin	Genistin	Glycitin	Total Glucosides
1	312.67±1.56[a]	81.97±1.86[a]	144.59±0.50[a]	539.23±3.92[a]	351.89±2.35[e]	98.18±1.49[d]	258.03±2.23[d]	708.10±3.08[d]
5	405.31±0.79[b]	100.93±0.59[b]	255.80±0.28[b]	762.03±1.09[b]	255.95±1.96[d]	62.90±1.89[c]	156.47±1.40[c]	475.32±5.25[c]
10	445.79±0.65[c]	110.43±1.11[c]	295.21±0.63[c]	851.43±1.13[c]	201.89±2.35[c]	51.55±0.45[b]	142.00±0.62[b]	395.44±2.18[b]
15	493.35±1.25[d]	117.99±2.06[d]	332.27±2.15[d]	943.61±1.34[d]	150.40±0.59[a]	34.84±1.05[a]	116.79±1.38[a]	302.03±0.26[a]
20	494.72±1.65[d]	114.96±0.86[c]	333.17±0.54[d]	938.84±1.34[d]	156.79±1.57[b]	33.57±0.46[a]	114.33±0.93[a]	304.69±0.17[a]
Non fermented soygerm	104.60±3.08	29.52±0.72	26.96±0.97	161.08±4.76	570.98±13.76	155.23±2.26	356.68±10.01	1,082.89±26.03

Note: Means within same column with different superscripts are significantly different (P<0.05).

Table 4.4 Isoflavone contents in fermented soygerm (SJ2 variety) at the different fermentation temperatures

Teperature (°c)	Aglycones content (mg/100 g dry weight)				Glucosides content (mg/100 g dry weight)			
	Daidzein	Genistein	Glycitein	Total Aglycones	Daidzin	Genistin	Glycitin	Total Glucosides
30	492.52±2.47^{c}	122.99±2.05^{c}	334.73±1.40^{c}	950.24±0.97^{d}	152.89±2.19^{a}	34.49±1.33^{a}	114.55±1.42^{a}	301.93±0.56^{a}
35	493.35±1.25^{c}	117.99±2.06^{c}	332.27±2.15^{c}	943.61c±1.34^{c}	150.40±0.59^{a}	34.84±1.05^{a}	116.79±1.38^{a}	302.03±0.26^{a}
40	474.30±1.49^{b}	98.61±1.32^{b}	315.26±0.70^{b}	888.17±0.87^{b}	171.94±1.96^{b}	54.05±1.68^{b}	131.05±0.72^{b}	357.04±1.01^{b}
45	434.45±1.56^{a}	81.99±2.06^{a}	276.79±1.70^{a}	793.23±1.92^{a}	215.95±1.96^{c}	62.90±1.89^{c}	176.47±1.40^{c}	455.32±5.25^{c}
Non fermented soygerm	104.60±3.08	29.52±0.72	26.96±0.97	161.08±4.76	570.98±13.76	155.23±2.26	356.68±10.01	1,082.89±26.03

Note: Means within same column with different superscripts are significantly different (P<0.05).

4.3.4 Optimal fermentation temperature for isoflavone aglycones production by *B.coagulans* PR03

The experiment in section 4.3.1 - 4.3.3 had found that the soygerm (SJ2 variety), aerobic fermentation and 15% inoculum concentration of *B. coagulans* PR03 were suitable for isoflevone aglycone production. This experiment studied optimal fermentation temperature for isoflavone aglycones production. The fermentation temperatures were changed as followed; 30, 35, 40 and 45 ^{o}C. The results are shown in Table 4.4. It was found that the fermentation temperatures were significantly affected the quantities of isoflavone aglycones and isoflavones glucosides (P<0.05). The number of daidzein, genistein, glycitein and total aglycones in non-fermented soygerm started at 104.60, 29.52, 26.96 and 161.08 mg/ 100 g, respectively. After fermentation process, it was found that the fermentation at 30 ^{o}C was greatly increased the quantities of isoflavone aglycones to 492.52, 122.99, 334.73 and 950.24 mg/100 g, respectively. Secondly, fermentation at 35 ^{o}C also significantly increased the amounts of the compounds to 493.35, 117.99, 332.27 and 943.61 mg/100 g, respectively. The fermentation at 40 ^{o}C had a smaller effect on the quantities of the compounds (474.30, 98.61, 315.26 and 888.17 mg/100 g, respectively) where the fermentation at 45 ^{o}C showed the least effect, which only increased the aglycone contents to 435.45, 81.99, 276.79 and 793.23 mg/100 g respectively. Moreover, fermentation at 30 ^{o}C decreased the values of daidzin, genistin, glycitin and total glucosides to 152.89, 34.49, 114.55 and 301.93 mg/100 g, respectively. However, when considering the amounts of isoflavone aglycones at 30 and 35 ^{o}C fermentations, it was shown that both conditions had similar quantities of daidzein, genistein and glycitein. So, at this temperature range, *B. coagulans* PR03 could grow well and effectively produce β-glucosidase to hydrolyze isoflavone glucosides during fermentation. Nonetheless, the low amounts of isoflavone aglycones was found when fermented at 40 and 45 ^{o}C might suggest the lower growth of *B. coagulans* PR03 in this temperature range. In conclusion, the most suitable fermentation temperature was 30 ^{o}C and was used in the next experiment.

4.3.5 The kinetic study of isoflavone aglycones fermentation production by *B. coagulans* PR03

The optimum conditions from section 4.3.1-4.3.4 were used to produce isoflavone aglycones from soygerm. For the fermented soygerm preparation, one kilogram of soygerm was washed 2 times with clean water and drained for 30 min at ambient temperature (30-35 °C). After draining, the soygerm was transferred to 5 L glass bottle (Duran®), filled with 2 L of water, sealed with cotton wool and sterilized at 121°C for 15 min. After that, the sterilized soygerm was inoculated with 15% (v/w) of *B. coagulans* PR03, which was cultured in nutrient broth (NB) and incubated at 37°C, 120 rpm for 18 h before used. The fermentation conditions were 30°C with 1 L/min of air flow rate for 10 days. The instrument of fermentation is shown in Figure 4.3.

Figure 4.3 The instrument of isoflavone aglycones fermentation.

During the fermentation period, fermented soygerm was daily analyzed for isoflavones content, β- glucosidase activity and total bacterial count. The results are shown in Table 4.5. It was found that, fermentation process had significant effects on the isoflavone aglycones, isoflavone glucosides, β-glucosidase activity and total bacterial count (P<0.05). The quantity of isoflavone aglycones tended to increase during the fermentation period. As a result, the quantities of daidzein, genistein, glycitein and total aglycones were increased from 105.65, 30.26, 26.72 and 162.62 mg/100 g to 474.57, 218.16, 399.55 and 1,092.27 mg/100 g, respectively. On the contrary, the quantity of isoflavone glucosides decreased during the fermentation which resulted in the decreasing values of daidzin, genistin, glycitin and total glucosides from 601.49, 166.39, 385.61 and 1,153.49 mg/100g to 133.61, 4.55, 13.33 and 151.49 mg/100 g, respectively. β-glucosidase activity increased from 8.26 mU/g to the highest value of 28.54 mU/g at the fifth day of fermentation process. After that, the value decreased to 13.48 mU/g at the last day of fermentation. Total bacterial count represented cell number of *B. coagulans* PR03 showed a similar trend as β-glucosidase activity. The value increased from 5.83 to 8.08 log cfu/g at the fifth day of fermentation and decreased to 7.35 log cfu/g at the last day of fermentation process. The changes of isoflavones, β-glucosidase activity and total bacterial count during soygerm fermentation by *B. coagulans* PR03 (Figure 4.4) were similar with soybean fermentation in section 3.3.3 (Figure 3.2). The increasing number of *B. coagulans* PR03 and β-glucosidase activity were relevant. For instance, it could be observed that *B. coagulans* PR03 showed logarithmic growth along with β-glucosidase activity until the fifth day of fermentation process. The increasing value of *B. coagulans* PR03 and β-glucosidase activity resulted in hydrolization of isoflavone glucosides in soygerm transforming it to isoflavones aglycones. As a result, the suitable time for isoflavone aglycones fermentation was 5 days. Therefore, the results during fermentation period of 0-5 days were used to calculate specific growth rate of *B. coagulans* PR03 (μ), product yield coefficient ($Y_{p/s}$), specific product usage rate (q_s) and specific product formation rate (q_p) of laboratory scale isoflavone aglycones production.

Table 4.5 Isoflavones contents, β-glucosidase activity and total bacterial count of isoflavone aglycone production in lab scale

Time (Day)	Isoflavones content (mg/100 g dry weight)								β-glucosidase activity (mU/g)	Total bacterial count (log CFU/g)
	Aglycones				Glucosides					
	Daidzein	Genistein	Glycitein	Total aglycones	Daidzin	Genistin	Glycitin	Total glucosides		
0	105.65±1.44^{a}	30.26±1.79^{a}	26.72±1.07^{a}	162.62±0.72^{a}	601.49±1.20^{g}	166.39±1.17^{g}	385.61±1.32^{f}	1153.49±1.34^{i}	8.26±0.28^{a}	5.83±0.05^{a}
1	214.85±0.74^{b}	90.12±1.25^{b}	139.77±1.26^{b}	444.74±0.76^{b}	460.26±1.22^{f}	98.95±0.70^{f}	248.23±1.11^{e}	807.44±0.59^{h}	10.46±0.48^{b}	6.18±0.03^{b}
2	346.51±1.57^{c}	155.99±1.10^{c}	273.32±1.00^{c}	775.82±0.53^{c}	335.49±1.01^{e}	34.95±1.48^{e}	121.88±1.88^{d}	492.32±1.41^{g}	14.81±0.21^{d}	6.71±0.05^{c}
3	374.58±1.08^{d}	167.85±1.14^{d}	299.69±0.45^{d}	842.12±0.38^{d}	285.22±1.10^{d}	27.28±1.48^{d}	81.89±1.25^{c}	394.38±0.87^{f}	17.43±0.10^{e}	7.24±0.01^{d}
4	431.55±1.71^{e}	199.22±0.77^{e}	360.43±0.79^{e}	991.21±0.15^{e}	221.61±1.66^{c}	11.59±0.91^{c}	39.47±1.30^{b}	272.66±0.55^{e}	20.29±0.23^{f}	7.49±0.02^{f}
5	471.64±1.08^{fg}	216.67±1.12^{f}	398.87±1.47^{fg}	1087.18±1.43^{f}	143.43±1.48^{b}	6.49±0.74^{b}	15.31±1.39^{a}	165.23±0.65^{d}	28.54±0.45^{h}	8.08±0.05^{i}
6	473.28±1.81^{fg}	220.44±1.36^{g}	402.48±0.89^{h}	1096.20±0.44^{i}	143.45±0.76^{b}	4.72±0.40^{ab}	13.72±0.39^{a}	161.90±0.02^{c}	28.42±0.12^{h}	7.98±0.04^{h}
7	473.53±0.97^{fg}	217.37±1.51^{fg}	399.56±0.97^{g}	1090.46±0.43^{g}	134.94±0.40^{a}	4.39±0.55^{ab}	14.27±0.38^{a}	153.60±0.53^{b}	24.50±0.38^{g}	7.89±0.03^{g}
8	474.12±1.73^{g}	220.26±1.10^{f}	403.76±1.09^{h}	1098.14±0.45^{j}	133.11±0.79^{a}	5.67±0.46^{ab}	13.60±0.23^{a}	152.37±0.56^{ab}	17.38±0.10^{e}	7.36±0.03^{e}
9	470.52±0.72^{f}	218.71±1.65^{fg}	396.50±1.82^{f}	1085.73±0.88^{f}	143.79±0.62^{b}	4.27±0.38^{a}	13.69±0.21^{a}	161.75±0.45^{c}	15.23±0.14^{d}	7.39±0.02^{e}
10	474.57±1.76^{g}	218.16±2.42^{fg}	399.55±0.76^{g}	1092.27±0.10^{h}	133.61±1.65^{a}	4.55±0.46^{ab}	13.33±0.46^{a}	151.49±1.65^{a}	13.48±0.21^{c}	7.35±0.02^{e}

Note: Means within same column with different superscripts are significantly different (P<0.05).

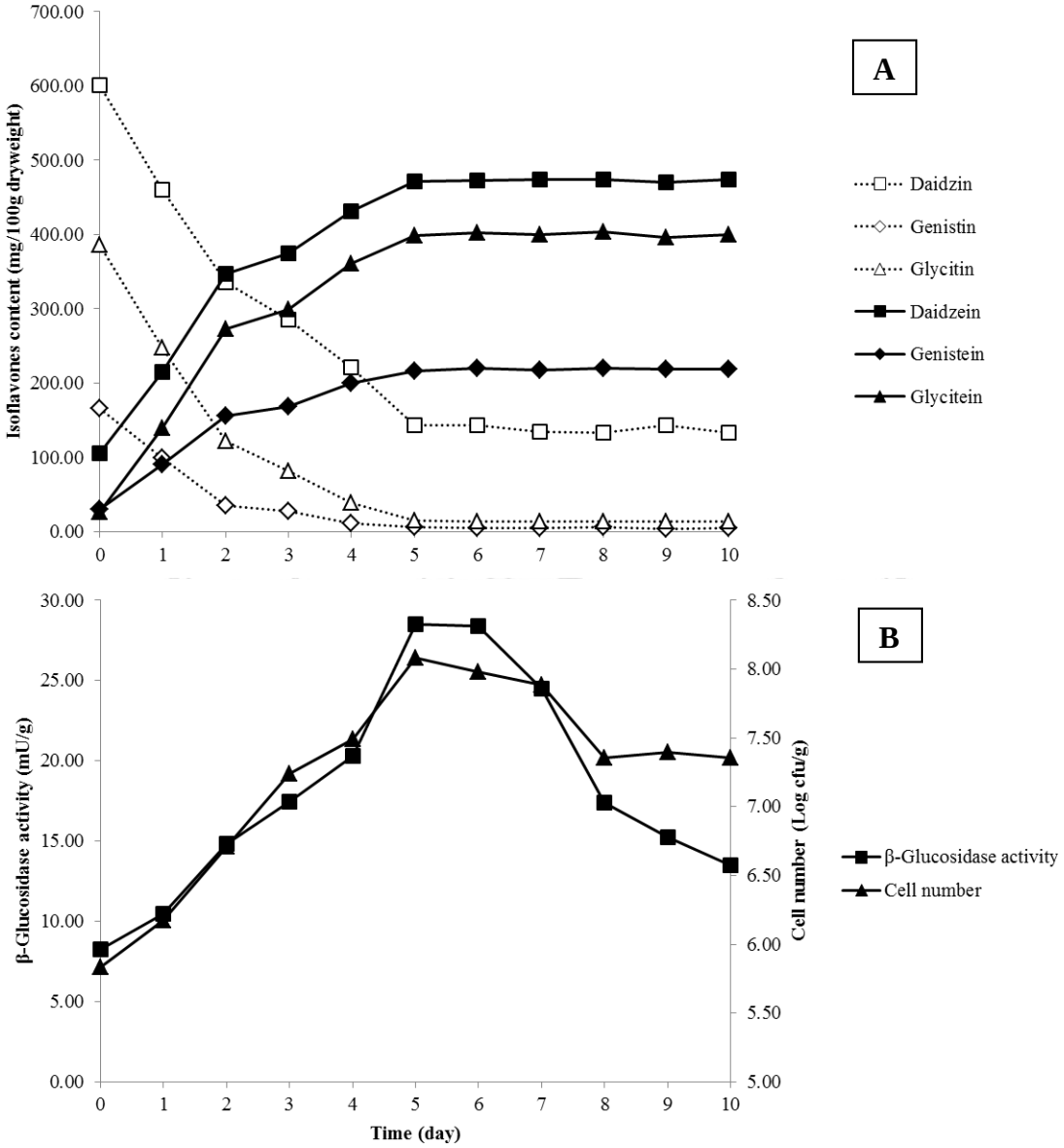

Figure 4.4 Changes of isoflavones contents (A), β-glucosidase activity and total bacterial count (B) of isoflavone aglycones production in lab scale.

4.3.5.1 Specific growth rate of *B. coagulans* PR03

From growth curve of *B. coagulans* PR03 in fermented soygerm (Figure 4.4), it was found that the log phase of microbial growth was in the period of 0–5 days. So, the cell numbers in term of cfu/g during that period (Table 4.5) were used to calculate specific growth rate of *B. coagulans* PR03 (μ)

$$\mu = \ln (X_t/X_0) / t$$

$$\mu_{PR03} = \ln (X_5/X_0) / t$$

$$= \ln (10^{8.08}/10^{5.83}) / (5-0)$$

$$= \ln (177.83) / 5$$

$$= 1.04 \text{ day}^{-1}$$

4.3.5.2 Product yield coefficient

Similarly, the amounts isoflavone aglycones and isoflavone glucosides during day 0 and day 5th of the fermentation (Table 4.5) were used to calculate product yield coefficient of lab scale isoflavone aglycones production ($Y_{agy/glu}$).

$$Y_{agy/glu} = (P_{agy}1 - P_{agy}0)/(S_{glu}0 - S_{glu}1)$$

$$= (1{,}087.18-162.62) / (1{,}153.49-165.23)$$

$$= 0.94 \text{ mg/100 g dry weight}$$

4.3.5.3 Kinetic of substrate usage

Specific substrate usage rate (q_s) was also calculated using the amounts of isoflavone glucosides from 0-5th day of the fermentation (Table 4.5).

$$q_s = (dS/dX)\ \mu$$

$$q_{din} = [(601.49-143.43) / (\ln 10^{8.08} - \ln 10^{5.83})] \times 1.04$$

$$= 91.95 \text{ mg/100 g-day}$$

q_{gin} = [(166.39-6.49) / (ln $10^{8.08}$ – ln $10^{5.83}$)] x 1.04

= 32.10 mg/100 g-day

q_{glin} = [(385.61-15.31) / (ln $10^{8.08}$ – ln $10^{5.83}$)] x 1.04

= 74.33 mg/100 g-day

q_{glu} = [(1,153.49-165.23) / (ln $10^{8.08}$ – ln $10^{5.83}$)] x 1.04

= 198.38 mg/100 g-day

4.3.5.4 Kinetic of product formation

From Table 4.5, the amounts of isoflavone aglycones in a period of 0-5 days of fermentation was used to determine specific product formation rate (q_p)

q_p = (dP/dX) μ

q_{dein} = [(471.64-105.65) / (ln $10^{8.08}$ – ln $10^{5.83}$)] x 1.04

= 73.46 mg/100 g-day

q_{gein} = [(216.67-30.26) / (ln $10^{8.08}$ – ln $10^{5.83}$)] x 1.04

= 37.42 mg/100 g-day

q_{glein} = [(398.87-26.72) / (ln $10^{8.08}$ – ln $10^{5.83}$)] x 1.04

= 74.71 mg/100 g-day

q_{agly} = [(1,087.18-162.62) / (ln $10^{8.08}$ – ln $10^{5.83}$)] x 1.04

= 185.60 mg/100 g-day

4.4 Conclusion

Soygerm of SJ2 variety was a suitable source of isoflavone glucosides. The optimum fermentation conditions were 15% (v/w) inoculum concentration of *B. coagulans* PR03 and aerobic fermentation with air flow rate of 1 L/min at 30 ºC for 5 days. The kinetic study of isoflavone aglycones production from soygerm had found that specific growth rate of *B. coagulans* PR03 (μ), product yield coefficient ($Y_{p/s}$), specific product usage rate of total glucosides (q_{glu}) and specific product formation rate of total aglycones (q_{agly}) were 1.04 day^{-1}, 0.99 mg/100 g dry weight, 198.38 mg/100 g-day and 185.60 mg/100 g-day, respectively.