รายงานโครงงานวิจัย

เงินงบประมาณประจำปี 2555

เรื่อง

การใช้ยีสต์ออโตไลเสทเป็นแหล่งไนโตรเจนทางเลือกสำหรับการผลิตกรดโพรพิโอนิกโดยเชื้อ *Propionibacterium acidipropionici* ATCC 422 ที่ถูกตรึงด้วยสารสกัดเพคตินหยาบจากใบกรุงเขมา (*Cissampelos pareira* L.)

Using Yeast Autolysate as an Alternative Nitrogen Source for Propionic Acid Production by Immobilized *Propionibacterium acidipropionici* ATCC 422 with Pectin Crude Extracts from Krung Kha Mao Leaves (*Cissampelos pareira* L.)

โดย

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สารบัญ

เรื่อง **หน้า**

บทคัดย่อภาษาไทย

งานวิจัยนี้เลือกใช้กากน้ำตาลเป็นแหล่งคาร์บอนราคาถูกและยีสต์ออโตไลเสทเพื่อเป็นแหล่งไนโตรเจนทางเลือกสำหรับศึกษาการผลิตกรดโพรพิโอนิกโดย *Propionibacterium acidipropionici* TISTR 422 และได้ใช้ Plackett-Burman Design ในการศึกษาสภาวะที่สามารถผลิตกรดได้สูงสุด การทดลองได้กระทำใน Erlenmeyer flasks ขนาด 125 มิลลิลิตร และมีปริมาณอาหาร 63 มิลลิลิตร ในอาหารเติมแคลเซียมคาร์บอเนตร้อยละ 1 (น้ำหนักต่อปริมาตร) เพื่อรักษาระดับของพีเอชในอาหาร หลังจากผ่านการหมัก เป็นเวลา 7 วัน ปริมาณกรดสูงสุดที่ได้ 30.84 กรัมต่อลิตร และผลผลิตของกรดโพรพิโอนิกได้ 4.4 กรัมต่อลิตรต่อวัน ซึ่งสภาวะที่เหมาะสม คือ ปริมาณน้ำตาลในกากน้ำตาล 20 กรัมต่อลิตร ปริมาณยีสต์สกัด 10 กรัมต่อลิตร และปริมาณยีสต์ออโตไลเสท 10 กรัมต่อลิตร

คำสำคัญ Plackett-Burman design กรดโพรพิโอนิก *Propionibacterium acidipropionici* TISTR 422 กากน้ำตาล ยีสต์ออโตไลเสท

ABSTRACT

The production of propionic acid by *Propionibacterium acidipropionici* TISTR 422 was investigated by using molasses as a cheap carbon source and yeast autolysate as an alternative nitrogen source. A Plackett-Burman design was used to determine maximum propionic acid production. The assay was performed in 125 ml Erlenmeyer flasks containing 63 ml of production medium, 1% (w/v) calcium carbonate was added to the production medium in order to maintain the pH constant. The maximum propionic acid concentration reached to 30.84 $g.l^{-1}$ and propionic productivity was 4.4 $g.l^{-1}d$ after 7 days, which corresponded to 20 $g.l^{-1}$ total sugar from molasses, 10 $g.l^{-1}$ of yeast extract and 10 $g.l^{-1}$ of yeast autolysate.

Keywords: Plackett-Burman design, Propionic acid, *Propionibacterium acidipropionici* TISTR 422, Molasses, Yeast autolysate

INTRODUCTION

Propionic acid is an important chemical that is widely used as a raw material in different industries (11, 19). Currently, almost propionic acids produce by petrochemical process, but propionic acid biosynthesis is expected to be a promising option due to its renewable raw sources and the overall increasing consumer demand. Although, there has been an interest to produce propionic acid from biomass by fermentation with propionibacteria, but the relatively low propionic acid concentration, yield and production rate from the fermentation have been the major barriers for economical applications (54).

A number of by-products and raw materials from the food and/or agriculture industries have been employed for microorganism growth due to their considerable availability and low cost (13). Several carbon sources have been patented for producing propionic acid by fermentation such as glucose (20, 21, 25, 34, 54), lactose (29), xylose (10), sucrose (45), and glycerol (6, 14, 16, 25, 31, 37, 58, 61). The cheap raw materials, such as whey (9, 11, 29,31), hemicelluloses (38, 48) hydrolyzed corn meal (27). All these were also applied for the propionic acid production. Furthermore molasses, the by-product of the sugar industry, contained about 50% (w/w) total sugar (sucrose, glucose and fructose) were also utilized for the propionic acid production as a cheap carbon source (19).

Yeast autolysate and yeast extract are the main nitrogen source used for the production of propionic acid, which is rich in free amino acid, proteins, vitamins, and fiber. Therefore, it is good for use as supplements in culture media (8). Furthermore, molasses and yeast autolysate are prominent composition for culture media in fermentative processes due to the high content of sugar and nitrogen respectively (7).

Response surface methodology (RSM) is the collection of statistical techniques for experiment design, model development, evaluation factor, and optimum condition search. Now it is extensively applied in the optimization of medium composition, conditions of enzymatic hydrolysis, fermentation, and food manufacturing processes. RSM design was used for further study of the influences of major factor and interaction between them on the response value, which is based on the result of sole-experiment and Plackett-Burman (PB) design. PB design is a method of choice for initial screening of medium components (51).

However, no research used statistically based experimental design for the screening of the media component to improve the propionic acid production from *P.acidipropionici* TISTR 422. The aim of this study was to investigate the use of molasses as a carbon sources in culture medium to obtain the optimum conditions for biomass production by *Candida utilis* TISTR 5046. In this study, we investigate the

suitable condition for propionic acid production by *P. acidipropionici* TISTR 422 using molasses and yeast autolysate as carbon and nitrogen sources in Plackett-Burman design method.

MATERIALS AND METHODS

Microorganisms

C. utilis TISTR 5046 obtains from microbiology laboratory of KMITL, was used in biomass production because of its ability to utilize a variety of carbon source and to support high protein (2, 18, 41, 46, 47). The culture was stored in YM medium at 4 °C and reactivated every 2 mounths (49, 50, 55, 60).

P. acidipropionici TISTR 422 obtains from Thailand Institute of Scientific and Technological Research (TISTR). This strain to produce propionic acid, under anaerobic condition (54, 58, 61). The culture was stored in MRS medium at 4 °C (11, 12, 22) and reactivated every 2 mounths.

Medium and growth conditions

Preculture medium was used as inoculum medium for *C. utilis* TISTR 5046 consisted of peptone 5 $g.l^{-1}$, yeast extract 3 $g.l^{-1}$, malt extract 3 $g.l^{-1}$ and glucose 3 $g.l^{-1}$ (55, 60). The inoculum was grown at 30 °C with pH 6.0 for 24 hours on a shaker at 150 rpm (15, 46, 47, 50, 60).

The fermentation medium for *C. utilis* TISTR 5046 consisted of the hydrolyzed molasses containing 1% (w/v), KH_2PO_4 5 $g.l^{-1}$, $(NH_4)_2SO_4$ 5 $g.l^{-1}$, $CaCl_2$ 0.13 $g.l^{-1}$, $MgSO_4.7H_2O$ 0.5 $g.l^{-1}$, and yeast extract 0.5 $g.l^{-1}$ (2, 46) at 30 °C, pH of the medium was adjusted to 4.5-5.5 and shaking at 150 rpm until the 48 h. The sample were measured total sugar content by the Dubois's process (17, 33 41), free amino acid by HPLC (15), total nitrogen and protein content by Kjeldaht method (41, 60).

The inoculum of *P. acidipropionici* TISTR 422, was prepared by transfer a loopfull to 50 ml of growth MRS medium in Erlenmeyer flasks 250 ml (11). The MRS growth medium was made up of peptone 10 $g.l^{-1}$, beef extract 10 $g.l^{-1}$, yeast extract 5 $g.l^{-1}$, glucose 20 $g.l^{-1}$, tween80 1 ml, K_2HPO_4 2 $g.l^{-1}$, sodium acetate 5 $g.l^{-1}$, tri-ammonium citrate 2 $g.l^{-1}$, $MgSO_4.7H_2O$ 0.2 $g.l^{-1}$and $MnSO_4.4H_2O$ 0.2 $g.l^{-1}$ (11, 12, 22, 43), pH of the medium was adjusted to 6.5 (23, 37, 54), the inoculated medium was inocubated at 30 °C for 48 hour (14, 23, 37, 38, 62) at static state. A total of 5% (v/v) (11,11,8) of the inoculum was transferred to 125 ml Erlenmeyer flasks containing 63 ml of production medium,1% (w/v) calcium carbonate was added to the production medium in order to maintain the pH constant (13). Sample were taken from culture 0 hour until the 360 hour were measured pH value, total sugar content by the Dubois's process and analyse for propionic acid and acetic acid by high performance liquid chromatography (HPLC) (11).

Molasses hydrolysis

Molasses were adjusted to pH 3.0 (32, 52, 57) by addition of 20% H_2SO_4 (w/v). Then the molasses was remained at 80 °C with water batch for 20 min (12), all of the sucroses were hydrolyzed for the glucose and fructose. The hydrolyzed molasses were centrifuged at 10,000×g for 10 min. The supernatants were collected and adjusted to pH 6.5 with 10 M NaOH (18, 32, 52) and supernatants sterilized at 121 °C for 15 min.

Analysis

The cell dry weight was determined by the following way: yeast cell were harvested by centrifugation, washed twice with distilled water and dryed at 105 °C overnight until constant weight was reached (15, 33, 50, 51), the total sugar content of the medium was determined by Dubois's process (17, 33, 51), free amino acid by HPLC (15), total nitrogen and protein content were determined with the Kjeldahl method (5, 41, 47, 60)

Cell growth was estimated by measuring the optical density of cell suspensions at 600 nm in a spectrophotometer (DR/4000, HACH Co., Ltd) (37, 54, 58, 61). The supernatants of the samples which had been centrifuged at 10,000×g for 10 min were analyzed by HPLC. Propionic acid and acetic acid were quantified by filtering the samples through 0.45 μm cellulose membranes on to an inerstsil C8-3 column (1.6×250 nm) and operated at room temperature, using 5mM H_2SO_4 (14, 23, 61) as the mobile phase. The wave length of the UV detector was 210 nm and the flow rate was 0.1 $ml.min^{-1}$ (11). Total sugar was determined by the Dubois's process (17) and measured pH value by pH meter.

Plackett-Burman design

The purpose of the first optimization step was used for screening of the factors that significantly influenced propionic acid production (3, 30). Base on Plackett-Burman design, each variable was examined in two levels: -1 for low level and +1 for high level (59). This design was used to evaluated the important factor that influence the response of twelve assigned factor, were screened including: molasse, temperature, pH, yeast autolysate (nanoproplus), yeast extract, trytic soy broth (TSB), K_2HPO_4, KH_2PO_4, $MnSO_4.4H_2O$, $MgSO_4.7H_2O$, $CaCl_2.6H_2O$ and $CoCl_2.6H_2O$.

RESULTS

The study of *Candida utilis* TISTR 5046 biomass and nitrogen composition analysis for an alternative nitrogen source.

In this study , we interested in the biomass and nitrogen composition in *C. utilis* for using as the candidate of nitrogen source and using for yeast autolysate production . We have been determined the growth rate of yeast cells that related to biomass and yield that is tend to be use as nitrogen source in propionic acid fermentation. At 48 hours, It is suitable for yeast biomass production, but in experiments we have to use the commercial yeast extract and yeast autolysate (nanoproplus™) as nitrogen source because we have to study the suitable conditions for propionic acid production and after that we will use yeast autolysate from *C. utilis* as nitrogen source instead.

C. utilis was studied for growth using molasses as a sole carbon for an alternative nitrogen source in propionic acid production. Ahmed, S. et al., (2010) reported that molasses, a cheap by-product is widely available from the sugar industry and consist of water, sucrose which is disaccharide most easily utilized by yeast cell, nitrogen source, proteins, vitamins, amino acids, organic acids and heavy metals, and found that 1% molasses gave higher microbial biomass production by sequential culture fermentation of *Arachniotus* sp. and *C. utilis*.

In this study, the pH of the medium during cultivation was maintained at 4.5-5.5, incubated at 30 °C, shaking at 150 rpm. After 48 hours were found suitable for maximum production of biomass (4.29 $g.l^{-1}$), productivity of biomass (2.14 $g.l^{-1}h$) and utilization of sugar (82.44 % w/w) as shown in **Figure 1**. *C. utilis* TISTR 5046 was cultivated in fermentation medium contain concentration molasses 10 $g.l^{-1}$. After 6 hours, the microbial cells started to adapting during lag phase state. Cells were rapidly grown until the 24 hours, then growth were slow and reaches a stationary phase. The total sugar was also determined and the results showed that the consumption of sugar were consistant with the cell dry weight (DCW). **Figure 1**, shows that highest amount of sugar consumption is 10 $g.l^{-1}$ at 4.5-5.5 and 30 °C. The experiment were initially investigation for sugar consumption at a constant retention time of 24 hours.

The part of autolysate experiment was done at pH 4.5-5.5, 30 °C for 48 hours. Yeast suspension was centrifuged at 10,000×g for 10 min to remove the supernatant, washed twice with distilled water and then, adjusted pH of yeast suspension at 5.5-6.0, incubated in water bath at 50 °C for 24 hours reaction time of autolysis, autolysed yeast extract of *C. utilis* TISTR 5046 was concentrated by rotary vacuum evaporator and analysed total nitrogen and protein content of samples as shown in **Table 1** and the analysis amino acid was showed in **Table 2.**

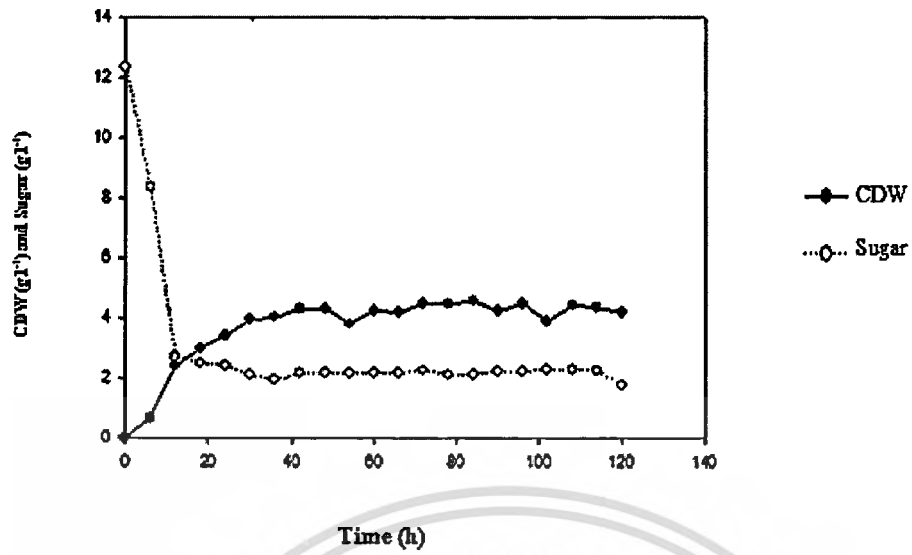

Figure 1. Growth curves of *C. utilis*TISTR 5046 in molasses

Table 1. Chemical compositions of yeast extract from *C. utilis* TISTR 5046, nanoproplus™ and commercials yeast extract

Chemical compositions	**Yeast extract form *C. utilis* TISTR 5046**	**Nanoproplus™**	***Commercials yeast extract**
Total nitrogen (%)	2.12	8.41	13.66
Protein content (%)	13.25	52.56	84.38

Nanoproplus™ from Specialty Biotech Co;Ltd.

*Commercials yeast extract from Himedia Laboratories Pvt.Ltd

Table 2. Amino acid compositions of yeast extract from *C. utilis* TISTR 5046, nanoproplus™ and commercials yeast extract

Amino acids	Compositions (mg.100 ml^{-1})		
	Yeast extract from *C.utilis* TISTR 5046	Nanoproplus™	*Commercials yeast extract
Aspartic acid	0.82	0.24	26.75
Threonine	0.06	0.56	25.83
Serine	0.09	0.10	26.48
Glutamic acid	0.87	8.42	85.85
Proline	0.14	2.44	13.62
Glycine	0.08	1.71	17.39
Alanine	0.13	8.49	53.18
Cystine	0.24	0.62	-
Valine	0.04	2.91	34.27
Methionine	0.20	0.54	7.86
Isoleucine	0.03	1.45	27.73
Leucine	0.03	2.56	47.63
Tyrosine	1.02	3.38	16.20
Phenylalanine	1.08	30.81	27.91
Histidine	0.15	0.51	3.41
Lysine	0.36	1.32	25.67
Arginine	0.11	0.29	20.94
Tryptophan	0.12	0.12	4.86

Nanoproplus™ from Specialty Biotech Co;Ltd.

*Commercials from Himedia Laboratories Pvt.Ltd

Screening of significant variables by Plackett-Burman design

Plackett-Burman design was used in this study for selecting the significant variables for propionic acid production. The concentration of molasses was 20 $g.l^{-1}$ (w/v), yeast autolysate 10 $g.l^{-1}$ and yeast extract 10 $g.l^{-1}$. Propionic acid production reached the maximal value of 30.84 $g.l^{-1}$ within 168 hours of fermentation, with the productivity of 4.4 $g.l^{-1}h$ (**Table 3**). While, molasses concentration 40 $g.l^{-1}$ (w/v),yeast autolysate 5 $g.l^{-1}$ and yeast extract 5 $g.l^{-1}$, gave propionic acid production of 36.42 $g.l^{-1}$ in 264 hours with the productivity of 3.3 $g.l^{-1}$ h, which is a lower production rate (data not shown). Moreover, when nitrogen concentration increase, it led to a reduction in fermentation time, and reached maximal value propionic acid production. **Table 3** displayed the Plackett-Burman design (coded values) of the 20 experiment with 12 variables (F_1 = molasses, F_2 = temperature, F_3 = pH, F_4= yeast autolysate (nanoproplus), F_5 = yeast extract, F_6 = TSB, F_7 = K_2HPO_4, F_8 = KH_2PO_4, F_9 = $MnSO_4.4H_2O$, F_{10} = $MgSO_4.7H_2O$, F_{11} = $CaCl_2.6H_2O$, F_{12} = $CoCl.6H_2O$).

ANOVA of the model is given in **Table 4**. The model F-value of 3.69 implies that the model is significant. The goodness of fit of the model was checked by determination coefficient (R^2). In this study, the R^2 value was calculated to be 0.8312. A regression model with R^2 colsed to 1.0 is considered as having a very high correlation, whereas Adjusted R^2 of 0.6061 implies that confirmed the significance of the mode as well.

Table 3. Plackett-Burman design (coded values) with the respective results

Run	Independent variables												Propionic acid (g. l^{-1})
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	
1	+1	-1	-1	-1	-1	+1	+1	-1	+1	+1	-1	-1	23.28
2	+1	-1	-1	+1	+1	+1	+1	-1	+1	-1	+1	-1	25.43
3	+1	-1	+1	-1	-1	-1	-1	+1	+1	-1	+1	+1	24.74
4	+1	+1	-1	+1	+1	-1	-1	+1	+1	+1	+1	-1	20.90
5	-1	-1	+1	+1	+1	+1	-1	+1	-1	+1	-1	-1	17.66
6	+1	+1	-1	-1	+1	+1	+1	+1	-1	+1	-1	+1	17.73
7	-1	-1	-1	+1	+1	-1	+1	+1	-1	-1	+1	+1	14.19
8	+1	+1	+1	+1	-1	+1	-1	+1	-1	-1	-1	-1	19.63
9	+1	-1	+1	+1	-1	-1	+1	+1	+1	+1	-1	+1	23.89
10	-1	+1	+1	-1	+1	+1	-1	-1	+1	+1	+1	+1	26.98
11	+1	+1	-1	+1	-1	+1	-1	-1	-1	-1	-1	-1	16.21
12	-1	-1	+1	+1	-1	+1	+1	-1	-1	+1	+1	+1	17.96
13	-1	+1	+1	-1	-1	+1	+1	+1	+1	-1	+1	-1	20.52
14	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	14.17
15	-1	+1	-1	+1	-1	-1	-1	-1	+1	+1	-1	+1	19.07
16	-1	+1	-1	-1	-1	-1	+1	+1	-1	+1	+1	-1	23.27
17	-1	+1	+1	+1	+1	-1	+1	-1	+1	-1	-1	-1	30.84
18	-1	-1	-1	-1	+1	-1	-1	+1	+1	-1	-1	+1	14.63
19	+1	-1	+1	-1	+1	-1	-1	-1	-1	+1	+1	-1	22.90
20	+1	+1	+1	-1	+1	-1	+1	-1	-1	-1	-1	+1	25.39

Table 4. ANOVA for Plackett-Burman.

Source	Sum of squares	df	Mean square	F-value	p-valuve Prob < F
Model	333.51	12	27.79	3.69	0.0289
F_1-Molasses	21.65	1	21.65	2.88	0.1241
F_2-Temperature	23.52	1	23.52	3.13	0.1109
F_3-pH	86.65	1	86.65	11.51	0.0080
F_4-Yeast autolysate	3.07	1	3.07	0.41	0.5392
F_5-Yeast extract	9.67	1	9.67	1.29	0.2862
F_6-TSB	18.68	1	18.68	2.48	0.1496
F_7-K_2HPO_4	32.79	1	32.79	4.36	0.0665
F_8-KH_2PO_4	31.43	1	31.43	4.18	0.0714
F_9-$MnSO_4.4H_2O$	84.75	1	84.75	11.26	0.0084
F_{10}-$MgSO_4.7H_2O$	3.11	1	3.11	0.41	0.5362
F_{11}-$CaCl_2.6H_2O$	2.32	1	2.32	0.31	0.5924
F_{12}-$CoCl_2.6H_2O$	15.86	1	15.86	2.11	0.1805
Cor Total	402.12	22			

Std. Dev: 2.74; R-Squared: 0.8312; Adj R-Squared: 0.6061; Pred R-Squared: -0.3750; PRESS: 552.93; Adeq: 6.696

Molasses, temperature, pH, yeast extract, K_2HPO_4, $MnSO_4.4H_2O$, $MgSO_4.7H_2O$, and $CaCl_2.6H_2O$ had positive coefficients while the other three variables showed negative coefficients. Molasses was used as the dominant nutrients in production of propionic acid. Yeast extract as the sole nitrogen source in medium, contains abundant of amino acid, minerals and vitamin, which are necessary for cell growth and increased propionic acid production. Altaf et al. (2006) reported that peptone and yeast extract are the main nitrogen sources used for the production of lactic acid and if alternative source were used, the final product would be smaller and the fermentation time would increase. The effect of yeast extract was kept constant at low level because of the supplement with yeast autolysate (nanoproplus™) in the medium. On the other hand, high concentration of nitrogen may lead to cell death and inhibition of the product.

K_2HPO_4 is phosphate source in fermentation medium plays a key role in enhancing of microorganism growth, Honorato et al. (2007) revealed that the use of K_2HPO_4 is reported to provide K^+ and phosphate (PO_4^-) for microorganism growth and also acts as a buffering agent in the medium.

The coefficient of $MnSO_4 4H_2O$, $MgSO_4.7H_2O$, and $CaCl_2.6H_2O$ were positive, suggested that it remain constant at high level, which mean that this chemical reagent is trace amount in culture medium but necessary and important for cell growth. Yeast autolysate (nanoproplus™) has negative coefficient, thus in the next study had to reduce or keep in contant level the concentration of mineral ion. Whereas, tryticase soy broth (TSB) showed a negative coeffient, in comparison with that of yeast extract and yeast autolysate (nanoproplus™), it contribution was the least significant in production of propionic acid and therefore TSB can be excluded from further experiment. Furthermore, KH_2PO_4 and $CoCl.6H_2O$ were showed negative coefficients, therefore their lower level may be suggested for further experiments shown in **Table 5**.

Table 5. Coefficient of each variable, confidence interval (CI) at 95% confidence level based on 't' statistic and sum of the squares as percentage (SS%) for production of propionic acid in 12 variable Plackett-Burman design.

Factor	Coefficient	95% CI Low	95% CI High
F_1-Molasses	1.04	-0.35	2.43
F_2-Temperature	1.08	-0.30	2.47
F_3-pH	2.08	0.69	3.47
F_4-Yeast	-0.39	-1.78	1.00
F_5-Yeast extract	0.70	-0.69	2.08
F_6-TSB	-0.97	-2.35	0.42
F_7-K_2HPO_4	1.28	-0.11	2.67
F_8-KH_2PO_4	-1.25	-2.64	0.13
F_9-$MnSO_4.4H_2O$	2.06	0.67	3.45
F_{10}-$MgSO_4.7H_2O$	0.39	-0.99	1.78
F_{11}-$CaCl_2.6H_2O$	0.34	-1.05	1.73
F_{12}-$CoCl_2.6H_2O$	-0.89	-2.28	0.50

DISCUSSION

According to the study of *C. utilis* TISTR 5046 growth rate, at 48 hours showed the highest growth rate and it is the suitable period for harvest yeast cells. At this state, yeast cell biomass reached 4.29 $g.l^{-1}$ which is the highest value and the percentage of sugar utilization is 82.44 %. Zhao, G. et al (2010) studied production of single cell protein using waste capsicum powder produced during capsanthin extraction, *C. utilis* 1769 was chosen as the biomass producer because of its highest SCP formation 6.8 $g.l^{-1}$.

C. utilis TISTR 5046 was chosen as the biomass producer because of its highest biomass formation. Nigam (2000) reported *C. utilis* has been frequently used in SCP production because of its ability to utilize a variety of carbon sources and to support high protein yield and used in yeast autolysate production by *C. utilis* were performed at 50 °C for 24 hours. After that yeast autolysate were harvested and concentrated for chemical composition analysis as shown in **Table 1** and **Table 2**. Ahmed, S. et al. (2010) revealed that the production of microbial biomass protein by sequential culture fermentation of *Arachiotus* sp. and *C. utilis* found that the mixed microbial biomass protein in fermentation contained 16.41% of true protein, 23.51% of crude protein, 19.9% of crude fiber, 12.11% of ash and 0.12% of RNA content, while the amino acid profile of final mixed microbial biomass protein showed that it was enriched with essential amino acids (aspartic acid, threonine, serine, gluatamic acid, proline, glycine, alanine, valine, methoinine, isoleucine, leucine, tyrosine, phynylalanine, lysine, histidine and arginine). Dimova, N.D. et al. (2010) studied production of candida biomass from hydrolysed agricultural biowaste, revealed that *C. tropicalis* and *C. utilis* grown on agricultural as the substrate, are promising yeast strains for the production of single cell protein and biomasses from *C.tropicalis* and *C. utilis* may be used as a source of protein after sulfur amino acid enrichment.

However, the study of yeast growth rate were useful in the experiment of yeast autolysate production in the next study, it is rich in free amino acid, protein and other, which can be a good source of supplement. From the second experiments, yeast autolysate is possible to be the alternative nitrogen sources because of their essential amino acids and suitable for use in propionic acid production, consistant with the research work.

Wood and Holzapfel (1995) found that the nitrogen source is a major factor of influence on the growth of Lactobacillus. Cristian J. et al. (2009) studied the production of D(-) lactic acid from *Lactobacillus* LMI8 sp. by using 2 low cost nitrogen sources: corn steep liquor (CSL) and yeast autolysate (YA). Maximal production of lactic acid was 41.42 $g.l^{-1}$ and a value located at the central point, which corresponded to 15 $g.l^{-1}$ of CSL and 5 $g.l^{-1}$ of YA. Selmer-Olsen and Sorhaug (1998) reported that yeast extract is an excellent source of B complex vitamin and often used to provide these factor to the bacteriological culture media, which are often considered indispensable to obtaining faster growth and production rate of lactic acid by lactic bacteria. Moreover, the increase in the nitrogen concentration in the fermentative medium led to a reduction in fermentation time. De Lima et al. (2009) reported about high concentration of nitrogen, Which can lead to cell death. The previous experiments, the result consistence with the report of the using alternative nitrogen for propionic acid production from others study.

Under optimized conditions of this studied, the best result for propionic acid production (30.84 $g.l^{-1}$) was obtained after 168 hours with 20 $g.l^{-1}$ of molasses, 10 $g.l^{-1}$ of yeast autolysate (nanoproplus™), 10 $g.l^{-1}$ yeast extract. Thus, the use of molasses for fermentation by *P.acidipropionici* TISTR 422 is feasible and yield considerable propionic acid production, requiring supplementation with a cheap nitrogen source (yeast autolysate)

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