CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Growth curve and EPS production from *P. urinae-equi* TISTR 1499 in MRS medium

The growth curve and EPS production of *P. urinae-equi* TISTR 1499 in MRS medium are shown in Fig. 4.1. It can been seen that maximum amount of EPS produced by *P. urinae-equi* TISTR 1499 was found to be 7.67 g/L after 6 h of incubation and EPS production was associated with their growth. The bacterial growth (in dry weight) at various time intervals is also shown. Apparently, the growth was excellent in MRS medium. Sucrose was also used in high quantity.

At the stationary phase, the EPS production rate was lower than that of during exponential growth phase. Although EPS is an extra-cellular polysaccharide, cells may not remain free form in the medium. The presence of polysaccharide matrix around the cells probably limit their mobility and

oxygen transfer (Dreveton *et al.*, 1994). Furthermore, the synthesis of the EPS is enhanced principally by cell proliferation and reduced by cell growth limitation (Lobas *et al.*, 1992).

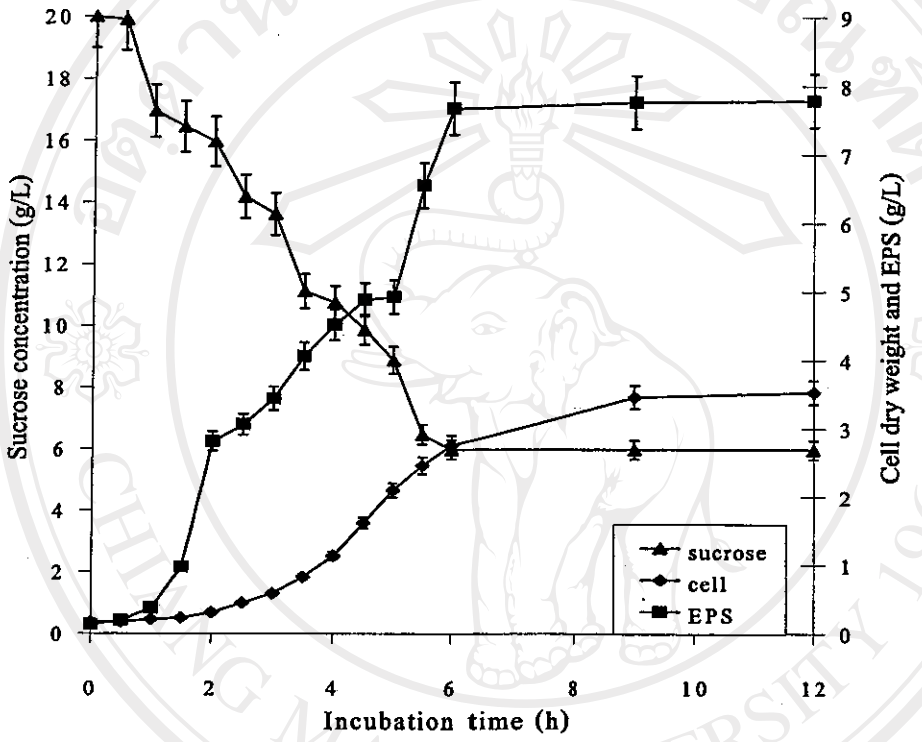

Fig. 4.1 Fermentation profile of *P. urinae-equi* TISTR 1499 in MRS medium

4.2 Growth curve and EPS production from *P. urinae-equi* TISTR 1499 on MRS agar medium plate culture

In this investigation, cultivation of *P. urinae-equi* TISTR 1499 on agar medium surface was used to predict its potential EPS productivity by SSF. The slime formed on the agar surface was

collected and used for EPS and cell yield quantification. EPS yields obtained from agar plates are shown in Fig. 4.2. It can been seen that the maximum amount of EPS was found to be 5.99 g/L after 21 h of incubation and the production of EPS was associated with growth. The EPS production rate on MRS agar was increased dramatically during 9-12 h. After that, the production rate was increased slightly. This is possibly the limitation of agar surface.

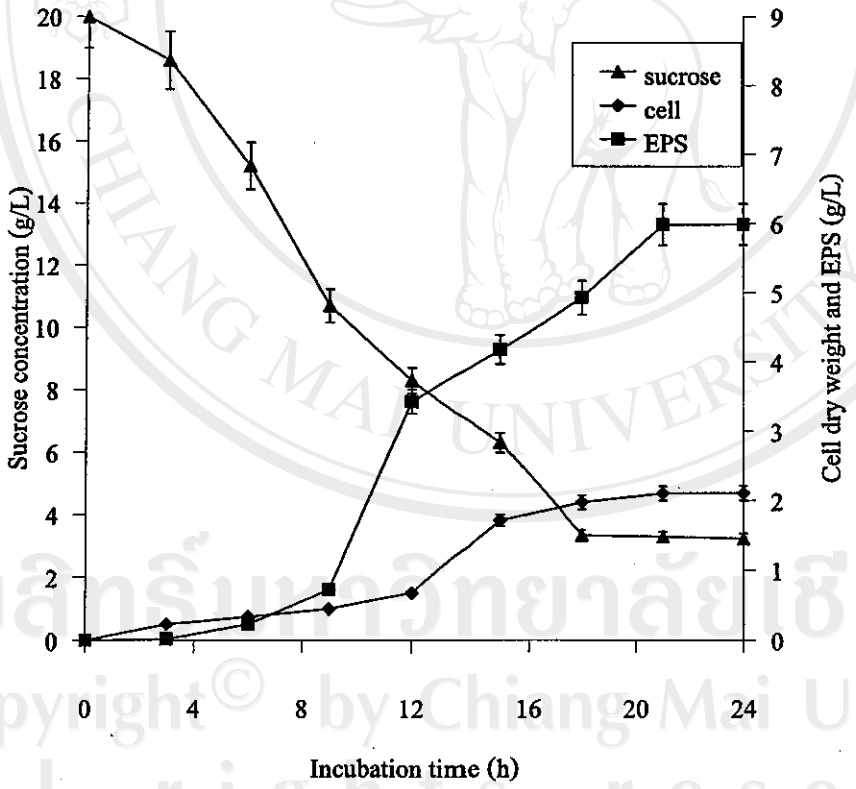

Fig. 4.2 Fermentation profile of *P. urinae-equi* TISTR 1499 on MRS agar plate culture

4.3 EPS production from *P. urinae-equi* TISTR 1499 on various inert solid supports

Terra-cotta, paper put on cellulose sponge, plastic beads and polystyrene sponge immerged with MRS medium were used as inert solid supporter for EPS production.

The growth curve and EPS production by *P. urinae-equi* TISTR 1499 are shown in Fig. 4.3 (a) and Fig. 4.3 (b), respectively. The cell growth (Fig. 4.3 a) was associated with EPS production (Fig. 4.3 b). The cell growth on polystyrene sponge was better than others probably because of the pore inside polystyrene sponge which has more space for bacterial growth. It was found that, the maximum EPS yields (Fig. 4.3 b) obtained from paper put on sponge, terra-cotta, plastic bead and polystyrene sponge were found to be 4.37, 0.26, 6.75 and 5.49 g/L, respectively. The EPS yield obtained from plastic bead was higher than other because the plastic bead has smooth surface for easily to extract EPS. Furthermore, the plastic bead, which is a small particle, has higher surface area than other. The sucrose concentration remained in medium is shown in Fig. 4.3 (c). It was indicated that, the residual sucrose concentration obtained from cultivation on paper put on cellulose sponge was higher than other conditions. This is the result of low mobility of MRS from cellulose sponge to the paper, which put on their surface.

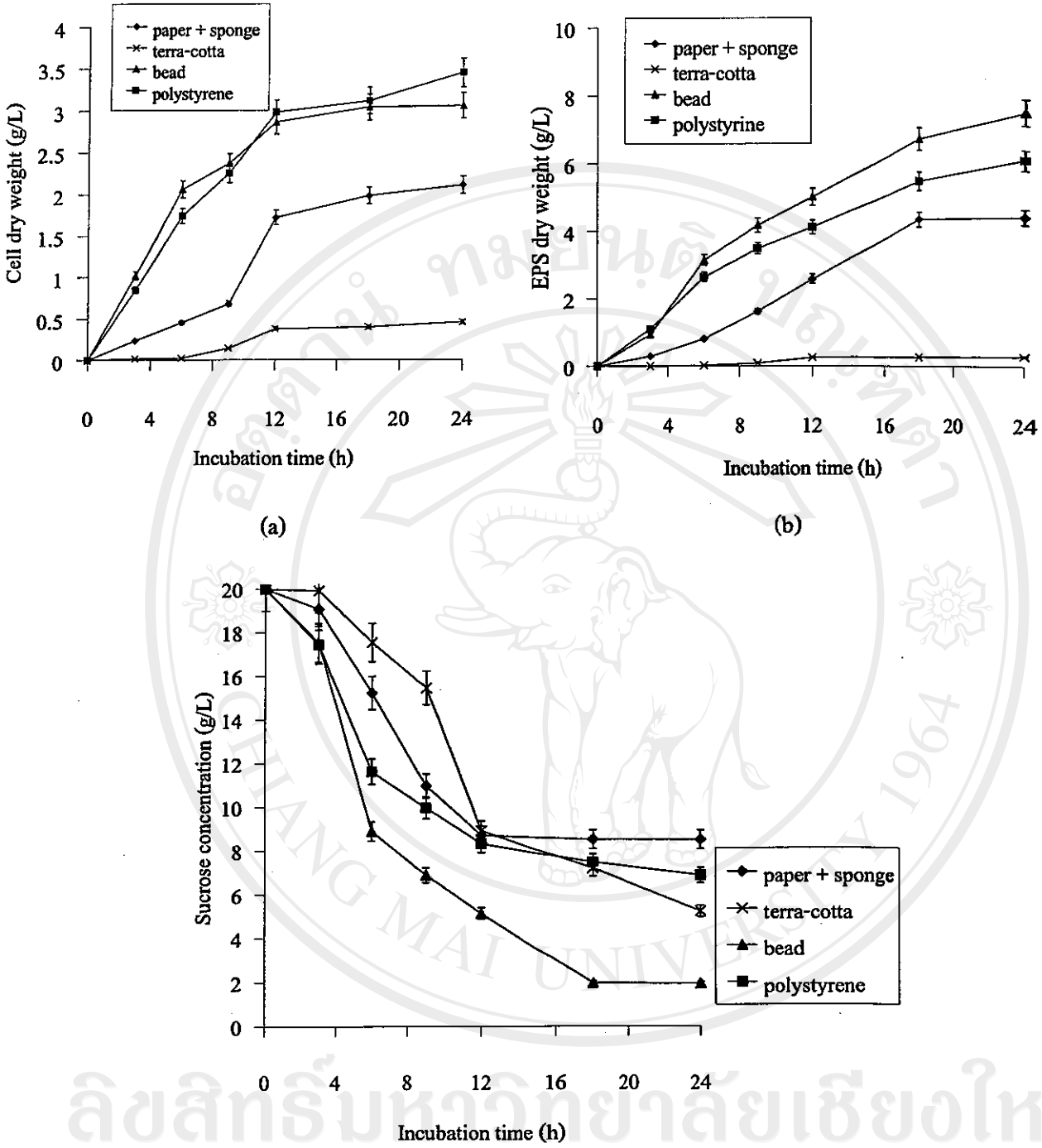

Fig. 4.3 Cell growth (a), EPS production (b) and sucrose concentration (c) after incubation of *P. urinae-equi* TISTR 1499 on various inert solid supports

The comparison of kinetic parameters of *P. urinae-equi* TISTR 1499 which were grown on various inert solid supports are shown in Table 4.1. The maximum specific growth rate (μ_{max}) of cultivation on plastic bead was higher than other systems. The kinetic parameters of EPS production were the yield coefficient of EPS from cell mass ($Y_{p/x}$) and the specific rate EPS formation (q_p). However, the $Y_{p/s}$ and q_p obtained from plastic bead support culture were higher than those obtained from other systems. It is indicated that the cell cultivated on plastic bead support culture could produce higher EPS than other systems.

Table 4.1 Kinetic parameters of *P. urinae-equi* TISTR 1499 cultured on various inert solid supports

Kinetic parameter	Paper put on sponge	Terra-cotta	Plastic bead	Polystyrene sponge
μ_{max} (h^{-1})	0.144	0.534	0.577	0.384
$Y_{X/S}$ (g cell/g substrate)	0.172	0.033	0.128	0.208
$Y_{P/S}$ (g EPS/ g substrate)	0.236	0.022	0.310	0.317
$Y_{P/X}$ (g EPS/g cell)	1.378	0.656	2.400	1.520
q_P (g EPS/g cell/ h)	0.206	0.027	0.545	0.440
q_S (g substrate/g cell/ h)	0.872	1.242	1.760	1.390

4.4 Optimization of fermentation process parameters for EPS production on agar plate culture

4.4.1 Effect of moist-air supplement for EPS production on agar plate culture

To study the influence of physical factors on EPS production upon prolonged fermentation, the additional fermentation was carried out with moist-air at a constant rate of 17 mL/min. The results, compared with the control fermentation (without moist-air supplement) are shown in Fig. 4.4.

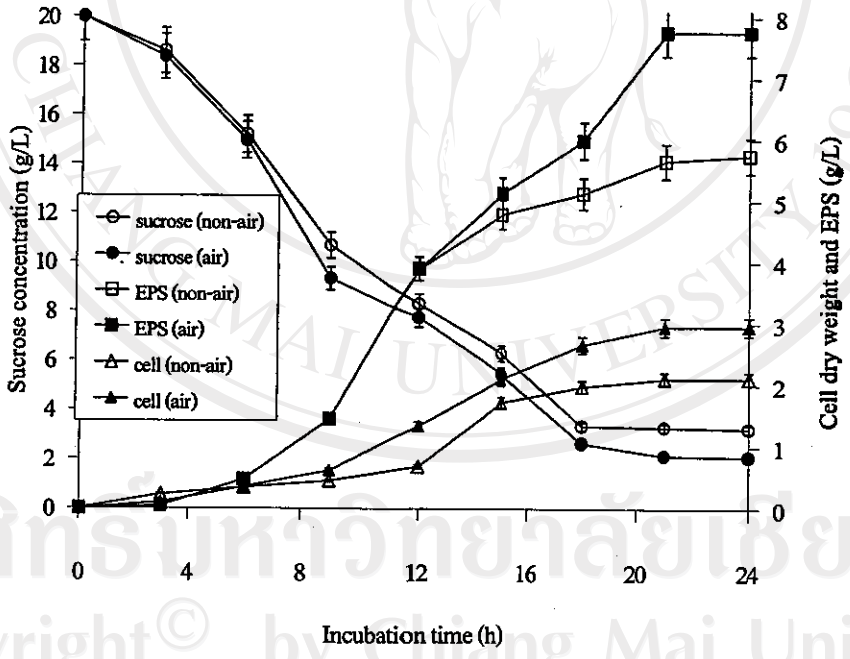

Fig. 4.4 Effect of moist-air supplement on fermentation profile of *P. urinae-equi* TISTR 1499 on agar plate culture

It can be seen that, the EPS yields obtained from control and moist-air fermentation were found to be 5.65 and 7.76 g/L in 18 h, respectively. The cell growth was associated with EPS production. Residual sucrose was decrease associated by the time. The higher productivity was observed in agar plate culture carried out by moist-air supplement (see Appendix B-1). Thus, the bacterial cells adhering to the substrate surface receive unlimited oxygen quantities to perform all metabolic functions.

However, facilitated oxygen diffusion alone would not account for the increased EPS productivity in agar plate culture because the moisture could help to protect the agar surface to be dry (Stredansky and Conti, 1999).

4.4.2 Effect of moist-air flowrate, MRS agar medium volume and incubation time on EPS production under agar plate culture

A response surface experimental design was employed to determine optimum of process parameters including moist-air flowrate, MRS agar medium volume and incubation time for EPS production by *P. urinae-equi* TISTR 1499. The results are presented in Table 4.2 and Fig. 4.5-4.7.

The coefficient of each variable from central composite design was used for simulation quadratic model. The program was employed to find out the quadratic mathematical model, which was showed as the following equation (R-squared = 0.9418);

$$Y = 0.31A^2 + 2.92B^2 + 0.93C^2 + 0.98AB - 0.51AC + 0.86BC + 0.81A - 3.27B + 0.39C + 4.23$$

Where Y is the EPS yield; A, B and C are MRS agar volume, incubation time and moist-air flowrate, respectively (see APPENDIX C).

Table 4.2 Results of fermentation experiments done to optimize EPS production by *P. urinae-equi* TISTR 1499 on agar plate culture

Std	Run	Block	Medium volume (mL)	Incubation time (h)	Moist-air flowrate (mL/min)	EPS[a] (g/L)	Cell dry weight[a] (g/L)	Sucrose[a] (g/L)
4	1	Block 1	18	31	7	4.88	2.48	1.34
5	2	Block 1	10	17	28	7.98	1.82	16.71
1	3	Block 1	10	17	7	3.11	1.86	16.96
12	4	Block 1	14	24	17	6.92	2.07	1.44
6	5	Block 1	18	17	28	7.31	1.37	11.41
10	6	Block 1	14	24	17	6.27	2.73	0.55
3	7	Block 1	10	31	7	3.65	1.45	0.89
8	8	Block 1	18	31	28	7.76	2	3.38
2	9	Block 1	18	17	7	4.48	2.64	16.9
9	10	Block 1	14	24	17	6.1	1.82	1.6
11	11	Block 1	14	24	17	6.79	2	7.15
7	12	Block 1	10	31	28	7.91	2.11	1.34
17	13	Block 2	14	24	0	8.24	4.37	3.94
14	14	Block 2	20	24	17	6.88	3.68	9.16
18	15	Block 2	14	24	35	8.18	2.86	9.04
19	16	Block 2	14	24	17	6.47	1.98	5.13
16	17	Block 2	14	35	17	6.59	2.01	15.23
13	18	Block 2	7	24	17	7.32	2.15	0.92
15	19	Block 2	14	12	17	6.04	2.83	9.72
20	20	Block 2	14	24	17	6.52	1.92	8.68

[a] Values represent the mean of the central composite design experiments.

The trend of suitable moist-air flowrate, MRS agar medium volume and incubation time were found to be 28 mL/min (Fig. 4.5), 10 mL (Fig. 4.6) and 24 h (Fig. 4.7), respectively, with a predicted maximum EPS production of 8.24 g/L. The results also indicated that higher EPS production yield might be obtained if the moist-air circulation was greater than 28 mL/min. The actual experiment using the optimum conditions obtained from the program calculation were examined, the EPS yield was obtained at 8.25 ± 0.62 g/L for 24 h-incubation time.

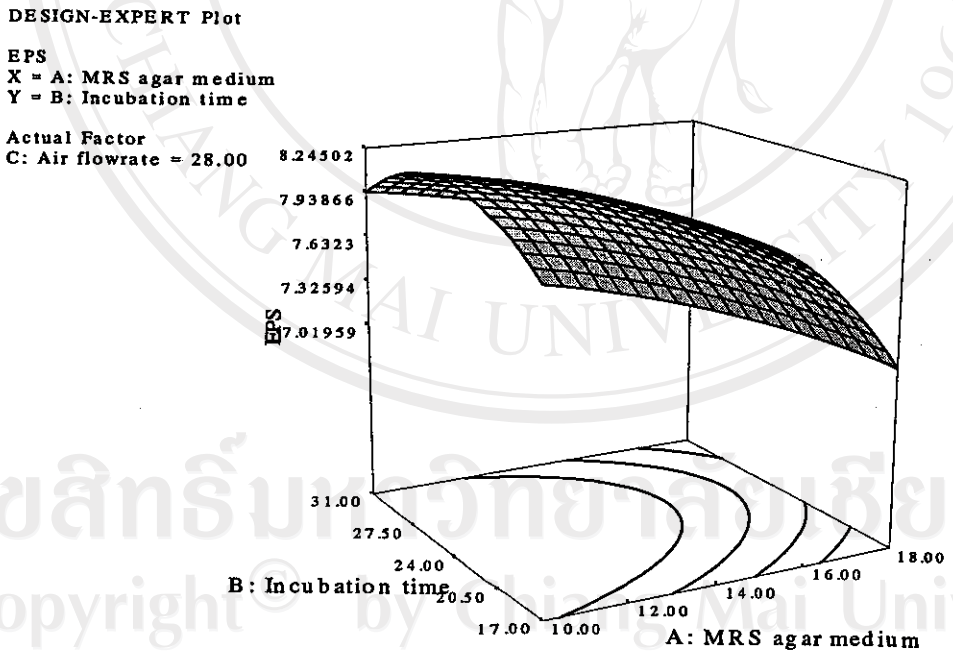

Fig. 4.5 Respond surface result, three-dimensional plots and contour plots of EPS production by *P. urinae-equi* TISTR 1499 at moist-air flowrate of 28 mL/min as a function of incubation time (h) and MRS agar medium volume (mL)

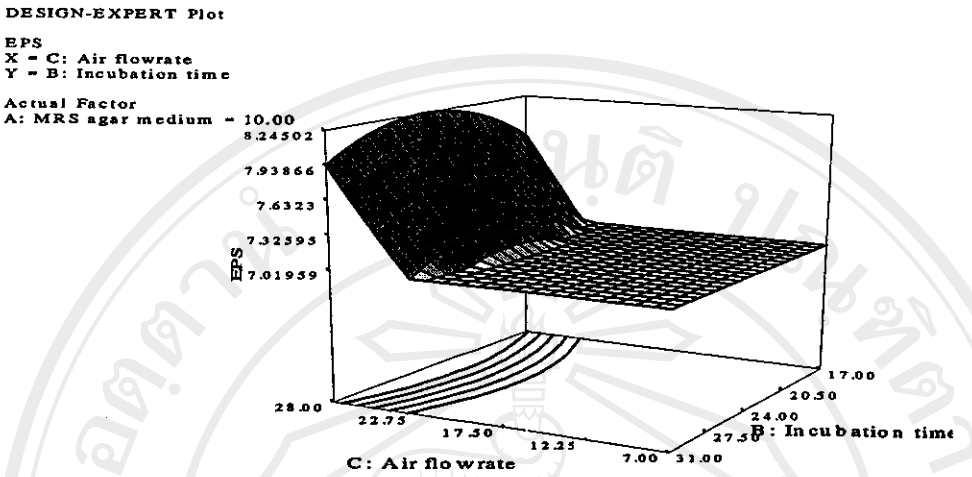

Fig. 4.6 Respond surface result, three-dimensional plots and contour plots of EPS production by *P. urinae-equi* TISTR 1499 at the MRS agar medium volume of 10 mL as a function of incubation time (h) and moist-air flowrate (mL/min)

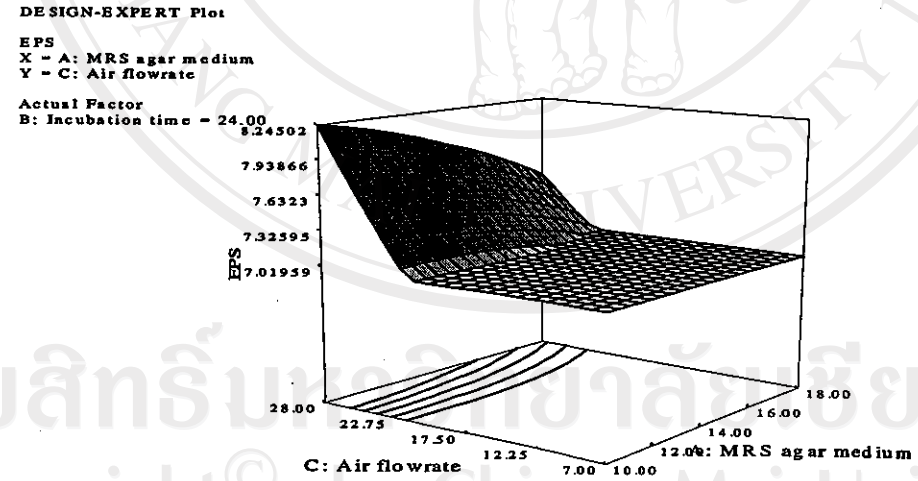

Fig. 4.7 Respond surface result, three-dimensional plots and contour plots of EPS production by *P. urinae-equi* TISTR 1499 at the incubation time at 24 h as a function of the MRS agar medium volume (mL) and moist-air flowrate (mL/min)

4.4.3 Effect of initial sucrose concentration on EPS production on agar plate culture

In this experiment, the effects of different sucrose concentrations on EPS production by *P. urinae-equi* TISTR 1499 were investigated. Each conditions were controlled at the temperature of 37 °C with moist-air flowrate of 28 mL/min and incubation time of 24 h. It can be seen from Fig. 4.8 that the optimal concentration of sucrose for EPS production by *P. urinae-equi* TISTR 1499 was 12 g/L, resulting in EPS yield of 8.76 ± 0.44 g/L which significant higher than others (see Appendix B-2). In contrast, Petronella *et al.* (1999) reported that *L. lactis* subsp. *cremoris* used glucose better than fructose because of enzyme activity. Indeed, it has been reported previously that an adequate amount of the carbohydrate source must be present for EPS production (Sutherland, 1990). Cerning *et al.* (1995) showed that the presence of excess sugar in the medium had a stimulating effect on EPS production by lactic acid bacteria. However, some strains of *Lb. delbrueckii* subsp. *bulgaricus* did not show this effect (Petry *et al.*, 2000).

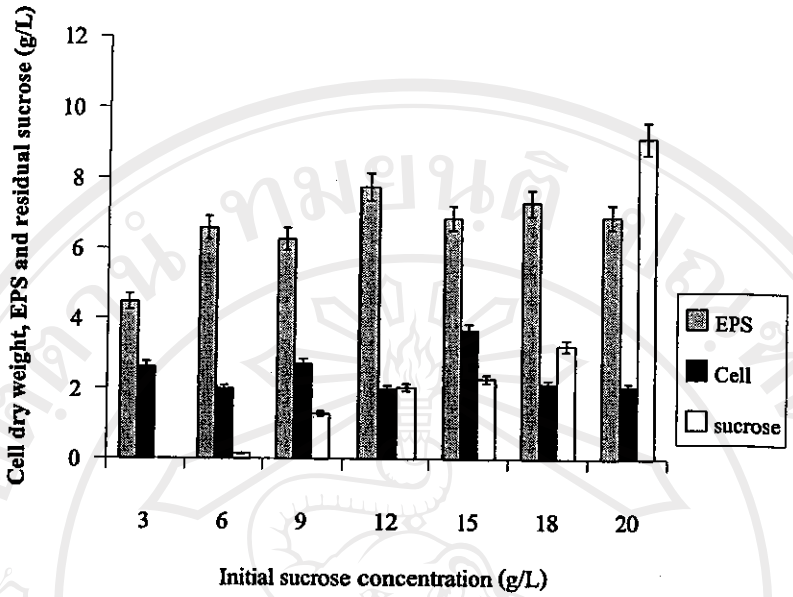

Fig. 4.8 Effect of various initial sucrose concentration on fermentation profile of *P. urinae-equi* TISTR 1499 on MRS agar plate cultivation

In summary, the optimum conditions for EPS production by *P. urinae-equi* TISTR 1499 on agar plate culture are shown in Table 4.3 with the significant highest EPS yield amount 8.76 ± 0.44 g/L.

Table 4.3 The optimum conditions for EPS production by *P. urinae-equi* TISTR1499 on agar plate culture at 37°C

Condition Factor	**Optimum Level**
Physical Factor	
- Moist-air flowrate	28 mL/min
- MRS agar volume	10 mL
- Incubation time	24 h
Nutrition Factor	
- Initial sucrose concentration	12 g/L MRS medium

4.5 EPS production from *P. urinae-equi* TISTR 1499 cultured on agricultural solid waste support

The growth curve of *P. urinae-equi* TISTR 1499 on rice husk, rice straw and sugarcane bagasse as solid support is shown in Fig 4.9 (a). The exponential growth was observed at 3 h. EPS production yields (Fig. 4.9 b) were associated with their growth. After 18 h of incubation, the maximum EPS yields obtained from rice straw, rice husk and sugarcane bagasse were 6.98, 7.30 and 6.87 g EPS /L MRS medium, respectively.

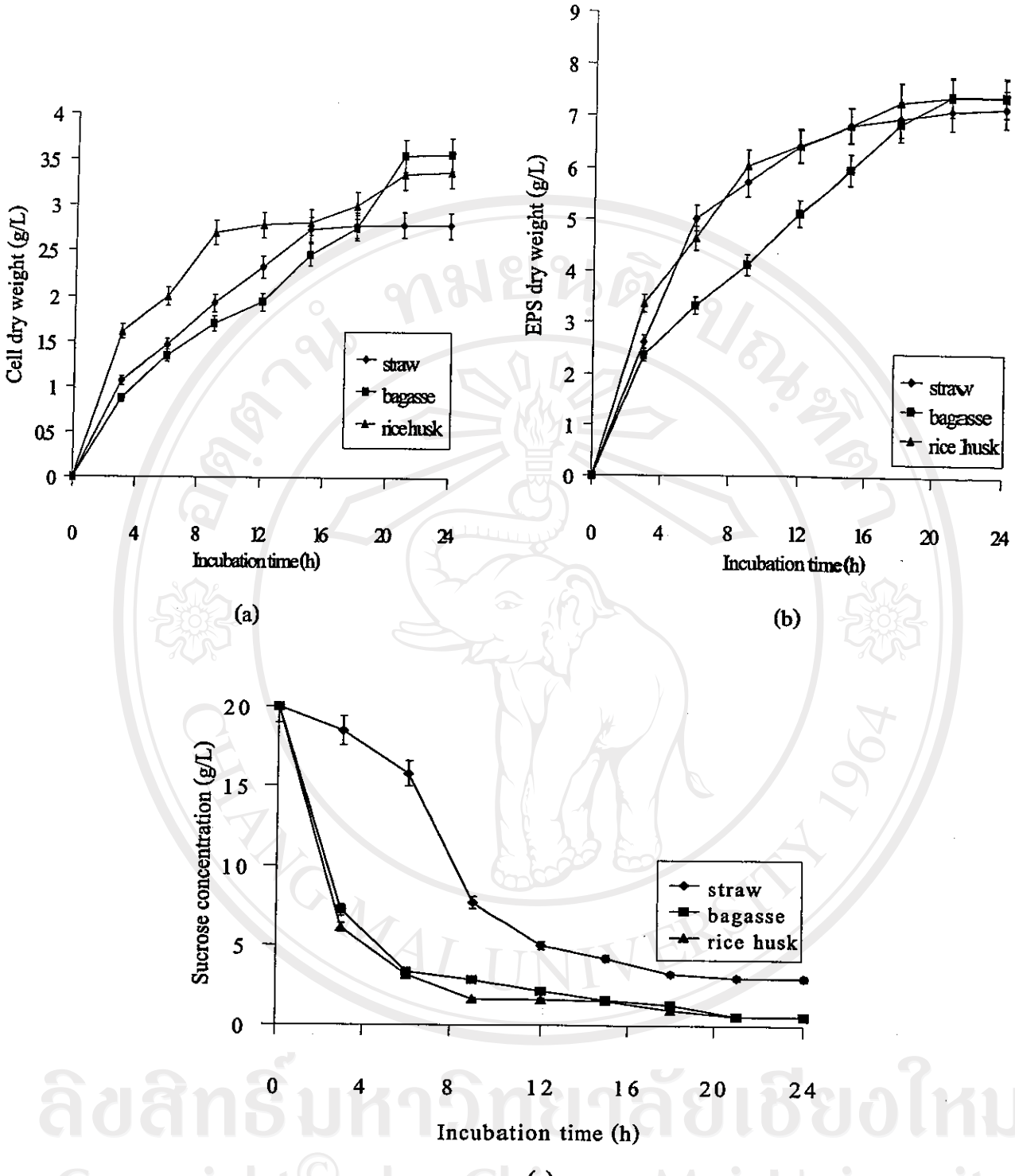

Fig. 4.9 Cell growth (a), EPS production (b) and sucrose concentration (c) after incubation of *P. urinae-equi* TISTR 1499 on various agricultural solid waste

The maximum EPS production from the cultivation by using rice husk as the solid support was higher than other supports because the rice husk is smaller particle, so they has the surface area for anchor of *P. urinae-equi* TISTR 1499 greater than rice straw and sugarcane bagasse, respectively. Furthermore, EPS was easily extracted out of their surface because the surfaces of rice husk are smoother than another agricultural wastes.

Fig 4.9 (c) showed the sucrose concentration remaining in the solid support. The rice straw cultivation showed higher residual sugar than other. This can be explained that hemicellulose in rice straw might be hydrolyzed by acid which produced from *P. urinae-equi* TISTR 1499. For instance, Michniewicz *et al.* (2003) mentioned that the rice straw after hydrolyzed with 2N HCl, it could be easily hydrolyzed by 0.5 N lactic acid at 40°C for 6 h. This may be interfere the result of residual sugar obtained. On the other hand, the same reaction did not occurred in rice husk, which contains silica and cellulose, and in sugarcane bagasse which contains mainly cellulose. In general, both silica and cellulose could be hydrolyzed hardly by acid, which produced from *P. urinae-equi* TISTR 1499. Indeed, Michniewicz *et al.* (2003) mentioned that rice husk and sugarcane bagasse, after hydrolyzed with 2N HCl, could be hydrolyzed by 0.5 N lactic acid at 40°C for 144 h.

The several kinetics parameters of *P. urinae-equi* TISTR 1499 grown on various agricultural solid waste supports are shown in Table 4.4. The maximum specific growth rate (μ_{max}) obtained from cultivation on rice husk was higher than other systems. The kinetic parameters of EPS production were the yield coefficient of EPS from cell mass ($Y_{p/x}$) and the specific rate EPS formation (q_p). The q_p obtained from rice husk culture was higher than other systems. It is indicated that the cell cultivated on rice husk could produce higher EPS productivity than other systems.

Stredansky and Conti (1998) mentioned that critical requirements of the solid support to be used were a high absorption capacity, which would prevent packing of the fermentation mass following slime formation by the growing cells, and size homogeneity of the solid particles. Rice husk meets both requirements, with a water sorption capacity of over 80%, an average particle size of 1–4 mm (George and Ghose, 1974). So, rice husk was considered to be the inert support in further studies.

Table 4.4 Kinetic parameters of *P. urinae-equi* TISTR 1499 cultured on various agricultural solid waste supports

Kinetic parameter	Rice straw	Sugarcane bagasse	Rice husk
μ_{max} (h^{-1})	0.177	0.209	0.277
$Y_{X/S}$ (g cell/g substrate)	0.096	0.068	0.101
$Y_{P/S}$ (g EPS/g substrate)	0.139	0.169	0.237
$Y_{P/X}$ (g EPS/g cell)	1.459	2.475	2.320
q_P (g EPS/g cell/ h)	0.209	0.557	0.779
q_S (g substrate/g cell/ h)	0.52	3.294	3.290

4.6 Optimization of fermentation process parameters for EPS production on rice husk support culture

4.6.1 Effect of rice husk to MRS medium ratio on EPS production

The medium absorption capacity of rice husk was investigated by studying the rice husk to MRS medium ratio for EPS production by *P. urinae-equi* TISTR 1499. Fig. 4.10 shows the cell growth and EPS production. The maximum EPS yields obtained from 1-to-3, 1-to-4 and 1-to-5 ratios were found to be 8.64, 8.71 and 8.85 g/L, respectively.

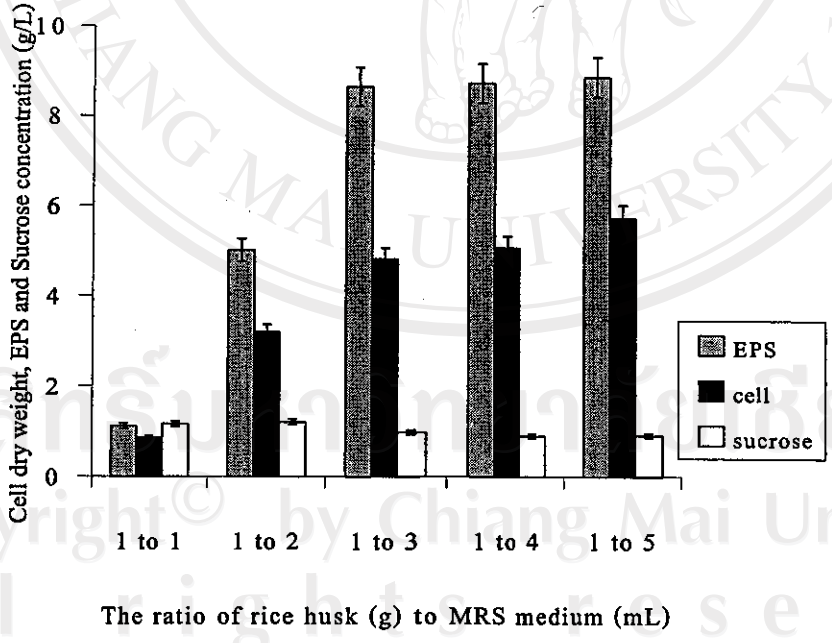

Fig. 4.10 Effect of rice husk to MRS medium ratio on fermentation profile of *P. urinae-equi* TISTR 1499

It can be seen that the optimum ratio of rice husk to MRS medium for EPS production was 1-to-3. Increasing the ratios were non-significant higher than 1-to-3 on the EPS yield. At ratios of 1-to-1 and 1-to-2, cell growth and EPS production were significant lower than other ratios because some rice husk particles could not be immerged in MRS medium (see Appendix B-3).

4.6.2 Effect of inoculum size on EPS production on rice husk support culture

The production medium was inoculated with different inoculum size of *P. urinae-equi* TISTR 1499 and the result is shown in Fig. 4.11. The microbial growth was increased following the inoculum size. However, the significant maximum EPS production (9.86 g/L) was obtained when 15 % (v/v) inoculum was used (see Appendix B-4). Since Meenakshi *et al.* (1995) mentioned that the high inoculum size about 20 % (v/v) gave the highest EPS yield *by A. vinelandii* MTCC 2460. Indeed, Triveni *et al.* (2001) also studied the optimum inoculum size for EPS production by *Agro. radiobacter*. It was found that, the 1.0 % (v/v) of inoculum size was suitable for EPS production.

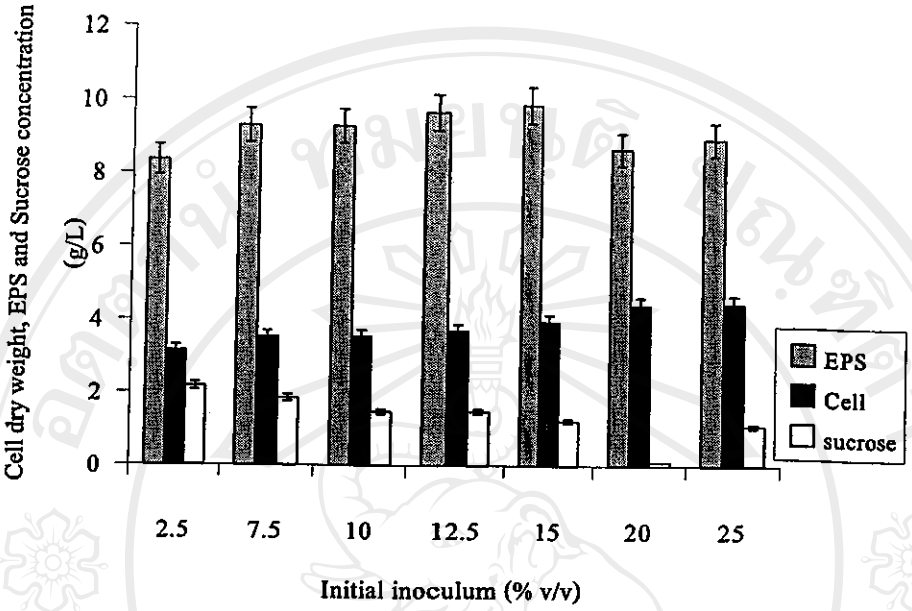

Fig. 4.11 Effect of inoculum size on fermentation profile of *P. urinae-equi* TISTR 1499 cultured on rice husk support

4.6.3 Effect of moist-air supplement on EPS production on rice husk support culture

The fermentation was carried out by supplying moist-air at a constant rate of 17 mL/min compared with non-supplying condition. The result is shown in Fig. 4.12. The cell growth was associated with EPS production and the exponential growth was observed at 3 h. The residual sucrose was associated decrease by the time. The residual sucrose was associated decrease by the time. The EPS yields obtained from the control and supplying moist-air were 9.86 and 9.28 g/L, respectively, within 18 h-incubation time.

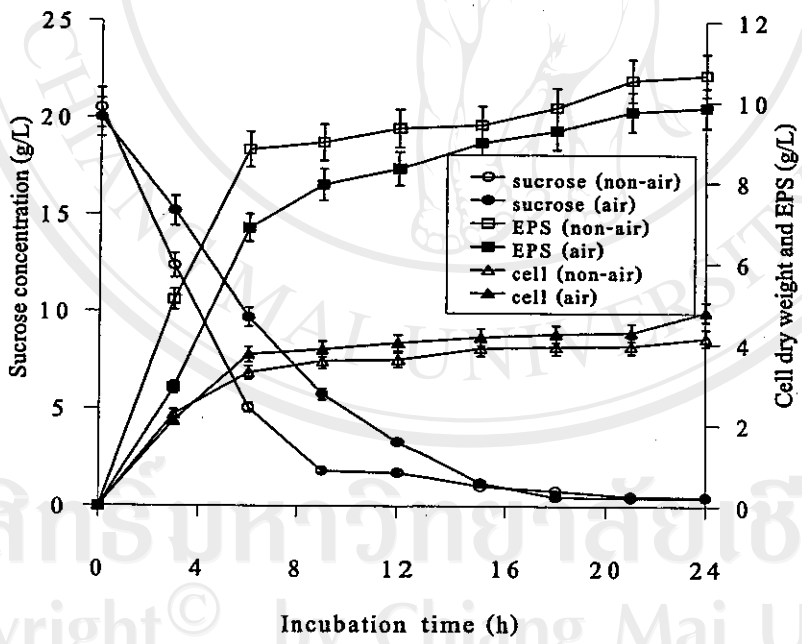

Fig. 4.12 Effect of moist-air supplement on fermentation profile of *P. urinae-equi* TISTR 1499 cultured on rice husk support

They gave non-significant EPS production yield (see Appendix B-5). So, it can be seen that the moist-air had no affect to enhance EPS production. This can be explained that, possibly, the rice husk particle has porousity for air supplement. The overload air may be gave the unsuitable condition to cell growth and EPS production.

4.6.4 Effect of initial sucrose concentration on EPS production on rice husk support culture

The effect of different sucrose concentrations on EPS production by *P. urinae-equi* TISTR 1499 was investigated. It can be seen from Fig. 4.13 that the microbial growth was high when cultured in the medium contained 50 and 60 g/L of initial sucrose concentration because *P.urinae-equi* TISTR 1499 used sucrose for maintenance their cell more than EPS production (de Vuyst and Degeest, 1999). The optimum concentration of sucrose for EPS production, when cultured on rice husk support, was 20 g/L resulting in EPS yield of 10.01 g/L which significant higher than others (see Appendix B-6).

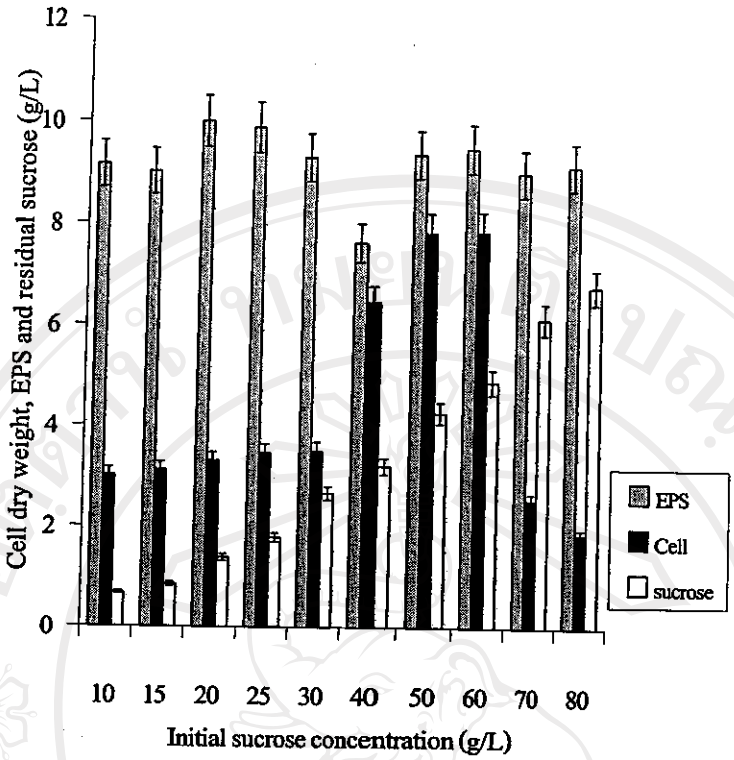

Fig. 4.13 Effect of various initial sucrose concentration on fermentation profile of *P. urinae-equi* TISTR 1499 cultured on rice husk support

4.6.5 Effect of nitrogen sources for EPS production on rice husk support culture

The major nitrogen sources, which have been used so far for EPS production by *P.urinae-equi* TISTR 1499, were yeast extract, bacto-peptone, meat extract and di-ammonium hydrogen citrate. To investigate their optimum concentrations, mixture design experiment was employed. The results are shown in Table 4.5 and Fig. 4.14-4.17.

Table 4.5 Results of mixture design for investigation of nitrogen source affecting EPS production by *P. urinae-equi* TISTR 1499 on rice husk as solid support

Std	Run	Yeast extract (%)*	Meat extract (%)*	Bacto-peptone (%)*	Diammonium hydrogen citrate (%)*	EPS[a] (g/L)	Cell[a] (g/L)	Sucrose[a] (g/L)
16	1	0.00	0.00	1.50	0.00	8.550	3.48	1.414
4	2	1.00	0.00	0.00	0.50	9.105	3.29	1.699
18	3	0.00	1.00	0.00	0.50	8.790	4.21	1.946
13	4	0.50	0.50	0.50	0.00	10.380	4.30	1.596
20	5	0.25	1.00	0.00	0.25	9.000	4.05	1.926
7	6	0.00	0.00	1.00	0.50	6.825	4.13	1.783
17	7	0.00	1.00	0.50	0.00	9.960	4.35	2.000
2	8	1.00	0.50	0.00	0.00	7.050	2.36	2.034
10	9	0.50	0.50	0.00	0.50	8.145	4.20	1.630
15	10	0.33	0.33	0.33	0.50	8.220	4.00	1.783
6	11	1.00	0.00	0.50	0.00	8.265	3.76	1.576
19	12	1.00	0.50	0.00	0.00	8.160	4.34	1.832
14	13	0.72	0.22	0.22	0.34	8.070	4.13	1.857
3	14	0.00	1.00	0.00	0.50	7.680	4.22	1.581
5	15	0.00	1.00	0.50	0.00	9.375	4.47	1.562
12	16	0.50	0.00	1.00	0.00	8.910	4.80	1.488
9	17	0.25	1.00	0.00	0.25	9.255	5.07	1.916
8	18	0.00	0.50	0.75	0.25	11.250	5.34	0.118
11	19	0.50	0.00	0.50	0.50	6.960	1.73	2.473
1	20	0.00	0.00	1.50	0.00	8.550	4.79	1.763

[a] Values represent the mean of the central composite design experiments

Fermentation conditions, incubation temperature of 37 °C for 18 h

*(%) defined by DESIGN-EXPERT version 5.0 program

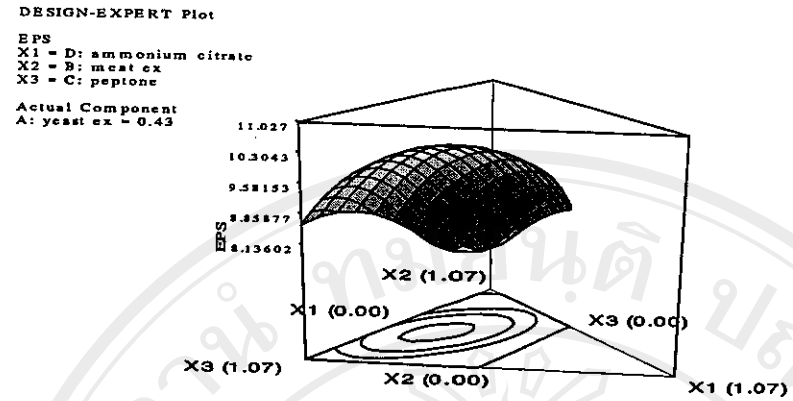

Fig. 4.14 Respond surface result, three-dimensional plots and contour plots of EPS production by *P. urinae-equi* TISTR 1499 at the yeast extract concentration of 0.43 % (w/v) as a function of X1, diammonium hydrogen citrate (% w/v); X2, meat extract concentration (% w/v) and X3, bacto-peptone concentration (% w/v)

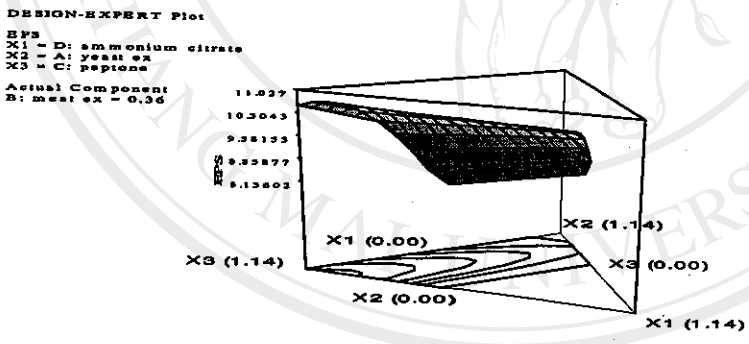

Fig. 4.15 Respond surface result, three-dimensional plots and contour plots of EPS production by *P. urinae-equi* TISTR 1499 at the meat extract concentration of 0.43 % (w/v) as a function of X1, diammonium hydrogen citrate (% w/v); X2, yeast extract concentration (% w/v) and X3, bacto-peptone concentration (% w/v)

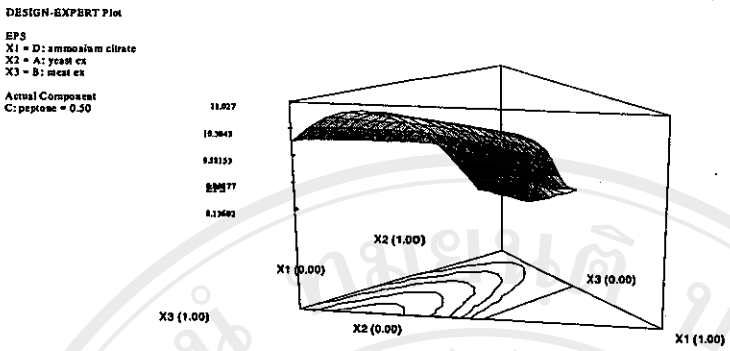

Fig. 4.16 Respond surface result, three-dimensional plots and contour plots of EPS production by *P. urinae-equi* TISTR 1499 at the bacto-peptone concentration of 0.43 % (w/v) as a function of X1, diammonium hydrogen citrate (% w/v); X2, yeast extract concentration (% w/v) and X3, meat extract concentration (% w/v)

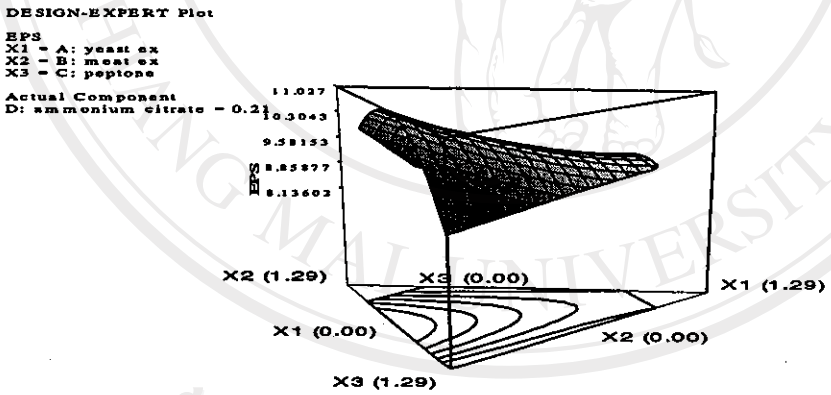

Fig. 4.17 Respond surface result, three-dimensional plots and contour plots of EPS production by *P. urinae-equi* TISTR 1499 at the diammonium hydrogen citrate of 0.21 % (w/v) as a function of X1, yeast extract concentration (% w/v); X2, meat extract concentration (% w/v) and X3, bacto-peptone concentration (% w/v)

The coefficient of each variable from central composite design was used for simulation quadratic model. The program was employed to find out the quadratic mathematical model at 24 h, which was showed as the following equation (R-squared = 0.9776);

$$Y = 1.03AB + 0.31AC + 42.02AD + 13.57BC + 48.50BD + 33.01CD + 8.21A + 6.15B + 8.71C - 19.83D$$

Where Y is the EPS yield; A, B, C and D are the concentration of yeast extract, meat extract, bacto-peptone and diammonium hydrogen citrate, respectively (see APPENDIX D).

From the computer program, it can be predicted that the optimal concentration of yeast extract, meat extract, bacto-peptone and diammonium hydrogen citrate were found to be 4.30, 3.60, 5.00 and 2.10 g/L, respectively, which the highest EPS yield as 10.30 g/L is approximately. The actual experiment using the suitable conditions obtained from program calculation were tested. It was found that, the maximum EPS yield was obtained at 10.27 $\pm$ 0.72 g/L for 24 h-incubation time.

Grobben *et al.* (1997) reported that *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772 required the nitrogen sources for the high EPS yield production. Also the amino acid complex nitrogen source might be yeast extract, meat extract, beef extract or bacto-peptone, etc. In this study, *P. urinae-equi* TISTR 1499 required the amino acid complex nitrogen sources, which were 4.30 g/L yeast extract, 3.60 g/L meat extract and 5.00 g/L bacto-peptone for the high EPS yield production amount 10.27 ± 0.72 g/L.

In summary, the optimum conditions for EPS production by *P. urinae-equi* TISTR 1499 on rice husk culture are shown in Table 4.6 with the highest EPS yield amount 10.27 ± 0.72 g/L.

Table 4.6 The optimum conditions for EPS production by *P. urinae-equi* TISTR1499 under rice husk culture

Condition Factor	**Optimum Level**
Physical Factor	
Ratio of rice husk to MRS medium	1 –to- 3 (w/v)
Incubation time	24 h
Inoculum size	15% (v/v)
Incubation temperature	37°C
Moist-air supplement	none
Nutrition Factor	
Initial sucrose concentration	20 g/L
Yeast extract	4.30 g/L
Meat extract	3.60 g/L
Bacto-peptone	5.00 g/L
Diammonium hydrogen citrate	2.10 g/L