สำนักงานวิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Operating Limits

The operating limits of the single-stage foam fractionator were determined by varying both air flow rates and solution volume. Two important operational constraints; foam formation and complete carry–over, are considered as the limits of the operation of foam fractionation. For the foam production, a sufficient air flow rate is needed to produce foam which can reach the foam outlet at the column. However, at a very high air flow rate and/or low liquid volume, complete carry–over of the liquid can occur in the foam fractionation column, leading to no biosurfactant separation. Figure 4.1 shows the operational regions with the two boundaries of the no-foam regions and the complete carry–over regions of the investigated foam fractionation column operated at an initial biosurfactant concentration of 362.16±0.13 mg rhamnose/l and the pore size of sintered glass disk ranging from 100 to 160 μm (No. 1) at different air flow rates, initial foam heights, and initial liquid volumes. To avoid both no-foam formation and complete carry–over, the maximum and minimum values of both the air flow rate and liquid volume were used at various column heights.

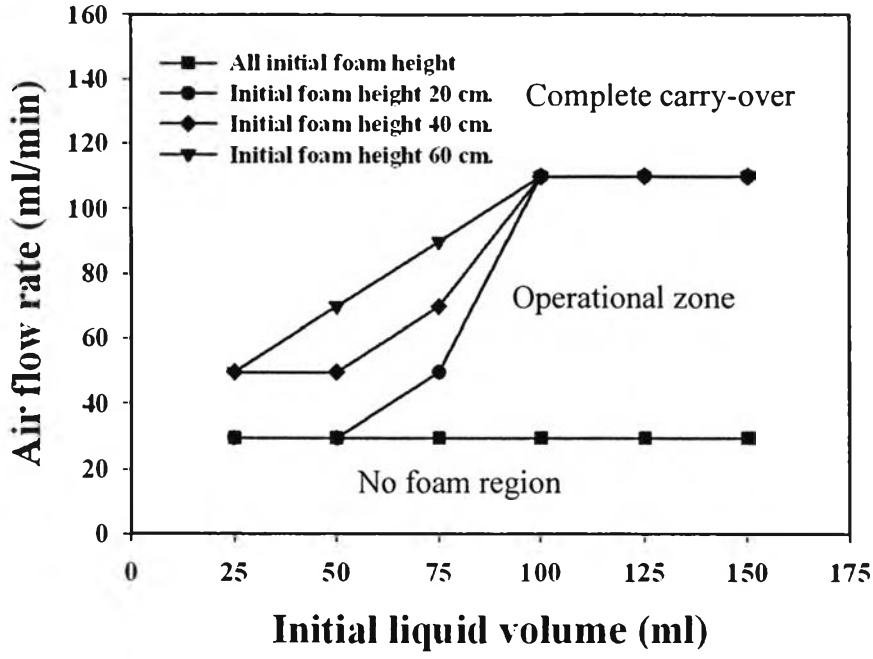

Figure 4.1 Operational zone under operational condition; sinter glass disk no.1, initial concentration 362.1632 µg/l.

4.2 Effect of Air Flow Rate

The effect of air flow rate on the biosurfactant recovery using a single stage foam fractionators, was investigated at the initial foam height of 20 cm, the pore size of sintered glass disk in the range of 100–160 µm (No. 1), and initial liquid volume of 100 ml. The air flow rate was varied at 30, 50, 70, 90, and 110 ml/min. The experiment were carefully designed such that the lowest air flow rate of 30 ml/min used in this part of the study was very close to the lowest flow rate possibly used for this single stage foam fractionator. Thus, the air flow rate lower than this resulted in such a low production of foam that did collapse before reaching the overflow pipe. As can be seen from figures 4.2, 4.3 and 4.4, an increasing in air flow rate results in a reduction in the enrichment ratio but increase in the percentage of biosurfactant recovery. For all condition, the percentage of biosurfactant recovery was in range of 81.33±1.16 to 94.62±1.02 as seen in the table 4.1. When compare among various

flow rate used, the enrichment ratio reduced from 2.98±0.02 to 1.07±0.01, the highest enrichment ratio was obtain at the lowest air flow rate used which was 30 ml/min. At this air flow rate the enrichment ratio as high as 2.98±0.02 was obtain when the collapse foamate was collect form highest column height (60 cm.) as seen in Figure 4.2. In contrast, when air flow rate was increased, better the percentage of biosurfactant recovery was observed as seen that the percentage of biosurfactant recovery as high as 94.62±1.02 could be achieved in almost all conditions studied. The wetness of foam were investigated as shown in the table 4.1 and figure 4.5 an increase in the air flow rate tends to produce wetter foam because of more bubble generated and lower the liquid drainage rate. The obtained results are in consistent with the previous works (Boonyasuwat, 2003, Burapatana, 2005). An increase in the air flow rate increased both transportation of bulk liquid into the foam and adsorption onto surfaces of the air bubbles. Hence, a volumetric rate of foam was higher and the foam became wetter, leading to a reduction of the enrichment ratio. In contrast, at a lower air flow rate, longer residence time of the generated air bubbles in the rising foam allows more liquid drainage, resulting in dryer foam with a higher biosurfactant concentration. Thus, the highest enrichment ratio was obtained at the lowest air flow rate. Meanwhile, at a lower air flow rate, the generated air bubbles move up slowly to the top of the foam fractionation column because of a low liquid entrainment rate. This effect was more dominant than that of an increase in the biosurfactant concentration in the dryer foam, so the biosurfactant recovery percentage decreased with reducing the air flow rate. Since it was not possible to get both high biosurfactant recovery percentage and high enrichment ratio at the same air flow rate, the optimum air flow rate for operating a single stage foam fractionator was selected by further considering the results integrated with other effects.

Furthermore, this work has investigated the effect of residue palm oil, which can remove together with foam and interfere in the foam fractionation process. The result shown that the percentage of oil removal was in range of 32.95±2.48 to 79.89±4.07 as seen in the table 4.2, among various flow rate studied, the percentage of oil removal lower than the percentage of biosurfactant recovery, but they had same trend as shown in the figure 4.2-4.4, the lowest percentage of oil removal as low as 32.95±2.48 was obtained at the lowest air flow rate used which was 30

ml/min same as the percentage of biosurfactant recovery. It indicated that as the air flow rate decrease, the lower volumetric rate of foam is occurred resulting in decreasing the percentage of oil removal. Therefore, the lowest air flow rate reduced the interference of palm oil residue in foam fractionation process.

Table 4.1 Effect of air flow rate on enrichment ratio, biosurfactant recovery (%) and wetness for the column height of 20, 40 and 60 cm.

Air flow rate (ml/min)	Column height 20 cm.			Column height 40 cm.			Column height 60 cm.		
	Enrichment ratio	Biosurfactant removal(%)	Wetness(g/l)	Enrichment ratio	Biosurfactant removal(%)	Wetness(g/l)	Enrichment ratio	Biosurfactant removal(%)	Wetness(g/l)
30	1.7223±0.0164	84.3841±0.9220	11.10±1.61	2.3391±0.0074	83.4362±1.5946	7.18±0.59	2.9758±0.0235	81.3299±1.1579	5.32±0.85
50	1.1330±0.0071	94.0360±0.9535	34.47±1.84	1.3238±0.0057	92.2218±0.3678	17.35±1.33	1.5033±0.0063	90.1985±1.7298	10.27±1.19
70	1.0815±0.0052	94.4531±1.0753	49.12±1.94	1.2101±0.0031	93.9822±0.7030	27.82±1.51	1.2199±0.0059	92.7111±0.8397	25.85±1.78
90	1.0702±0.0029	93.8227±0.3736	51.76±1.22	1.1596±0.0036	93.9248±1.1415	47.79±1.30	1.1726±0.0085	92.6344±1.6257	41.90±1.92
110	1.0712±0.0027	94.6236±1.0200	52.81±1.76	1.1174±0.0056	94.2410±1.0961	49.10±0.75	1.1399±0.0058	93.8547±1.0690	44.04±1.45

Table 4.2 Effect of air flow rate on oil removal (%) for the column height of 20, 40 and 60 cm.

Air flow rate (ml/min)	Oil removal(%)		
	Column height 20 cm.	Column height 40 cm.	Column height 60 cm.
30	53.8681±1.6911	51.9318±3.5626	32.9490±2.4757
50	78.8851±2.5163	68.7331±1.8901	65.9651±2.6920
70	79.0210±1.4423	65.8418±2.4376	63.6304±4.1242
90	79.8908±4.0736	67.4112±4.0070	64.0020±3.6511
110	79.3847±1.8369	67.6061±3.3091	65.7643±2.3452

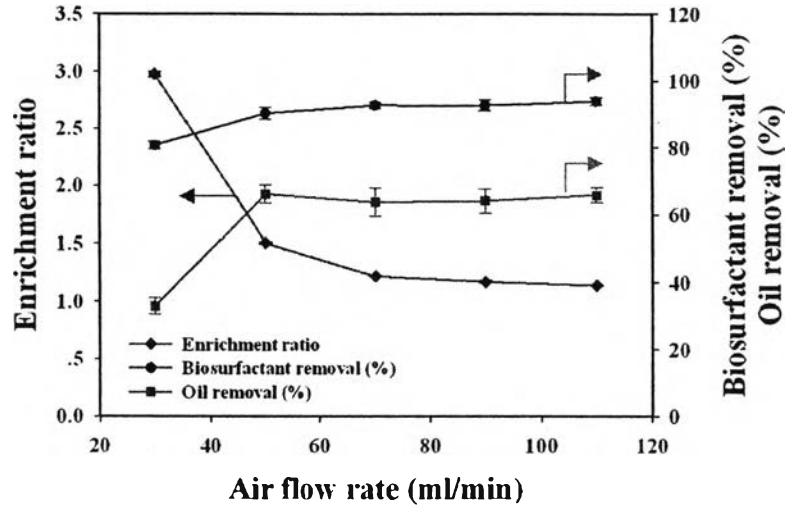

Figure 4.2 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 60 cm, sinter glass disk No.1, initial concentration 362.1632 µg/l.

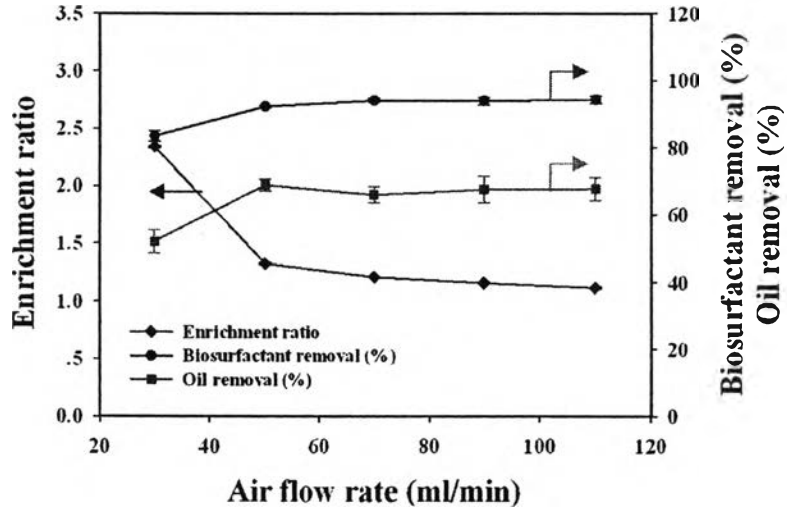

Figure 4.3 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 40 cm, sinter glass disk No.1, initial concentration 362.1632 µg/l.

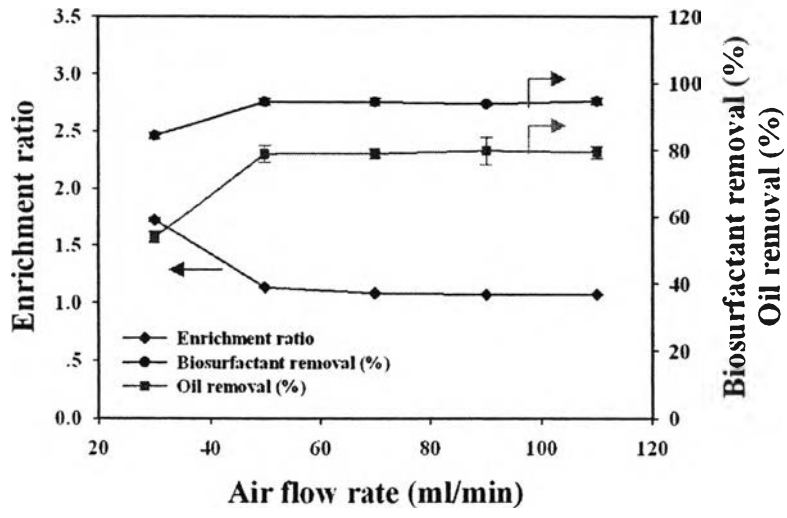

Figure 4.4 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 20 cm, sinter glass disk No.1, initial concentration 362.1632 μg/l.

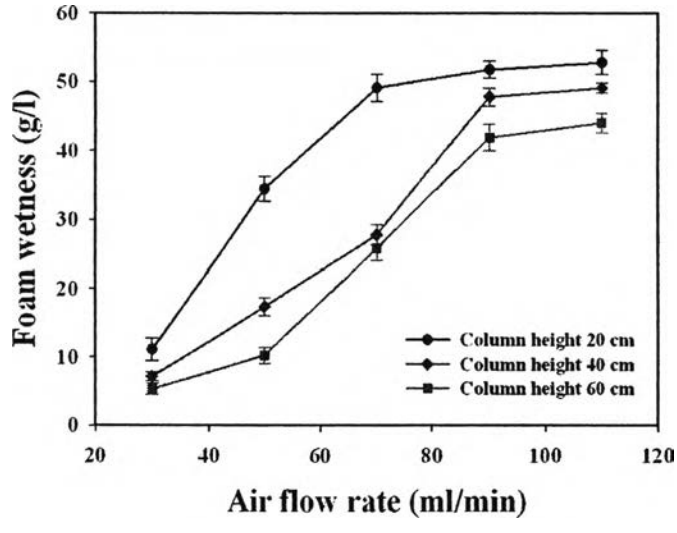

Figure 4.5 Wetness of foam in the effect of air flow rate under operational conditions of foam height 20, 40 and 60 cm, sinter glass disk No.1, initial concentration 362.1632 μg/l.

4.3 Effect of Column Height

The effect of the initial foam height on the process performance is shown in Figure 4.6-4.7. The foam fractionation experiment was carried out at the pore size of sintered glass disk in the range of 100–160 μm (No. 1) and initial liquid volume of 100 ml at five different air flow rates, while the initial foam height was varied at 20, 40, and 60 cm. It can see that increasing column height resulted in an increase in the enrichment ratio but has little effect on the percentage of biosurfactant recovery. For all conditions, the percentage of biosurfactant recovery was approximately 81.33±1.16 to 94.62±1.02, whereas the enrichment ratio was in the range of 2.98±0.02 to 1.07±0.01 as seen in the table 4.1. Among three column height studied (20, 40, 60 cm), the foam height at 60 cm. gave the highest enrichment ratio for all condition studied as shown in figure 4.6. Increasing column height lead to longer residence time, which allowed more drainage of the liquid in the films, resulted in dryer foam as shown in Table 4.1 and figure 4.5. The results agree well with the previous work (Triroj, 2005), the concentration of the adsorbed biosurfactant molecules increase as foam height increase, resulting in higher enrichment ratio. On a contrary within the range of foam height investigated here, the percentage of biosurfactant recovery decreased slightly with increasing foam height because of the increased rate of foam collapse due to foam drainage (Qu, 2008). From this study, the biosurfactant recovery percentage and the enrichment ratio were found to be optimized at the initial foam height of 60 cm.

For the oil removal studied, The result shown that the percentage of oil removal was in range of 32.95±2.48 to 79.89±4.07as seen in the table 4.2 among various column height investigated, the result had same trend with the percentage of biosurfactant recovery, as shown in the figure 4.2-4.5, the lowest percentage of oil removal as low as 32.9490 was obtained at the highest column height which was 60 cm as shown in figure 4.8. When increased the column height result in a decrease in the percentage of oil removal, because the foam had longer residence time to drain the liquid from the films and increased rate of foam collapse.

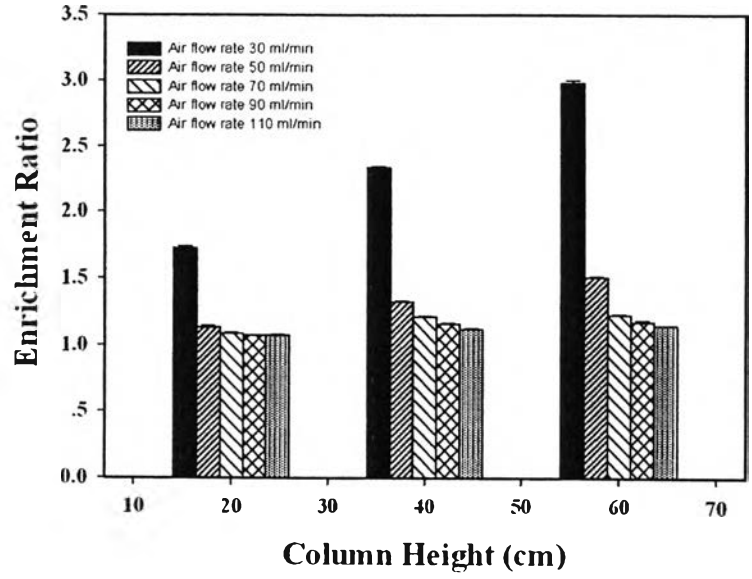

Figure 4.6 Effect of foam height on enrichment ratio of Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min, sinter glass disk No.1, initial concentration 362.1632 μg/l.

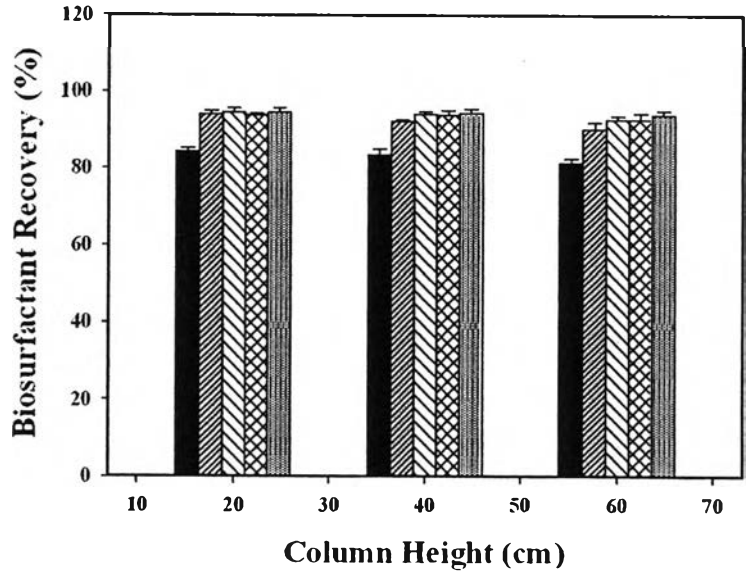

Figure 4.7 Effect of foam height on %biosurfactant recovery of Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min, sinter glass disk No.1, initial concentration 362.1632 μg/l.

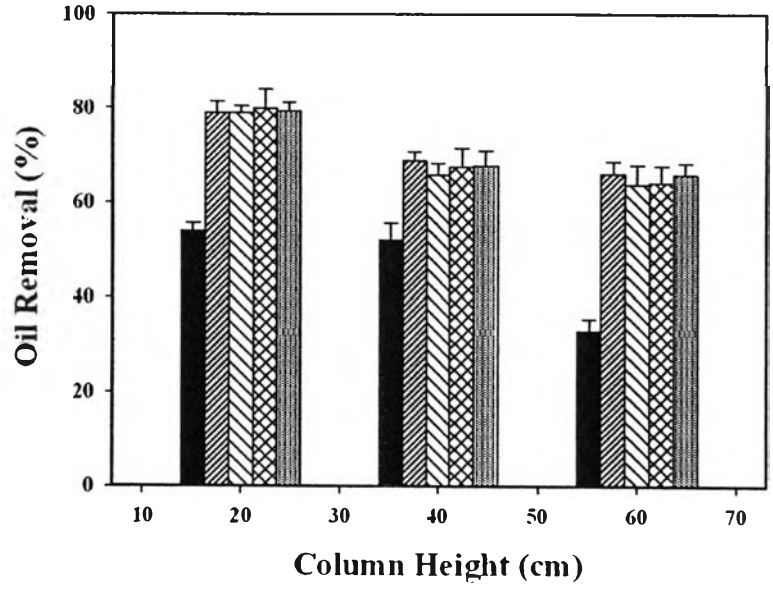

Figure 4.8 Effect of foam height on % oil removal with Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min, sinter glass disk No.1, initial concentration 362.1632 μg/l.

4.4 Effect of Pore Size of Sinter Glass Disk

The effect of porosity of the sintered glass disk was performed at the initial foam height of 60 cm and initial liquid volume of 100 ml at five different air flow rates, while the pore size of sintered glass disk was varied to be in the range of 160–250 (No. 0), 100–160 (No. 1), and 16–40 μm (No. 3). As shown in the figure 4.9-4.11 the result found that both enrichment ratio and the percentage of biosurfactant recovery had the similar trend as the result that mansion above. For the studied in the effect of porosity of the sintered glass disk, it can seen that decreasing pore size of sinter glass disk resulted in an decrease in the enrichment ratio but it led to increased in the percentage of biosurfactant recovery as shown in figure 4.13-4.14 For all conditions, the enrichment ratio was in the range of 1.03±0.004 to 4.44±0.019, whereas the percentage of biosurfactant recovery was approximately 77.03±2.24 to 96.16±0.88 as shown in Table 4.3 Among three sinter glass disk studied (No.0, 1, 3), the highest enrichment ratio of 4.44±0.02 was achieved from the sinter glass disk No.0. The foam wetness has been studied as shown in Table 4.3 and figure 4.12, as the pore size increases, the greater bubbles yield dryer foam and higher the liquid drainage rate. This effect decreased surface area of the bubble formed, resulting in a decreased in the percentage of biosurfactant recovery. The percentage of biosurfactant recovery is highest when used the lowest pore size of sinter glass disk No.3, because an increase in surface area of the air bubbles provided better biosurfactant removal from the free-cell culture medium, resulting in an increase in the biosurfactant recovery percentage, moreover, these results is in agreement with some work done by Tharapiwattananon (1995).

The effect of porosity of the sintered glass disk on the oil removal has shown that the percentage of oil removal was in range of 30.38±1.84 to 80.30±3.39 as seen in the table 4.4. Among various porosity of the sintered glass disk studied, the percentage of oil removal lower than the percentage of biosurfactant recovery, but they had same trend when varied the air flow rate as shown in the figure 4.9-4.11, the lowest percentage of oil removal as low as 30.38±1.84 was achieved at the greatest pore size of sintered glass disk No.0 as shown in figure 4.15, this result

similar to the percentage of biosurfactant recovery. When porosity increased lead to lower surface area of bubble can contain and remove oil with the biosurfactant solution, therefore, the interference from palm oil was reduced by used sinter glass disk No.0.

Table 4.3 Effect of air flow rate on enrichment ratio, biosurfactant recovery (%) and wetness for the sinter glass disk No.0, 1 and 3.

Air flow rate (ml/min)	Sinter glass disk No. 0			Sinter glass disk No. 1			Sinter glass disk No. 3		
	Enrichment ratio	Biosurfactant removal(%)	Wetness(g/l)	Enrichment ratio	Biosurfactant removal(%)	Wetness(g/l)	Enrichment ratio	Biosurfactant removal(%)	Wetness(g/l)
30	4.4444±0.0190	77.0288±2.2365	2.77±0.35	2.7949±0.0237	78.2413±2.1618	7.60±1.00	1.2119±0.0087	94.5218±0.5315	31.69±0.77
50	1.3675±0.0028	86.6071±0.7668	7.96±0.46	1.2646±0.0094	90.6175±1.2895	15.22±2.33	1.1039±0.0024	94.9383±1.2073	56.48±0.79
70	1.1739±0.0052	88.4306±1.2490	12.88±0.72	1.1237±0.0030	91.0188±0.9216	21.76±1.55	1.0340±0.0045	96.1610±0.8780	56.73±1.00
90	1.1309±0.0048	93.4836±0.3902	19.02±1.62	1.0958±0.0051	95.3291±0.7825	32.17±1.79	1.0377±0.0095	95.4590±0.1783	66.49±0.92
110	1.0985±0.0092	94.8286±1.1437	31.73±0.68	1.0755±0.0044	94.2852±1.9376	45.62±0.81	1.0314±0.0041	93.8569±0.7757	71.87±0.26

Table 4.4 Effect of air flow rate on oil removal (%) for the sinter glass disk No.0, 1 and 3

Air flow rate (ml/min)	Oil removal(%)		
	Sinter glass disk No. 0	Sinter glass disk No. 1	Sinter glass disk No. 3
30	30.3819±1.8427	43.7492±5.0028	79.0764±2.8445
50	67.4404±1.8021	74.9772±2.3633	79.2818±1.4021
70	66.5975±1.8288	74.2983±2.2902	80.3049±3.3895
90	66.5546±0.9895	75.9217±1.8692	77.0082±1.3255
110	68.0895±3.2609	74.1223±1.2280	75.6509±2.1325

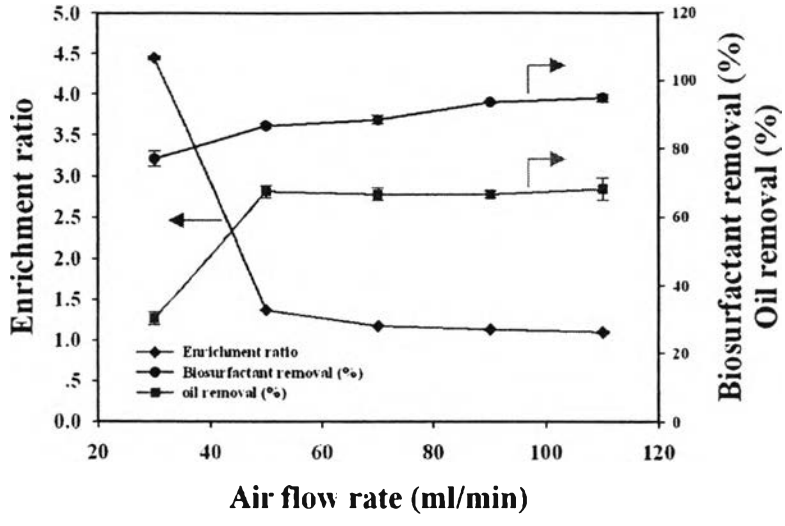

Figure 4.9 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 60 cm, sinter glass disk No.0, Initial concentration 434.4238 µg/l.

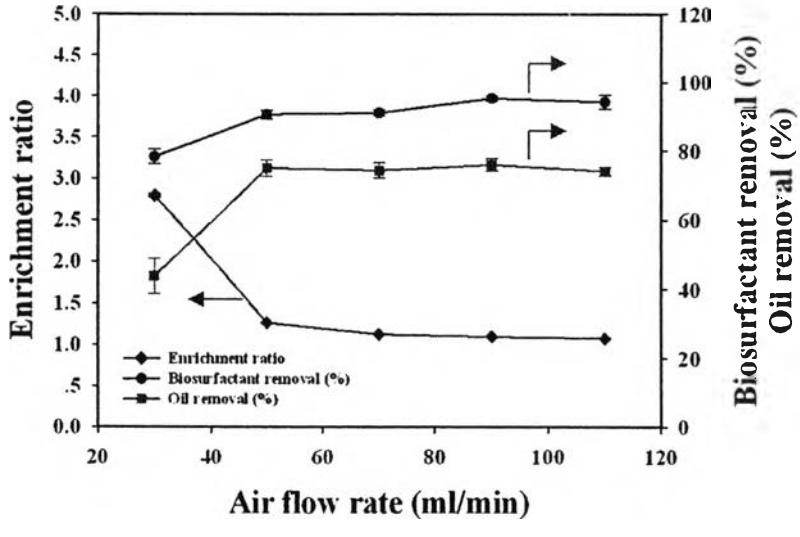

Figure 4.10 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 60 cm, sinter glass disk No.1, Initial concentration 434.4238 µg/l.

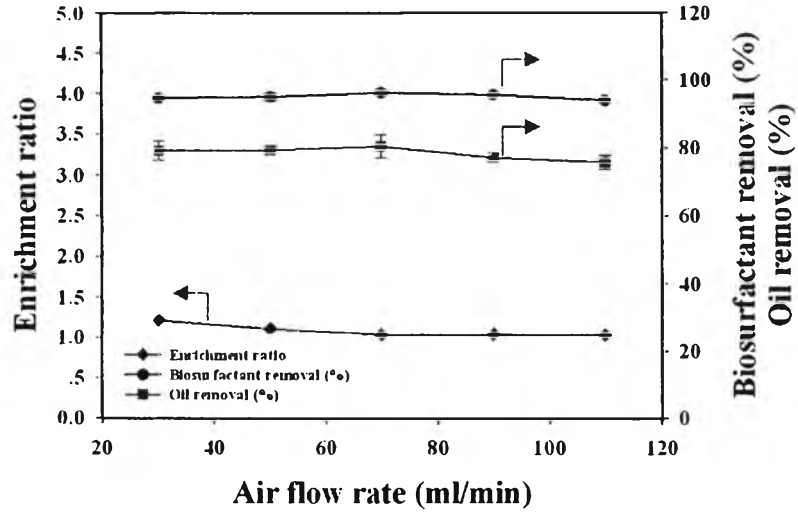

Figure 4.11 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution and oil removal (%) in the effect of air flow rate under operational conditions of foam height 60 cm, sinter glass disk No.3, Initial concentration 434.4238 µg/l.

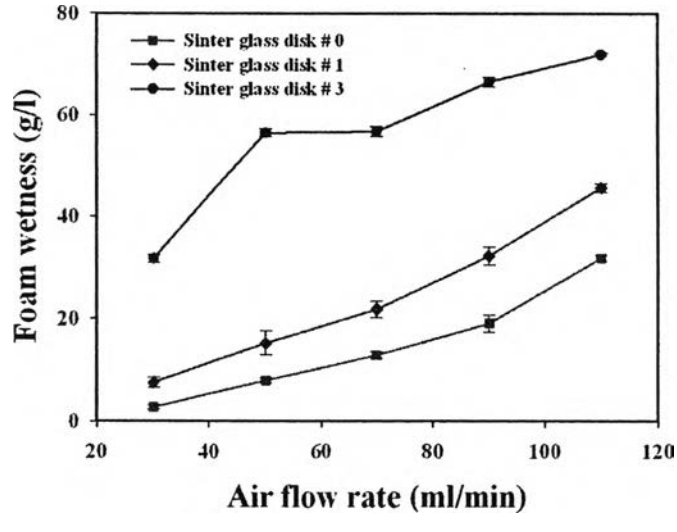

Figure 4.12 Wetness of foam in the effect of air flow rate under operational conditions of sinter glass disk No.0, 1 and 3.

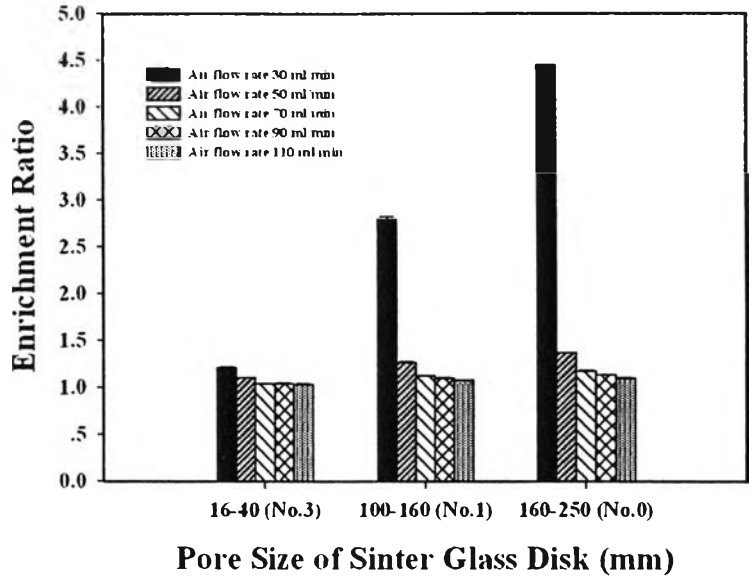

Figure 4.13 Effect of porosity of the sintered glass disk on enrichment ratio of Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min; initial concentration 434.4238 µg/l.

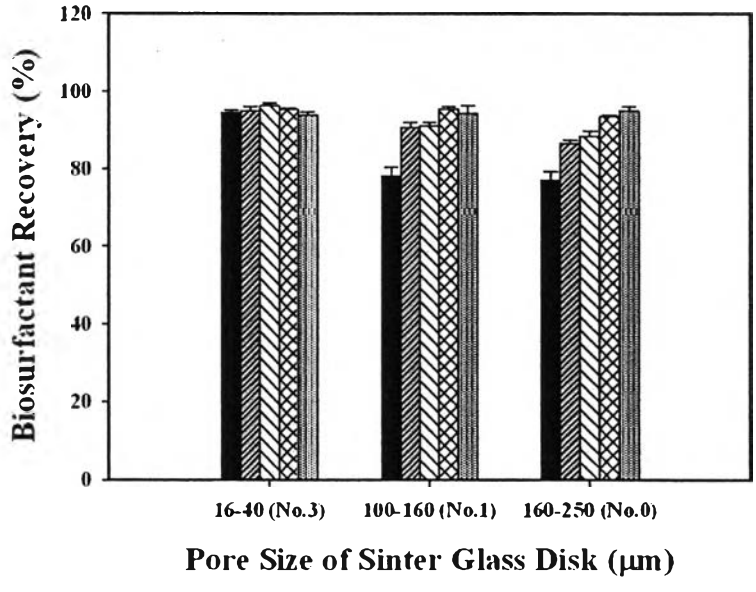

Figure 4.14 Effect of porosity of the sintered glass disk on biosurfactant recovery (%) of Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min; initial concentration 434.4238 µg/l.

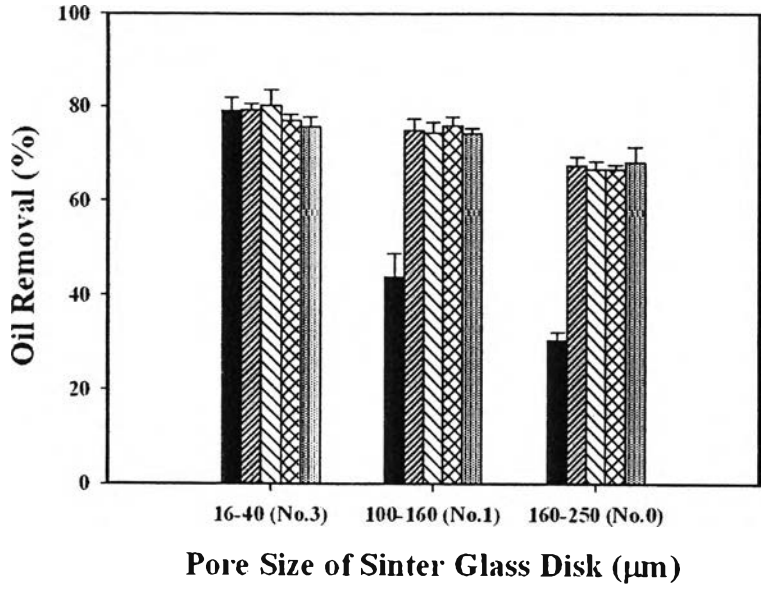

Figure 4.15 Effect of porosity of the sintered glass disk on oil removal (%) with Rhamnolipid biosurfactant solution under operational conditions of air flow rate 30, 50, 70, 90 and 110 ml/min; initial concentration 434.4238 μg/l.

4.5 Effect of Liquid Volume

The effect of liquid volume on biosurfactant recovery percentage and enrichment ratio was shown in figure 4.16. The foam fractionation experiment was done at the air flow rate of 30 ml/min, the initial foam height of 60 cm, and the sintered glass disk No. 0, while initial volume of the free-cell culture medium was varied at 25, 50, 75, 100, and 125 ml. The results indicated that an increase in initial liquid volume strongly reduced the biosurfactant recovery percentage, but slightly affected the enrichment ratio. As initial liquid volume increased from 25 to 125 ml, the biosurfactant recovery percentage decreased from 94.80±1.91 to 58.98±2.12 while the enrichment ratio slightly reduced from 3.88±0.10 to 3.72±0.01. Furthermore, when increase solution volume lead to shorter residence time to drain the liquid in the films, resulted in an increase in foam wetness as shown in figure 4.17. At low initial liquid volume, the air bubbles could be generated easily, and a large volumetric of foam was produced. Therefore, the highest biosurfactant recovery percentage was obtained at the lowest initial liquid volume. From the results, the optimum initial liquid volume for the foam fractionation experiment was found to be at 25 ml.

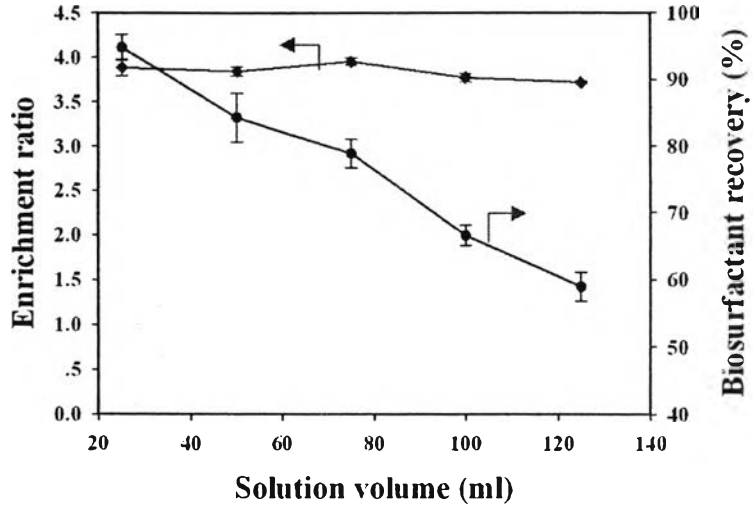

Figure 4.16 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution in the effect of solution volume under operational conditions of air flow rate 30 ml/min, foam height 60 cm, sinter glass disk No.0, Initial concentration 385.4343 µg/l.

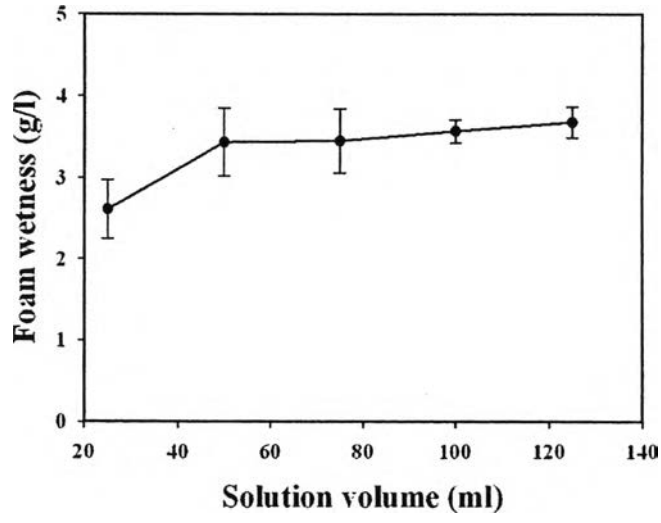

Figure 4.17 Wetness of foam in the effect of solution volume under operational conditions of air flow rate 30 ml/min, foam height 60 cm, sinter glass disk No.0, Initial concentration 385.4343 µg/l.

4.6 Effect of Operation Time

The effect of operation time was investigated at the air flow rate of 30 ml/min, the initial foam height of 60 cm, the sintered glass disk No. 0, and initial liquid volume of 25 ml. To let the foam outflow from the top of the column, the effect of operation time was investigated after 1 h. Figure 4.18 shows the effect of operation time on process performance in terms of the biosurfactant recovery percentage and the enrichment ratio. The results indicated that the biosurfactant recovery significantly increased with increasing operation time, while the enrichment ratio remained stable after 1.5 h. As operation time increased from 1 to 4 h, the biosurfactant recovery percentage increased from 81.93±1.51 to 96.82±0.65. An increase in operation time led to a reduction of liquid volume in the foam fractionation column—remaining liquid volume. Since the air bubbles was generated easier in low liquid volume, a larger volumetric of foam was produced with increasing operation time. Hence, the biosurfactant recovery percentage increased. From the obtained results, the optimum operating conditions of the single stage foam fractionator used in this present study was at the air flow rate of 30 ml/min, the initial foam height of 60 cm, the pore size of sintered glass disk in the range of 160–250μm (No. 0), initial liquid volume of 25 ml, and operation time of 4 h, corresponding to the biosurfactant recovery percentage of 96.82±0.65 and the enrichment ratio of 3.79±0.05. The foamate (collapsed foam) obtained from the foam fractionator operated at the optimum conditions will be collected before analyzed for the rhamnolipid composition and concentration, compared to those of the free-cell culture medium (initial solution).

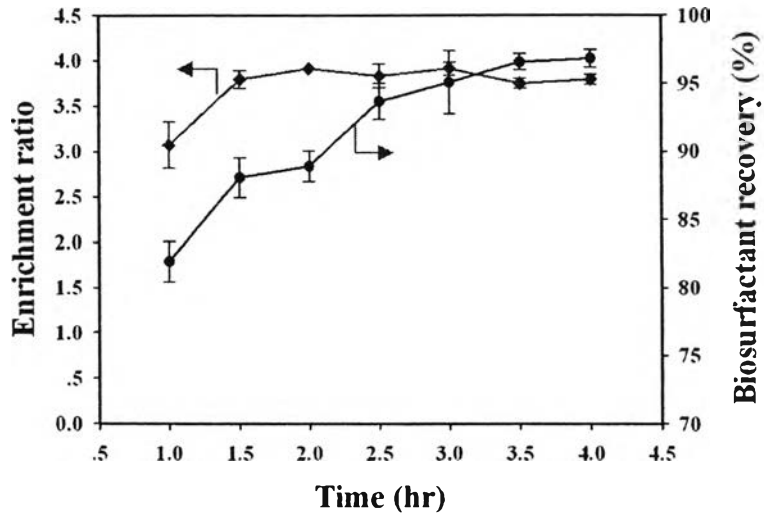

Figure 4.18 Enrichment ratio, Biosurfactant recovery (%) of Rhamnolipid biosurfactant solution in the effect of collection time under operational conditions of air flow rate 30 ml/min, foam height 60 cm, sinter glass disk No.0, solution volume 25 ml, Initial concentration 385.4343 µg/l.

4.7 Diameter of Bubbles

The measurement of foam diameter was performed under the optimum condition of column height 60 cm., air flow rate 30 ml/min., sinter glass disk No.0, however, in this studied solution volume was used 100 ml for convenient to take the photo in the solution. This study was observed at different level of column: in the free-cell culture medium, at the interface of the free-cell culture medium, in the middle of column and at the top of column. The averaged diameter of the generated air bubbles in the free-cell culture medium, at the interface of the free-cell culture medium, in the middle of the column, and at the top of the column was found to be 1.12±0.07, 1.53±0.15, 1.67±0.15, and 1.88±0.14 mm, respectively. As shown in Figure 4.19, the greatest average diameter of bubble was achieved at the top of the column and the lowest average diameter of bubble was obtained in the free-cell culture medium as shown in the figure 4.20. The result consequence to the studied of column height, Increasing height lead to longer residence time, which allow more drainage of the liquid in the films resulted in a dryer foam. The drainage of foam results from competition between gravitational forces and the capillary pressure in channels separating adjacent bubbles. The drainage-capillary effects imply that the top of the foam becomes dry while the bottom of the column remains wet. The spherical air bubbles were observed in the free-cell culture medium and at the interface, while the polyhedral air bubbles were in the middle and at the top of the foam fractionation column. As the initial foam height increased, residence time increased and foam became dryer due to an increase in a liquid drainage, leading to a bigger air bubbles in the foam. Generally, dry foam is composed of polyhedral air bubbles meeting at thin edge, while wet foam contains spherical air bubbles which can sometimes move freely (Weaire,1999). Thus, bigger polyhedral air bubbles were observed at the top of the foam, while smaller spherical ones were found at the bottom of the foam fractionation column where foam was still wet.

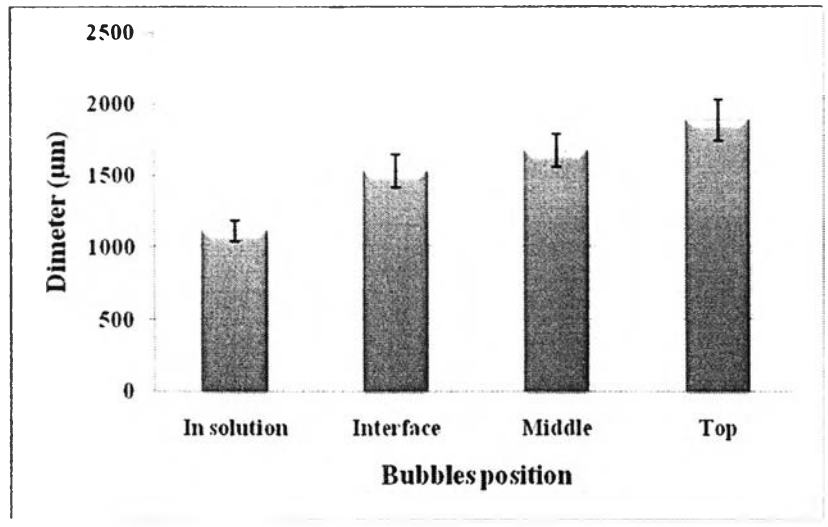

Figure 4.19 Diameter of bubble at difference position of column, Column height 60 cm, Air flow rate 30 ml/min, sinter glass disk No.0 and solution volume 100 ml.

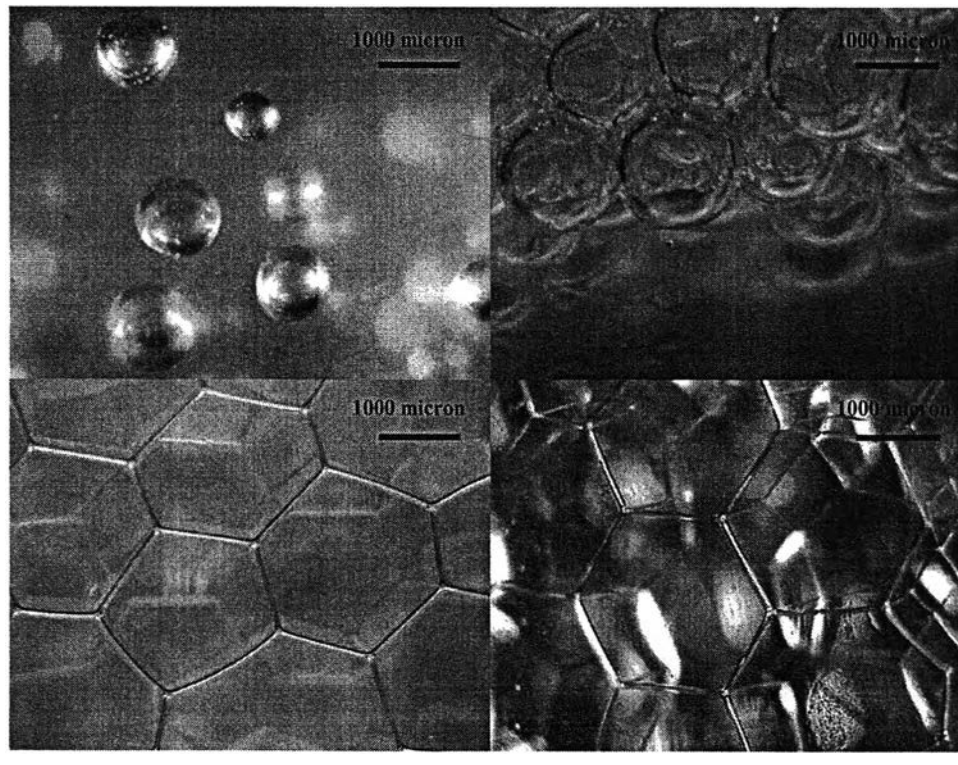

Figure 4.20 Images of air bubbles in different position of column (a) in the free-cell culture medium, (b) at interface of the free-cell culture medium, (c) in the middle of column and (d) at the top of column.

4.8 Fractions of Crude Biosurfactant

The fraction of crude biosurfactant, initial biosurfactant solution and the collapsed foam biosurfactant obtained from the liquid culture of *Pseudomonas aeruginosa* strain SP4 were compared by using HPLC-ELSD technique. The HPLC patterns of crude biosurfactant, the initial biosurfactant solution and the collapsed foam biosurfactant are identical as seen in figure 4.22-4.24. The key components of the crude biosurfactant were separated into six main fractions eluted from the HPLC column at different retention times. As shown in the figure 4.21 and the table 4.5, the crude biosurfactant contains those six fractions as shown in figure 4.28, named A, B, C, D, E, and F, about 7.45%, 1.98%, 64.98%, 10.73%, 14.18%, and 0.68%, respectively. In the present study, either the free-cell culture medium or the foamate was subjected to the HPLC analysis without further purification by the solvent extraction. The freshly-prepared nutrient broth, culture medium, was used as a control. It was found that fractions A and F were diluted in both the free-cell culture medium and the foamate (less than 1%) (data not shown), so only the four main rhamnolipid components (fractions B,C, D, and E) in either the free-cell culture medium or the foamate will be quantified based on the HPLC peak area. Among the fractionated components, fraction C is the predominant component in those biosurfactant, this result is in agreement with work done by Pornsunthorntawee (2007). In addition, the peak intensities of the biosurfactant in the collapsed foam were higher than those of the initial biosurfactant solution. When compare the peak area between initial biosurfactant and the biosurfactant in the collapsed foam, the peak area ratio of Collapsed foam biosurfactant/Initial biosurfactant was shown in table 4.6 and figure 4.25, The rhamnolipid composition in the foamate is also quite comparable to those of the crude extract. Therefore, the foam fractionation method is not selective to any rhamnolipid components. Based on the peak area ratio of each fraction, it was found that fractions B, C, D, and E in the foamate were concentrated to 4.78, 3.64, 10.04, and 10.04 times of those in the initial solution, respectively. It can be seen that the peak area of the fraction B, C, D and E of biosurfactants in the

collapsed foam was higher than the initial biosurfactant solution, indicating the higher biosurfactant concentration.

Furthermore, the peak areas of fraction C and E of the biosurfactants in the collapsed foam were 1.1404 and 1.4815 times higher than the crude biosurfactants, which show the efficiency of foam fractionation technique is better than solvent extraction, to separate fraction C and E of biosurfactants from the initial solution. Apart from that, the nutrient broth has been performed by HPLC-ELSD as shown in figure 4.25, the result show peak at retention time 2.900 and 3.700, which also shown in both the initial biosurfactant solution and the collapsed foam biosurfactant HPLC graphs but those peak were not shown in crude biosurfactnts, indicating the interference from component in nutrient broth occured when the biosurfactant was not purified by solvent extraction. Based on the total peak area ratio, the rhamnolipid biosurfactant in the foamate was concentrated to 4.44±0.31 times of that in the free-cell culture medium. The obtained HPLC results also agree well with the enrichment ratio of the foamate which was found to be about 4. Hence, the single stage foam fractionator used in the present study could successfully recover and primarily concentrate the rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* strain SP4 from the free-cell culture medium.

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Table 4.5 HPLC-ELSD isolated fractions of the crude biosurfactant produced by *Pseudomonas aeruginosa* SP4.

Fraction	Avg. Retention Time (min)	Peak Area (%)
A	4.6387	7.6902
B	13.3499	2.4694
C	18.0499	65.8504
D	21.0222	10.4086
E	23.7944	13.4382
F	27.3998	0.1433

Table 4.6 HPLC peak area ratio of isolated fractions produced by *Pseudomonas aeruginosa* SP4.

Fraction	Peak area ratio	
	Collapsed foam biosurfactant/ Initial biosurfactant	Collapsed foam biosurfactant/ Crude biosurfactant
B	4.7822	0.3290
C	3.6359	1.1404
D	10.0412	0.9590
E	10.0404	1.4815

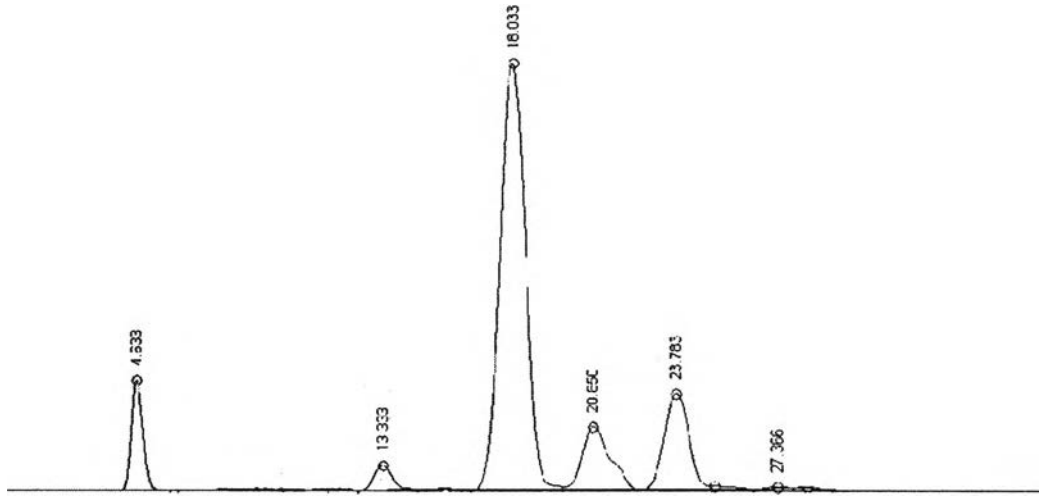

Figure 4.21 HPLC fraction of crude biosurfactant.

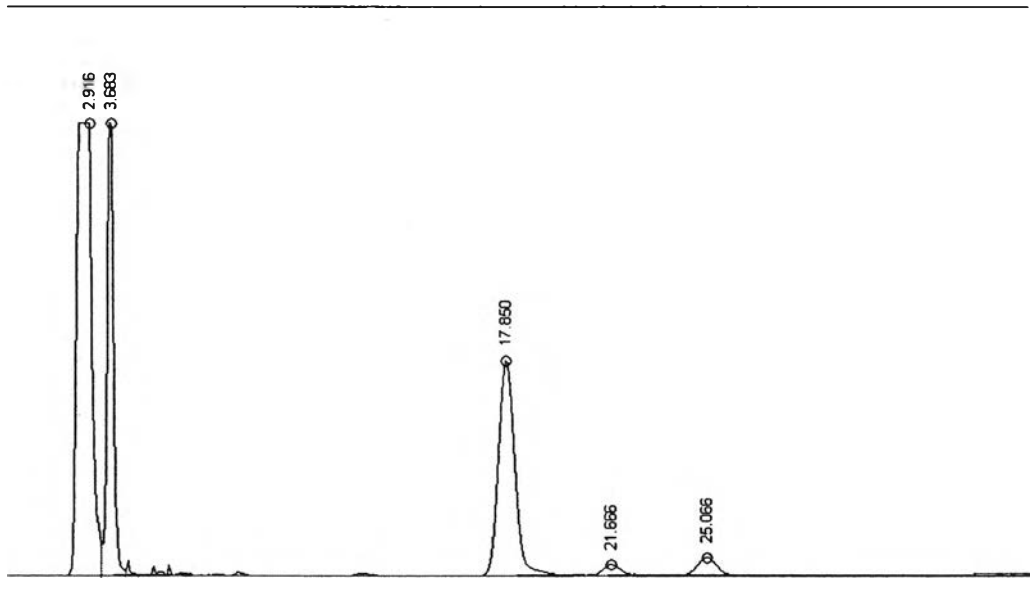

Figure 4.22 HPLC fraction of initial biosurfactant solution.

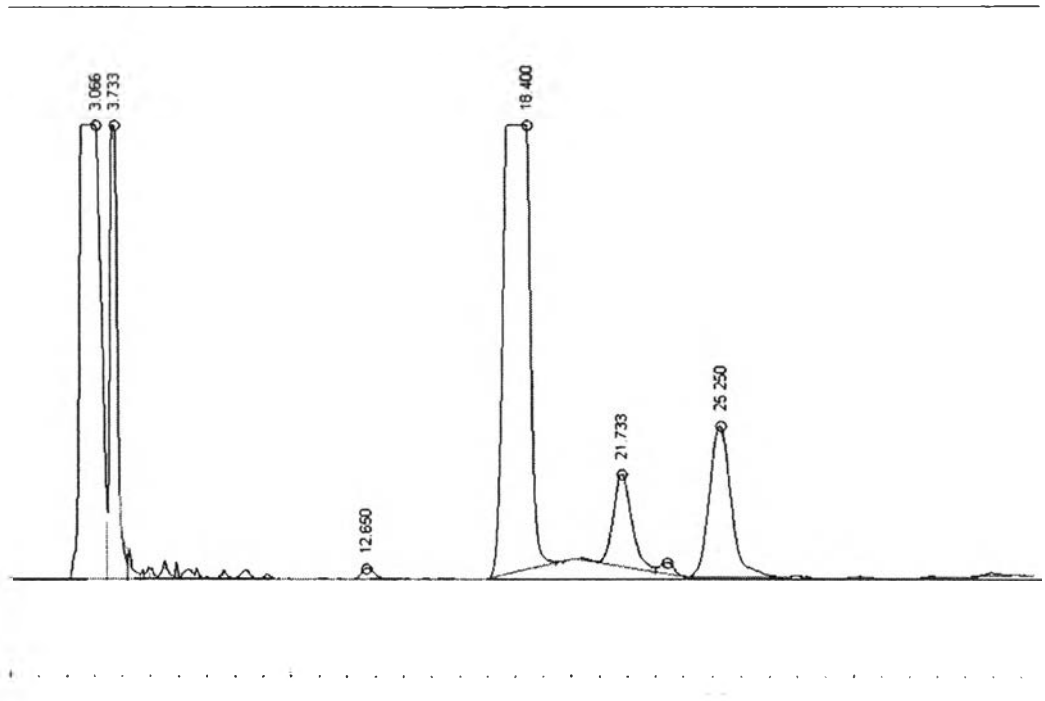

Figure 4.23 HPLC fraction of collapsed foam from foam fractionation process

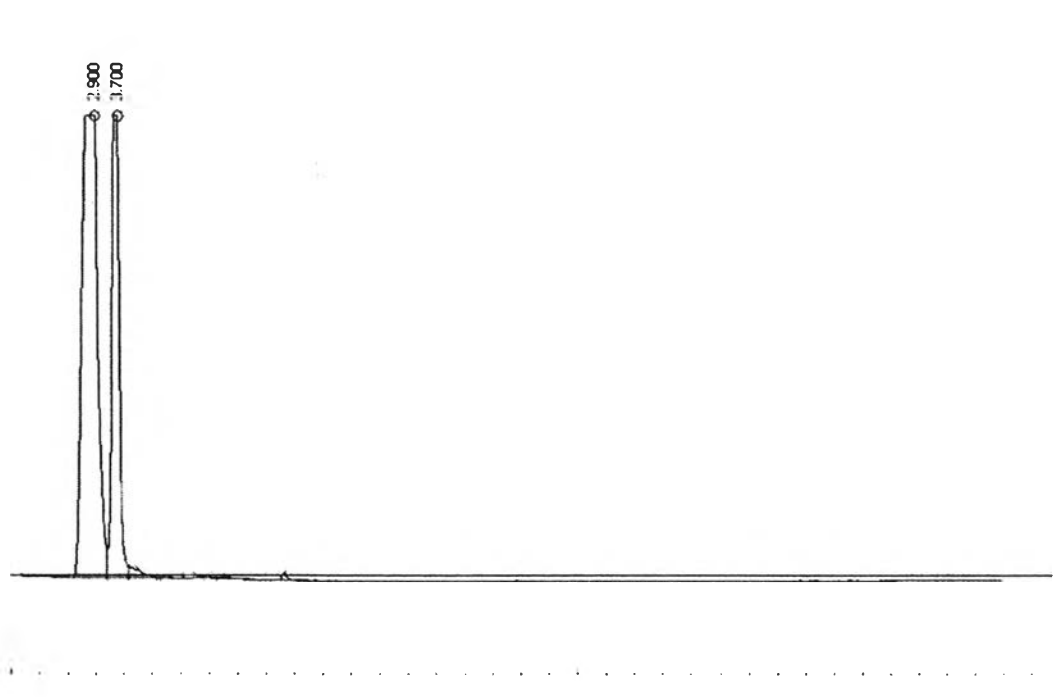

Figure 4.24 HPLC fraction of nutrient broth

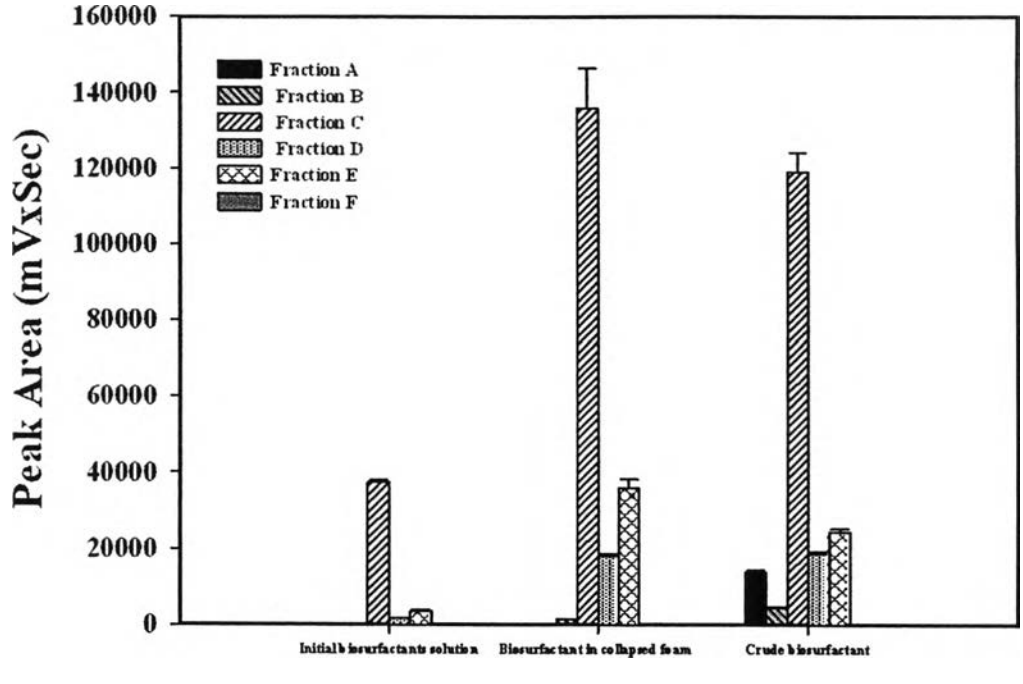

Figure 4.25 Comparison of the peak area of initial biosurfactants solution, the collapsed foam biosurfactants and crude biosurfactants from HPLC histogram.

4.9 Chemical Structures of Biosurfactants

From the HPLC results, the fraction C of foam fractionation and solvent extraction method is interesting because the predominant component in those biosurfactant, so these fractions were continued observed by MS in order to investigate the molecular weight, and to identify the structure by using previous work (Pornsunthorntawee, 2007) as the references for interpret the structure of these fractions. The MS analysis was studied in the positive electrospray ionization mode, therefore positively charged molecules were observed. Under these conditions, the molecules having an organic function like rhamnolipids tend to form sodium or potassium adducts; thus the signals appearing in the mass spectra are assigned to the sodiated or potassiated ions (Rendell et al., 1990; Déziel et al., 1999). From the mass spectra, the possible molecular weights for the fraction C, which is the predominant component in the crude biosurfactant produced by *P. aeruginosa strain* SP4 were identified as 504. The mass spectrum of fraction C of the crude biosurfactants shows the peak signal at m/z 504, furthermore, the peak shows the intense sodiated and dipotassiated molecular ions at m/z 527 and 582, respectively, these spectrum are consistent with the structure of monorhamnolipid (Rha–C10–C10). For the mass spectrum of fraction C of the biosurfactants in collapsed foam, the peak of spectrum also shows ion at m/z 527 and 582, consistent with the structure of monorhamnolipid (Rha–C10–C10). According to the data indicated that both the fractions have the similar structure, which was observed in the previous work (Pornsunthorntawee, 2007).