REFERENCES

Ahn, H., Lee, H., Lee, S., and Lee, Y. Pervaporation of an aqueous ethanol solution through hydrophilic zeolite membranes. Desalination 193(2006) 244-251.

Ball, J.I., Huang, S.-C., Wolf, R. A., Shimano, Y., and Kaner, R. B. Pervaporation studies with polyaniline membranes and blends. Journal of Membrane Science 174 (2000) 161–176.

Brunauer, S., Emmett, P.H. and Teller, E., Adsorption of gases in multimolecular layers. Journal of the American Chemical Society 60 (1938) 309-319.

Chen, J.H., Liu, Q.L., Zhang, X.H., and Zhang, Q.G. Pervaporation and characterization of chitosan membranes cross-linked by 3-aminopropyltriethoxysilane. Journal of Membrane Science 292 (2007) 125–132.

Chen, S.-H., Liou, R.-M., Hsu, C.-S., Chang, D.-J., Yu, K.-H., and Chang, C.-Y. Pervaporation separation water/ethanol mixture through lithiated polysulfone membrane. Journal of Membrane Science 193 (2001) 59–67.

Dubey, V., Saxena, C., Singh, L., Ramana, K.V., andChauhan ,R.S. Pervaporation of binary water-ethanol mixtures through bacterial cellulose membrane. Separation and Purification Technology 27(2001) 163-171.

Dubey, V., Pandey, L. K., and Saxena, C. Pervaporative separation of ethanol/water azeotrope using a novel chitosan-impregnated baterial cellulose membrane and chitosan-poly(vinyl alcohol) blends. Journal of Membrane Science 251(2004) 131-136.

Dubey, V., Pandey, L.K., and Saxena, C. Pervaporative separation of ethanol/water azeotrope using a novel chitosan-impregnated bacterial cellulose membrane and chitosan–poly(vinyl alcohol) blends. Journal of Membrane Science 251 (2005) 131-136.

Gonzalez-Velasco, J.R., Gonzalez-Marcos , J.A.,and Lopez-Dehesa, C. Pervaporation of ethanol-water mixtures through poly(1-trimethylsilyl-1-propyne) (PTMSP) membranes. Desalination 149(2002) 61-65.

Jiraratananon, R., Chanachai, A., Huang, R.Y.M., and Uttapap, D. Pervaporation dehydration of ethanol-water mixtures with chitosan/hydroxyethylcellulose (CS/HEC) composite membranes. I.Effect of operating conditions. Journal of Membrane Science 195(2002) 143-151.

Kalyani, S., Smitha, B., Sridhat, S., and Krishnaiah, A. Pervaporation separation of ethanol-water mixtures through sodium alginate membranes. Desalination 229 (2008) 68-81.

Kanti, P., Srigowri, K., Madhuri, J., Smitha, B., and Sridhar, S. Dehydration of ethanol through blend membranes of chitosan and sodium alginate by pervaporation. Separation and Purification Technology 40 (2004) 259-266.

Pandey, L.K., Saxena, C., and Dubey, V. Studies on pervaporative characteristics of bacterial cellulose membrane. Separation and Purification Technology 42 (2005) 213-218.

Kujawski, W., and Krajewski. Influence of inorganic salt on the effectiveness of liquid mixtures separation by pervaporation. Separation and Purification Technology 57(2007) 495-501.

Li, B.-B., Xu, Z.-L., Qusay , F.Q., and Li, R. Chitosan-poly (vinyl alcohol)/poly (acrylonitrile) (CS–PVA/PAN) composite pervaporation membranes for the separation of ethanol–water solutions. Desalination 193 (2006) 171–181.

Lee, Y.M., Kim, S.H., and Kim, S.J. Preparation and characterization of β-chitin and poly (vinyl alchol) blend. Polymer 37 (1994) 5897-5905.

Lee, K.H., Yeom, C.K. and Jegal, J.G., Study on permeation behavior of liquid mixture through sodium alginate membranes. Division of Polymeric Materials Science and Engineering of the American Chemical Society 77 (1997) 345.

Nomura, M., Bin, and Nakao, T. Selective ethanol extraction from fermentation broth using a silicalite membrane. Separation and Purification Technology 27(2002) 59-66.

Nunes, S.P., and Peinemann, K.-V. Membranes.Membrane technology in the chemical industry (2001) 149-150.

Ohshima, T., Kogami, Y., Miyata, T., and Uragami, T. Pervaporation characteristics of cross-linked poly(dimethylsiloxane)membranes for removal of various volatile organic compounds from water. Journal of Membrane Science 260 (2005) 156–163.

Phisalaphong, M., Suwanmajo, T., and Sangtherapitikul, P. Novel nanoporous membranes from regenerated bacterial cellulose. Journal of Applied Polymer Science 107 (2008) 292-299.

Sanchavanakit, N., Sangrungraungroj, W., Kaomongkolgit, R., Banaprasert, T., Pavasant, P., and Phisalaphong, M. Growth of human keratinocytes and fibroblasts on bacterial cellulose film. Biotechnol.Prog. 22 (2006) 1194-1199.

Shieh, J.J., and Huang Y.M. Chitosan/*N*-methylol nylon 6 blend membranes for the pervaporation separation of ethanol-water mixtures. Journal of Membrane Science 148 (1998) 243-255.

Smitha, B., Suhanya, D., Sridhar, S., and Ramakrishna, M. Separation of organic organic mixtures by pervaporation. Journal of Membrane Science 241 (2004) 1–21.

Yang, G., Zhang, L., Peng, T., and Zhong, W. Effects of Ca^{2+} bridge cross-linking on structure and pervaporation of cellulose/alginate blend membranes. Journal of Membrane Science 175 (2000) 53-60.

Zhang, L., Zhou, D., Wang, H. and Cheng, S. Ion exchange membrane blended by cellulose cuoxam with alginate. Journal of Membrane Science 124 (1997) 195-201.

Zhou, J. and Zhang, L. Structure and properties of blend membranes prepared from cellulose and alginate in NaOH/Urea aqueous solution. Journal of Polymer Science 39 (2001) 451-458

Zhou, L.L., Sun, D.P., Hu, L.Y., Li, Y.W., and Yang, J.Z. Effect of addition of sodium alginate on bacterial cellulose production by *Acetobacter xylinum*. Journal of Industrial Microbiology and Biotechnology 34 (2007) 483-489.

APPENDICES

APPENDIX A

DATA OF MEMBRANE CHARACTERIZATION

Table A1 Data of Figure 5.3

Alginate content (%)	Tensile strength (MPa)						
	1	2	3	4	5	Average	S.D.
0	5.48	5.62	5.29	5.11	5.01	5.30	0.25
0.5	4.82	4.71	4.84	4.67	4.51	4.71	0.13
0.75	4.26	4.38	4.09	4.12	4.04	4.18	0.14
1	3.98	3.74	3.62	3.81	3.84	3.80	0.13

Table A2 Data of Figure 5.4

Alginate content (%)	Young's Modulus (MPa)						
	1	2	3	4	5	Average	S.D.
0	184	170	167	168	175	172.80	6.98
0.5	171	147	165	158	167	161.60	9.42
0.75	154	159	142	150	144	149.80	7.01
1	152	148	137	143	142	144.40	5.77

Table A3 Data of Figure 5.5

Alginate content (%)	Elongation at break (%)						
	1	2	3	4	5	Average	S.D.
0	3.99	3.67	3.57	3.76	3.79	3.76	0.16
0.5	3.62	3.31	3.46	3.36	3.42	3.43	0.12
0.75	2.71	2.98	2.96	2.74	2.92	2.86	0.13
1	2.46	2.67	2.51	2.68	2.72	2.61	0.12

Table A4 Data of Figure 5.6

Alginate content (%)	Degree of swelling (%) at pure water						
	1	2	3	4	5	Average	SD
0	531	501	518	543	528	524	15.74
0.5	631	592	616	645	628	622	19.88
0.75	674	689	660	698	688	682	14.91
1	716	724	702	694	696	706	13.07

Table A4 Data of Figure 5.6

Alginate content (%)	Degree of swelling (%) at 95% ethanol (v/v)						
	1	2	3	4	5	Average	SD
0	238	267	254	241	232	246	14.05
0.5	284	273	288	256	294	279	14.97
0.75	286	325	312	291	294	302	16.35
1	312	336	342	302	327	324	16.62

Table A6 Data of Figure 5.7

Alginate content (%)	WVTR (g/m^2day)			
	1	2	Average	SD
0	2124	2088	2106	25.5
0.5	1924	1929	1926	3.2
0.75	1942	1886	1914	40.0
1	1894	1736	1815	111.7

Table A7 Data of Figure 5.8

Alginate content (%)	OTR (cc/m^2day)			
	1	2	Average	SD
0	37630	35457	36544	1536.5
0.5	13549	14071	13810	369.1
0.75	104	98	101	4.1
1	51	63	57	8.1

APPENDIX B

DATA OF PERVAPORATION EXPERIMENTS

Table B1 Data of Figure 5.8 (a) and (b)

Alginate content (%)	Selectivity	Total Flux $(g/(m^2h))$	Ethanol Flux $(g/(m^2h))$	Water Flux $(g/(m^2h))$
0	1.01	27159.24	25790.60	1368.64
0.5	1.11	26598.73	25136.27	1462.45
0.75	1.19	19490.45	18345.98	1144.46
1	1.64	14904.46	13716.96	1187.50

Table B2 Data of Figure 5.9 (a) and (b)

Ethanol Feed (%)	Selectivity	Total Flux $(g/(m^2h))$	Ethanol Flux $(g/(m^2h))$	Water Flux $(g/(m^2h))$
70	1.48	18063.69	11053.82	7009.87
80	1.93	16993.63	11467.51	5526.12
90	1.81	15566.88	12965.70	2601.17
95	1.64	14904.46	13716.96	1187.50

Table B3 Data of Figure 5.10 (a) and (b)

Temperature (^oC)	Selectivity	Total Flux (g/(m^{2}h))	Ethanol Flux (g/(m^{2}h))	Water Flux (g/(m^{2}h))
30	1.48	18063.69	11053.82	7009.87
40	1.36	18290.56	11548.34	6742.23
50	1.24	18540.25	12103.20	6437.05
60	1.20	18731.11	12356.48	6374.63

Table B4 Data of Figure 5.10 (c) and (d)

Temperature (^oC)	Selectivity	Total Flux (g/(m^{2}h))	Ethanol Flux (g/(m^{2}h))	Water Flux (g/(m^{2}h))
30	1.81	15566.88	12965.70	2601.17
40	1.58	16204.17	13780.33	2423.84
50	1.47	16866.24	14503.82	2362.43
60	1.34	17321.02	15081.60	2239.42

Table B5 Data of Figure 5.10 (e) and (f)

Temperature (^{o}C)	Selectivity	Total Flux ($g/(m^2h)$)	Ethanol Flux ($g/(m^2h)$)	Water Flux ($g/(m^2h)$)
30	1.64	14904.46	13716.96	1187.50
40	1.54	15541.40	14378.44	1162.96
50	1.33	16411.46	15337.14	1074.33
60	1.22	17095.54	16066.13	1029.41

Table B6 Data of Figure 5.11 (a) and (b)

Permeate Pressure (mmHg)	Selectivity	Total Flux ($g/(m^2h)$)	Ethanol Flux ($g/(m^2h)$)	Water Flux ($g/(m^2h)$)
5	2.34	17402.86	15491.02	1911.84
7.5	2.10	16048.25	14447.94	1600.32
10	1.64	14904.46	13716.96	1187.50

Table B7 Data of Figure 5.12 (a) and (b)

Thickness (μm)	Selectivity	Total Flux (g/(m^{2}h))	Ethanol Flux (g/(m^{2}h))	Water Flux (g/(m^{2}h))
40	1.64	14904.46	13716.96	1187.50
60	2.95	12826.41	11100.26	1726.15
90	4.05	11964.41	9862.04	2102.37

APPENDIX C

NOMENCLATURE

Greek symbols

α = activity factor [-]

β = enrichment factor [-]

μ_i = chemical potential of a component i [-]

Symbols

a_i = activity of a component i [-]

c_i = molar concentration of a component i [mol kg^{-1}]

J_i = membrane flux of a component i [kg m^{-2}h^{-1}]

l = membrane thickness [m]

L_i = phenomehological transport coefficient [mol s^{-1}m^{-3}]

P = permeability coefficient [mol s^{-1}m^{-2}]

R = gas constant [J mol^{-1}K^{-1}]

T = absolute temperature [K]

Subscripts

P = permeate

F = feed

Superscript

i = component

m = membrane phas

CURRICULUM VITAE

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To : Nitisak Kanjanamosit, Chirakarn Muangnapoh, Muenduen Phisalaphong

Date : September 17, 2008

Dear Sir or Madam,

It is our pleasure to invite you to participate in the 18th Thailand Chemical Engineering and Applied Chemistry conference (TIChE 18) which will be held in Pattaya from October 20th to 21st, 2008 .

Your contribution entitled "Biosynthesis and Characterization of Bacterial Cellulose-Alginate Film" has been accepted as oral presentation.

Yours sincerely,

N. Yoswathana

Prof. Dr. Nuttawan Yoswathana
Head of Chemical Engineering Department,
Faculty of engineering ,
Mahidol University.

Biosynthesis and Characterization of Bacterial Cellulose-Alginate Membrane

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Bacterial cellulose film was modified by adding 0.5-1 (% w/v) of alginate in the culture medium during biosynthesis by *Acetobacter xylinum*. In this study, BCA and BC film refer to bacterial cellulose film with and without the addition of alginate in culture medium, respectively. The surface structures of film analyzed by scanning electron microscopy (SEM) showed that the BCA film was significantly denser compared to the BC film. The water absorption capacity (WAC) increased with the increase of alginate content. The WAC of BC film was 542%. Supplementation of 0.5%, 0.75%, 1% (w/v) caused the increase in WAC to 622%, 682% and 706% respectively. The total surface area and average pore size of the entire BC film determined by BET demonstrated that the average pore diameter of film decreased with increasing alginate content; whereas, the surface area was slightly decreased.

1. Introduction

Cellulose is Earth's major biopolymer from plants and other living systems such as plankton, algae, fungi, and bacterial. Bacterial cellulose (BC), produced by *Acetobacter xylinum*, is distinctly different from cellulose derived from plants and the others. Its unique structural features and properties, including high degree of crystallinity, superior mechanical properties and high water absorption capacity (WVC) has been previously reported [1]. Several applications of BC have been proposed such as artificial skin for human with extensive burns [2], diaphragms in speakers for audio-communication and filter membrane [3].

The cellulose membranes could be blended with other natural polymer such as alginate, konjac glucomannan, chitosan and chitin to modify their properties for applications in separation fields [4]. Alginate (Al), a heteropolysaccharide extracted from marine brown algae, has been widely explored as a substrate material for blend membrane. The blend membrane from cellulose (cotton linter) and Al showed improved performance for the dehydration of ethanol-water in a pervaporation process [5]. Therefore, in this work, we focused on developing a new blend membrane from BC and Al.

2. Materials and methods

2.1 Microbial strain

The *A. xylinum* strain was isolated from *nata de coco*. The stock culture was kindly supplied by Pramote Tammarat, the Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand.

2.2 Culture media and method

The medium for the inoculum was coconut-water supplemented with 5.0% sucrose, 0.5% ammonium sulfate $(NH_4)_2SO_4$, 1.0% acetic acid, and different alginate concentration. The experiment was designed to test effects of supplementation of alginate (0%, 0.5%, 0.75%, 1% w/v). Precultures were prepared by a transfer of 50 ml stock culture to 1000 ml in 1500 ml bottle and incubated statically at 30 °C

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for 7 days. After the surface pellicle was removed, the 5% (v/v) preculture broth was added to the main culture a medium with different alginate content. The 75 ml of activated medium was inoculated in a Preti-dish and kept at 30 ^{0}C for 7 days.

All sample films were first purified by washing with DI water and then was treated with NaOH at room temperature to remove bacterial cells followed by a rinse with DI water until pH came to 7. Afterward, the BC film was air-dried at room temperature (30^{0}C) and stored in plastic film at room temperature.

2.3 Characterization of membranes

2.3.1 Scanning electron microscope (SEM)

The films were frozen in liquid nitrogen, immediately snapped, vacuum-dried and then sputtered with gold and photographed. Images were taken on JOEL (Tokyo, Japan) JSM-5410LV scanning electron microscope.

2.3.2 Water absorption capacity (WAC)

To determine the WAC, the dried membranes were immersed in DI water at room temperature until equilibration. After that the membranes were removed from the water and excess water at the surface of the membranes was blotted out with kimwipes paper. The weights of the swollen membranes were measured, and the procedure was repeated until no further weight change was observed. The water content was calculated with the following formula:

$$WAC\ (\%) = \frac{W_h - W_d}{W_d}\ x\ 100 \qquad (1)$$

Where, W_h and W_d are the weights of hydrate and dry membrane respectively.

2.3.3 BET Surface analysis

The pore size and surface area of the membranes were measured by a Brunauer-Emmett-Teller (BET) surface area analyzer (Model ASAP 2020). The samples were placed in the sample cell, which was then heated up to 75 ^oC and held at this temperature for 2 hours. The samples were cooled down to room temperature and ready to measure the surface area. There were three steps to measure the surface area: adsorption step, desorption step and calibration step.

3. Results and discussion

3.1 Surface morphology

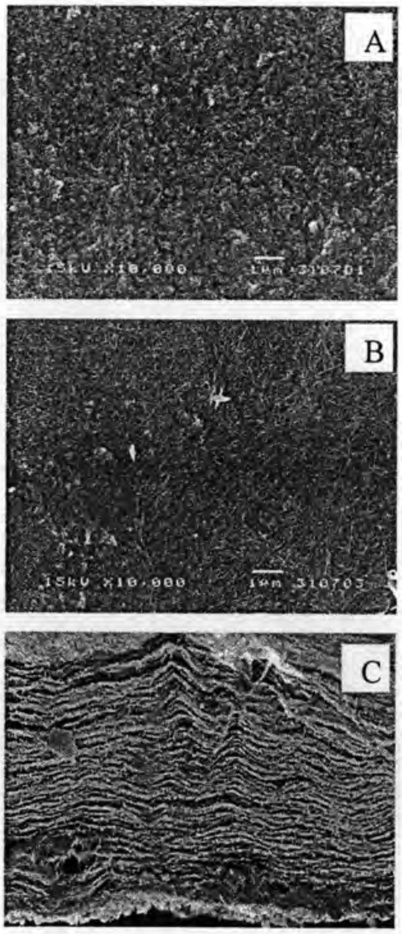

Figure 1 SEM images of surface morphology at 0% alginate: (A) Top view, (B) Bottom view and (C) Cross-section

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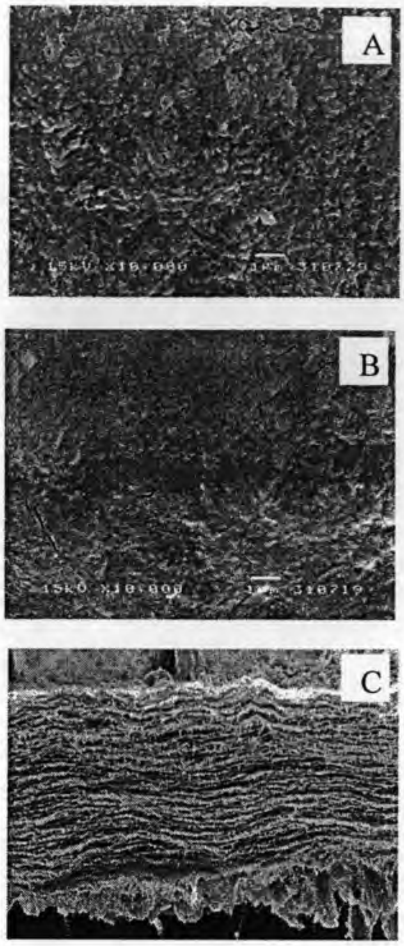

Figure 2 SEM images of surface morphology at 1% alginate: (A) Top view, (B) Bottom view and (C) Cross-section

Bacterial cellulose-alginate film was developed by means of adding alginate into the culture medium during the synthesis by *A. xylinum*. The surface structures of film were then analyzed by scanning electron microscopy (SEM). In this study, BCA and BC film refers to BC film with and without the addition of alginate in culture medium, respectively. Figure1 and Figure 2 reveal the fibril network of BC and BCA films at 1%Al respectively. The cross-section of the membrane illustrated that the organic material composed a multi-layered thin film. It was found that the BCA film was significantly denser compared to the BC film

3.2 Water absorption capacity (WAC)

From Figure 3, the water absorption capacity of BC was 542%; WAC increased with the increase of alginate content. Supplementation of 0.5%, 0.75%, 1% (w/v) caused the increase in WAC to 622%, 682% and 706% respectively. Because alginate is hydrophilic and can be well incorporated into the cellulose network structure, the increasing alginate content in culture medium increased WAC of the film.

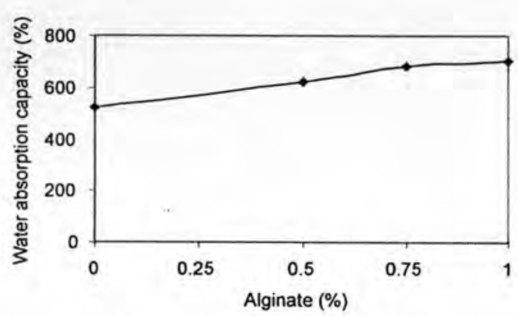

Figure 3 The water absorption capacity (WAC) of BCA as a function of alginate content (% w/v)

3.3 Porosity

Table1 Average pore size and surface area of the BC and BCA analyzed with BET analyzer

Alginate contente (%)	Average Pore diameter (A°)	Surface area (m^2/g)
0	224	12.6
0.5	97	12.1
0.75	64	11.8
1	39	11.2

The total surface area and average pore size of the entire BC film determined by BET were 12.62 m^2/g and 224 Å [6]. As shown in Table1, the average pore diameter of film decreased with increasing alginate content; whereas, the surface area was slightly decreased.

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4. Conclusion

Modifying bacterial cellulose by means of adding 0.5-1 (% w/v) of alginate in the culture medium during biosynthesis resulted in the denser film structure with the smaller average pore diameter. The BCA films demonstrate superior water absorption capacity to that of BC film. The developed hydrogel bacterial cellulose-alginate nano-composite membrane is expected to be suitable for the application in membrane separation processes. The evaluation of the modified film as a separation membrane in pervaporation processes is ongoing.

5. References

[1] Brown, Jr. R.M. Advances in Cellulose Biosynthesis Polymers from Biobased Materials. New Jersey, 1991.

[2] Fontana, J. D., De Souza, A. M., Fontana, C. K., Torriani, I. L., Moreschi, j. C., Gallotti, B.J. et al. Acetobacter Cellulose Pellicle as a Temporary Skin Substitute, 1990.

[3] Dubey, V., Saxena, C., Singh, L., Ramana, K.V., and Chauhan ,R.S. Pervaporation of Binary Water-Ethanol Mmixtures Through Bacterial Cellulose Membrane. Separation and Purification Technology 27(2001) 163-171.

[4] Dubey, V., Pandey, L.K., and Saxena, C. Pervaporative Separation of Ethanol/Water Azeotrope Using a Novel Chitosan-Impregnated Bacterial Cellulose Membrane and Chitosan–Poly(vinyl Alcohol) Blends. Journal of Membrane Science 251 (2005) 131-136.

[5] Phisalaphong, M., Suwanmajo, T., and Tammarate, P. Synthesis and Characterization of Bacterial Cellulose/Alginate Blend Membranes. Journal of Applied Polymer Science 107(2008), 3419-3424.

[6] Phisalaphong, M., Suwanmajo, T., and Sangtherapitikul, P. Novel Nanoporous Membranes from Regenerated Bacterial Cellulose. Journal of Applied polymer Science 107(2008), 292-299.

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