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## APPENDICES

### Appendix A The Bionanocomposite Sponge Codes at Various Weight Ratios of SF to CLWs

**Table A1** The non-methanol treated bionanocomposite sponge codes at various weight ratios of SF to CLWs

Bionanocomposite sponge codes	SF (wt%)	CLWs (wt%)
SF100	100	0
SF90	90	10
SF80	80	20
SF70	70	30
SF60	60	40
SF50	50	50

**Table A2** The methanol-treated bionanocomposite sponge codes at various weight ratios of SF to CLWs

Bionanocomposite sponge codes	SF (wt%)	CLWs (wt%)
MSF100	100	0
MSF90	90	10
MSF80	80	20
MSF70	70	30
MSF60	60	40
MSF50	50	50

### Appendix B Weight Loss of Silk Fibroin/Cellulose Whiskers Bionanocomposite Sponges at Various Weight Ratios of SF/CLWs

Weight loss (%) =  $(W_i - W_f) / W_i \times 100$  ; where  $W_i$  and  $W_f$  represent the initial dry weight and final dry weight of bionanocomposite sponge, respectively.

**Table B1** Weight loss behavior of non-methanol treated SF100 sponge

Time(hr)	Initial dry weight, $W_i$ (g)	Final dry weight, $W_f$ (g)	Weight loss (%)	Average weight loss (%)	SD
16	0.0216	0.0154	28.7037	31.1375	2.1700
	0.0216	0.0145	32.8704		
	0.0223	0.0152	31.8386		
24	0.0216	0.0140	35.1852	34.2556	0.8592
	0.0220	0.0145	34.0909		
	0.0212	0.0141	33.4906		
48	0.0218	0.0153	29.8165	32.2120	2.0760
	0.0189	0.0126	33.3333		
	0.0218	0.0145	33.4862		
72	0.0394	0.0265	32.7411	33.3123	2.1734
	0.0216	0.0148	31.4815		
	0.0224	0.0144	35.7143		

**Table B2** Weight loss behavior of non-methanol treated SF90 sponge

Time(hr)	Initial dry	Final dry	Weight loss (%)	Average	SD
	weight, W <sub>i</sub> (g)	weight, W <sub>f</sub> (g)		weight loss (%)	
16	0.0195	0.0154	21.0256	20.8487	2.4404
	0.0191	0.0156	18.3246		
	0.0194	0.0149	23.1959		
24	0.0195	0.0157	19.4872	20.3743	0.9814
	0.0196	0.0154	21.4286		
	0.0193	0.0154	20.2073		
48	0.0191	0.0155	18.8482	20.3261	2.0979
	0.0198	0.0153	22.7273		
	0.0201	0.0162	19.4030		
72	0.0199	0.0158	20.6030	20.0916	2.7007
	0.0200	0.0155	22.5000		
	0.0198	0.0164	17.1717		

**Table B3** Weight loss behavior of non-methanol treated SF80 sponge

Time(hr)	Initial dry	Final dry	Weight loss (%)	Average	SD
	weight, $W_i$ (g)	weight, $W_f$ (g)		weight loss (%)	
16	0.0175	0.0154	12.0000	10.3672	1.4150
	0.0177	0.0160	9.6045		
	0.0179	0.0162	9.4972		
24	0.0182	0.0159	12.6374	12.2212	1.0916
	0.0173	0.0154	10.9827		
	0.0184	0.0160	13.0435		
48	0.0168	0.0145	13.6905	12.4657	1.4070
	0.0180	0.0157	12.7778		
	0.0183	0.0163	10.9290		
72	0.0181	0.0156	13.8122	12.5596	1.0932
	0.0178	0.0157	11.7978		
	0.0174	0.0153	12.0690		



**Table B4** Weight loss behavior of non-methanol treated SF70 sponge

Time(hr)	Initial dry weight, $W_i$ (g)	Final dry weight, $W_f$ (g)	Weight loss (%)	Average weight loss (%)	SD
16	0.0164	0.0148	9.7561	8.8404	0.8195
	0.0163	0.0149	8.5890		
	0.0159	0.0146	8.1761		
24	0.0166	0.0148	10.8434	9.6935	0.9962
	0.0165	0.0150	9.0909		
	0.0164	0.0149	9.1463		
48	0.0169	0.0155	8.2840	8.5368	1.0991
	0.0145	0.0134	7.5862		
	0.0154	0.0139	9.7403		
72	0.0152	0.0135	11.1842	12.4707	1.1891
	0.0170	0.0147	13.5294		
	0.0126	0.0110	12.6984		

**Table B5** Weight loss behavior of non-methanol treated SF60 sponge

Time(hr)	Initial dry weight, $W_i$ (g)	Final dry weight, $W_f$ (g)	Weight loss (%)	Average weight loss (%)	SD
16	0.0138	0.0133	3.6232	4.6310	0.9253
	0.0147	0.0139	5.4422		
	0.0145	0.0138	4.8276		
24	0.0116	0.0110	5.1724	4.8521	0.7207
	0.0149	0.0143	4.0268		
	0.0112	0.0106	5.3571		
48	0.0119	0.0113	5.0420	4.6904	0.4366
	0.0119	0.0114	4.2017		
	0.0145	0.0138	4.8276		
72	0.0129	0.0121	6.2016	5.8602	0.4906
	0.0148	0.0139	6.0811		
	0.0151	0.0143	5.2980		

**Table B6** Weight loss behavior of non-methanol treated SF50 sponge

Time(hr)	Initial dry weight, $W_i$ (g)	Final dry weight, $W_f$ (g)	Weight loss (%)	Average weight loss (%)	SD
16	0.0104	0.0101	2.8846	1.8976	1.0786
	0.0097	0.0095	2.0619		
	0.0134	0.0133	0.7463		
24	0.0125	0.0121	3.2000	2.2501	1.2399
	0.0111	0.0108	2.7027		
	0.0118	0.0117	0.8475		
48	0.0129	0.0125	3.1008	2.7124	0.7154
	0.0127	0.0123	3.1496		
	0.0106	0.0104	1.8868		
72	0.0133	0.0127	4.5113	3.8096	0.7053
	0.0131	0.0126	3.8168		
	0.0129	0.0125	3.1008		

**Table B7** Weight loss behavior of methanol-treated MSF100 sponge

Time(hr)	Initial dry weight, $W_i$ (g)	Final dry weight, $W_f$ (g)	Weight loss (%)	Average weight loss (%)	SD
16	0.0211	0.0209	0.9479	1.1066	0.2672
	0.0209	0.0207	0.9569		
	0.0212	0.0209	1.4151		
24	0.0210	0.0208	0.9524	1.1149	0.7314
	0.0209	0.0208	0.4785		
	0.0209	0.0205	1.9139		
48	0.0214	0.0213	0.4673	1.0962	1.0836
	0.0211	0.0210	0.4739		
	0.0213	0.0208	2.3474		
72	0.0219	0.0209	4.5662	4.7587	0.3515
	0.0213	0.0202	5.1643		
	0.0220	0.0210	4.5455		

**Table B8** Weight loss behavior of methanol-treated MSF90 sponge

Time(hr)	Initial dry	Final dry	Weight loss (%)	Average	SD
	weight, $W_i$ (g)	weight, $W_f$ (g)		weight loss (%)	
16	0.0191	0.0188	1.5707	1.0536	0.5194
	0.0189	0.0187	1.0582		
	0.0188	0.0187	0.5319		
24	0.0187	0.0186	0.5348	1.1317	0.6336
	0.0188	0.0186	1.0638		
	0.0167	0.0164	1.7964		
48	0.0190	0.0186	2.1053	2.1164	0.0112
	0.0188	0.0184	2.1277		
	0.0189	0.0185	2.1164		
72	0.0199	0.0189	5.0251	5.2189	0.2926
	0.0198	0.0187	5.5556		
	0.0197	0.0187	5.0761		

**Table B9** Weight loss behavior of methanol-treated MSF80 sponge

Time(hr)	Initial dry	Final dry	Weight loss (%)	Average	SD
	weight, $W_i$ (g)	weight, $W_f$ (g)		weight loss (%)	
16	0.0169	0.0168	0.5917	0.9724	0.4598
	0.0172	0.0169	1.7442		
	0.0172	0.0171	0.5814		
24	0.0169	0.0167	1.1834	0.9681	0.3439
	0.0175	0.0174	0.5714		
	0.0174	0.0172	1.1494		
48	0.0169	0.0165	2.3669	2.3355	0.0543
	0.0169	0.0165	2.3669		
	0.0176	0.0172	2.2727		
72	0.0180	0.0173	3.8889	4.1913	0.5611
	0.0186	0.0177	4.8387		
	0.0182	0.0175	3.8462		

**Table B10** Weight loss behavior of methanol-treated MSF70 sponge

Time(hr)	Initial dry	Final dry	Weight loss (%)	Average	SD
	weight, W <sub>i</sub> (g)	weight, W <sub>f</sub> (g)		weight loss (%)	
16	0.0165	0.0164	0.6061	1.2322	0.6174
	0.0160	0.0158	1.2500		
	0.0163	0.0160	1.8405		
24	0.0170	0.0165	2.9412	2.2436	0.8663
	0.0159	0.0155	2.5157		
	0.0157	0.0155	1.2739		
48	0.0174	0.0169	2.8736	2.5598	0.2741
	0.0169	0.0165	2.3669		
	0.0164	0.0160	2.4390		
72	0.0164	0.0158	3.6585	3.9729	0.2747
	0.0168	0.0161	4.1667		
	0.0171	0.0164	4.0936		

**Table B11** Weight loss behavior of methanol-treated MSF60 sponge

Time(hr)	Initial dry	Final dry	Weight loss (%)	Average	SD
	weight, $W_i$ (g)	weight, $W_f$ (g)		weight loss (%)	
16	0.0152	0.0150	1.3158	0.8804	0.3775
	0.0155	0.0154	0.6452		
	0.0147	0.0146	0.6803		
24	0.0149	0.0148	0.6711	0.8821	0.3536
	0.0155	0.0153	1.2903		
	0.0146	0.0145	0.6849		
48	0.0143	0.0141	1.3986	0.8904	0.4411
	0.0150	0.0149	0.6667		
	0.0165	0.0164	0.6061		
72	0.0149	0.0143	4.0268	3.6011	0.4117
	0.0156	0.0151	3.2051		
	0.0140	0.0135	3.5714		



**Table B12** Weight loss behavior of methanol-treated MSF50 sponge

Time(hr)	Initial dry	Final dry	Weight loss (%)	Average	SD
	weight, $W_i$ (g)	weight, $W_f$ (g)		weight loss (%)	
16	0.0127	0.0126	0.7874	0.7440	0.0398
	0.0136	0.0135	0.7353		
	0.0141	0.0140	0.7092		
24	0.0134	0.0133	0.7463	0.7726	0.0409
	0.0133	0.0132	0.7519		
	0.0122	0.0121	0.8197		
48	0.0138	0.0137	0.7246	0.7354	0.0093
	0.0135	0.0134	0.7407		
	0.0135	0.0134	0.7407		
72	0.0138	0.0136	1.4493	2.1698	0.9955
	0.0114	0.0112	1.7544		
	0.0121	0.0117	3.3058		

**Appendix C Shrinkage of Silk Fibroin/Cellulose Whiskers Bionanocomposite Sponges after Methanol Treatment at Various Weight Ratios of SF/CLWs**

Volume ( $V$ ) =  $\pi r^2 h$  ; where  $r$  and  $h$  represent the radius and height of bionanocomposite sponge, respectively.

Shrinkage (%) =  $(V_i - V_f) / V_i \times 100$  ; where  $V_i$  and  $V_f$  represent the volume of the bionanocomposite sponge before and after methanol treatment, respectively.

**Table C1** The volume of silk fibroin/cellulose whiskers bionanocomposite sponges before methanol treatment ( $V_i$ )

Bionanocomposite sponge	Radius, $r$ (cm)	Height, $h$ (cm)	Volume, $V_i$ (cm <sup>3</sup> )
SF100	0.6925	0.5550	0.8365
	0.7000	0.5200	0.8008
	0.6750	0.5200	0.7446
SF90	0.6650	0.5400	0.7505
	0.6875	0.5800	0.8616
	0.6825	0.5450	0.7979
SF80	0.6875	0.5200	0.7725
	0.7000	0.5250	0.8085
	0.6700	0.5150	0.7266
SF70	0.7100	0.5100	0.8080
	0.6975	0.5200	0.7951
	0.7100	0.5200	0.8238
SF60	0.7200	0.5200	0.8472
	0.7050	0.5000	0.7810
	0.7025	0.5500	0.8531
SF50	0.7450	0.5300	0.9245
	0.7325	0.5100	0.8600
	0.7225	0.5200	0.8531

**Table C2** The volume of silk fibroin/cellulose whiskers bionanocomposite sponges after methanol treatment ( $V_f$ )

Bionanocomposite sponge	Radius, r (cm)	Height, h (cm)	Volume, $V_f$ (cm <sup>3</sup> )
MSF100	0.6175	0.4500	0.5393
	0.6075	0.4500	0.5220
	0.6300	0.4400	0.5489
MSF90	0.6275	0.4450	0.5507
	0.6225	0.4700	0.5724
	0.6250	0.4800	0.5893
MSF80	0.6400	0.4800	0.6179
	0.6525	0.4600	0.6155
	0.6325	0.4500	0.5658
MSF70	0.6775	0.4500	0.6492
	0.6675	0.4800	0.6722
	0.6475	0.4450	0.5864
MSF60	0.7000	0.4900	0.7546
	0.6900	0.4400	0.6584
	0.6700	0.4750	0.6701
MSF50	0.6950	0.5250	0.7970
	0.7050	0.5050	0.7888
	0.6925	0.5000	0.7536

**Table C3** Shrinkage (%) of silk fibroin/cellulose whiskers bionanocomposite sponges after methanol treatment

Bionanocomposite sponge	$V_i$ (cm <sup>3</sup> )	$V_f$ (cm <sup>3</sup> )	Shrinkage (%)	Average shrinkage (%)	SD
MSF100	0.8365	0.5393	35.5306	32.2142	5.1422
	0.8008	0.5220	34.8213		
	0.7446	0.5489	26.2906		
MSF90	0.7505	0.5507	26.6246	28.7768	4.1529
	0.8616	0.5724	33.5640		
	0.7979	0.5893	26.1417		
MSF80	0.7725	0.6179	20.0069	22.0015	1.9340
	0.8085	0.6155	23.8687		
	0.7266	0.5658	22.1288		
MSF70	0.8080	0.6492	19.6577	21.3153	6.8346
	0.7951	0.6722	15.4620		
	0.8238	0.5864	28.8263		
MSF60	0.8472	0.7546	10.9316	16.0263	5.2628
	0.7810	0.6584	15.7048		
	0.8531	0.6701	21.4425		
MSF50	0.9245	0.7970	13.7934	11.2449	2.7828
	0.8600	0.7888	8.2757		
	0.8531	0.7536	11.6655		

**Appendix D Compression Modulus of Methanol-treated Silk Fibroin/Cellulose Whiskers Bionanocomposite Sponges at Various Weight Ratios of SF/CLWs**

**Table D** Compression modulus of methanol-treated bionanocomposite sponges at various weight ratios of SF/CLWs

Bionanocomposite sponge	Compression modulus(kPa)	Average Compression modulus(kPa)	SD
MSF100	284.0102	256.2477	24.0775
	243.6537		
	241.0791		
MSF90	357.9443	349.5800	14.6500
	358.1318		
	332.6640		
MSF80	509.4235	566.2392	71.2469
	646.1748		
	543.1192		
MSF70	880.3890	833.0517	52.6153
	776.4019		
	842.3642		
MSF60	1160.3652	1038.8923	105.9872
	991.0611		
	965.2507		
MSF50	1203.0449	1188.5147	117.5832
	1298.1574		
	1064.3416		

**Appendix E Growth Curve of *Saccharomyces Cerevisiae* Burgundy KY 11**

**Table E1** The number of yeast cells at difference times

Time (h)	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Total cell	Total volume (ml)	cell/ml	Dilution	Actual cell/ml
0	7	1	5	2	2	17	0.00002	850000	10	$0.85 \times 10^7$
	2	2	2	4	4	14	0.00002	700000	10	$0.70 \times 10^7$
	3	1	3	1	1	9	0.00002	450000	10	$0.45 \times 10^7$
4	5	1	4	8	8	26	0.00002	1300000	10	$1.30 \times 10^7$
	3	8	4	8	1	24	0.00002	1200000	10	$1.20 \times 10^7$
	4	8	1	9	3	25	0.00002	1250000	10	$1.25 \times 10^7$
8	19	5	14	16	32	86	0.00002	4300000	10	$4.30 \times 10^7$
	42	15	20	20	10	107	0.00002	5350000	10	$5.35 \times 10^7$
	17	26	27	35	19	124	0.00002	6200000	10	$6.20 \times 10^7$
12	9	4	5	8	7	33	0.00002	1650000	100	$1.65 \times 10^8$
	3	11	5	10	11	40	0.00002	2000000	100	$2.00 \times 10^8$
	8	9	7	8	8	40	0.00002	2000000	100	$2.00 \times 10^8$
16	11	5	15	10	7	48	0.00002	2400000	100	$2.40 \times 10^8$
	4	10	8	8	9	39	0.00002	1950000	100	$1.95 \times 10^8$
	11	8	10	15	10	54	0.00002	2700000	100	$2.70 \times 10^8$
20	9	6	6	11	11	43	0.00002	2150000	100	$2.15 \times 10^8$
	6	9	12	7	6	40	0.00002	2000000	100	$2.00 \times 10^8$
	6	9	7	8	15	45	0.00002	2250000	100	$2.25 \times 10^8$

Time (h)	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Total cell	Total volume (ml)	cell/ml	Dilution	Actual cell/ml
28	11	6	13	7	6	43	0.00002	2150000	100	$2.15 \times 10^8$
	15	6	4	13	4	42	0.00002	2100000	100	$2.10 \times 10^8$
	8	4	13	6	9	40	0.00002	2000000	100	$2.00 \times 10^8$
32	7	11	8	2	10	38	0.00002	1900000	100	$1.90 \times 10^8$
	6	9	6	9	12	42	0.00002	2100000	100	$2.10 \times 10^8$
	5	7	10	8	10	40	0.00002	2000000	100	$2.00 \times 10^8$

**Table E2** The average number of yeast cells at difference times

Time (h)	Actual cell/ml	Average actual cell/ml
0	$0.85 \times 10^7$	$0.67 \times 10^7$
	$0.70 \times 10^7$	
	$0.45 \times 10^7$	
4	$1.30 \times 10^7$	$1.25 \times 10^7$
	$1.20 \times 10^7$	
	$1.25 \times 10^7$	
8	$4.30 \times 10^7$	$5.28 \times 10^7$
	$5.35 \times 10^7$	
	$6.20 \times 10^7$	
12	$1.65 \times 10^8$	$1.88 \times 10^8$
	$2.00 \times 10^8$	
	$2.00 \times 10^8$	
16	$2.40 \times 10^8$	$2.35 \times 10^8$
	$1.95 \times 10^8$	
	$2.70 \times 10^8$	

Time (h)	Actual cell/ml	Average actual cell/ml
20	2.15 x 10 <sup>8</sup> 2.00 x 10 <sup>8</sup> 2.25 x 10 <sup>8</sup>	2.07 x 10 <sup>8</sup>
28	2.15 x 10 <sup>8</sup> 2.10 x 10 <sup>8</sup> 2.00 x 10 <sup>8</sup>	2.08 x 10 <sup>8</sup>
32	1.90 x 10 <sup>8</sup> 2.10 x 10 <sup>8</sup> 2.00 x 10 <sup>8</sup>	2.00 x 10 <sup>8</sup>

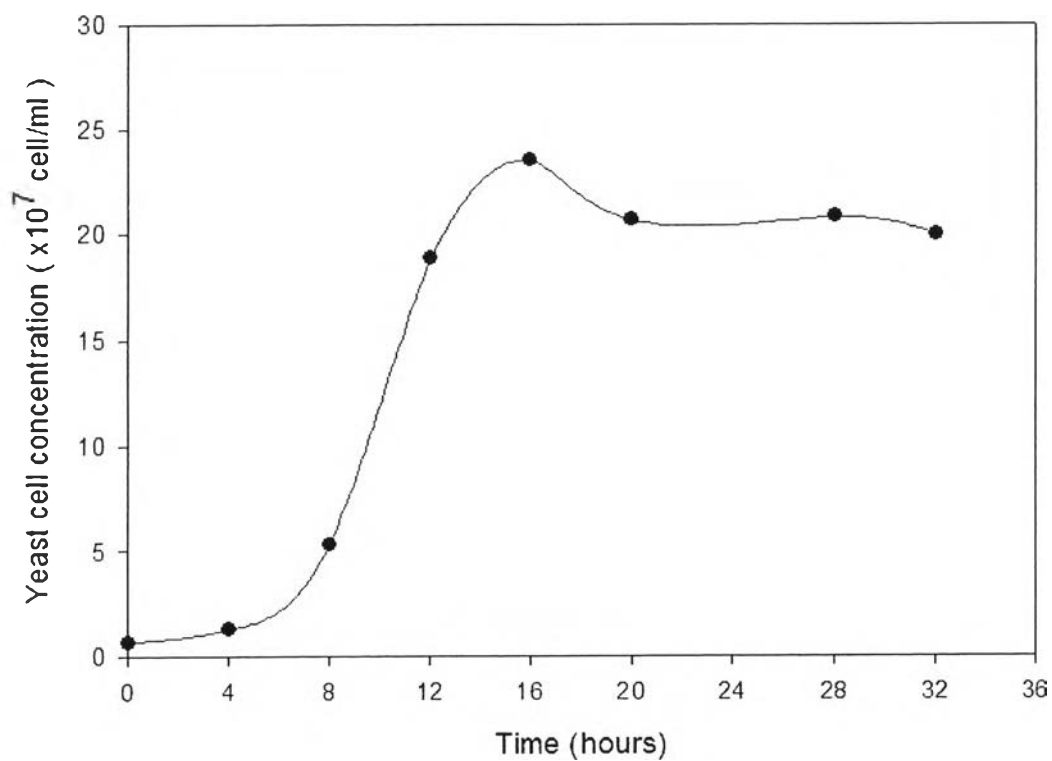


Figure E Growth curve of *Saccharomyces cerevisiae burgundy* KY 11.



**Appendix F Yeast Cells in Methanol-treated Silk Fibroin/Cellulose Whiskers Bionanocomposite Sponges at Various Weight Ratios of SF/CLWs**

**Table F1** The number of yeast cells in methanol-treated MSF100 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge
1	2	3	4	5	Total						
5	7	6	8	8	34	0.00002	10	$1.70 \times 10^7$	$1.70 \times 10^8$	0.0201	$8.46 \times 10^9$
3	4	2	1	6	16	0.00002	10	$8.00 \times 10^6$	$8.00 \times 10^7$	0.0201	$3.98 \times 10^9$
7	5	9	5	5	31	0.00002	10	$1.55 \times 10^7$	$1.55 \times 10^8$	0.0201	$7.71 \times 10^9$
										Average	$6.72 \times 10^9$
										SD	$2.40 \times 10^9$

**Table F2** The number of yeast cells in methanol-treated MSF90 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge
1	2	3	4	5	Total						
13	9	12	8	9	51	0.00002	10	$2.55 \times 10^7$	$2.55 \times 10^8$	0.0188	$1.36 \times 10^{10}$
3	6	7	15	8	39	0.00002	10	$1.95 \times 10^7$	$1.95 \times 10^8$	0.0188	$1.04 \times 10^{10}$
4	19	13	16	8	60	0.00002	10	$3.00 \times 10^7$	$3.00 \times 10^8$	0.0188	$1.60 \times 10^{10}$
										average	$1.33 \times 10^{10}$
										SD	$2.80 \times 10^9$

**Table F3** The number of yeast cells in methanol-treated MSF80 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge	
1	2	3	4	5	Total							
12	5	6	5	9	37	0.00002	10	$1.85 \times 10^7$	$1.85 \times 10^8$	0.0176	$1.05 \times 10^{10}$	
4	15	12	7	11	49	0.00002	10	$2.45 \times 10^7$	$2.45 \times 10^8$	0.0176	$1.39 \times 10^{10}$	
11	10	8	15	20	64	0.00002	10	$3.20 \times 10^7$	$3.20 \times 10^8$	0.0176	$1.82 \times 10^{10}$	
											average	$1.42 \times 10^{10}$
											SD	$3.84 \times 10^9$

**Table F4** The number of yeast cells in methanol-treated MSF70 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge	
1	2	3	4	5	Total							
17	17	13	15	17	79	0.00002	10	$3.95 \times 10^7$	$3.95 \times 10^8$	0.0172	$2.30 \times 10^{10}$	
10	20	11	16	17	74	0.00002	10	$3.70 \times 10^7$	$3.70 \times 10^8$	0.0172	$2.15 \times 10^{10}$	
17	12	19	21	7	76	0.00002	10	$3.80 \times 10^7$	$3.80 \times 10^8$	0.0172	$2.21 \times 10^{10}$	
											average	$2.22 \times 10^{10}$
											SD	$7.32 \times 10^8$

**Table F5** The number of yeast cells in methanol-treated MSF60 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge	
1	2	3	4	5	Total							
21	13	15	21	15	85	0.00002	10	$4.25 \times 10^7$	$4.25 \times 10^8$	0.0158	$2.69 \times 10^{10}$	
10	17	20	8	19	74	0.00002	10	$3.70 \times 10^7$	$3.70 \times 10^8$	0.0158	$2.34 \times 10^{10}$	
22	15	17	11	9	74	0.00002	10	$3.70 \times 10^7$	$3.70 \times 10^8$	0.0158	$2.34 \times 10^{10}$	
											average	$2.46 \times 10^{10}$
											SD	$2.01 \times 10^9$

**Table F6** The number of yeast cells in methanol-treated MSF50 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge	
1	2	3	4	5	Total							
16	33	28	17	23	117	0.00002	10	$5.85 \times 10^7$	$5.85 \times 10^8$	0.0147	$3.98 \times 10^{10}$	
25	21	30	21	20	117	0.00002	10	$5.85 \times 10^7$	$5.85 \times 10^8$	0.0147	$3.98 \times 10^{10}$	
19	15	25	16	26	101	0.00002	10	$5.05 \times 10^7$	$5.05 \times 10^8$	0.0147	$3.44 \times 10^{10}$	
											average	$3.80 \times 10^{10}$
											SD	$3.14 \times 10^9$

**Appendix G Leaked Yeast Cells from Methanol-treated Silk Fibroin/Cellulose Whiskers Bionanocomposite Sponges at Various Weight Ratios of SF/CLWs after Immersed in Distilled Water with 150 rpm Shaking at 30°C for 24 Hours**

**Table G1** The number of leaked yeast cells of methanol-treated MSF100 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge	
1	2	3	4	5	Total							
4	7	4	6	7	28	0.00002	1	1400000	$1.40 \times 10^7$	0.0201	$6.97 \times 10^8$	
3	8	5	4	5	25	0.00002	1	1250000	$1.25 \times 10^7$	0.0201	$6.22 \times 10^8$	
6	8	6	5	6	31	0.00002	1	1550000	$1.55 \times 10^7$	0.0201	$7.71 \times 10^8$	
											average	$6.97 \times 10^8$
											SD	$7.46 \times 10^7$

**Table G2** The number of leaked yeast cells of methanol-treated MSF90 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge	
1	2	3	4	5	Total							
2	9	4	2	6	23	0.00002	1	1150000	$1.15 \times 10^7$	0.0188	$6.12 \times 10^8$	
6	3	3	7	4	23	0.00002	1	1150000	$1.15 \times 10^7$	0.0188	$6.12 \times 10^8$	
3	2	8	10	0	23	0.00002	1	1150000	$1.15 \times 10^7$	0.0188	$6.12 \times 10^8$	
											average	$6.12 \times 10^8$
											SD	0

**Table G3** The number of leaked yeast cells of methanol-treated MSF80 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge
1	2	3	4	5	Total						
3	6	6	5	1	21	0.00002	1	1050000	$1.05 \times 10^7$	0.0176	$5.97 \times 10^8$
1	2	5	5	2	15	0.00002	1	750000	$7.50 \times 10^6$	0.0176	$4.26 \times 10^8$
1	2	2	3	4	12	0.00002	1	600000	$6.00 \times 10^6$	0.0176	$3.41 \times 10^8$
										average	$4.55 \times 10^8$
										SD	$1.30 \times 10^8$

**Table G4** The number of leaked yeast cells of methanol-treated MSF70 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge
1	2	3	4	5	Total						
3	6	2	2	1	14	0.00002	1	700000	$7.00 \times 10^6$	0.0172	$4.07 \times 10^8$
2	7	1	3	2	15	0.00002	1	750000	$7.50 \times 10^6$	0.0172	$4.36 \times 10^8$
3	2	5	4	6	20	0.00002	1	1000000	$1.00 \times 10^7$	0.0172	$5.81 \times 10^8$
										average	$4.75 \times 10^8$
										SD	$9.34 \times 10^7$

**Table G5** The number of leaked yeast cells of methanol-treated MSF60 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge	
1	2	3	4	5	Total							
1	5	0	2	0	8	0.00002	1	400000	$4.00 \times 10^6$	0.0158	$2.53 \times 10^8$	
4	3	5	4	2	18	0.00002	1	900000	$9.00 \times 10^6$	0.0158	$5.70 \times 10^8$	
1	4	6	4	0	15	0.00002	1	750000	$7.50 \times 10^6$	0.0158	$4.75 \times 10^8$	
											average	$4.32 \times 10^8$
											SD	$1.62 \times 10^8$

**Table G6** The number of leaked yeast cells of methanol-treated MSF50 sponge

Cell						Total volume (ml)	Dilution	cell/ml	Actual cell in 10 ml	Average weight of sponge(g)	Cells/1 g sponge	
1	2	3	4	5	Total							
2	0	1	2	0	5	0.00002	1	250000	$2.50 \times 10^6$	0.0147	$1.70 \times 10^8$	
2	4	1	3	2	12	0.00002	1	600000	$6.00 \times 10^6$	0.0147	$4.08 \times 10^8$	
2	4	3	7	0	16	0.00002	1	800000	$8.00 \times 10^6$	0.0147	$5.44 \times 10^8$	
											average	$3.74 \times 10^8$
											SD	$1.89 \times 10^8$

**Appendix H % Yeast Cell Immobilization Efficiency and % Yeast Cell Leakage of Methanol-treated Silk Fibroin/Cellulose Whiskers Bionanocomposite Sponges at Various Weight Ratios of SF/CLWs after Immersed in Distilled Water with 150 rpm Shaking at 30°C for 24 Hours**

**Table H1** % Yeast cell immobilization efficiency of methanol-treated silk fibroin/cellulose whiskers bionanocomposite sponges at various weight ratios of SF/CLWs after immersed in distilled water

Bionano composite sponge	Immobilized cells/1g sponge	Immobilized cells after immersed in water/1g sponge	Immobilization efficiency (%)	Average immobilization efficiency (%)	SD
MSF100	$8.46 \times 10^9$	$7.76 \times 10^9$	91.76	88.71	3.86
	$3.98 \times 10^9$	$3.36 \times 10^9$	84.38		
	$7.71 \times 10^9$	$6.94 \times 10^9$	90.00		
MSF90	$1.36 \times 10^{10}$	$1.30 \times 10^{10}$	95.49	95.25	1.05
	$1.04 \times 10^{10}$	$9.76 \times 10^9$	94.10		
	$1.60 \times 10^{10}$	$1.53 \times 10^{10}$	96.17		
MSF80	$1.05 \times 10^{10}$	$9.91 \times 10^9$	94.32	96.46	1.94
	$1.39 \times 10^{10}$	$1.35 \times 10^{10}$	96.94		
	$1.82 \times 10^{10}$	$1.78 \times 10^{10}$	98.13		
MSF70	$2.30 \times 10^{10}$	$2.26 \times 10^{10}$	98.23	97.86	0.44
	$2.15 \times 10^{10}$	$2.11 \times 10^{10}$	97.97		
	$2.21 \times 10^{10}$	$2.15 \times 10^{10}$	97.37		
MSF60	$2.69 \times 10^{10}$	$2.66 \times 10^{10}$	99.06	98.20	0.77
	$2.34 \times 10^{10}$	$2.28 \times 10^{10}$	97.57		
	$2.34 \times 10^{10}$	$2.29 \times 10^{10}$	97.97		
MSF50	$3.98 \times 10^{10}$	$3.96 \times 10^{10}$	99.57	98.99	0.58
	$3.98 \times 10^{10}$	$3.94 \times 10^{10}$	98.97		
	$3.44 \times 10^{10}$	$3.38 \times 10^{10}$	98.42		

**Table H2** % Yeast cells leakage of methanol-treated silk fibroin/cellulose whiskers bionanocomposite sponges at various weight ratios of SF/CLWs after immersed in distilled water

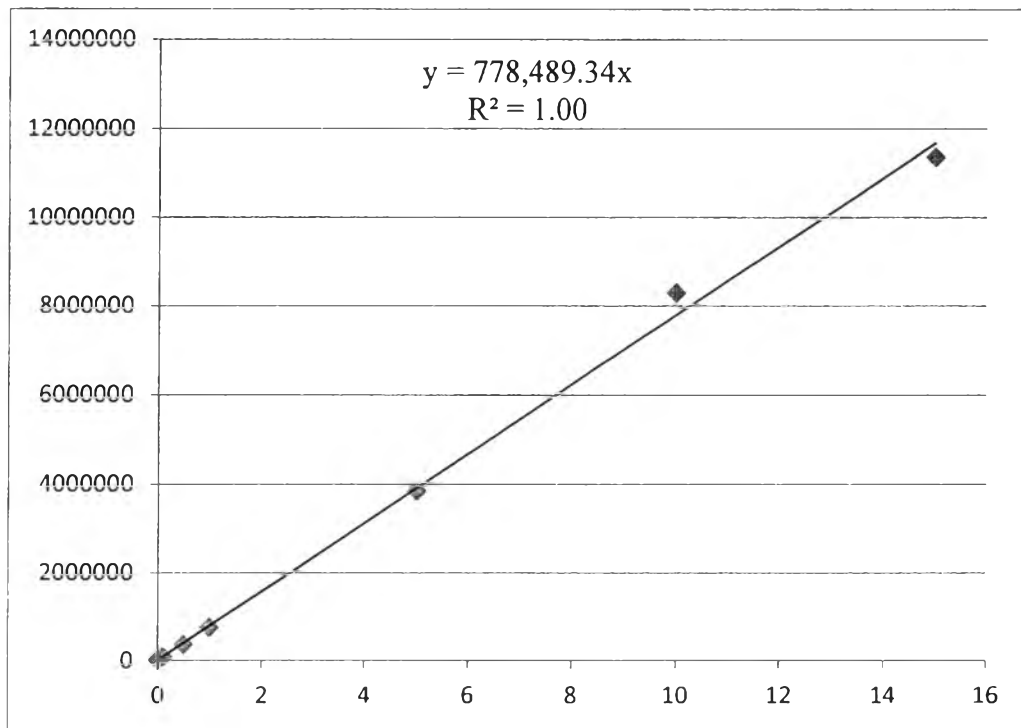
Bionanocomposite sponge	Immobilized cells/1g sponge	Leaked Cell in medium/1g sponge	Cell leakage (%)	Average cell leakage (%)	SD
MSF100	$8.46 \times 10^9$	$6.97 \times 10^8$	8.24	11.29	3.86
	$3.98 \times 10^9$	$6.22 \times 10^8$	15.63		
	$7.71 \times 10^9$	$7.71 \times 10^8$	10.00		
MSF90	$1.36 \times 10^{10}$	$6.12 \times 10^8$	4.51	4.75	1.05
	$1.04 \times 10^{10}$	$6.12 \times 10^8$	5.90		
	$1.60 \times 10^{10}$	$6.12 \times 10^8$	3.83		
MSF80	$1.05 \times 10^{10}$	$5.97 \times 10^8$	5.68	3.54	1.94
	$1.39 \times 10^{10}$	$4.26 \times 10^8$	3.06		
	$1.82 \times 10^{10}$	$3.41 \times 10^8$	1.88		
MSF70	$2.30 \times 10^{10}$	$4.07 \times 10^8$	1.77	2.14	0.44
	$2.15 \times 10^{10}$	$4.36 \times 10^8$	2.03		
	$2.21 \times 10^{10}$	$5.81 \times 10^8$	2.63		
MSF60	$2.69 \times 10^{10}$	$2.53 \times 10^8$	0.94	1.80	0.77
	$2.34 \times 10^{10}$	$5.70 \times 10^8$	2.43		
	$2.34 \times 10^{10}$	$4.75 \times 10^8$	2.03		
MSF50	$3.98 \times 10^{10}$	$1.70 \times 10^8$	0.43	1.01	0.58
	$3.98 \times 10^{10}$	$4.08 \times 10^8$	1.03		
	$3.44 \times 10^{10}$	$5.44 \times 10^8$	1.58		



**Appendix I Determination of Ethanol Concentration in Continuous Ethanol Fermentation by Immobilized Yeast Cell.**

**Table II** Standard curve of ethanol concentration in the concentration range of 0.01 to 15 (%v/v)

<b>Ethanol concentration (% v/v)</b>	<b>Peak area</b>
0	0
0.01	11515
0.1	71437
0.5	369286
1	753101
5	3843802
10	8293023
15	11357200



**Figure I** Standard curve of ethanol

**Table I2** Determination of ethanol production in continuous ethanol fermentation by immobilized yeast cell at 100 g/l of feed glucose concentration

Dilution rate ( $\text{hr}^{-1}$ )	Times	Peak area	Ethanol (%v/v)	Ethanol (g/l)	Average ethanol (g/l)	SD
0.15	1	4255674	5.4666	43.1313	41.2831	1.7974
		3901452	5.0116	39.5413		
		4062824	5.2189	41.1768		
	2	3958902	5.0854	40.1235	41.1498	1.1082
		4045451	5.1965	41.0007		
		4176120	5.3644	42.3250		
	3	4002366	5.1412	40.5640	41.7183	1.9483
		4008187	5.1487	40.6230		
		4338204	5.5726	43.9678		
	4	3951815	5.0763	40.0517	41.6432	1.7470
		4081432	5.2428	41.3654		
		4293281	5.5149	43.5125		
	5	3980933	5.1137	40.3468	40.7467	0.3468
		4038383	5.1875	40.9291		
		4041864	5.1919	40.9643		
0.20	1	3402984	4.3713	34.4893	35.2220	0.6353
		3514588	4.5146	35.6204		
		3508260	4.5065	35.5563		
	2	3540703	4.5482	35.8851	36.6822	0.9209
		3598524	4.6224	36.4711		
		3718823	4.7770	37.6903		
	3	3734238	4.7968	37.8466	36.8650	0.8833
		3612638	4.6406	36.6141		
		3565285	4.5797	36.1342		

Dilution rate (hr <sup>-1</sup> )	Times	Peak area	Ethanol (%v/v)	Ethanol (g/l)	Average ethanol (g/l)	SD
0.20	4	3788942	4.8670	38.4010	36.6870	1.6558
		3462872	4.4482	35.0963		
		3607675	4.6342	36.5638		
	5	3470962	4.4586	35.1782	37.6508	2.4096
		3945931	5.0687	39.9921		
		3727884	4.7886	37.7822		
0.25	1	2927207	3.7601	29.6673	30.6832	0.8798
		3076912	3.9524	31.1845		
		3078205	3.9541	31.1976		
	2	3196124	4.1055	32.3928	32.0313	0.3448
		3156897	4.0552	31.9952		
		3128370	4.0185	31.7061		
	3	3167018	4.0682	32.0978	31.2029	0.9288
		2984059	3.8331	30.2435		
		3085087	3.9629	31.2674		
	4	3528225	4.5321	35.7586	36.2594	1.7127
		3438866	4.4174	34.8530		
		3765820	4.8373	38.1666		
	5	3590795	4.6125	36.3928	35.7732	0.7601
		3552232	4.5630	36.0019		
		3445974	4.4265	34.9250		

**Table I3** Determination of ethanol production in continuous ethanol fermentation by immobilized yeast cell at 150 g/l of feed glucose concentration

Dilution rate (hr <sup>-1</sup> )	Times	Peak area	Ethanol (%v/v)	Ethanol (g/l)	Average ethanol (g/l)	SD
0.15	1	3901201	5.0112	39.5387	40.2936	0.6641
		4001401	5.1400	40.5543		
		4024442	5.1696	40.7878		
	2	4139271	5.3171	41.9516	41.5692	0.7421
		4017152	5.1602	40.7139		
		4148194	5.3285	42.0420		
	3	3838624	4.9309	38.9045	41.2946	2.3507
		4302301	5.5265	43.6039		
		4082425	5.2440	41.3754		
	4	4089466	5.2531	41.4468	43.0350	1.3758
		4327858	5.5593	43.8629		
		4321177	5.5507	43.7952		
5	4154260	5.3363	42.1035	44.1352	2.1460	
	4333748	5.5669	43.9226			
	4576174	5.8783	46.3796			
0.20	1	4225400	5.4277	42.8245	41.5419	1.5152
		4137271	5.3145	41.9313		
		3933888	5.0532	39.8700		
	2	4148052	5.3283	42.0406	41.4338	0.8445
		4123484	5.2968	41.7916		
		3993020	5.1292	40.4693		
	3	3963154	5.0908	40.1666	40.3944	1.2802
		3872063	4.9738	39.2434		
		4121675	5.2945	41.7732		

Dilution rate (hr <sup>-1</sup> )	Times	Peak area	Ethanol (%v/v)	Ethanol (g/l)	Average ethanol (g/l)	SD
0.20	4	3672899	4.7180	37.2249	39.1490	1.7921
		3892612	5.0002	39.4517		
		4022743	5.1674	40.7706		
	5	3882456	4.9872	39.3487	39.7305	0.7301
		4003190	5.1423	40.5724		
		3874726	4.9772	39.2704		
0.25	1	2805240	3.6034	28.4311	30.5589	2.3068
		2983218	3.8321	30.2350		
		3257084	4.1839	33.0106		
	2	3691042	4.7413	37.4088	36.8615	0.5244
		3632174	4.6657	36.8121		
		3587908	4.6088	36.3635		
	3	3828653	4.9181	38.8034	39.0002	2.0655
		4060865	5.2163	41.1569		
		3654666	4.6946	37.0401		
	4	3252890	4.1785	32.9681	33.0066	0.0390
		3260578	4.1883	33.0460		
		3256597	4.1832	33.0057		
	5	3266470	4.1959	33.1057	32.7718	0.8578
		3137367	4.0301	31.7973		
		3296730	4.2348	33.4124		

**Table I4** Determination of ethanol production in continuous ethanol fermentation by immobilized yeast cell at 200 g/l of feed glucose concentration

Dilution rate (hr <sup>-1</sup> )	Times	Peak area	Ethanol (%v/v)	Ethanol (g/l)	Average ethanol (g/l)	SD
0.15	1	4845688	6.2245	49.1111	51.7530	2.8230
		5073521	6.5171	51.4202		
		5399858	6.9363	54.7276		
	2	4809173	6.1776	48.7410	52.0464	5.0635
		5710485	7.3353	57.8758		
		4886271	6.2766	49.5224		
	3	5290797	6.7962	53.6223	49.0596	3.9634
		4645940	5.9679	47.0867		
		4585090	5.8897	46.4699		
	4	5610901	7.2074	56.8666	53.0578	3.3178
		5082478	6.5286	51.5110		
		5011930	6.4380	50.7960		
5	4837192	6.2136	49.0250	49.9714	2.7476	
	4718497	6.0611	47.8220			
	5236012	6.7259	53.0671			
0.20	1	4313553	5.5409	43.7179	46.0479	2.0941
		4603159	5.9129	46.6531		
		4713637	6.0549	47.7728		
	2	4263470	5.4766	43.2103	46.2831	2.7038
		4765470	6.1214	48.2981		
		4671036	6.0001	47.3410		
	3	4804181	6.1712	48.6904	46.1059	2.2518
		4397330	5.6485	44.5670		
		4446009	5.7111	45.0604		

Dilution rate (hr <sup>-1</sup> )	Times	Peak area	Ethanol (%v/v)	Ethanol (g/l)	Average ethanol (g/l)	SD
0.20	4	5011171	6.4370	50.7883	49.4245	2.6659
		4573515	5.8749	46.3526		
		5045151	6.4807	51.1327		
	5	4618858	5.9331	46.8122	46.6633	0.4508
		4639437	5.9595	47.0208		
		4554200	5.8500	46.1569		
0.25	1	4013196	5.1551	40.6738	40.4811	0.1695
		3981746	5.1147	40.3550		
		3987604	5.1222	40.4144		
	2	3909687	5.0221	39.6247	42.1539	2.5740
		4417409	5.6743	44.7705		
		4150596	5.3316	42.0663		
	3	4085988	5.2486	41.4115	42.1965	2.3766
		3977464	5.1092	40.3117		
		4426859	5.6865	44.8663		
	4	4376033	5.6212	44.3512	43.5698	0.7217
		4235638	5.4408	42.9282		
		4285157	5.5045	43.4301		
	5	4413556	5.6694	44.7314	42.7111	1.7974
		4073940	5.2331	41.2894		
		4155138	5.3374	42.1124		



**Appendix J Determination of Volumetric Ethanol Productivity ( $Q_p$ ) in Continuous Ethanol Fermentation by Immobilized Yeast Cell.**

**Table J1** Determination of volumetric ethanol productivity ( $Q_p$ ) in continuous ethanol fermentation by immobilized yeast cell at 100 g/l of feed glucose concentration

$$\text{Volumetric ethanol productivity } (Q_p) = P/H \text{ or } PD$$

P = Ethanol concentration (g/l) at outlet

H = Hydraulic retention time (hr)

D = Dilution rate ( $\text{hr}^{-1}$ ) = 1/H

Dilution rate ( $\text{hr}^{-1}$ )	Times	Ethanol (g/l)	$Q_p$ ( $\text{gl}^{-1}\text{hr}^{-1}$ )	Average $Q_p$ ( $\text{gl}^{-1}\text{hr}^{-1}$ )	SD
0.15	1	41.2831	6.1925	6.1962	0.0591
	2	41.1498	6.1725		
	3	41.7183	6.2577		
	4	41.6432	6.2465		
	5	40.7467	6.1120		
0.20	1	35.2220	7.0444	7.3243	0.1757
	2	36.6822	7.3364		
	3	36.8650	7.3730		
	4	36.6870	7.3374		
	5	37.6508	7.5302		
0.25	1	30.6832	7.6708	8.2975	0.6575
	2	32.0313	8.0078		
	3	31.2029	7.8007		
	4	36.2594	9.0649		
	5	35.7732	8.9433		

**Table J2** Determination of volumetric ethanol productivity ( $Q_p$ ) in continuous ethanol fermentation by immobilized yeast cell at 150 g/l of feed glucose concentration

Dilution rate ( $\text{hr}^{-1}$ )	Times	Ethanol (g/l)	$Q_p$ ( $\text{gl}^{-1}\text{hr}^{-1}$ )	Average $Q_p$ ( $\text{gl}^{-1}\text{hr}^{-1}$ )	SD
0.15	1	40.2936	6.0440	6.3098	0.2275
	2	41.5692	6.2354		
	3	41.2946	6.1942		
	4	43.0350	6.4553		
	5	44.1352	6.6203		
0.20	1	41.5419	8.3084	8.0900	0.2091
	2	41.4338	8.2868		
	3	40.3944	8.0789		
	4	39.1490	7.8298		
	5	39.7305	7.9461		
0.25	1	30.5589	7.6397	8.6100	0.8529
	2	36.8615	9.2154		
	3	39.0002	9.7501		
	4	33.0066	8.2517		
	5	32.7718	8.1930		

**Table J3** Determination of volumetric ethanol productivity ( $Q_p$ ) in continuous ethanol fermentation by immobilized yeast cell at 200 g/l of feed glucose concentration

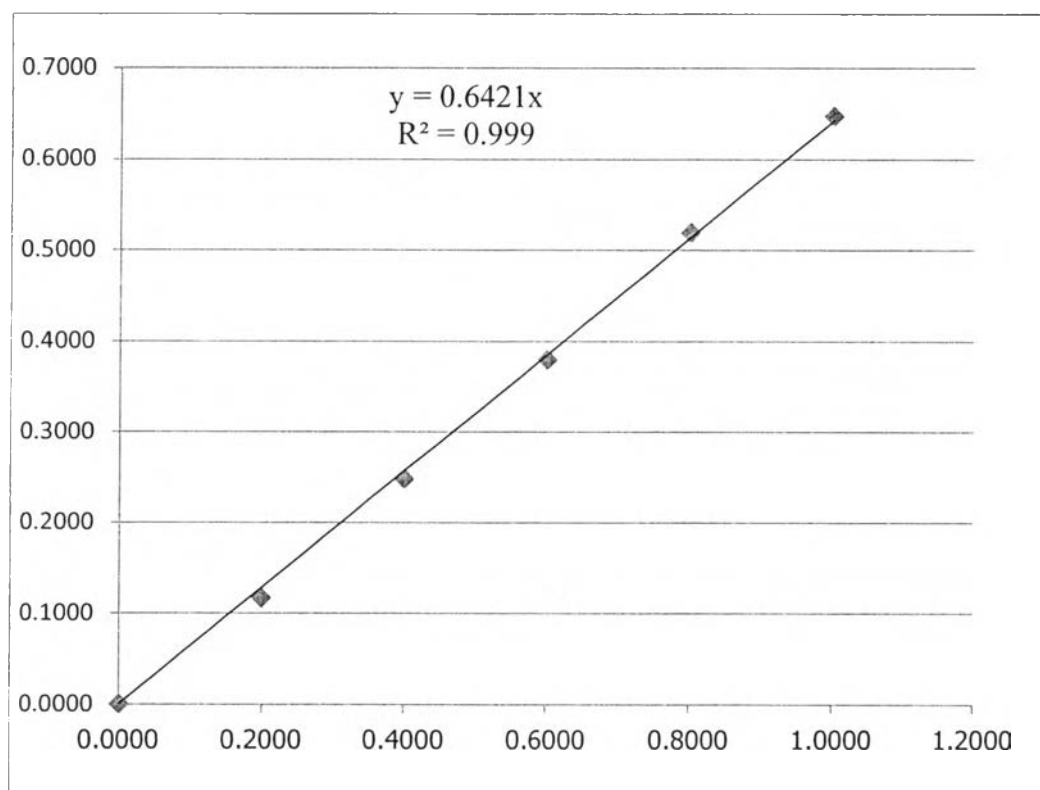
Dilution rate ( $\text{hr}^{-1}$ )	Times	Ethanol (g/l)	$Q_p$ ( $\text{g l}^{-1} \text{hr}^{-1}$ )	Average $Q_p$ ( $\text{g l}^{-1} \text{hr}^{-1}$ )	SD
0.15	1	51.7530	7.7630	7.6766	0.2437
	2	52.0464	7.8070		
	3	49.0596	7.3589		
	4	53.0578	7.9587		
	5	49.9714	7.4957		
0.20	1	46.0479	9.2096	9.3810	0.2858
	2	46.2831	9.2566		
	3	46.1059	9.2212		
	4	49.4245	9.8849		
	5	46.6633	9.3327		
0.25	1	40.4811	10.1203	10.5556	0.2821
	2	42.1539	10.5385		
	3	42.1965	10.5491		
	4	43.5698	10.8925		
	5	42.7111	10.6778		

**Appendix K Determination of Sugar Concentration in Continuous Ethanol Fermentation by Immobilized Yeast Cell.**

**Table K1** Standard curve of D-glucose in the concentration range of 0.2 to 1.0 g/l

Glucose concentration (g/l)	Absorbance at 575 nm	Average absorbance	SD
0.2	0.1179	0.1167	0.0010
	0.1160		
	0.1157		
	0.1161		
	0.1177		
0.4	0.2519	0.2479	0.0029
	0.2452		
	0.2451		
	0.2492		
	0.2483		
0.6	0.3843	0.3790	0.0042
	0.3733		
	0.3768		
	0.3797		
	0.3809		
0.8	0.5282	0.5191	0.0068
	0.5176		
	0.5136		
	0.5121		
	0.5239		

Glucose concentration (g/l)	Absorbance at 575 nm	Average absorbance	SD
1.0	0.6565	0.6474	0.0065
	0.6407		
	0.6416		
	0.6478		
	0.6503		



**Figure K** Standard curve of D-glucose

**Table K2** Determination of glucose concentration (g/l) in continuous ethanol fermentation by immobilized yeast cell at 100 g/l of feed glucose concentration

Dilution rate (hr <sup>-1</sup> )	Times	Absorbance at 575 nm	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.15	1	0.0778	12.1184	12.0187	0.1189
		0.0765	11.9159		
		0.0764	11.9003		
		0.0770	11.9938		
		0.0781	12.1651		
	2	0.0738	11.4953	11.4673	0.1665
		0.0734	11.4330		
		0.0724	11.2773		
		0.0753	11.7290		
		0.0732	11.4019		
	3	0.0919	14.3146	14.1402	0.1053
		0.0906	14.1121		
		0.0901	14.0343		
		0.0908	14.1433		
		0.0905	14.0966		
	4	0.0900	14.0187	13.6386	0.2293
		0.0872	13.5826		
		0.0874	13.6137		
		0.0860	13.3956		
		0.0872	13.5826		
5	0.0929	14.4704	14.2150	0.1798	
	0.0916	14.2679			
	0.0901	14.0343			
	0.0902	14.0498			
	0.0915	14.2523			

Dilution rate (hr <sup>-1</sup> )	Times	Absorbance at 575 nm	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.20	1	0.1188	18.5047	17.9751	0.3693
		0.1170	18.2243		
		0.1138	17.7259		
		0.1138	17.7259		
		0.1136	17.6947		
	2	0.1124	17.5078	17.7695	0.4487
		0.1191	18.5514		
		0.1121	17.4611		
		0.1130	17.6012		
		0.1138	17.7259		
	3	0.1075	16.7445	16.5171	0.1723
		0.1068	16.6355		
		0.1059	16.4953		
		0.1050	16.3551		
		0.1050	16.3551		
	4	0.0999	15.5607	15.3364	0.1673
		0.0977	15.2181		
		0.0982	15.2959		
		0.0973	15.1558		
		0.0992	15.4517		
5	0.1086	16.9159	16.6044	0.2238	
	0.1072	16.6978			
	0.1067	16.6199			
	0.1049	16.3396			
	0.1056	16.4486			

Dilution rate (hr <sup>-1</sup> )	Times	Absorbance at 575 nm	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.25	1	0.1195	18.6137	17.7477	1.0857
		0.1019	15.8723		
		0.1178	18.3489		
		0.1155	17.9907		
		0.1150	17.9128		
	2	0.1165	18.1464	18.0374	0.4127
		0.1200	18.6916		
		0.1135	17.6791		
		0.1153	17.9595		
		0.1137	17.7103		
	3	0.1259	19.6106	18.9875	0.3783
		0.1223	19.0498		
		0.1197	18.6449		
		0.1205	18.7695		
		0.1211	18.8629		
	4	0.0986	15.3583	16.6947	1.4444
		0.0981	15.2804		
		0.1164	18.1308		
		0.1056	16.4486		
		0.1172	18.2555		
5	0.1265	19.7041	17.9875	1.0516	
	0.1147	17.8660			
	0.1136	17.6947			
	0.1146	17.8505			
	0.1080	16.8224			



**Table K2 (cont.)** Conclusion of glucose concentration (g/l) in continuous ethanol fermentation by immobilized yeast cell at 100 g/l of feed glucose concentration

Dilution rate (hr <sup>-1</sup> )	Times	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.15	1	12.0187	13.0960	1.2699
	2	11.4673		
	3	14.1402		
	4	13.6386		
	5	14.2150		
0.20	1	17.9751	16.8405	1.0692
	2	17.7695		
	3	16.5171		
	4	15.3364		
	5	16.6044		
0.25	1	17.7477	17.8910	0.8193
	2	18.0374		
	3	18.9875		
	4	16.6947		
	5	17.9875		

**Table K3** Determination of glucose concentration (g/l) in continuous ethanol fermentation by immobilized yeast cell at 150 g/l of feed glucose concentration

Dilution rate (hr <sup>-1</sup> )	Times	Absorbance at 575 nm	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.15	1	0.3048	47.4766	45.9477	1.0237
		0.2957	46.0592		
		0.2963	46.1526		
		0.2892	45.0467		
		0.2886	44.9533		
	2	0.2623	40.8567	40.9502	0.5493
		0.2603	40.5452		
		0.2675	41.6667		
		0.2654	41.3396		
		0.2590	40.3427		
	3	0.2525	39.3302	39.5981	0.3774
		0.2557	39.8287		
		0.2533	39.4548		
		0.2577	40.1402		
		0.2519	39.2368		
	4	0.2556	39.8131	39.1215	0.4747
		0.2501	38.9564		
		0.2489	38.7695		
		0.2483	38.6760		
		0.2529	39.3925		
5	0.2321	36.1526	35.4019	0.4833	
	0.2277	35.4673			
	0.2274	35.4206			
	0.2240	34.8910			
	0.2252	35.0779			

Dilution rate (hr <sup>-1</sup> )	Times	Absorbance at 575 nm	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.20	1	0.2827	44.0343	43.0343	0.7108
		0.2751	42.8505		
		0.2791	43.4735		
		0.2726	42.4611		
		0.2719	42.3520		
	2	0.2760	42.9907	42.6417	0.5251
		0.2730	42.5234		
		0.2715	42.2897		
		0.2700	42.0561		
		0.2783	43.3489		
	3	0.2664	41.4953	41.1122	0.3424
		0.2609	40.6386		
		0.2627	40.9190		
		0.2655	41.3551		
		0.2642	41.1526		
	4	0.3082	48.0062	46.7165	0.8539
		0.3020	47.0405		
		0.2988	46.5421		
		0.2940	45.7944		
		0.2966	46.1994		
5	0.3016	46.9782	46.9003	0.5144	
	0.3026	47.1340			
	0.2987	46.5265			
	0.2971	46.2773			
	0.3055	47.5857			

Dilution rate (hr <sup>-1</sup> )	Times	Absorbance at 575 nm	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.25	1	0.4245	66.1215	66.1277	0.6798
		0.4274	66.5732		
		0.4170	64.9533		
		0.4270	66.5109		
		0.4268	66.4797		
	2	0.3245	50.5452	50.0280	0.4043
		0.3227	50.2648		
		0.3178	49.5016		
		0.3212	50.0312		
		0.3197	49.7975		
	3	0.3030	47.1963	46.7383	0.4007
		0.2967	46.2150		
		0.2984	46.4797		
		0.3020	47.0405		
		0.3002	46.7601		
	4	0.3711	57.8037	56.3302	1.0622
		0.3651	56.8692		
		0.3612	56.2617		
		0.3536	55.0779		
		0.3572	55.6386		
5	0.4229	65.8723	65.8255	0.5865	
	0.4239	66.0280			
	0.4235	65.9657			
	0.4163	64.8442			
	0.4264	66.4174			

**Table K3 (cont.)** Conclusion of glucose concentration (g/l) in continuous ethanol fermentation by immobilized yeast cell at 150 g/l of feed glucose concentration

Dilution rate (hr <sup>-1</sup> )	Times	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.15	1	45.9377	40.2019	3.8089
	2	40.9502		
	3	39.5981		
	4	39.1215		
	5	35.4019		
0.20	1	43.0343	44.0810	2.5921
	2	42.6417		
	3	41.1122		
	4	46.7165		
	5	46.9003		
0.25	1	66.1277	57.0099	8.8821
	2	50.0280		
	3	46.7383		
	4	56.3302		
	5	65.8255		

**Table K4** Determination of glucose concentration (g/l) in continuous ethanol fermentation by immobilized yeast cell at 200 g/l of feed glucose concentration

Dilution rate (hr <sup>-1</sup> )	Times	Absorbance at 575 nm	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.15	1	0.4943	76.9938	75.4517	1.2079
		0.4845	75.4673		
		0.4838	75.3583		
		0.4727	73.6293		
		0.4867	75.8100		
	2	0.4649	72.4143	72.2399	1.0882
		0.4697	73.1620		
		0.4709	73.3489		
		0.4547	70.8255		
		0.4587	71.4486		
	3	0.4916	76.5732	74.6573	1.2069
		0.4799	74.7508		
		0.4765	74.2212		
		0.4704	73.2710		
		0.4781	74.4704		
	4	0.4592	71.5265	71.7321	2.4453
		0.4545	70.7944		
		0.4521	70.4206		
		0.4490	69.9377		
		0.4878	75.9813		
5	0.5105	79.5171	76.9813	1.4389	
	0.4904	76.3863			
	0.4926	76.7290			
	0.4892	76.1994			
	0.4884	76.0748			

Dilution rate (hr <sup>-1</sup> )	Times	Absorbance at 575 nm	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.20	1	0.5749	89.5483	86.4704	2.3627
		0.5674	88.3801		
		0.5398	84.0810		
		0.5452	84.9221		
		0.5484	85.4206		
	2	0.5746	89.5016	87.1090	1.4928
		0.5586	87.0093		
		0.5533	86.1838		
		0.5495	85.5919		
		0.5602	87.2586		
	3	0.5091	79.2991	77.4704	1.1955
		0.4887	76.1215		
		0.4937	76.9003		
		0.4955	77.1807		
		0.4998	77.8505		
	4	0.5132	79.9377	77.6542	1.6507
		0.5028	78.3178		
		0.4946	77.0405		
		0.4844	75.4517		
		0.4977	77.5234		
5	0.5190	80.8411	78.0592	2.5000	
	0.4760	74.1433			
	0.5099	79.4237			
	0.5013	78.0841			
	0.4995	77.8037			

Dilution rate (hr <sup>-1</sup> )	Times	Absorbance at 575 nm	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.25	1	0.5886	91.6822	89.8287	1.3147
		0.5766	89.8131		
		0.5808	90.4673		
		0.5690	88.6293		
		0.5685	88.5514		
	2	0.6124	95.3894	93.1994	1.3618
		0.5889	91.7290		
		0.5956	92.7726		
		0.5996	93.3956		
		0.5952	92.7103		
	3	0.6201	96.5888	92.8941	2.3791
		0.5992	93.3333		
		0.5791	90.2025		
		0.5950	92.6791		
		0.5885	91.6667		
	4	0.5532	86.1682	83.6978	1.7785
		0.5438	84.7040		
		0.5345	83.2555		
		0.5237	81.5732		
		0.5315	82.7882		
5	0.6641	103.4424	94.9564	5.1336	
	0.5771	89.8910			
	0.6034	93.9875			
	0.5929	92.3520			
	0.6106	95.1090			



**Table K4 (cont.)** Conclusion of glucose concentration (g/l) in continuous ethanol fermentation by immobilized yeast cell at 200 g/l of feed glucose concentration

Dilution rate ( $\text{hr}^{-1}$ )	Times	Glucose concentration (g/l)	Average glucose concentration (g/l)	SD
0.15	1	75.4517	74.2125	2.2047
	2	72.2399		
	3	74.6573		
	4	71.7321		
	5	76.9813		
0.20	1	86.4704	81.3526	4.9730
	2	87.1090		
	3	77.4704		
	4	77.6542		
	5	78.0592		
0.25	1	89.8287	90.9153	4.4367
	2	93.1994		
	3	92.8941		
	4	83.6978		
	5	94.9564		

**Appendix L Determination of % Sugar Consumption in Continuous Ethanol Fermentation by Immobilized Yeast Cell.**

**Table L1** Determination of % sugar consumption in continuous ethanol fermentation by immobilized yeast cell at 100 g/l of feed glucose concentration

$$\text{Sugar consumption (\%)} = \frac{(S_0 - S) \times 100}{S_0}$$

S = Residual glucose concentration (g/l) at outlet

S<sub>0</sub> = Glucose concentration (g/l) in feed

Dilution rate (hr <sup>-1</sup> )	Times	S <sub>0</sub> (g/l)	S (g/l)	S <sub>0</sub> - S	Sugar consumption (%)	Average sugar consumption (%)	SD
0.15	1	100	12.0187	87.9813	87.9813	86.9040	1.2699
	2	100	11.4673	88.5327	88.5327		
	3	100	14.1402	85.8598	85.8598		
	4	100	13.6386	86.3614	86.3614		
	5	100	14.2150	85.7850	85.7850		
0.20	1	100	17.9751	82.0249	82.0249	83.1595	1.0692
	2	100	17.7695	82.2305	82.2305		
	3	100	16.5171	83.4829	83.4829		
	4	100	15.3364	84.6636	84.6636		
	5	100	16.6044	83.3956	83.3956		
0.25	1	100	17.7477	82.2523	82.2523	82.1090	0.8193
	2	100	18.0374	81.9626	81.9626		
	3	100	18.9875	81.0125	81.0125		
	4	100	16.6947	83.3053	83.3053		
	5	100	17.9875	82.0125	82.0125		

**Table L2** Determination of % sugar consumption in continuous ethanol fermentation by immobilized yeast cell at 150 g/l of feed glucose concentration

Dilution rate (hr <sup>-1</sup> )	Times	S <sub>0</sub> (g/l)	S (g/l)	S <sub>0</sub> - S	Sugar consumption (%)	Average sugar consumption (%)	SD
0.15	1	150	45.9377	104.0623	69.3749	73.1987	2.5393
	2	150	40.9502	109.0498	72.6999		
	3	150	39.5981	110.4019	73.6013		
	4	150	39.1215	110.8785	73.9190		
	5	150	35.4019	114.5981	76.3987		
0.20	1	150	43.0343	106.9657	71.3105	70.6127	1.7280
	2	150	42.6417	107.3583	71.5722		
	3	150	41.1122	108.8878	72.5919		
	4	150	46.7165	103.2835	68.8557		
	5	150	46.9003	103.0997	68.7331		
0.25	1	150	66.1277	83.8723	55.9149	61.9934	5.9214
	2	150	50.0280	99.9720	66.6480		
	3	150	46.7383	103.2617	68.8411		
	4	150	56.3302	93.6698	62.4465		
	5	150	65.8255	84.1745	56.1163		

**Table L3** Determination of % sugar consumption in continuous ethanol fermentation by immobilized yeast cell at 200 g/l of feed glucose concentration

Dilution rate (hr <sup>-1</sup> )	Times	S <sub>0</sub> (g/l)	S (g/l)	S <sub>0</sub> - S	Sugar consumption (%)	Average sugar consumption (%)	SD
0.15	1	200	75.4517	124.5483	62.2742	62.8938	1.1024
	2	200	72.2399	127.7601	63.8801		
	3	200	74.6573	125.3427	62.6714		
	4	200	71.7321	128.2679	64.1340		
	5	200	76.9813	123.0187	61.5094		
0.20	1	200	86.4704	113.5296	56.7648	59.3237	2.4865
	2	200	87.1090	112.8910	56.4455		
	3	200	77.4704	122.5296	61.2648		
	4	200	77.6542	122.3458	61.1729		
	5	200	78.0592	121.9408	60.9704		
0.25	1	200	89.8287	110.1713	55.0857	54.5424	2.2183
	2	200	93.1994	106.8006	53.4003		
	3	200	92.8941	107.1059	53.5530		
	4	200	83.6978	116.3022	58.1511		
	5	200	94.9564	105.0436	52.5218		

**Appendix M Determination of Ethanol Yield ( $Y_{P/S}$ ) in Continuous Ethanol Fermentation by Immobilized Yeast Cell.**

**Table M1** Determination of ethanol yield ( $Y_{P/S}$ ) in continuous ethanol fermentation by immobilized yeast cell at 100 g/l of feed glucose concentration

$$\text{Ethanol yield} = P/S_0-S$$

P = Ethanol concentration (g/l) at outlet

$S_0-S$  = Sugar consumption (g/l)

Dilution rate ( $\text{hr}^{-1}$ )	Times	P (g/l)	$S_0 - S$	Ethanol yield	Average ethanol yield	SD
0.15	1	41.2831	87.9813	0.4692	0.4754	0.0088
	2	41.1498	88.5327	0.4648		
	3	41.7183	85.8598	0.4859		
	4	41.6432	86.3614	0.4822		
	5	40.7467	85.7850	0.4750		
0.20	1	35.2220	82.0249	0.4294	0.4404	0.0090
	2	36.6822	82.2305	0.4461		
	3	36.8650	83.4829	0.4416		
	4	36.6870	84.6636	0.4333		
	5	37.6508	83.3956	0.4515		
0.25	1	30.6832	82.2523	0.3730	0.4041	0.0296
	2	32.0313	81.9626	0.3908		
	3	31.2029	81.0125	0.3852		
	4	36.2594	83.3053	0.4353		
	5	35.7732	82.0125	0.4362		

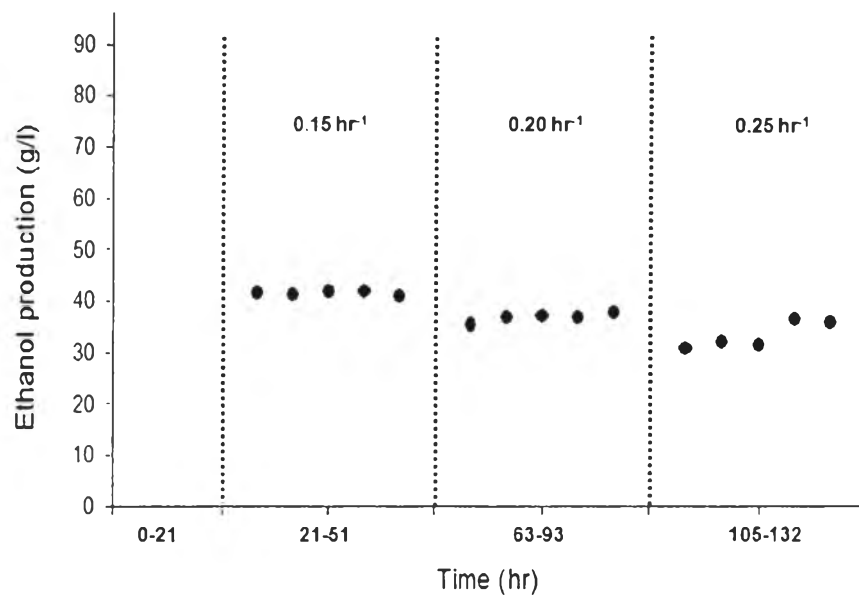
**Table M2** Determination of ethanol yield ( $Y_{P/S}$ ) in continuous ethanol fermentation by immobilized yeast cell at 150 g/l of feed glucose concentration

Dilution rate ( $\text{hr}^{-1}$ )	Times	P (g/l)	$S_0 - S$	Ethanol yield	Average ethanol yield	SD
0.15	1	40.2936	104.0623	0.3872	0.3831	0.0057
	2	41.5692	109.0498	0.3812		
	3	41.2946	110.4019	0.3740		
	4	43.0350	110.8785	0.3881		
	5	44.1352	114.5981	0.3851		
0.20	1	41.5419	106.9657	0.3884	0.3819	0.0070
	2	41.4338	107.3583	0.3859		
	3	40.3944	108.8878	0.3710		
	4	39.1490	103.2835	0.3790		
	5	39.7305	103.0997	0.3854		
0.25	1	30.5589	83.8723	0.3644	0.3705	0.0139
	2	36.8615	99.9720	0.3687		
	3	39.0002	103.2617	0.3777		
	4	33.0066	93.6698	0.3524		
	5	32.7718	84.1745	0.3893		

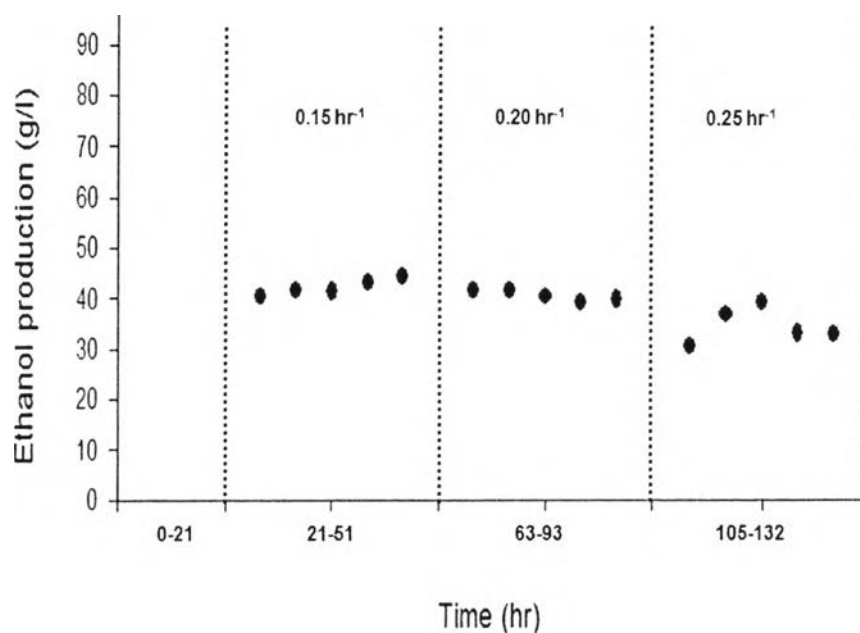
**Table M3** Determination of ethanol yield ( $Y_{P/S}$ ) in continuous ethanol fermentation by immobilized yeast cell at 200 g/l of feed glucose concentration

Dilution rate ( $\text{hr}^{-1}$ )	Times	P (g/l)	$S_0 - S$	Ethanol yield	Average ethanol yield	SD
0.15	1	51.7530	124.5483	0.4155	0.4068	0.9995
	2	52.0464	127.7601	0.4074		
	3	49.0596	125.3427	0.3914		
	4	53.0578	128.2679	0.4136		
	5	49.9714	123.0187	0.4062		
0.20	1	46.0479	113.5296	0.4056	0.3957	0.0151
	2	46.2831	112.8910	0.4100		
	3	46.1059	122.5296	0.3763		
	4	49.4245	122.3458	0.4040		
	5	46.6633	121.9408	0.3827		
0.25	1	40.4811	110.1713	0.3674	0.3875	0.0160
	2	42.1539	106.8006	0.3947		
	3	42.1965	107.1059	0.3940		
	4	43.5698	116.3022	0.3746		
	5	42.7111	105.0436	0.4066		

**Appendix N Plot Graph between Ethanol Production (g/l) and Time (hr) in Continuous Ethanol Fermentation by Immobilized Yeast Cell.**

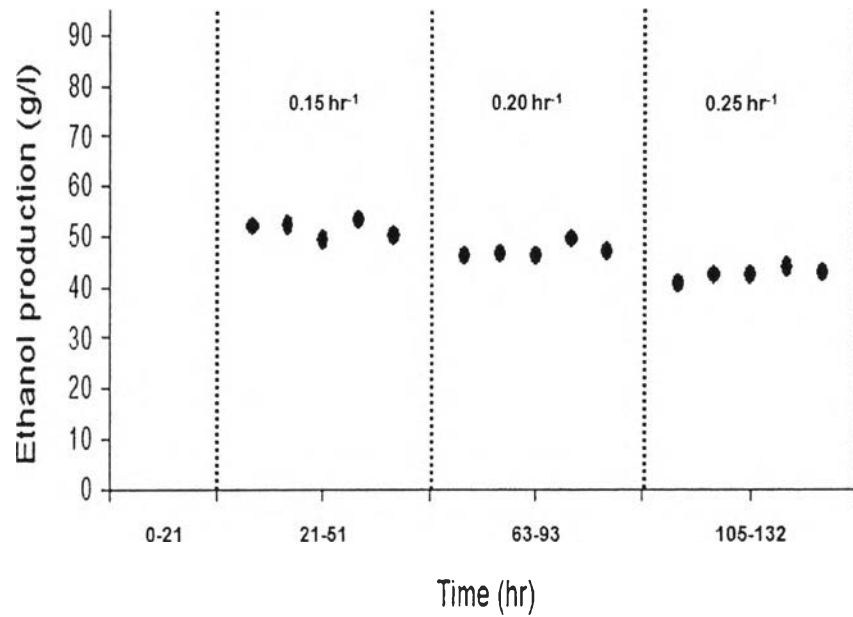


**Figure N1** Ethanol production (g/l) vs Time (hr) in continuous ethanol fermentation by immobilized yeast cell at 100 g/l of feed glucose concentration.



**Figure N2** Ethanol production (g/l) vs Time (hr) in continuous ethanol fermentation by immobilized yeast cell at 150 g/l of feed glucose concentration.

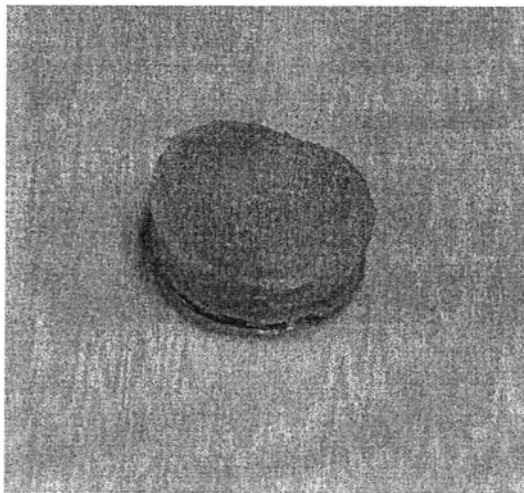




**Figure N3** Ethanol production (g/l) vs Time (hr) in continuous ethanol fermentation by immobilized yeast cell at 200 g/l of feed glucose concentration.

**Appendix O Photograph of MSF50 Bionanocomposite Sponge after Used in Continuous Ethanol Fermentation.**

The photograph in Figure N was MSF50 bionanocomposite sponge after used in continuous ethanol fermentation.



**Figure O** MSF50 bionanocomposite sponge after used in continuous ethanol fermentation.

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**Proceedings:**

1. Jampa, S.; Tokura, S.; and Rujiravanit, R. (2013, April 23<sup>rd</sup>) Preparation of Silk Fibroin/Cellulose Whiskers Bionanocomposite Sponges for Yeast Cell Immobilization Used in Continuous Ethanol Production by Packed Bed Bioreactor. Proceeding of the 4<sup>th</sup> Research Symposium on Petrochemical and Materials Technology and the 19<sup>th</sup> PPC Symposium on Petroleum, Petrochemicals and Polymers, Bangkok, Thailand.

**Presentations:**

1. Jampa, S., Tokura, S., and Rujiravanit, R. (2013, March 23<sup>rd</sup>) Preparation of Silk Fibroin/Cellulose Whiskers Bionanocomposite Sponges for Yeast Cell Immobilization Used in Continuous Ethanol Fermentation. Oral presentation at the 8<sup>th</sup> Science and Technology Conference for Youths, Bangkok, Thailand.
2. Jampa, S., Tokura, S., and Rujiravanit, R. (2013, April 23<sup>rd</sup>) Preparation of Silk Fibroin/Cellulose Whiskers Bionanocomposite Sponges for Yeast Cell Immobilization Used in Continuous Ethanol Production by Packed Bed Bioreactor. Paper presented at the 4<sup>th</sup> Research Symposium on Petrochemical and Materials Technology and the 19<sup>th</sup> PPC Symposium on Petroleum, Petrochemicals and Polymers, Bangkok, Thailand.