

Appendix B

Table 1 Time course of growth and biosurfactant production of *Bacillus* MUV4 in Mckeen medium under shake-flask culture

Time (h)	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)
0	7.00 ^{bc}	0 ⁱ	0 ^g	0 ^d
6	6.88 ^{bc}	0.29 ^h	0 ^g	0 ^d
12	6.66 ^c	2.05 ^g	0 ^g	0 ^d
18	6.71 ^{bc}	4.97 ^d	0.12 ^f	0.66 ^c
24	6.81 ^{bc}	5.57 ^c	4.63 ^e	0.76 ^b
36	7.26 ^a	6.82 ^a	6.15 ^d	0.74 ^b
48	7.13 ^{bc}	5.73 ^b	9.76 ^a	0.89 ^a
60	6.98 ^{bc}	4.98 ^e	9.45 ^b	0.80 ^b
72	6.65 ^c	4.74 ^h	9.07 ^c	0.65 ^c

Table 2 Growth, final pH and biosurfactant production of *Bacillus* MUV4 at 48 h cultivation in the Mckeen medium with various carbon sources (2.0%) on shaker (200 rpm) at 30°C

Types of carbon source	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)
Glucose	7.13 ^b	5.73 ^b	9.76 ^a	0.89 ^a
Sucrose	7.19 ^b	7.52 ^a	7.88 ^b	0.64 ^b
Mollases	8.98 ^a	5.86 ^b	1.01 ^c	0.21 ^c
Glutamate	8.79 ^a	3.69 ^c	0.87 ^d	0.11 ^d

Note : The same supscript-letter means not significantly different (p<0.05) These values are the average of triplicate for shake-flask cultures

Table 3 Growth, final pH and biosurfactant production of *Bacillus* MUV4 at 48 h cultivation in the Mckeen medium with various hydrophobic carbon sources (0.1%) on shaker (200 rpm) at 30°C

Types of hydrophobic carbon source	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)
Weathered oil	8.26 ^b	3.13 ^b	0.50 ^a	0.15 ^a
Palm oil	8.52 ^a	3.59 ^a	0.38 ^b	0.05 ^b
n-hexadecane	8.53 ^a	2.77 ^c	0.50 ^a	0.14 ^a

Note : The same supscript-letter means not significantly different (p<0.05) These values are the average of triplicate for shake-flask cultures

Table 4 Growth, final pH and biosurfactant production of *Bacillus* MUV4 at 48 h cultivation in the Mckeen medium with various weathered oil concentration on shaker (200 rpm) at 30°C

Conc. of weathered oil (%)	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)
0.1	8.15 ^a	2.98 ^a	0.50 ^b	0.14 ^a
0.3	8.18 ^a	2.41 ^a	1.33 ^a	0.19 ^a
0.5	8.26 ^a	2.47 ^a	0.79 ^b	0.13 ^a

Note : The same supscript-letter means not significantly different (p<0.05) These values are the average of triplicate for shake-flask cultures

Table 5 Growth, final pH and biosurfactant production of *Bacillus* MUV4 at 48 h cultivation in the Mckeen medium with various glucose concentration on shaker (200 rpm) at 30°C

Glucose concentration (%)	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)
1.0	7.44 ^{ab}	2.09 ^e	8.16 ^c	0.61 ^c
2.0	7.78 ^a	5.45 ^c	10.36 ^b	0.67 ^c
2.5	7.12 ^b	5.89 ^a	10.74 ^a	0.93 ^a
3.0	6.61 ^c	5.71 ^b	6.75 ^d	0.78 ^b
3.5	6.65 ^c	5.25 ^{cd}	6.16 ^e	0.83 ^b
4.0	6.70 ^c	5.12 ^d	5.56 ^f	0.64 ^c

Note : The same subscript-letter means not significantly different (p<0.05) These values are the average of triplicate for shake-flask cultures

Table 6 Growth, final pH and biosurfactant production of *Bacillus* MUV4 at 48 h cultivation in the Mckeen medium contained 2.5% as carbon source with various nitrogen sources on shaker (200 rpm) at 30°C

Type of nitrogen source	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)
KNO ₃	6.49 ^b	0.76 ^c	0 ^f	0 ^g
NH ₄ NO ₃	4.30 ^e	2.53 ^e	0 ^f	0 ^g
(NH ₄) ₂ SO ₄	4.49 ^e	1.45 ^f	0.10 ^f	0.12 ^f
(NH ₄) ₂ HPO ₄	5.20 ^d	7.32 ^a	4.91 ^c	1.24 ^c
(NH ₄)H ₂ PO ₄	5.77 ^c	5.24 ^b	3.71 ^d	0.89 ^d
NH ₄ HCO ₃	5.75 ^c	3.81 ^d	2.41 ^e	0.45 ^e
NaNO ₃	6.37 ^b	0.54 ^g	0.10 ^f	0.16 ^f
DL-glutamic acid	7.67 ^a	4.99 ^{bc}	10.75 ^b	2.30 ^b
L-glutamic acid	7.59 ^a	5.09 ^b	11.35 ^a	2.60 ^a
MSG	7.57 ^a	5.28 ^b	11.25 ^a	2.58 ^a

Note : The same supscript-letter means not significantly different (p<0.05) These values are the average of triplicate for shake-flask cultures

Table 7 Growth, final pH and biosurfactant production of *Bacillus* MUV4 at 48 h cultivation in the Mckeen medium contained 2.5% as carbon source with various monosodium glutamate (MSG) concentration (%) on shaker (200 rpm) at 30 °C

MSG concentration (%)	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)	EA (%)
0.1	6.64 ^d	1.66 ^d	1.54 ^e	0.77 ^d	67.82 ^d
0.3	6.65 ^d	4.11 ^{cd}	5.89 ^{de}	1.67 ^c	73.33 ^c
0.5	7.30 ^c	6.73 ^{abc}	10.75 ^c	2.61 ^b	74.70 ^b
1.0	7.70 ^a	8.23 ^a	44.28 ^a	2.94 ^a	80.00 ^a
1.5	7.63 ^b	7.31 ^{ab}	34.94 ^b	2.53 ^b	74.60 ^b

Note : The same subscript-letter means not significantly different (p<0.05) These values are the average of triplicate for shake-flask cultures

Table 8 pH change and cell growth and biosurfactant production from *Bacillus* MUV4 when cultivated in the medium containing yeast extract and bacto peptone at various concentrations for 48 hrs (M+Y1=Medium+ 0.1% yst extract, M+Y3=Medium+0.3% yst extract, M+Y5=Medium+ 0.5 % yst extract, A+P1= Medium+0.1% bacto peptone, M+P3=Medium+ 0.3% bacto peptone and M+P5=Medium+0.5% bacto peptone, M=Medium contained 2.5% as carbon source and 1.0% monosodium glutamate)

Type and concentration of yeast extract and bacto peptone	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)	EA (%)
M	7.21 ^a	8.18 ^f	44.28 ^c	2.74 ^f	80.00 ^a
M+Y1	6.52 ^{bc}	9.18 ^e	60.79 ^{bc}	2.93 ^e	81.00 ^a
M+Y3	6.65 ^b	9.49 ^d	78.50 ^a	4.72 ^a	80.13 ^a
M+Y5	7.10 ^a	9.84 ^c	66.44 ^{ab}	3.35 ^d	81.00 ^a
M+P1	6.40 ^c	10.28 ^a	42.70 ^{bc}	2.49 ^g	78.18 ^a
M+P3	6.63 ^b	10.19 ^{ab}	47.73 ^c	3.69 ^b	81.00 ^a
M+P5	7.44 ^a	9.93 ^{bc}	45.22 ^c	3.47 ^c	80.00 ^a

Note : The same supscript-letter means not significantly different (p<0.05) These values are the average of triplicate for shake-flask cultures

Table 9 Time course of growth and biosurfactant production of *Bacillus* MUV4 in optimal Mckeen medium under shake-flask culture (200 rpm) at 30°C

Time (h)	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)	EA (%)
0	7.00 ^d	0 ^e	0 ^g	0.09 ^g	0 ^e
6	5.89 ^h	1.38 ^d	0 ^g	0.11 ^g	0 ^e
12	6.17 ^g	9.34 ^c	3.40 ^f	0.61 ^f	57.64 ^d
18	6.87 ^e	9.51 ^c	7.10 ^e	1.41 ^e	63.64 ^c
24	7.17 ^c	10.43 ^b	40.69 ^d	1.89 ^d	71.93 ^b
36	7.69 ^a	12.96 ^a	66.44 ^c	4.38 ^b	79.42 ^a
48	7.62 ^b	12.62 ^b	78.50 ^a	4.29 ^b	81.13 ^a
60	6.65 ^{ab}	10.10 ^{bc}	78.50 ^a	5.18 ^a	81.82 ^a
72	6.63 ^f	9.96 ^{bc}	72.35 ^b	4.06 ^c	78.18 ^a

Note : The same supscript-letter means not significantly different (p<0.05) These values are the average of triplicate for shake-flask cultures

Table 10 Time course of growth and biosurfactant production of *Bacillus* MUV4 in optimal medium in 2.0-l fermentor culture under uncontrolled pH (pH 7.0) at 30°C

Time (h)	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)	EA (%)
0	7.10 ^c	0 ^f	0 ^e	0 ^e	0 ^e
6	6.26 ^g	1.41 ^e	0 ^e	0 ^e	0 ^e
12	6.49 ^f	2.66 ^d	0.79 ^e	0.23 ^e	12.96 ^d
18	6.71 ^e	3.76 ^c	3.46 ^e	1.14 ^d	56.60 ^c
24	6.79 ^{de}	4.24 ^c	5.31 ^e	1.54 ^c	62.62 ^{bc}
36	6.83 ^{de}	5.44 ^b	11.30 ^d	1.77 ^c	63.64 ^b
48	6.91 ^d	7.41 ^a	32.10 ^c	3.50 ^b	72.72 ^a
60	7.95 ^a	5.36 ^b	60.79 ^a	4.21 ^a	74.54 ^a
72	7.41 ^b	5.89 ^b	50.24 ^b	4.10 ^a	72.72 ^a

Note : The same supscript-letter means not significantly different (p<0.05) These values are the average of triplicate for fermentor cultures

Table 11 Time course of growth and biosurfactant production of *Bacillus* MUV4 in optimal medium under 2.0-l fermentor culture under controlled pH (pH 7.0) at 30°C for 72 h

Time (h)	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)	EA (%)
0	7.05 ^a	0 ^l	0 ^g	0 ^f	0 ^e
6	6.94 ^b	1.37 ^h	0 ^g	0 ^f	0 ^e
12	7.04 ^b	2.00 ^g	0.79 ^g	0.10 ^f	36.36 ^d
18	9.96 ^b	2.64 ^f	3.14 ^f	0.92 ^e	36.36 ^d
24	9.94 ^b	4.19 ^e	4.90 ^e	1.13 ^{de}	47.27 ^c
36	7.02 ^a	5.14 ^d	7.07 ^d	1.25 ^d	60.00 ^b
48	7.05 ^a	6.69 ^a	9.07 ^c	1.72 ^c	72.72 ^a
60	7.03 ^a	6.46 ^b	32.10 ^a	2.80 ^a	72.72 ^a
72	7.04 ^a	6.21 ^c	28.26 ^b	2.50 ^b	63.63 ^b

Note : The same subscript-letter means not significantly different (p<0.05) These values are the average of triplicate for fermentor cultures

Table 12 pH change, cell growth and biosurfactant production from *Bacillus* MUV4 during cultivation in fermentor at various aeration rates for 60 h

Aeration rates (vvm)	pH	Cell growth (OD660nm)	ODA (cm ²)	EC (%)	EA (%)
0	4.89 ^d	1.19 ^d	0.57 ^d	0.53 ^c	9.09 ^d
0.5	7.95 ^a	5.36 ^c	60.79 ^{bc}	4.00 ^b	73.23 ^b
1.0	7.74 ^b	7.16 ^b	72.34 ^a	4.49 ^a	81.48 ^a
1.5	7.38 ^c	7.98 ^a	55.39 ^c	3.70 ^b	60.00 ^c

Note : The same subscript-letter means not significantly different ($p < 0.05$) These values are the average of triplicate for fermentor culture

Table 13 Time course of growth and biosurfactant production of *Bacillus* MUV4 under optimal condition (uncontrolled pH, aeration rate 1.0 vvm, agitation rate 200 rpm, pH 7.0) at 30°C

Time (h)	pH	Cell growth (OD _{660nm})	ODA (cm ²)	EC (%)	EA (%)
0	7.01 ^c	0 ^h	0 ^c	0 ^g	0 ^f
6	6.26 ^f	1.52 ^g	0 ^c	0 ^g	0 ^f
12	6.62 ^e	2.10 ^f	1.77 ^c	0.54 ^f	12.70 ^e
18	6.85 ^d	3.76 ^e	4.91 ^c	0.62 ^f	50.91 ^d
24	7.03 ^c	3.91 ^e	6.15 ^c	0.85 ^e	55.55 ^c
36	7.01 ^c	5.00 ^d	45.34 ^b	2.28 ^d	74.07 ^b
48	7.85 ^a	6.62 ^b	47.75 ^b	3.57 ^c	81.48 ^a
60	7.74 ^b	7.13 ^a	72.34 ^a	4.49 ^a	81.48 ^a
72	7.88 ^a	6.26 ^c	50.25 ^b	4.09 ^b	8036 ^a

Note : The same subscript-letter means not significantly different ($p < 0.05$) These values are the average of triplicate for fermentor cultures

Table 14 Effect of pH on stability of culture broth biosurfactant from *Bacillus* MUV4 (controlled pH =7.74)

pH	Parameters		
	ODA relative (%)	EA relative (%)	EC relative (%)
2	6.25	57.77	17.45
4	7.29	74.69	24.50
6	77.44	98.72	97.31
7.74 (control)	100	100	100
8	84.64	99.58	95.97
10	82.1	98.18	94.70
12	64.00	86.67	25.50
14	51.83	82.22	29.36

Table 15 Effect of pH on stability of acid precipitated biosurfactant from *Bacillus* MUV4 (controlled pH =7.44)

pH	Parameters		
	ODA relative (%)	EA relative (%)	EC relative (%)
2	7.29	0	23.53
4	19.55	15.79	52.29
6	82.08	80.28	82.35
7.44 (control)	100	100	100
8	89.61	91.13	83.88
10	84.52	90.13	82.16

12	75.15	85.71	71.91
14	57.84	67.89	62.75

Table 16 Effect of NaCl concentration on stability of culture broth biosurfactant from *Bacillus* MUV4

NaCl concentration (%)	Parameters		
	ODA relative (%)	EA relative (%)	EC relative (%)
0	100	100	100
5	95.95	74.27	95.94
10	69.33	0	67.57
15	69.33	0	56.42
20	9.22	0	63.51
25	6	0	58.11
30	6	0	55.74
35	6	0	55.74

Table 17 Effect of NaCl concentrations on stability of acid precipitated biosurfactant from *Bacillus* MUV4

NaCl concentration (%)	Parameters		
	ODA relative (%)	EA relative (%)	EC relative (%)
0	100	100	100
5	93.02	73.23	90.20
10	33.22	0	39.21
15	9.80	0	26.67
20	5.15	0	20.39

25	1.32	0	18.48
30	0	0	16.86
35	0	0	10.58

Table 18 Effect of temperature on stability (%ODA relative)of culture broth biosurfactant from *Bacillus* MUV4

Incubation times (h)	Temperature (°C)				
	4	30	55	80	100
0	100	100	100	100	100
6	97.64	97.64	86.87	89.97	86.97
12	95.31	92.25	79.57	77.44	64.00
18	95.31	92.50	77.44	64.00	64.00
24	91.49	82.64	64.00	64.00	59.59
36	84.81	77.69	67.90	67.90	50.12
48	84.81	77.66	64.00	64.00	47.87

Table 19 Effect of temperature on stability (%EA relative)of culture broth biosurfactant from *Bacillus* MUV4

Incubation times (h)	Temperature (°C)				
	4	30	55	80	100

0	100	100	100	100	100
6	99.34	99.58	97.90	97.89	97.15
12	98.51	98.96	96.41	97.15	94.12
18	98.51	98.51	94.51	90.37	88.54
24	97.92	97.58	94.73	86.04	86.04
36	96.59	95.67	78.87	78.04	77.28
48	96.43	90.78	78.43	77.28	77.28

Table 20 Effect of temperature on stability (%EC relative)of culture broth biosurfactant from *Bacillus* MUV4

Incubation times (h)	Temperature (°C)				
	4	30	55	80	100
0	100	100	100	100	100
6	95.75	96.25	92.52	90.16	91.80
12	81.30	80.98	84.42	80.32	80.00
18	69.12	66.57	71.96	69.84	69.72
24	67.76	60.52	57.32	49.84	38.80
36	65.44	56.14	41.43	39.68	38.80
48	58.73	41.21	35.82	30.79	25.87

Table 21 Effect of temperature on stability (%ODA relative) of acid precipitated biosurfactant from *Bacillus* MUV4

Incubation times (h)	Temperature (°C)				
	4	30	55	80	100

0	100	100	100	100	100
6	97.55	94.25	91.80	84.02	91.50
12	89.58	76.56	75.62	75.62	70.41
18	74.68	75.62	75.62	70.41	63.31
24	63.07	62.67	63.31	54.39	61.62
36	61.13	56.24	59.23	50.17	49.61
48	61.13	56.24	50.17	44.44	42.53

Table 22 Effect of temperature on stability (%EA) of acid precipitated biosurfactant from *Bacillus* MUV4

Incubation times (h)	Temperature (°C)				
	4	30	55	80	100
0	100	100	100	100	100
6	98.18	99.82	98.82	98.85	98.85
12	98.18	98.18	98.18	98.85	98.20
18	99.79	98.18	95.86	88.22	82.35
24	95.40	87.66	76.46	73.52	73.52
36	80.00	83.31	73.52	73.52	73.52
48	74.28	77.35	73.72	58.81	58.81

Table 23 Effect of temperature on stability (%EC relative) of acid precipitated biosurfactant from *Bacillus* MUV4

Incubation times (h)	Temperature (°C)				
	4	30	55	80	100
0	100	100	100	100	100
6	95.24	94.61	88.80	87.90	91.06
12	93.65	95.38	64.80	67.74	69.92
18	58.73	56.14	47.20	48.38	47.15
24	57.94	55.38	32.60	39.52	34.15
36	57.14	55.38	35.20	24.19	20.32
48	57.14	55.38	32.80	23.39	20.32