

APPENDIX A

Media

1. Media composition and Preparation

1.1 De Man, Rogasa, and Sharpe agar (MRS broth and agar)

Composition per liter:

Glucose	20.0	g
Beef extract	10.0	g
Yeast extract	5.0	g
Sodium acetate (CH ₃ COONa 3H ₂ O)	5.0	g
K ₂ HPO ₄	2	g
Ammonium citrate	2	g
MgSO ₄ .7H ₂ O	0.2	g
MnSO ₄ .4H ₂ O	0.2	g
Tween 80	1.0	g
Agar	15.0	g
Distilled water	1	L

pH 6.2±0.2 at 25°C

Preparation of Medium: Add component to distilled/deionized water and adjust volume to 1.0 L. Mix thoroughly and sterile at 121 °C for 15 min.

1.2 Glucose Yeast Peptone (GYP broth and agar)

Composition per liter:

Glucose	10.0	g
Yeast extract	10.0	g
Peptone	10.0	g
Salt solution (see below)	5.0	ml
Agar	15.0	g
Distilled water	1	L
pH 6.2±0.2 at 25°C		

Salt solution:

MgSO ₄ .7 H ₂ O	40.0	g
MnSO ₄ .4 H ₂ O	2.0	g
FeSO ₄ .7 H ₂ O	2.0	g
NaCl	2.0	g
Distilled water	1	L

Preparation of Medium: Add component to distilled/deionized water and adjust volume to 1.0 L. Mix thoroughly and sterile at 121 °C for 15 min.

APPENDIX B

1. Composition of chemical solution and Preparation

1.1 2% of Sodium alginate solution (w/v)

Composition per 100 millitre:

Sodium alginate	2.0 g
H ₂ O	100 ml

Preparation: Add sodium alginate to distilled/deionized water and adjust volume to 100 ml. Mix thoroughly and heat to dissolve. Sterile at 121 °C for 15 min.

1.2 0.5% of κ-carrageenan solution (w/v)

Composition per 100 millitre:

κ-carrageenan	0.5 g
NaCl	0.9 % (w/v)
H ₂ O	100 ml

Preparation: Add NaCl to distilled/deionized water and adjust volume to 100 ml. Mix thoroughly, Then add NaCl solution to κ-carrageenan and heat to dissolve. Sterile at 121 °C for 15 min.

1.3 0.1 M of Calcium chloride (w/v)

Composition per 0.5 liter:

Calcium chloride	7.35 g
H ₂ O	500 ml

Preparation: Add calcium chloride to distilled/deionized water and adjust volume to 0.5 L. Mix thoroughly and sterile at 121 °C for 15 min.

1.4 0.3 M of Potassium chloride (w/v)**Composition per 0.5 liter:**

Potassium chloride	11.10 g
H ₂ O	500 ml

Preparation: Add potassium chloride to distilled/deionized water and adjust volume to 0.5 L. Mix thoroughly and sterile at 121 °C for 15 min.

1.5 Glycerol solution**Composition per 0.5 liter:**

Glycerol	325 ml
MgSO ₄ .7H ₂ O	6.02 g
Tris HCl	1.51 g
H ₂ O	500 ml
pH 8.0 at 25 °C	

Preparation: Add component to distilled/deionized water and adjust volume to 0.5 L. Mix thoroughly and sterile at 121 °C for 15 min.

1.6 Simulated Gastric Juices Solution (Rao *et al.*, 1989)**Composition per 100 millitre:**

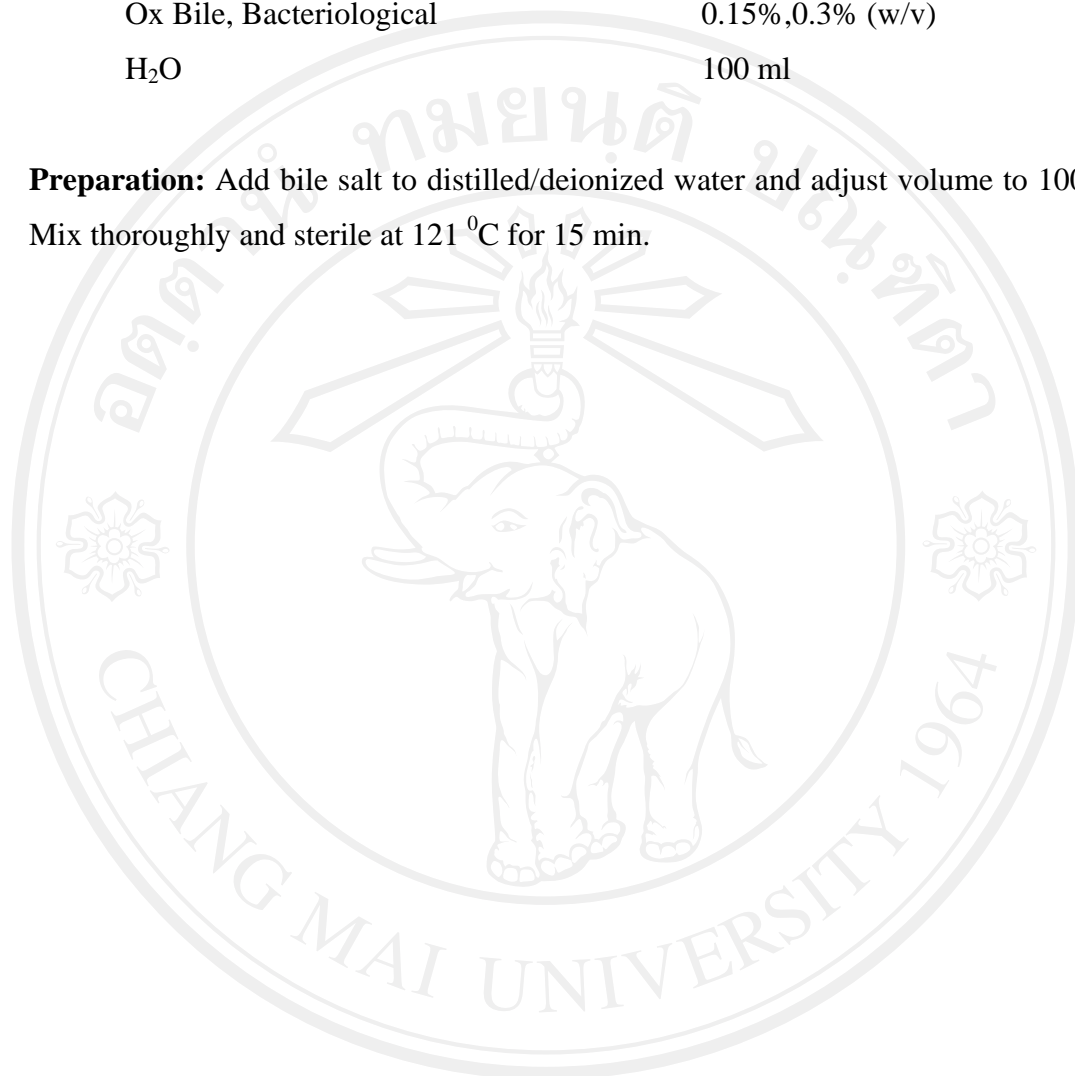
HCl solution	0.08 M
NaCl	0.2% (w/v)
pH 1.5 at 25 °C	

Preparation: Add NaCl to distilled/deionized water, Then add HCl solution to NaCl solution and adjust volume to 100 ml. Mix thoroughly and sterile at 121 °C for 15 min.

1.7 Bile Salt Solution (Modified by Clark and Martin, 1994)**Composition per 100 millitre:**

Ox Bile, Bacteriological	0.15%,0.3% (w/v)
H ₂ O	100 ml

Preparation: Add bile salt to distilled/deionized water and adjust volume to 100 ml. Mix thoroughly and sterile at 121 °C for 15 min.



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APPENDIX C

Lactobacillus fermentum 2311M

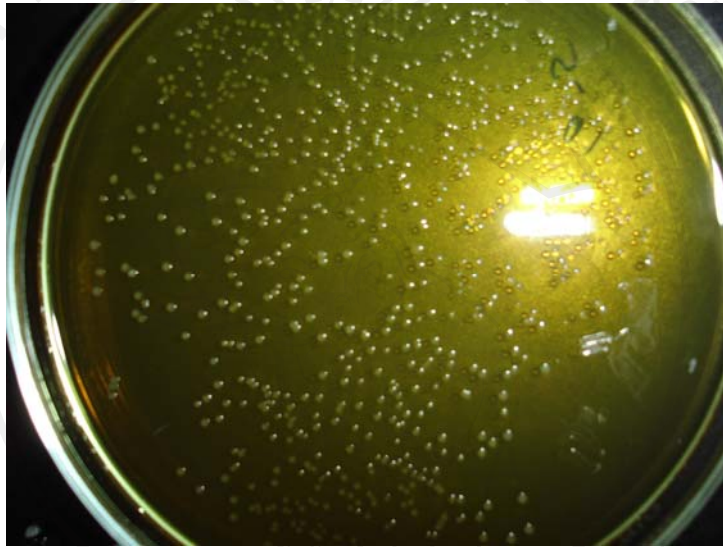


Figure 24 The morphology of *L. fermentum* 2311M on MRS agar + Bromocresol purple (oval shape with yellow zone around colony)

Table 9 Absorbance at 600 nm by *L. fermentum* 2311M after incubated at 37 °C for 24 h

Viable cell (cfu/ml)	A ₆₀₀
0	0
0.22	0.079
0.55	0.101
0.58	0.125
0.74	0.180
1.56	0.276
1.82	0.305
2.24	0.428
2.56	0.558

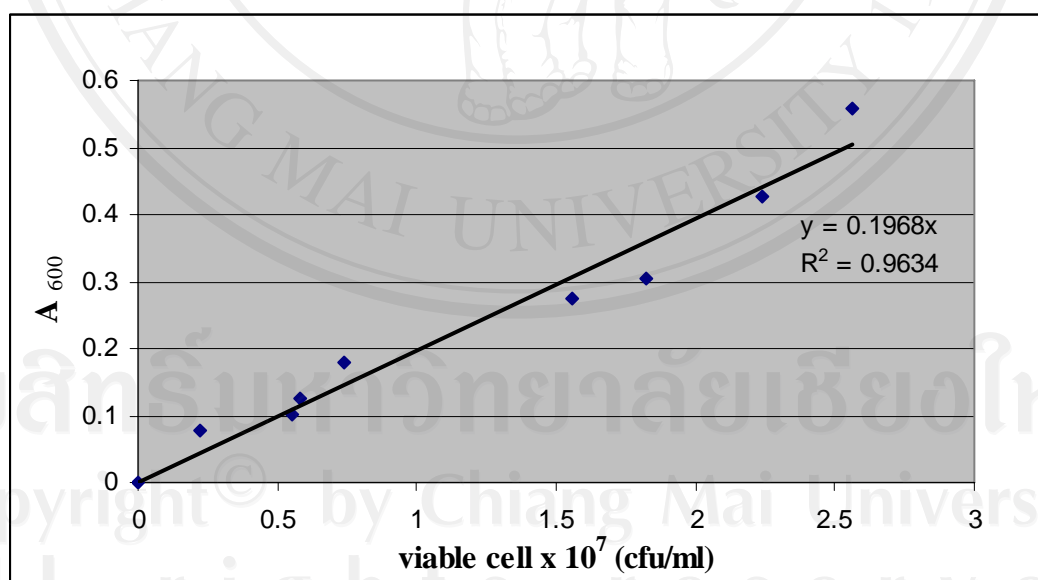


Figure 25 Standard curve of viable cells (*L. fermentum* 2311M) at OD₆₀₀ after incubated at 37 °C for 24 h

APPENDIX D

Data of statistical analysis

1. Alginate beads

1.1 Encapsulated cells and free cells at pH 6.5 for 3 h. (0, 1, and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	5	0.00159	3.189E-04	0.41	0.8299
REF (B)					
A*B	12	0.00923	7.693E-04		
TOTAL	17	0.01083			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	MEAN	HOMOGENEOUS GROUPS
E0	9.4170	I
E1	9.4120	I
E3	9.4107	I
F0	9.4030	I
F1	9.3947	I
F3	9.3910	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL T VALUE 2.179 REJECTION LEVEL 0.050

CRITICAL VALUE FOR COMPARISON 0.0493

STANDARD ERROR FOR COMPARISON 0.0226

ERROR TERM USED: TRT*REF, 12 DF

1.2 Encapsulated cells and free cells at pH 1.5 for 3 h. (0, 1 and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	5	84.9192	16.9838	2848.05	0.0000
REF (B)					
A*B	12	0.07156	0.00596		
TOTAL	17	84.9908			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	HOMOGENEOUS	
	MEAN	GROUPS
E0	9.3243	I
F0	9.2007	I
E1	5.5910	.. I
E3	5.2197 I
F1	4.8110 I
F3	3.6810 I

THERE ARE 5 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL T VALUE 2.179 REJECTION LEVEL 0.050
 CRITICAL VALUE FOR COMPARISON 0.1374
 STANDARD ERROR FOR COMPARISON 0.0631

ERROR TERM USED: TRT*REF, 12 DF

1.3 Encapsulated cells and free cells at bile salt 0% for 3 h. (0, 0.5, 1, 1.5, 2, 2.5 and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	13	0.01538	0.00118	0.35	0.9754
REF (B)					
A*B	28	0.09485	0.00339		
TOTAL	41	0.11023			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	HOMOGENEOUS GROUPS	
	MEAN	
F0	9.1771	I
E0	9.1748	I
E0.5	9.1633	I
F0.5	9.1605	I
E1.5	9.1469	I
E1	9.1446	I
F1	9.1422	I
F1.5	9.1398	I
E2.5	9.1378	I
E2	9.1368	I
F2	9.1287	I
E3	9.1282	I
F2.5	9.1179	I
F3	9.1100	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL T VALUE 2.048 REJECTION LEVEL 0.050

CRITICAL VALUE FOR COMPARISON 0.0973

STANDARD ERROR FOR COMPARISON 0.0475

ERROR TERM USED: TRT*REF, 28 DF

1.4 Encapsulated cells and free cells at bile salt 0.15% for 3 h. (0, 0.5, 1, 1.5, 2, 2.5 and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	13	98.7540	7.59646	1197.83	0.0000
REF (B)					
A*B	28	0.17757	0.00634		
TOTAL	41	98.9316			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	HOMOGENEOUS	
	MEAN	GROUPS
F0	9.1470	I
E0	9.1413	II
E0.5	9.0137	.. II
E1	8.9193 II
E1.5	8.8097 II
E2	8.7227 I
F0.5	8.6813 I
E2.5	8.3683 I
E3	8.1647 I
F1	7.6513 I
F1.5	6.4637 I
F2	5.8230 I
F2.5	5.3743 I
F3	4.3507 I

THERE ARE 12 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL T VALUE 2.048 REJECTION LEVEL 0.050
 CRITICAL VALUE FOR COMPARISON 0.1332
 STANDARD ERROR FOR COMPARISON 0.0650

ERROR TERM USED: TRT*REF, 28 DF

1.5 Encapsulated cells and free cells at bile salt 0.3% for 3 h. (0, 0.5, 1, 1.5, 2, 2.5 and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	13	321.399	24.7230	4022.01	0.0000
REF (B)					
A*B	28	0.17211	0.00615		
TOTAL	41	321.571			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	HOMOGENEOUS	
	MEAN	GROUPS
E0	9.1233	I
F0	8.7577	.. I
E0.5	8.3000 I
F0.5	8.2163 I
E1	6.8983 I
E1.5	6.4720 I
E2	6.3517 I
F1	6.3513 I
E2.5	5.6093 I
E3	5.5040 I
F1.5	5.0013 I
F2	3.5587 I
F2.5	0.0000 I
F3	0.0000 I

THERE ARE 9 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL T VALUE 2.048 REJECTION LEVEL 0.050

CRITICAL VALUE FOR COMPARISON 0.1311

STANDARD ERROR FOR COMPARISON 0.0640

ERROR TERM USED: TRT*REF, 28 DF

2. κ -carrageenan beads

2.1 Encapsulated cells and free cells at pH 6.5 for 3 h. (0, 1, and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	5	0.00263	5.258E-04	0.21	0.9525
REF (B)					
A*B	12	0.03026	0.00252		
TOTAL	17	0.03289			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	MEAN	HOMOGENEOUS GROUPS
E0	9.2369	I
F0	9.2229	I
E1	9.2214	I
E3	9.2178	I
F1	9.2107	I
F3	9.1972	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL T VALUE 2.179 REJECTION LEVEL 0.050
 CRITICAL VALUE FOR COMPARISON 0.0893
 STANDARD ERROR FOR COMPARISON 0.0410

ERROR TERM USED: TRT*REF, 12 DF

2.2 Encapsulated cells and free cells at pH 1.5 for 3 h. (0, 1 and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	5	81.5307	16.3061	4117.94	0.0000
REF (B)					
A*B	12	0.04752	0.00396		
TOTAL	17	81.5782			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	HOMOGENEOUS	
	MEAN	GROUPS
E0	9.0797	I
F0	9.0707	I
E1	5.5463	.. I
E3	5.1440 I
F1	4.7587 I
F3	3.5497 I

THERE ARE 5 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL T VALUE 2.179 REJECTION LEVEL 0.050
 CRITICAL VALUE FOR COMPARISON 0.1119
 STANDARD ERROR FOR COMPARISON 0.0514

ERROR TERM USED: TRT*REF, 12 DF

2.3 Encapsulated cells and free cells at bile salt 0% for 3 h. (0, 0.5, 1, 1.5, 2, 2.5 and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	13	0.01477	0.00114	0.31	0.9858
REF (B)					
A*B	28	0.10369	0.00370		
TOTAL	41	0.11846			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	HOMOGENEOUS	
	MEAN	GROUPS
E0	9.2493	I
E0.5	9.2300	I
F0	9.2296	I
E1	9.2291	I
F0.5	9.2194	I
E1.5	9.2143	I
F1	9.2086	I
E2.5	9.2073	I
E2	9.2043	I
F1.5	9.1996	I
F2	9.1977	I
E3	9.1975	I
F3	9.1828	I
F2.5	9.1795	I

THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL T VALUE 2.048 REJECTION LEVEL 0.050

CRITICAL VALUE FOR COMPARISON 0.1018

STANDARD ERROR FOR COMPARISON 0.0497

ERROR TERM USED: TRT*REF, 28 DF

2.4 Encapsulated cells and free cells at bile salt 0.15% for 3 h. (0, 0.5, 1, 1.5, 2, 2.5 and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	13	93.0383	7.15679	2130.56	0.0000
REF (B)					
A*B	28	0.09406	0.00336		
TOTAL	41	93.1323			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	MEAN	HOMOGENEOUS GROUPS
E0	9.1493	I
F0	8.9857	.. I
E0.5	8.6180 I
F0.5	8.5370 I
E1	8.0783 I
F1	7.5327 I
E1.5	7.3720 I
E2	6.9737 I
E2.5	6.5667 I
E3	6.1927 I
F1.5	6.1787 I
F2	5.7377 I
F2.5	5.0513 I
F3	3.9923 I

THERE ARE 12 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL T VALUE 2.048 REJECTION LEVEL 0.050
 CRITICAL VALUE FOR COMPARISON 0.0969
 STANDARD ERROR FOR COMPARISON 0.0473

ERROR TERM USED: TRT*REF, 28 DF

2.5 Encapsulated cells and free cells at bile salt 0.3% for 3 h. (0, 0.5, 1, 1.5, 2, 2.5 and 3)

ANALYSIS OF VARIANCE TABLE FOR LOG

SOURCE	DF	SS	MS	F	P
TRT (A)	13	318.432	24.4947	7594.26	0.0000
REF (B)					
A*B	28	0.09031	0.00323		
TOTAL	41	318.522			

LSD (T) COMPARISON OF MEANS OF LOG BY TRT

TRT	HOMOGENEOUS	
	MEAN	GROUPS
E0	8.8437	I
F0	8.6683	.. I
E0.5	8.5123 I
F0.5	8.0557 I
E1	6.9110 I
F1	6.2363 I
E1.5	6.1387 I
E2	5.0353 I
E2.5	4.8920 I
F1.5	4.8920 I
E3	4.4583 I
F2	3.5283 I
F2.5	0.0000 I
F3	0.0000 I

THERE ARE 12 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL T VALUE 2.048 REJECTION LEVEL 0.050

CRITICAL VALUE FOR COMPARISON 0.0950

STANDARD ERROR FOR COMPARISON 0.0464

ERROR TERM USED: TRT*REF, 28 DF

APPENDIX E

1. Alginate beads

1.1 Initial cells for encapsulation = 2.00×10^{10} cfu/ml

1.2 NaCl + Cells = 2.23×10^{10} cfu/ml

1.3 Alginate+ Cells = 2.32×10^{10} cfu/ml

1.4 Cells of alginate beads/ 1 g of alginate bead

= 2.60×10^8 cfu/1 ml of phosphate buffer

*Phosphate buffer 9 ml: bead 1 g

* Bead 0.120 g, phosphate buffer = 1.080 ml \longrightarrow ($2.60 \times 10^8 \times 1.080$)
= 2.80×10^8 cfu/1.080 ml of phosphate buffer

* Phosphate buffer 1.080 ml = Bead 0.120 g
= 2.80×10^8 cfu/0.120 g

* Total of alginate bead from process = 5.130 g

= $\frac{2.80 \times 10^8 \times 5.130}{0.120}$ cfu/5.130 g

Cells of alginate beads = 1.19×10^{10} cfu/5.130 g

Total of beads in process

Cells of alginate beads
1 g of alginate bead = 2.31×10^9 cfu/ g

1.5 Cells leak in CaCl₂ solution = 1.81×10^7 cfu/1 ml of CaCl₂

* Total of CaCl₂ solution = 50 ml \longrightarrow ($1.81 \times 10^7 \times 50$)

$$= 9.05 \times 10^8 \text{ cfu/50 ml of CaCl}_2$$

* Total of initial cells in process = 2 ml

$$= 9.05 \times 10^8 \text{ cfu/50 ml of CaCl}_2$$

$$= 9.05 \times 10^8 \text{ cfu/2 ml of cells}$$

Cells leak in CaCl₂ solution = 4.52×10^8 cfu/1 ml of cell

* Weight of alginate beads leak in container = 1.250 g

* Bead 1.00 g \longrightarrow 2.31×10^9 cfu

$$\text{Bead 1.250 g} \longrightarrow \frac{2.31 \times 10^9 \times 1.250}{1} = 2.89 \times 10^9 \text{ cfu}$$

* Cells of alginate beads leak in container = 2.89×10^9 cfu/1.250 g

Total of beads leak in container

* Cells of alginate beads in process + Cells of alginate beads leak in container

Total of beads in process + Total of bead leak in container

$$= 1.19 \times 10^{10} \text{ cfu/5.130 g} + 2.89 \times 10^9 \text{ cfu/1.250 g}$$

$$= 1.48 \times 10^{10} \text{ cfu/6.380 g}$$

Total of cells of alginate bead = 1.48×10^{10} cfu/6.380 g

Total of alginate bead

* Total of initial cells in process = 2 ml

$$= 1.48 \times 10^{10} \text{ cfu/6.380 g}$$

$$= 1.48 \times 10^{10} \text{ cfu/2 ml of cells}$$

* **1.48×10^{10} cfu** \longrightarrow **cell 2 ml**

1.6 Cells of alginate beads leak in container

$$* 1.48 \times 10^{10} \text{ cfu} \longrightarrow \text{cell 2 ml}$$

$$2.89 \times 10^9 \text{ cfu} \longrightarrow \frac{2 \times 2.89 \times 10^9}{1.48 \times 10^{10}} = 0.39 \text{ ml}$$

$$* 0.39 \text{ ml} \longrightarrow 2.89 \times 10^9 \text{ cfu}$$

$$1 \text{ ml} \longrightarrow \frac{2.89 \times 10^9 \times 1}{0.39} = 7.18 \times 10^9 \text{ cfu}$$

$$\text{Cells of alginate beads leak in container} = 7.18 \times 10^9 \text{ cfu/1 ml of cell}$$

1.7 Cells of alginate beads in process

$$* 1.48 \times 10^{10} \text{ cfu} \longrightarrow \text{cell 2 ml}$$

$$1.19 \times 10^{10} \text{ cfu} \longrightarrow \frac{2 \times 1.19 \times 10^{10}}{1.48 \times 10^{10}} = 1.61 \text{ ml}$$

$$* 1.61 \text{ ml} \longrightarrow 1.19 \times 10^{10} \text{ cfu}$$

$$1 \text{ ml} \longrightarrow \frac{1.19 \times 10^{10} \times 1}{1.61} = 7.39 \times 10^9 \text{ cfu}$$

$$\text{Cells of alginate beads in process} = 7.39 \times 10^9 \text{ cfu/ 1 ml of cell}$$

$$\begin{aligned} \text{1.8 Initial cells for encapsulation} &= \text{Cells in Alginate beads} + \text{Cells in alginate} \\ &\quad \text{beads leak in container} + \text{Cells leak in} \\ &\quad \text{CaCl}_2 \text{ solution} \end{aligned}$$

$$2.00 \times 10^{10} \text{ cfu/ ml} = 7.39 \times 10^9 + 7.18 \times 10^9 + 4.52 \times 10^8 \text{ cfu/ ml}$$

$$2.00 \times 10^{10} \text{ cfu/ ml} = 1.50 \times 10^{10} \text{ cfu/}$$

2. κ -carrageenan beads

2.1 Initial cells for encapsulation = 2.00×10^{10} cfu/ml

2.2 NaCl + Cells = 2.14×10^{10} cfu/ml

2.3 κ -carrageenan + Cells = 2.08×10^{10} cfu/ml

2.4 Cells of κ -carrageenan beads/ 1 g of κ -carrageenan bead

= 2.02×10^8 cfu/1 ml of 0.9% NaCl

* 0.9% NaCl 9 ml: bead 1 g

* Bead 0.1624 g, 0.9% NaCl = 1.460 ml \longrightarrow ($2.02 \times 10^8 \times 1.460$)

= 2.94×10^8 cfu/1.460 ml of 0.9% NaCl

* 0.9% NaCl 1.460 ml = Bead 0.1624 g

= 2.94×10^8 cfu/0.1624 g

* Total of κ -carrageenan bead from process = 6.050 g

= $\frac{2.94 \times 10^8 \times 6.050}{0.1624}$ cfu/6.050 g

Cells of κ -carrageenan beads = 1.09×10^{10} cfu/6.050 g

Total of beads in process

Cells of κ -carrageenan beads = 1.80×10^9 cfu/ g

1 g of κ -carrageenan bead

2.5 Cells leak in KCl solution = 1.38×10^7 cfu/1 ml of KCl

* Total of KCl solution = 50 ml \longrightarrow $(1.38 \times 10^7 \times 50)$

$$= 6.90 \times 10^8 \text{ cfu/50 ml of KCl}$$

* Total of initial cells in process = 2 ml

$$= 6.90 \times 10^8 \text{ cfu/50 ml of KCl}$$

$$= 6.90 \times 10^8 \text{ cfu/2 ml of cells}$$

Cells leak in KCl solution = 3.45×10^8 cfu/1 ml of cell

* Weight of κ -carrageenan beads leak in container = 0.500 g

* Bead 1.00 g \longrightarrow 1.80×10^9 cfu

$$\text{Bead 0.500 g} \longrightarrow \frac{1.80 \times 10^9 \times 0.500}{1} = 9.00 \times 10^8 \text{ cfu}$$

* Cells of κ -carrageenan beads leak in container = 9.00×10^8 cfu/0.500 g

Total of beads leak in container

* Cells of κ -carrageenan beads in process + Cells of κ -carrageenan beads leak in container

Total of beads in process Total of bead leak in container

$$= 1.09 \times 10^{10} \text{ cfu/6.050 g} + 9.00 \times 10^8 \text{ cfu/0.500 g}$$

$$= 1.18 \times 10^{10} \text{ cfu/6.550 g}$$

Total of cells of κ -carrageenan bead = 1.18×10^{10} cfu/6.550 g

Total of κ -carrageenan bead

$$\begin{aligned}
 * \text{ Total of initial cells in process} &= 2 \text{ ml} \\
 &= 1.18 \times 10^{10} \text{ cfu} / 6.550 \text{ g} \\
 &= 1.18 \times 10^{10} \text{ cfu} / 2 \text{ ml of cells} \\
 * 1.18 \times 10^{10} \text{ cfu} &\longrightarrow \text{cell 2 ml}
 \end{aligned}$$

2.6 Cells of κ -carrageenan beads leak in container

$$\begin{aligned}
 * 1.18 \times 10^{10} \text{ cfu} &\longrightarrow \text{cell 2 ml} \\
 9.00 \times 10^8 \text{ cfu} &\longrightarrow \frac{2 \times 9.00 \times 10^8}{1.18 \times 10^{10}} = 0.15 \text{ ml} \\
 * 0.15 \text{ ml} &\longrightarrow 9.00 \times 10^8 \text{ cfu} \\
 1 \text{ ml} &\longrightarrow \frac{9.0 \times 10^8 \times 1}{0.15} = 6.00 \times 10^9 \text{ cfu}
 \end{aligned}$$

Cells of κ -carrageenan beads leak in container = 6.00×10^9 cfu/1 ml of cell

2.7 Cells of κ -carrageenan beads in process

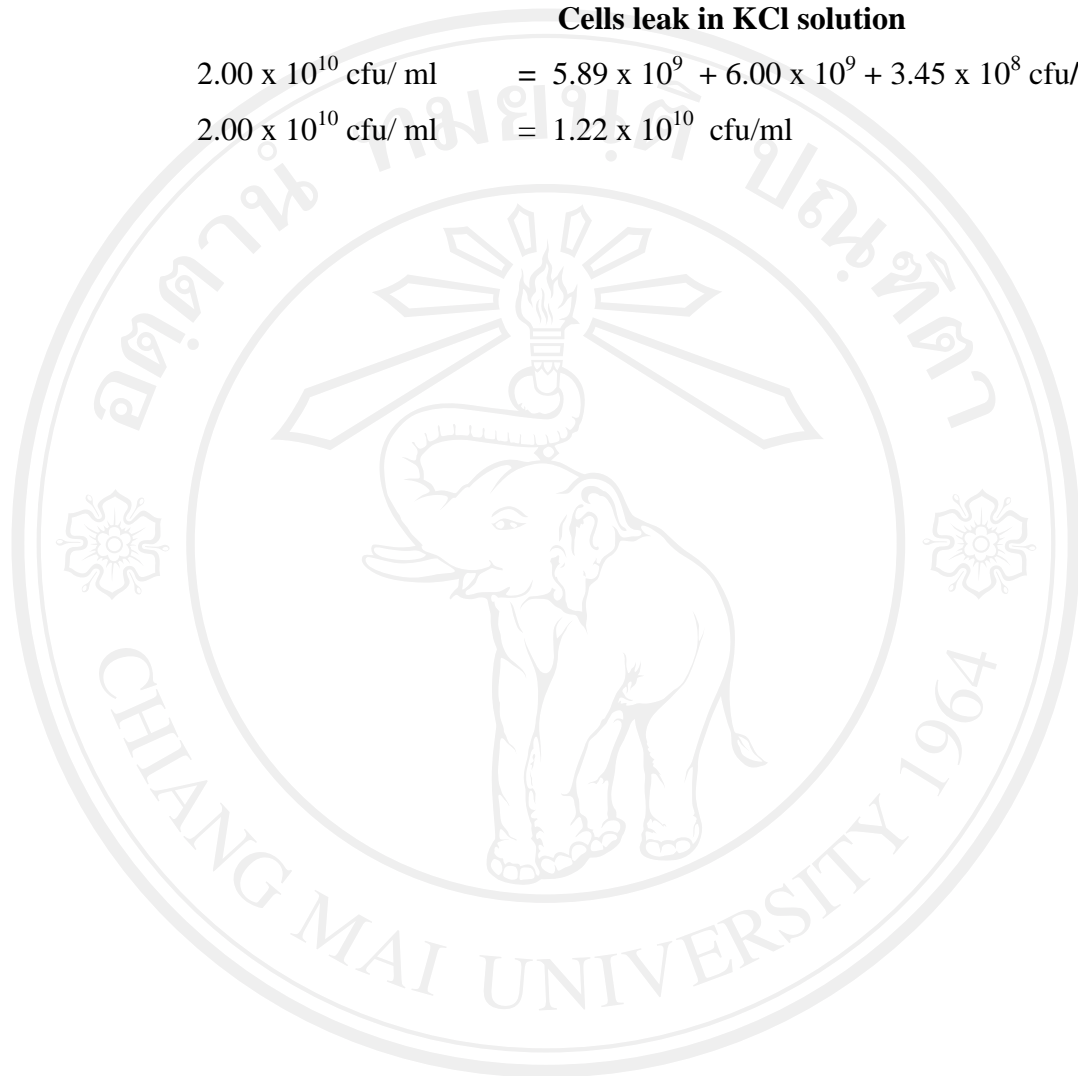
$$\begin{aligned}
 * 1.18 \times 10^{10} \text{ cfu} &\longrightarrow \text{cell 2 ml} \\
 1.09 \times 10^{10} \text{ cfu} &\longrightarrow \frac{2 \times 1.09 \times 10^{10}}{1.18 \times 10^{10}} = 1.85 \text{ ml} \\
 * 1.85 \text{ ml} &\longrightarrow 1.09 \times 10^{10} \text{ cfu} \\
 1 \text{ ml} &\longrightarrow \frac{1.09 \times 10^{10} \times 1}{1.85} = 5.89 \times 10^9 \text{ cfu}
 \end{aligned}$$

Cells of κ -carrageenan beads in process = 5.89×10^9 cfu/ 1 ml of cell

2.8 Initial cells for encapsulation = Cells of κ -carrageenan beads + Cells of κ -carrageenan beads leak in container + Cells leak in KCl solution

$$2.00 \times 10^{10} \text{ cfu/ml} = 5.89 \times 10^9 + 6.00 \times 10^9 + 3.45 \times 10^8 \text{ cfu/ml}$$

$$2.00 \times 10^{10} \text{ cfu/ml} = 1.22 \times 10^{10} \text{ cfu/ml}$$



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