MODELLING DAIRY BIOFILMS OF TARGETED CONTROL OF THERMOPHILIC BACTERIA

Additional Appendices

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Table of Appendices

Appendix 1 Culture Management System Used

1. Culture Management System Currently Using

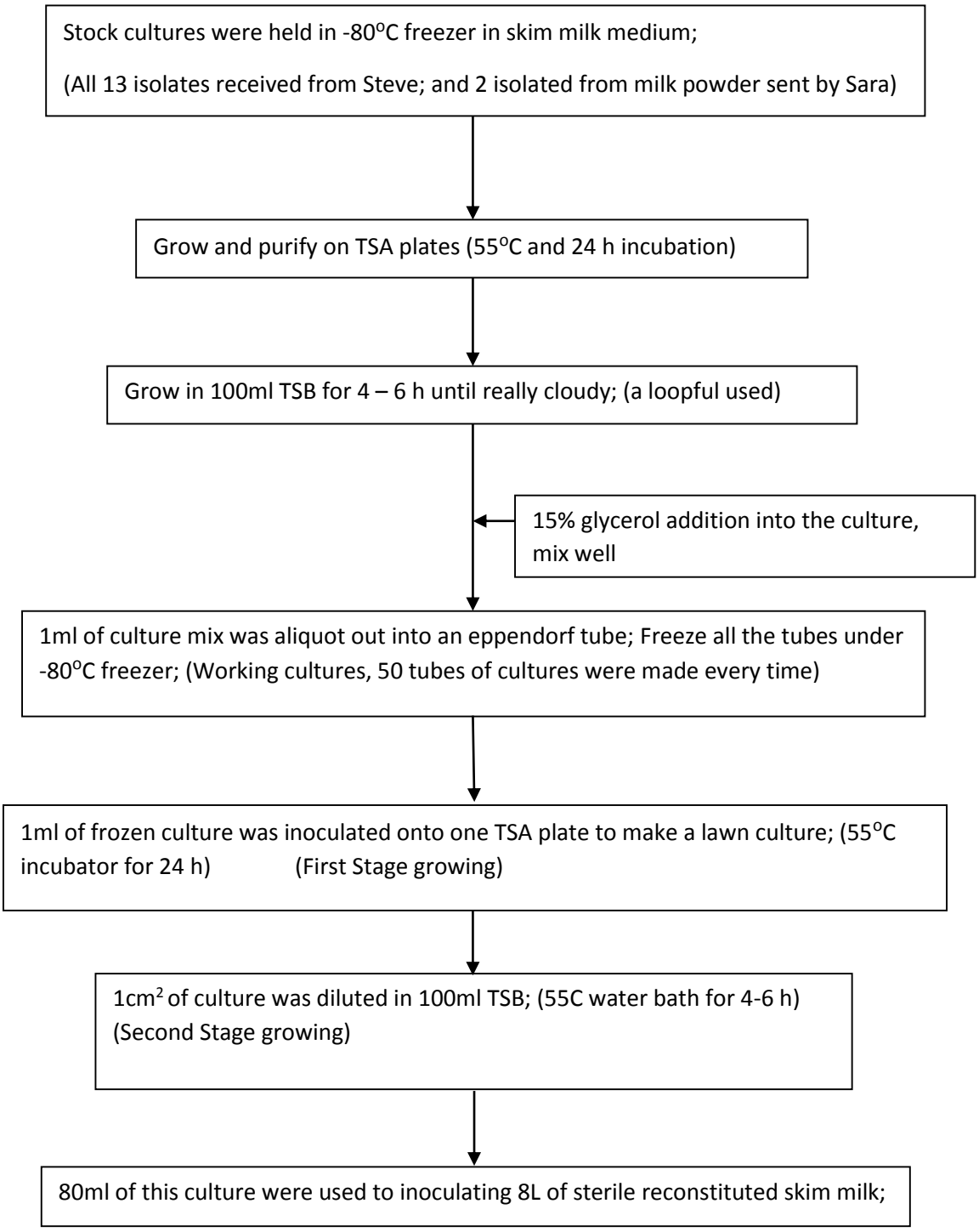

Figure 1.1: Flow Chart of the Current Culture Management Procedures;

2. Definitions:

Museum Culture: These cultures referred to as the culture received at the start of the project. They might be pure or mixed. They should be preserved immediately after receiving them. They should be stored at a condition aiming long term storage, such as – 80^{o}C freezing on level 6 of WS. A separate set of stock cultures should be purified from these cultures. (What I do is I freeze both the mixed and pure culture, in case you might purify the wrong bacteria. Keep the original culture as untouched as much as possible. Make subcultures of them when you received them. Freeze some of the subcultures as well since the received amount might be quite small.)

Stock Culture: These cultures should be purified from the 'museum culture' set at the beginning of the project. They should be pure and ready to use. They should be kept in a condition aiming mid-long term storage, i.e. freezing at -20^{o}C on level 2 of WN. Sufficient amount of stock culture should be made up, such as 50 vials. The purity of the bacteria should be double checked before making up a big batch of subcultures or stock cultures. They should be stored in small aliquots or quantities as a large batch, for example, 50 vials of 1ml aliquots. Therefore, if any contamination occurs, the small aliquot of stock culture can be thrown out without interfering with the project.

Working Culture: These cultures should be consistent with the stock cultures. They should be subcultured with stock cultures. They can be kept in broth or plates in the cold room or incubator just for daily use. They can be thrown away at any point if there are any concerns with them. Don't sub-culture too many times in sequence since genomic changes may occur within the bacteria? If you are concerning about this, just subculture from stock culture again.

3. Procedure

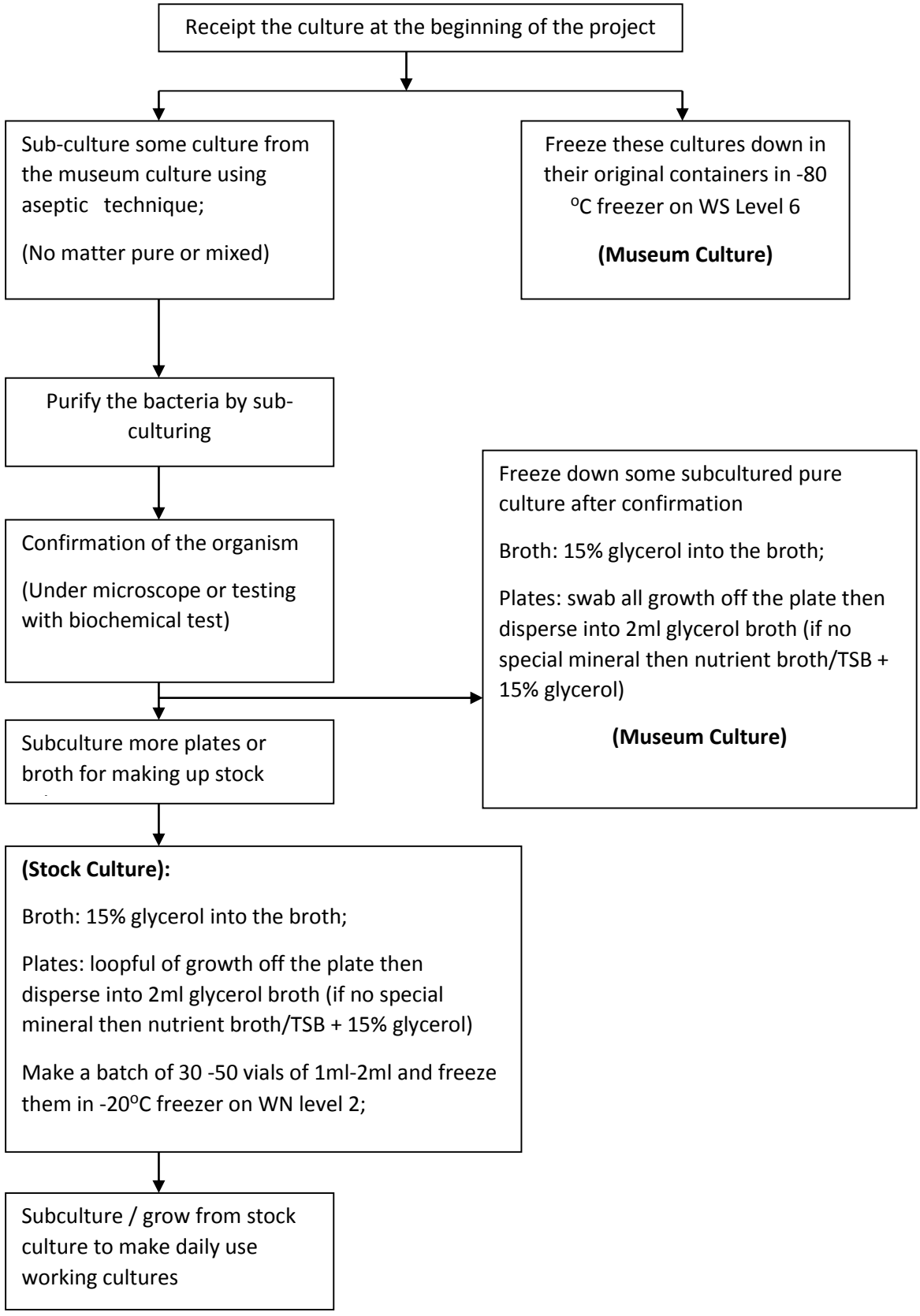

Figure 1.2: Culture Management Procedures;

Appendix 2 BacTrac Calibration curves for All 13 Massey Samples (TSB)

Sample 1: DSM 2641, Reference Strain, *Anoxybacillus flavithermus*			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 1 d0	6.56	4.56	6.16
Sample 1 d-1	5.93	3.25	5.16
Sample 1 d-2	7.52	4.78	4.16
Sample 1 d-3	6.32	**	3.16
Sample 1 d-4	8.63	6.25	2.16
Sample 1 d-5	8.2	6.62	1.16
Sample 1 d-6	11.4	**	0.16
Sample 1 d-7	9.64	**	-0.84
Sample 1D d0	8.44	4.95	5.20
Sample 1D d-1	6.77	4.46	4.20
Sample 1D d-2	6.35	4.5	3.20
Sample 1D d-3	**	**	2.20
Sample 1D d-4	8.89	7.05	1.20
Sample 1D d-5	10.24	**	0.20
Sample 1D d-6	7.38	8.63	-0.80
Sample 1D d-7	8.31	**	-1.80

Table 2.1: Sample 1 and duplicate 1D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

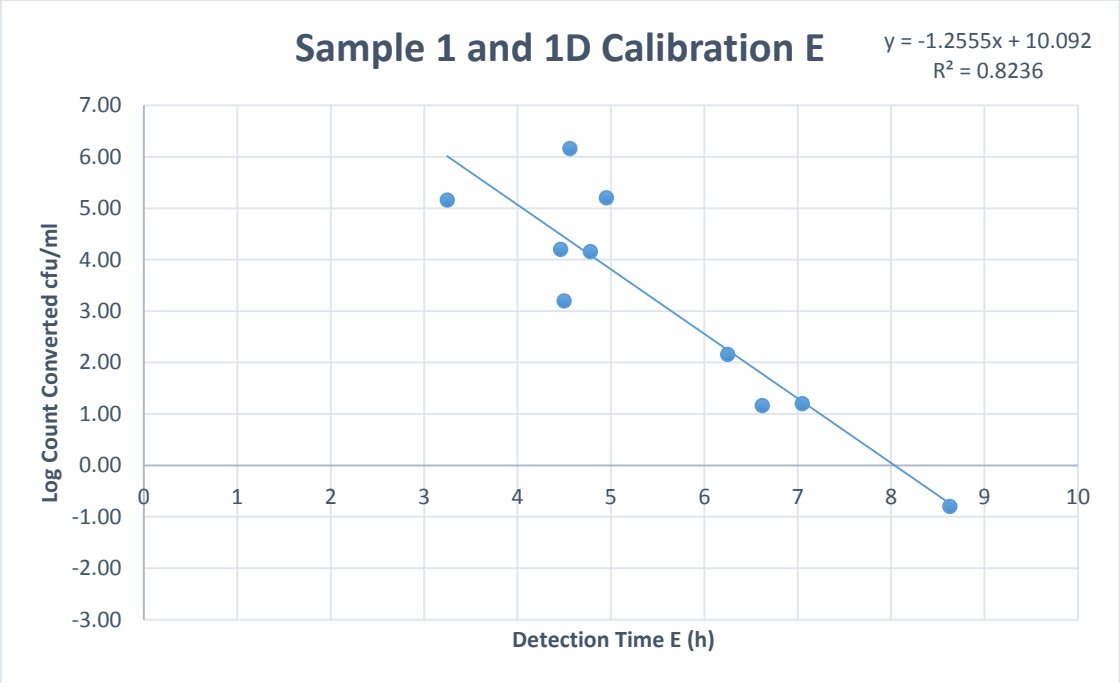

Figure 2.1: Sample 1 and duplicate 1D (DSM 2641) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 2: DSM 14791, Reference Strain, *Geobacillus thermolevorans*			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 2 d0	11.77	0.99	8.07
Sample 2 d-1	12.97	1.68	7.07
Sample 2 d-2	9.87	2.63	6.07
Sample 2 d-3	9.83	3.81	5.07
Sample 2 d-4	14.27	5.05	4.07
Sample 2 d-5	14.81	6.18	3.07
Sample 2 d-6	12	6.61	2.07
Sample 2 d-7	12.06	8.13	1.07
Sample 2D d0	9.99	0.71	8.08
Sample 2D d-1	13.74	2.16	7.08
Sample 2D d-2	13.94	2.42	6.08
Sample 2D d-3	12.21	4.35	5.08
Sample 2D d-4	12.94	4.7	4.08
Sample 2D d-5	13.61	5.69	3.08
Sample 2D d-6	0.4	9.22	2.08
Sample 2D d-7	2.95	7.33	1.08

Table 2.2: Sample 2 and duplicate 2D BacTrac calibration data under 55°C incubation using TSB as medium in the BacTrac cells;

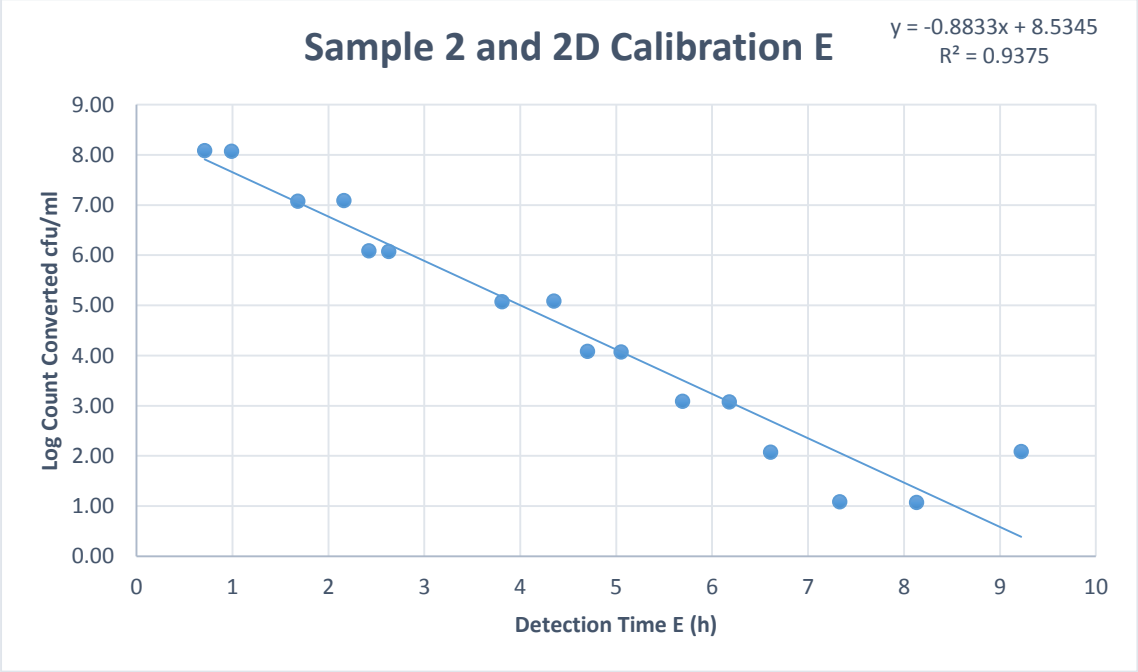

Figure 2.2: Sample 2 and duplicate 2D (DSM 14791) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 3: DSM 11667, Reference Strain, *Geobacillus thermoleovorans*			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 3 d0	8	0.52	7.12
Sample 3 d-1	7.79	5.34	6.12
Sample 3 d-2	9.25	4.49	5.12
Sample 3 d-3	8.93	**	4.12
Sample 3 d-4	13.31	8.55	3.12
Sample 3 d-5	12.19	10.96	2.12
Sample 3 d-6	11.07	12.38	1.12
Sample 3 d-7	1.41	**	0.12
Sample 3D d0	9.61	1.45	7.44
Sample 3D d-1	11.28	2.1	6.44
Sample 3D d-2	11.81	5.68	5.44
Sample 3D d-3	10.54	6.11	4.44
Sample 3D d-4	11.93	8.57	3.44
Sample 3D d-5	10.75	15.96	2.44
Sample 3D d-6	9.91	**	1.44
Sample 3D d-7	9.73	14.07	0.44

Table 2.3: Sample 3 and duplicate 3D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

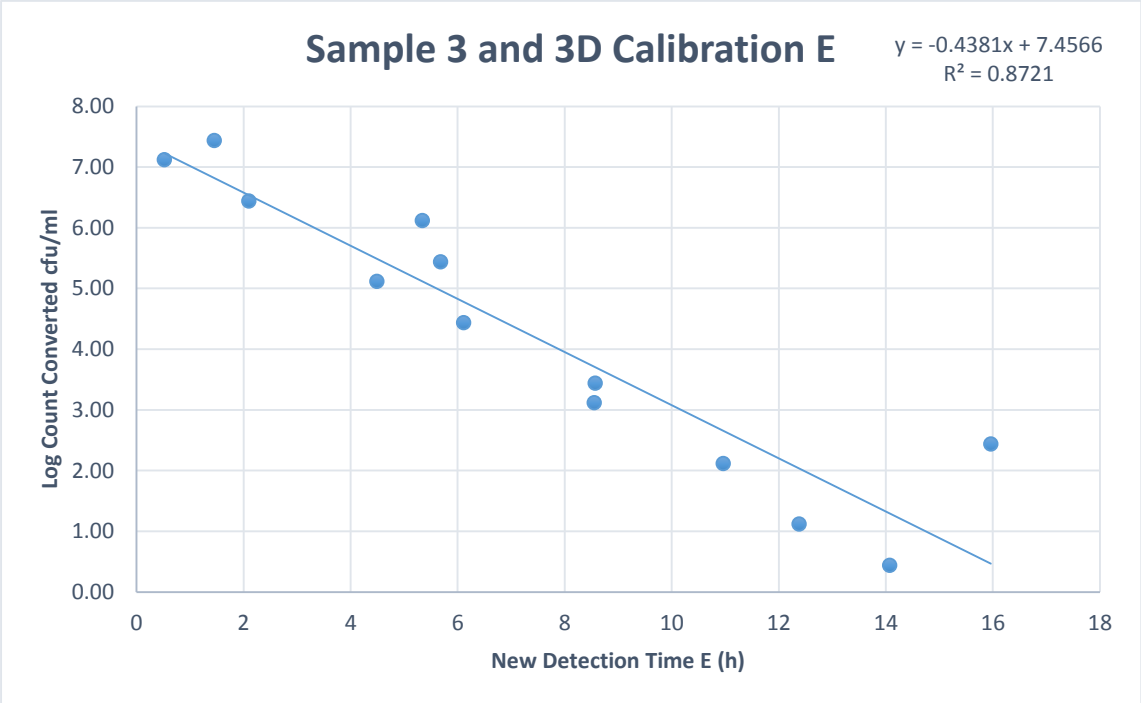

Figure 2.3: Sample 3 and duplicate 3D (DSM 11667) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 4: DSM 5934, Reference Strain, *Geobacillus stearothermophilus*			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 4 d0	1.75	0.32	8.14
Sample 4 d-1	3.66	1.29	7.14
Sample 4 d-2	4.39	2.59	6.14
Sample 4 d-3	2.01	3.96	5.14
Sample 4 d-4	6.56	4.6	4.14
Sample 4 d-5	7.66	6.11	3.14
Sample 4 d-6	9.23	7.74	2.14
Sample 4 d-7	9.58	9.53	1.14
Sample 4D d0	1.55	0.24	8.22
Sample 4D d-1	3.01	0.59	7.22
Sample 4D d-2	4.1	1.33	6.22
Sample 4D d-3	4.4	4.26	5.22
Sample 4D d-4	6.64	4.18	4.22
Sample 4D d-5	**	6.97	3.22
Sample 4D d-6	8.07	7.06	2.22
Sample 4D d-7	6.91	8.05	1.22

Table 2.4: Sample 4 and duplicate 4D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

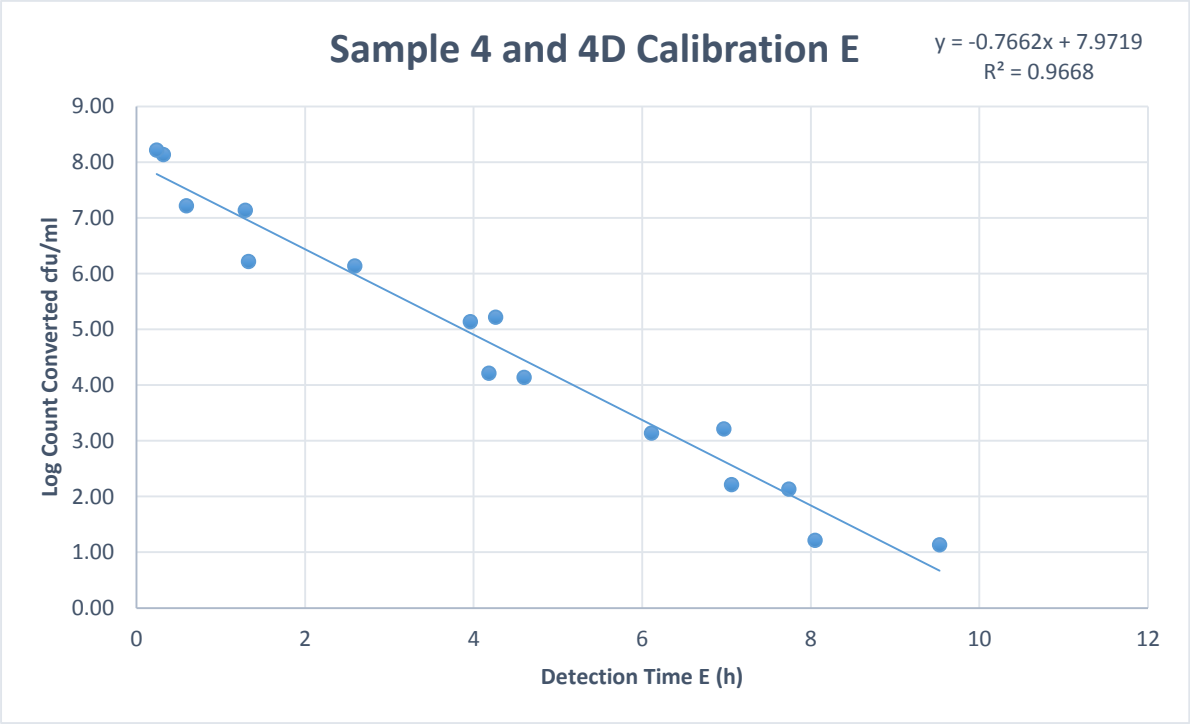

Figure 2.4: Sample 4 and duplicate 4D (DSM 5934) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 5: DSM 1550, Reference Strain, *Geobacillus stearothermophilus*		
INFO1	**Detection Time M (h)**	**Log Count Converted (cfu/ml)**
Sample 5 d0	**1.45**	**6.25**
Sample 5 d-1	**3.1**	**5.25**
Sample 5 d-2	**3.82**	**4.25**
Sample 5 d-3	**5.65**	**3.25**
Sample 5 d-4	**6.84**	**2.25**
Sample 5 d-5	**8.25**	**1.25**
Sample 5 d-6	**9.24**	**0.25**
Sample 5 d-7	**9.54**	**-0.75**
Sample 5 d-8	**9.28**	**-1.75**
Sample 5D d0	**1.94**	**7.39**
Sample 5D d-1	**3.31**	**6.39**
Sample 5D d-2	**3.54**	**5.39**
Sample 5D d-3	**4.54**	**4.39**
Sample 5D d-4	**4.22**	**3.39**
Sample 5D d-5	**7.58**	**2.39**
Sample 5D d-6	**7.45**	**1.39**
Sample 5D d-7	**9.96**	**0.39**
Sample 5D d-8	**12.27**	**-0.61**
Sample 5 d0	**1.33**	**7.28**
Sample 5 d-1	**3.7**	**6.28**
Sample 5 d-2	**4.44**	**5.28**
Sample 5 d-3	**5.22**	**4.28**
Sample 5 d-4	**6.16**	**3.28**
Sample 5 d-5	**7.24**	**2.28**
Sample 5 d-6	**8.41**	**1.28**
Sample 5 d-7	**8.64**	**0.28**
Sample 5 d-8	**11.86**	**-0.72**

Table 2.5: Sample 5 and duplicates 5D BacTrac calibration data under 55°C incubation using TSB as medium in the BacTrac cells;

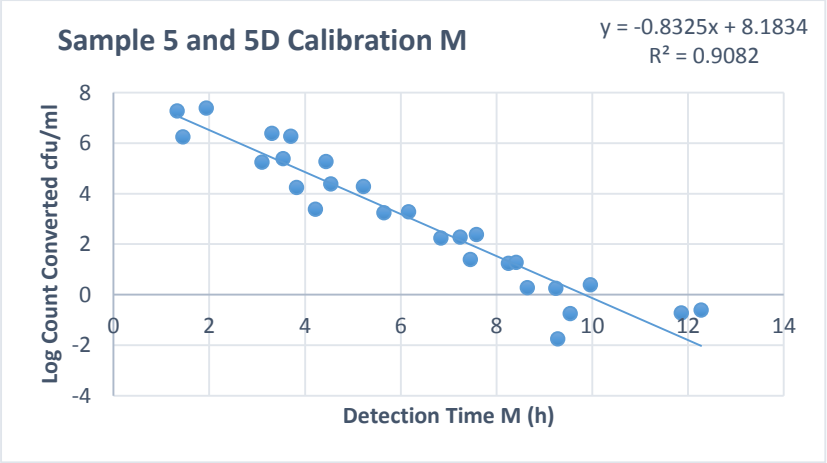

Figure 2.5: Sample 5 and duplicate 5D (DSM 1550) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 6: CBF 4, Wild Strain, *Anoxybacillus flavithermus*, Clandeboye, 2003			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 6 d0	2.16	0.51	7.37
Sample 6 d-1	2.73	0.81	6.37
Sample 6 d-2	4.27	2.17	5.37
Sample 6 d-3	5.52	2.93	4.37
Sample 6 d-4	5.84	3.59	3.37
Sample 6 d-5	6.75	4.57	2.37
Sample 6 d-6	7.29	5.21	1.37
Sample 6 d-7	8.62	7.3	0.37
Sample 6 d-8	10.32	**	-0.63
Sample 6D d0	3.16	1.36	6.74
Sample 6D d-1	3.64	1.3	5.74
Sample 6D d-2	4.26	2.23	4.74
Sample 6D d-3	5.11	2.92	3.74
Sample 6D d-4	5.87	4.02	2.74
Sample 6D d-5	6.6	4.81	1.74
Sample 6D d-6	6.63	5.3	0.74
Sample 6D d-7	7.45	**	-0.26
Sample 6D d-8	10.98	**	-1.26

Table 2.6: Sample 6 and duplicate 6D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

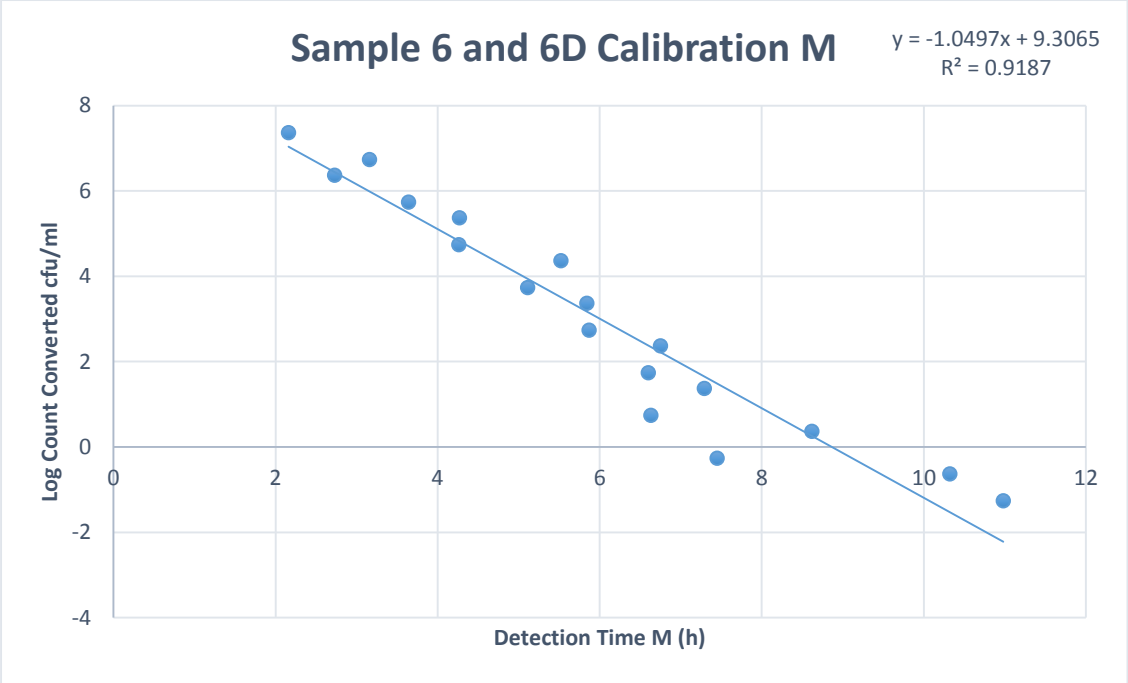

Figure 2.6: Sample 6 and duplicate 6D (CBF4) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 7: CBF 5, Wild Strain, *Anoxybacillus flavithermus*, Clandeboye, 2003			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 7 d0	3.13	0.24	6.34
Sample 7 d-1	3.17	0.75	5.34
Sample 7 d-2	3.82	1.59	4.34
Sample 7 d-3	5.1	2.63	3.34
Sample 7 d-4	5.84	3.67	2.34
Sample 7 d-5	6.2	4.24	1.34
Sample 7 d-6	7.33	5.29	0.34
Sample 7 d-7	7.87	**	-0.66
Sample 7 d0	3.25	0.67	7.92
Sample 7 d-1	4.71	1.27	6.92
Sample 7 d-2	4.99	2.28	5.92
Sample 7 d-3	5	2.91	4.92
Sample 7 d-4	6.05	3.86	3.92
Sample 7 d-5	6.81	4.59	2.92
Sample 7 d-6	7.81	1.25	1.92
Sample 7 d-7	8.13	**	0.92
Sample 7D d0	3.27	0.77	7.11
Sample 7D d-1	3.56	0.85	6.11
Sample 7D d-2	3.81	1.57	5.11
Sample 7D d-3	4.61	2.27	4.11
Sample 7D d-4	5.75	3.29	3.11
Sample 7D d-5	6.41	4.16	2.11
Sample 7D d-6	7.28	5	1.11
Sample 7D d-7	8.04	6.22	0.11
Sample 7D d0	3.47	1.05	7.96
Sample 7D d-1	3.16	0.63	6.96
Sample 7D d-2	4.99	2.13	5.96
Sample 7D d-3	5.85	3.34	4.96
Sample 7D d-4	5.28	3.26	3.96
Sample 7D d-5	12.81	**	2.96
Sample 7D d-6	6.51	4.6	1.96
Sample 7D d-7	9.43	7.15	0.96

Table 2.7: Sample 7 and duplicates 7D BacTrac calibration data under 55°C incubation using TSB as medium in the BacTrac cells; (means 'not detected', red number was an outlier, which was not drawn onto the graph below.)**

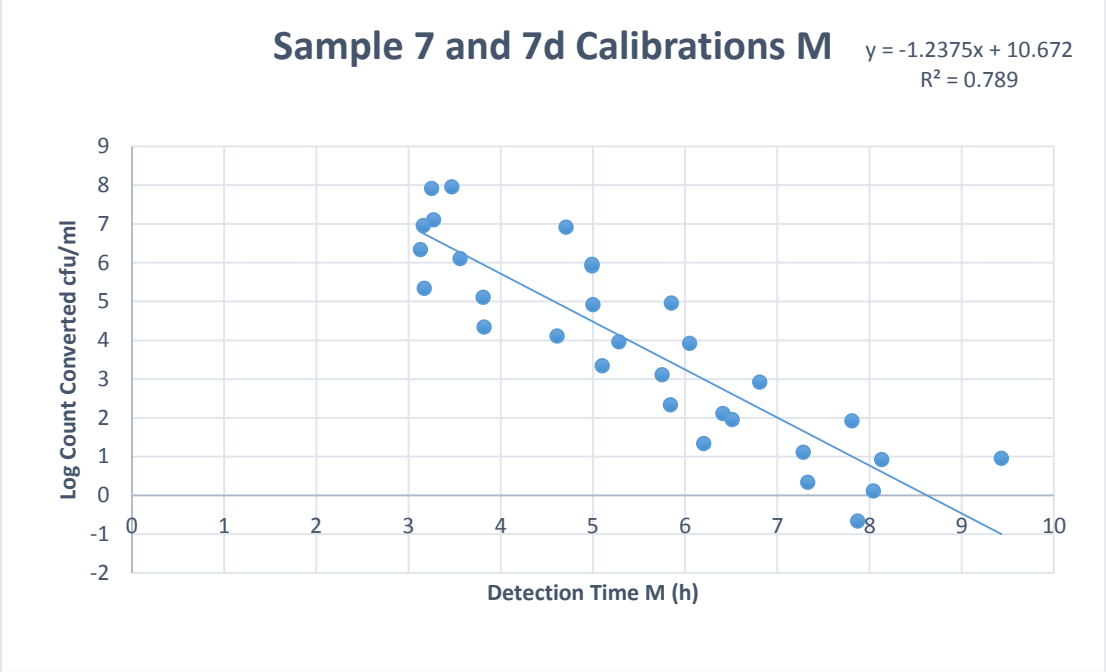

Figure 2.7: Sample 7 and duplicate 7D (CBF 5) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 8: TT 1, Wild Strain, *Geobacillus species*, Clandeboye, ditto			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 8 d0	4.08	0.33	8.06
Sample 8 d-1	4.24	1.81	7.06
Sample 8 d-2	4.63	4.01	6.06
Sample 8 d-3	5.62	4.46	5.06
Sample 8 d-4	7.2	5.84	4.06
Sample 8 d-5	2.12	6.84	3.06
Sample 8 d-6	9.55	8.85	2.06
Sample 8 d-7	10.19	**	1.06
Sample 8D d0	2.25	0.55	8.12
Sample 8D d-1	4.35	1.7	7.12
Sample 8D d-2	4.5	3.11	6.12
Sample 8D d-3	6.2	5.18	5.12
Sample 8D d-4	7.11	5.47	4.12
Sample 8D d-5	8.77	7.45	3.12
Sample 8D d-6	9.54	8.47	2.12
Sample 8D d-7	10.12	9.84	1.12

Table 2.8: Sample 8 and duplicate 8D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

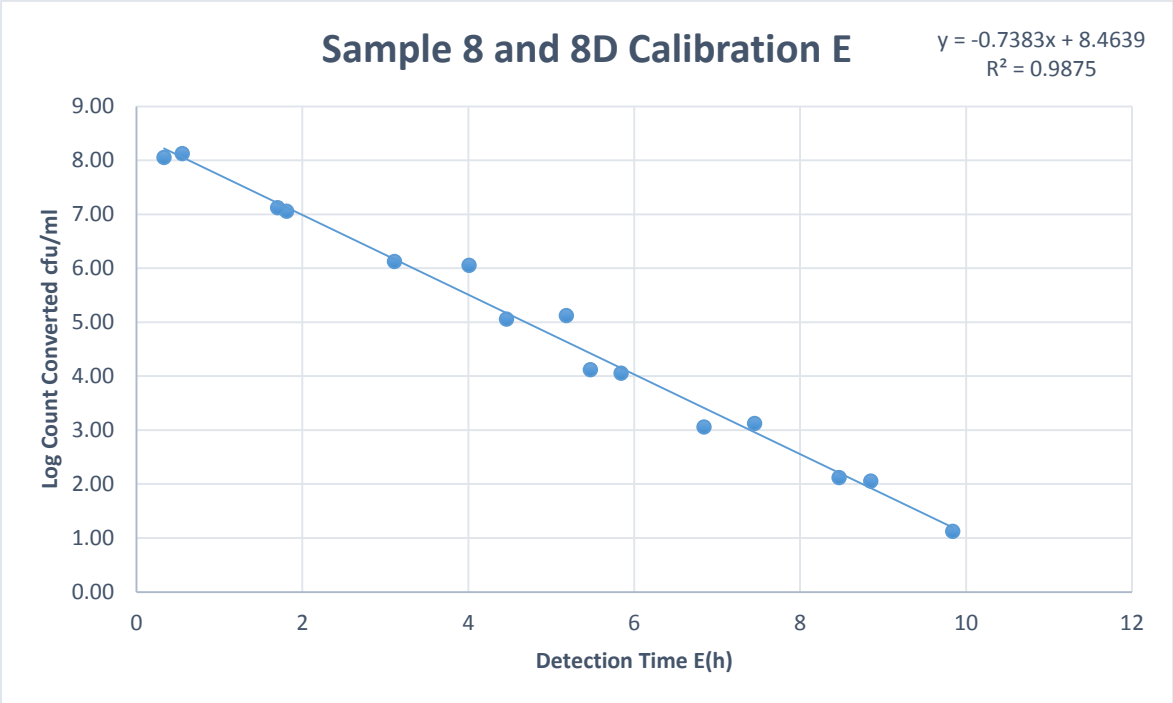

Figure 2.8: Sample 8 and duplicate 8D (TT1) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 9: TRGT 6, Wild Strain, *Geobacillus specie*, Te Rapa, powder, August 2008			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 9 d0	3.45	1.04	8.01
Sample 9 d-1	4.87	2.32	7.01
Sample 9 d-2	5.12	2.27	6.01
Sample 9 d-3	5.82	3.36	5.01
Sample 9 d-4	19.19	**	4.01
Sample 9 d-5	1.13	**	3.01
Sample 9 d-6	8.87	7.17	2.01
Sample 9D d0	3.77	0.87	8.08
Sample 9D d-1	1.5	1.48	7.08
Sample 9D d-2	7.91	2.25	6.08
Sample 9D d-3	8.2	3.21	5.08
Sample 9D d-4	7.2	5.38	4.08
Sample 9D d-5	7.96	5.74	3.08
Sample 9D d-6	9.65	8.55	2.08
Sample 9D d-7	9.92	8.64	1.08

Table 2.9: Sample 9 and duplicate 9D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

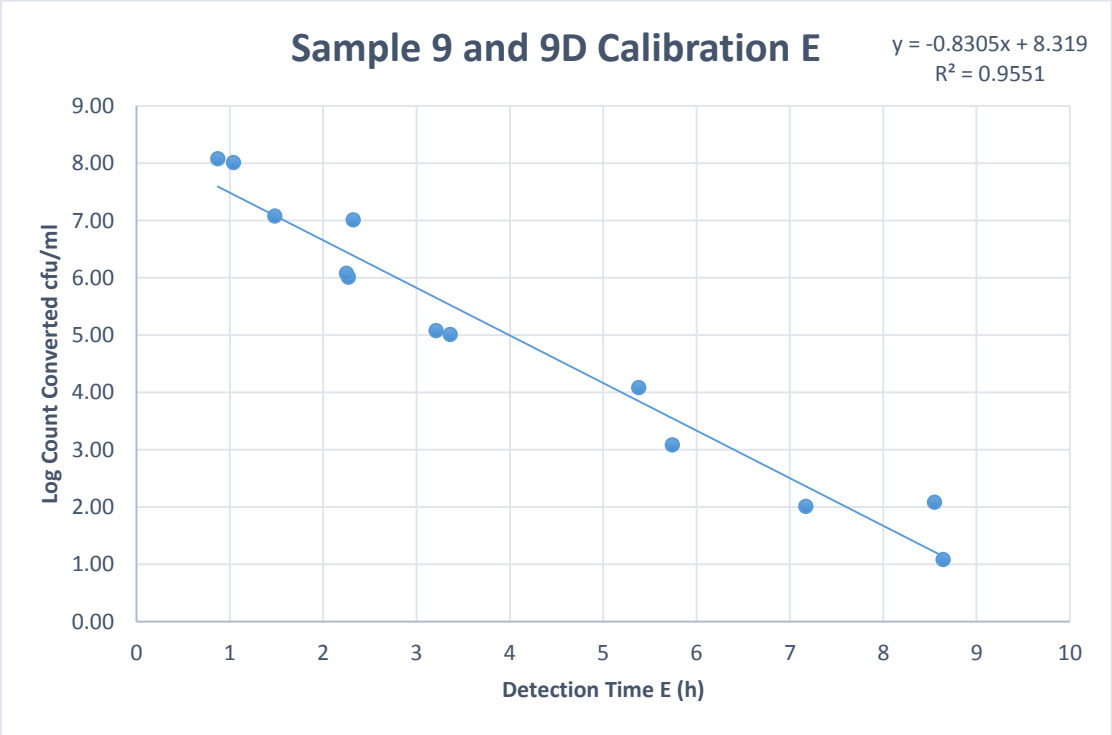

Figure 2.9: Sample 9 and duplicate 9D (TRGT 6) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 10: TRGT 7, Wild Strain, *Geobacillus species*, Te Rapa, powder, August 2008			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 10 d0	2.81	0.54	8.05
Sample 10 d-1	3.86	1.77	7.05
Sample 10 d-2	9.88	3.37	6.05
Sample 10 d-3	6.45	4.57	5.05
Sample 10 d-4	7.48	5.53	4.05
Sample 10 d-5	19.24	6.09	3.05
Sample 10 d-6	9.48	7.49	2.05
Sample 10 d-7	9.79	8.4	1.05
Sample 10D d0	4.73	1.19	7.73
Sample 10D d-1	4.65	1.9	6.73
Sample 10D d-2	5.36	3	5.73
Sample 10D d-3	6.3	4.17	4.73
Sample 10D d-4	7.23	5.3	3.73
Sample 10D d-5	8.6	6.75	2.73
Sample 10D d-6	9.2	7.67	1.73
Sample 10D d-7	10.71	9.69	0.73

Table 2.10: Sample 10 and duplicate 10D BacTrac calibration data under 55°C incubation using TSB as medium in the BacTrac cells;

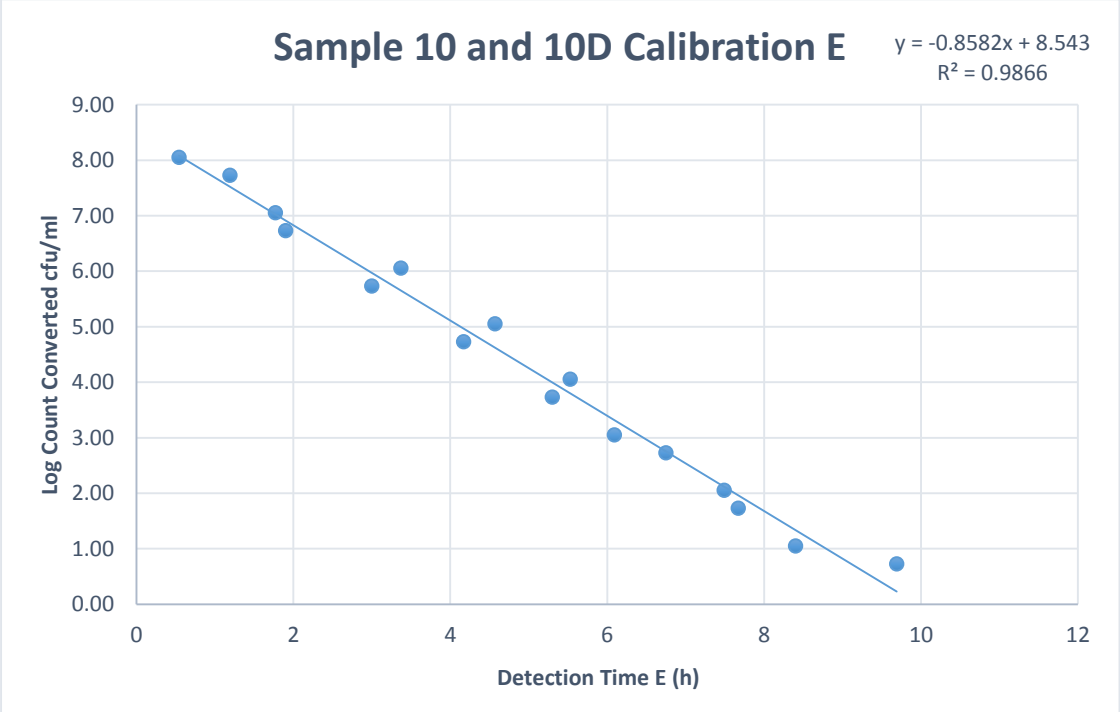

Figure 2.10: Sample 10 and duplicate 10D (TRGT 7) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 11: TRGT 8, Wild Strain, *Geobacillus species*, Te Rapa, powder, August 2008			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 11 d0	2.97	1.39	7.83
Sample 11 d-1	4.36	1.65	6.83
Sample 11 d-2	5.27	2.45	5.83
Sample 11 d-3	6.72	4.45	4.83
Sample 11 d-4	1.26	5.7	3.83
Sample 11 d-5	8.45	5.76	2.83
Sample 11 d-6	7.89	5.87	1.83
Sample 11 d-7	7.42	6.51	0.83
Sample 11D d0	3.5	1.09	7.86
Sample 11D d-1	4.32	1.95	6.86
Sample 11D d-2	5.31	2.86	5.86
Sample 11D d-3	0.82	**	4.86
Sample 11D d-4	7.14	5.1	3.86
Sample 11D d-5	8.13	6.26	2.86
Sample 11D d-6	9.05	7.5	1.86
Sample 11D d-7	9.69	8.52	0.86

Table 2.11: Sample 11 and duplicate 11D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

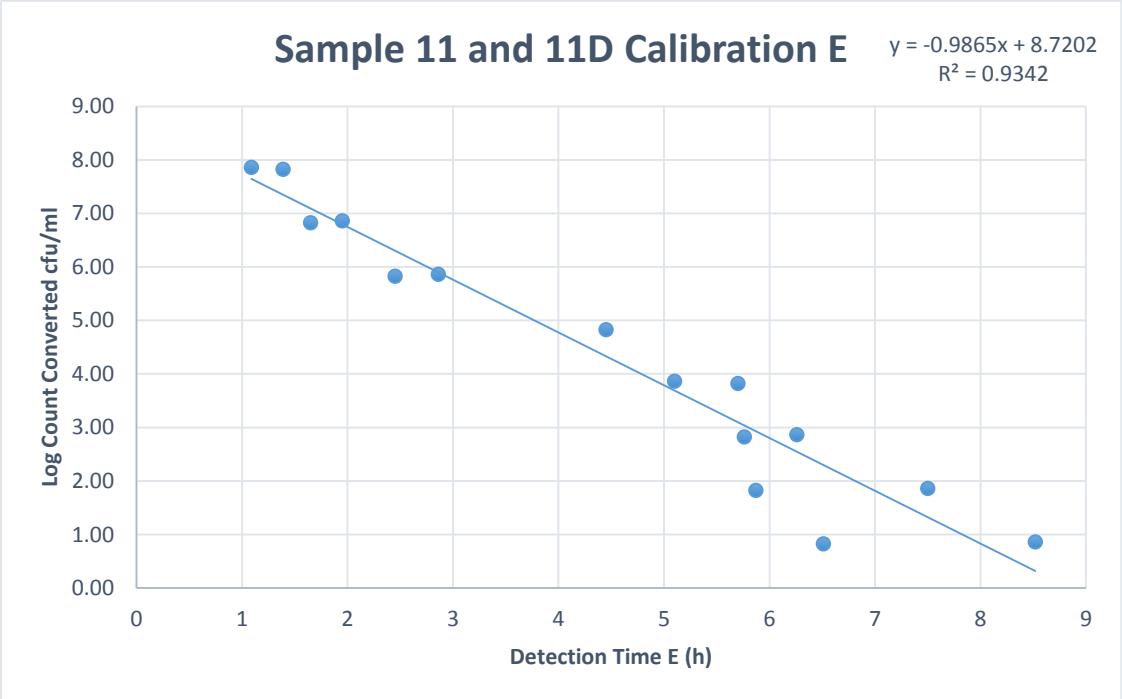

Figure 2.11: Sample 11 and duplicate 11D (TRGT8) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 12: TRGT 9, Wild Strain, *Geobacillus species*, Te Rapa, powder, August 2008			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 12 d0	2.39	1.49	6.32
Sample 12 d-1	5.23	2.93	5.32
Sample 12 d-2	6.36	4.57	4.32
Sample 12 d-3	7.57	5.9	3.32
Sample 12 d-4	8.56	6.79	2.32
Sample 12 d-5	9.43	8.04	1.32
Sample 12 d-6	10.6	9.22	0.32
Sample 12 d-7	9.62	**	-0.68
Sample 12 d0	1.56	0.22	8.22
Sample 12 d-1	3.37	1.53	7.22
Sample 12 d-2	4.22	2.26	6.22
Sample 12 d-3	4.83	3.06	5.22
Sample 12 d-4	9.92	9.3	4.22
Sample 12 d-5	7.83	6.04	3.22
Sample 12 d-6	7.53	6.15	2.22
Sample 12 d-7	9.14	8.57	1.22
Sample 12D d0	4.28	1.14	6.39
Sample 12D d-1	5.35	2.73	5.39
Sample 12D d-2	6.34	4.24	4.39
Sample 12D d-3	6.3	4.28	3.39
Sample 12D d-4	7.06	5.23	2.39
Sample 12D d-5	9.01	7.66	1.39
Sample 12D d-6	9.47	8.83	0.39
Sample 12D d-7	7.33	10.2	-0.61
Sample 12D d0	1.7	0.41	7.86
Sample 12D d-1	3.94	1.53	6.86
Sample 12D d-2	5.93	2.28	5.86
Sample 12D d-3	5.22	3.36	4.86
Sample 12D d-4	8.27	7.15	3.86
Sample 12D d-5	7.2	5.67	2.86
Sample 12D d-6	8.01	6.6	1.86
Sample 12D d-7	8.64	7.68	0.86

Table 2.12: Sample 12 and duplicates 12D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

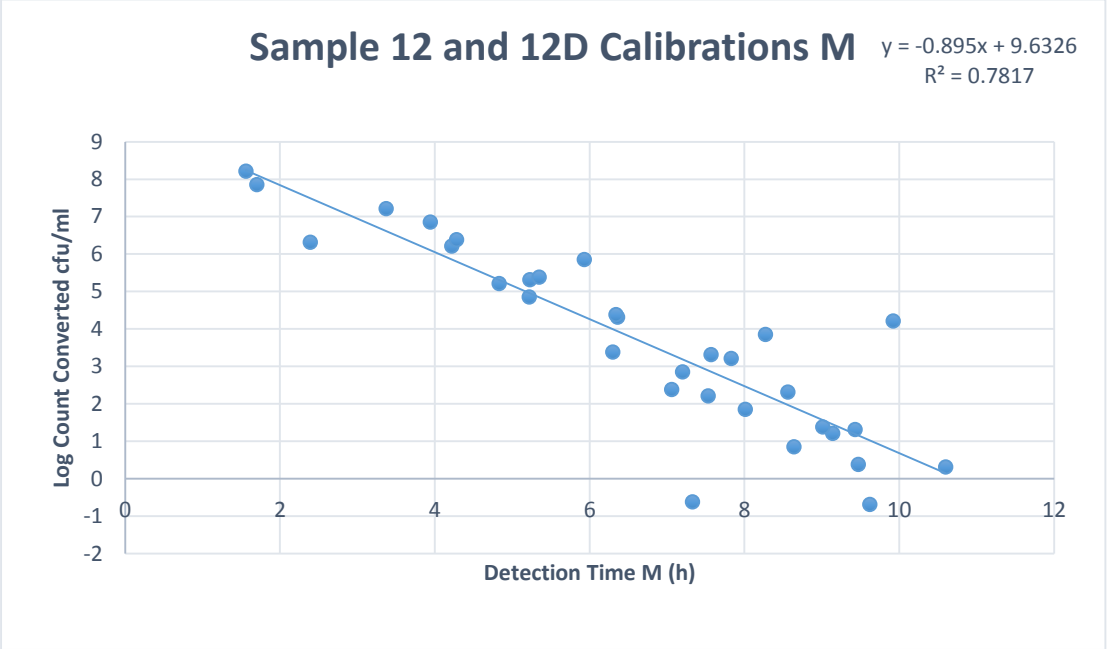

Figure 2.12: Sample 12 and duplicate 12D BacTrac calibration curve (TRGT 9) under 55°C incubation using TSB as medium in the BacTrac cells;

Sample 13: CGT 1, Wild Strain, *Geobacillus species*, Complaint from Taiwan			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Sample 13 d0	0.57	0.81	7.53
Sample 13 d-1	4.86	2.06	6.53
Sample 13 d-2	4.61	2.74	5.53
Sample 13 d-3	6.33	4.38	4.53
Sample 13 d-4	6.9	4.94	3.53
Sample 13 d-5	7.69	6.69	2.53
Sample 13 d-6	8.81	7.17	1.53
Sample 13 d-7	11.33	10.8	0.53
Sample 13D d0	3.38	0.55	7.65
Sample 13D d-1	4.42	1.56	6.65
Sample 13D d-2	5.54	2.89	5.65
Sample 13D d-3	5.96	3.63	4.65
Sample 13D d-4	6.82	5.08	3.65
Sample 13D d-5	8.82	6.88	2.65
Sample 13D d-6	8.77	7.47	1.65
Sample 13D d-7	11.24	10.43	0.65

Table 2.13: Sample 13 and duplicate 13D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

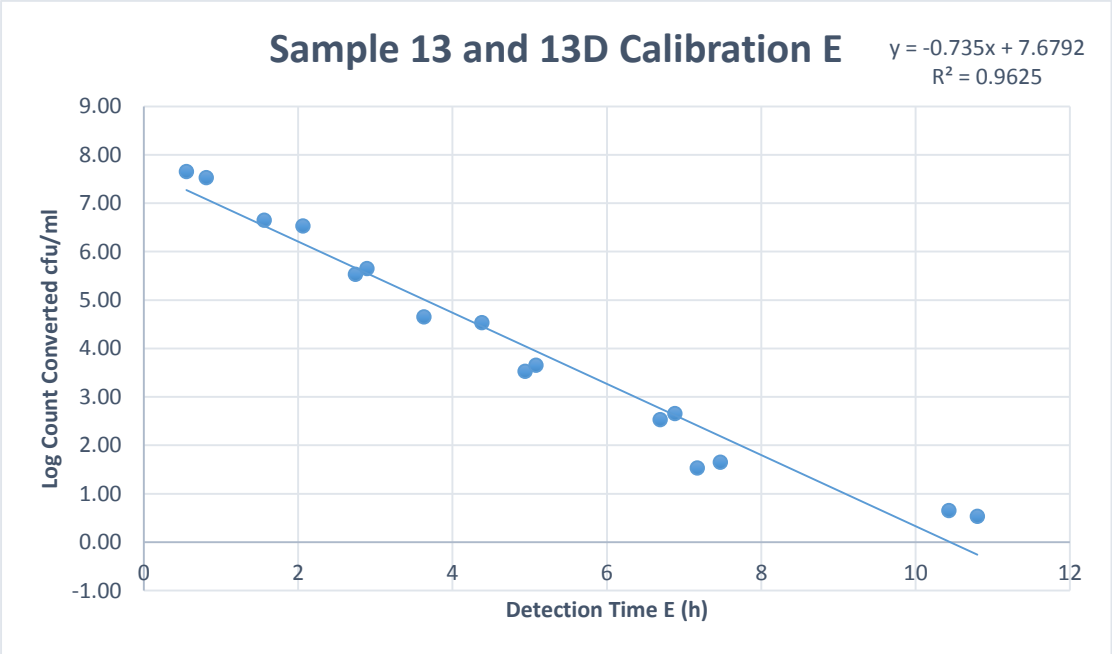

Figure 2.13: Sample 13 and duplicate 13D (CGT 1) BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Appendix 3 BacTrac Calibration curves for Milk Isolate 1 and Isolate 2(TSB)

Isolate 1: Wild Strain, *Geobacillus species*, Skim Milk Powder, AUT, 2010			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Isolate 1 d0	3.71	1.17	8.46
Isolate 1 d-1	4.93	**	7.46
Isolate 1 d-2	5.84	1.65	6.46
Isolate 1 d-3	6.9	2.86	5.46
Isolate 1 d-4	7.5	4.3	4.46
Isolate 1 d-5	8.3	5.01	3.46
Isolate 1 d-6	8.8	5.9	2.46
Isolate 1 d-7	10.61	8.66	1.46
Isolate 1 d-8	12	10.83	0.46
Isolate 1D d0	2.68	0.33	7.85
Isolate 1D d-2	5.47	3.03	5.85
Isolate 1D d-3	6.58	4.27	4.85
Isolate 1D d-4	8.23	7.18	3.85
Isolate 1D d-5	8.95	15.3	2.85
Isolate 1D d-7	11.44	7.6	0.85

Table 3.1: Isolate 1 and duplicate 1D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

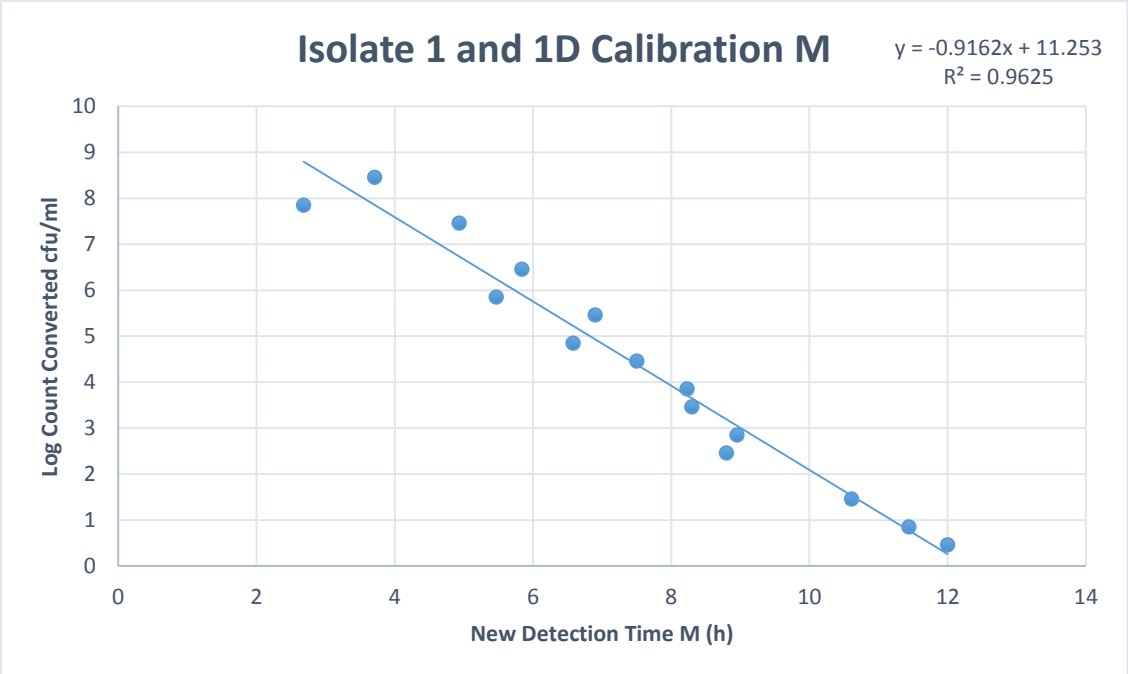

Figure 3.1: Isolate 1 and duplicate 1D BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Isolate 2: Wild Strain, *Anoxybacillus flavithermus*, Skim Milk Powder, AUT, 2010			
INFO1	**Detection Time M (h)**	**Detection Time E (h)**	**Log Count Converted (cfu/ml)**
Isolate 2 d0	2.6	0.88	8.62
Isolate 2 d-1	4.05	0.3	7.62
Isolate 2 d-2	6.32	1.25	6.62
Isolate 2 d-3	6.98	2.83	5.62
Isolate 2 d-4	8.16	4.16	4.62
Isolate 2 d-5	8.63	5.29	3.62
Isolate 2 d-6	10.54	7.29	2.62
Isolate 2 d-7	11.89	9.25	1.62
Isolate 2 d-8	12.15	11.3	0.62
Isolate 2D d0	3.4	1.16	8.64
Isolate 2D d-1	3.47	0.11	7.64
Isolate 2D d-2	5.44	1.34	6.64
Isolate 2D d-3	6.47	2.97	5.64
Isolate 2D d-4	8.31	4.96	4.64
Isolate 2D d-5	10.21	6.17	3.64
Isolate 2D d-6	8.95	0.09	2.64
Isolate 2D d-7	15.12	**	1.64
Isolate 2D d-8	12.8	10.88	0.64

Table 3.2: Isolate 2 and duplicate 2D BacTrac calibration data (means 'not detected') under 55°C incubation using TSB as medium in the BacTrac cells;**

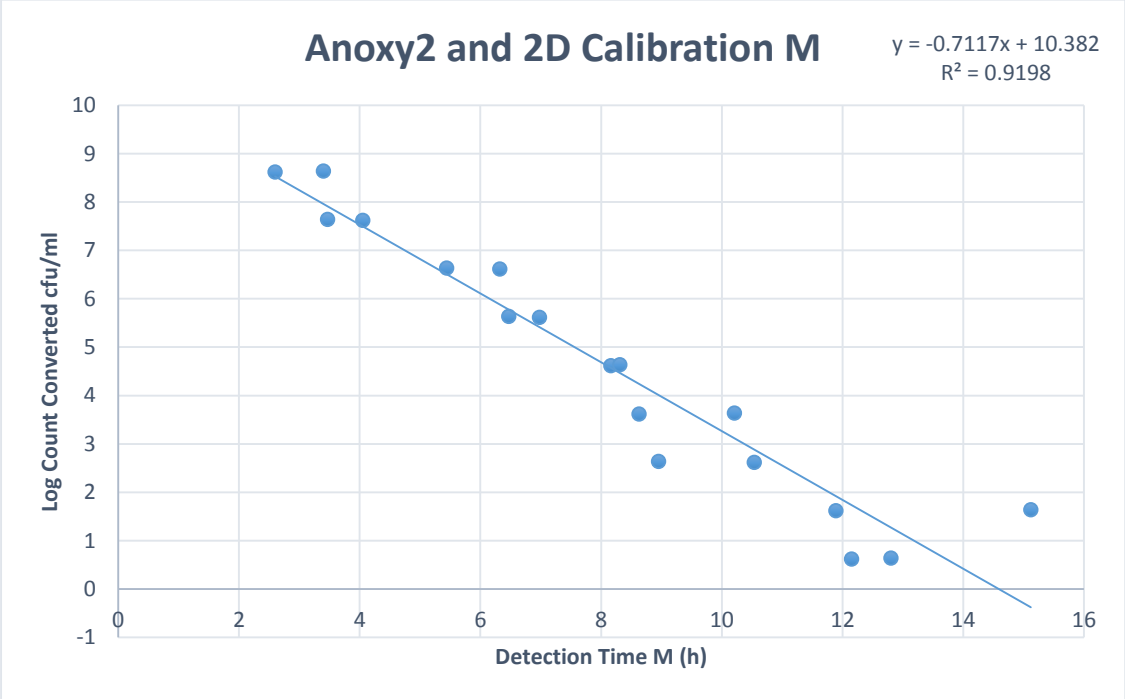

Figure 3.2: Isolate 2 and duplicate 2D BacTrac calibration curve under 55°C incubation using TSB as medium in the BacTrac cells;

Appendix 4 30 Min Coupon Attachment Assay in TSB (Raw data)

INFO1	Detection Time M (h)	Detection Time E (h)
Sample 1 TSB Attachment 1	4.7	3.15
Sample 1 TSB Attachment 2	5.16	3.56
Sample 1 TSB Attachment 3	4.59	3.12
Sample 1 TSB Attachment 4	4.06	2.65
Sample 1 TSB Attachment 5	4.37	2.81
Sample 1 TSB Attachment 6	4.52	2.88
Sample 2 TSB Attachment 1	5.63	2.54
Sample 2 TSB Attachment 2	5.7	3.42
Sample 2 TSB Attachment 3	3.63	3.02
Sample 2 TSB Attachment 4	4.87	3.25
Sample 2 TSB Attachment 5	4.5	2.91
Sample 2 TSB Attachment 6	6.39	3.06
Sample 3 TSB Attachment 1	8.71	6.39
Sample 3 TSB Attachment 2	7.86	6.91
Sample 3 TSB Attachment 3	6.19	4.87
Sample 3 TSB Attachment 4	6.89	5.75
Sample 3 TSB Attachment 5	11.87	8.03
Sample 3 TSB Attachment 6	9.28	6.25
Sample 4 TSB Attachment 1	5.52	4.2
Sample 4 TSB Attachment 2	5.62	3.9
Sample 4 TSB Attachment 3	5.24	3.8
Sample 4 TSB Attachment 4	5.36	3.72
Sample 4 TSB Attachment 5	5.21	3.3
Sample 4 TSB Attachment 6	5.45	3.77
Sample 5 TSB Attachment 1	8.23	7.2
Sample 5 TSB Attachment 2	8	7.54
Sample 5 TSB Attachment 3	7.9	6.67
Sample 5 TSB Attachment 4	7.24	7.64
Sample 5 TSB Attachment 5	8.25	7.41
Sample 5 TSB Attachment 6	7.91	7.55
Sample 6 TSB Attachment 1	5.34	3.66
Sample 6 TSB Attachment 2	5.34	3.88
Sample 6 TSB Attachment 3	5.88	4.42
Sample 6 TSB Attachment 4	5.59	4.05
Sample 6 TSB Attachment 5	5.82	3.89
Sample 6 TSB Attachment 6	6.35	4.93
Sample 7 TSB Attachment 1	4.7	2.72
Sample 7 TSB Attachment 2	4.65	2.82
Sample 7 TSB Attachment 3	4.79	3.08
Sample 7 TSB Attachment 4	4.59	2.77

Sample 7 TSB Attachment 5	4.98	2.89
Sample 7 TSB Attachment 6	4.64	3.02
Sample 8 TSB Attachment 1	6.46	5.31
Sample 8 TSB Attachment 2	5.27	5.09
Sample 8 TSB Attachment 3	5.54	4.88
Sample 8 TSB Attachment 4	6.21	4.74
Sample 8 TSB Attachment 5	1.94	4.51
Sample 8 TSB Attachment 6	5.88	4.61
Sample 9 TSB Attachment 1	5.84	4.78
Sample 9 TSB Attachment 2	5.61	4.21
Sample 9 TSB Attachment 3	6.12	5.05
Sample 9 TSB Attachment 4	6.51	5.22
Sample 9 TSB Attachment 5	6.15	4.97
Sample 9 TSB Attachment 6	5.67	4.26
Sample 10 TSB Attachment 1	6.31	4.98
Sample 10 TSB Attachment 2	6.56	5
Sample 10 TSB Attachment 3	6.48	5.24
Sample 10 TSB Attachment 4	6.09	5.84
Sample 10 TSB Attachment 5	6.62	5.19
Sample 10 TSB Attachment 6	6.04	4.47
Sample 11 TSB Attachment 1	7.09	5.52
Sample 11 TSB Attachment 2	7.01	5.16
Sample 11 TSB Attachment 3	7.09	5.16
Sample 11 TSB Attachment 4	7.14	6.13
Sample 11 TSB Attachment 5	7.16	4.73
Sample 11 TSB Attachment 6	7.49	5.16
Sample 12 TSB Attachment 1	5.66	4.53
Sample 12 TSB Attachment 2	5.61	4.61
Sample 12 TSB Attachment 3	5.19	4.74
Sample 12 TSB Attachment 4	5.13	4
Sample 12 TSB Attachment 5	5.18	4.01
Sample 12 TSB Attachment 6	5.35	3.96
Sample 13 TSB Attachment 1	5.6	4.54
Sample 13 TSB Attachment 2	5.54	4.97
Sample 13 TSB Attachment 3	5.31	4.03
Sample 13 TSB Attachment 4	5.86	5.44
Sample 13 TSB Attachment 5	5.12	3.78
Sample 13 TSB Attachment 6	5.81	5.19
Isolate 1 TSB Attachment 1	7.82	5.07
Isolate 1 TSB Attachment 2	6.16	3.87
Isolate 2 TSB Attachment 1	7.23	6.03
Isolate 2 TSB Attachment 2	8.24	**

Table 4.1: 30 min stainless steel square coupon attachment assay in TSB raw data
(Note: the mean and SD values are calculated using highlighted M or E values. ** means 'not detected');

Appendix 5 30 Min Coupon Attachment Assay in Milk (RSM) (Raw data)

INFO1	Detection Time M (h)	Detection Time E (h)
Sample 1 Milk Attachment 1	6.51	5.27
Sample 1 Milk Attachment 2	5.77	4.67
Sample 1 Milk Attachment 3	12.78	20.29
Sample 1 Milk Attachment 4	9.24	7.4
Sample 1 Milk Attachment 5	10.3	**
Sample 1 Milk Attachment 6	6.33	5.06
Sample 2 Milk Attachment 1	6.03	4.24
Sample 2 Milk Attachment 2	5.17	4.41
Sample 2 Milk Attachment 3	8.56	4.05
Sample 2 Milk Attachment 4	6.55	4.1
Sample 2 Milk Attachment 5	7.9	4.52
Sample 2 Milk Attachment 6	7.23	4.11
Sample 3 Milk Attachment 1	8.61	6.6
Sample 3 Milk Attachment 2	7.23	6.18
Sample 3 Milk Attachment 3	12.16	12.26
Sample 3 Milk Attachment 4	12.44	15.35
Sample 3 Milk Attachment 5	8.58	6.11
Sample 3 Milk Attachment 6	7.65	6.77
Sample 4 MILK Attachment 1	6.1	4.28
Sample 4 MILK Attachment 2	5.86	4.17
Sample 4 MILK Attachment 3	6	4.11
Sample 4 MILK Attachment 4	5.99	3.93
Sample 4 MILK Attachment 5	5.85	3.91
Sample 4 MILK Attachment 6	5.89	3.84
Sample 5 MILK Attachment 1	10.34	9.24
Sample 5 MILK Attachment 2	6.16	4.94
Sample 5 MILK Attachment 3	10.17	10.95
Sample 5 MILK Attachment 4	7.16	5.99
Sample 5 MILK Attachment 5	7.54	6.15
Sample 5 MILK Attachment 6	6.23	4.68
Sample 6 MILK Attachment 1	7.06	5.6
Sample 6 MILK Attachment 2	7.1	6.08
Sample 6 MILK Attachment 3	6.83	5.45
Sample 6 MILK Attachment 4	6.77	5.44
Sample 6 MILK Attachment 5	7.34	6
Sample 6 MILK Attachment 6	6.24	4.64
Sample 7 MILK Attachment 1	4.9	3.1
Sample 7 MILK Attachment 2	4.24	2.78
Sample 7 MILK Attachment 3	5.14	3.08
Sample 7 MILK Attachment 4	5.08	3.09

Sample 7 MILK Attachment 5	5.18	3.14
Sample 7 MILK Attachment 6	4.98	2.95
Sample 8 MILK Attachment 1	7.8	6.56
Sample 8 MILK Attachment 2	8.68	9.29
Sample 8 MILK Attachment 3	8.57	7.31
Sample 8 MILK Attachment 4	9	7.37
Sample 8 MILK Attachment 5	8.52	8.05
Sample 8 MILK Attachment 6	8.19	7.96
Sample 9 MILK Attachment 1	9.53	8.47
Sample 9 MILK Attachment 2	8.61	7.38
Sample 9 MILK Attachment 3	7.61	7.36
Sample 9 MILK Attachment 4	10.02	8.25
Sample 9 MILK Attachment 5	7.8	6.68
Sample 9 MILK Attachment 6	9.79	10.29
Sample 10 MILK Attachment 1	9.67	8.48
Sample 10 MILK Attachment 2	6.56	12.09
Sample 10 MILK Attachment 3	9	7.31
Sample 10 MILK Attachment 4	8.05	6.28
Sample 10 MILK Attachment 5	9.54	8.94
Sample 10 MILK Attachment 6	9.1	12.41
Sample 11 MILK Attachment 1	6.95	5.59
Sample 11 MILK Attachment 2	7.12	5.77
Sample 11 MILK Attachment 3	6.84	5.43
Sample 11 MILK Attachment 4	6.98	5.76
Sample 11 MILK Attachment 5	7.11	5.7
Sample 11 MILK Attachment 6	7.17	**
Sample 12 MILK Attachment 1	7.29	6.29
Sample 12 MILK Attachment 2	7.07	6.4
Sample 12 MILK Attachment 3	6.88	5.49
Sample 12 MILK Attachment 4	7.25	6.21
Sample 12 MILK Attachment 5	7.5	6.4
Sample 12 MILK Attachment 6	6.9	5.94
Sample 13 MILK Attachment 1	8.92	7.96
Sample 13 MILK Attachment 2	7.35	7.5
Sample 13 MILK Attachment 3	8.37	6.86
Sample 13 MILK Attachment 4	8.45	7.99
Sample 13 MILK Attachment 5	8.79	8.21
Sample 13 MILK Attachment 6	8.46	7.54
Isolate 1 MILK Attachment 1	8.75	6.67
Isolate 1 MILK Attachment 2	7.86	9.9
Isolate 2 MILK Attachment 1	9.42	7.57
Isolate 2 MILK Attachment 2	7.36	7.46

Table 5.1: 30 min stainless steel square coupon attachment assay in RSM raw data
(Note: the average is calculated using highlighted M or E values. ** means 'not detected');

Appendix 6 30 Min Coupon Attachment Assay in TSB vs. RSM

Lab ID	Mean Log Count Converted Attachment in TSB (cfu/cm^2)	Standard Deviation	Mean Log Count Converted Attachment in RSM (cfu/cm^2)	Standard Deviation
Sample 1	6.99	0.40	4.51	0.38
Sample 2	6.55	0.27	5.49	0.17
Sample 3	5.37	0.47	4.27	1.73
Sample 4	5.77	0.22	5.58	0.13
Sample 5	2.29	0.31	3.24	0.57
Sample 6	4.00	0.40	2.77	0.40
Sample 7	5.52	0.18	5.28	0.43
Sample 8	5.58	0.22	3.44	0.68
Sample 9	5.07	0.35	2.68	0.61
Sample 10	4.85	0.38	2.59	1.03
Sample 11	4.18	0.47	3.85	0.14
Sample 12	5.54	0.21	3.93	0.22
Sample 13	4.95	0.48	2.74	0.21
Geo1	5.55	1.08	4.34	0.58
Anoxy2	5.58	0.51	5.11	1.04

Table 6.1: Comparison between 30 min bacterial attachments in TSB vs. RSM of different isolates onto stainless steel coupons (Sample 1-13 are Massey samples. Geo1 and Anoxy2 are the new isolates isolated at AUT from skim milk powder in 2010.);

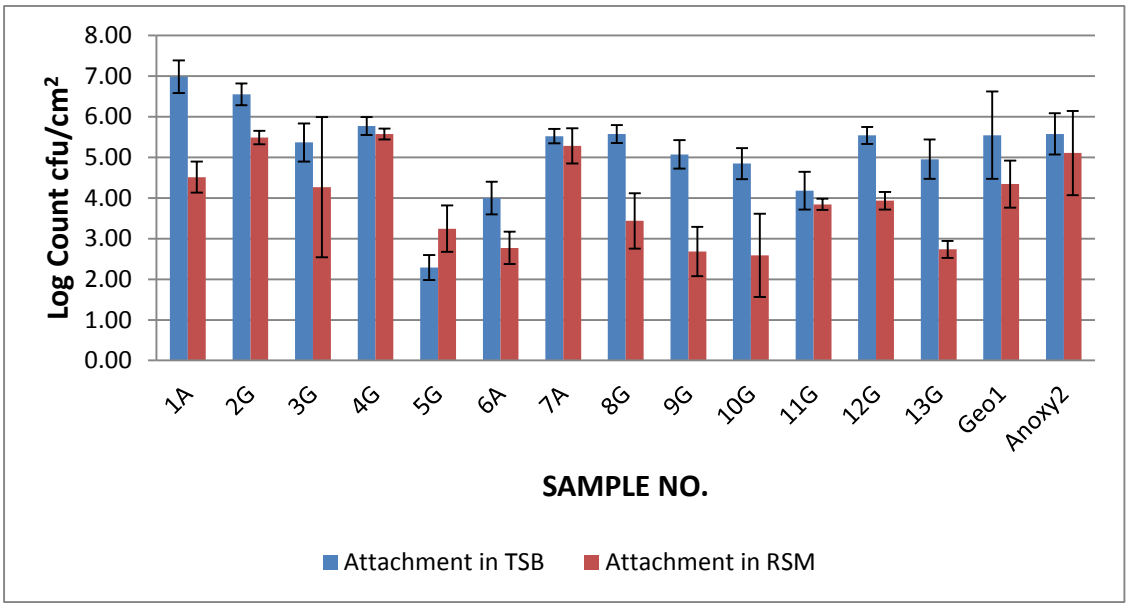

Figure 6.1: Comparison between 30 min bacterial attachments in TSB vs. RSM of different isolates onto 1 cm^2 stainless steel coupons (Sample 1-13 are Massey samples. Geo1 and Anoxy 2 are the new isolates isolated at AUT from skim milk powder in 2010, attachment is enumerated using beads dislodging method in TSB, then the TSB concentrations cfu/ml are converted into attachment data cfu/cm^2 using surface area of coupons and TSB volume; error bars are representing +/- one standard deviation of the data);

Appendix 7 Microtitre Plate Assay for Attachment in TSB for Sample 1-13

User: ISABEL			**Path: C:\Program Files\BMG\Omega\Isabel\data**					**File Name: 5.dbf**					
Test Name: CRYSTAL VIOLET						Date: 16/08/2009 Time: 8:36:18 p.m.							
ID1: PLATE 3 SAMPLE 1-7 ATTACHMENT TSB			Absorbance					Absorbance values are displayed as OD					
	1. Blank corrected raw data (550)												
Lab No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Mean**	**Standard Deviation**
Sample 1	-0.012	0.187	0.168	0.162	0.318	0.12	0.182	0.175	0.149	0.081	0	0.113	0.137	0.088
Sample 2	0.195	0.2	0.191	0.234	0.154	0.14	0.138	0.117	0.241	0.147	0.181	0.146	0.174	0.039
Sample 3	0.168	0.129	0.125	0.094	0.112	0.132	0.079	0.007	0.093	0.103	0.107	0.012	0.097	0.047
Sample 4	0.134	0.13	0.119	0.118	0.085	0.081	0.055	0.032	0.039	0.077	0.107	-0.008	0.081	0.044
Sample 5	-0.005	0.077	0.082	0.112	0.069	0.053	0.035	0.008	-0.008	0.048	0.092	-0.009	0.046	0.042
Sample 6	-0.005	-0.005	-0.007	-0.008	-0.008	-0.007	-0.006	-0.007	-0.005	-0.009	-0.001	-0.004	-0.006	0.002
Sample 7	-0.004	0.002	-0.002	0.002	0.002	-0.002	-0.002	-0.011	-0.004	-0.009	-0.007	0.002	-0.003	0.004
	2. Blank corrected raw data (595)												
Lab No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Mean**	**Standard Deviation**
Sample 1	-0.014	0.19	0.172	0.163	0.321	0.125	0.184	0.175	0.149	0.085	0	0.115	0.139	0.089
Sample 2	0.198	0.203	0.193	0.251	0.155	0.141	0.14	0.118	0.243	0.149	0.182	0.148	0.177	0.042
Sample 3	0.168	0.131	0.126	0.093	0.113	0.13	0.078	0.009	0.096	0.104	0.106	0.013	0.097	0.046
Sample 4	0.138	0.13	0.119	0.118	0.083	0.081	0.055	0.032	0.039	0.078	0.107	-0.009	0.081	0.045
Sample 5	-0.007	0.076	0.081	0.111	0.068	0.055	0.037	0.009	-0.009	0.049	0.096	-0.009	0.046	0.042
Sample 6	-0.005	-0.007	-0.009	-0.009	-0.009	-0.008	-0.007	-0.009	-0.005	-0.01	0	-0.005	-0.007	0.003
Sample 7	-0.005	0.003	-0.002	0.003	0.001	-0.001	-0.003	-0.012	-0.004	-0.01	-0.008	0.002	-0.003	0.005

Table 7.1: Microtitre plate attachment assay using TSB as medium, stained after incubating in 55°C incubator for 30min (10^{5}cfu/ml cell concentration, Sample 1-7, 550 and 595 nm, crystal violet stained; sterile TSB as blank);

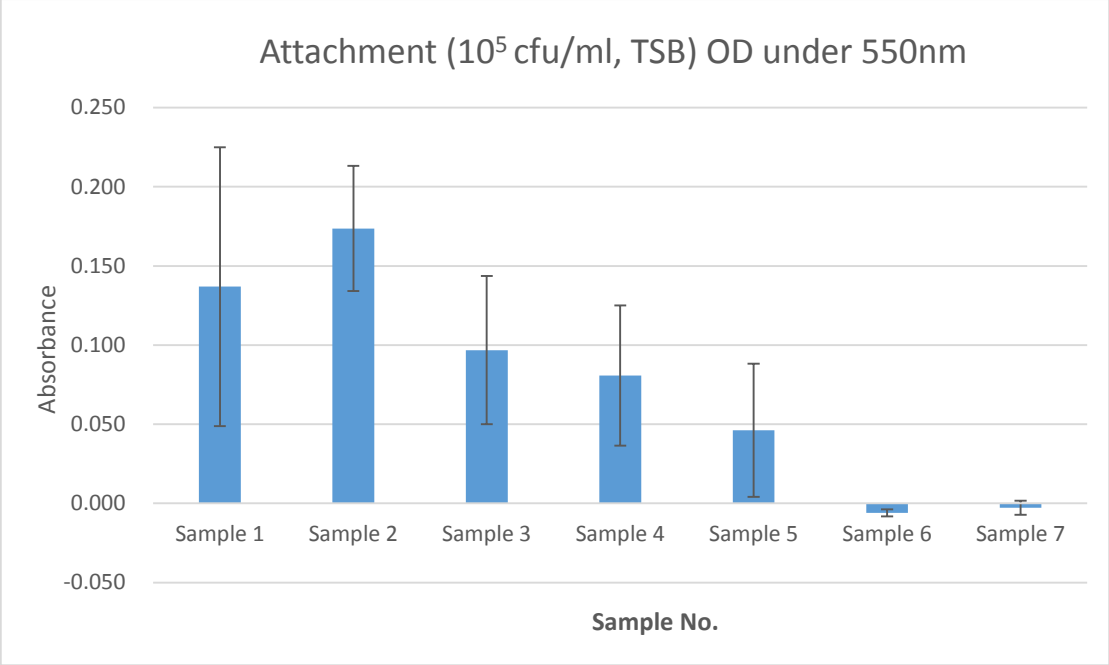

Figure 7.1: Microtitre plate attachment assay using TSB as medium, stained after incubating in 55°C incubator for 30min (**10^5cfu/ml cell concentration, Sample 1-7, 550nm, crystal violet stained, error bars are representing +/– one standard deviation; sterile TSB as blank);**

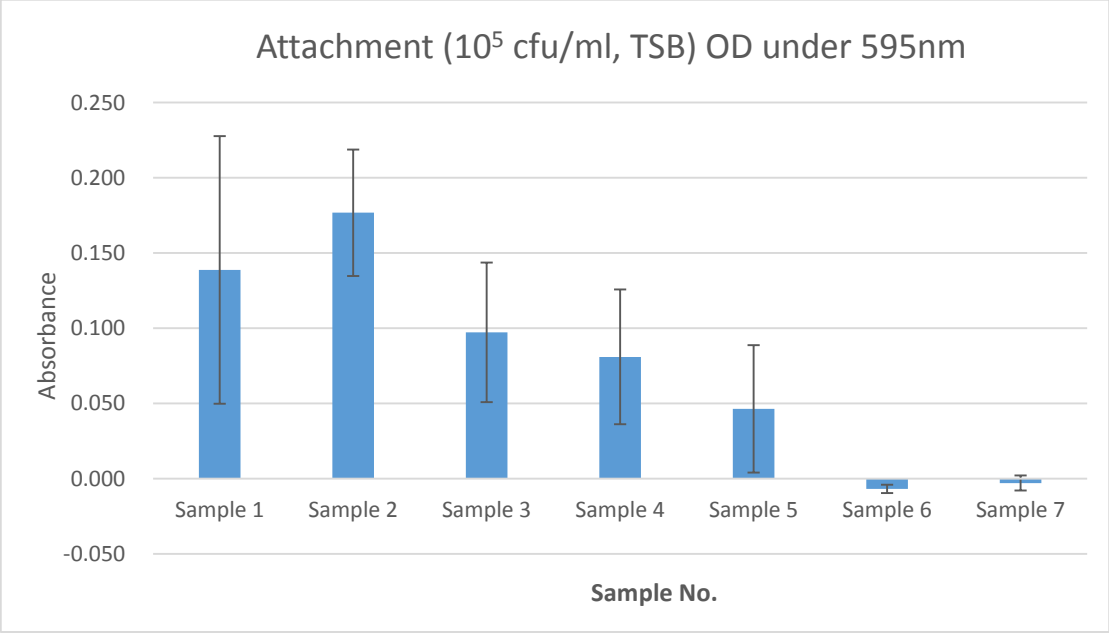

Figure 7.2: Microtitre plate attachment assay using TSB as medium, stained after incubating in 55°C incubator for 30min (**10^5cfu/ml cell concentration, Sample 1-7, 595nm, crystal violet Stained, error bars are representing +/– one standard deviation; sterile TSB as blank);**

User: ISABEL	**Path: C:\Program Files\BMG\Omega\Isabel\data**					**File Name: 7.dbf**		
Test Name: CRYSTAL VIOLET	Date: 19/08/2009					Time: 12:10:31 a.m.		
ID1: PLATE 5 SAMPLE 8-13,8 ATTACHMENT(TSB)	Absorbance					Absorbance values are displayed as OD		
	1. Blank corrected raw data (550)							
Lab No.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 8	0.16	0.56	0.187	0.221	0.15	0.192	0.245	0.156
Sample 9	0.273	0.272	0.209	-0.01	-0.012	-0.03	0.117	0.149
Sample 10	-0.005	-0.023	-0.006	-0.034	-0.027	-0.016	-0.019	0.012
Sample 11	-0.006	0.002	-0.019	-0.028	-0.029	-0.048	-0.021	0.018
Sample 12	0.013	0.008	0.03	-0.01	0	0.007	0.008	0.013
Sample 13	0.031	-0.001	0.016	0.023	-0.018	0.001	0.009	0.018
	2. Blank corrected raw data (595)							
Lab No.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 8	0.197	0.615	0.221	0.252	0.176	0.225	0.281	0.166
Sample 9	0.287	0.276	0.212	-0.01	-0.014	-0.032	0.120	0.154
Sample 10	-0.006	-0.026	-0.008	-0.037	-0.031	-0.019	-0.021	0.012
Sample 11	-0.008	-0.001	-0.021	-0.032	-0.032	-0.05	-0.024	0.018
Sample 12	0.017	0.008	0.03	-0.011	0	0.005	0.008	0.014
Sample 13	0.035	0	0.018	0.025	-0.017	0.002	0.011	0.019

Table 7.2: Microtitre plate attachment assay using TSB as medium, stained after incubating in 55°C incubator for 30min (10^{7}cfu/ml cell concentration, Sample 8-13, 550nm and 595nm, crystal violet stained, Sterile TSB as blank);

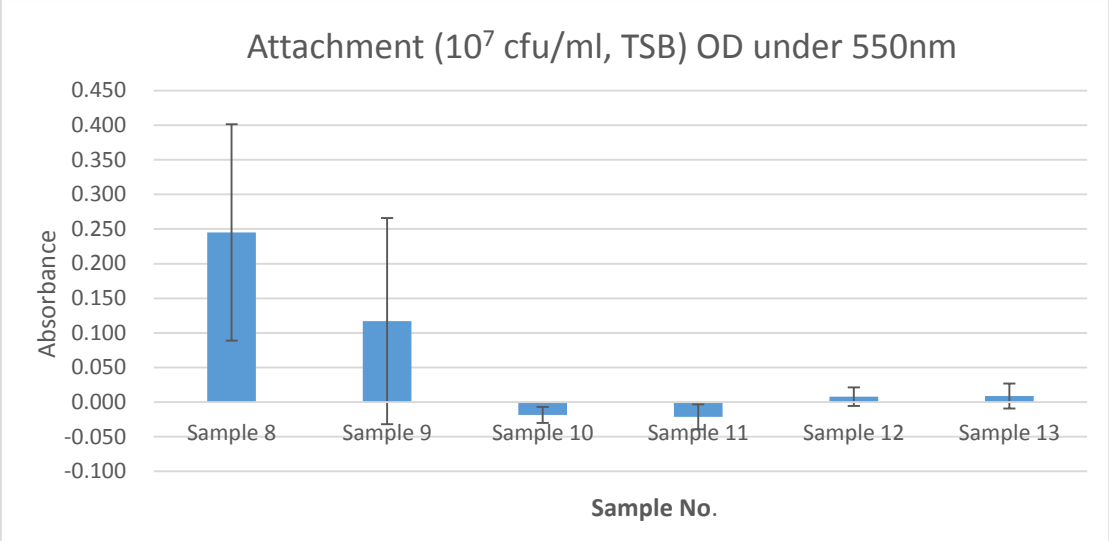

Figure 7.3: Microtitre plate attachment assay using TSB as medium, stained after incubating in 55°C incubator for 30min (**10^7cfu/ml cell concentration, Sample 8-13, 550nm, crystal violet stained, error bars are representing +/– one standard deviation; sterile TSB as blank);**

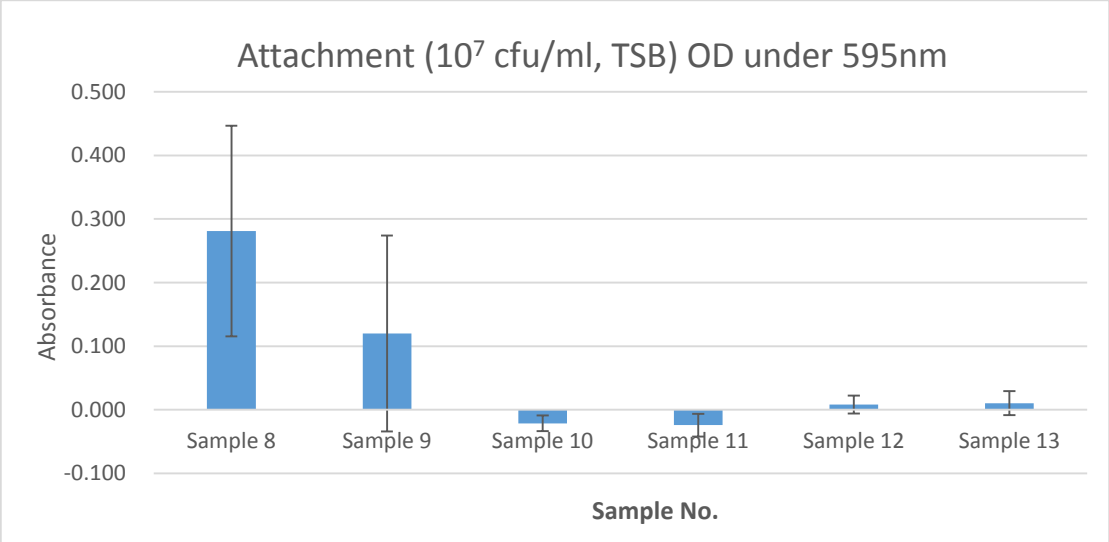

Figure 7.4: Microtitre plate attachment assay using TSB as medium, stained after incubating in 55°C incubator for 30min (**10^7cfu/ml cell concentration, Sample 8-13, 595nm, crystal violet stained, error bars are representing +/– one standard deviation; sterile TSB as blank);**

Appendix 8 Microtitre Plate Assay for Attachment in UHT Milk for Sample 1-13

User: ISABEL	**Path: C:\Program Files\BMG\Omega\Isabel\data**					**File Name: 10.dbf**	
Test Name: CRYSTAL VIOLET	Date: 21/08/2009		Time: 7:49:02 p.m.				
ID1: PLATE 8 SAMPLE 1-7 ATTACHMENT(MILK)	Absorbance		Absorbance values are displayed as OD				
	1. Blank corrected raw data (550)						
Lab No.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 1	-0.127	-0.099	-0.045	-0.018	-0.088	-0.058	-0.073	0.040
Sample 2	-0.016	-0.083	-0.041	-0.1	-0.055	-0.039	-0.056	0.031
Sample 3	-0.054	-0.061	0.009	-0.074	-0.057	-0.042	-0.047	0.029
Sample 4	-0.103	-0.003	0.005	-0.011	-0.032	-0.057	-0.034	0.041
Sample 5	-0.006	-0.005	0.01	0.003	-0.018	0.006	-0.002	0.010
Sample 6	-0.015	-0.003	-0.042	0.044	0.029	0.025	0.006	0.032
Sample 7	-0.044	-0.074	-0.074	-0.061	-0.048	-0.01	-0.052	0.024
	2. Blank corrected raw data (595)						
Lab No.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 1	-0.135	-0.106	-0.049	-0.019	-0.094	-0.055	-0.076	0.043
Sample 2	-0.023	-0.091	-0.047	-0.108	-0.062	-0.045	-0.063	0.032
Sample 3	-0.058	-0.069	0.003	-0.082	-0.064	-0.051	-0.054	0.030
Sample 4	-0.109	-0.007	0	-0.017	-0.038	-0.063	-0.039	0.041
Sample 5	-0.005	-0.009	0.005	-0.002	-0.025	0.007	-0.005	0.012
Sample 6	-0.019	-0.007	-0.046	0.047	0.028	0.025	0.005	0.035
Sample 7	-0.049	-0.082	-0.081	-0.068	-0.056	-0.016	-0.059	0.025

Table 8.1: Microtitre plate attachment assay using UHT milk as medium, stained after incubating in 55°C incubator for 30min (10^{7}cfu/ml cell concentration 1:1 diluted into milk, Sample 1-7, 550nm and 595nm, crystal violet stained, sterile UHT milk as blank);

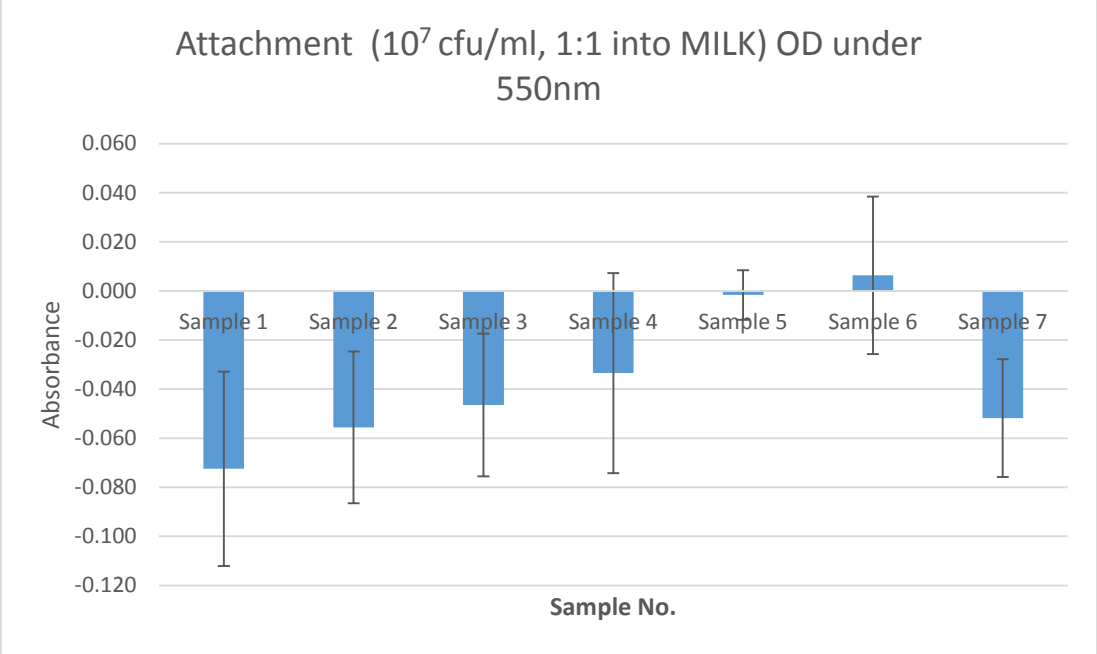

Figure 8.1: Microtitre plate attachment assay using UHT milk as medium, stained after incubating in 55°C incubator for 30min (**10^7cfu/ml cell concentration 1:1 diluted into milk, Sample 1-7, 550nm, crystal violet stained, error bars are representing + / – one standard deviation; sterile UHT milk as blank);**

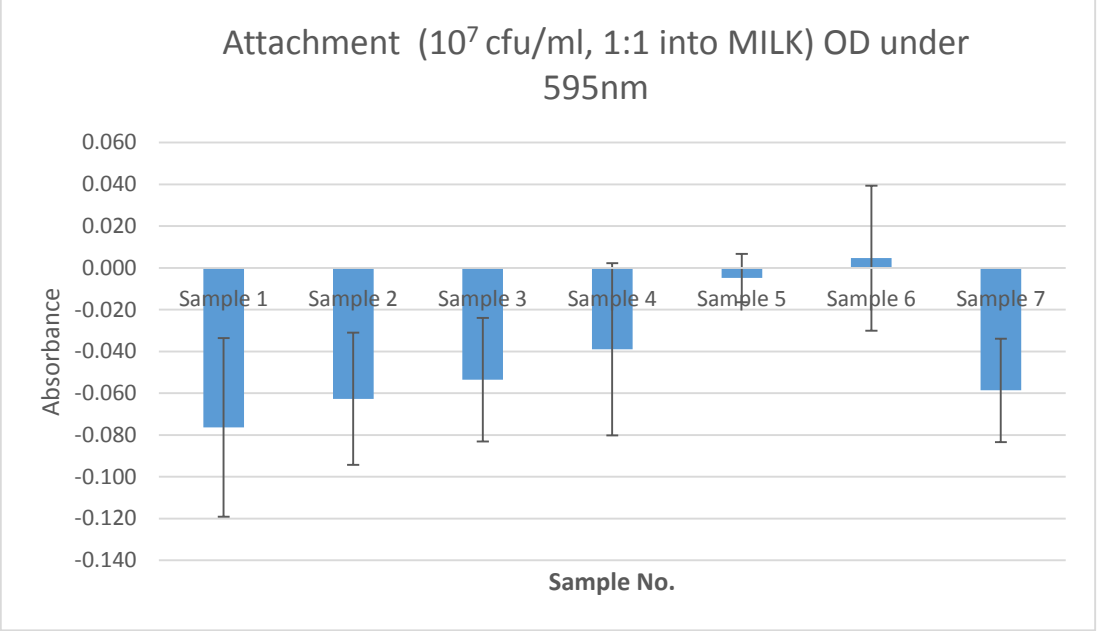

Figure 8.2: Microtitre plate attachment assay using UHT milk as medium, stained after incubating in 55°C incubator for 30min (**10^7cfu/ml cell concentration 1:1 diluted into milk, Sample 1-7, 595nm, crystal violet stained, error bars are representing + / – one standard deviation; sterile UHT milk as blank);**

User: ISABEL	**Path: C:\Program Files\BMG\Omega\Isabel\data**					**File Name: 12.dbf**		
Test Name: CRYSTAL VIOLET		Date: 26/08/2009		Time: 11:28:43 p.m.				
ID1: PLATE 10-2 SAMPLE 8-13, ATTACHMENT(MILK)		Absorbance		Absorbance values are displayed as OD				
	1. Blank corrected raw data (550)							
Lab No.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 8	0.033	0.049	0.001	0.009	0.071	0.02	0.031	0.026
Sample 9	0.057	0.089	0	0.066	0.043	0.041	0.049	0.030
Sample 10	0.059	0.012	-0.016	0.008	0.084	0.027	0.029	0.037
Sample 11	0.09	0.016	-0.031	0.006	-0.003	-0.069	0.002	0.053
Sample 12	-0.127	-0.02	0.002	-0.025	-0.079	-0.039	-0.048	0.047
Sample 13	0.033	0.011	-0.009	0	-0.034	-0.02	-0.003	0.024
	2. Blank corrected raw data (595)							
Lab No.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 8	0.037	0.051	0.001	0.012	0.063	0.021	0.031	0.024
Sample 9	0.062	0.099	0.001	0.071	0.049	0.047	0.055	0.032
Sample 10	0.067	0.009	-0.016	0.006	0.089	0.026	0.030	0.040
Sample 11	0.099	0.013	-0.036	0.002	-0.009	-0.073	-0.001	0.058
Sample 12	-0.122	-0.023	0.002	-0.031	-0.084	-0.043	-0.050	0.045
Sample 13	0.035	0.007	-0.013	-0.002	-0.038	-0.022	-0.006	0.025

Table 8.2: Microtitre plate attachment assay using UHT milk as medium, stained after incubating in 55°C incubator for 30min (10^{7}cfu/ml cell concentration 1:1 diluted into milk, Sample 8-13, 550nm and 595nm, crystal violet stained, sterile UHT milk as blank);

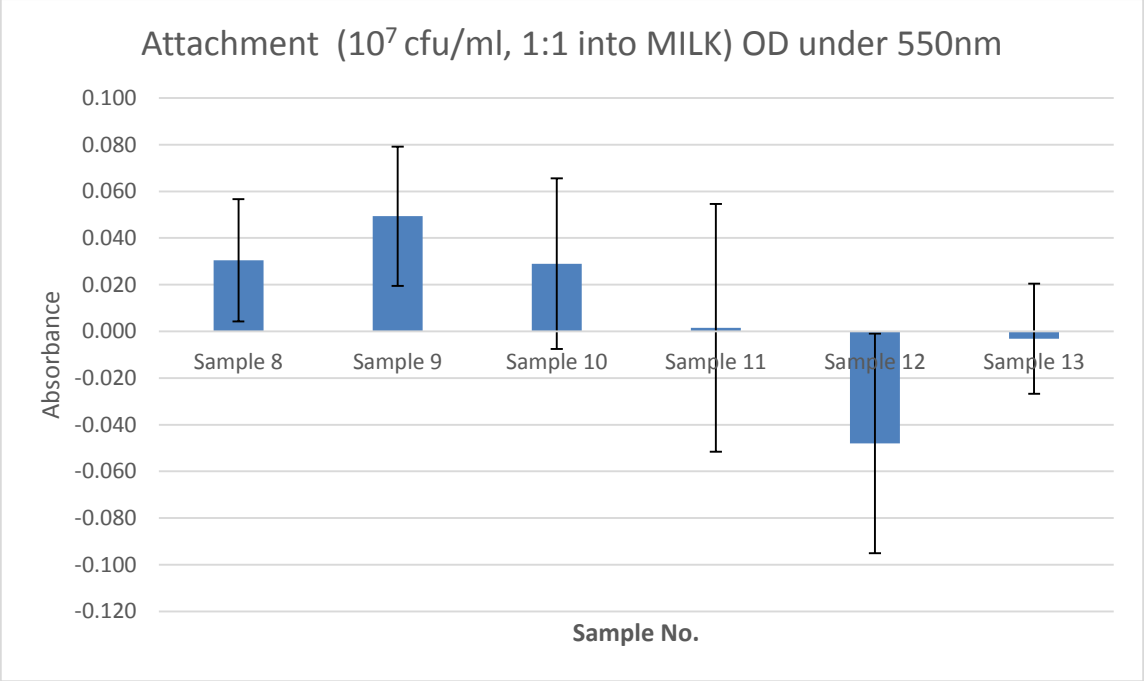

Figure 8.3: Microtitre plate attachment assay using UHT milk as medium, stained after incubating in 55°C incubator for 30min (**10^7cfu/ml cell concentration 1:1 diluted into milk, Sample 8-13, 550nm, crystal violet stained, error bars are representing + / – one standard deviation; sterile UHT milk as blank);**

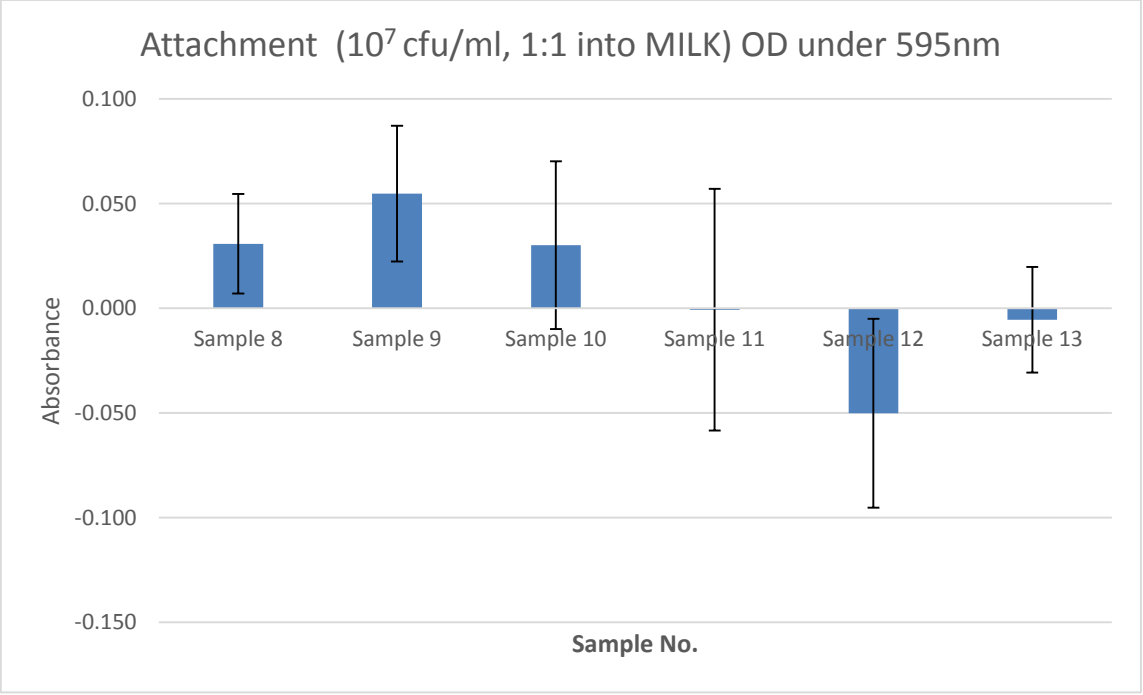

Figure 8.4: Microtitre plate attachment assay using UHT milk as medium, stained after incubating in 55°C incubator for 30min (**10^7cfu/ml cell concentration 1:1 diluted into milk, Sample 8-13, 595nm, crystal violet stained, error bars are representing + / – one standard deviation; sterile UHT milk as blank);**

Appendix 9 Microtitre Plate Assay for Growth in Milk for Sample 1-13

User: ISABEL	**Path: C:\Program Files\BMG\Omega\Isabel\data**						**File Name: 14.dbf**	
Test Name: CRYSTAL VIOLET	Date: 4/09/2009		Time: 9:34:01 a.m.					
ID1: PLATE 11 SAMPLE 1-13 GROWTH AND ATTACHMENT MILK CRYSTAL				Absorbance			Absorbance values are displayed as OD	
	1. Blank corrected raw data (550) WATER AS BLANK;							
Lab ID.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 1	1.299	0.051	0.137	0.3	0.228	0.224	0.373	0.462
Sample 2	0.319	0.267	0.226	0.111	0.054	0.27	0.208	0.103
Sample 3	1.283	0.322	0.076	0.315	0.369	0.256	0.437	0.427
Sample 4	0.968	0.287	0.38	0.193	0.317	0.127	0.379	0.302
Sample 5	1.455	0.261	0.126	0.208	0.296	0.488	0.472	0.496
Sample 6	2.381	2.296	2.496	2.712	2.629	2.683	2.533	0.170
Sample 7	2.584	1.302	0.797	1.125	1.135	1.375	1.386	0.620
Sample 8	1.482	0.786	0.572	0.62	0.15	0.567	0.696	0.439
Sample 9	0.24	0.472	0.337	0.26	0.258	0.503	0.345	0.116
Sample 10	0.233	0.25	0.282	0.524	0.454	0.431	0.362	0.123
Sample 11	1.86	1.107	0.688	0.63	0.976	0.696	0.993	0.464
Sample 12	2.807	0.658	2.555	1.061	0.63	0.668	1.397	1.011
Sample 13	1.08	0.602	0.852	0.684	0.64	0.502	0.727	0.208
TSB+MILK	1.087	0.559	0.884	0.984	0.472	0.627	0.769	0.250

Table 9.1: Microtitre plate assay: growth and attachment for all 13 samples in UHT milk as medium, stained after incubating in 55°C incubator for 8 h (Absorbance, Sample 1-13, 550nm, crystal violet stained, water as blank corrected data);

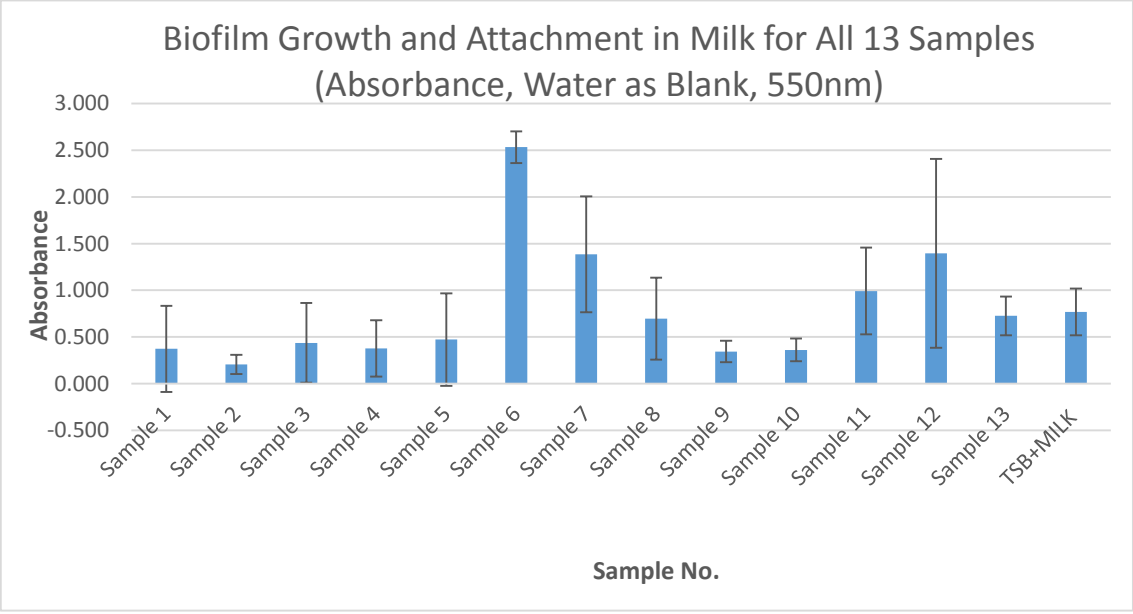

Figure 9.1: Microtitre plate assay: growth and attachment for all 13 samples in UHT milk as medium, stained after incubating in 55°C incubator for 8 h (Absorbance, Sample 1-13, 550nm, crystal violet stained, water as blank, error bars are representing + / – one standard deviation ;);

User: ISABEL	**Path: C:\Program Files\BMG\Omega\Isabel\data**					**File Name: 14.dbf**		
Test Name: CRYSTAL VIOLET	Date: 4/09/2009		Time: 9:34:01 a.m.					
ID1: PLATE 11 SAMPLE 1-13 GROWTH AND ATTACHMENT MILK CRYSTAL				Absorbance		Absorbance values are displayed as OD		
	2. Blank corrected raw data (595) WATER AS BLANK;							
Lab No.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 1	1.646	0.056	0.165	0.372	0.276	0.276	0.465	0.589
Sample 2	0.394	0.323	0.279	0.13	0.066	0.332	0.254	0.128
Sample 3	1.626	0.399	0.084	0.387	0.455	0.313	0.544	0.546
Sample 4	1.226	0.352	0.465	0.237	0.39	0.154	0.471	0.386
Sample 5	1.826	0.32	0.146	0.244	0.362	0.601	0.583	0.628
Sample 6	2.973	2.859	3.347	3.281	3.21	3.288	3.160	0.197
Sample 7	3.399	1.604	0.97	1.424	1.391	1.696	1.747	0.847
Sample 8	1.891	0.981	0.709	0.767	0.174	0.699	0.870	0.567
Sample 9	0.292	0.581	0.411	0.313	0.319	0.62	0.423	0.144
Sample 10	0.28	0.301	0.339	0.645	0.563	0.521	0.442	0.154
Sample 11	2.335	1.383	0.851	0.767	1.217	0.866	1.237	0.589
Sample 12	3.301	0.814	3.033	1.32	0.792	0.839	1.683	1.169
Sample 13	1.364	0.75	1.064	0.846	0.796	0.623	0.907	0.266
TSB+MILK	1.364	0.692	1.12	1.246	0.587	0.774	0.964	0.321

Table 9.2: Microtitre plate assay: growth and attachment for all 13 samples in UHT milk as medium, stained after incubating in 55°C incubator for 8 h (Absorbance, Sample 1-13, 595nm, crystal violet stained, water as blank corrected data);

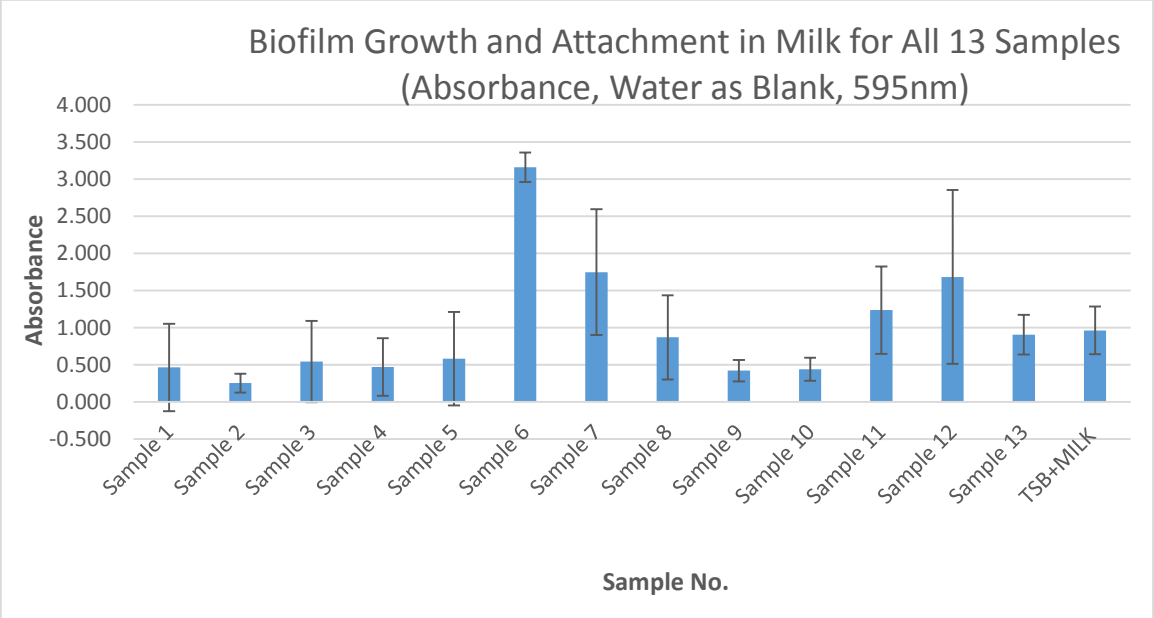

Figure 9.2: Microtitre plate assay: growth and attachment for all 13 samples in UHT milk as medium, stained after incubating in 55°C incubator for 8 h (Absorbance, Sample 1-13, 595nm, crystal violet stained, water as blank, error bars are representing + / – one standard deviation ;) ;

User: ISABEL	**Path: C:\Program Files\BMG\Omega\Isabel\data**					**File Name: 14.dbf**	
Test Name: CRYSTAL VIOLET	Date: 4/09/2009			Time: 9:34:01 a.m.			
ID1: PLATE 11 SAMPLE 1-13 GROWTH AND ATTACHMENT MILK CRYSTAL				Absorbance	Absorbance values are displayed as OD		
3. Blank corrected raw data (550) TSB + MILK+ WATER AS BLANK;							
Lab ID.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 1	0.212	-0.508	-0.747	-0.684	-0.244	-0.403	-0.396	0.350
Sample 2	-0.768	-0.292	-0.658	-0.873	-0.418	-0.357	-0.561	0.238
Sample 3	0.196	-0.237	-0.808	-0.669	-0.103	-0.371	-0.332	0.369
Sample 4	-0.119	-0.272	-0.504	-0.791	-0.155	-0.5	-0.390	0.256
Sample 5	0.368	-0.298	-0.758	-0.776	-0.176	-0.139	-0.297	0.430
Sample 6	1.294	1.737	1.612	1.728	2.157	2.056	1.764	0.312
Sample 7	1.497	0.743	-0.087	0.141	0.663	0.748	0.618	0.553
Sample 8	0.395	0.227	-0.312	-0.364	-0.322	-0.06	-0.073	0.320
Sample 9	-0.847	-0.087	-0.547	-0.724	-0.214	-0.124	-0.424	0.326
Sample 10	-0.854	-0.309	-0.602	-0.46	-0.018	-0.196	-0.407	0.299
Sample 11	0.773	0.548	-0.196	-0.354	0.504	0.069	0.224	0.452
Sample 12	1.72	0.099	1.671	0.077	0.158	0.041	0.628	0.828
Sample 13	-0.007	0.043	-0.032	-0.3	0.168	-0.125	-0.042	0.159

Table 9.3: Microtitre plate assay: growth and attachment for all 13 samples in UHT milk as medium, stained after incubating in 55°C incubator for 8 h (Absorbance, Sample 1-13, 550nm, crystal violet stained, TSB + milk + water as blank corrected data);

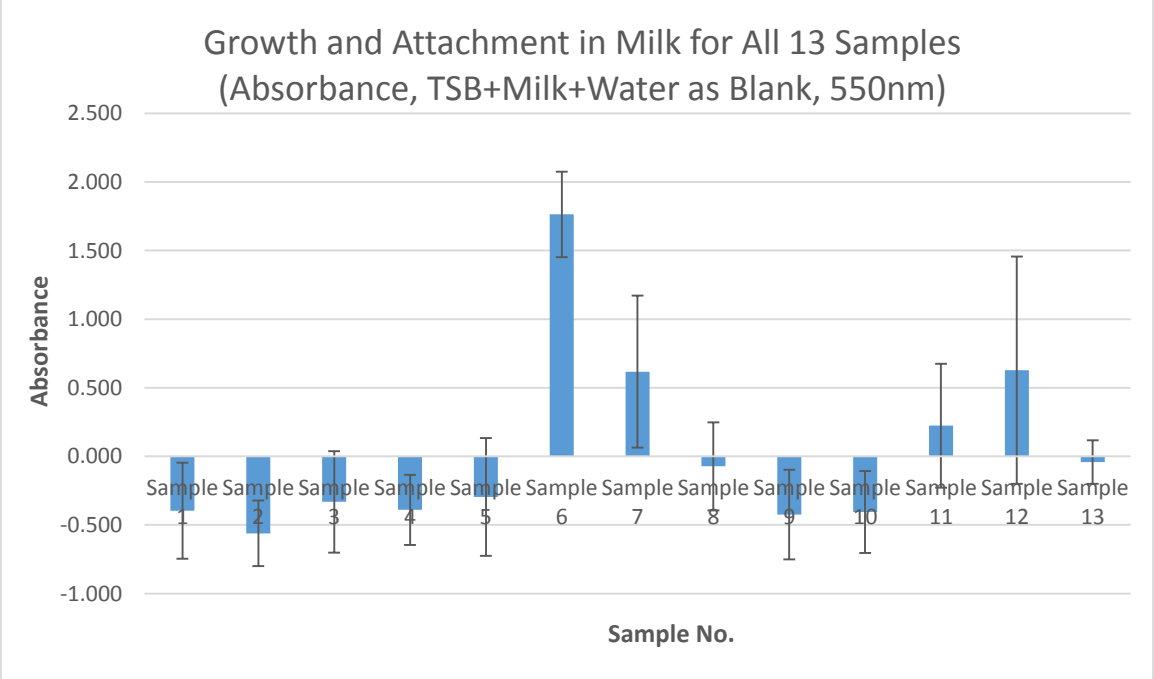

Figure 9.3: Microtitre plate assay: growth and attachment for all 13 samples in UHT milk as medium, stained after incubating in 55°C incubator for 8 h (Absorbance, Sample 1-13, 550nm, crystal violet stained, TSB + milk + water as blank, error bars are representing + / – one standard deviation ;) ;

User: ISABEL	**Path: C:\Program Files\BMG\Omega\Isabel\data**					**File Name: 14.dbf**		
Test Name: CRYSTAL VIOLET	Date: 4/09/2009		Time: 9:34:01 a.m.					
ID1: PLATE 11 SAMPLE 1-13 GROWTH AND ATTACHMENT MILK CRYSTAL			Absorbance			Absorbance values are displayed as OD		
	4. Blank corrected raw data (595) TSB + MILK + WATER AS BLANK;							
Lab ID.	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
Sample 1	0.282	-0.636	-0.955	-0.874	-0.311	-0.498	-0.499	0.450
Sample 2	-0.97	-0.369	-0.841	-1.116	-0.521	-0.442	-0.710	0.308
Sample 3	0.262	-0.293	-1.036	-0.859	-0.132	-0.461	-0.420	0.477
Sample 4	-0.138	-0.34	-0.655	-1.009	-0.197	-0.62	-0.493	0.330
Sample 5	0.462	-0.372	-0.974	-1.002	-0.225	-0.173	-0.381	0.551
Sample 6	1.609	2.167	2.227	2.035	2.623	2.514	2.196	0.362
Sample 7	2.035	0.912	-0.15	0.178	0.804	0.922	0.784	0.754
Sample 8	0.527	0.289	-0.411	-0.479	-0.413	-0.075	-0.094	0.420
Sample 9	-1.072	-0.111	-0.709	-0.933	-0.268	-0.154	-0.541	0.418
Sample 10	-1.084	-0.391	-0.781	-0.601	-0.024	-0.253	-0.522	0.381
Sample 11	0.971	0.691	-0.269	-0.479	0.63	0.092	0.273	0.580
Sample 12	1.937	0.122	1.913	0.074	0.205	0.065	0.719	0.935
Sample 13	0	0.058	-0.056	-0.4	0.209	-0.151	-0.057	0.207

Table 9.4: Microtitre plate assay: growth and attachment for all 13 samples in UHT milk as medium, stained after incubating in 55°C incubator for 8 h (Absorbance, Sample 1-13, 595nm, crystal violet stained, TSB + milk + water as blank corrected data);

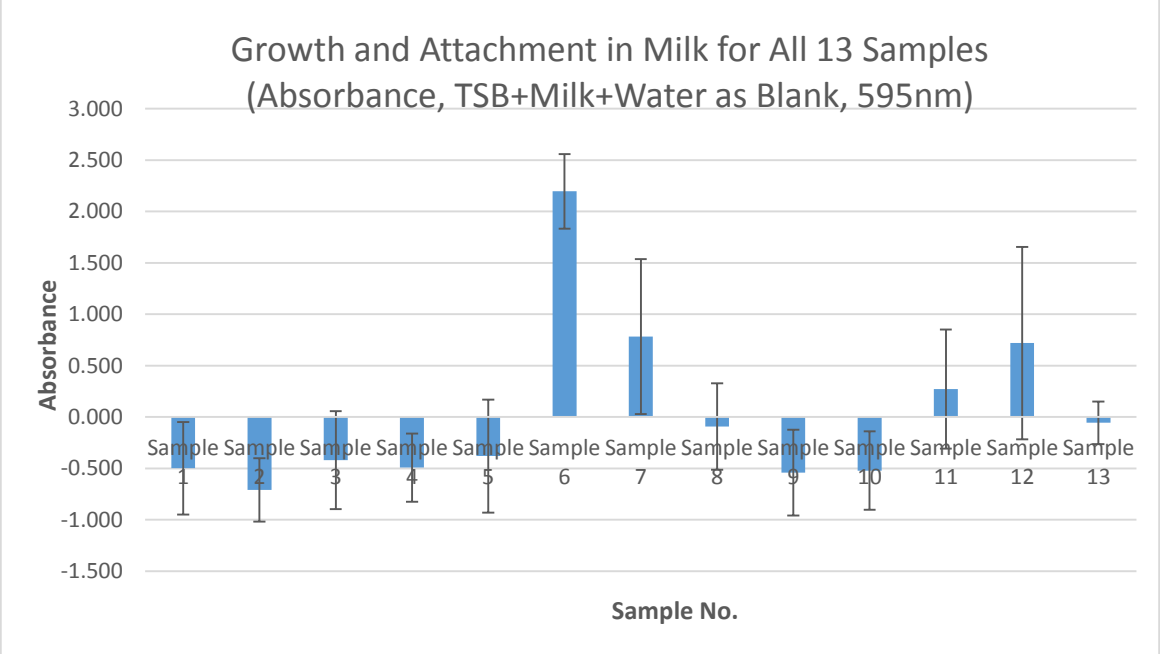

Figure 9.4: Microtitre plate assay: growth and attachment for all 13 samples in UHT milk as medium, stained after incubating in 55°C incubator for 8 h (Absorbance, Sample 1-13, 595nm, crystal violet stained, TSB + milk + water as blank, error bars are representing + / – one standard deviation ;);

Appendix 10 Planktonic Growth Rate in TSB (Sample 7 and 12)

Original data		Absorbance						
Time	1	2	3	4	5	6	7	8
Blank	0.092	0.169	0.162	0.154	0.155	0.112	0.13	0.096
Time 0	0.093	0.142	0.165	0.169	0.139	0.098	0.095	0.098
Time 30 min	0.307	0.311	0.316	1.416	0.361	0.369	0.395	0.399
Time 1hr	0.1	0.212	0.244	0.308	0.295	0.304	0.323	0.32
Time 90min	0.163	0.201	0.097	0.246	0.312	0.344	0.364	0.369
Time 2hr	0.204	0.241	0.246	0.378	0.365	0.405	0.393	0.434
Time 150 min	0.216	0.35	0.429	0.583	0.422	0.427	0.433	0.544
Time 3hr	0.255	0.35	0.407	0.75	0.399	0.574	0.447	0.632
Time 210 min	0.278	0.445	0.575	0.931	0.32	0.558	0.366	0.736
Time 4hr	0.279	0.578	0.693	1.073	0.359	0.829	0.477	1.02
Time 270 min	0.398	0.972	0.969	1.098	0.533	1.299	0.718	1.154
Time 5hr	0.421	0.875	0.819	0.727	0.517	0.838	0.673	0.822

Table 10.1: Absorbance reading at 30 min interval for testing planktonic growth rate in TSB for Sample 7 and 7D, Sample 12 and 12 D with different cell concentrations (10^3 cfu/ml and 10^4 cfu/ml) under 55°C for 5 h;

Calculated Ln (absorbance)								
Time	1	2	3	4	5	6	7	8
Blank	-2.386	-1.778	-1.820	-1.871	-1.864	-2.189	-2.040	-2.343
Time 0	-2.375	-1.952	-1.802	-1.778	-1.973	-2.323	-2.354	-2.323
Time 30 min	-1.181	-1.168	-1.152	0.348	-1.019	-0.997	-0.929	-0.919
Time 1hr	-2.303	-1.551	-1.411	-1.178	-1.221	-1.191	-1.130	-1.139
Time 90min	-1.814	-1.604	-2.333	-1.402	-1.165	-1.067	-1.011	-0.997
Time 2hr	-1.590	-1.423	-1.402	-0.973	-1.008	-0.904	-0.934	-0.835
Time 150 min	-1.532	-1.050	-0.846	-0.540	-0.863	-0.851	-0.837	-0.609
Time 3hr	-1.366	-1.050	-0.899	-0.288	-0.919	-0.555	-0.805	-0.459
Time 210 min	-1.280	-0.810	-0.553	-0.071	-1.139	-0.583	-1.005	-0.307
Time 4hr	-1.277	-0.548	-0.367	0.070	-1.024	-0.188	-0.740	0.020
Time 270 min	-0.921	-0.028	-0.031	0.093	-0.629	0.262	-0.331	0.143
Time 5hr	-0.865	-0.134	-0.200	-0.319	-0.660	-0.177	-0.396	-0.196

Table 10.2: Calculated ln(Absorbance) reading for each 30 min interval for testing planktonic growth rate in TSB for Sample 7 and 7D and Sample 12 and 12D with different cell concentrations (10^3 cfu/ml and 10^4 cfu/ml) under 55°C for 5 h;

Glossary & Growth Rate		Maximum Specific Growth Rate (/h)
1	Sample 7 (10^3 cfu/ml)	0.3102
2	Sample 7 (10^4 cfu/ml)	0.4798
3	Sample 7d (10^3 cfu/ml)	0.4937
4	Sample 7d (10^4 cfu/ml)	0.5897
5	Sample 12 (10^3 cfu/ml)	0.1812
6	Sample 12 (10^4 cfu/ml)	0.3767
7	Sample 12d (10^3 cfu/ml)	0.2529
8	Sample 12d (10^4 cfu/ml)	0.3739

Table 10.3: Glossary and summary table for the planktonic growth rate in TSB data for Sample 7 and 7D with different cell concentrations (10^3 cfu/ml and 10^4 cfu/ml) under 55^{o}C for 5 h;

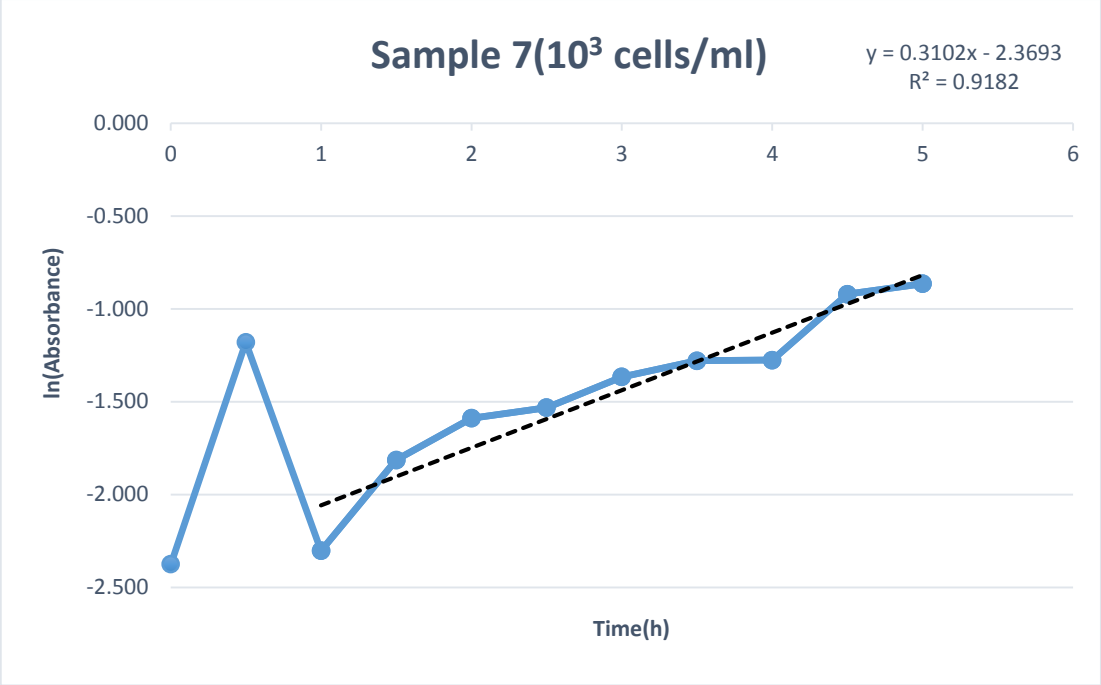

Figure 10.1: Absorbance reading at 30 min interval for testing planktonic growth rate in TSB for Sample 7 with 10^3 cells/ml cell concentration under 55°C for 5 h. The dashed line is the trend line for calculating the growth rate;

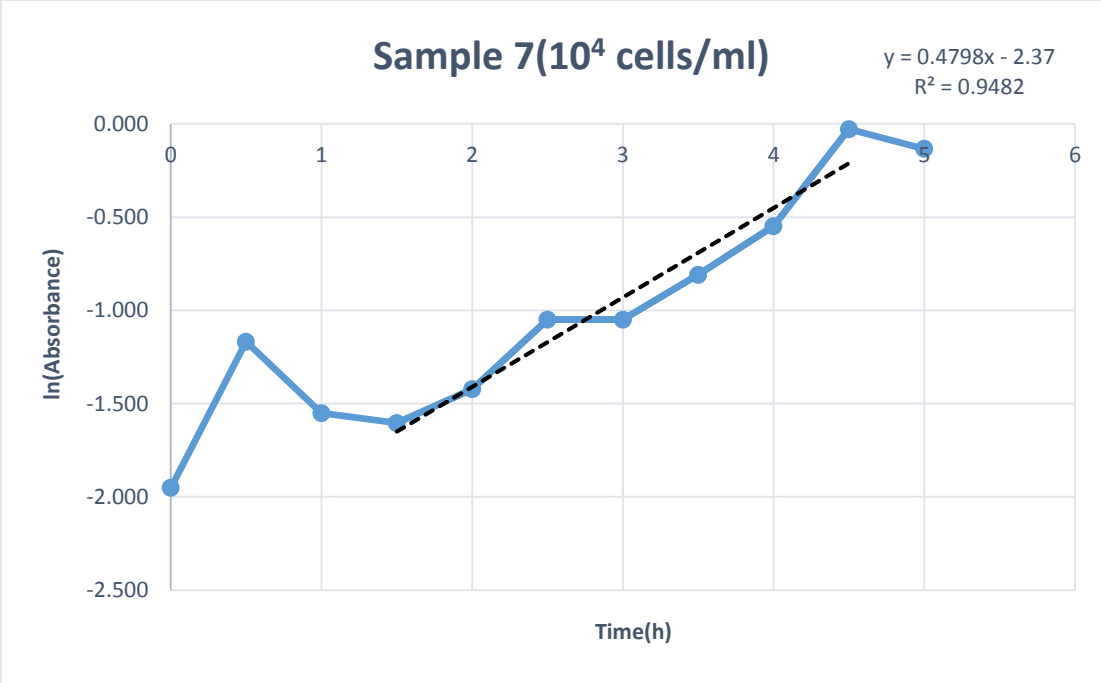

Figure 10.2: Absorbance reading at 30 min interval for testing planktonic growth rate in TSB for Sample 7 with 10^4 cells/ml cell concentration under 55°C for 5 h. The dashed line is the trend line for calculating the growth rate;

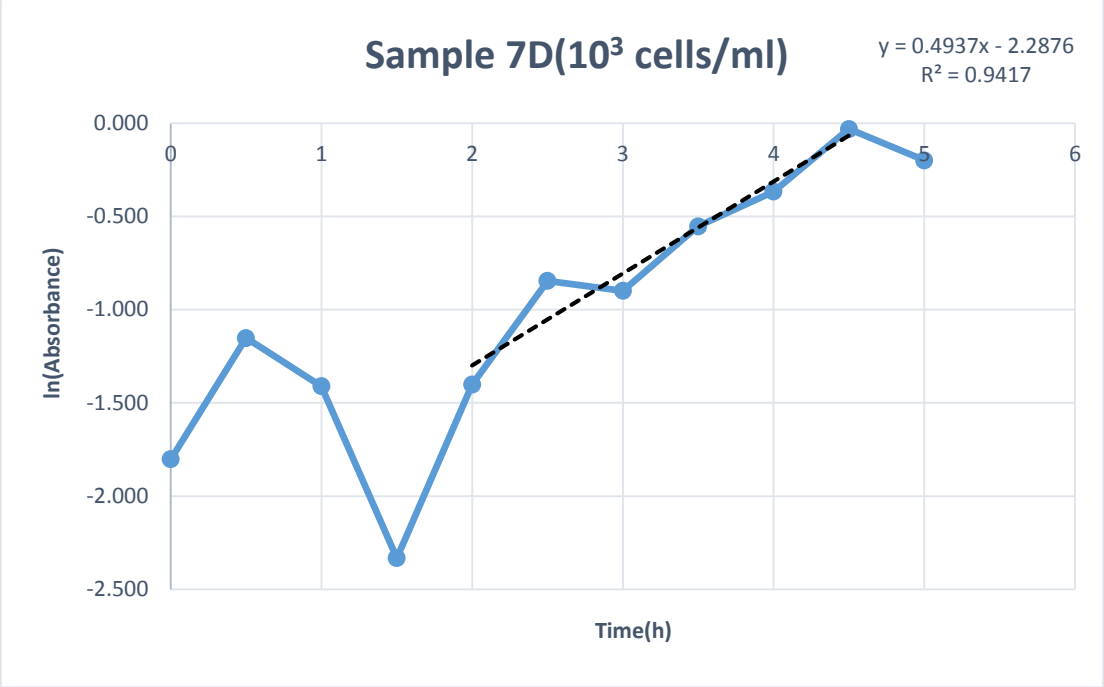

Figure 10.3: Absorbance reading at 30 min interval for testing planktonic growth rate in TSB for Sample 7D with 10^3 cells/ml cell concentration under 55°C for 5 h.
The dashed line is the trend line for calculating the growth rate;

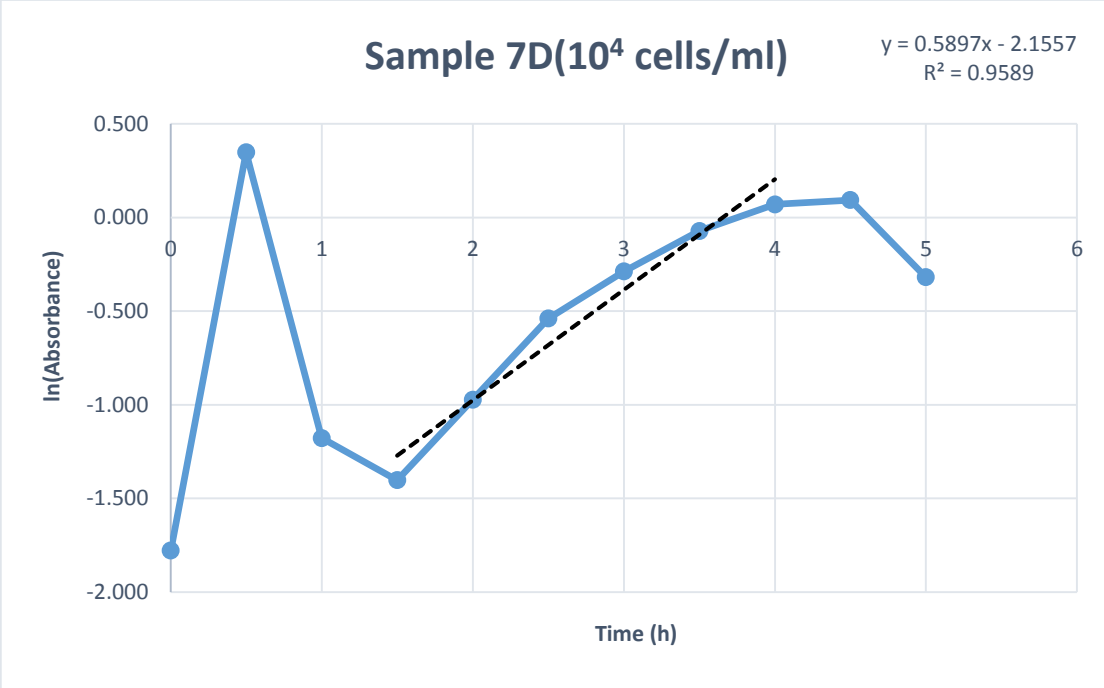

Figure 10.4: Absorbance reading at 30 min interval for testing planktonic growth rate in TSB for Sample 7D with 10^4 cells/ml cell concentration under 55°C for 5 h.
The dashed line is the trend line for calculating the growth rate;

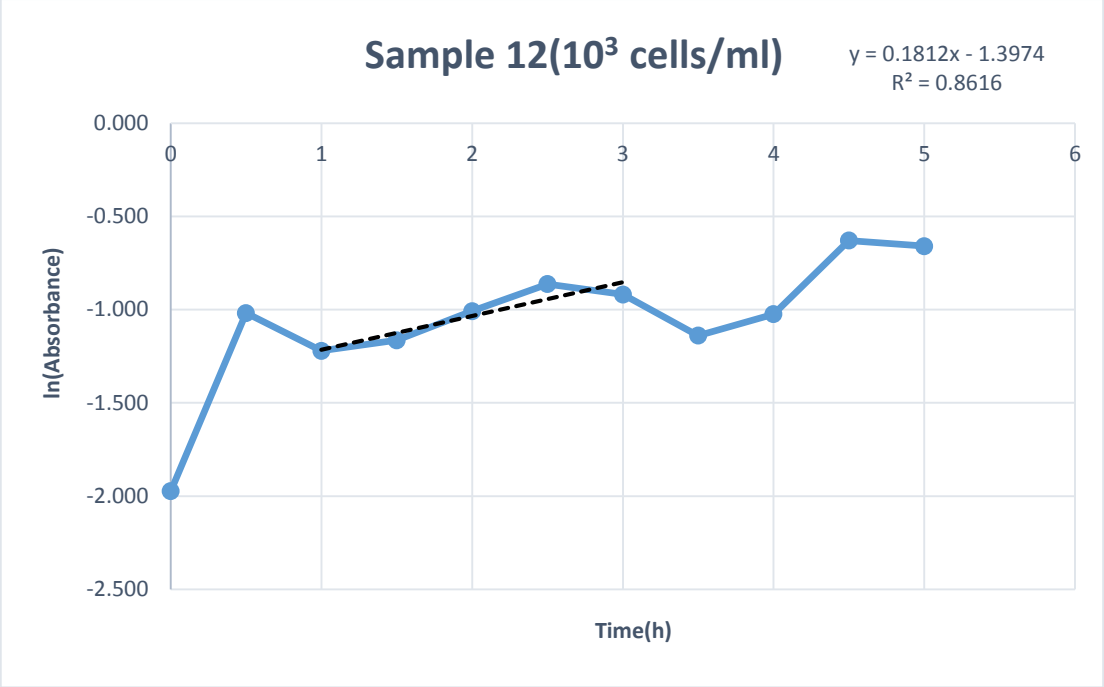

Figure 10.5: Absorbance reading at 30 min interval for testing planktonic growth rate in TSB for Sample 12 with 10^3 cells/ml cell concentration under 55°c for 5 h.
The dashed line is the trend line for calculating the growth rate;

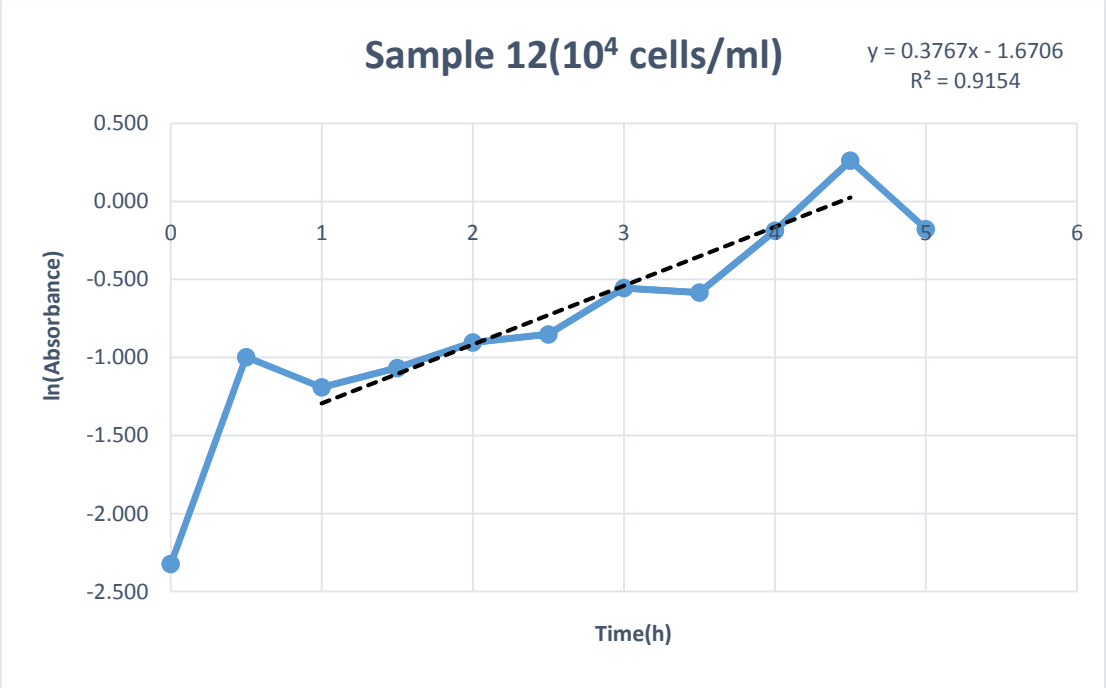

Figure 10.6: Absorbance reading at 30 min interval for testing planktonic growth rate in TSB for Sample 12 with 10^4 cells/ml cell concentration under 55°c for 5 h.
The dashed line is the trend line for calculating the growth rate;

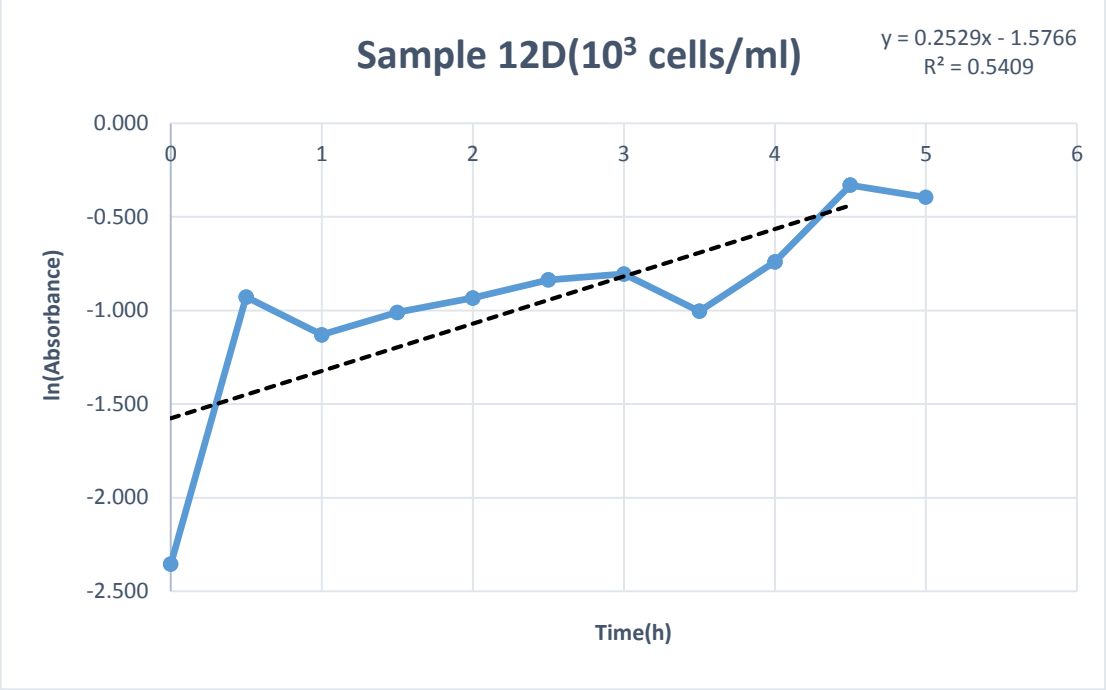

Figure 10.7: Absorbance reading at 30 min interval for testing planktonic growth rate in TSB for Sample 12D with 10^3 cells/ml cell concentration under 55°c for 5 h.
The dashed line is the trend line for calculating the growth rate;

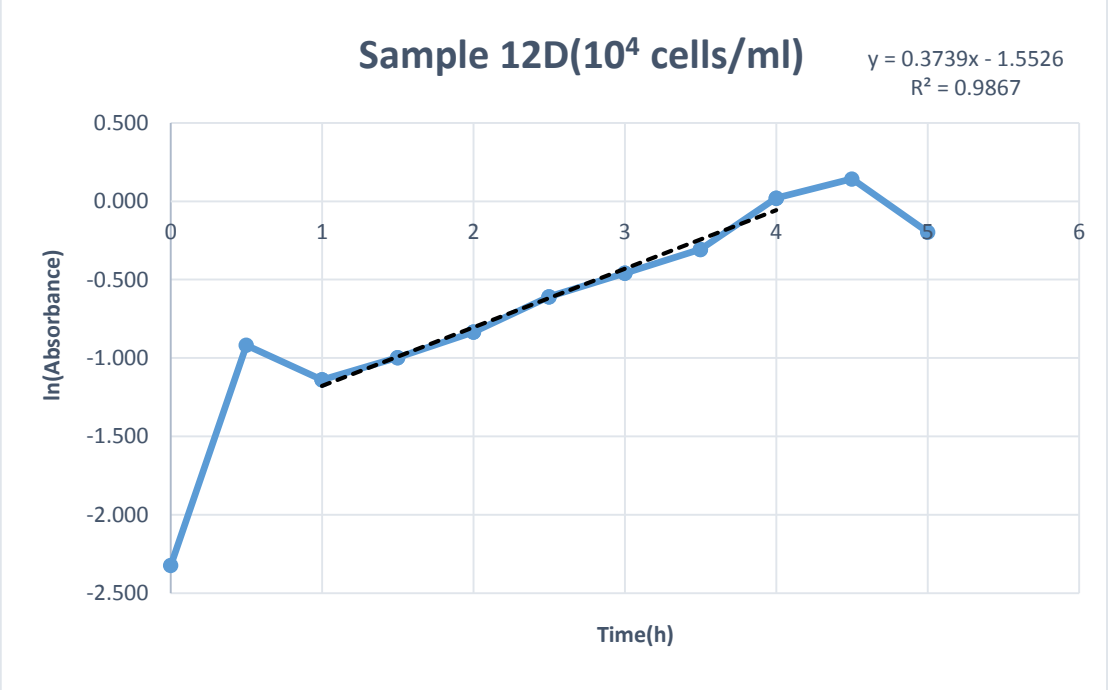

Figure 10.8: Absorbance reading at 30 min interval for testing planktonic growth rate in TSB for Sample 12D with 10^4cells/ml cell concentration under 55°c for 5 h.
The dashed line is the trend line for calculating the growth rate;

Appendix 11 Planktonic Growth Rate in UHT Milk (Sample 7, 7D and 12, 12D)

INFO1	Time (h)	Detection Time M (h)	Log Count Converted (cfu/ml)
Sample 7 T0	0	4.38	5.24
Sample 7 T1	1	3.48	6.49
Sample 7 T2	2	2.46	7.91
Sample 7 T2.5	2.5	1.61	9.10
Sample 7 T3	3	1.22	9.64
Sample 7 T3.5	3.5	1.22	9.64
Sample 7 T4	4	1.33	9.49
Sample 7 T4.5	4.5	1.05	9.88
Sample 7 T5	5	1.05	9.88
Sample 7 T6.5	6.5	1.18	9.70
Sample 7 T8.5	8.5	1.44	9.33
Sample 7D T0	0	4.43	5.17
Sample 7D T1	1	3.53	6.42
Sample 7D T2	2	2.41	7.98
Sample 7D T2.5	2.5	1.39	9.40
Sample 7D T3	3	1.45	9.32
Sample 7D T3.5	3.5	0.95	10.02
Sample 7D T4	4	1.32	9.50
Sample 7D T4.5	4.5	1.05	9.88
Sample 7D T5	5	1.07	9.85
Sample 7D T6.5	6.5	1.19	9.68
Sample 7D T8.5	8.5	1.46	9.31

Table 11.1: Sample 7 and duplicate 7D BacTrac readings (Detection Time M) at 30 min intervals for testing planktonic growth rate in UHT milk under 55°c water bath for 8.5 h;

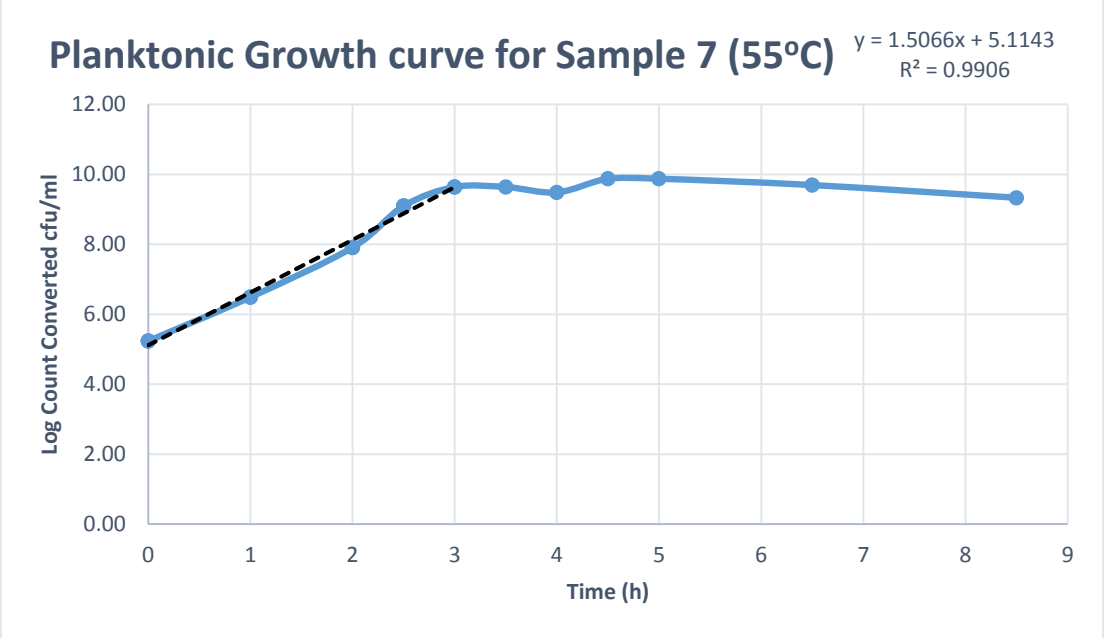

Figure 11.1: Sample 7 BacTrac readings (Detection Time M) at 30 min intervals for testing planktonic growth rate in UHT milk under 55°C water bath for 8.5 h. The trend line is for calculation of the maximum specific growth rate;

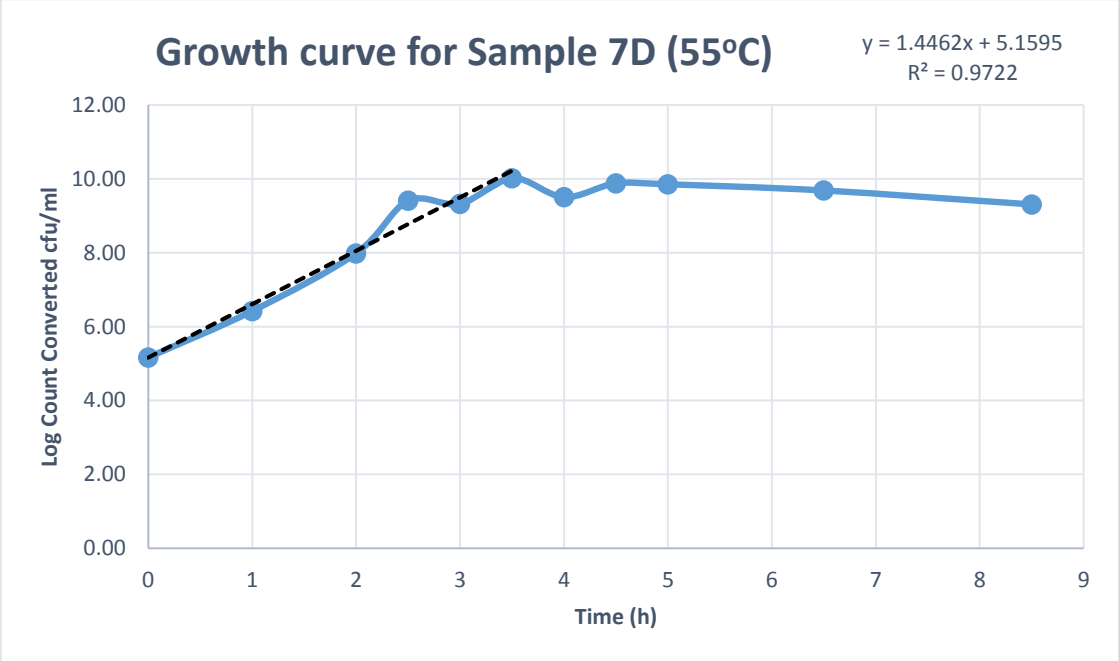

Figure 11.2: Sample 7D BacTrac readings (Detection Time M) at 30 min intervals for testing planktonic growth rate in UHT milk under 55°C water bath for 8.5 h. The trend line is for calculation of the maximum specific growth rate;

INFO1	Time (h)	Detection Time M (h)	Log Count Converted (cfu/ml)
Sample 12 T0	0	7.91	2.55
Sample 12 T1	1	6.01	4.25
Sample 12 T2	2	4.84	5.30
Sample 12 T2.5	2.5	4.61	5.51
Sample 12 T3	3	4.45	5.65
Sample 12 T3.5	3.5	3.81	6.22
Sample 12 T4	4	3.64	6.37
Sample 12 T4.5	4.5	2.61	7.30
Sample 12 T5	5	2.75	7.17
Sample 12 T6.5	6.5	2.26	7.61
Sample 12 T8.5	8.5	2.18	7.68
Sample 12D T0	0	7.92	2.54
Sample 12D T2	2	5.04	5.12
Sample 12D T2.5	2.5	4.46	5.64
Sample 12D T3	3	4.05	6.01
Sample 12D T3.5	3.5	3.72	6.30
Sample 12D T4	4	4.21	5.86
Sample 12D T4.5	4.5	2.43	7.46
Sample 12D T5	5	2.26	7.61
Sample 12D T6.5	6.5	2.2	7.66
Sample 12D T8.5	8.5	1.98	7.86

Table 11.3: Sample 12 and duplicate 12D BacTrac readings (Detection Time M) at 30 min intervals for testing planktonic growth rate in UHT milk under 55^{o}c water bath for 8.5 h;

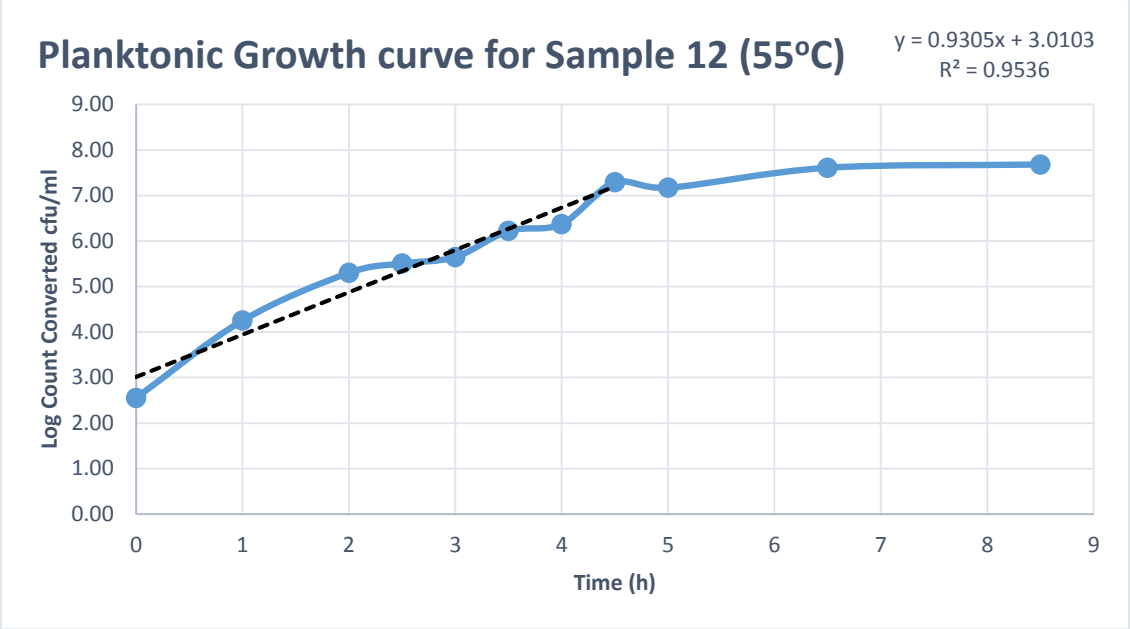

Figure 11.3: Sample 12 BacTrac readings (Detection Time M) at 30 min intervals for testing growth rate in UHT milk under 55°C water bath for 8.5 h. **The trend line is for calculation of the maximum specific growth rate;**

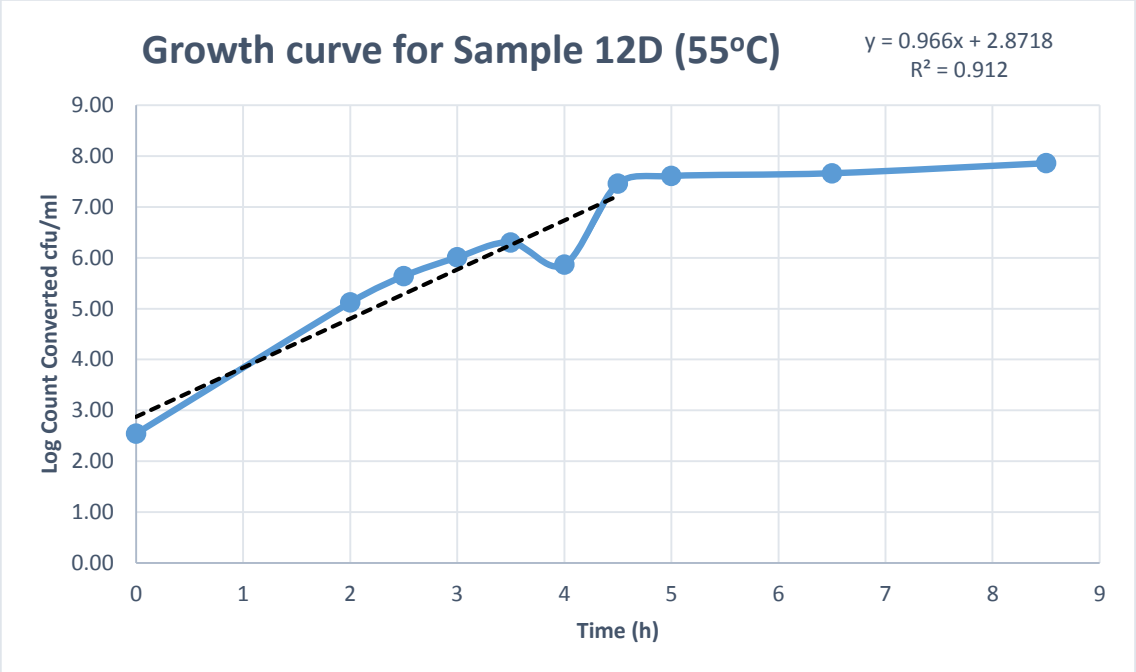

Figure 11.4: Sample 12D BacTrac readings (Detection Time M) at 30 min intervals for testing growth rate in UHT milk under 55°C water bath for 8.5 h. **The trend line is for calculation of the maximum specific growth rate;**

Appendix 12 Growth Rate in UHT Milk for Isolate 1 and Isolate 2

INFO1	Time (h)	Detection Time M (h)	Log Count Converted (cfu/ml)
Milk Isolate 1 T0	0	5.76	5.98
Milk Isolate 1 T1	1	4.88	6.78
Milk Isolate 1 T2	2	3.68	7.88
Milk Isolate 1 T2.5	2.5	3.12	8.39
Milk Isolate 1 T3	3	2.49	8.97
Milk Isolate 1 T3.5	3.5	1.92	9.49
Milk Isolate 1 T4	4	1.73	9.67
Milk Isolate 1 T4.5	4.5	1.18	10.17
Milk Isolate 1 T5	5	1.07	10.27
Milk Isolate 1 T6	6	1.13	10.22
Milk Isolate 1 T8	8	1.08	10.26
Milk Isolate 1D T0	0	5.7	6.03
Milk Isolate 1D T1	1	4.85	6.81
Milk Isolate 1D T2	2	4.13	7.47
Milk Isolate 1D T2.5	2.5	3.29	8.24
Milk Isolate 1D T3	3	2.66	8.82
Milk Isolate 1D T3.5	3.5	2.11	9.32
Milk Isolate 1D T4	4	1.81	9.59
Milk Isolate 1D T4.5	4.5	1.11	10.24
Milk Isolate 1D T5	5	0.97	10.36
Milk Isolate 1D T6	6	1.13	10.22
Milk Isolate 1D T8	8	2	9.42

Table 12.1: Isolate 1 and duplicate 1D BacTrac readings (Detection Time M) for testing planktonic growth rate in UHT milk under 55°C water bath for 8 h; Milk Isolate 1/1D = Geo1;

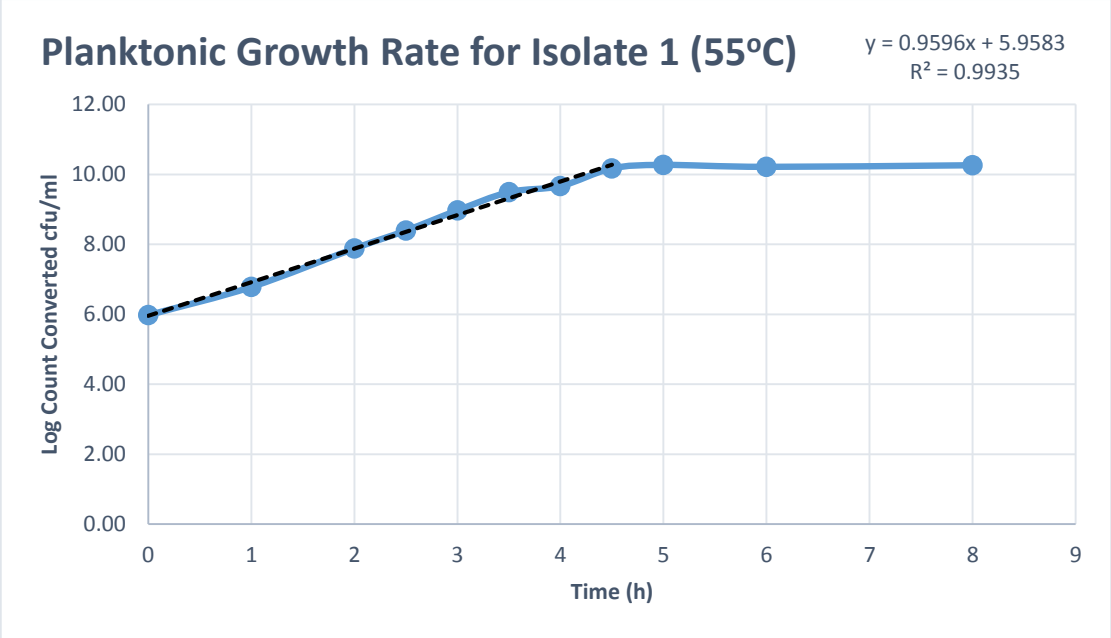

Figure 12.1: Growth curve for Isolate 1 growth in UHT Milk under 55°C water bath for 8 h. **The trend line is for calculation of the maximum specific growth rate; Isolate 1/1D = Geo1;**

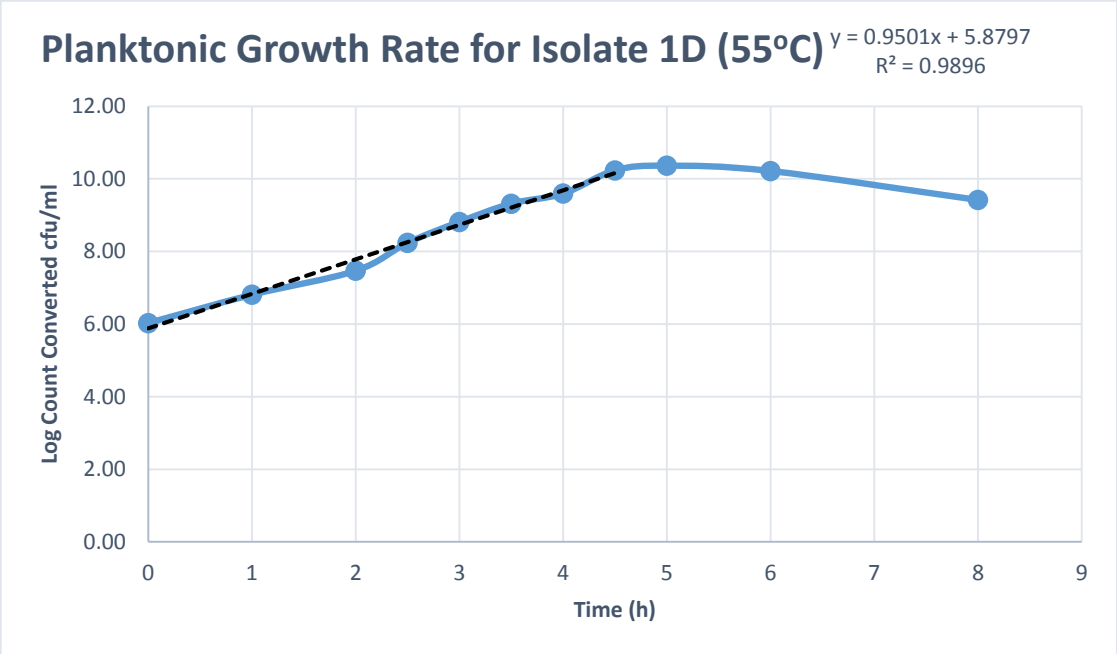

Figure 12.2: Growth curve for Isolate 1D growth in UHT milk under 55°C water bath for 8 h. **The trend line is for calculation of the maximum specific growth rate; Isolate 1/1D = Geo1;**

INFO1	Time (h)	Detection Time M (h)	Log Count Converted (cfu/ml)
Milk Isolate 2 T0	0	6.21	5.96
Milk Isolate 2 T1	1	5.19	6.69
Milk Isolate 2 T2	2	3.77	7.70
Milk Isolate 2 T2.5	2.5	3.45	7.93
Milk Isolate 2 T3	3	2.95	8.28
Milk Isolate 2 T3.5	3.5	2.24	8.79
Milk Isolate 2 T4	4	1.83	9.08
Milk Isolate 2 T4.5	4.5	1.32	9.44
Milk Isolate 2 T5	5	0.99	9.68
Milk Isolate 2 T6	6	1.04	9.64
Milk Isolate 2 T8	8	1.03	9.65
Milk Isolate 2D T0	0	6.11	6.03
Milk Isolate 2D T1	1	5.18	6.70
Milk Isolate 2D T2	2	4.14	7.44
Milk Isolate 2D T2.5	2.5	3.5	7.89
Milk Isolate 2D T3	3	2.88	8.33
Milk Isolate 2D T3.5	3.5	2.11	8.88
Milk Isolate 2D T4	4	1.82	9.09
Milk Isolate 2D T4.5	4.5	0.36	10.13
Milk Isolate 2D T5	5	1.03	9.65
Milk Isolate 2D T6	6	1.09	9.61
Milk Isolate 2D T8	8	0.98	9.68

Table 12.2: Isolate 2 and duplicate 2D BacTrac readings (Detection Time M) for testing growth rate in UHT milk under 55^{o}c water bath for 8 h; Milk Isolate 2/2D = Anoxy2;

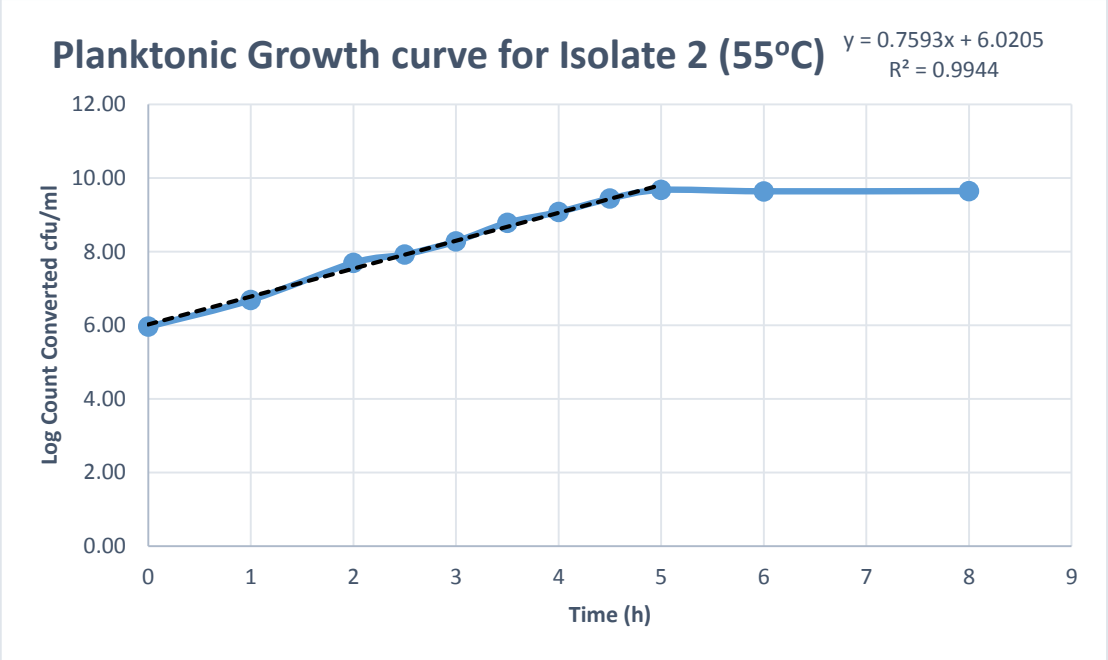

Figure 12.3: Growth curve for Isolate 2 Growth in UHT milk under 55°C water bath for 8 h. **The trend line is for calculation of the maximum specific growth rate; Isolate 2/2D = Anoxy2;**

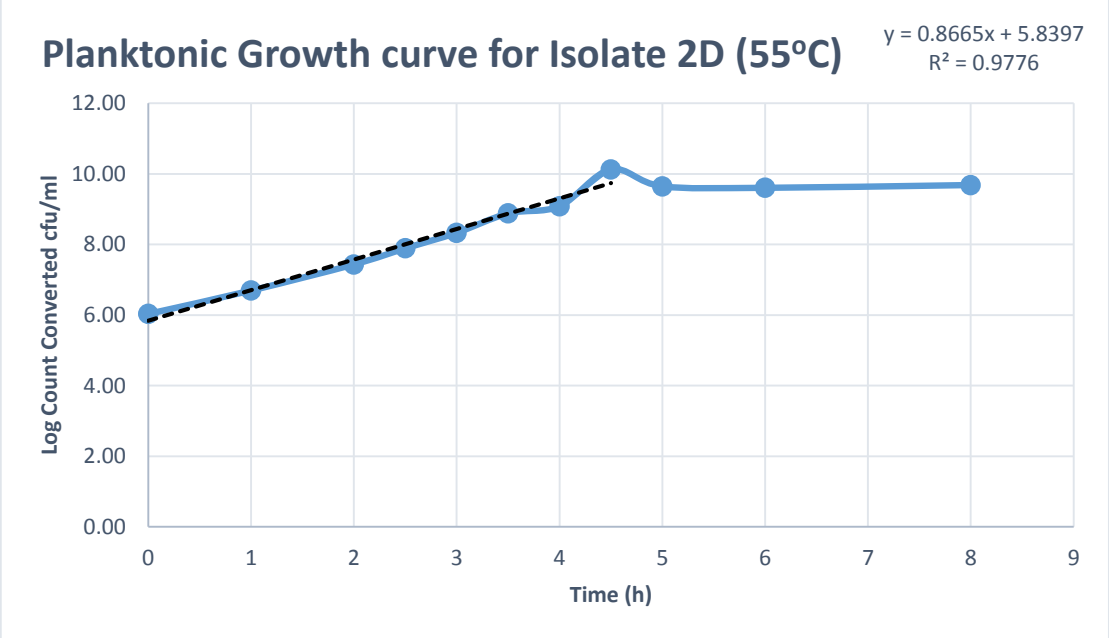

Figure 12.4: Growth curve for Isolate 2D growth in UHT Milk under 55°C water bath for 8 h. **The trend line is for calculation of the maximum specific growth rate; Isolate 2/2D = Anoxy 2**

Appendix 13 Testing Boundary conditions of experiments using BacTrac

Testing the boundary conditions of experiments of testing planktonic growth of different isolates in different % of solids of RSM and different temperatures by comparing BacTrac readings at time 0 and time 8 h. A decrease in Detection Time M reading means 'growth' whereas an increase in the Detection Time M reading means 'no growth'.

TESTING BOUNDARY CONDITIONS OF EXPERIMENTS 1 (40°C, 70°C, 10%, 40%)			
	Detection Time M (h)		
SAMPLE	T0	T8	Growth of not
Geo1 40°C 10%	6.42	5.64	YES
Geo1 40°C 40%	5.64	7.3	NO
Geo1 70°C 10%	6.45	10.21	NO
Geo1 70°C 40%	5.72	10.56	NO
Anoxy2 40°C 10%	9.66	5.88	YES
Anoxy2 40°C 40%	5.87	0.48	YES
Anoxy2 70°C 10%	6.57	2.3	YES
Anoxy2 70°C 40%	6.09	7.65	NO

Table 13.1: Testing boundary conditions of experiments for Geo1 and Anoxy2 growth in RSM with 10% and 40% of solids under 40°C and 70°C for 8 h;

TESTING BOUNDARY CONDITIONS OF EXPERIMENTS 2 (40°C, 65°C, 10%, 35%)			
	Detection Time M(h)		
SAMPLES	T0	T8	Growth or not
Geo1 40°C 10%	3.98	1.89	YES
Geo1 40°C 35%	4.79	6.62	NO
Geo1 65°C 10%	3.98	3.34	YES
Geo1 65°C 35%	4.37	5	NO
Anoxy2 40°C 10%	4.01	1.79	YES
Anoxy2 40°C 35%	4.26	6.03	NO
Anoxy2 65°C 10%	4.08	2.78	YES
Anoxy2 65°C 35%	4.24	5.15	NO

Table 13.2: Testing boundary conditions of experiments for Geo1 and Anoxy2 growth in RSM with 10% and 35% of solids under 40°C and 65°C for 8 h; (means 'not detected');**

TESTING BOUNDARY CONDITIONS OF EXPERIMENTS 3 (40°C, 60°C, 10%, 30%)			
	Detection Time M (h)		
SAMPLES	T0	T8	Growth or not
Geo1 40°C 10%	3.66	2.16	YES
Geo1 40°C 30%	4.23	7.4	NO
Geo1 60°C 10%	1.85	2.72	NO
Geo1 60°C 30%	4.19	4.11	YES
Anoxy2 40°C 10%	4.01	1.77	YES
Anoxy2 40°C 30%	4.51	1.43	YES
Anoxy2 60°C 10%	4.12	2.62	YES
Anoxy2 60°C 30%	4.31	4.3	NO

Table 13.3: Testing boundary conditions of experiments for Geo1 and Anoxy2 growth in RSM with 10% and 30% of solids under 40°C and 60°C for 8 h; (means 'not detected');**

TESTING BOUNDARY CONDITIONS OF EXPERIMENTS 4 (40°C, 60°C, 10%, 30%)			
	Detection Time M(h)		
SAMPLES	T0	T8	Growth or not
Geo1 40°C 10%	4.16	2.39	YES
Geo1 40°C 30%	3.54	6.57	NO
Geo1 60°C 10%	3.71	2.65	YES
Geo1 60°C 30%	3.59	3.74	NO
Anoxy2 40°C 10%	3.71	1.51	YES
Anoxy2 40°C 30%	3.87	6.73	NO
Anoxy2 60°C 10%	3.7	2.58	YES
Anoxy2 60°C 30%	13.78	5.2	YES

Table 13.4: Testing boundary conditions of experiments for Geo1 and Anxoy2 growth in RSM with 10% and 30% of solids under 40°C and 60°C for 8 h;

TESTING BOUNDARY CONDITIONS OF EXPERIMENTS 5 (40°C, 60°C, 10%, 20%, 30%)			
	New Detection Time M		
SAMPLES	T0	T8	GROWTH
Anoxy2 60°C 30% R1	6.1	10.36	NO
Anoxy2 60°C 30% R2	5.92	8.61	NO
Anoxy2 60°C 30% R3	6.18	8.26	NO
Anoxy2 40°C 30% R1	5.63	8.43	NO
Anoxy2 40°C 30% R2	5.85	9.24	NO
Anoxy2 40°C 30% R3	5.7	8.95	NO
Geo1 40°C 20% R1	5.69	6.11	NO
Geo1 40°C 20% R2	5.75	5.73	YES
Geo1 40°C 20% R3	5.52	5.92	NO
Geo1 60°C 20% R1	5.81	**	?
Geo1 60°C 20% R2	5.54	6.82	NO
Geo1 60°C 20% R3	5.73	5.14	YES
Anoxy2 40°C 20% R1	4.95	6.05	NO
Anoxy2 40°C 20% R2	5.34	5.97	NO
Anoxy2 40°C 20% R3	4.98	5.91	NO
Anoxy2 60°C 20% R1	5.39	4.02	YES
Anoxy2 40°C 20% R2	5.24	4.91	YES
Anoxy2 40°C 20% R3	5.44	**	?
Geo1 60°C 10% R1	5.46	2.88	YES
Geo1 60°C 10% R2	5.32	2.47	YES
Geo1 60°C 10% R3	5.46	2.97	YES

Table 13.5: Testing boundary conditions of experiments for Geo1 and Anxy2 growth in RSM with 10%, 20% and 30% of solids under 40°C and 60°C for 8 h; (means 'not detected');**

TESTING BOUNDARY CONDITIONS OF EXPERIMENTS 6 (40°C, 60°C, 15%, 30%)			
	Detection Time M (h)		
SAMPLES	T0	T8	Growth or not
Geo1 40°C 15% R1	5.82	2.91	YES
Geo1 40°C 15% R2	5.11	2.8	YES
Geo1 40°C 15% R3	5.12	5.74	NO
Geo1 60°C 15% R1	5.29	2.38	YES
Geo1 60°C 15% R2	5.15	2.5	YES
Geo1 60°C 15% R3	2.22	2.51	NO
Isolate 12 40°C 15% R1	6.88	7.07	NO
Isolate 12 40°C 15% R2	7.72	7.47	YES
Isolate 12 40°C 30% R1	13.8	**	?
Isolate 12 40°C 30% R2	2.52	**	?
Isolate 12 60°C 15% R1	7.72	1.95	YES
Isolate 12 60°C 15% R2	8.09	2.35	YES
Isolate 12 60°C 30% R1	1.39	8.35	NO
Isolate 12 60°C 30% R2	11.05	9.2	YES
Anoxy2 40°C 15% R1	2.46	2.7	NO
Anoxy2 40°C 15% R2	5.14	2.65	YES
Anoxy2 40°C 15% R3	5.22	2.59	YES
Anoxy2 60°C 15% R1	5.35	2.46	YES
Anoxy2 60°C 15% R2	4.9	2.42	YES
Anoxy2 60°C 15% R3	5.1	2.48	YES
Isolate 7 40°C 15% R1	3.75	2.03	YES
Isolate 7 40°C 15% R2	3.66	1.67	YES
Isolate 7 40°C 30% R1	4.66	7.23	NO
Isolate 7 40°C 30% R2	4.26	5.85	NO
Isolate 7 60°C 15% R1	3.75	2.57	YES
Isolate 7 60°C 15% R2	3.73	2.65	YES
Isolate 7 60°C 30% R1	4.12	4.6	NO
Isolate 7 60°C 30% R2	3.76	4.42	NO

Table 13.6: Testing boundary conditions of experiments for Geo1 and Anoxy2, Sample 7 and 12 growth in RSM with 15% and 30% of solids under 40°C and 60°C for 8 h; (means 'not detected');**

TESTING BOUNDARY CONDITIONS OF EXPERIMENTS 7 (40°C, 60°C, 70°C, 18%, 25%)			
	Detection Time M (h)		
SAMPLES	T0	T8	Growth or not
Geo1 40°C 18% R1	3.81	2.25	YES
Geo1 40°C 18% R2	4.22	2.34	YES
Geo1 40°C 18% R3	4.25	2.03	YES
Geo1 60°C 18% R1	4.1	10.18	NO
Geo1 60°C 18% R2	4.3	2.8	YES
Geo1 60°C 18% R3	0.32	2.48	NO
Anoxy2 40°C 18% R1	0.7	2.1	NO
Anoxy2 40°C 18% R2	3.08	2.1	YES
Anoxy2 40°C 18% R3	3.15	2.15	YES
Anoxy2 60°C 18% R1	3	0.43	YES
Anoxy2 60°C 18% R2	2.92	2.69	YES
Anoxy2 60°C 18% R3	3.15	2.83	YES
Anoxy2 70°C 18% R1	3.99	9.92	NO
Anoxy2 70°C 18% R2	3.34	9.87	NO
Anoxy2 70°C 18% R3	2.94	**	?
Isolate 7 40°C 25% R1	3.09	2.73	YES
Isolate 7 40°C 25% R2	3.18	2.76	YES
Isolate 7 40°C 25% R3	3.19	2.78	YES
Isolate 7 60°C 25% R1	3.04	3.98	NO
Isolate 7 60°C 25% R2	3.47	3.99	NO
Isolate 7 60°C 25% R3	3.63	4.5	NO
Isolate 12 40°C 25% R1	4.81	6.82	NO
Isolate 12 40°C 25% R2	4.7	7.2	NO
Isolate 12 40°C 25% R3	4.53	6.52	NO
Isolate 12 60°C 25% R1	4.63	2.34	YES
Isolate 12 60°C 25% R2	0.98	2.35	NO
Isolate 12 60°C 25% R3	4.77	2.63	YES

Table 13.7: Testing boundary conditions of experiments for Isolate 1 and Anoxy2, Sample 7 and 12 growth in Milk with 18% and 25% of solids under 40°C, 60°C and 70°C for 8 h; (means 'not detected');**

TESTING BOUNDARY CONDITIONS OF EXPERIMENTS 8 (40°C, 60°C, 70°C, 10%, 20%)			
	Detection Time M (h)		
SAMPLES	T0	T8	Growth or not
Isolate 7 40°C 20% R1	3.78	2.47	YES
Isolate 7 40°C 20% R2	3.77	2.5	YES
Isolate 7 40°C 20% R3	3.63	0.25	YES
Isolate 7 60°C 20% R1	3.56	2.77	YES
Isolate 7 60°C 20% R2	6.42	2.77	YES
Isolate 7 60°C 20% R3	3.6	2.78	YES
Isolate 12 40°C 20% R1	5.88	9.14	NO
Isolate 12 40°C 20% R2	5.4	7.69	NO
Isolate 12 40°C 20% R3	4.21	3.66	YES
Isolate 12 60°C 20% R1	5.87	2.12	YES
Isolate 12 60°C 20% R2	5.19	2.49	YES
Isolate 12 60C° 20% R3	5.59	2.49	YES
Isolate 12 70C° 20% R1	4.7	3.14	YES
Isolate 12 70°C 20% R2	5.62	3.24	YES
Isolate 12 70°C 20% R3	5.5	3.6	YES

Table 13.8: Testing boundary conditions of experiments for Sample 7 and 12 growth in Milk with 10% and 20% of solids under 40°C, 60°C and 70°C for 8 h; (means 'not detected');**

Appendix 14 CDC Factorial Runs Geo1

Reading 1, 2 and 3 are triplicate plates plated from the same coupon. Two Coupons (A and B) were sampled at each time interval. The coupons were dislodged using glass beads in TSB. Then the TSB is enumerated using spiral plater. The table are the calculated ln (actual plate count) data. The graph is the incubation time vs. the mean of the ln (mean three readings).

Geo1 CDC Reactor Run 40°C / 10% of Solids						
ln(Actual Plate Count)	**A SAMPLE**			**B SAMPLE**		
Time	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
(h)	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml
1.5	2.30	2.30	2.30	2.30	3.00	2.30
3	2.30	3.00	2.30	3.00	3.00	2.30
4	3.40	3.00	3.40	3.69	3.40	3.40
5	4.09	4.09	3.91	3.91	4.25	3.91
6	4.61	4.50	4.70	4.38	4.50	4.61
7	5.30	5.30	4.61	5.70	5.30	4.61
8	5.30	5.70	5.30	5.70	5.70	5.30
9	6.91	7.60	6.91	6.91	7.60	7.60
10	9.21	9.21	9.21	9.21	9.90	9.21
11	9.21	9.90	9.90	9.90	9.21	9.21
12	10.31	9.90	9.21	9.90	9.90	9.21

Table 14.1: Mean ln (Plate count of the coupon) of Geo1 CDC Reactor Run under 40°C with 10% of Solids RSM for 12 h.

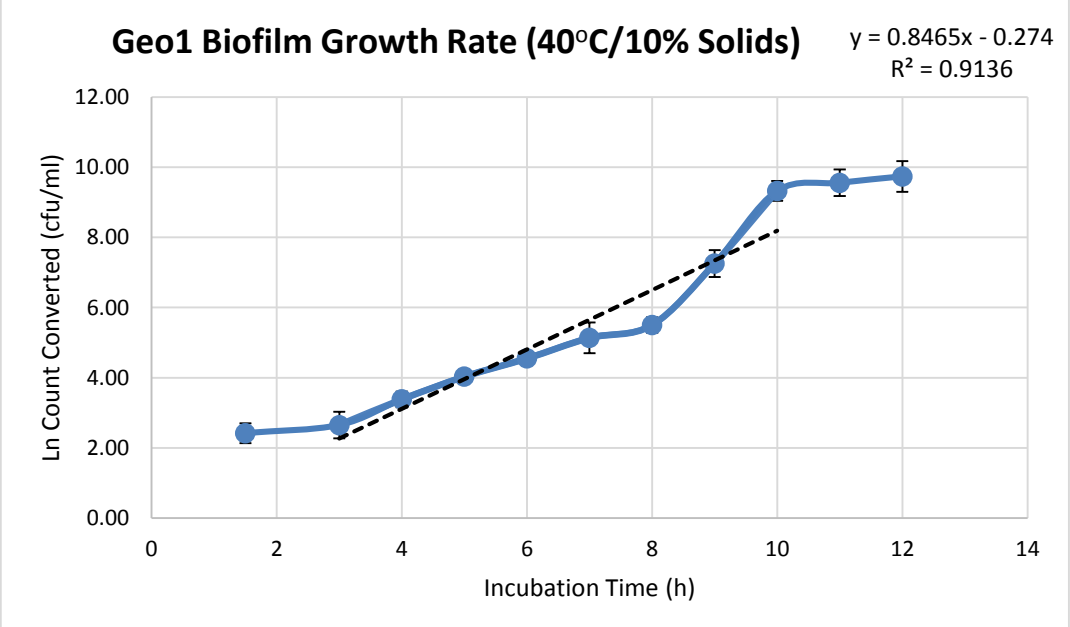

Figure 14.1: Mean ln (Plate count of the coupon) of Geo1 CDC Reactor Run under 40°C with 10% of Solids RSM for 12 h. (Error bars are representing +/– one standard deviation of 6 readings of the same sampling time, dashed line is for calculating the growth rate);

Geo1 CDC Reactor Run 50°C / 10% of Solids						
ln (Actual Plate Count)			**A SAMPLE**	**B SAMPLE**		
Time	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
(h)	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml
1.5	<1	3.69	3.00	<1	2.30	2.30
3	6.04	6.23	6.11	6.21	6.09	5.70
4	7.31	7.19	7.42	7.24	7.28	6.85
5	8.79	8.68	8.88	8.85	8.90	8.92
6	8.85	9.10	9.39	9.55	8.99	9.31
7	12.61	12.90	9.90	12.21	11.51	12.21
8	13.08	13.13	13.13	12.99	12.96	13.18
9	14.29	14.21	13.91	13.91	14.08	14.05
10	15.82	15.73	15.78	15.80	15.75	15.78
11	16.00	16.21	16.20	16.00	16.03	16.08
12	16.13	15.89	15.98	16.03	16.01	15.96

Table 14.2: Mean ln (Plate count of the coupon) of Geo1 CDC Reactor Run under 50°C with 10% of Solids RSM for 12 h.

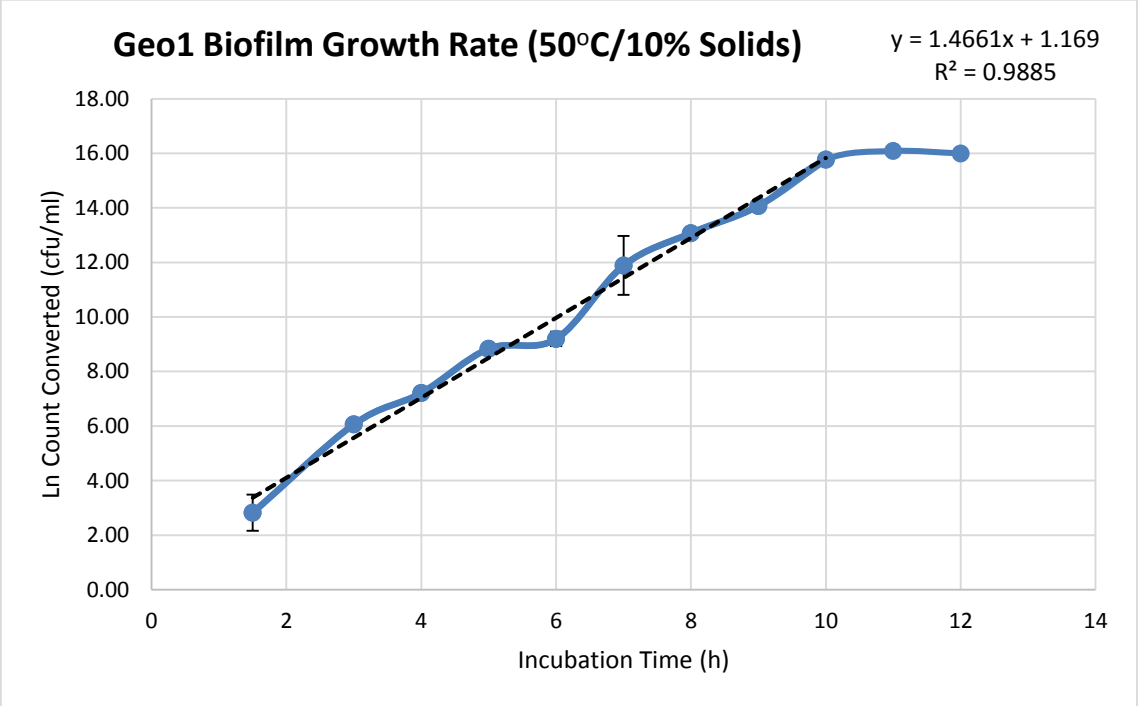

Figure 14.2: Mean ln (Plate count of the coupon) of Geo1 CDC Reactor Run under 50°C with 10% of Solids Milk for 12 h. (Error bars are representing +/– one standard deviation of 6 readings of the same sampling time, dashed line is for calculating the growth rate)

Geo1 CDC Reactor Run 60°C / 10% of Solids						
ln(Actual Plate Count)			**A SAMPLE**		**B SAMPLE**	
Time	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
(h)	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml
1.5	7.66	7.64	7.58	7.61	7.64	7.53
3	10.86	11.05	10.97	10.80	10.85	10.90
4	11.18	11.33	11.23	11.28	11.24	11.26
5	11.81	11.96	11.82	12.25	12.35	12.15
6	13.47	13.50	13.43	13.45	13.52	13.49
7	14.22	14.14	14.17	14.33	14.30	14.25
8	14.69	14.56	14.69	14.54	14.46	14.69
9	15.12	15.15	14.68	15.09	15.05	15.12
10	15.07	14.98	15.01	14.98	15.12	14.88
11	15.23	15.07	15.12	15.15	15.25	14.91
12	15.25	14.91	14.98	15.20	15.04	15.25

Table 14.3: Mean ln (Plate count of the coupon) of Geo1 CDC Reactor Run under 60°C with 10% of Solids RSM for 12 h.

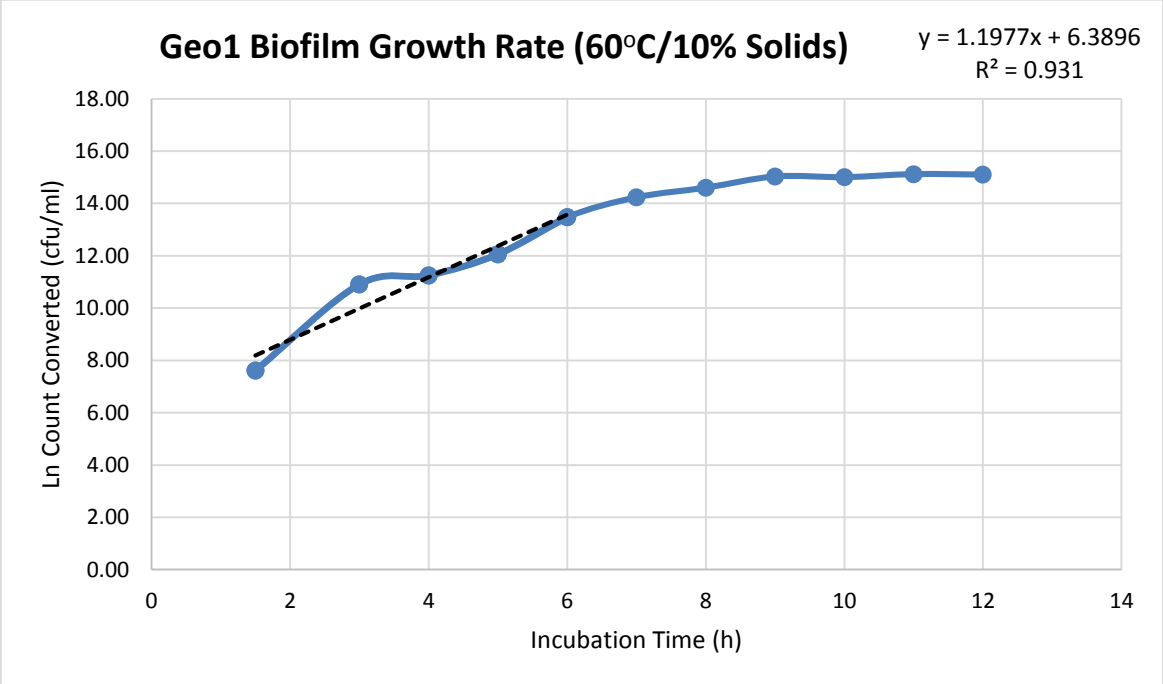

Figure 14.3: Mean ln(Plate count of the coupon) of Geo1 CDC Reactor Run under 60°C with 10% of Solids RSM for 12 h. (Error bars are representing +/– one standard deviation of 6 readings of the same sampling time, dashed line is for calculating the growth rate);

Geo1 CDC Reactor Run 50°C / 14% of Solids						
ln (Actual Plate Count)		**A SAMPLE**		**B SAMPLE**		
Time	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
(h)	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml
1.5	5.01	5.02	4.77	5.35	4.70	4.87
3	4.28	5.28	5.71	5.28	5.33	5.72
4	5.36	5.17	5.21	5.34	5.32	5.91
5	6.48	6.52	6.52	6.48	6.47	6.53
6	9.13	9.24	9.46	9.06	9.73	9.41
7	8.81	8.87	8.84	8.68	8.79	8.82
8	11.83	11.63	11.57	12.17	12.14	12.03
9	11.36	11.30	11.26	11.37	11.23	11.42
10	13.10	13.27	13.32	10.97	13.37	13.22
11	13.14	13.08	13.18	13.10	13.37	12.95
12	13.57	13.62	13.70	13.60	13.57	13.72

Table 14.4: Mean ln (Plate count of the coupon) Geo1 CDC Reactor Run under 50°C with 14% of Solids RSM for 12 h.

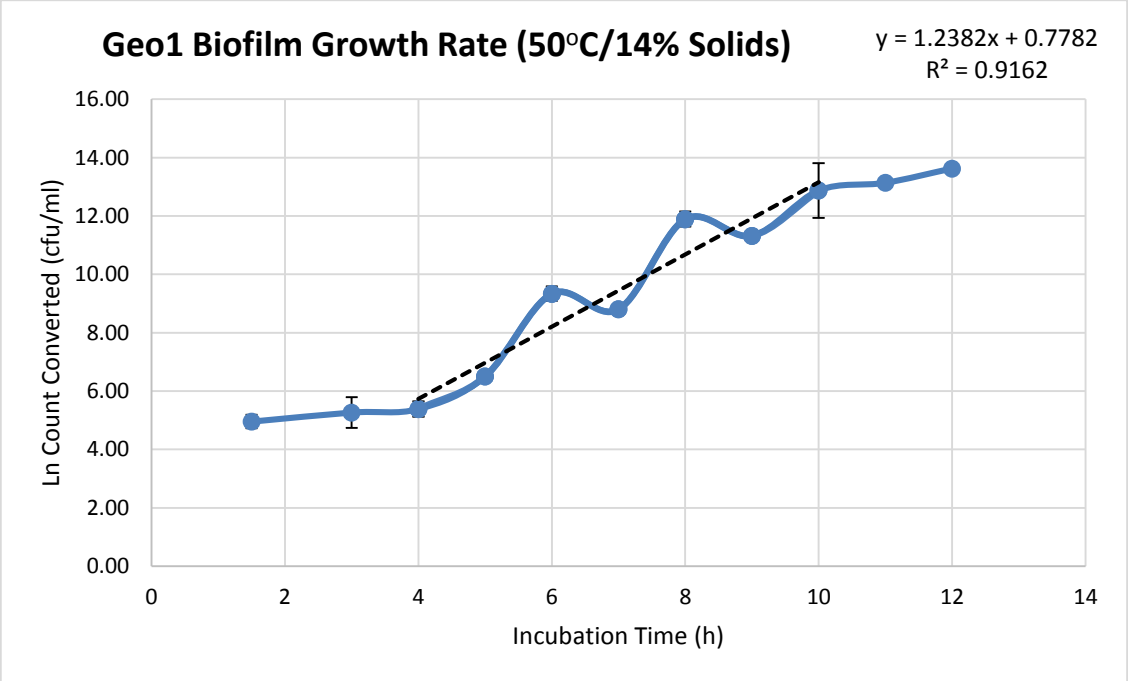

Figure 14.4: Mean ln (Plate count of the coupon) of Geo1 CDC Reactor Run under 50°C with 14% of Solids RSM for 12 h. (Error bars are representing +/– one standard deviation of 6 readings of the same sampling time, dashed line is for calculating the growth rate);

Geo1 CDC Reactor Run 60°C / 14% of Solids						
ln(Actual Plate Count)			**A SAMPLE**	**B SAMPLE**		
Time	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
(h)	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml
1.5	6.17	6.43	6.48	6.52	6.57	6.46
3	7.50	7.74	7.86	8.07	7.55	7.82
4	8.67	8.75	8.78	8.84	8.92	8.52
5	10.31	9.95	9.21	9.80	10.13	10.37
6	10.09	10.55	10.95	9.95	9.62	10.24
7	11.78	12.30	11.51	12.47	11.98	11.70
8	14.14	14.05	13.95	13.85	14.14	13.96
9	14.41	14.47	14.23	14.36	14.41	14.26
10	12.95	13.20	13.34	13.08	12.95	13.16
11	13.43	13.34	13.55	13.27	13.50	13.34
12	14.11	13.80	14.05	14.01	13.99	13.82

Table 14.5: Mean ln (Plate count of the coupon) of Geo1 CDC Reactor Run under 60°C with 14% of Solids RSM for 12 h.

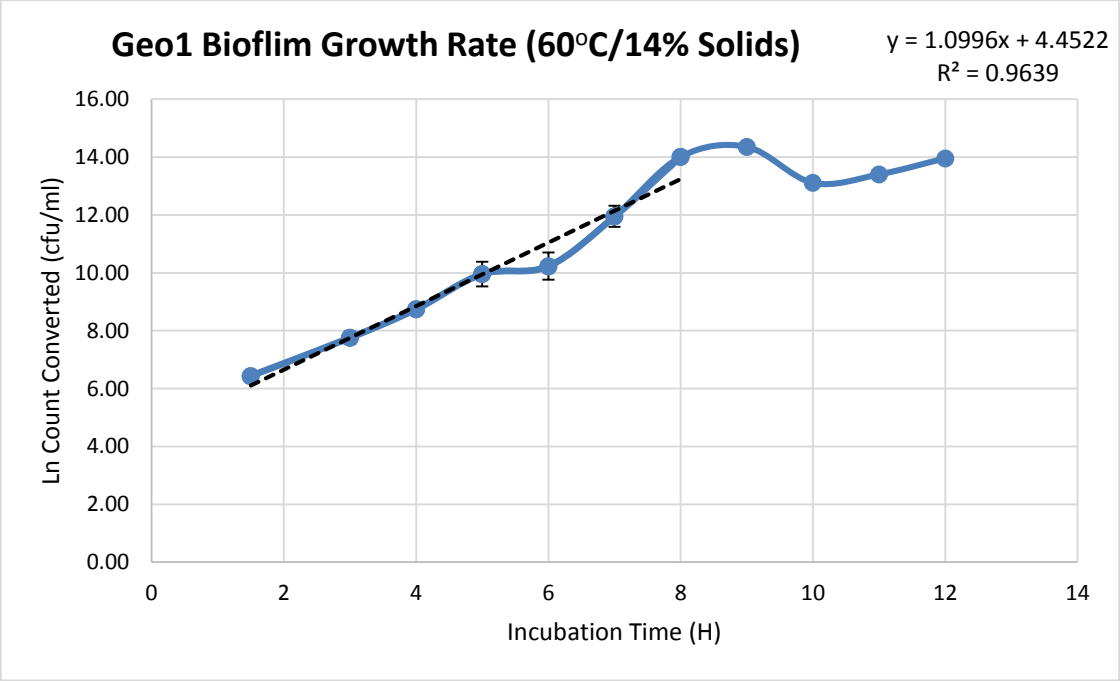

Figure 14.5: Mean ln (Plate count of the coupon) of Geo1 CDC Reactor Run under 60°C with 14% of Solids RSM for 12 h. (Error bars are representing +/– one standard deviation of 6 readings of the same sampling time, dashed line is for calculating the growth rate);

Appendix 15 Minitab Output of Geo1 (Factorial Regression)

Factorial Fit: Growth Rate versus Temperature C, Solids%

```
* NOTE * data in the worksheet do not appear to match the
center point column.
* NOTE * This design has some botched runs.  It will be
analyzed using a
         regression approach.

Estimated Effects and Coefficients for Growth Rate (coded
units)

Term                    Effect     Coef  SE Coef      T      P
Constant                         0.6498   0.1339   4.85  0.005
Temperature C           0.4836   0.2418   0.1640   1.47  0.201
Solids%                -1.1700  -0.5850   0.1640  -3.57  0.016
Temperature C*Solids%  -0.1756  -0.0878   0.2009  -0.44  0.680


S = 0.401812   R-Sq = 75.10%   R-Sq(adj) = 60.16%


Analysis of Variance for Growth Rate (coded units)

Source              DF   Seq SS   Adj SS   Adj MS     F      P
Main Effects         2  2.40411  2.40411  1.20205  7.45  0.032
2-Way Interactions   1  0.03084  0.03084  0.03084  0.19  0.680
Residual Error       5  0.80727  0.80727  0.16145
Total                8  3.24221


Estimated Coefficients for Growth Rate using data in uncoded
units

Term                         Coef
Constant                  -0.04808
Temperature C            0.0549083
Solids%                  -0.036500
Temperature C*Solids%  -0.00219500

* NOTE * Some factors have more than 2 levels, no alias table
was printed.
```

Appendix 16 Minitab Output of Geo1 (Response Regression)

Response Surface Regression: Growth Rate versus Temperature C, Solids%

The analysis was done using coded units.

Estimated Regression Coefficients for Growth Rate

Term	Coef	SE Coef	T	P
Constant	1.03093	0.2877	3.584	0.037
Temperature C	0.24178	0.1576	1.534	0.222
Solids%	-0.58500	0.1576	-3.713	0.034
Temperature C*Temperature C	-0.37745	0.2729	-1.383	0.261
Solids%*Solids%	-0.19420	0.2729	-0.712	0.528
Temperature C*Solids%	-0.08780	0.1930	-0.455	0.680

S = 0.3860 R-Sq = 86.2% R-Sq(adj) = 63.2%

Analysis of Variance for Growth Rate

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	2.79530	2.79530	0.55906	3.75	0.153
Linear	2	2.40411	2.40411	1.20205	8.07	0.062
Square	2	0.36036	0.36036	0.18018	1.21	0.412
Interaction	1	0.03084	0.03084	0.03084	0.21	0.680
Residual Error	3	0.44690	0.44690	0.14897		
Total	8	3.24221				

Estimated Regression Coefficients for Growth Rate using data in uncoded units

Term	Coef
Constant	-11.4822
Temperature C	0.4324
Solids%	0.3034
Temperature C*Temperature C	-0.0038
Solids%*Solids%	-0.0121
Temperature C*Solids%	-0.0022

Appendix 17 CDC Factorial Runs Anoxy2

Reading 1, 2 and 3 are triplicate plates plated from the same coupon. Two Coupons (A and B) were sampled at each time interval. The coupons were dislodged using glass beads in TSB. Then the TSB is enumerated using spiral plater. The table are the calculated ln (actual plate count) data. The graph is the incubation time vs. the mean of the ln (mean three readings).

Anoxy2 CDC Reactor Run 50°C / 10% of Solids						
ln(Actual Plate Count)		**A SAMPLE**		**B SAMPLE**		
Time	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
(h)	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml
1.5	4.09	4.50	4.94	4.38	3.69	3.91
3	6.62	6.51	6.78	6.79	6.27	6.13
4	5.14	5.30	5.44	5.30	5.19	5.74
5	5.30	4.61	5.30	5.30	5.70	5.30
6	5.99	5.70	5.30	5.70	5.30	5.70
7	10.04	10.71	10.43	10.43	10.11	10.46
8	10.78	10.93	11.20	11.18	11.13	10.90
9	11.70	11.90	11.98	11.80	11.96	11.85
10	12.77	12.71	12.58	13.02	12.69	12.62
11	11.78	11.16	11.92	12.04	12.25	11.85
12	12.68	12.35	12.30	12.21	12.31	12.71

Table 17.1: Mean ln (Plate count of the coupon) of Anoxy2 CDC Reactor Run under 50°C with 10% of Solids RSM for 12 h;

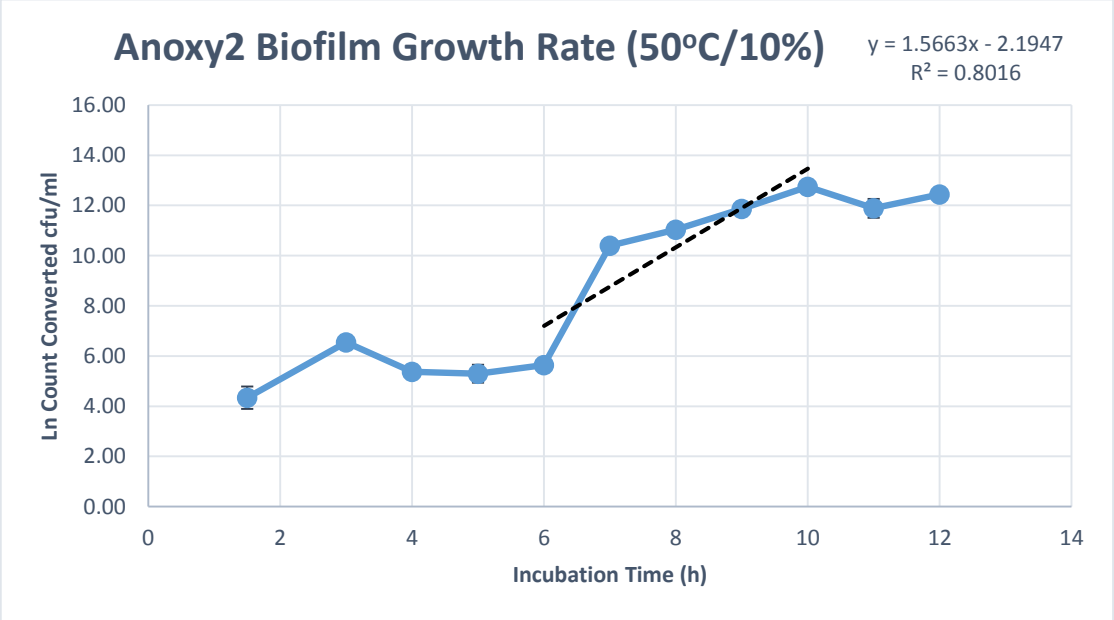

Figure 17.1: Mean ln (Plate count of the coupon) of Anoxy2 CDC Reactor Run under 50°C with 10% of Solids RSM for 12 h. (Error bars are representing +/– one standard deviation of 6 readings of the same sampling time, dashed line is for calculating the growth rate)

Anoxy2 CDC Reactor Run 60°C / 10% of Solids						
ln(Actual Plate Count)			A SAMPLE	B SAMPLE		
Time	Reading 1	Reading 2	Reading 3	Reading 1	Reading 2	Reading 3
(h)	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml	cfu/ml
1.5	<1	<1	<1	<1	<1	<1
3	5.52	5.56	5.67	5.74	4.94	5.08
4	5.99	7.09	5.99	6.91	6.68	7.09
5	5.30	5.99	5.70	6.11	5.83	5.44
6	5.99	6.40	6.11	5.77	6.06	5.48
7	8.52	8.01	8.29	8.29	8.70	8.29
8	11.07	10.74	10.86	10.74	10.95	10.55
9	11.84	11.35	11.60	11.48	11.74	11.53
10	11.51	11.00	11.00	11.70	11.61	11.29
11	11.92	12.43	12.10	12.43	12.25	12.15
12	12.74	12.58	12.64	12.54	12.47	12.61

Table 17.2: Mean ln (Plate count of the coupon) of Anoxy2 CDC Reactor Run under 60°C with 10% of Solids RSM for 12 h;

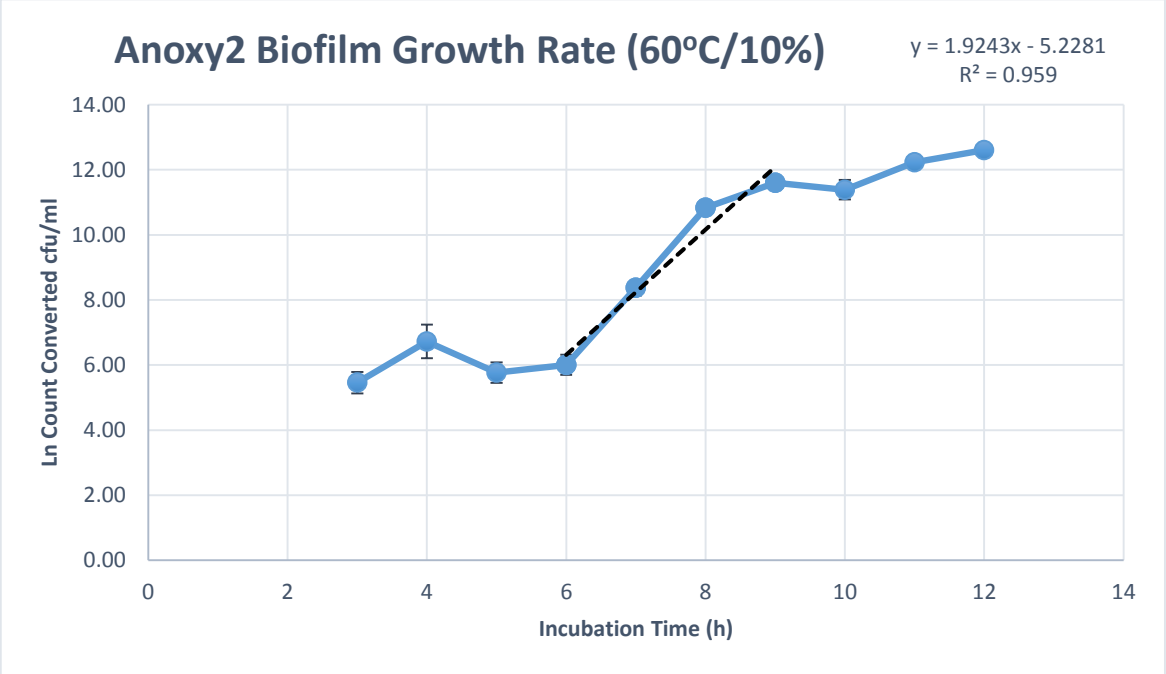

Figure 17.2: Mean ln (Plate count of the coupon) of Anoxy2 CDC Reactor Run under 60°C with 10% of Solids RSM for 12 h. (Error bars are representing +/– one standard deviation of 6 readings of the same sampling time, dashed line is for calculating the growth rate)

Appendix 18 Geo1 Thermocycling (Sine Wave) 30°C

RAW DATA SET (log count converted, cfu/ml)										
	RUN 4 -55-30°C, 10MIN		RUN 3 -55-30°C, 20MIN		RUN 2 -55°C, control		RUN 1 - 55-30°C, 50MIN		RUN 5 -55°C, control	
Time(h)	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
0	3.45	3.45	4.04	4.04	4.40	4.40	5.27	5.27	4.37	4.37
2	3.42	3.41	4.05	4.01	3.90	4.00	5.04	5.05	4.21	4.23
4	3.42	3.45	4.11	4.07	4.53	4.54	4.90	4.93	5.05	5.05
6	3.46	3.54	3.85	3.95	5.30	5.25	5.14	5.22	5.53	5.56
8	3.58	3.60	4.17	4.20	6.37	6.40	6.01	5.99	6.39	6.55
12	4.56	4.55	5.64	5.78	6.79	6.80	6.64	6.51	6.37	6.59
24	6.14	6.19	6.78	6.78	7.26	7.39	6.76	6.68	6.55	6.67
NORMALIZED AND ANALYZED DATA SET(log count converted, cfu/ml)										
	RUN 4 -55-30°C, 10MIN		RUN 3 -55-30°C, 20MIN		RUN 2 -55°C, control		RUN 1 - 55-30°C, 50MIN		RUN 5 -55°C, control	
Time(h)	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00
2	3.40	0.03	3.44	0.12	3.10	0.06	3.30	0.03	3.33	0.04
4	3.42	0.03	3.48	0.14	3.56	0.04	3.22	0.05	3.99	0.05
6	3.48	0.07	3.33	0.08	4.14	0.04	3.39	0.04	4.38	0.07
8	3.57	0.05	3.56	0.12	5.01	0.04	3.93	0.02	5.11	0.10
12	4.54	0.05	4.87	0.07	5.33	0.06	4.31	0.08	5.12	0.11
24	6.14	0.05	5.79	0.10	5.74	0.09	4.41	0.08	5.22	0.08

Table 18.1: Bacterial concentration of the outflowing milk from experiment: Geo1 under different periods of sine waves 55-30°C Thermocycling conditions for 24 h in 10% of solids RSM (period = 10 min, 20 min, and 50 min);

The mean and standard deviation of triplicate plate readings of the same sampling point were calculated and converted into log counts then tabulated above. The points on the below graphs are the mean of upper and lower reactors of either raw data or the normalized data. The raw data was normalized to the lowest T0 (milk-in) according to get the normalized data. +/- one SD was plotted error bars.

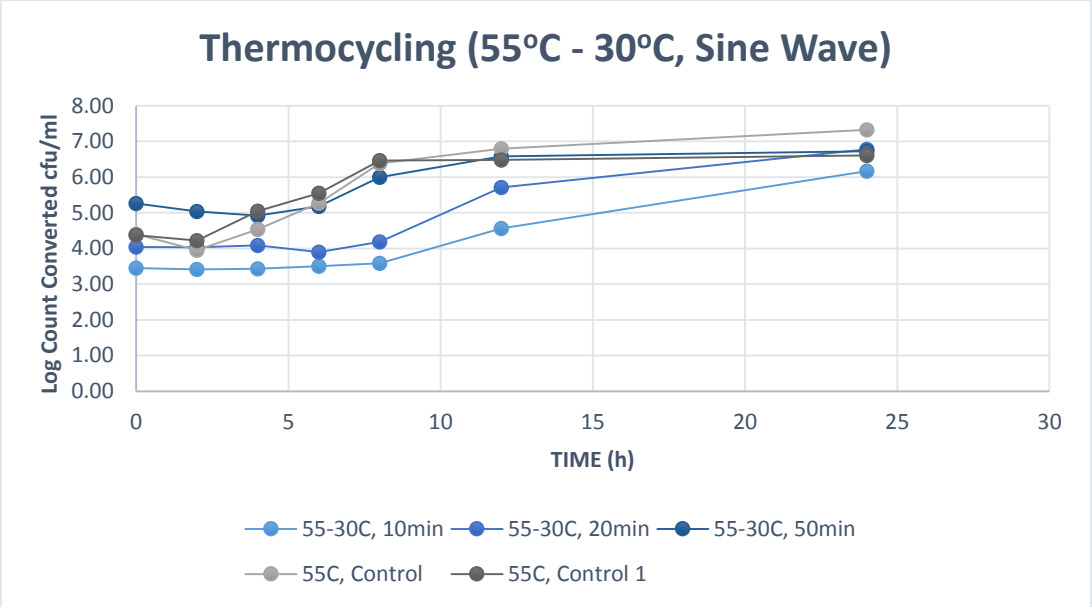

Figure 18.1: Bacterial concentration of the outflowing milk from experiment: Geo1 under different periods of sine waves 55-30°C thermocycling conditions for 24 h in 10% of solids RSM (Raw data);

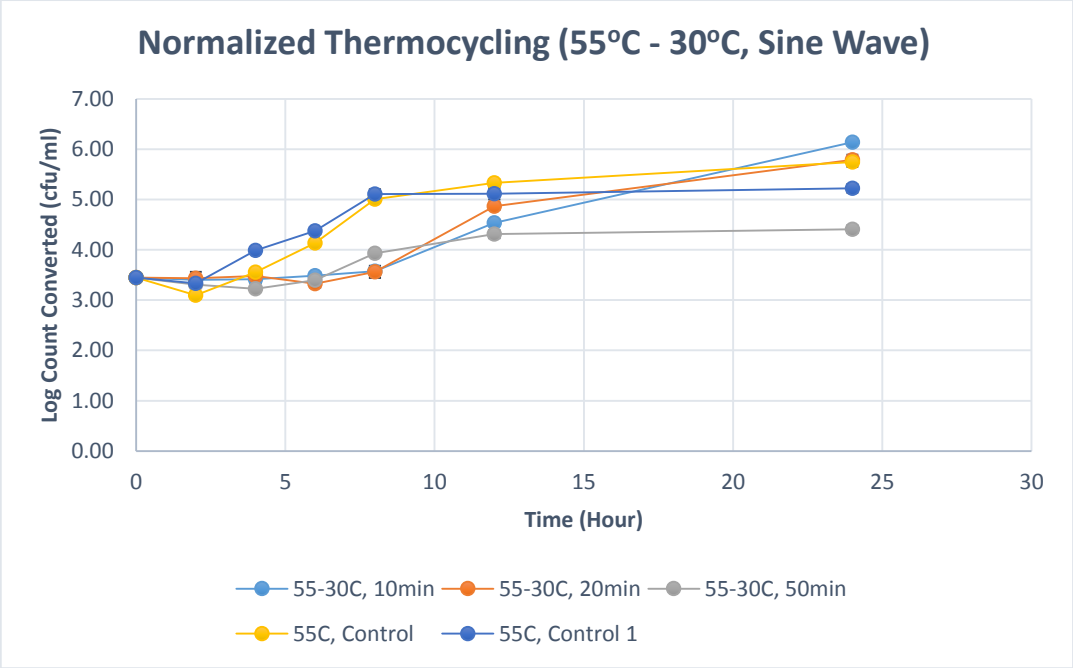

Figure 18.2: Bacterial concentration of the outflowing milk from experiment: Geo1 under different periods of sine waves 55-30°C thermocycling conditions for 24 h in 10% of solids RSM (Normalized and analyzed data);

Appendix 19 Geo1 Thermocycling (Sine Wave) 35°C

RAW DATA SET(Log Count Converted, cfu/ml)										
Log(Count)	RUN 7 -55-35°C, 10MIN		RUN 8 -55-35°C, 20MIN		RUN 2 -55°C, control		RUN 6 - 55-35°C, 50MIN		RUN 5 -55°C, control	
Time(h)	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
0	4.80	4.80	3.56	3.56	4.40	4.40	5.21	5.21	4.37	4.37
2	4.25	4.31	3.67	3.67	3.90	4.00	4.90	4.81	4.21	4.23
4	3.78	3.64	4.05	4.26	4.53	4.54	4.57	4.51	5.05	5.05
6	3.43	3.39	5.40	5.36	5.30	5.25	4.88	4.80	5.53	5.56
8	3.85	3.59	5.46	5.50	6.37	6.40	5.48	5.57	6.39	6.55
12	4.28	4.29	6.46	6.51	6.79	6.80	7.09	7.02	6.37	6.59
24	7.47	7.45	6.23	6.20	7.26	7.39	7.45	7.51	6.55	6.67
NORMALIZED AND ANALYZED DATA SET(Log Count Converted, cfu/ml)										
Log(Count)	RUN 7 -55-35°C, 10MIN		RUN 8 -55-35°C, 20MIN		RUN 2 -55°C, control		RUN 6 - 55-35°C, 50MIN		RUN 5 -55°C, control	
Time (h)	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00
2	3.08	0.05	3.55	0.08	3.10	0.06	3.21	0.04	3.33	0.04
4	2.67	0.06	4.03	0.15	3.56	0.04	3.00	0.04	3.99	0.05
6	2.45	0.06	5.22	0.10	4.14	0.04	3.20	0.05	4.38	0.07
8	2.68	0.12	5.32	0.11	5.01	0.04	3.65	0.05	5.11	0.10
12	3.08	0.04	6.30	0.16	5.33	0.06	4.67	0.05	5.12	0.11
24	5.37	0.04	6.04	0.15	5.74	0.09	4.95	0.02	5.22	0.08

Table 19.1: Bacterial concentration of the outflowing milk from experiment: Geo1 under different periods of sine waves 55-35°C Thermocycling conditions for 24 h in 10% of solids RSM (period = 10 min, 20 min, and 50 min);

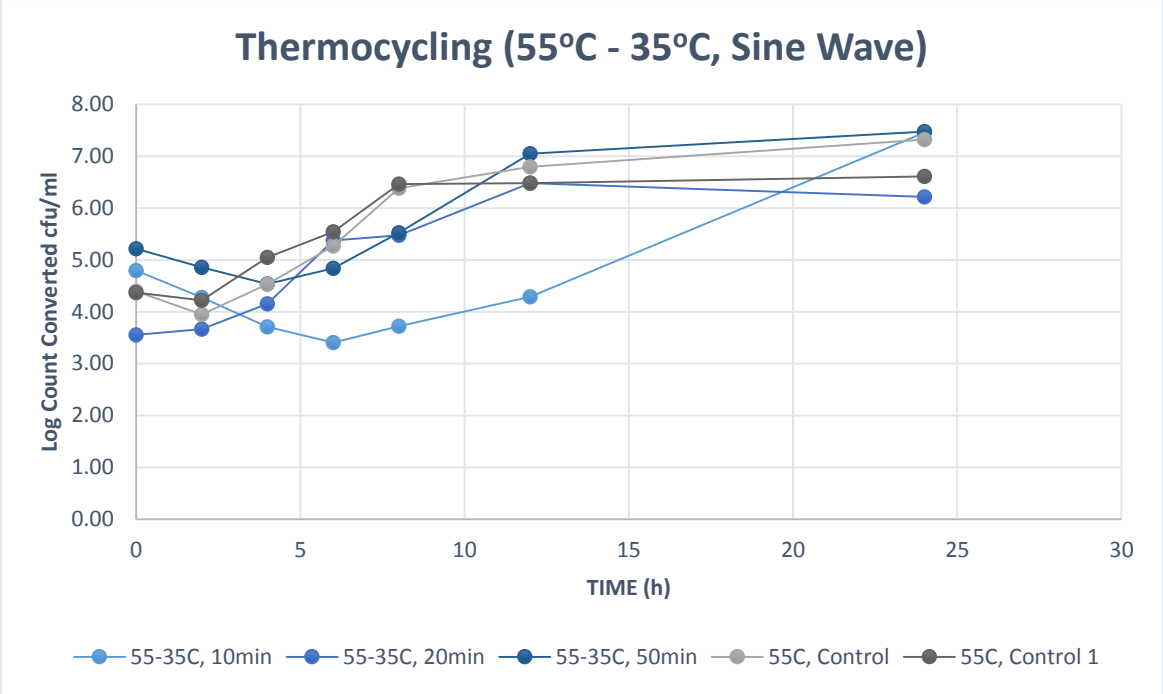

Figure 19.1: Bacterial concentration of the outflowing milk from experiment: Geo1 *Geobacillus* under different periods of sine waves 55-35°C Thermocycling conditions for 24 h in 10% of solids RSM (Raw data);

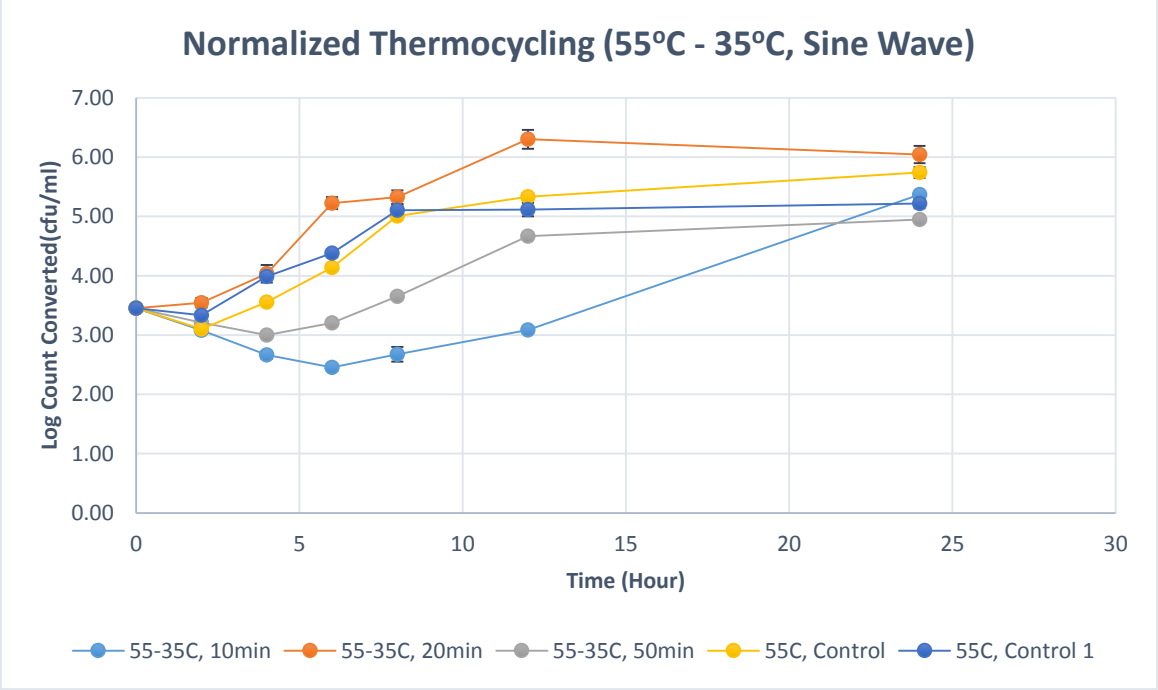

Figure 19.2: Bacterial concentration of the outflowing milk from experiment: Geo1 *Geobacillus* under different periods of sine waves 55-35°C Thermocycling conditions for 24 h in 10% of solids RSM (Normalized and analyzed data);

Appendix 20 Geo1 Thermospiking (square wave) 30°C

RAW DATA SET (Log Count Converted, cfu/ml)												
	CONTROL		RUN 1		RUN 2		RUN 3		RUN 4		CONTROL 1	
Log(Count)	55C, no ramp, control		55C/5m, 30C/15m		55C/15m, 30C/35m		55C/5m, 30C/35m		55C/15m, 30C/15m		55C, no ramp, control	
Time(h)	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
0	4.40	4.40	5.05	5.05	4.77	4.77	5.05	5.05	4.34	4.34	4.37	4.37
2	3.90	4.00	4.95	4.99	4.50	4.56	5.00	4.66	3.70	3.78	4.21	4.23
4	4.53	4.54	5.06	5.00	4.18	4.15	4.74	4.40	3.64	3.64	5.05	5.05
6	5.30	5.25	4.77	3.95	4.43	4.47	5.54	5.62	4.37	4.52	5.53	5.56
8	6.37	6.40	4.56	4.54	5.05	4.91	5.91	5.86	4.30	4.48	6.39	6.55
12	6.79	6.80	5.38	5.80	5.19	5.10	6.81	6.91	5.03	4.90	6.37	6.59
24	7.26	7.39	6.56	6.49	6.25	6.24	7.72	7.79	6.51	6.19	6.55	6.67
NORMALIZED AND ANALYZED DATA SET (Log Count Converted, cfu/ml)												
	CONTROL		RUN 1		RUN 2		RUN 3		RUN 4		CONTROL 1	
Log(Count)	55C, no ramp, control		55C/5m, 30C/15m		55C/15m, 30C/35m		55C/5m, 30C/35m		55C/15m, 30C/15m		55C, no ramp, control	
Time(h)	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00
2	3.10	0.06	3.39	0.03	3.28	0.09	3.30	0.13	2.95	0.13	3.33	0.04
4	3.56	0.04	3.44	0.03	3.01	0.06	3.12	0.13	2.88	0.09	3.99	0.05
6	4.14	0.04	3.66	0.31	3.22	0.07	3.81	0.05	3.52	0.18	4.38	0.07
8	5.01	0.04	3.78	0.05	3.60	0.09	4.02	0.04	3.42	0.26	5.11	0.10
12	5.33	0.06	4.49	0.17	3.72	0.08	4.69	0.04	3.95	0.07	5.12	0.11
24	5.74	0.09	5.48	0.35	4.51	0.08	5.30	0.05	5.06	0.19	5.22	0.08

Table 20.1: Bacterial concentration of the outflowing milk from experiment: Geo1 under different square waves 55-30°C Thermospiking conditions for 24 h in 10% of solids RSM;

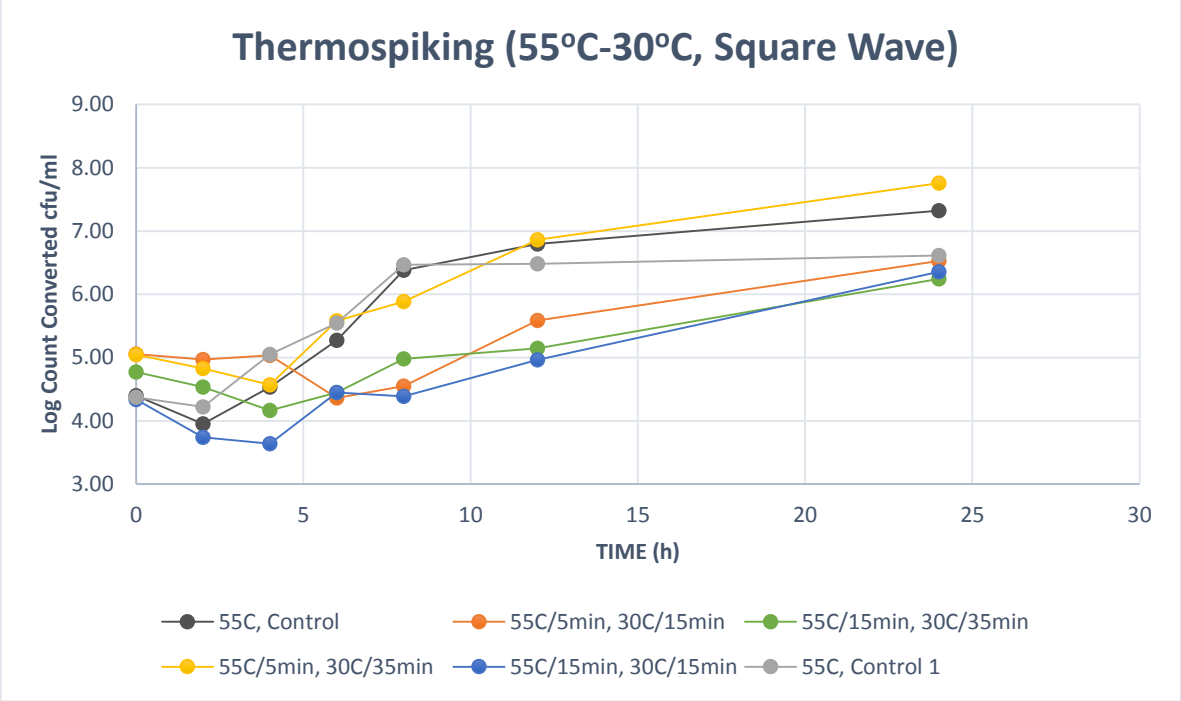

Figure 20.1: Bacterial concentration of the outflowing milk from experiment: Geo1 *Geobacillus* under different square waves 55-30°C Thermospiking conditions for 24 h in 10% of solids RSM (Raw data);

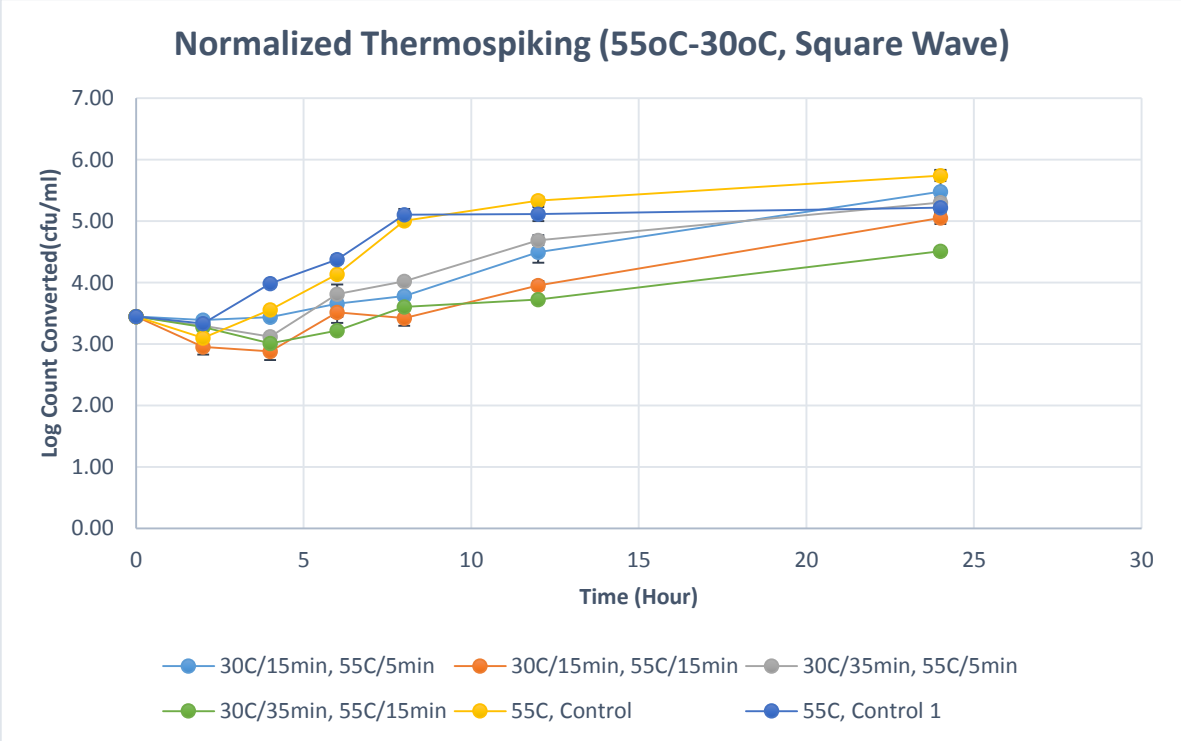

Figure 20.2: Bacterial concentration of the outflowing milk from experiment: Geo1 *Geobacillus* under different square waves 55-30°C Thermospiking conditions for 24 h in 10% of solids RSM (Normalized and Analyzed data);

Appendix 21 Geo1 Thermospiking (square wave) 35ºC

RAW DATA SET (Log Count Converted, cfu/ml)												
	CONTROL		RUN 5		RUN 6		RUN 7		RUN 8		CONTROL 1	
Log(Count)	55C, no ramp, control		55C/5m, 35C/15m		55C/15m, 35C/35m		55C/5m, 35C/35m		55C/15m, 35C/15m		55C, no ramp, control	
Time (h)	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
0	4.40	4.40	4.47	4.47	4.39	4.39	4.32	4.32	3.95	3.95	4.37	4.37
2	3.90	4.00	3.64	3.70	3.82	3.73	3.75	3.85	3.43	3.73	4.21	4.23
4	4.53	4.54	3.70	3.60	3.70	3.67	3.48	3.70	3.67	3.52	5.05	5.05
6	5.30	5.25	3.64	3.60	3.12	3.64	3.37	3.12	3.30	3.48	5.53	5.56
8	6.37	6.40	3.22	3.70	3.12	3.37	3.56	3.64	3.43	3.43	6.39	6.55
12	6.79	6.80	3.95	3.87	3.90	3.97	3.75	3.80	3.70	3.52	6.37	6.59
24	7.26	7.39	6.69	6.65	6.39	6.85	6.76	6.60	6.51	6.87	6.55	6.67
NORMALIZED AND ANALYZED DATA SET (Log Count Converted, cfu/ml)												
	CONTROL		RUN 5		RUN 6		RUN 7		RUN 8		CONTROL 1	
Log(Count)	55C, no ramp, control		55C/5m, 35C/15m		55C/15m, 35C/35m		55C/5m, 35C/35m		55C/15m, 35C/15m		55C, no ramp, control	
Time (h)	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00	3.45	0.00
2	3.10	0.06	2.82	0.10	2.98	0.15	3.03	0.12	3.08	0.30	3.33	0.04
4	3.56	0.04	2.80	0.14	2.89	0.16	2.86	0.14	3.14	0.10	3.99	0.05
6	4.14	0.04	2.79	0.06	2.61	0.28	2.57	0.19	2.93	0.21	4.38	0.07
8	5.01	0.04	2.63	0.28	2.53	0.20	2.87	0.15	2.94	0.21	5.11	0.10
12	5.33	0.06	3.00	0.13	3.12	0.13	3.01	0.09	3.14	0.11	5.12	0.11
24	5.74	0.09	5.15	0.03	5.24	0.27	5.34	0.12	5.85	0.19	5.22	0.08

Table 21.1: Bacterial concentration of the outflowing milk from experiment: Geo1 *Geobacillus* under different square waves 55-35ºC Thermospiking conditions for 24 h in 10% of solids RSM;

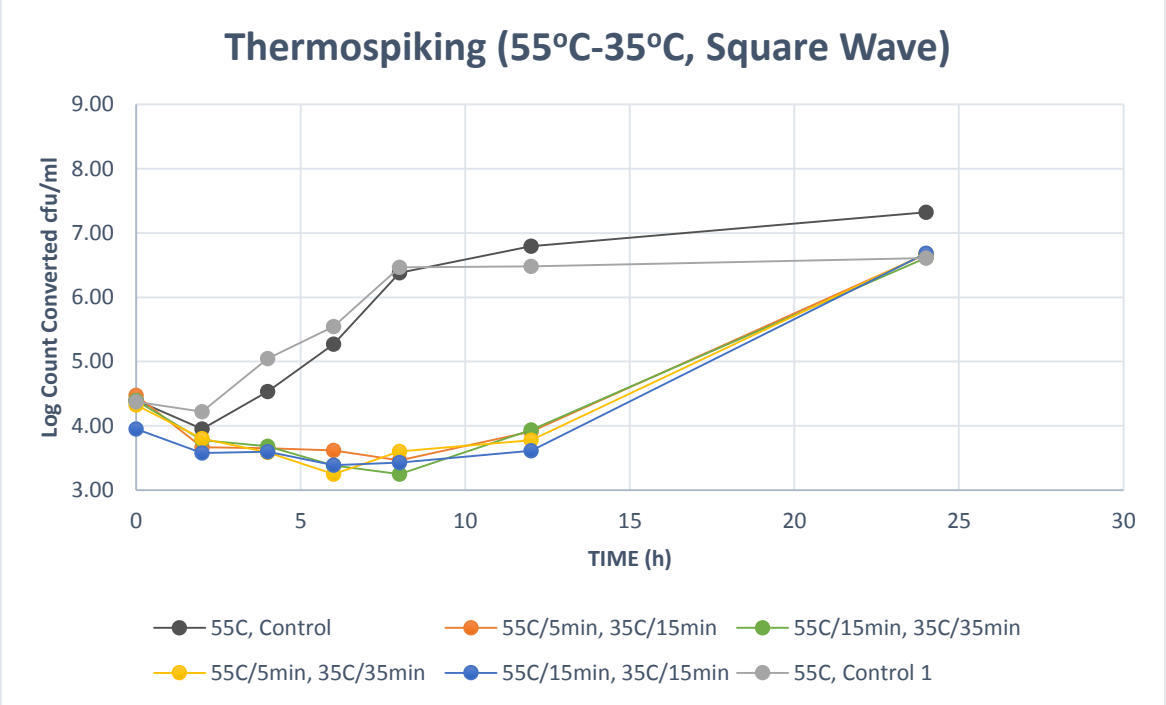

Figure 21.1: Bacterial concentration of the outflowing milk from experiment: Geo1 *Geobacillus* under different square waves 55-35ºC Thermospiking conditions for 24 h in 10% of solids RSM (Raw data);

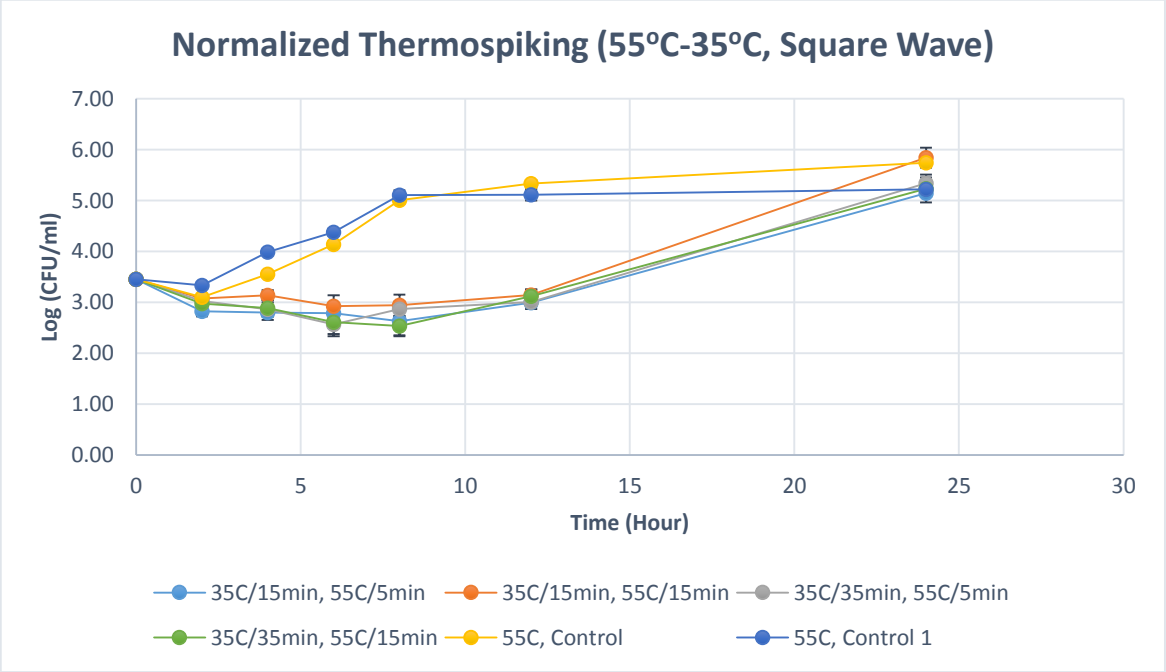

Figure 21.2: Bacterial concentration of the outflowing milk from experiment: Geo1 *Geobacillus* under different square waves 55-35ºC Thermospiking conditions for 24 h in 10% of solids RSM (Normalized and Analyzed data);

Appendix 22 Geo1 Reactor Biofilm after 24 h Thermocycling Run

Thermocycling Runs	Upper Reactor	Lower Reactor	Mean Reading (cfu/ml)	Converted Mean Reading (cfu/cm²)	Standard Deviation
55-30°C, 10min	5.08	5.11	5.10	5.97	0.02
55-30°C, 20min	6.27	6.27	6.27	7.14	0.00
55-30°C, 50min	4.63	4.40	4.51	5.39	0.17
55-35°C, 10min	3.96	4.11	4.04	4.91	0.10
55-35°C, 20min	7.49	7.26	7.38	8.25	0.16
55-35°C, 50min	4.56	4.56	4.56	5.44	0.00
55°C Control	5.63	5.63	5.63	6.51	0.00
55°C Control 1	5.68	5.78	5.73	6.61	0.07

Table 22.1: Geo1 Bacterial attachment sampled after 24 h Thermocycling Runs from the hexagonal reactors;

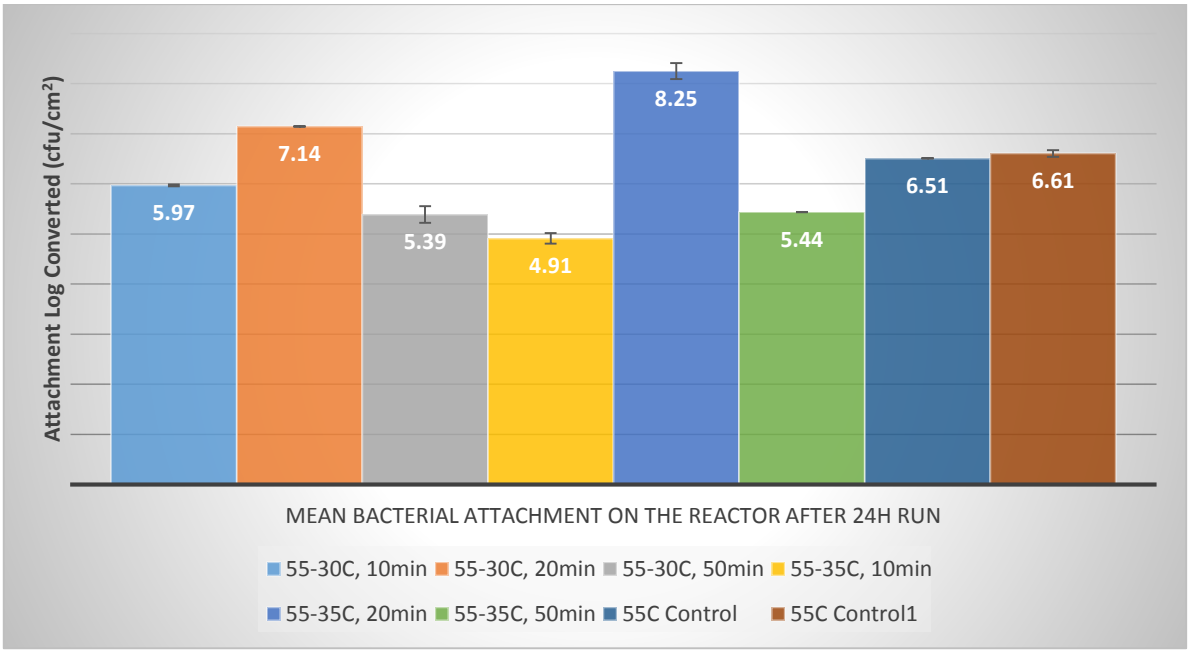

Figure 22.1: Geo1 Bacterial attachment sampled after 24 h Thermocycling Runs from the hexagonal reactors;

Biofilm formed on the inner surfaces of the stainless steel reactors. The inner surfaces were sampled by dislodging the bacterial cells in 60 ml of TSB using glass beads and vortex mixing at maximum speed for 2 min. The bacterial concentration of the TSB was enumerated, normalized according to the lowest T_0 value then converted to the bacterial attachment cfu/cm^2. Mean and standard deviation of the attachment plotted. Bacterial concentration of the TSB (cfu/ml) is converted to attachment (cfu/cm^2) = the concentration of the TSB x TSB volume / reactor surface area = Concentration x 60ml / 8cm^2

Appendix 23 Geo1 Reactor Biofilm after 24 h Thermospiking Run

Thermospiking Runs	Upper Reactor	Lower Reactor	Mean Reading (cfu/ml)	Converted Mean Reading (cfu/cm^2)	Standard Deviation
55°C/5m, 30°C/15m	4.20	5.19	4.69	5.57	0.70
55°C/15m, 30°C/35m	4.56	4.64	4.60	5.47	0.06
55°C/5m, 30°C/35m	5.01	5.06	5.04	5.91	0.03
55°C/15m, 30°C/15m	4.49	4.53	4.51	5.39	0.03
55°C/5m, 35°C/15m	4.67	4.99	4.83	5.71	0.23
55°C/15m, 35°C/35m	3.94	4.42	4.18	5.05	0.34
55°C/5m, 35°C/35m	4.09	3.91	4.00	4.88	0.12
55°C/15m, 35°C/15m	5.24	5.28	5.26	6.14	0.03
55°C Control	5.63	5.63	5.63	6.51	0.00
55°C Control 1	5.68	5.78	5.73	6.61	0.07

Table 23.1: Geo1 Bacterial attachment sampled after 24 h Thermospiking Runs from the hexagonal reactors;

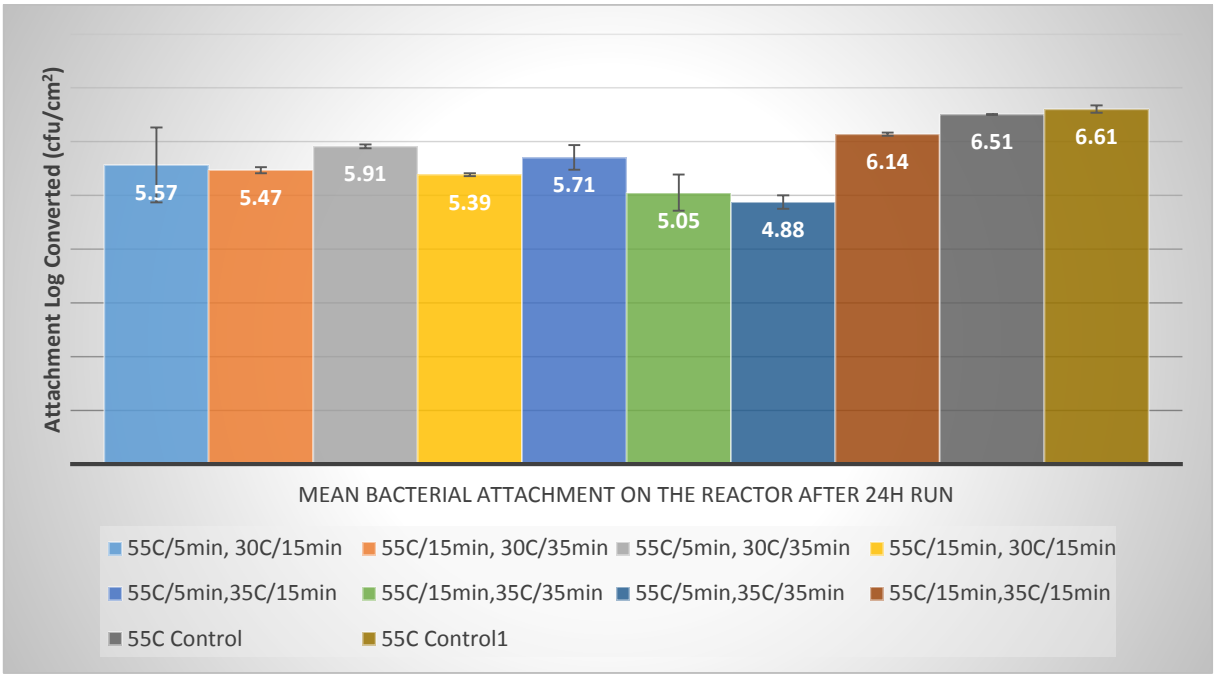

Figure 23.1: Geo1 Reactor Biofilm Bacterial attachment sampled after 24 h Therospiking Runs from the hexagonal reactors;

Biofilm formed on the inner surfaces of the stainless steel reactors. The inner surfaces were sampled by dislodging the bacterial cells in 60 ml of TSB using glass beads and vortex mixing at maximum speed for 2 min. The bacterial concentration of the TSB was enumerated, normalized according to the lowest T_0 value then converted to the bacterial attachment cfu/cm^2. Mean and standard deviation of the attachment plotted. (Detailed data in Appendix 23 in the data CD) Bacterial concentration of the TSB (cfu/ml) is converted to attachment (cfu/cm^2) = the concentration of the TSB x TSB volume / reactor surface area = Concentration x 60ml / 8cm^2

Appendix 24 55°C - 30°C - 55°C Temperature Step Change (24 h Duration)

In this part of the study, sterile milk was used together with inoculated hexagonal coupon reactors in the temperature step change experiments. Hexagonal coupon reactors were inoculated with 10^7 cfu/ml Geo1 *Geobacillus* culture for an h prior to the experiment. Temperature step change experiments consisted two steps, one step of temperature decrease and one step of temperature increase. The holding time in between each step was 24 h. The temperature step change regime used was the square wave design. The table below was the log10 (plate count). The plate count was the average of three triplicate plates from the same sampling point.

55°C (24hr)-temperature change -30°C (24h)-temperature change-55°C (24h)			
Temperature = 55°C (24h) - 30°C (24h) - 55°C (24h)			
Inoculation onto metal reactors, sterile milk, LOG Count Converted, cfu/ml			
Time(h)	Mean Concentration (log)	Standard Deviation	Temp Profile (°C)
2	2.36	0.20	55
4	2.74	0.03	55
6	3.16	0.05	55
8	3.88	0.19	55
12	5.20	0.07	55
24	6.28	0.20	30
26	6.10	0.19	30
28	5.90	0.24	30
30	5.60	0.21	30
32	5.40	0.14	30
48	4.60	0.24	30
50	5.95	0.06	55
52	6.07	0.08	55
54	6.16	0.12	55
56	6.34	0.02	55
72	6.48	0.02	55

Table 24.1: Bacterial concentration of the outflowing milk from experiment: Temperature step change experiment testing bacterial viability after 55°C - 30°C - 55°C for Geo1 (24 h duration each temperature, using inoculated hexagonal reactors and sterile 10% RSM. Samples were taken before temperature changed at 24h and 48h)

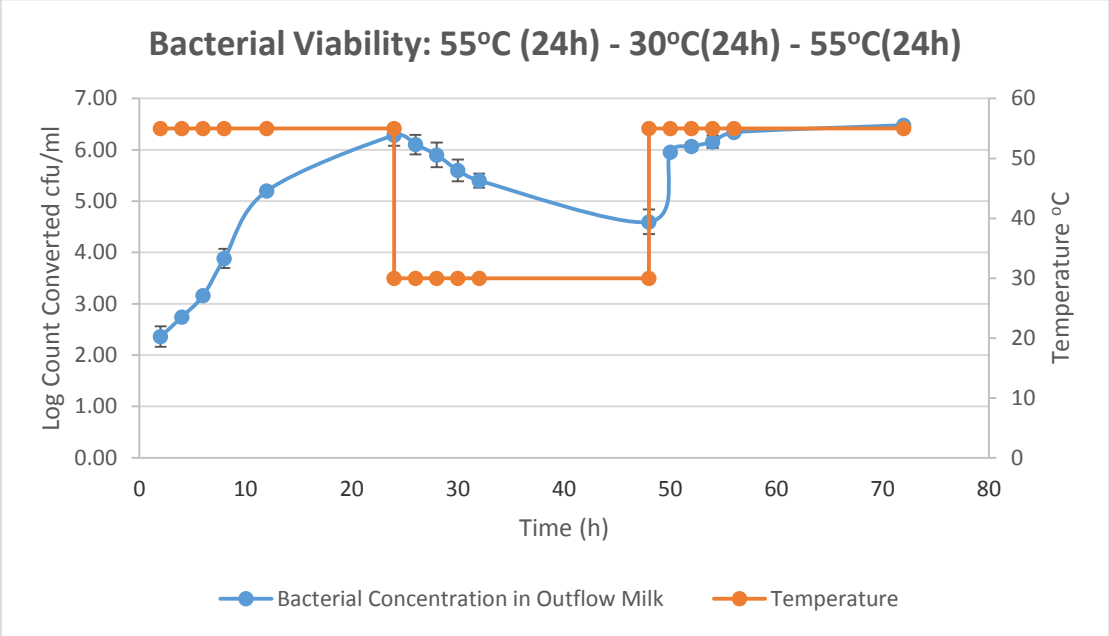

Figure 24.1: Bacterial concentration of the outflowing milk from experiment: Temperature step change experiment testing bacterial viability after 55°C - 30°C - 55°C for Geo1 (24 h duration each temperature, using inoculated hexagonal reactors and sterile 10% RSM. Y-axis = log count of the milk-out sample (cfu/ml); X-axis = time (h). Mean bacterial concentration of outflowing milk from upper and lower reactors was plotted. One +/- standard deviation as plotted as error bar)

Appendix 25 55°C - 35°C - 55°C Temperature Step Change (24 h Duration)

In this part of the study, sterile milk was used together with inoculated hexagonal reactors in the temperature step change experiments. Hexagonal reactors were inoculated with 10^7 cfu/ml Geo1 *Geobacillus* culture for an h prior to the experiment. Temperature step change experiments consisted two steps, one step of temperature decrease and one step of temperature increase. The holding time in between each step was 24 h. The temperature step change regime used was the square wave design. The table below was the log10 (plate count). The plate count was the average of three triplicate plates from the same sampling point.

55°C (25h)-temperature change -35°C (24h)-temperature change-55°C (24h)			
Temperature = 55°C (25h) - 35°C (24hr) - 55°C (24h)			
Inoculation onto metal reactors, sterile milk, LOG Count Converted, cfu/ml			
Time(h)	Mean Concentration (log)	Standard Deviation	Temp Profile (°C)
2	1.00	0.00	55
4	1.00	0.00	55
6	1.00	0.00	55
8	1.00	0.00	55
12	1.00	0.00	55
23	2.07	0.14	55
25	2.83	0.10	55
27	2.36	0.06	35
29	2.15	0.11	35
31	2.14	0.09	35
33	2.11	0.15	35
49	1.72	0.16	35
51	2.89	0.05	55
53	3.41	0.06	55
55	3.77	0.08	55
57	4.72	0.05	55
73	7.19	0.06	55

Table 25.1: Bacterial concentration of the outflowing milk from experiment: Temperature step change experiment testing bacterial viability after 55°C - 35°C - 55°C for Geo1 (24 h duration each temperature, using inoculated hexagonal reactors and sterile 10% RSM. Samples were taken before temperature changed at 25h and 49h. Extra hour was applied in the first 55°C step due to the low count in bacterial concentration)

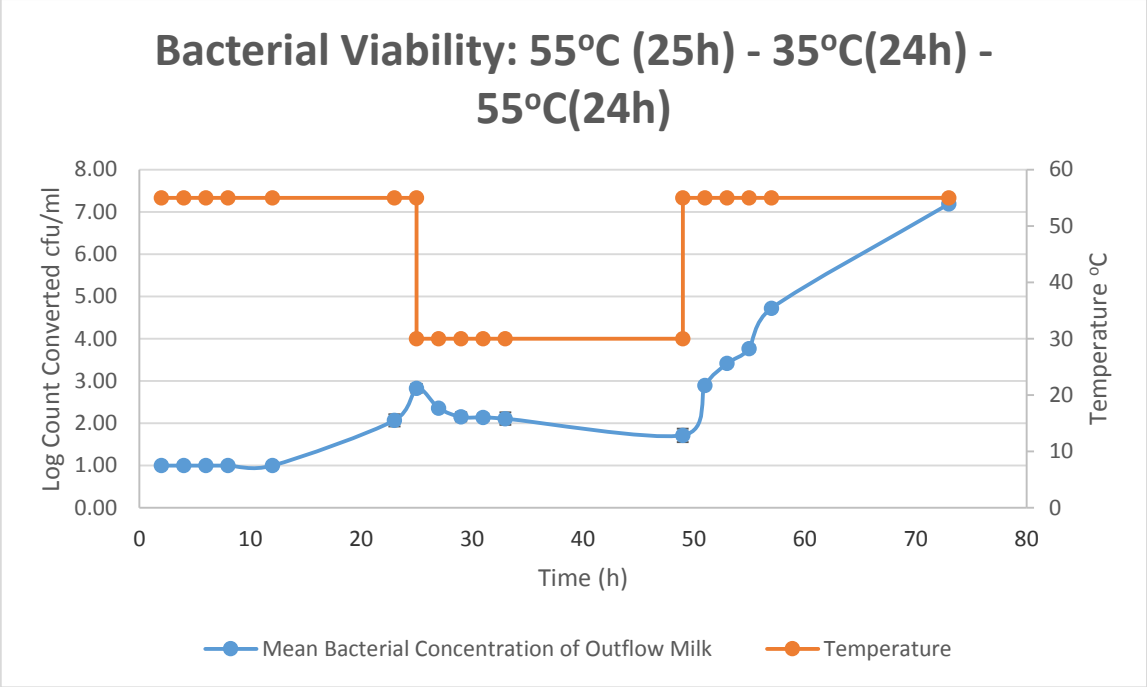

Figure 25.1: Bacterial concentration of the outflowing milk from experiment: Temperature step change experiment testing bacterial viability after 55°C - 35°C - 55°C for Geo1 (24 h duration each temperature, except 1 hour extra for first cycle, using inoculated hexagonal reactors and sterile 10% RSM. Y-axis = log count of the milk-out sample (cfu/ml); X-axis = time (h). Mean bacterial concentration of outflowing milk from upper and lower reactors was plotted. One +/- standard deviation as plotted as error bar)

Appendix 26 Geo1 Preheating Pipe Only 55°C Control Run (No Reactor Linked)

Only preheating pipes were used in this experiment, without linking to the hexagonal coupon reactors before pump and entering the waste. This experiment was to test the effect of biofilm attachment onto preheating pipe and its overall influence to the thermocycling and thermospiking results. Inoculated milk and sterile preheating pipes were used. The experiment was run using constant 55°C for 24 h. The table below included the mean and standard deviation based on the log10 (plate count) data. The plate count was the average of three triplicate plates from the same sampling point. All the milk-out from each half-an-h sampling interval was collected and homogenized before plating.

Preheating Pipe Control Run 24 h				
Pipe without the metal reactors; **Milk-out collected from the whole 1/2 h, homogenized then sampled;**				
Temperature = 55°C constant				
Tube Length = 60 cm each, Log Count Converted, cfu/ml				
Time (h)	Mean Concentration of Pipe 1	Standard Deviation	Mean Concentration of Pipe 2	Standard Deviation
0	5.26	0.04	5.26	0.04
0.5	5.08	0.01	4.97	0.02
1	5.06	0.07	5.03	0.05
1.5	5.07	0.00	5.08	0.05
2	4.84	0.00	4.87	0.09
2.5	4.90	0.05	4.81	0.04
3	4.66	0.01	4.78	0.02
3.5	4.85	0.03	4.84	0.01
4	4.85	0.03	4.83	0.03
4.5	4.65	0.11	4.69	0.03
5	4.09	0.07	4.30	0.17
5.5	4.46	0.00	4.44	0.11
6	4.26	0.08	4.43	0.09
6.5	4.78	0.06	4.76	0.01
7	4.39	0.01	4.88	0.06
7.5	4.57	0.05	4.42	0.06
8	4.74	0.02	4.45	0.01
8.5	4.11	0.10	4.86	0.04
9	4.45	0.05	4.91	0.05
9.5	4.62	0.04	5.01	0.02
10	4.65	0.06	5.09	0.04
10.5	5.43	0.02	5.12	0.00
11	5.55	0.07	5.45	0.02
11.5	5.15	0.04	5.59	0.00

12	5.56	0.04	5.62	0.04
12.5	5.62	0.04	6.01	0.04
13	5.69	0.09	6.05	0.10
13.5	5.39	0.06	5.91	0.02
14	5.65	0.05	5.84	0.08
14.5	5.71	0.07	5.90	0.05
15	5.77	0.08	6.12	0.10
15.5	5.90	0.07	5.99	0.19
16	6.14	0.03	6.12	0.05
16.5	6.31	0.05	6.48	0.04
17	6.41	0.02	6.52	0.06
17.5	6.59	0.01	6.44	0.07
18	6.15	0.00	7.08	0.01
18.5	6.30	0.06	6.71	0.08
19	6.28	0.03	6.42	0.08
19.5	6.39	0.04	6.29	0.16
20	6.06	0.09	6.56	0.04
20.5	5.89	0.08	6.56	0.12
21	5.92	0.02	6.53	0.05
21.5	5.83	0.04	6.58	0.06
22	5.96	0.02	6.53	0.09
22.5	5.93	0.05	6.43	0.05
23	6.16	0.02	6.57	0.04
23.5	6.18	0.04	6.64	0.03
24	6.36	0.03	7.38	0.05

Table 26.1 : Bacterial concentration of the outflowing milk from experiment: Geo1 preheating pipe only 55°C control run (no reactor linked) using inoculated 10% RSM and sterile hexagonal reactors for 24 h with 0.5 h sampling interval (All the milk-out from each 0.5 h sampling interval was collected and homogenized before plating);

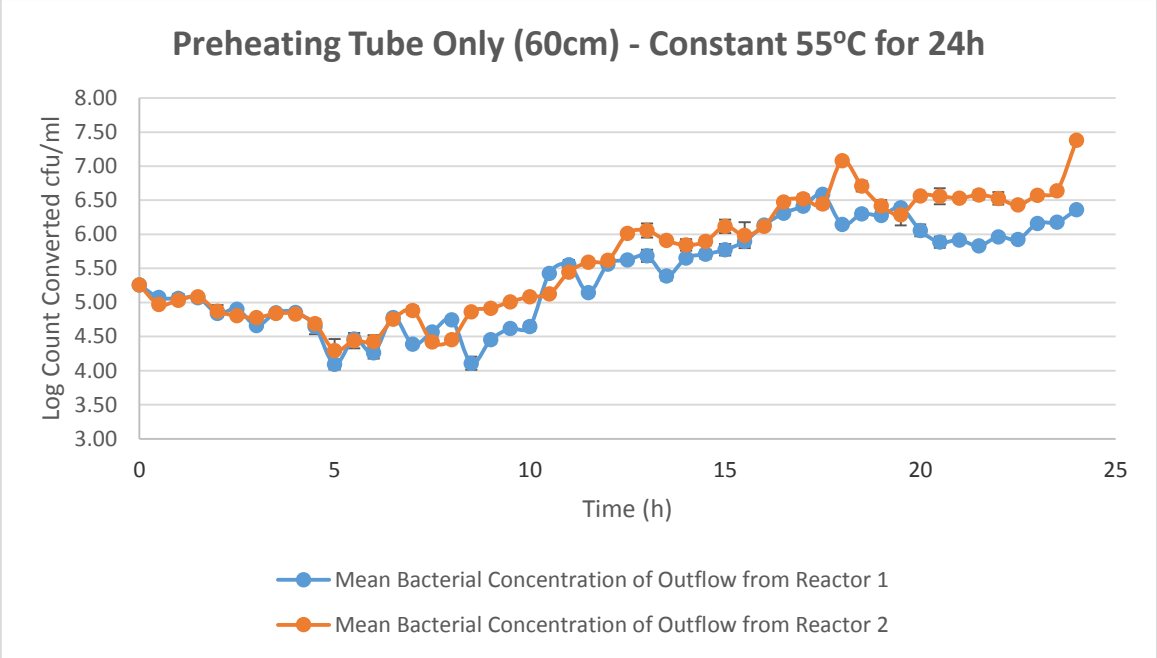

Figure 26.1: Bacterial concentration of the outflowing milk from experiment: Geo1 preheating pipe only 55°C control run (no reactor linked) using inoculated 10% RSM and sterile hexagonal reactors for 24 h with 0.5 h sampling interval (All the milk-out from each 0.5 h sampling interval was collected and homogenized before plating);

Appendix 27 Cold Storage Impact on Spores and Bacterial Counts

SPORE COUNT: Geobacillus Cold Shock Spore Study							
Sample No.	Conc.	Reading 1	Reading 2	Log Count R1 (cfu/ml)	Log Count R2 (cfu/ml)	Mean Log Count (cfu/ml)	Standard Deviation
Inoculum T0	1.00E+04	378	402	7.58	7.60	7.59	0.02
Inoculum T0 Spore	1.00E+02	208	230	5.32	5.36	5.34	0.03
Bacteria T2	1.00E+02	120	147	5.08	5.17	5.12	0.06
Spore T2	1.00E+01	26	31	3.41	3.49	3.45	0.05
Bacteria T4	1.00E+02	140	143	5.15	5.16	5.15	0.01
Spore T4	1.00E+01	56	59	3.75	3.77	3.76	0.02
Bacteria T24	1.00E+02	84	93	4.92	4.97	4.95	0.03
Spore T24	1.00E+01	39	44	3.59	3.64	3.62	0.04

Table 27.1: Geo1 bacterial and spore counts before (Inoculum), during (Time at 2 h and 4 h) and after (Time 24 h) the cold storage at 4 °C in 10% RSM over 24 h period;

Appendix 28 Geo1 Preheating Pipe Model

```
library(deSolve)

# all volumes are in ml and all times are in seconds.

flowrate_min <- 1.5  #NB:  ml/min

flowrate <- flowrate_min / 60

incoming_milk_conc <- 1e5  # NB: bacteria per ml
Vol_pipe <- pi*0.3^2 * 60  # NB: volume in ml

parameters <- c(t0      = 3800,    # settling efolding time, sec
                tG      = 8800,    # growth   efolding time, sec
                k       = 1e8,     # carrying capacity of the FILM of the pipe
                inflow  = incoming_milk_conc * flowrate
                )

## first specify the _start_ state, remember that 'M' is the number of
## CFU in the free-flowing mil in the pipe.
state <- c(M = Vol_pipe * incoming_milk_conc , P = 0)


bact <- function(t, state, parameters) {
 with(as.list(c(state, parameters)),{

 settling <- M / t0
 slough <- P^2/(k*tG)
 growth <- P/tG



 outflow <- M/Vol_pipe  * flowrate

 # rate of change
 dM = (
   +inflow
   -outflow
   -settling
   +slough
   )

 dP = (
   +settling
   -slough
   +growth
   )

# return the rate of change
```

```
 list(c(dM, dP))
}) # end with(as.list ...
}


times <- seq(0, 24*3600, by = 30*60)

out <- ode(y = state, times = times, func = bact, parms = parameters)
head(out)
```

Appendix 29 Optimization of the Geo1 Preheating Pipe Model

```
source("try.R")

a <- read.table("observations.txt",header=T)


## convert to seconds:
a$time <- a$time * 3600



parameters <- c(t0      = 1800,    # settling efolding time, sec
                tG      = 7800,    # growth   efolding time, sec
                k       = 1e7,     # carrying capacity of the FILM of the pipe
                inflow  = incoming_milk_conc * flowrate
                )


# First, plot observational data.
plot  ((log10(Vol_pipe) + c1)~time,data=a,type='b',col='red')
points((log10(Vol_pipe) + c2)~time,data=a,type='b',col='blue')

points(out[,1],log10(out[,2]),col='green')

mismatch1  <- log10(out[,2]) - (a$c1 + log10(Vol_pipe))
mismatch2  <- log10(out[,2]) - (a$c2 + log10(Vol_pipe))

badness <- sum(mismatch1[-1]^2) + sum(mismatch2[-1]^2)

bad <- function(parameters,giveall=FALSE){

  out <- ode(y = state, times = a$time, func = bact, parms = parameters)
  if(giveall){return(out)}
  mismatch1  <- log10(out[,2]) - (a$c1 + log10(Vol_pipe))
  mismatch2  <- log10(out[,2]) - (a$c2 + log10(Vol_pipe))

  badness <- sum(mismatch1[-1]^2) + sum(mismatch2[-1]^2)
  return(badness)
}

objective <- function(x,giveall=FALSE){
  print(x)
  if(any(x<0)){return(1e99)}
  params <- c(x,inflow=2500)
  bad(params,giveall=giveall)
}

startvalue <- c(t0=1800,tG=7800,k=4e7)
```

```
ans <- optim(par = startvalue, fn=objective)
```

Note: The observation.txt is the observed 24 h tube-only run data;

Appendix 30 Hexagonal Reactor Model for Geo1 Temperature-cycling System

```
#This file does the coupon reactor growth as well.

library(deSolve)

                                        # all volumes are in ml and all times
are in seconds.


source("usefulfuncs.R")   # this defines f() which gives the growth rate in the
coupon as a function of Temp.
flowrate_min <- 1.5  #NB:  ml/min
flowrate <- flowrate_min / 60
incoming_milk_conc <- 1e4  # NB: bacteria per ml



Vol_pipe <- pi*0.3^2 * 60  # NB: volume in ml

Vol_coupon <- 4  # NB: volume in (milliliters)

Area_coupon <- Vol_coupon / 0.5     # 0.5 = height of coupon.

Area_pipe <- 2*pi*0.3*60  # curved surface area in square centimeters

settling_velocity <- Vol_pipe /(Area_pipe*48526)


carrying_capacity_perunitarea <- 4e8/Area_pipe


parameters <- c(t0         = Vol_pipe/(Area_pipe*settling_velocity),    #
settling efolding time, sec
                tG         = 6473,    # growth efolding time, sec
                k          = carrying_capacity_perunitarea * Area_pipe,     #
carrying capacity of the FILM of the pipe
                inflow     = incoming_milk_conc * flowrate,
              t0_coupon  = Vol_coupon /(Area_coupon *settling_velocity),
              k_coupon   = carrying_capacity_perunitarea * Area_coupon
                )


## first specify the _start_ state, remember that 'M' is the number of
## CFU in the free-flowing mil in the pipe.
state <- c(M = Vol_pipe * incoming_milk_conc , P = 0, N_coupon = Vol_coupon *
incoming_milk_conc, R_coupon=0)

bact <- function(t, state, parameters) {
 with(as.list(c(state, parameters)),{
```

```
 settling <- M / t0
 slough <- P^2/(k*tG)
 growth <- P/tG


 outflow <- M/Vol_pipe  * flowrate

 # rate of change
 dM <-  (
   +inflow
   -outflow
   -settling
   +slough
   )

 dP <-  (
   +settling
   -slough
   +growth
   )


 if(FALSE){
   tG_coupon <- growthrate_T(sine_wave(t,Tmin=35,Tmax=55,period=20))   # period
in *minutes*
 } else {
   tG_coupon <- growthrate_T(square_wave(t,Tmin=30,Tmax=55,t_low=35,t_high=15))
# 't_low' is time at low temperature
 }



out_conc <- M / Vol_pipe   # concentration in outflow from pipe = inflow to
coupon.

 inflow_coupon <-  out_conc*flowrate
 outflow_coupon <- (N_coupon / Vol_coupon) * flowrate
 settling_coupon <- N_coupon / t0_coupon

 slough_coupon <- R_coupon^2/(k_coupon*tG_coupon)
 growth_coupon <- R_coupon/tG_coupon

dN_coupon <- (
   +inflow_coupon
   -outflow_coupon
   -settling_coupon
   +slough_coupon
   )

 dR_coupon  <-        (
```

```
   +settling_coupon
   -slough_coupon
   +growth_coupon
   )


                                        # return the rate of change
 list(c(dM, dP, dN_coupon, dR_coupon))
}) # end with(as.list ...
}


times <- seq(0, 86400, by = 7200)

out <- ode(y = state, times = times, func = bact, parms = parameters)
head(out)
```

Appendix 31 Geo 1 Hexagonal Reactor Model Wave Function

```
jj <- read.table("data.txt",header=T)


growthrate_T <- approxfun(jj$temp,(1/jj$growth)*3600,yleft=1e-6,yright=1e-6)
# divide by 3600 to give growth rate per second, not h.
rm(jj)



sine_wave <- function(time,Tmin,Tmax,period){    # time in seconds,
                                                 # period in minutes,
                                                 # Tmin and Tmax min
                                                 # and max temperatures
                                                 # respectively
  p <- period*60
  (Tmin+Tmax)/2 - (Tmax-Tmin)*0.5*cos(2*pi*time/p)     # cosine because time=0
means minimum temperature.
}


square_wave_basic <- function(time,t_high,t_low){
  time <- time %% (t_high + t_low)
  ifelse(time < t_high, 1,0)
}


square_wave <- function(time, Tmin, Tmax, t_low, t_high){
 # time in seconds, period in minutes, Tmin and Tmax min and max
 # temperatures respectively.  t_high and t_low are TIMES at high temp
 # and low temp respectively.

  t_low <-  t_low  * 60
  t_high <- t_high * 60

  Tmin +  (Tmax-Tmin)* square_wave_basic(time,t_high,t_low)
}
```

Appendix 32 Geo 1 Hexagonal Coupon Reactor Model data.txt

temp growth

30 1e-6

35 1e-6

40 0.8465

50 1.4661

60 1.1997

70 1.052

80 1e-6

Appendix 33 Estimated Outflow vs. Actual Outflow of Geo1 Temperature-cycling System using the Reactor Model

Thermocycling 55-30°C, 20min								
Time	M	P	N_coupon	R_coupon	N_coupon/volume	Log_{10} (N_coupon)	Log_{10} (Actual)	Time
(h)	cfu	cfu	cfu	cfu	converted, cfu/ml	converted, cfu/ml	converted, cfu/ml	h
0	169646	0	40000	0	10000.00	4.00	4.04	0
2	167306.9	45645.92	39409.48	3857.817	9852.37	3.99	4.03	2
4	167313.5	184340.3	39411.09	19557.774	9852.77	3.99	4.09	4
6	167384.4	605636.4	39429.06	83359.237	9857.27	3.99	3.90	6
8	168063.7	1881743	39616.05	340422.231	9904.01	4.00	4.19	8
10	174310.5	5714260	41555.07	1341342.639	10388.77	4.02	5.71	12
12	229134.7	16936135	60655.12	4774316.371	15163.78	4.18	6.78	24
14	659612.5	47495932	204910.89	12817336.03	51227.72	4.71		
16	3219136	1.17E+08	896718.51	21852290.88	224179.63	5.35		
18	11914093	2.23E+08	2986527.91	26451580.89	746631.98	5.87		
20	25386818	3.2E+08	6186256.6	27954488.97	1546564.15	6.19		
22	35396451	3.73E+08	8583743.79	28411825.23	2145935.95	6.33		
24	40050292	3.94E+08	9705451.49	28561215.83	2426362.87	6.38		

Table 33.1: Comparison between estimated outflow bacterial concentrations vs. observed outflow bacterial concentrations (cfu/ml) from the Geo1 temperature-cycling system for Thermocycling 55-30°C with period of 20 min;

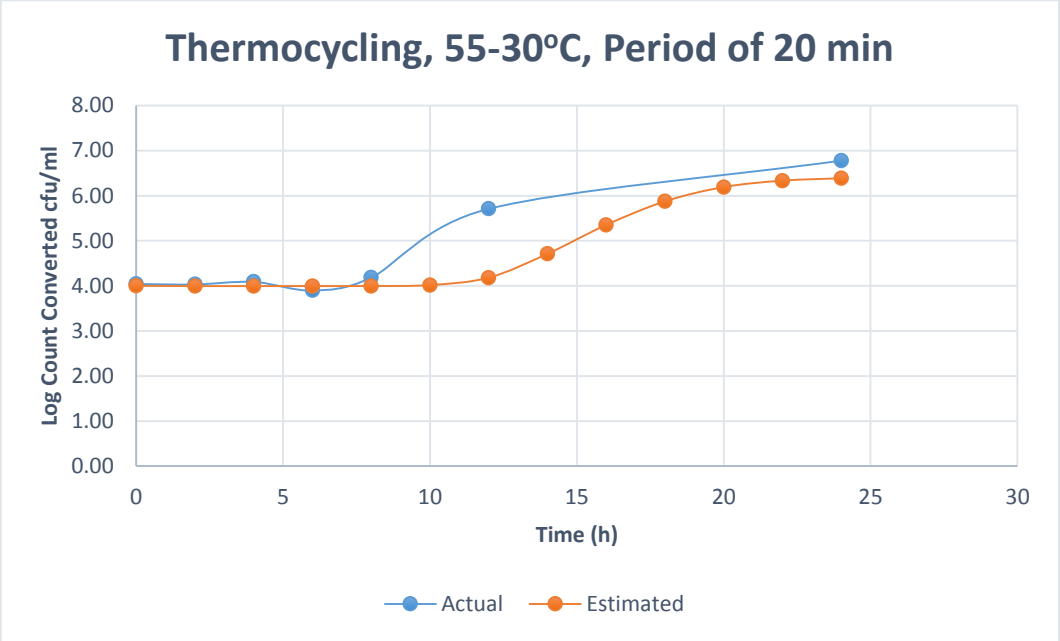

Figure 33.1: Comparison between estimated outflow bacterial concentrations (orange line) vs. observed outflow bacterial concentrations (blue line) (cfu/ml) from the Geo1 temperature-cycling system for Thermocycling 55-30°C with period of 20 min;

Thermocycling 55-35°C, 20min								
Time	M	P	N_coupon	R_coupon	N_coupon/volume	Log_{10} (N_coupon)	Log_{10} (Actual)	Time
(h)	cfu	cfu	cfu	cfu	converted, cfu/ml	converted, cfu/ml	converted, cfu/ml	h
0	169646	0	40000	0	10000	4.00	3.56	0
2	167306.9	45645.93	39409.49	4485.229	9852.3725	3.99	3.67	2
4	167313.5	184339.6	39411.31	27719.403	9852.8275	3.99	4.15	4
6	167384.4	605633.8	39436.77	147651.337	9859.1925	3.99	5.38	6
8	168063.7	1881728	39838.35	753919.33	9959.5875	4.00	5.48	8
10	174310.4	5714215	46691.6	3522594.888	11672.9	4.07	6.49	12
12	229133.8	16936007	121795.21	12018326.42	30448.8025	4.48	6.22	24
14	659605.6	47495601	399107.09	22454153.4	99776.7725	5.00		
16	3219101	1.17E+08	1098422.66	26974739.15	274605.665	5.44		
18	11914006	2.23E+08	3140500.41	28094051.93	785125.1025	5.89		
20	25386727	3.2E+08	6318720.39	28382510.49	1579680.098	6.20		
22	35396401	3.73E+08	8710174.69	28498866.57	2177543.673	6.34		
24	40050272	3.94E+08	9830405.52	28554518.83	2457601.38	6.39		

Table 33.2: Comparison between estimated outflow bacterial concentrations vs. observed outflow bacterial concentrations (cfu/ml) from the Geo1 temperature-cycling system for Thermocycling 55-35°C with period of 20 min;

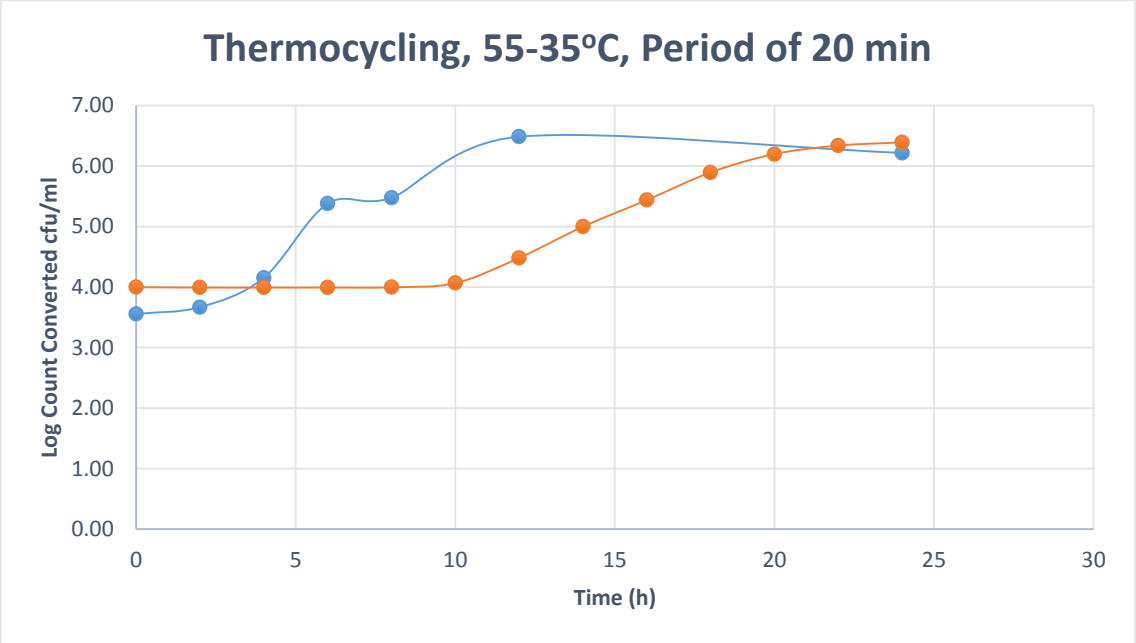

Figure 33.2: Comparison between estimated outflow bacterial concentrations (orange line) vs. observed outflow bacterial concentrations (blue line) (cfu/ml) from the Geo1 temperature-cycling system for Thermocycling 55-35°C with period of 20 min;

Thermospiking 55°C/15min, 30°C/35min								
Time	M	P	N_coupon	R_coupon	N_coupon/volume	Log_{10} (N_coupon)	Log_{10} (Actual)	Time
(h)	cfu	cfu	cfu	cfu	converted, cfu/ml	converted, cfu/ml	converted, cfu/ml	h
0	169646	0	40000	0	10000	4.00	4.77	0
2	167306.9	45645.58	39409.48	2904.334	9852.37	3.99	4.53	2
4	167313.5	184336.3	39410.96	8163.755	9852.74	3.99	4.17	4
6	167384.4	605618.8	39427.78	22481.394	9856.945	3.99	4.45	6
8	168063.6	1881687	39579.29	51190.452	9894.8225	4.00	4.98	8
10	174310.2	5714132	40982.11	101294.841	10245.5275	4.01	5.15	12
12	229132.5	16935828	53332.29	274125.762	13333.0725	4.12	6.24	24
14	659596.3	47495153	150466.61	530686.352	37616.6525	4.58		
16	3219054	1.17E+08	736040.64	1269147.245	184010.16	5.26		
18	11913890	2.23E+08	2742573.69	2699526.285	685643.4225	5.84		
20	25386608	3.2E+08	5912274.94	5026094.169	1478068.735	6.17		
22	35396345	3.73E+08	8334471.06	10785918.28	2083617.765	6.32		
24	40050254	3.94E+08	9419043.24	15790838.17	2354760.81	6.37		

Table 33.3: Comparison between estimated outflow bacterial concentrations vs. observed outflow bacterial concentrations (cfu/ml) from the Geo1 temperature-cycling system for square wave Thermospiking 55°C/15min, 30°C/35min;

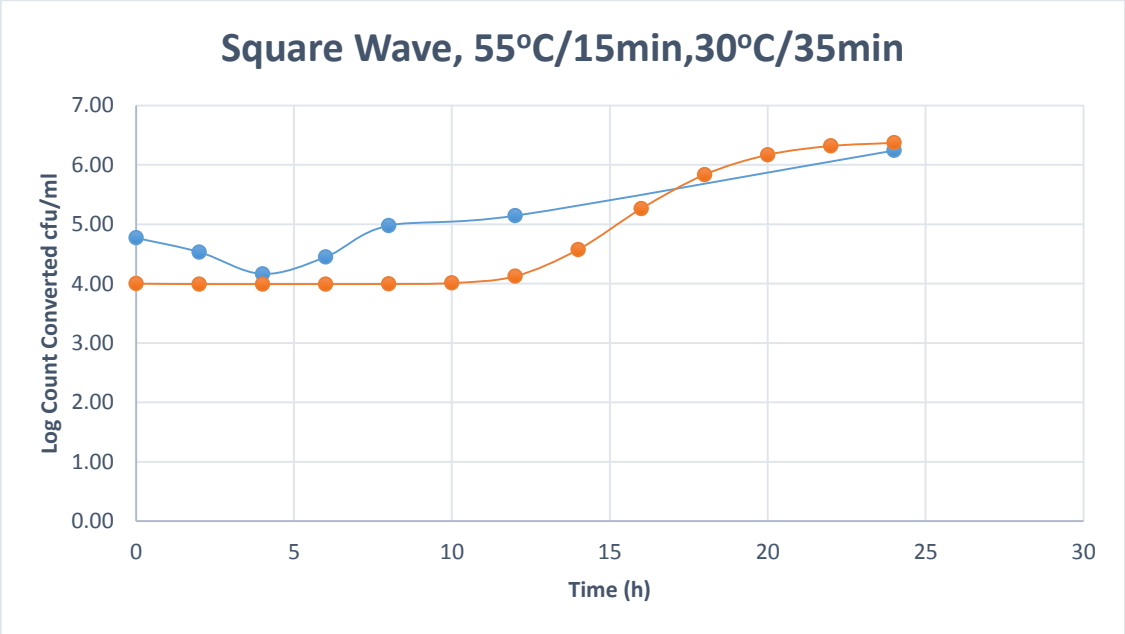

Figure 33.3: Comparison between estimated outflow bacterial concentrations (orange line) vs. observed outflow bacterial concentrations (blue line) (cfu/ml) from the Geo1 temperature-cycling system for square wave Thermospiking 55°C/15min, 30°C/35min;

Thermospiking 55°C/15min, 35°C/35min								
Time	M	P	N_coupon	R_coupon	N_coupon/volume	Log_{10} (N_coupon)	Log_{10} (Actual)	Time
(h)	cfu	cfu	cfu	cfu	converted, cfu/ml	converted, cfu/ml	converted, cfu/ml	(h)
0	169646	0	40000	0	10000	4.00	4.39	0
2	167306.9	45645.58	39409.48	2904.334	9852.37	3.99	3.78	2
4	167313.5	184336.3	39410.96	8163.755	9852.74	3.99	3.68	4
6	167384.4	605618.8	39427.78	22481.394	9856.945	3.99	3.38	6
8	168063.6	1881687	39579.29	51190.452	9894.8225	4.00	3.25	8
10	174310.2	5714132	40982.11	101294.841	10245.5275	4.01	3.94	12
12	229132.5	16935828	53332.29	274125.762	13333.0725	4.12	6.62	24
14	659596.3	47495153	150466.61	530686.352	37616.6525	4.58		
16	3219054	1.17E+08	736040.64	1269147.245	184010.16	5.26		
18	11913890	2.23E+08	2742573.69	2699526.285	685643.4225	5.84		
20	25386608	3.2E+08	5912274.94	5026094.169	1478068.735	6.17		
22	35396345	3.73E+08	8334471.06	10785918.28	2083617.765	6.32		
24	40050254	3.94E+08	9419043.24	15790838.17	2354760.81	6.37		

Table 33.4: Comparison between estimated outflow bacterial concentrations vs. observed outflow bacterial concentrations (cfu/ml) from the Geo1 temperature-cycling system for square wave Thermospiking 55°C/15min, 35°C/35min;

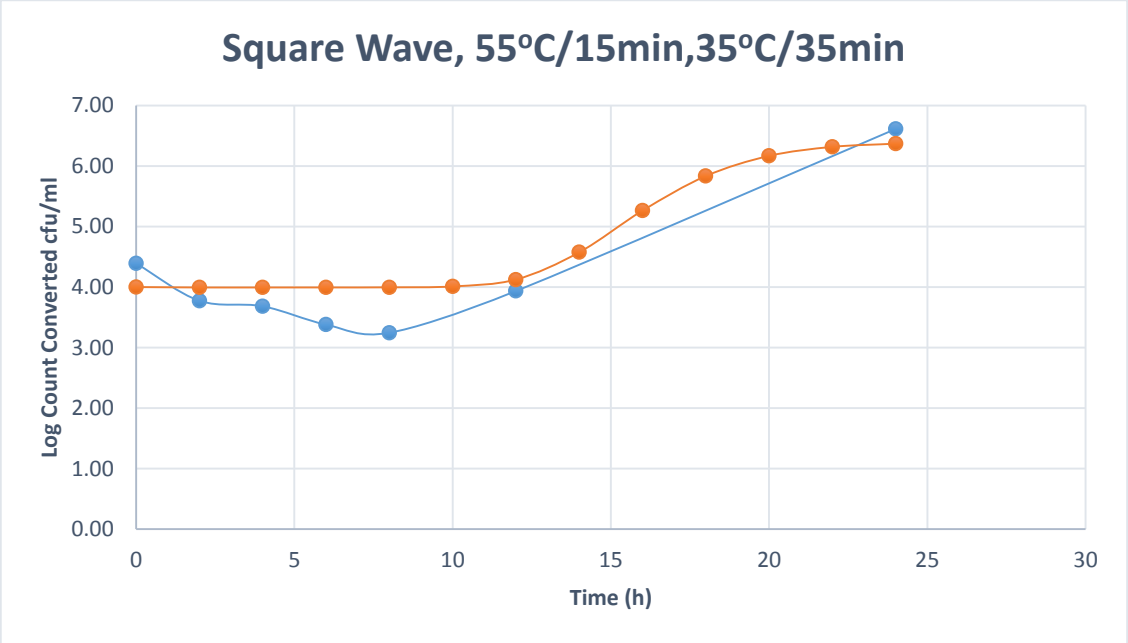

Figure 33.4: Comparison between estimated outflow bacterial concentrations (orange line) vs. observed outflow bacterial concentrations (blue line) (cfu/ml) from the Geo1 temperature-cycling system for square wave Thermospiking 55°C/15min, 35°C/35min;