APPENDICES

APPENDIX A

Cultivation medium

Cultivation medium

1. Nutrient broth (NB) per Littre

Beef extract	3.0 g
Peptone	5.0 g

Add the components to distilled water and make a volume to 1 L. Mix throughly and adjust pH to be 7.0 $\pm$ 0.2. Autoclave for 15 min at 15 psi pressure 121˚C before use.

2. Nutrient agar (NA) per Littre

Beef extract	3.0 g
Peptone	5.0 g
Agar	15.0 g

Add the components to distilled water and make a volume to 1 L. Mix throughly and adjust pH to 7.0 $\pm$ 0.2. Autoclave for 15 min at 15 psi pressure 121˚C. Pour the mixture into sterile petri dishes.

3. Sucrose yeast extract medium per Littre

Sucrose	20.0 g
Yeast extract	4.0 g
K_2HPO_4	2.5 g
$MgSO_4.7H_2O$	1.0 g
Trace element solution	4 mL

Trace element solution per Littre

$ZnSO_4.7H_2O$	18.0 g
$FeSO_4.7H_2O$	1.46 g
$CoCl_2.6H_2O$	0.90 g
$MnSO_4.4H_2O$	3.0 g
$CuSO_4.5H_2O$	0.80 g
H_2SO_4 (concentrated)	5.0 mL

Trace elements solution: Add all components to distilled water and adjust volume to 900 mL and mix throughly. Then, add dissolved ferric into 100 mL distilled water.

Medium: Add all components, except ferric and trace element solution, to distilled water, adjust volume to 996 mL and mix throughly. Autoclave for 15 min at 15 psi pressure, -121˚C. Leave it cool down, then add 4 mL of sterile trace element solution and 100 µL ferric solution into the mixture and mix throughly. (Atlas, 2006)

4. Minimal medium (Modified from Grothe et al, 1999)

Medium per Littre

Glucose or Sucrose	10.00 g
$(NH_4)_2SO_4$	1.40 g
KH_2PO_4	1.50 g
$Na_2HPO_4.12H_2O$	4.54 g
$MgSO_4.7H_2O$	0.20 g
Trace element solution	1.0 mL

Trace element solution per Littre

$FeSO_4$	1.46 g
$CaCl_2$	4.70 g
H_3BO_3	0.03 g
$CoCl_2.6H_2O$	0.20 g
$ZnSO_4.7H_2O$	0.10 g
$MnCl_2.4H_2O$	0.03 g
$Na_2MoO_4.4H_2O$	0.03 g
$CuSO_4.5H_2O$	0.01 g

Trace elements solution: Add the components to distilled and adjust volume to 900 L. Mix thoroughly, then and dissolved ferric into 100 mL distilled water.

Medium: Add the components, except ferric and trace element solution, to distilled water, mix throughly and adjust volume to 999 mL. Then, autoclave for 15 min at 15 psi pressure, -121˚C. Leave it cool down. Then, add 900 µL of sterile trace element solution and 100 µL ferric solution using aseptic technique.

APPENDIX B

Analytical methods

Analytical methods

1. Total sugar (Phenol sulfuric acid method) (Dubois et al, 1956)

Color reactions in strong acids qualitative test for sugars are based on

1) color reactions effected by the condensation of degradation products of sugars in strong mineral acids with various organic compounds;

2) the reducing properties of the carbonyl group

3) on oxidative cleavage of neighboring hydroxyl groups.

Many qualitative tests are determined on fractions separated by paper, thin-layer, or column chromatography (Pomeranz, Meloan, 1994). The phenol-sulfuric acid method (Dubois et al, 1956) is simple, rapid, sensitive, accurate, specific and widely applicable for carbohydrates. Virtually all classes of sugars including sugar derivatives, oligosaccharides and polysaccharides can be determined. The reagents are inexpensive, readily available and stable. A stable color is produced and the results are reproducible. The method is excellent for determining sugars separated by chromatography. In the direct determination of lactose in milk and cheese, normal amounts of casein, amino acid and organic acids do not interfere.

Reagents:

5% (w/v) phenol (analytical grade)

96% (v/v) sulfuric: H_2SO_4 (analytical grade)

Procedure:

1. Prepare a calibration sugar standard by using 100 µg mL^{-1} sugar standard solutions of the same sugar contained in the sample into distilled water. Vary sugar standard concentration in rage 5 to 100 µg mL^{-1}.

2. Add 500 µL of different sugar standard concentrations to clean and dry test tubes.

3. Add 500 µL of 5% (w/v) phenol and mix throughly, then add 2.5 mL concentration H_2SO_4 and wait 10 min.

4. Mix throughly and wait 10 min then, transfer the solutions from test tubes to the cuvettes and measure the absorbance at 490 nm. (Blank is a distilled water mixed with reagent)

5. To calculate the concentration of sugar present in the sample, make a graph plotting absorbance 490 nm versus sugar concentration (µg) of the sugar calibration standards.
6. Unknown samples were prepared and measure the same as sugar standard concentration.
7. Calculate the unknown sample concentration using the equation from standard sugar concentration versus A_{490} nm plot.

2. Optical density

Bacterial growth was monitored by measuring the optical density of cell culture at 600 nm after dilution with distilled water.

3. Dry cell weight

Dry cell mass was determined by gravimetry. The culture medium (20 mL) was centrifuged (10000×g, 5 min), the cell pellet was washed in distilled water, recovered (10000×g, 5 min) and dried to constant weight at 70°C, cooled in a desiccators and weighed.

4. Assay of poly-β-hydroxybutyric acid (PHB) content by crotonic acid method
(Law, Slepecky, 1961)

This method was used to estimate the production of poly-β-hydroxybutyric acid. The quantitative conversion of poly-β-hydroxybutyric acid to crotonic acid by heating in concentrated sulfuric acid and determination of the ultraviolet absorption of the produce permits an accurate determination of this material.

Reagents:

Poly- β-hydroxybutyrate (analytical grade)
96% (v/v) sulfuric: H_2SO_4 (analytical grade)
Chloroform: $CHCl_3$ (analytical grade)
6% Sodium hypochlorite: NaClO (commercial grade)
Procedure:
1) Dry cell mass was resuspended in a sodium hypochlorite solution equal to original volume of medium then was incubated at 37 ºC for 1 hr.

2) After 1 hr, then was centrifuged and washed with distilled water.

3) The polymer was dissolved by extraction with hot chloroform.

4) The chloroform was evaporated and 10 mL of concentrated H_2SO_4 was added, the test tube was capped with glass beat and heated to 100ºC for 10 min in a water bath.

5) The solution was cooled and after mixing transferred to cuvette for measured the absorbance at 235 nm.

6) The amount of crotonic acid is calculated from standard graph between poly-β-hydroxybutyric acid concentration (μg mL^{-1}) and absorbance at 235 nm.

5. Extraction of polyhydroxyalkanoates (PHAs) by gravimetric method

Gravimetrical determination of PHAs was modified from Gouda et al. (2001) and Zhaolin and Xuenan (2000). It was simple method. The sequent steps are explained ad follow:

1) Cell pellet was treated with 6% (v/v) NaClO (1:1) and incubate at 37°C and shake for 3 hr on incubator shaker.

2) Chloroform was added into mixed solution (2:1) then, incubated at 55°C for 6 hr.

3) After 6 h, the was separated chloroform phase to precipitate with chilled ethanol.

4) The white powder/ solid was obtained when was dried for 24-48 hr at room temperature to constant weight.

APPENDIX C

Data collection

Growth profiles of isolated strains

Table 1 Growth profile of OD600nm in nutrient broth

Time (h)	SC1 14	SC1 26	SV1 3	SV1 9
0	0.140	0.002	0.016	0.025
3	0.044	0.053	1.187	0.094
6	0.079	0.110	1.434	0.670
9	0.205	0.238	1.735	1.678
12	0.412	0.466	1.924	2.007
15	0.583	0.738	1.866	2.067
21	0.737	0.933	1.495	2.082
25	0.956	1.223	1.579	2.050
29	0.969	0.926	1.716	2.079
33	1.040	1.145	2.010	2.014
37	1.055	1.313	2.026	2.010
41	1.052	1.306	1.987	1.983
45	1.026	1.273	1.919	1.965
49	1.013	1.225	1.947	1.956
53	0.958	1.187	1.943	1.878

Table 2 Growth profile of isolated strain SV13 in minimal medium

Time (h)	OD600nm	OD490	TS (gL^{-1})	DCW (gL^{-1})	OD235nm	PHAs (μgmL^{-1})
0	0.234	0.249	31.859	2.500	0	0
4	1.032	0.248	31.731	2.700	-	-
8	1.422	0.212	27.137	4.700	-	-
12	1.624	0.203	25.983	4.700	0.012	0.805
16	1.696	0.204	26.154	5.600	0.029	1.946
20	1.722	0.198	25.385	4.300	0.035	2.349
24	1.652	0.179	22.991	5.100	0.041	2.752
28	1.768	0.164	21.068	4.600	0.285	19.128
32	1.762	0.164	21.026	3.700	0.132	8.859
36	1.692	0.166	21.282	6.700	0.140	9.396
40	1.606	0.162	20.769	6.500	0.223	14.966
44	1.644	0.158	20.192	5.600	0.191	12.819
48	1.758	0.160	20.513	6.200	0.126	8.456

Table 3 Growth profile of isolated strain SV19 in minimal medium

Time (h)	OD600nm	OD490	TS (gL^{-1})	DCW (gL^{-1})	OD235nm	PHAs (μgmL^{-1})
0	0.436	0.250	32.051	0.0034	3.200	0.005
4	1.394	0.252	32.350	0.0032	3.400	0.032
8	1.634	0.228	29.274	0.0038	3.800	
12	1.68	0.217	27.821	0.0033	3.300	0.329
16	1.66	0.212	27.179	0.0052	5.200	
20	1.648	0.212	27.222	0.0042	4.200	0.229
24	1.646	0.201	25.769	0.0030	3.000	
28	1.622	0.200	25.641	0.0045	4.500	0.126
32	1.55	0.208	26.667	0.0051	5.100	0.156
36	1.486	0.194	24.872	0.0056	5.600	0.107
40	1.554	0.193	24.744	0.0037	3.700	0.155
44	1.574	0.178	22.821	0.0040	4.000	0.097
48	1.592	0.152	19.530	0.0036	3.600	0.075

Table 4 Growth profile of isolated strain SC114 in minimal medium

Time (h)	OD600nm	OD490	TS (gL^{-1})	DCW (gL^{-1})	OD235nm	PHAs (μgmL^{-1})
0	0.272	0.243	31.154	0.0030	3.000	0.403
4	0.962	0.247	31.667	0.0018	1.800	0.403
8	1.296	0.246	31.581	0.0027	2.700	0.470
12	1.53	0.243	31.154	0.0039	3.900	2.416
16	1.688	0.220	28.141	0.0033	3.300	1.275
20	1.704	0.216	27.692	0.0028	2.800	1.477
24	1.75	0.215	27.607	0.0037	3.700	1.544
28	1.718	0.214	27.436	0.0033	3.300	1.275
32	1.75	0.215	27.521	0.0057	5.700	1.342
36	1.664	0.211	26.987	0.0037	3.700	0.604
40	1.744	0.206	26.410	0.0044	4.400	3.221
44	1.776	0.200	25.684	0.0039	3.900	0.403
48	1.626	0.198	25.385	0.0031	3.100	2.013

Table 5 Growth profile of isolated strain SC126 in minimal medium

Time (h)	OD600nm	OD490	TS (gL^{-1})	DCW (gL^{-1})	OD235nm	PHAs (μgmL^{-1})
0	0.422	0.207	26.581	0.0032	3.200	0.007
4	1.396	0.177	22.650	0.0023	2.300	0.018
8	1.592	0.160	20.556	0.0034	3.400	0.044
12	1.604	0.147	18.889	0.0031	3.100	0.107
16	1.582	0.145	18.547	0.0031	3.100	0.105
20	1.648	0.130	16.667	0.0034	3.400	0.199
24	1.74	0.127	16.282	0.0038	3.800	0.188
28	1.738	0.113	14.487	0.0038	3.800	0.168
32	1.644	0.100	12.821	0.0042	4.200	0.150
36	1.672	0.100	12.821	0.0051	5.100	0.276
40	1.718	0.095	12.222	0.0042	4.200	0.231
44	1.77	0.089	11.368	0.0043	4.300	0.149
48	1.684	0.095	12.179	0.0038	3.800	0.167

Table 6 Compare production of PHAs by SV13 and SV19 in minimal medium

Time (h)	SV13		SV19	
	Replication No.1 PHAs ($\mu g\ mL^{-1}$)	Replication No.2 PHAs ($\mu g\ mL^{-1}$)	Replication No.1 PHAs ($\mu g\ mL^{-1}$)	Replication No.2 PHAs ($\mu g\ mL^{-1}$)
0	0.000	0.000	0.000	0.403
4	22.361	16.720	37.470	9.871
8	65.270	48.550	15.310	10.677
12	71.112	84.811	7.454	4.633
16	31.426	68.896	4.432	3.022
20	19.339	34.247	4.835	5.641
24	25.383	55.600	0.806	2.216
28	24.778	50.161	24.980	1.007
32	41.700	51.571	22.764	3.425
36	-	63.255	3.425	-
40	9.468	46.938	8.461	2.619

Table 7 Compare total sugar consumed by SV13 and SV19 in minimal medium

Time (h)	SV13		SV19	
	Replication No.1 Total sugar (gL^{-1})	Replication No.2 Total sugar (gL^{-1})	Replication No.1 Total sugar (gL^{-1})	Replication No.2 Total sugar (gL^{-1})
0	8.547	9.530	9.744	8.932
4	6.346	6.966	9.103	7.949
8	6.346	6.752	9.615	7.692
12	6.154	7.009	7.564	7.265
16	6.538	7.949	7.436	7.564
20	-	6.966	8.077	7.436
24	5.983	5.983	7.821	8.205
32	6.368	7.051	7.308	8.718
36	6.603	7.265	-	8.077
40	6.282	6.368	-	8.932

Table 8 Growth profile of PHAs production by SV13 in minimal medium

Time (h)	OD600nm	Total sugar (gL^{-1})	DCW (gL^{-1})	PHAs ($\mu g\ mL^{-1}$)
0	0.156	9.422	0.0483	4.36476
4	2.673	6.786	0.7667	11.01262
8	3.522	5.918	1.1950	57.01048
12	4.11	5.740	1.2583	40.89444
16	4.413	5.918	1.5483	59.49503
20	4.683	5.612	1.5583	177.0078
24	4.824	5.357	1.5183	116.3041
28	4.641	4.898	1.5383	97.50201
32	4.689	5.000	1.5444	73.73086
36	4.695	5.204	1.5017	49.95971
40	4.608	5.408	1.4750	4.297609

Table 9 Growth profile in nutrient broth by *Alcaligenes latus* TISTR 1403

Time (h)	OD 600nm	
	Replication No. 1	Replication No. 2
0	0	0
3	0.002	0.003
6	0.017	0.027
10	0.352	0.457
14	1.001	1.073
18	1.063	0.978
22	1.004	0.981
26	0.914	0.936
30	0.895	0.915

Table 10 Preliminary growth profile of PHAs production in minimal medium by *Alcaligenes latus* TISTR 1403

Time	OD600nm	TS (gL^{-1})	PHAs ($\mu g\ mL^{-1}$)
0	0.081	11.966	1.410
4	0.465	11.923	2.417
8	0.786	11.410	4.835
12	1.707	10.256	-
16	2.757	8.803	3.022
20	2.856	7.692	-
24	2.766	7.115	3.022
28	2.781	7.308	6.044
32	2.724	6.752	4.432
36	2.631	6.923	3.828
40	2.655	7.051	2.216

Table 11 Growth profile of PHAs production in minimal medium by *Alcaligenes latus* TISTR 1403

Time (h)	OD600nm	Total sugar (gL^{-1})	DCW (gL^{-1})	PHAs ($\mu g\ mL^{-1}$)
0	0.09	9.693878	0.055557	0
4	0.111	8.647959	0.057778	0.402901
8	0.123	7.92517	0.057778	4.49906
12	2.424	6.292517	0.722222	12.08703
16	2.922	5.969388	1.208889	26.25571
20	3.867	5.178571	1.306667	39.08139
24	4.479	5.034014	1.646667	138.7322
28	4.983	4.158163	1.608889	116.5055
32	5.373	4.115646	-	-
36	5.748	3.112245	1.826667	-
40	5.28	2.729592	1.462222	5.170561

Table 12.1 Plackett and Burman production of PHAs profile by SV13 (Replication No.1)

Run	Total sugar concentrations			DCW (gL^{-1})	PHAs (μgL^{-1})
	Initial (0h)	Final (20h)	Total sugar consumed (gL^{-1})		
1	52.270	25.995	26.276	3.930	444.668
2	43.707	28.367	15.340	1.7300	14.370
3	49.031	36.803	12.228	2.3500	17.325
4	9.388	3.699	5.689	1.1900	106.702
5	47.891	41.276	6.616	2.3100	23.100
6	9.056	2.730	6.327	0.3800	5.305
7	9.048	1.888	7.160	1.0800	9.334
8	8.027	5.689	2.338	1.0500	62.114

Table 12.2 Plackett and Burman production of PHAs profile by SV13 (Replication No.2)

Run	Total sugar concentrations			DCW (gL^{-1})	PHAs (μgL^{-1})
	Initial (0h)	Final (20h)	Total sugar consumed (gL^{-1})		
1	47.245	26.497	20.748	3.7300	461.456
2	47.109	28.980	18.129	1.4900	7.991
3	45.714	37.270	8.444	2.1000	30.553
4	10.587	3.503	7.083	0.9600	81.655
5	46.276	33.231	13.044	1.9700	27.733
6	9.082	2.781	6.301	1.6200	9.804
7	9.796	1.964	7.832	1.0500	29.412
8	8.435	5.612	2.823	0.4600	70.373

Table 13 Central composite design of 3 factors (agitation rate, pH and nitrogen) for process optimization of PHAs production by strain SV13 profile

Run	Agitation rate (rpm)	pH	Nitrogen (gL^{-1})	PHAs ($\mu g\ mL^{-1}$)	Total sugar consumed (gL^{-1})
1	140.55	6.60	1.00	231.440	24.620
2	259.45	6.60	1.00	431.938	25.349
3	140.55	8.40	1.00	66.613	5.367
4	259.45	8.40	1.00	357.370	22.419
5	140.55	6.60	3.00	161.698	14.749
6	259.45	6.60	3.00	560.032	24.024
7	140.55	8.40	3.00	89.847	10.323
8	259.45	8.40	3.00	436.980	28.118
9	100.02	7.50	2.00	22.020	11.740
10	299.98	7.50	2.00	551.303	17.420
11	200.00	5.99	2.00	491.940	29.460
12	200.00	9.01	2.00	134.430	26.490
13	200.00	7.50	0.32	291.160	27.820
14	200.00	7.50	3.68	164.518	23.890
15	200.00	7.50	2.00	415.250	14.760
16	200.00	7.50	2.00	416.060	19.400
17	200.00	7.50	2.00	443.997	15.206

Table 14 ANOVA for response surface quadratic model analysis of variance of 3 factors by strain SV13 (Partial sum of squares)

Std. Dev.	59.89201	R-Squared	0.949631
Mean	309.7997	Adj R-Squared	0.884871
C.V.	19.33249	Pred R-Squared	0.615163
PRESS	191845	Adeq Precision	11.91307

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	423.4816	1	34.511	341.8761	505.0872	
A-Agitation	155.7362	1	16.20666	117.4135	194.0588	1
B-pH	-75.8268	1	16.20666	-114.149	-37.5041	1
C-Nitrogen	-3.79224	1	16.20666	-42.1149	34.53042	1
A^2	-43.3638	1	17.83779	-85.5435	-1.18415	1.155714
B^2	-33.9863	1	17.83779	-76.166	8.193374	1.155714
C^2	-64.1607	1	17.83779	-106.34	-21.981	1.155714
AB	4.882219	1	21.17502	-45.1888	54.95319	1
AC	31.77656	1	21.17502	-18.2944	81.84753	1
BC	5.561544	1	21.17502	-44.5094	55.63251	1

Table 15 Central composite design of 2 factors (pH and nitrogen) for process optimization of PHAs production by strain SV13 profile (Replication No.1)

Run	pH	Nitrogen (gL^{-1})	PHAs ($\mu g\ mL^{-1}$)	DCW (gL^{-1})	Total sugar consumed (gL^{-1})
1	5.00	0.50	348.619	3.835	22.527
2	6.50	0.50	538.122	4.890	21.855
3	5.00	2.75	325.691	4.2225	20.168
4	6.50	2.75	570.994	4.3975	17.366
5	4.69	1.63	236.188	3.2325	19.543
6	6.81	1.63	440.608	4.2950	24.695
7	5.75	0.03	585.635	4.1800	23.844
8	5.75	3.22	565.193	3.8925	19.059
9	5.75	1.63	687.840	-	11.194
10	5.75	1.63	736.460	3.0800	11.273
11	5.75	1.63	686.740	4.0025	21.604
12	5.75	1.63	676.519	3.2000	20.968

Table 16 ANOVA for response surface quadratic model analysis of variance of 2 factors by strain SV13 on Replication No.1 (Partial sum of squares)

Std. Dev.	29.58768	R-Squared	0.981793
Mean	533.2174	Adj R-Squared	0.96662
C.V.	5.548895	Pred R-Squared	0.910563
PRESS	25801.37	Adeq Precision	23.5156

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	696.8898	1	14.79384	660.6905	733.089	
A-pH	90.48744	1	10.46082	64.89073	116.0842	1
B-Nitrogen	-2.37067	1	10.46082	-27.9674	23.22604	1
A^2	-182.008	1	11.69556	-210.626	-153.39	1.041667
B^2	-63.5003	1	11.69556	-92.1182	-34.8823	1.041667
AB	13.95	1	14.79384	-22.2492	50.14922	1

Table 17 Central composite design of 2 factors (pH and nitrogen) for process optimization of PHAs production by strain SV13 profile (Replication No.2)

Run	pH	Nitrogen (gL^{-1})	PHAs ($\mu g\ mL^{-1}$)	DCW (gL^{-1})	Total sugar consumed (gL^{-1})
1	5.00	0.50	378.453	3.915	23.172
2	6.50	0.50	482.044	4.6525	22.097
3	5.00	2.75	323.632	3.6325	20.412
4	6.50	2.75	474.586	4.2250	17.124
5	4.69	1.63	248.895	3.6900	21.048
6	6.81	1.63	416.851	3.0050	26.685
7	5.75	0.03	595.027	4.0175	22.527
8	5.75	3.22	559.669	3.9075	17.948
9	5.75	1.63	626.51	3.3040	17.284
10	5.75	1.63	626.52	2.8000	12.763
11	5.75	1.63	652.49	3.2320	12.497
12	5.75	1.63	654.69	3.3040	20.423

Table 18 ANOVA for response surface quadratic model analysis of variance of 2 factors by strain SV13 on Replication No.2 (Partial sum of squares)

Std. Dev.	26.01272	R-Squared	0.980525
Mean	503.2806	Adj R-Squared	0.964296
C.V.	5.168633	Pred R-Squared	0.880327
PRESS	24948.37	Adeq Precision	22.52838

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	640.0525	1	13.00636	608.2271	671.8779	
A-pH	61.50883	1	9.196887	39.00486	84.0128	1
B-Nitrogen	-14.0353	1	9.196887	-36.5393	8.468625	1
A^2	-163.698	1	10.28243	-188.858	-138.537	1.041667
B^2	-41.4602	1	10.28243	-66.6204	-16.3	1.041667
AB	11.84075	1	13.00636	-19.9847	43.66617	1

Calculations

1. Biomass yield ($Y_{X/S}$)
2. PHAs yield ($Y_{P/S}$)
3. Productivity
4. Specific productivity

For example (PHAs production in flask scale data in the first row in Table 4.16)

Scale	Total sugar consumed (gL^{-1})	DCW (gL^{-1})	PHAs ($g\ L^{-1}$)	$Y_{P/X}$	$Y_{P/S}$	Productivity ($g\ L^{-1}\ h^{-1}$)	Specific productivity ($g\ g\ h^{-1}$)
	17.35	3.1710	0.6174	0.1947	0.0356	0.0309	0.009736
Flask	27.50	4.035	0.7913	0.196	0.0288	0.0396	0.009802
	31.41	4.211	0.7907	0.188	0.0252	0.0395	0.00938

1. Biomass yield ($Y_{X/S}$)

(gram Biomass produced per gram substrate consumed, $g\ g^{-1}$)

DCW (X) is 3.1710 gL^{-1} and Total sugar consumed (S) is 17.35 gL^{-1}

$$\left(Y_{X/S}\right) = \frac{\text{DCW}}{\text{Total sugar consumed}} \frac{gL^{-1}}{gL^{-1}}$$

$$\text{Biomass yield } \left(Y_{X/S}\right) = \frac{3.1710}{17.35} \frac{g}{g}$$

Biomass yield ($Y_{X/S}$) = 0.1828 $g\ g^{-1}$

2. PHAs yield ($Y_{P/S}$)

(gram PHAs produced per gram substrate consumed, g g^{-1})

PHAs (P) is 0.6174 gL^{-1}, Total sugar consumed (S) is 17.35 gL^{-1}

$$\left(Y_{P/S}\right) = \frac{\text{PHAs}}{\text{Total sugar consumed}} \frac{gL^{-1}}{gL^{-1}}$$

$$\text{PHAs yield } \left(Y_{P/S}\right) = \frac{0.6174}{17.35} \frac{g}{g}$$

PHAs yield ($Y_{P/S}$) = 0.0356

3. Productivity

(gram PHAs produced per hour, g L^{-1} h^{-1})

PHAs yield was produced at 0.6174 gL^{-1} under 20 hour cultivation time.

$$\text{Productivity} = \frac{\text{PHAs}}{\text{Time}} \frac{gL^{-1}}{h}$$

$$\text{Productivity} = \frac{0.6174}{20} \frac{gL^{-1}}{h}$$

Productivity = 0.0309 g L^{-1} h^{-1}

4. Specific productivity

(gram PHAs produced per gram DCW per hour, g g^{-1} h^{-1})

PHAs yield was produced at 0.6174 gL^{-1} and Biomass yield (DCW) was produced at 3.1710 gL^{-1} under 20 hour cultivation time.

$$\text{Specific productivity} = \frac{\text{PHAs yield}}{\text{Biomass yield (DCW)} \cdot \text{Time}} \quad \frac{gL^{-1}}{gL^{-1} \cdot h}$$

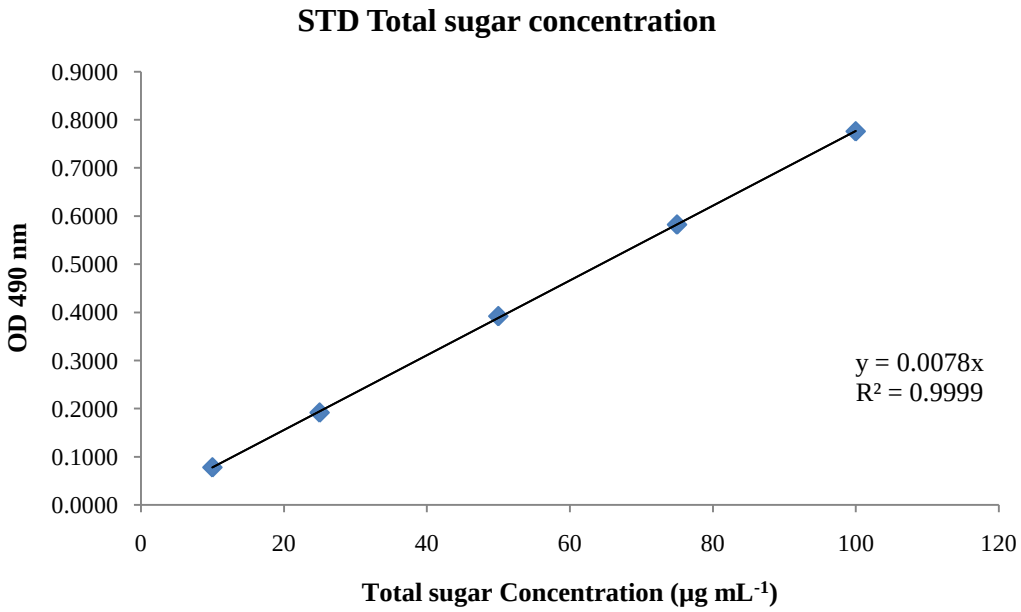
$$\text{Specific productivity} = \frac{0.6174}{3.1710 \cdot 20} \frac{gL^{-1}}{gL^{-1} \cdot h}$$

Specific productivity = 0.009736 g g^{-1} h^{-1}

Example standard graphs

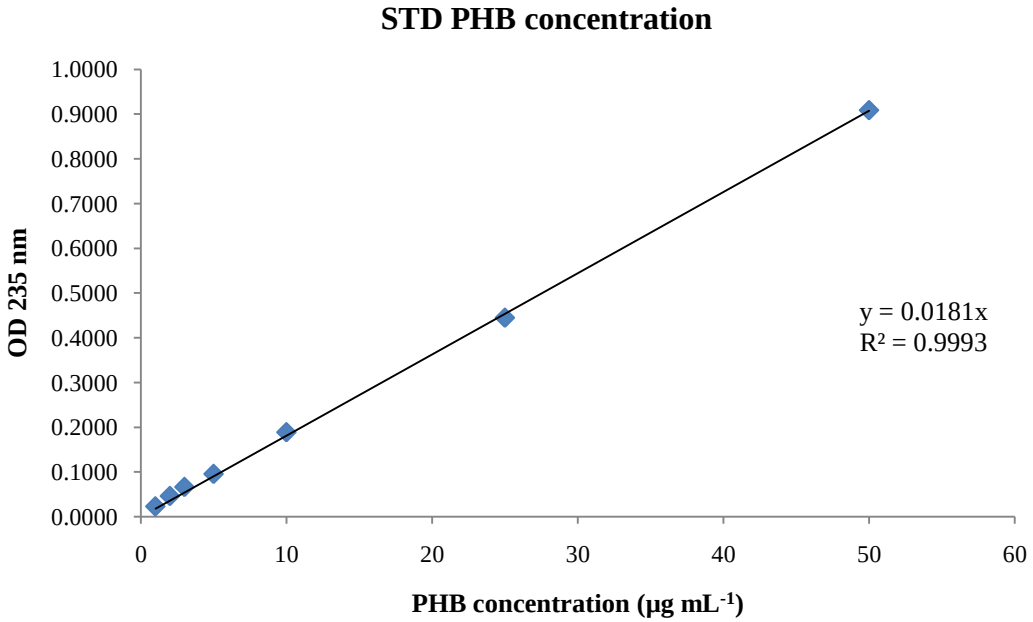
Standard Total sugar concentration

Total sugar ($\mu g\ mL^{-1}$)	OD490nm					
	1	2	3	4	5	Average
0	0.000	0.000	0.000	-	-	0.000
10	0.061	0.075	0.066	0.101	0.088	0.0782
25	0.172	0.175	0.236	0.195	0.182	0.1920
50	0.379	0.396	0.381	0.402	0.403	0.3922
75	0.572	0.591	0.59	0.598	0.561	0.5824
100	0.717	0.749	0.760	0.850	0.804	0.7760



Standard PHB concentration

PHB ($\mu g\ mL^{-1}$)	OD235nm					
	1	2	3	4	5	Average
1	0.020	-	0.024	0.026	0.022	0.0230
2	0.048	0.049	0.044	0.044	0.046	0.0462
3	0.062	0.075	0.076	0.059	0.06	0.0664
5	0.097	0.095	0.096	0.094	0.096	0.0956
10	0.182	0.193	0.190	0.187	0.192	0.1888
25	0.445	0.420	-	0.448	0.466	0.44475
50	-	0.921	0.907	0.904	0.903	0.90875



วว.

คำขอบริการที่ 2554/3-011 ที่ ฝวช.

รายงานผลการทดสอบและวิเคราะห์

ให้แก่

ภาควิชาเทคโนโลยีชีวภาพ คณะเทคโนโลยี

มหาวิทยาลัยขอนแก่น

การทดสอบ / วิเคราะห์ การพิสูจน์เอกลักษณ์เชื้อด้วยการวิเคราะห์ลำดับเบส 16S rDNA ของตัวอย่าง SV13

วิธีทดสอบ / วิเคราะห์ การวิเคราะห์ลำดับเบส 16S rDNA

ภาวะการทดสอบ / วิเคราะห์ : อุณหภูมิ - ^{o}C **ความชื้นสัมพัทธ์** - %

วันที่ทดสอบ / วิเคราะห์ 28 กุมภาพันธ์ 2554

ผลการทดสอบ / วิเคราะห์

การพิสูจน์เอกลักษณ์เชื้อด้วยการวิเคราะห์ลำดับเบส 16S rDNA ของตัวอย่าง SV13 พบว่ามีความคล้ายคลึงกับ *Bacillus cereus* ที่ระดับ 99%

ผู้ทดสอบ / วิเคราะห์

1. นางสาวขนิษฐา นิวาศะบุตร

ผู้ตรวจสอบ

(ดร. โสภณ สิริศรัทธา)

ผู้รับรอง

(ดร. ภูษิตา วรรณิสสร)

รักษาการผู้อำนวยการฝ่ายวิทยาศาสตร์ชีวภาพ

วันที่ 10 มี.ค. 2554

สถาบันวิจัยวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย
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APPENDIX D

Research contributions

Research contributions

During study, 5 articles have been made and contributed. The details showed ad follow;

1) **Waranya Suwannasing**. Pakawadee Kaewkannetra. Isolation of bacteria from the environments to produce biopolymer, **Academic Service Center, Khon Kaen University** 2010; 18(3-4); 9-14. (Review article in Thai)

2) **Waranya Suwannasing**, Samart Moonamart, Pakawadee Kaewkannetra. **Feasibility of sugar cane juice as a sole carbon for polyhydroxy-alkanoates (PHAs) production via batch fermentation by *Alcaligenes latus* 1403 and *Alcaligenes eutrophus* TISTR 1095.** Poster presentation in the 14th International Biotechnology Symposium and Exhibition (IBS 2010). September 14-18, 2010. Rimini, Italy.

3) **Waranya Suwannasing**, Pakawadee Keawkannetra, Samart Mooamart. Feasibility of sugar cane juice as a sole carbon for polyhydroxyalkanoates (PHAs) production via batch fermentation by *Alcaligenes latus* TISTR 1043 and *Alcaligenes eutrophus* TISTR 1095. **Journal of Biotechnology** 2010 Nov; 150S: 229.

4) Samart Moonamart, **Waranya Suwannasing**, Pakawadee Kaewkannetra. Batch fermentation of sugar cane juice for producing biopolymer of PHAs. **KKU Research Journal** 2011; In press. (Research article in Thai)

5) **Waranya Suwannasing**, Samart Moonamart, Pakawadee Kaewkannetra. Yields of polyhydroxyalkanoates (PHAs) during batch fermentation of sugar cane syrup by *Alcaligenes latus* and *Alcaligenes eutrophus*. **Journal of Life Sciences** 2011; In press.