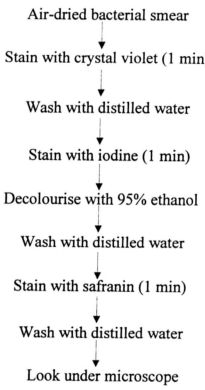
APPENDICES

APPENDIX

APPENDIX A : ANALYTICAL METHODS

1. Gram Staining (Collins and Lyne, 1984)



(Note : Gram + cells are stained purple)

Gram - cells are stained red)

2. Cell Dry Weight

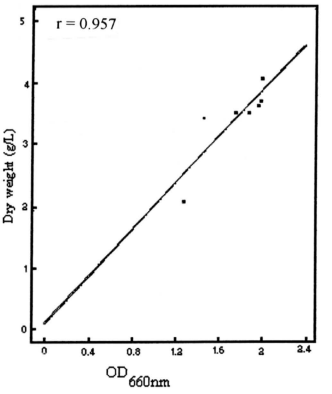
The cell dry weight was determined by filtering 10 mL of thoroughly mixed culture sample through Whatman filter paper No. 4 which was previously dried and weighed. The cell mass in the filter paper was oven-dried at 60 °C overnight, then cooled in a dessicator and finally weighed. Measurements were carried out in duplicates.

3. Cell Mass Concentration (Noparatnaraporn *et al.*, 1983)

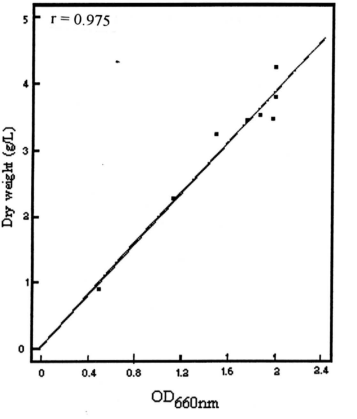
The cell mass concentration was determined by measuring the optical density at 660 nm with a spectrophotometer (Shimadzu UV-160A). The OD_{660nm} values were then converted to cell dry weight by interpolating from the standard curve of cell dry weight (g/L) against OD_{660nm}.

(a) Regression of *R. palustris* strain B1 dry cell weight against OD_{660nm}

(Appendix C32)



(b) Regression of *R. palustris* ATCC 17001 Dry Cell Weight Against OD_{660nm} (Appendix C33)



4. Electron Microscopy (Vijaya, N., Personal Communication)

2 to 3 drops of culture were transferred to a nucleopore membrane (size 0.45 μm)

↓

The membranes were subsequently fixed in 2% aqueous osmium tetraoxide overnight at 4 °C

↓

The sample were brought to room temperature

↓

Samples were washed 2 to 3 times in distilled water for 15 min per wash

↓

They were dehydrated in an ascending series of ethanol from 10% to 100%

↓

The samples were transferred to intermediate fluids of ethanol-acetone mixtures with ratios of 3:1, 1:1 and 1:3 for 15 min each before transferring to 100% acetone for one hour.

↓

Samples were critical point dried in liquid carbon dioxide before mounting on aluminium stubs with conductive carbon cement (Agar Aids)

↓

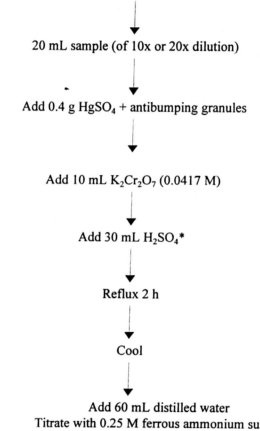
The stubs were dried overnight in a vacuum dessicator and coated with gold (Biorad cool sputter coater E5100)

↓

The stubs were viewed in a Phillips Scanning Electron Microscope 515

5. Chemical Oxygen Demand (COD) Analysis (APHA, AWWA and WPCF, 1989)

The COD determination is a measure of the oxygen equivalent of that portion of organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant.



(*Add Ag_2SO_4 to concentrated H_2SO_4)

The end-point is a sharp colour change from blue-green to reddish-brown.

The amount of oxidizable organic matter, measured as oxygen equivalent, is proportional to the potassium dichromate used.

The COD of the sample was calculated based on the formula:

$$\text{COD (mg/L)} = \frac{(A - B) \times M \times 8000}{\text{mL sample}}$$

where, A = mL FAS used for the titration of blank

B = mL FAS used for the titration of sample

M = molarity of FAS (Appendix B2)

APPENDIX B : MEDIA,REAGENTS AND BUFFERS

1. Media

a. Glutamate-malate medium, GMM (Noparatnaraporn, 1994)

	DL - malic acid	3.0 g
	Sodium glutamate	2.0 g
	$(NH_4)_2 PHO_4$	1.0 g
A	K_2HPO_4	0.5 g
	$KH_2 PO_4$	0.5 g
	$CaCl_2.2H_2O$	0.6 g
	$MgSO_4.7H_2O$	0.2 g
	$MnSO_4.5H_2O$ (1 mg/mL)	1.3 mL
	$CoCl_2.6H_2O$ (1 mg/mL)	1.0 mL
	Ferric citrate (10 mg/mL) in 30% NH_4OH	0.25 mL
	Nicotinic acid (1 mg/mL)	1.0 mL
	Thiamine - HCl (1 mg/mL)	1.0 mL
	Biotin (100 mg/mL)	1.0 mL
	Yeast extract	1.0 g

The organic substrates were added before the vitamins. The solution was made up to 1000 mL in distilled water. pH was adjusted to pH 6.8-7.2 by adding 3M NaOH prior to autoclaving. The medium may conveniently be made double-strength and stored at 4 °C with a few drops of toluene before sterilization.

b. GM-salt (1) Medium

DL - malic acid	3.0 g
Sodium chloride	5.0 g
p-Aminobenzoic acid (50 μg/mL)	1.0 mL
Biotin (100μg/mL)	1.0 mL
Distilled water	1000 mL

Add ingredients *A* and adjust pH to 7.0 prior to autoclaving.

c. Glutamate Starch (Medium)

Potato starch	1.0 g
Sodium chloride	5.0 g
p-Aminobenzoic acid (50 μg/mL)	1.0 mL
Biotin (100 μg/mL)	1.0 mL
Yeast extract	1.0 g
Distilled water	1000 mL

Add ingredients *A* and adjust pH to 7.0 prior to autoclaving.

d. GM-salt (2) Medium

DL - malic acid	3.0 g
Sodium chloride	5.0 g
Yeast extract	1.0 g
$MnSO_4.5H_2O$ (1 mg/mL)	1.3 mL
$CoCl_2.6H_2O$ (1 mg/mL)	1.0 mL
Ferric citrate (10 mg/mL) in 30% NH_4OH	0.25 mL
p-Aminobenzoic acid (50 μg/mL)	1.0 mL
Nicotinic acid (1 mg/mL)	1.0 mL
Biotin (100 μg/mL)	1.0 mL
Thiamine-HCl (1 mg/mL)	1.0 mL
Distilled water	1000mL

Add ingredients ***A*** and adjust the pH was adjsuted to 7.0 prior to autoclaving.

2. Reagents

a. Gram Strain

Crystal Violet:

A - Crystal violet	2 g/ 20 mL 95% ethanol
B - Ammonium oxalate	0.8 g / 80 mL distilled water

(solution mixed, stood for 24 h and filtered

Iodine Solution:

Iodine	1 g
Potassium iodide	2 g
Distilled water	300 mL

Safranin :

0.25 g safranin is ground in a mortar with
10 mL 95% ethanol before making it upto
100 mL with distilled water

b. Standard potassium dichromate solution, 0.0417 M

Dissolve 12.259 g $K_2Cr_2O_7$ previously dried at 103 °C for 2 h in distilled water and dilute to 1000 mL.

c. Sulfuric acid reagent (H_2SO_4*)

Add 10.5 g Ag_2SO_4 to 2.5 L concentrated H_2SO_4. Let stand for 1 to 2 days for dissolution.

d. Ferroin indicator solution

Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4.7H_2O$ in distilled water and dilute to 100 mL.

e. Standard ferrous ammonium sulfate (FAS) titrant, approximately 0.25 M

Dissolve 98 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 20 mL concentrated H_2SO_4. Cool and dilute to 1000 mL. Standardize this solution daily against standard $K_2Cr_2O_7$ solution as follows:-

Dilute 10.0 mL standard $K_2Cr_2O_7$ to about 100 mL. Add 30 mL concentrated H_2SO_4* and cool. Titrate with FAS titrant using 2 to 3 drops ferroin indicator.

Molarity of FAS solution:

$$M = \frac{\text{Volume 0.0417 M } K_2Cr_2O27 \text{ solution tritrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.25$$

f. Mercury sulphate, $HgSO_4$, crystals or powder

3. Buffers

a. Phosphate buffer saline (pH 7.0) (Grist *et al.*, 1979)

NaCl	8.0 g
KCl	0.2 g
Na_2HPO_4	1.15 g
KH_2PO_4	0.2 g
Distilled water	1000 ml

Adjust pH to 7.0 prior to autoclaving.

APPENDIX C : EXPERIMENTAL AND STATISTICAL DATA

1. OD_{660} nm and cell dry weight of *R. palustris* strain B1 grown in GM medium under anaerobic-light and aerobic-dark conditions

Time	Anaerobic-light		Aerobic-dark	
(Day)	OD_{660} nm*	Cell dry wt* (g/L)	OD_{660} nm*	Cell dry wt.* (g/L)
0	0.496	1.03	0.602	0.56
1	1.277	2.05	1.626	1.37
2	1.884	3.46	1.518	1.21
3	2.002	3.66	1.459	2.13
4	2.009	4.02	1.229	2.40
5	1.979	3.59	1.362	2.23
6	1.775	3.48	1.359	1.33
7	1.482	3.40	1.302	1.23

*Mean of three values

2. OD_{660} nm and cell dry weight of *R. palustris* ATCC 17001 grown in GM medium under anaerobic-light and aerobic-dark conditions

Time	Anaerobic-light		Aerobic-dark	
(day)	CD_{660} nm	Cell dry wt. (g/L)	CD_{660} nm*	Cell dry wt. (g/L)
0	0.490	0.89	0.602	0.57
1	1.131	2.26	1.607	0.77
2	1.871	3.53	1.581	1.04
3	2.006	3.79	1.445	3.00
4	2.009	4.23	1.229	2.40
5	1.979	3.59	1.310	2.31
6	1.780	3.47	1.419	1.74
7	1.492	3.24	1.403	1.69

*Mean of three values

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3. Optical density at 660 nm after 72 h at anaerobic-light conditions of *R. palustris* strain B1 and ATCC 17001 grown on different carbon sources

Carbon source	OD_{660nm}*	
	Strain B1	ATCC 17001
DL-malic acid	1.965	2.020
Sodium succinate	1.967	1.900
Sodium tartrate	1.921	1.696
Sodium formate	1.724	1.401
Sodium benzoate	1.694	1.732
Sodium sulfite	1.602	1.148
Methanol 1%	1.598	1.541
Pyruvic acid 0.3%	1.719	1.766
Starch	1.915	1.325
Amylopectin	1.882	1.379
Amylose	0.827	0.793

*Mean of three values

4. ANOVA (Effect of Carbon Source on Day 3 Growth of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Carbon source	10	.7387727	38.117	.0000*
Residual	11	.0193818		
Total	21			

*$p < 0.05$

Multiple Range Analysis Using Tukey HSD at 95% Confidence Intervals (Strain B1)

Carbon source	Average	Homogenous group*
Succinate	3.7750000	a
Malic acid	3.7750000	a
Starch	3.6750000	b
Tartrate	3.6750000	b
Amylopectin	3.6000000	b
Pyruvic acid 0.3%	3.3500000	b
Formate	3.3500000	b
Benzoate	3.3150000	c
Methanol 1%	3.1250000	c
Sulfite	3.1250000	c
Amylose	1.6250000	d

*Means followed by a common letter are not significantly different at 5% level.

5. ANOVA (Effect of Carbon Source on Day 3 Growth of ATCC 17001)

Sources of variation	df	Mean square	F-ratio	Sign. level
Carbon source	10	.9694882	18.185	.0000*
Residual	11	.0533136		
Total	21			

*$p < 0.05$

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals (ATCC 17001)

Carbon source	Average	Homogenous group*
Malic acid	4.0500000	a
Succinate	3.8500000	a
Pyruric acid 0.3%	3.5750000	b
Benzoate	3.5500000	b
Tartrate	3.4750000	b
Methanol 1%	3.1500000	b
Formate	2.8750000	c
Amylopectin	2.8400000	c
Starch	2.7300000	c
Sulfite	2.3750000	d
Amylose	1.6750000	e

*Means followed by a common letter are not significantly different at 5% level.

6. Optical density at 660 nm after 72 h at 30° ± 2 °C, 3 klux of *R. palustris* strain B1 grown on various types of starch

No.	Type of starch	OD_{660nm}*
1	Potato	1.888
2	Starch (soluble)	1.825
3	Sago	1.814
4	Tapioca	1.811
5	Corn	1.442
6	Wheat	1.375
7	Glutinous rice	1.315
8	Rice	1.103
9	Starch (soluble) without the yeast extract	0.808
10	Raw starch (soluble) +	0.311

*Mean of three values

+Sterilized at 120 °C for 2 h.

7. ANOVA (Effect of various types of starch on Day 3 Growth of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Carbon source	9	2.4223569	999.999	.0000*
Residual	12	.0003444		
Total	21			

*$p < 0.05$

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals (Strain B1)

Types of starch	Average	Homogenous group*
Potato	3.6000000	a
Sago	3.5000000	a
Soluble starch	3.5000000	a
Tapioca	3.4500000	a
Corn	2.8000000	b
Wheat	2.7000000	b
Glutinous rice	2.5500000	b
Rice	2.1500000	b
Soluble starch - YE^{+}	1.5666667	c
Raw starch (soluble)	0.6333333	d

*Means followed by a common letter are not significantly different at 5% level.

$^{+}$Yeast extract

8. Optical density at 660 nm after 72 h at 30° ± 2 °C, 3 klux of *R. palustris* strain B1 grown on various concentrations of potato starch

No.	Potato starch concentration (%)	OD_{660nm}*
1	0.3	1.952
2	0.5	1.979
3	1.0	2.342
4	2.0	2.356
5	3.0	2.369
6	4.0	2.371
7	5.0	2.362

*Mean of three values

9. ANOVA (Effect of potato starch concentrations on Day 3 Growth of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Potato starch concentration	6	.2647167	999.999	.0000*
Residual	7	.0001286		
Total	13			

*$p < 0.05$

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals (Strain B1)

Potato starch concentration	Average	Homogenous group*
4.0	4.5550000	a
3.0	4.5500000	a
5.0	4.5300000	a
2.0	4.5150000	a
1.0	4.4600000	b
0.5	3.8100000	c
0.3	3.7500000	d

*Means followed by a common letter are not significantly different at 5% level.

10. ANOVA (Effect of *p*-aminobenzoic acid (PABA) concentrations on Day 3 Growth of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
PABA concentration	4	.0113400	.344	.8381*
Residual	5	.0329300		
Total	9			

*$p < 0.05$

11. ANOVA (Effect of *p*-aminobenzoic acid (PABA) concentrations on Day 3 Growth of ATCC 17001)

Sources of variation	df	Mean square	F-ratio	Sign. level
PABA concentration	4	.0041150	.031	.9974*
Residual	5	.1311000		
Total	9			

*$p < 0.05$

12. Effect of temperature on the growth of *R. palustris* strain B1 and ATCC 17001

	OD_{660nm}*	
Temperature (°C)	Strain B1	ATCC 17001
25	1.892	1.819
30	1.831	1.775
35	1.739	1.745
37	1.722	2.009
40	1.715	1.484
45	0.513	0.305
50	-	-
55	-	-

*Mean of three values

13. ANOVA (Effect of Temperature on Day 3 Growth of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Temperature	7	5.3554286	181.233	.0000*
Residual	8	.0295500		
Total	15			

*$p < 0.05$

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals (Strain B1)

Temperature (^{o}C)	Average	Homogenous group*
25	3.6350000	a
30	3.5050000	a
35	3.37500000	a
37	3.3350000	a
40	3.3100000	a
45	1.0000000	b
55	.0000000	c
50	.0000000	c

*Means followed by a common letter are not significantly different at 5% level.

14. ANOVA (Effect of Temperature on Day 3 Growth of ATCC 17001)

Sources of variation	df	Mean square	F-ratio	Sign. level
Temperature	7	6.3389107	892.804	.0000*
Residual	8	.0071000		
Total	15			

*$p < 0.05$

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals (ATCC 17001)

Temperature (^{O}C)	Average	Homogenous group*
37	4.0700000	a
25	3.690000	b
30	3.600000	b
35	3.540000	b
40	3.050000	c
45	.6400000	d
55	.0000000	e
50	.0000000	e

*Means followed by a common letter are not significantly different at 5% level.

15. Effect of initial pH on the growth of *R. palsutris* strain B1 and ATCC 17001

pH	OD 660 nm*	
	Strain B1	ATCC 17001
4.0	0.765	0.758
4.5	1.330	1.175
5.0	1.842	1.842
5.5	2.357	2.416
6.0	2.275	2.323
6.5	2.084	2.087
7.0	2.080	2.087
7.5	2.063	2.064
8.0	2.163	1.995
8.5	1.902	1.902

*Mean of three values

16. ANOVA (Effect of pH on Day 3 Growth of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
pH	9	1.6906689	31.454	.0000*
Residual	10	.0537500		
Total	19			

*$p < 0.05$

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals (Strain B1)

pH	Average	Homogenous group*
5.5	4.510000	a
6.0	4.360000	a
8.0	4.140000	a
6.5	4.010000	a
7.0	4.000000	a
7.5	3.965000	a
8.5	3.660000	a
5.0	3.550000	b
4.5	2.590000	c
4.0	1.4950000	d

*Means followed by a common letter are not significantly different at 5% level.

17. ANOVA (Effect of pH on Day 3 Growth of ATCC 17001)

Sources of variation	df	Mean square	F-ratio	Sign. level
pH	9	2.1213894	16.923	.0001*
Residual	10	.1253550		
Total	19			

*$p < 0.05$

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals (ATCC 17001)

pH	Average	Homogenous group*
5.5	4.875000	a
6.0	4.7000000	a
7.0	4.2200000	a
6.5	4.2200000	a
7.5	4.1850000	a
8.0	4.0350000	a
8.5	3.8500000	a
5.0	3.7300000	a
4.5	2.4000000	b
4.0	1.5500000	c

*Means followed by a common letter are not significantly different at 5% level.

18. Effect of light intensity on growth of *R. palustris* strain B1 and ATCC 17001

light intensity (klux)	OD 660 nm*	
	Strain B1	ATCC 17001
1000	1.690	1.736
2000	1.709	1.768
3000	1.769	1.855
4000	1.897	1.906
5000	1.906	1.906
6000	1.906	1906

*Mean of three values

19. ANOVA (Effect of light intensity on Day 3 Growth of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Light intensity	5	.0682133	1.165	.4217*
Residual	6	.0585333		
Total	11			

*$p < 0.05$

20. ANOVA (Effect of light intensity on Day 3 Growth of ATCC 17001)

Sources of variation	df	Mean square	F-ratio	Sign. level
Light intensity	5	.0431083	22.789	.0008*
Residual	6	.0018917		
Total	11			

*$p < 0.05$

Multiple Range analysis Using Newman-Keuls at 95% Confidence Intervals (ATCC 17001)

Light intensity (klux)	Average	Homogenous group*
6	3.8600000	a
5	3.8600000	a
4	3.8600000	a
3	3.7550000	a
2	3.6050000	b
1	3.5250000	b

*Means followed by a common letter are not significantly different at 5% level.

21. Effect of Salinity on Growth

% NaCl	OD 660 nm*	
	Strain B1	ATCC 17001
0.0	1.875	1.875
0.5	1.951	1.935
1.0	1.936	1.375
1.5	1.478	1.316
2.0	1.302	1.145
2.5	1.152	0.944
3.0	0.693	0.874

*Mean of three values

22. ANOVA (Effect of NaCl Concentration on Day 3 Growth of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
NaCl concentration	6	1.5952833	10.459	.0034*
Residual	7	.1525286		
Total	13			

*$p < 0.05$

Multiple Range analysis Using Newman-Keuls at 95% Confidence Intervalsn (Strain B1)

NaCl conc. (%)	Average	Homogenous group*
3.0	1.3800000	c
2.5	2.2350000	b
2.0	2.5300000	a
1.5	2.8650000	a
0.0	3.6000000	a
1.0	3.7300000	a
0.5	3.7500000	a

*Means followed by a common letter are not significantly different at 5% level.

23. ANOVA (Effect of NaCl Concentration on Day 3 Growth of ATCC 17001)

Sources of variation	df	Mean square	F-ratio	Sign. level
NaCl concentration	6	1.4975071	3.233	.0751*
Residual	7	.4632429		
Total	13			

*$p < 0.05$

24. ANOVA (Effect of Inoculum Size on Day 3 Growth rate of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Inoculum size	3	8.05000E-005	7.318	.0422*
Residual	4	1.10000E-005		
Total	7			

*$p < 0.05$

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals (Strain B1)

Inoculum size (%)	Average	Homogenous group*
5	.0560000	a
10	.0520000	a
15	.0500000	a
20	.0410000	b

*Means followed by a common letter are not significantly different at 5% level.

25. ANOVA (Effect of Inoculum Size on Day 3 Growth rate of ATCC 17001)

Sources of variation	df	Mean square	F-ratio	Sign. level
Inoculum size	3	2.74583E-005	12.922	.0159*
Residual	4	2.12500E-006		
Total	7			

*$p < 0.05$

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals (ATCC 17001)

Inoculum size (%)	Average	Homogenous group*
10	.0540000	a
15	.0505000	a
5	.0500000	a
20	.0450000	b

*Means followed by a common letter are not significantly different at 5% level.

26. ANOVA (Effect of Inoculum Age on Day 3 Growth rate of Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Inoculum age	5	3.73333E-006	.974	.5012*
Residual	6	3.83333E-006		
Total	11			

*$p < 0.05$

27. ANOVA (Effect of Inoculum Age on Day 3 Growth rate of ATCC 17001)

Sources of variation	df	Mean square	F-ratio	Sign. level
Inoculum age	5	1.06833E-005	2.981	.1080*
Residual	6	3.58333E-006		
Total	11			

*$p < 0.05$

28. COD Removal of Settled and Unsettled Sago Effluent by Free and Immobilized Cells of *R. palustris* Strain B1

	COD removal (%)*			
	Settled effluent		Unsettled effluent	
	Free cells	Agar-entrapped cells	Free cells	Agar-entrapped cells
Day 1	34.8	16.6	73.6	70.8
Day 2	58.3	27.0	75.7	68.2
Day 3	60.4	39.5	86.4	79.5
Day 4	64.2	44.3	85.1	81.8
Day 5	70.8	55.2	74.4	79.0

*Mean of three values

29. ANOVA (Effect of Sago Effluent Concentration on COD Removal by Immobilied Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Sago effluent conc.	3	174.42917	10.157	.0242*
Residual	4	17.17252		
Total	7			

*p < 0.05

Multiple Range analysis Using Tukey HSD at 95% Confidence Intervals

Sago effluent conc. (%)	Average	Homogenous group*
100	57.590000	a
75	49.615000	a
50	39.665000	b
25	37.390000	b

*Means followed by a common letter are not significantly different at 5% level.

30. ANOVA (Effect of Mixing on the COD Removal of Sago Effluent by Immobilized Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Effect of mixing	3	128.04661	3.701	.1193*
Residual	4	34.59774		
Total	7			

*$p < 0.05$

31. ANOVA (Effect of Inoculum Size on the COD Removal of Sago Effluent by Immobilized Strain B1)

Sources of variation	df	Mean square	F-ratio	Sign. level
Inoculum size	2	.792067	.079	.9259*
Residual	3	10.022800		
Total	5			

*$p < 0.05$

32. Regression of *R. palustris* Strain B1 Dry Cell Weight Against OD_{660nm}

r = 0.957

OD at 660 nm	Mean dry weight (g/L)
0.496	1.03
1.277	2.05
1.482	3.40
1.775	3.48
1.884	3.46
1.979	3.59
2.002	3.66
2.009	4.02

33. Regression of *R. palustris* ATCC 17001 Dry Cell Weight Against OD_{660nm}

r = 0.975

OD at 660 nm	Mean dry weight (g/L)
0.490	0.89
1.131	2.26
1.492	3.24
1.780	3.47
1.871	3.53
1.979	3.47
2.006	3.79
2.009	4.23