



The Open Biotechnology Journal

Supplementary Material

Content list available at: www.benthamopen.com/TOBIOTJ/

DOI: 10.2174/18740707016100102203



Biosurfactant Production and Biodegradation of Leather Dust from Tannery

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SUPPLEMENTARY MATERIAL

Table 1: Concentration of chromium detected in the cell free supernatant during incubation with *Bacillus subtilis* SA-6 for 10 days (mg/L) for repeat 1

Sample repeat 1	Time	NBBSCR		NBBSLD		NBBSCR		NBLD		NBCR		NBBSS		NB	
		Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)
0		0.040	1.319	0.010	0.042	0.050	1.563	0.010	0.120	0.040	1.035	0.001	0.0014	0.001	0.491
	24	1.700	97.625	1.260	48.910	0.050	1.697	0.080	2.585	0.040	1.263	0.001	0.006	0.000	0.564

48	7.70	5.20	4.500	3.600	4.50	4.600
120	274.240	181.760	157.040	126.850	158.870	163.060
144	4.90	5.500	5.500	5.500	5.70	5.700
168	173.210	192.280	194.300	192.900	200.630	200.760
192	0.050	0.060	0.050	0.050	0.060	0.060
216	1.595	1.736	1.709	1.638	1.829	1.895
	0.130	0.260	0.290	0.330	0.380	0.420
	4.326	8.966	10.233	11.622	13.168	14.804
	0.050	0.050	0.050	0.050	0.050	0.050
	1.404	1.349	1.338	1.361	1.407	1.443
	0.002	0.001	0.001	0.001	0.001	0.001
	0.0242	0.0062	0.0006	0.0116	0.0076	0.0011
	0.010	0.003	0.005	0.006	0.007	0.012
	0.3246	0.0604	0.1563	0.1707	0.2173	0.3922

Table 2: Concentration of chromium detected in the cell free supernatant during incubation with *Bacillus subtilis* SA-6 for 10 days (mg/L) for repeat 2

Sample repeat 2	Time	NBBSCR		NBBSLD		NBBSCR		NBLD		NBCR		NBBBS		NB	
		Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)
0		0.060	1.772	0.010	0.220	0.050	1.570	0.000	0.080	0.050	1.764	0.001	0.0192	0.000	0.0083
24		1.820	72.184	1.000	35.967	0.050	1.539	0.090	3.114	0.050	1.760	0.001	0.0246	0.002	0.0439
48		5.500	163.270	4.600	134.870	0.050	1.525	0.140	5.008	0.050	1.782	0.001	0.0292	0.001	0.028

120	0.0313	0.001	0.036	0.001	1.881	0.050	10.40	0.280	1.640	0.060	169.930	5.700	150.350	5.100
144	0.0243	0.001	0.027	0.001	1.822	0.050	12.164	0.330	1.656	0.060	201.189	6.800	152.470	5.100
168	0.0263	0.001	0.026	0.001	1.830	0.050	13.054	0.350	1.709	0.060	178.800	6.000	152.520	5.100
192	0.0366	0.002	0.0293	0.001	1.862	0.050	19.840	0.540	1.726	0.060	181.610	6.100	157.680	5.300
216	0.0567	0.002	0.0231	0.001	1.990	0.060	21.523	0.580	1.757	0.060	193.660	6.500	160.360	5.400

Table 3: Concentration of chromium detected in the cell free supernatant during incubation with *Bacillus subtilis* SA-6 for 10 days (mg/L) for repeat 3

Sample repeat 3	Time	NBBSCLR		NBBSLD		NBBSCLR		NBBLD		NBCCR		NBBS		NB	
		Abs	Cr conc	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)	Abs	Cr conc (mg/L)
0		0.040	1.692	0.003	0.1369	0.040	1.530	0.003	0.126	0.030	2.369	0.001	0.0656	0.001	0.0479
		1.620	78.321	1.020	48.362	0.040	1.584	0.060	2.517	0.030	2.500	0.001	0.0664	0.000	0.0779
		4.100	183.090	4.300	191.760	0.040	1.600	0.090	3.994	0.030	2.493	0.001	0.0664	0.001	0.0582
24		1.620	78.321	1.020	48.362	0.040	1.584	0.060	2.517	0.030	2.500	0.001	0.0664	0.000	0.0779
		4.100	183.090	4.300	191.760	0.040	1.600	0.090	3.994	0.030	2.493	0.001	0.0664	0.001	0.0582
		1.620	78.321	1.020	48.362	0.040	1.584	0.060	2.517	0.030	2.500	0.001	0.0664	0.000	0.0779
48		1.620	78.321	1.020	48.362	0.040	1.584	0.060	2.517	0.030	2.500	0.001	0.0664	0.000	0.0779
		4.100	183.090	4.300	191.760	0.040	1.600	0.090	3.994	0.030	2.493	0.001	0.0664	0.001	0.0582
		1.620	78.321	1.020	48.362	0.040	1.584	0.060	2.517	0.030	2.500	0.001	0.0664	0.000	0.0779

120	0.0647	0.001	0.0638	0.001	2.735	0.040	8.639	0.200	1.637	0.040	210.210	4.700	159.670	3.600
144	0.0696	0.001	0.0657	0.001	2.686	0.040	9.996	0.230	1.797	0.040	209.880	4.700	203.600	4.600
168	0.0612	0.001	0.0672	0.001	2.618	0.040	11.242	0.250	1.786	0.040	209.640	4.700	136.570	3.100
192	0.0666	0.001	0.070	0.001	2.733	0.040	12.773	0.290	1.731	0.040	217.420	4.900	217.190	4.900
216	0.0719	0.001	0.0798	0.000	2.858	0.040	12.924	0.290	1.409	0.030	230.03	5.200	193.810	4.300

*Nutrient broth (NB), Nutrient broth+Bacillus subtilis (NBBS), Nutrient broth+Chromium (NBCR), Nutrient broth+Leather dust (NBLD), Nutrient broth+Bacillus subtilis+Chromium (NBBS CR), Nutrient broth+Bacillus subtilis+Leather dust (NBBSLD), Nutrient broth+Bacillus subtilis+Chromium+Leather dust (NBBS CR LD).

Table 4: Descriptive statistics for comparisons of detoxification of leather dust experimental groups on the rate of chromium concentration increase between 0-24 h

Descriptives

Rate (mg/L/h)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NB	3	.001750	.0010897	.0006292	-.000957	.004457	.0010	.0030
NBBS	3	.000143	.0000981	.0000567	-.000100	.000387	.0000	.0002
NBCR	3	.004833	.0047522	.0027437	-.006972	.016638	.0000	.0095
NBLD	3	.409667	.5079590	.2932703	-.852173	1.671507	.1030	.9960
NBBS CR	3	.009500	.0116512	.0067268	-.019443	.038443	.0000	.0225
NBBSLD	3	1.846667	.3092464	.1785435	1.078456	2.614877	1.4900	2.0400
NBBS CR LD	3	3.376667	.5636784	.3254399	1.976412	4.776921	2.9300	4.0100

Total	21	.807032	1.2768275	.2786266	.225827	1.388237	.0000	4.0100
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Table 5: One-way ANOVA analysis of all experimental groups on the rate of chromium concentration increase between 0-24 h

ANOVA

Rate (mg/L/h)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.263	6	5.210	54.312	.000
Within Groups	1.343	14	.096		
Total	32.606	20			

Table 6: One-way ANOVA analysis of NBLD, NBBSLD, NBBSCLRD experimental groups on the rate of chromium concentration increase between 0-24 h

ANOVA

mg/L/h

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.209	2	6.604	29.511	.001
Within Groups	1.343	6	.224		
Total	14.552	8			

Table 7: Comparison of means between NBLD, NBBSLD and NBBSCLRD groups between 0-24 h using the Dunnett test

Multiple Comparisons

Dependent Variable: mg/L/h

Dunnett t (2-sided)^a

(I) Culture	(J) Culture	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NBBSLD	NBLD	1.43700*	.38626	.017	.3312	2.5428
NBBSCLRD	NBLD	2.96700*	.38626	.000	1.8612	4.0728

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 8: Descriptive statistics for comparisons of detoxification of leather dust experimental groups on the rate of chromium concentration increase between 24-48 h

Descriptives

Rate (mg/L/h)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
					NB	3		
NBBS	3	.0067	.01149	.00663	-.0218	.0353	.00	.02
NBCR	3	.0029	.00441	.00254	-.0080	.0139	.00	.01
NBLD	3	.0700	.01000	.00577	.0452	.0948	.06	.08
NBBSCR	3	-.0014	.00248	.00143	-.0076	.0047	.00	.00
NBBSLD	3	5.0967	.92781	.53567	2.7919	7.4015	4.13	5.98
NBBSCRLD	3	5.2967	1.84641	1.06603	.7099	9.8834	3.80	7.36
Total	21	1.4954	2.48683	.54267	.3634	2.6274	-.01	7.36

Table 9: One-way ANOVA analysis of all experimental groups on the rate of chromium concentration increase between 24-48 h

ANOVA

Rate (mg/L/h)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	115.146	6	19.191	31.458	.000
Within Groups	8.541	14	.610		
Total	123.686	20			

Table 10: One-way ANOVA analysis of NBLD, NBBSLD, NBBSCRLD experimental groups on the rate of chromium concentration increase between 24-48 h

ANOVA

Rate (mg/L/h)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	52.625	2	26.313	18.486	.003

Within Groups	8.540	6	1.423		
Total	61.166	8			

Table 11: Comparison of means between NBLD, NBBSLD and NBBSCRLD groups between 24-48 h using the Dunnett test

Multiple Comparisons

Dependent Variable: Rate (mg/L/h)

Dunnett t (2-sided)^a

(I) Culture	(J) Culture	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NBBSLD	NBLD	5.02667*	.97413	.004	2.2380	7.8154
NBBSCRLD	NBLD	5.22667*	.97413	.003	2.4380	8.0154

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

*Nutrient broth (NB), Nutrient broth+*Bacillus subtilis* (NBBS), Nutrient broth+Chromium (NBCR), Nutrient broth+Leather dust (NBLD), Nutrient broth+*Bacillus subtilis*+Chromium (NBBSCR), Nutrient broth+*Bacillus subtilis*+Leather dust (NBBSLD), Nutrient broth+*Bacillus subtilis*+Chromium+Leather dust (NBBSCRLD).

Table 12: Rate of chromium concentration increase between 0-24 h and 24-48 h

0-24 h	Rate mg/L/h (Average±Standard deviation)	24-28 h	Rate mg/L/h (Average±Standard deviation)
NB	0.0018±0.0011	NB	0.0037±0.0055
NBBS	0.0001±0.0001	NBBS	0.0067±0.0114
NBCR	0.0048±0.0048	NBCR	0.0029±0.0044
NBLD	0.4097±0.5079	NBLD	0.07±0.01
NBBSCR	0.0028±0.0030	NBBSCR	0.0014±0.0024
NBBSLD	1.8467±0.3092	NBBSLD	5.097±0.9278
NBBSCRLD	3.3767±0.5637	NBBSCRLD	5.1767±1.9122