Supplementary material

Agronomy

Optimized Rhamnolipid Production by *Pseudomonas marginalis* C9 Strain Isolated from a Biopurification System to Enhance Pesticide Solubilization

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ANOVA table for different carbon and nitrogen sources concentration for surface tension and bacterial growth.

Table S1a. ANOVA table for homogeneous subsets by Tukey´s test (P ≤ 0.05). Comparison between groups (carbon sources concentration) for biosurfactant production by *P. marginalis* C9.

Responses	**CSC**	**n**	**Subsets for alpha= 0.05**			
			1	**2**	**3**	**4**
Surface	3	3	29.0250			
tension	4	3		31.0500		
(mN m^{-1})	5	3		31.2800		
	2	3			45.1600	
	1	3				54.780
	Sig.		0.345	0.783	1.000	1.000
			1	**2**	**3**	**4**
Biomass	3	3	1.5117			
(g L^{-1})	4	3	1.5373	1.5373		
	5	3		1.6691		
	2	3			1.9510	
	1	3				2.5549
	Sig.		0.993	0.843	1.000	1.000

ANOVA was performed comparing between groups (carbon sources concentration) for biosurfactant synthesis by *P. marginalis* C9. *CSC: Carbon sources concentration; n: numbers of samples.

Table S1b. ANOVA table for homogeneous subsets by Tukey´s test ($P \leq 0.05$). Comparison between groups (nitrogen sources concentration) for biosurfactant production by *P. marginalis* C9.

Responses	**NSC**	**n**	**Subsets for alpha= 0.05**			
			1	**2**	**3**	**4**
Surface	1	3	29.8000			
tension	2	3		35.0000		
(mN m^{-1})	0.5	3			42.4500	
	4	3				46.0500
			1	**2**	**3**	**4**
Biomass	4	3	1.1255			
(g L^{-1})	1	3		1.5362		
	2	3			1.7515	
	0.5	3				1.9019

ANOVA was performed comparing between groups (nitrogen sources concentration) for biosurfactant synthesis by *P. marginalis* C9. *NSC: Nitrogen sources concentration; n: numbers of samples.

Model Summary Statistics and ANOVA for a fitted model for surface tension and biomass as a function of studied variables (pH, temperature, and agitation).

Table S2a. Quadratic model and ANOVA for a fitted model for surface tension (mN m^{-1}) as a function of studied variables (pH, temperature, and agitation).

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4234.49	9	470.50	20.33	0.0003	significant
A-pH	808.02	1	808.02	34.91	0.0006	
B-Temperature	817.40	1	817.40	35.32	0.0006	
C-Agitation	40.16	1	40.16	1.74	0.2292	
AB	518.90	1	518.90	22.42	0.0021	
AC	33.91	1	33.91	1.47	0.2654	
BC	9.48	1	9.48	0.4098	0.5425	
A^2	18.74	1	18.74	0.8099	0.3980	
B^2	19.03	1	19.03	0.8222	0.3947	
C^2	1288.52	1	1288.52	55.68	0.0001	
Residual	162.00	7	23.14			
Lack of Fit	159.77	5	31.95	28.63	0.0341	
Pure Error	2.23	2	1.12			
Cor Total	4396.49	16				

Fit Statistics			
Std. Dev.	4.81	**R^2**	0.9632
Mean	52.23	**Adjusted R^2**	0.9158
C.V. %	9.21	**Predicted R^2**	0.8380
		Adeq Precision	12.1246

Table S2b. Quadratic model and ANOVA for a fitted model for biomass (g L^{-1}) as a function of studied variables (pH, temperature, and agitation).

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.64	9	0.5159	18.74	0.0004	significant
A-pH	1.42	1	1.42	51.65	0.0002	
B-Temperature	1.15	1	1.15	41.77	0.0003	
C-Agitation	0.1250	1	0.1250	4.54	0.0706	
AB	0.8561	1	0.8561	31.09	0.0008	
AC	0.0168	1	0.0168	0.6115	0.4598	
BC	0.0416	1	0.0416	1.51	0.2586	
A^2	0.2914	1	0.2914	10.58	0.0140	
B^2	0.0005	1	0.0005	0.0196	0.8925	
C^2	0.1719	1	0.1719	6.24	0.0411	
Residual	0.1927	7	0.0275			
Lack of Fit	0.1904	5	0.0381	32.93	0.0297	
Pure Error	0.0023	2	0.0012			
Cor Total	4.84	16				
Fit Statistics						
Std. Dev.	0.1659	**R^2**	0.9601			
Mean	0.7985	**Adjusted R^2**	0.9089			
C.V. %	20.78	**Predicted R^2**	0.7669			
		Adeq Precision	13.9581			

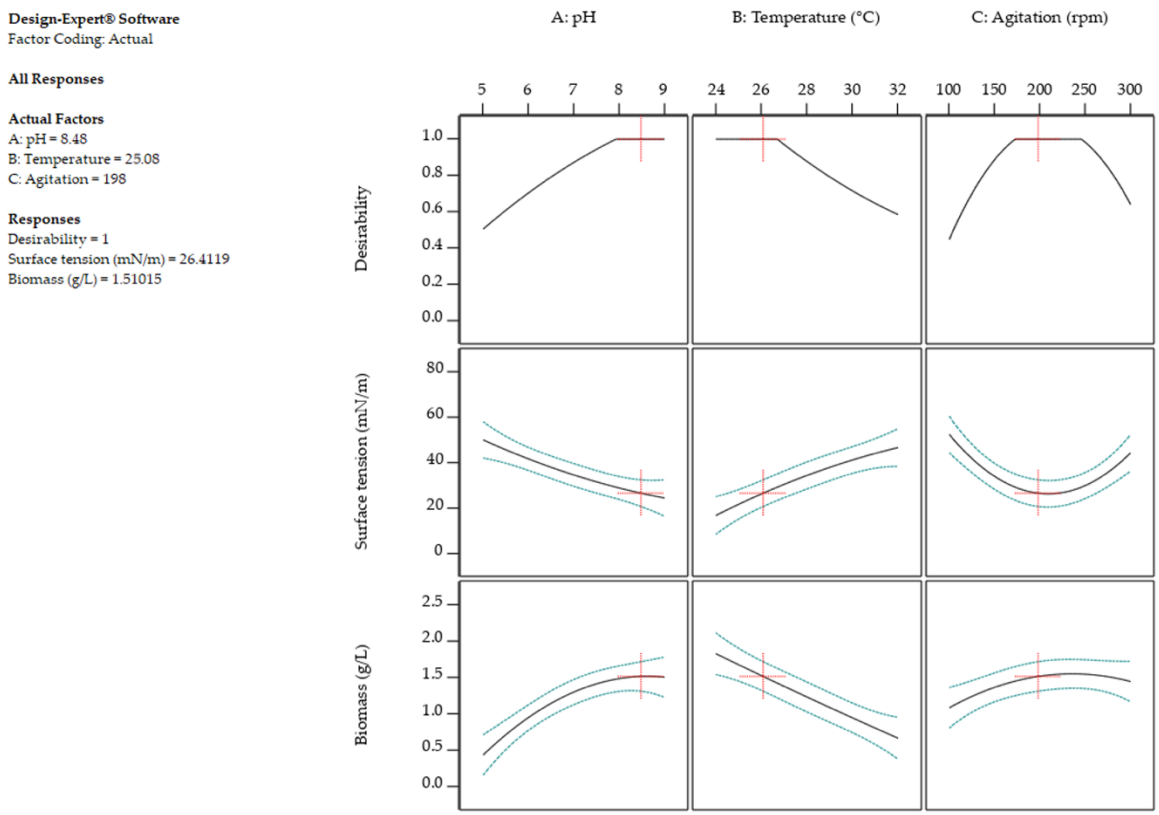


Figure S1. Interaction effects on surface tension (mN m^{-1}) and biomass (g L^{-1}) as a function of the independent variables pH (A), temperature (B), and agitation (C) from a quadratic model.

Table S3. Surface tension (mN m-1), oil displacement (%), extraction yield (g L-1), biomass (g L-1), and pH profiles of *P. marginalis* C9 in Bushnell-Haas medium supplemented with 3% olive oil incubated at 25 °C, and 200 rpm for 96 h.

Time (h)	pH	Surface tension (mN m^{-1})	Extraction yield (g L^{-1})	Oil displacement (%)	Biomass (g L^{-1})
0	8.50 ± 0.04	68.55 ± 0.35	0.02 ± 0.00	0.38 ± 0.15	0.09 ± 0.00
3	7.89 ± 0.00	68.70 ± 0.40	0.06 ± 0.00	11.13 ± 1.50	0.14 ± 0.00
6	7.79 ± 0.05	67.45 ± 1.25	0.27 ± 0.04	14.81 ± 1.00	0.29 ± 0.02
9	7.77 ± 0.02	56.15 ± 0.55	0.45 ± 0.04	24.13 ± 0.90	0.66 ± 0.01
24	7.76 ± 0.13	32.70 ± 0.10	1.24 ± 0.01	84.77 ± 0.50	1.18 ± 0.03
48	6.94 ± 0.06	29.25 ± 0.25	4.80 ± 0.05	96.49 ± 0.45	2.89 ± 0.14
72	6.80 ± 0.03	28.55 ± 0.35	6.49 ± 0.04	100.00 ± 0.00	3.63 ± 0.10
96	6.78 ± 0.02	27.45 ± 0.45	7.40 ± 0.15	100.00 ± 0.00	4.16 ± 0.28

Results are expressed as the mean ± standard deviation of three independent readings.

Table S4. ANOVA for a fitted model for surface tension as a function of studied variables (aeration and agitation) in a mini bioreactor system.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	839.94	3	279.98	248.87	0.0466	significant
A-Aeration (AR)	176.23	1	176.23	156.65	0.0508	
B-Agitation (AG)	490.40	1	490.40	435.91	0.0305	
AB	173.32	1	173.32	154.06	0.0512	
Curvature	92.02	1	92.02	81.80	0.0701	
Pure Error	1.12	1	1.12			
Cor Total	933.09	5				

			Fit Statistics
Std. Dev.	1.06	R^2	0.9987
Mean	34.77	Adjusted R^2	0.9946
C.V. %	3.05	Predicted R^2	NA(1)
		Adeq Precision	36.5816

Significant factors at 95% confidence level for biosurfactant production by *P. marginalis* C9 strain.

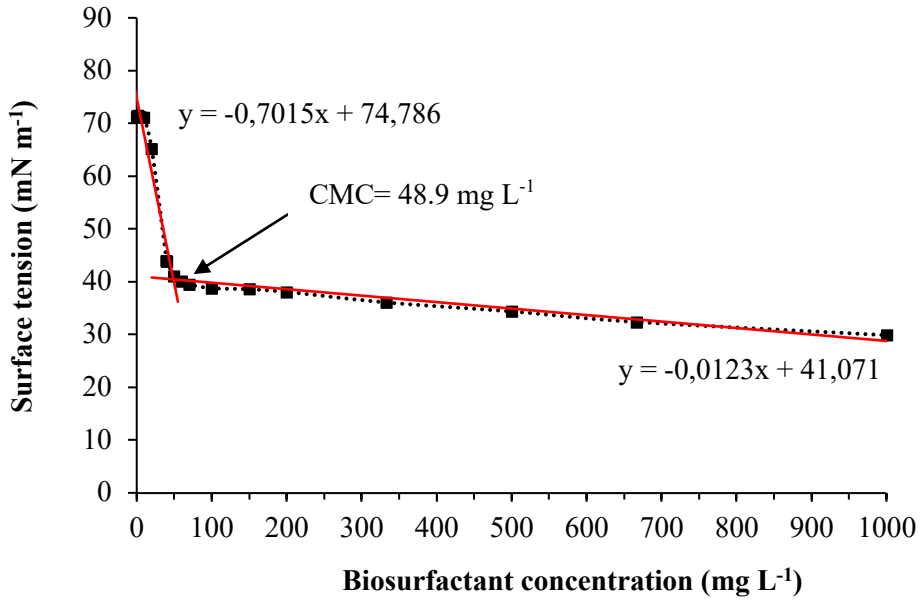


Figure S2. Determination of critical micelle concentration (CMC) value of biosurfactant produced by *P. marginalis* C9.