Supplementary Information

Cleaning carbohydrate impurities from lignin using Pseudomonas fluorescens

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Effect of yeast extract on lignin bio-cleaning process

To confirm the role of yeast extract, we performed three additional set of experiments: LBM medium only (with 0.1 gL^{-1} yeast extract and without lignin biomass), KL-A-LBM without yeast extract and KL-B-LBM without yeast extract. The growth curves (Figure S1) showing the bacterial population (CFU/ml) for all three experimental sets are compared with the growth curves of KL-A-LBM and KL-B-LBM in presence of yeast extract (data re-plotted from Figure 1a).

In absence of yeast extract (line-iii and iv) *P. fluorescens* suffered delayed growth represented by a prolonged lag phase in the first 9 hours of the biodegradation process. In absence of lignin biomass (line-v), the amount of yeast extract (0.1 gL^{-1}) added to the LBM media supported initial growth of *P. fluorescens* up to 12 hours and the bacteria subsequently entered the death phase due to the lack of nutrients. When both yeast extract and lignin biomass was supplemented (line-i and ii), the growth showed an initial lag phase of about 3-4 hours accompanied by a diauxic lag around 12-15 hours of growth. Comparing lines i and ii to iii and iv, similar population densities were obtained at late log phase. The only difference is the initial delayed growth in iii and iv, which indicates that yeast extract can only boost initial growth by providing essential amino acids and vitamins when bacteria prepare for cellulose and hemicellulose degradation. The presence of the diauxic lags in curves (i) and (ii) signifies the exhaustion of yeast extract at around 12 hours, which correlates with the initiation of death phase in line v.

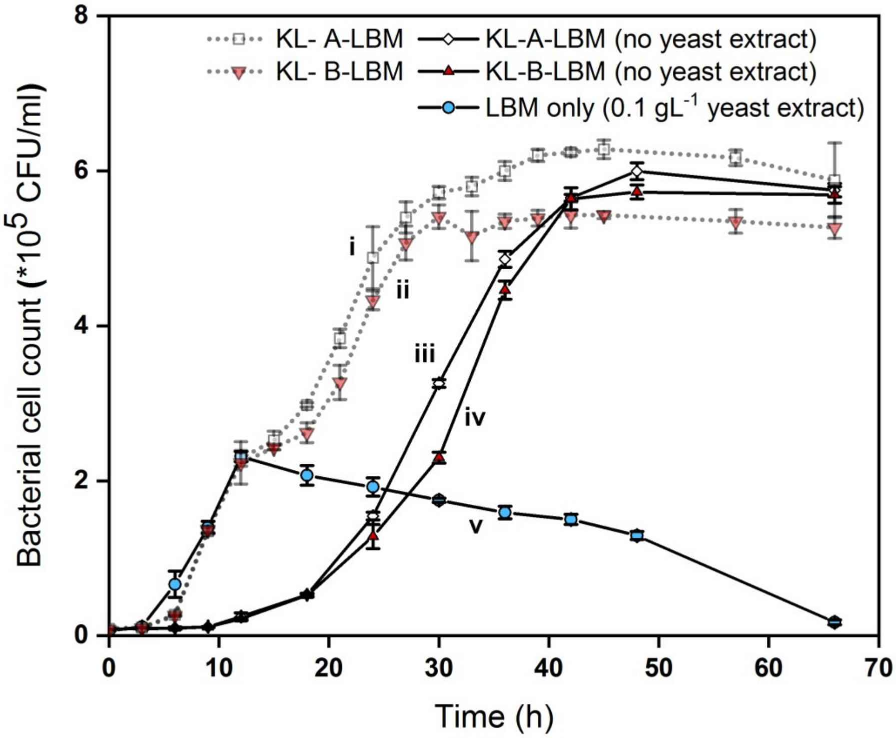

Figure S1. Growth curves of *P. fluorescens* in different LBM showing effect of yeast extract during lignin bio-cleaning process. Watermarked lines (i) and (ii) are reproduced from Figure 1a for comparison.

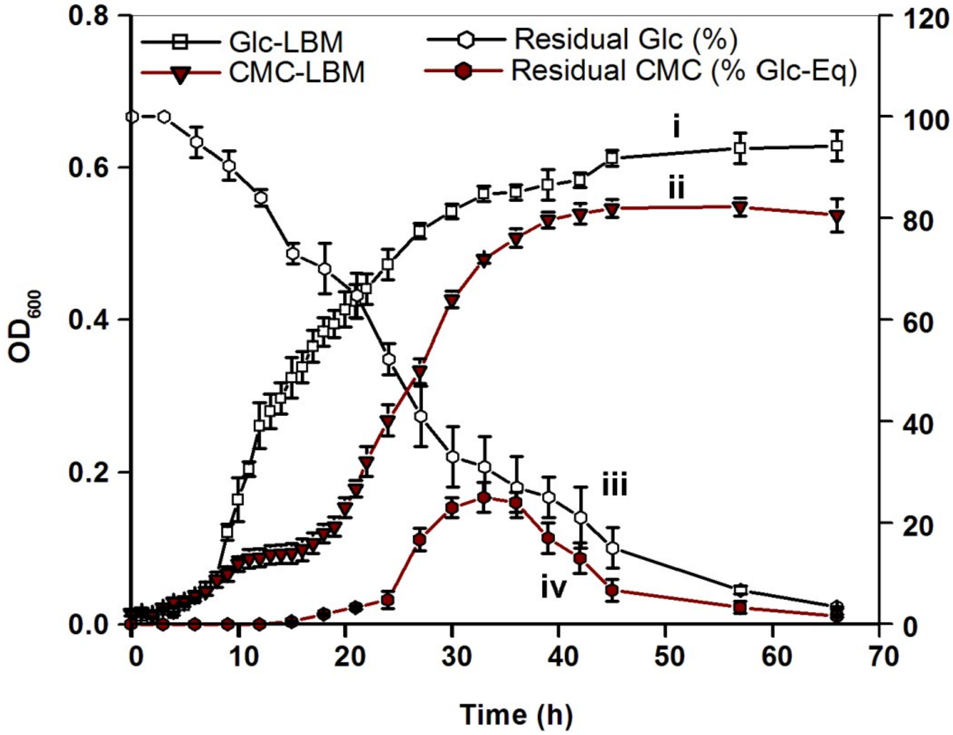

Figure S2. Time function of P. fluorescens growth and total reducing sugar in Glc-LBM and CMC-LBM.

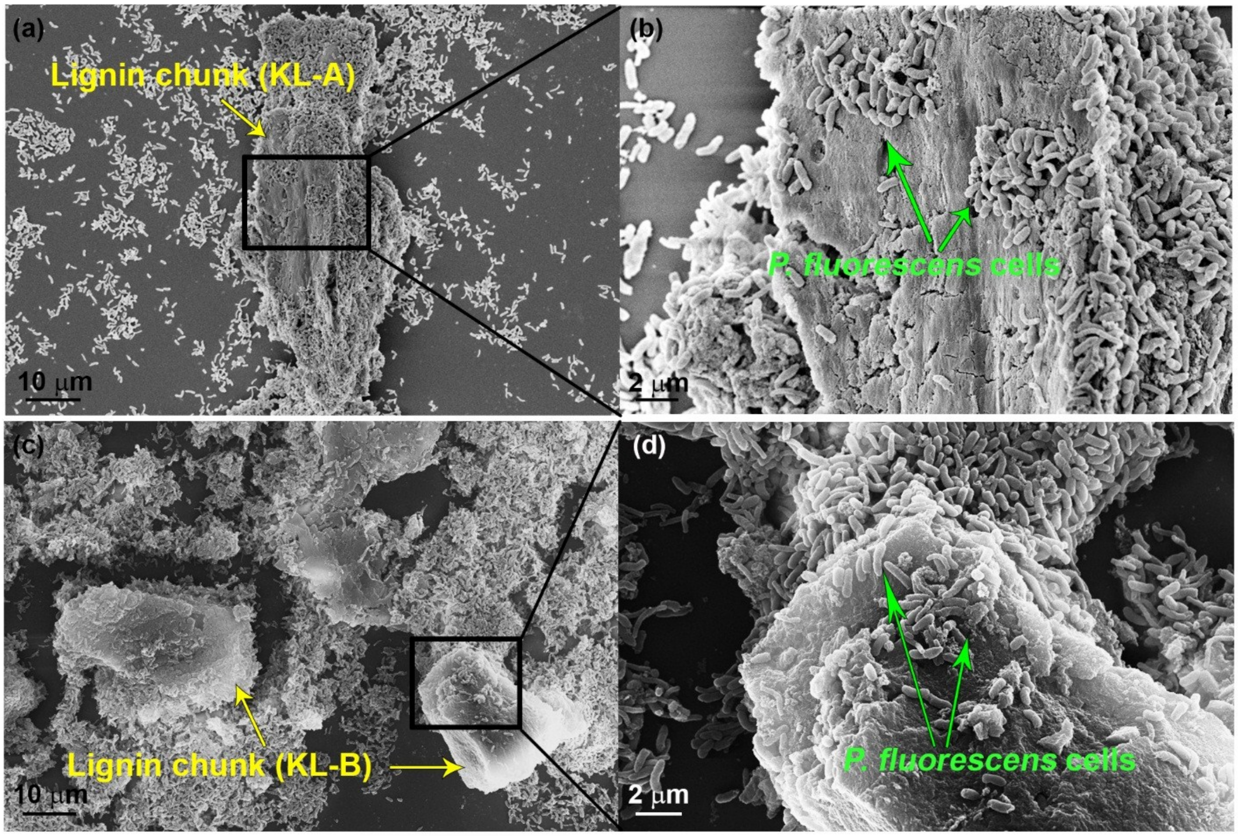

Figure S3. SEM images of *P. fluorescens* on KL-A (a-b) and KL-B (c-d) during bio-cleaning showing lignin chunks and bacterial attachment on the lignin surface.

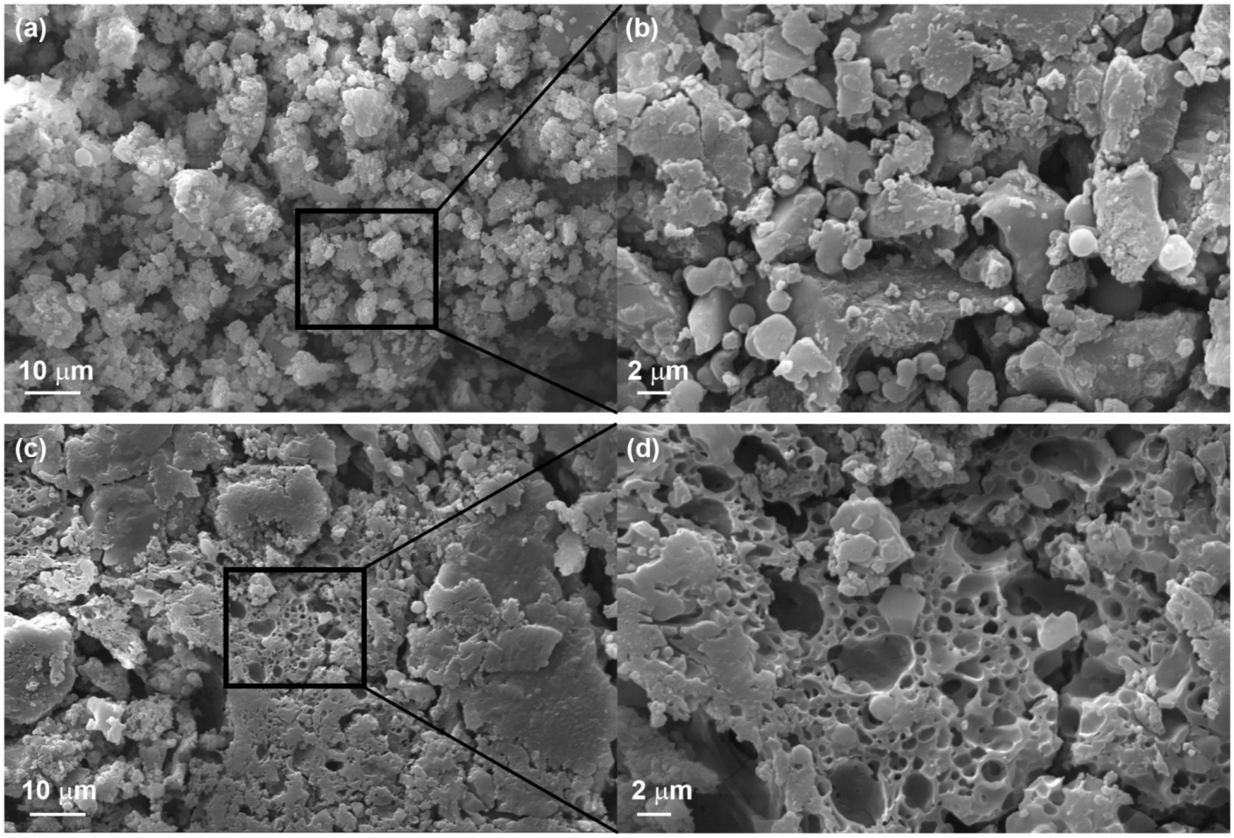

Figure S4. SEM images of KL-A and KL-B after bio-cleaning and purification showing complete removal of bacterial cells from lignin surface. (a) Purified KL-A, (b) Zoomed in KL-A surface, (c) Purified KL-B, (d) Zoomed in KL-B surface showing no traces of bacterial cells.

Table S1. Lignin (L) and carbohydrate (C) peak list assigned to ToF-SIMS ion signatures[1].

Nominal mass	Exact mass	Contributing ion	Assigned origin
51	51.021	$C_4H_3^+$	L
44	44.023	$C^{13}CH_3O^+$	C
47	47.013	$CH_3O_2^+$	C
59	59.015	$C_2H_3O_2^+$	C
60	60.021	$C_2H_4O_2^+$	C
61	61.03	$C_2H_5O_2^+$	C
63	63.022	$C_5H_3^+$	L
65	65.038	$C_5H_5^+$	L
67	67.056	$C_5H_7^+$	L
71	71.014	$C_3H_3O_2^+$	C
77	77.037	$C_6H_5^+$	L
79	79.056	$C_6H_7^+$	L
81	81.036	$C_5H_5O^+$	C
83	83.013	$C_4H_3O_2^+$	C
85	85.034	$C_4H_5O_2$	C
87	87.051	$C_4H_7O_2$	C
91	91.051	$C_7H_7^+$	L
93	93.073	$C_7H_9^+$	L
95	95.092	$C_7H_{11}^+$	L
97	97.032	$C_5H_5O_2^+$	C
99	99.049	$C_5H_7O_2^+$	C
101	101.029	$C_4H_5O_3^+$	C
105	105.071	$C_8H_9^+$	L
107	107.044	$C_7H_7O^+$	L
109	109.033	$C_6H_5O_2$	C
113	113.026	$C_5H_5O_3$	C
115	115.045	$C_5H_7O_3^+$ / $C_9H_7^+$	L
121	121.023	$C_7H_5O_2^+$ / $C_8H_9O^+$	L
127	127.041	C_6H7O_3	C
128	128.051	$C_6H_8O_3^+$	L
131	131.049	$C_5H_7O_4^+$ / $C_9H_7O^+$	L
137	137.063	$C_8H_9O_2^+$	L
145	145.061	$C_6H_9O_4^+$	C
147	147.068	$C_9H_7O_2^+$	L
		$C_6H_{11}O_4^+$	L
		$C_{10}H_{11}O^+$	L

151	151.049	$C_8H_7O_3^+$	L
		$C_5H_{11}O_5^+$	L
152	152.05	$C_8H_8O_3^+$	L
153	153.049	$C_8H_9O_3^+$	L
165	165.059	$C_9H_9O_3^+$	L
167	167.071	$C_9H_{11}O_3^+$	L
181	181.05	$C_9H_9O_4^+$	L
189	189.059	$C_{11}H_9O_3^+$	L

Table S2. Protein contents of KL-A-LBM and KL-B-LBM during biodegradation.

Time (h)	KL-A-LBM			KL-B-LBM
	Soluble protein (μg/ml)	Lignin-bound protein (μg/ml)	Total protein (μg/ml)	Total protein (μg/ml)
24	21.8 ± 2.5	0.8 ± 0.2	22.6 ± 2.6	65.9 ± 3.1
30	83.0 ± 1.2	129.3 ± 4.5	212.3 ± 4.3	238.0 ± 2.5
36	76.3 ± 1.9	129.7 ± 6.4	206.0 ± 7.6	225.9 ± 1.9
42	80.1 ± 1.9	123.4 ± 6.1	203.5 ± 4.3	232.6 ± 2.6
48	77.2 ± 3.8	130.1 ± 6.4	207.3 ± 5.7	233.0 ± 2.5

Reference

1. A. Tolbert and A. J. Ragauskas, *Energy Science & Engineering*, 2017, **5**, 5-20.