Supplementary material

Integrated extractive fermentation of laccase using aqueous biphasic systems: From production to functional coatings

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Materials and Methods

Culture media composition

Table S1 – Culture media composition applied at different stages of this work for *K. phaffi* manipulation, transformation and laccase production.

Culture medium	Application	Composition
Yeast peptone dextrose (YPD)	Growth and storage	1 % (w/v) yeast extract, 2 % (w/v) peptone and 2 % (w/v) glucose
Yeast peptone dextrose sorbitol (YPDS)	Selection of transformants	1 % (w/v) yeast extract, 2 % (w/v) peptone, 2 % (w/v) glucose, 2 % (w/v) agar, and 1 M sorbitol)
Buffered Glycerol-complex Medium (BMGY)	Biomass production (inoculum preparation)	1 % (w/v) yeast extract, 2 % (w/v) peptone, 100 mM phosphate (pH 6.0), 1.34 % (w/v) yeast nitrogen base (YNB), 0.00004 % (w/v) biotin and 1 % (v/v) glycerol
Buffered Methanol-complex Medium (BMMY)	Inducing secreted expression (fermentation)	1 % (w/v) yeast extract, 2 % (w/v) peptone, 100 mM potassium phosphate (pH 6.0), 1.34 % (w/v) yeast nitrogen base (YNB), 0.00004 % (w/v) biotin and 1 % (v/v) methanol

Results and Discussion

K. phaffii growth in ABS phase-forming compounds

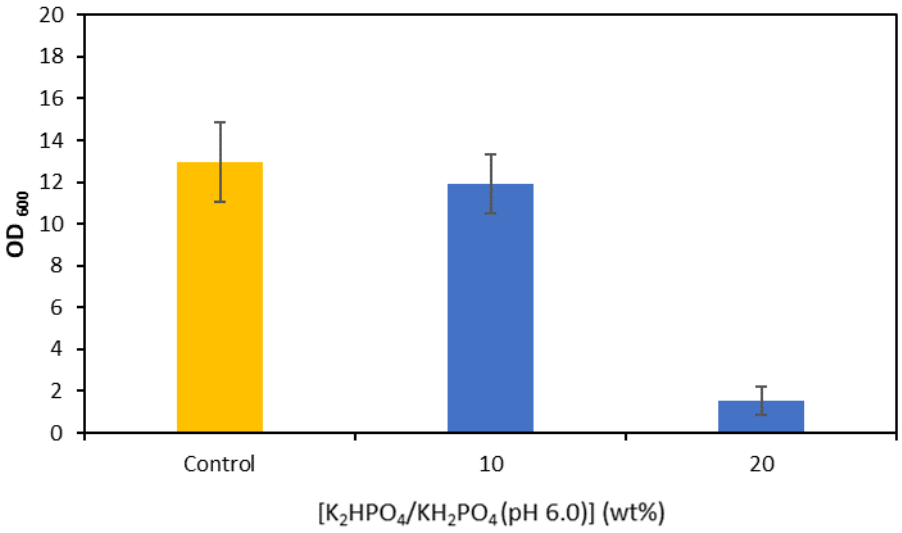


Figure S1. Effect of different potassium phosphate buffer concentrations on the growth of *K. phaffii*. Control was fermentation medium with addition of 10 wt% water.

Laccase stability in ABS phase-forming compounds

Table S2 – Enzymatic activity (U/L) at room temperature in fermentation broth with addition of ABS phase-forming compounds over time. Control was fermentation broth with addition of 10.0 or 15.0 wt% water.

Compound	**Enzymatic activity (U/L)**			
	Time (h)			
	0	**1**	**5**	**72**
Control 15.0 wt% water	110.6±2.1	111.3±0.8	113.9±0.2	88.1±3.2
Control 10.0 wt% water	116.0±1.1	119.6±0.6	123.6±0.9	93.9±0.9
15.0 wt% PEG 8000	-	82.6±0.0	86.6±0.0	71.4±7.4
15.0 wt% K_4HPO_4/KH_2PO_4 (pH 6.0)	-	111.8±1.7	114.3±0.1	88.8±5.8
10.0 wt% PEG 8000	-	97.1±0.2	100.5±0.4	91.6±1.1
10.0 wt% dextran 40	-	117.2±1.0	117.8±0.9	101.2±1.5

Extractive fermentation for laccase production in polymer-salt ABS

Table S3 – Partition coefficient (*K*) of cells, methanol and laccase in ABS composed of PEG 8000 and K_2HPO_4/KH_2PO_4 (pH 6.0).

PEG 8000 (wt%)	**K_2HPO_4/KH_2PO_4 (pH 6.0) (wt%)**	K_{cell}	K_{Lac}	K_{Meth}
10.0	15.0	0.05±0.00	0.00*	1.08
15.0	10.0	0.06±0.01	0.00	1.12
15.0	7.0	0.12±0.02	0.00	1.47

*only detected in bottom phase

Table S4 – Partition coefficient (*K*) of cells, methanol and laccase in ABS composed of PEG 8000 and dextran 40.

PEG 8000 (wt%)	**Dextran 40 (wt%)**	K_{Lac}	K_{cell}	K_{Meth}
5.0	10.0	0.05±0.01	0.00*	3.12
7.5	7.5	0.05±0.01	0.00	3.79
10.0	5.0	0.02±0.00	0.00	ND**

*only detected in bottom phase

**not determined

Table S5 – Initial and final pH values of the fermentative fermentation media for the polymer-salt ABS.

ABS	**Phase**	**pH**	
		Initial	Final
Control		5.70±0.10	4.93±0.09
10.0 wt% PEG 8000 + 15.0 wt% K_4HPO_4/KH_2PO_4 (pH 6.0)	Top	5.97±0.01	5.66±0.01
	Bottom	6.07±0.09	5.45±0.03
15.*0* wt% PEG 8000 + 10.0 wt% K_4HPO_4/KH_2PO_4 (pH 6.0)	Top	5.85±0.01	5.44±0.01
	Bottom	6.01±0.01	5.40±0.02
15.0 wt% PEG 8000 + 7.*0* wt% K_4HPO_4/KH_2PO_4 (pH 6.0)	Top	5.84±0.03	5.20±0.39
	Bottom	6.05±0.05	5.62±0.04

Extractive fermentation for laccase production in polymer-polymer ABS

Table S6 – Initial and final pH values of the fermentative fermentation media for the polymer-polymer ABS.

ABS	Phase	pH	
		Initial	Final
Control		5.77±0.04	5.01±0.00
5.0 wt% PEG 8000+ 10.0 wt% Dex 40	Top	5.87±0.04	5.21±0.04
	Bottom	5.89±0.02	5.23±0.04
7.5 wt% PEG 8000+ 7.5 wt% Dex 40	Top	5.78±0.02	5.10±0.04
	Bottom	5.85±0.04	5.13±0.04
10.0 wt% PEG 8000+ 5.0 wt% Dex 40	Top	5.75±0.04	5.08±0.03
	Bottom	5.84±0.04	5.09±0.02