Exploring Design Space and Optimization of nutrient factors for maximizing lipid production in *Metchnikowia pulcherrima* with Design of Experiments

SUPPLEMENTARY MATERIAL

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SECTION S1: SAMPLE PREPARATION

Section S1.1: Lipid extraction

The entire culture in a flask was harvested by centrifugation at 5000 rpm for 10 min to separate the culture into cell pellets and supernatant. The cell pellets were dried and weight measured. Dried cells were placed in 25 ml Duran bottles for lipid extraction. 10 ml of 6 HCl was added and the sample stirred for 1 h at 80 °C to dissolve the solid particles. After cooling the samples to the room temperature, 10ml of methanol/chloroform (1:1 v/v) was added and stirred overnight to mix the two solvent phases. Lipids were then gravimetrically separated. Lipid content was determined as the percentage of lipid weight per dry cell weight after chloroform evaporation [1].

Section S.1.2: Glucose concentration quantification

Glucose concentration was quantified by High-performance liquid chromatography (HPLC) using an UltiMate® 3000 system with an Aminex HPX-87H column. The samples were filtered (0.22 µm, Millipore) and diluted with deionised water by 50%. 5 µL sample injection volume with isocratic elution at 0.6 ml/min 5 mM H_2SO_4 were used, with the column at temperature of 50°C. A RefractoMax 520 refractive index detector was used to analyse the chromatograph (Thermo Fisher Scientific, UK). The sample peak areas were compared with a calibration curve of glucose [2]. Measurements of each sample were done in duplicate.

SECTION S2: SENSITIVITY TEST

An eight-run Plackett-Burman design was constructed [3] with the level of factors in Table 1 for the sensitivity test as shown in Table S1.

Table S1: Eight-run Plackett-Burman design for Sensitivity Test.

Run	Glucose (g/L)	Glycerol (g/L)	Yeast Extract (g/L)	$(NH_4)_2SO_4$ (g/L)	Na_2HPO_4 (g/L)	$MgSO_4.7H_2O$ (g/L)	KH_2PO_4 (g/L)
1	80	9	4	2	4	1.5	7
2	40	9	4	8	1	6	7
3	40	0	4	8	4	1.5	15.75
4	80	0	1	8	4	6	7
5	40	9	1	2	4	6	15.75
6	80	0	4	2	1	6	15.75
7	80	9	1	8	1	1.5	15.75
8	40	0	1	2	1	1.5	7

SECTION S3: EXPLORING THE DESIGN SPACE

A three-factor three-level Box-Behnken design was constructed [4] with the level of factors in Table S2 for exploring the design space as shown in Table S3.

Table S2: Levels of factors for Exploring the design space

Factors (g/L)	Symbol	Level (-1)	Level (0)	Level (+1)
Glucose	x_1	40	60	80
Yeast Extract	x_2	1	2.5	4
$(NH_4)_2SO_4$	x_3	2	5	8

Table S3: Box-Behnken design for Exploring the design space.

Run	Glucose (g/L)	Yeast Extract (g/L)	$(NH_4)_2SO_4$ (g/L)
1	40	1	1
2	40	4	2
3	80	1	3
4	80	4	4
5	40	2.5	5
6	40	2.5	6
7	80	2.5	7
8	80	2.5	8
9	60	1	9
10	60	1	10
11	60	4	11
12	60	4	12
13	60	2.5	13
14	60	2.5	14
15	60	2.5	15

SECTION S4: OPTIMIZATION

A three-factor three-level Box-Behnken design was constructed [4] with the level of factors for the optimization in Table 2 as shown in Table S4.

Table S4: Box-Behnken design for Optimization.

Run	Glucose (g/L)	Yeast Extract (g/L)	$(NH_4)_2SO_4$ (g/L)
1	5	1	8
2	5	7	8
3	50	1	8
4	50	7	8
5	5	4	2
6	5	4	14
7	50	4	2
8	50	4	14
9	27.5	1	2
10	27.5	1	14
11	27.5	7	2
12	27.5	7	14
13	27.5	4	8
14	27.5	4	8
15	27.5	4	8

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