

Fig. S1. Response surfaces plots showing the mutual effects of interactions between factors



Fig. S2. Fed-batch setup

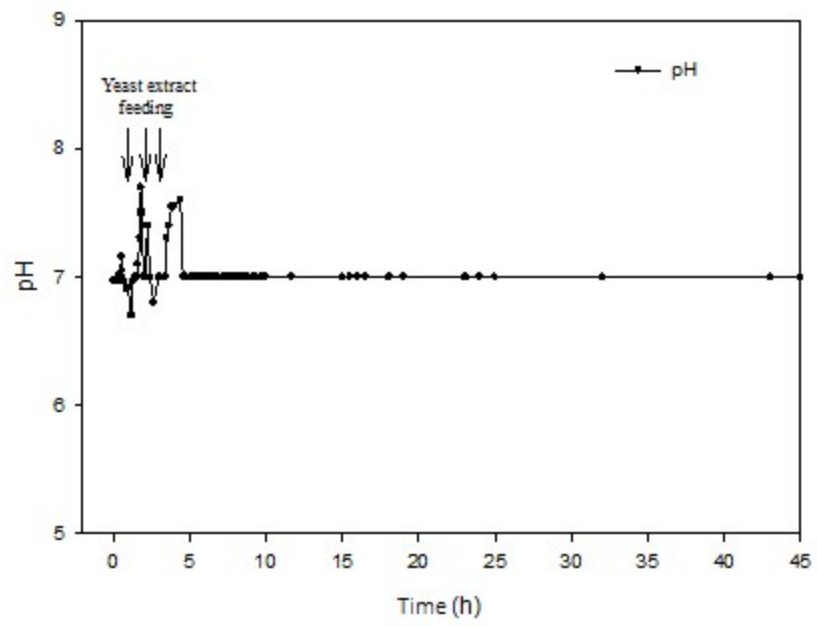


Fig. S3. Variation in pH over time in pH-stat strategy

Table S1. Central composite design matrix with experimental values of BS production

Run Order	Oil(gL ⁻¹)	MgSO ₄ (gL ⁻¹)	NaNO ₃ (gL ⁻¹)	Yeast extract(gL ⁻¹)	BS (gL ⁻¹)
1	15	0.5	3	1.25	5.15
2	20	0.2	4	0.5	4.75
3	20	0.8	4	0.5	6.2
4	15	0.5	3	1.25	5.15
5	10	0.8	2	2	4.35
6	20	0.2	4	2	5.15
7	15	0.5	3	2.225	5.2
8	10	0.8	4	2	4.9
9	20	0.8	2	2	4.5
10	10	0.8	2	0.5	4.95
11	15	0.5	1.7	1.25	5.15
12	20	0.2	2	0.5	4.7
13	20	0.8	4	2	4.2
14	15	0.11	3	1.25	4.7
15	15	0.5	3	1.25	6
16	15	0.5	3	0.275	5.3
17	10	0.2	4	2	6.6
18	15	0.5	3	1.25	5.3
19	15	0.5	3	1.25	5.1
20	15	0.5	3	1.25	5.2
21	15	0.89	3	1.25	5.15
22	20	0.2	2	2	4.6
23	21.5	0.5	3	1.25	4.8
24	15	0.5	4.3	1.25	5.45
25	10	0.8	4	0.5	5.6
26	20	0.8	2	0.5	7.1
27	10	0.2	2	2	4.95
28	10	0.2	4	0.5	4.4
29	8.5	0.5	3	1.25	4.4
30	15	0.5	3	1.25	5
31	10	0.2	2	0.5	3.4

Table S2. Results of Oil Spreading Test for identification of BS producer bacteria, (140 bacteria is listed here in two different media with glucose (g) or oil (o) as the sole carbon source). Selected strains have been written in Bold.

Bacterium #	Oil spreading test (mm)			Bacterium #	Oil spreading test (mm)			Bacterium #	Oil spreading test (mm)		
	1st day	2nd day	3rd day		1st day	2nd day	3rd day		1st day	2nd day	3rd day
1-o		4	4	21-o	5	5	2	42-o	2	4	3
1-g		9	1	21-g	2	2	2	42-g	0	0	2
2-o		3	3	22-o		10	3	43-o	90	90	90
2-g		1	5	22-g		3	1	43-g	3	15	2
3-o		4	3	23-o	2	1	4	44-o	1	3	1
3-g		1	0	23-g	0	0	1	44-g	0	1	0
4-o		3	5	24-o		0	1	45-o	7	5	3
4-g		4	1	24-g		2	1	45-g	2	2	1
6-o		1	0	25-o	6	0	3	46-o	2	2	5
6-g		0	0	25-g	0	1	1	46-g	1	4	2
8-o	2	3	3	26-o	0	4	6	47-o	90	100	90
8-g	0	2	5	26-g	0	0	0	47-g	4	7	3
9-o		3	2	28-o	1	3	5	50-o	2	0	0
9-g		0	0	28-g	0	0	3	50-g	0	0	0
10-o		10	5	29-o	5	5	5	55-o	4	2	2
10-g		0	0	29-g	0	0	0	55-g	0	2	1
11-o		4	7	30-o		1	0	56-o	3	17	30
11-g		4	2	30-g		3	2	56-g	2	2	1
12-o		50	20	31-o	50	100	90	57-o	3	5	7
12-g		?	0	31-g	3	10	8	57-g	2	3	4
13-o		5	3	32-o	0	0	0	58-o	2	5	10
13-g		2	0	32-g	0	0	1	58-g	2	3	4
14-o		5	0	33-o	10	5	2	59-o	2	5	2
14-g		0	0	33-g	2	2	3	59-g	2	1	2
15-o		0	4	34-o		5	5	61-o	3	7	3
15-g		2	2	34-g		0	0	61-g	3	2	5
16-o		4	2	35-o	2	0	2	63-o	1	2	2
16-g		2	4	35-g	4	2	4	63-g	2	3	2
18-o	1	4	4	36-o	0	5	3	64-o	1	0	2
18-g	0	2	0	36-g	0	0	3	64-g	2	3	2
19-o	2	3	4	37-o	0	2	1	65-o	1	3	8
19-g	2	3	3	37-g	2	5	7	65-g	1	3	5
20-o	2	3	6	41-o	2	2	1	68-o	4	5	5
20-g	0	0	1	41-g	2	2	2	68-g	4	2	3

Table S2. Continued

Bacterium #	Oil spreading test (mm)			Bacterium #	Oil spreading test (mm)			Bacterium #	Oil spreading test (mm)		
	1st day	2nd day	3rd day		1st day	2nd day	3rd day		1st day	2nd day	3rd day
69-o	2	2	4	104-o		2	2	123-o		2	1
69-g	1	1	2	104-g		3	0	123-g		3	2
70				105-o		2	4	124-o		3	3
71-o	0	1	5	105-g		3	2	124-g		2	0
71-g	5	5	5	106-o		6	3	125-o		4	3
72-o		0	0	106-g		3	3	125-g		0	2
72-g		3	4	107-o		3	0	126-o		0	0
75-o	1	1	5	107-g		3	3	126-g		3	0
75-g	5	5	6	108-o		3	3	127-o		55	45
77-o	2	0		108-g		30	40	127-g		3	4
77-g	0	0		109-o		3	3	128-o		0	2
78-o	5	5	11	109-g		20	20	128-g		1	4
78-g	5	5	5	110-o		0	5	130-o		0	3
84-o	3	2		110-g		0	3	130-g		3	3
84-g	2	3		111-o		5	5	131-o		4	2
91-o	30	90	70	111-g		2	2	131-g		0	0
91-g	1	3	5	112-o		4	3	132-o		2	2
92-o	1	2	2	112-g		2	2	132-g		2	2
92-g	2	5	3	113-o		3	3	133-o		3	3
93-o	2	3	5	113-g		3	4	133-g		0	1
93-g	2	0	1	114-o		3	3	134-o		3	3
94-o	20	30	45	114-g		2	0	134-g		3	3
94-g	3	3	5	115-o		6	0	135-o		4	3
95-o	2	2	2	115-g		4	0	135-g		3	3
95-g	3	2	2	116-o		0	0	136-o		2	3
96-o	35	45	90	116-g		2	2	136-g		3	0
96-g	10	20	40	117-o		2	7	137-o		4	0
97-o	2	4	11	117-g		2	4	137-g		0	0
97-g	3	4	4	118-o		2	3	138-o		2	4
98-o	1	2	5	118-g		10	5	138-g		0	0
98-g	5	3	3	119-o		4	3	139-o		0	0
99-o	2	2	1	119-g		3	0	139-g		1	1
99-g	3	1	5	120-o		40	4	140-o		1	1
100-o	3	1	1	120-g		2	3	140-g		6	4
100-g	15	5	6	121-o		7	10	74-o	3	1	11
101-o	1	2	2	121-g		3	3	74-g	3	2	6
101-g	1	2	4	122-o		2	1	81-o	2	40	40
102-o	1	1	3	122-g		0	4	81-g	5	2	3

Table S3. Analysis of RSM model variance (ANOVA) for BS production

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	14	14.2900	14.2900	1.02071	17.58	>0.001
Linear	4	1.9857	4.5069	1.12673	19.40	>0.001
oil	1	0.3408	1.6954	1.69539	29.19	>0.001
MgSO4	1	0.7589	1.0519	1.05190	18.11	0.001
NaNO3	1	0.6837	0.0405	0.04046	0.70	0.416
yeast	1	0.2023	0.8762	0.87619	15.09	0.001
Square	4	0.8559	0.8559	0.21396	3.68	0.026
oil*oil	1	0.6525	0.6246	0.62464	10.76	0.005
MgSO4* MgSO4	1	0.0191	0.0795	0.07947	1.37	0.259
NaNO ₃ *NaNO ₃	1	0.1330	0.0929	0.09287	1.60	0.224
yeast*yeast	1	0.0513	0.0513	0.05131	0.88	0.361
Interaction	6	11.4484	11.4484	1.90807	32.85	0.000
oil* MgSO4	1	0.3452	0.3452	0.34516	5.94	0.027
oil*NaNO3	1	1.2377	1.2377	1.23766	21.31	0.000
oil*yeast	1	2.8477	2.8477	2.84766	49.03	0.000
MgSO4*NaNO3	1	0.6602	0.6602	0.66016	11.37	0.004
MgSO4*yeast	1	6.1877	6.1877	6.18766	106.54	0.000
NaNO3*yeast	1	0.1702	0.1702	0.17016	2.93	0.106
Residual Error	16	0.9292	0.9292	0.05808		
Lack-of-Fit	10	0.2599	0.2599	0.02599	0.23	0.979
Pure Error	6	0.6693	0.6693	0.11155		
Total	30	15.2192				