

Experiment	Block	Ammonium chloride	Ammonium nitrate	Ammonium sulphate	Potassium sulphate	Potassium nitrate	Sodium carbonate	Sodium chloride	Dipotassium hydrogen phosphate	Sodium dihydrogen phosphate	Urea	Citric acid	MOPS	Calcium chloride	Iron sulphate	Magnesium sulphate	NTA	EDTA	Trace metals 500x	Vitamins 100x	Amino acids 100x	Yeast extract
1	1	0	50	50	50	0	1	0	0	17	0	0	50	1	0	1	0	0	0	1	0	0.1
2	1	50	50	50	50	0	0	8.6	33	0	0	10	0	0	0.1	1	0	1	1	1	0	0
3	1	50	0	50	0	50	0	8.6	0	17	0	10	0	1	0	0	1	0	1	0	0	0.1
4	1	0	0	50	0	50	1	0	33	0	0	0	50	0	0.1	0	1	1	0	0	0	0
5	1	0	50	0	0	50	0	8.6	33	0	50	0	50	1	0	1	0	0	1	0	1	0
6	1	50	0	0	50	0	1	0	33	0	50	10	0	1	0	0	1	0	0	1	1	0
7	1	50	50	0	0	50	1	0	0	17	50	10	0	0	0.1	1	0	1	0	0	1	0.1
8	1	0	0	0	50	0	0	8.6	0	17	50	0	50	0	0.1	0	1	1	1	1	1	0.1
9	2	0	0	50	50	50	1	8.6	33	17	50	0	0	0	0	0	0	0	1	1	1	0.1
10	2	50	50	50	0	0	0	0	33	17	50	10	50	0	0	1	0	0	0	0	1	0.1
11	2	50	0	0	0	0	1	8.6	33	17	0	10	50	1	0.1	0	0	1	1	0	0	0.1
12	2	50	50	0	0	50	2	8.6	0	0	0	10	50	0	0	1	0	0	1	1	0	0
13	2	0	50	50	0	0	1	8.6	0	0	50	0	0	1	0.1	1	1	1	1	0	1	0
14	2	0	50	0	0	50	0	0	33	17	0	0	0	1	0.1	1	1	1	0	1	0	0.1
15	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	2	50	0	50	50	50	0	0	0	0	50	10	50	1	0.1	0	0	1	0	1	1	0
17	3	50	0	50	50	0	0	8.6	33	0	0	0	50	1	0	1	0	1	0	0	1	0.1
18	3	50	50	50	0	50	0	8.6	0	17	0	0	50	0	0.1	0	1	0	0	1	1	0
19	3	0	50	50	0	50	1	0	33	0	0	10	0	1	0	0	1	1	1	1	1	0.1
20	3	0	0	50	50	0	1	0	0	17	0	10	0	0	0.1	1	0	0	1	0	1	0
21	3	50	50	0	50	0	1	0	33	0	50	0	50	0	0.1	0	1	0	1	0	0	0.1
22	3	50	0	0	0	50	1	0	0	17	50	0	50	1	0	1	0	1	1	1	0	0
23	3	0	0	0	0	50	0	8.6	33	0	50	10	0	0	0.1	1	0	0	0	1	0	0.1
24	3	0	50	0	50	0	0	8.6	0	17	50	10	0	1	0	0	1	1	0	0	0	0
25	4	0	50	0	0	0	0	0	0	0	0	10	50	1	0.1	0	0	0	1	1	1	0.1
26	4	50	0	50	0	0	0	0	33	17	50	0	0	1	0.1	1	1	0	1	1	0	0
27	4	0	0	50	0	0	1	8.6	0	0	50	10	50	0	0	1	1	1	0	1	0	0.1
28	4	0	0	0	50	50	0	0	33	17	0	10	50	0	0	1	1	1	1	1	1	0
29	4	50	50	0	0	0	1	8.6	33	17	0	0	0	0	0	0	0	1	0	1	1	0
30	4	50	0	0	50	50	1	8.6	0	0	0	0	0	1	0.1	1	1	0	0	0	1	0.1
31	4	0	50	50	50	50	1	8.6	33	17	50	10	50	1	0.1	0	0	0	0	0	0	0
32	4	50	50	50	50	50	0	0	0	0	50	0	0	0	0	0	0	1	1	0	0	0.1
33	5	50	0	0	50	0	0	8.6	0	17	50	0	50	0	0.1	1	0	0	0	0	0	0
34	5	50	0	50	0	50	1	0	33	0	0	0	50	0	0.1	1	0	0	1	1	1	0.1
35	5	0	50	50	50	0	0	8.6	33	0	0	10	0	0	0.1	0	1	0	0	0	1	0.1
36	5	0	0	0	50	0	1	0	33	0	50	10	0	1	0	1	0	1	1	0	0	0.1
37	5	50	50	0	0	50	0	8.6	33	0	50	0	50	1	0	0	1	1	0	1	0	0.1
38	5	0	50	0	0	50	1	0	0	17	50	10	0	0	0.1	0	1	0	1	1	0	0
39	5	50	50	50	50	0	1	0	0	17	0	0	50	1	0	0	1	1	1	0	1	0
40	5	0	0	50	0	50	0	8.6	0	17	0	10	0	1	0	1	0	1	0	1	1	0
41	6	0	50	50	0	0	0	0	33	17	50	10	50	0	0	0	0	1	1	1	0	0
42	6	50	50	0	50	50	0	0	33	17	0	0	0	1	0.1	0	0	0	1	0	1	0
43	6	50	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0.1
44	6	0	0	50	50	50	0	0	0	0	50	10	50	1	0.1	1	1	0	1	0	0	0.1
45	6	50	0	50	50	50	1	8.6	33	17	50	0	0	0	0	1	1	1	0	0	0	0
46	6	0	0	0	0	0	1	8.6	33	17	0	10	50	1	0.1	1	1	0	0	1	1	0
47	6	0	50	0	50	50	1	8.6	0	0	0	10	50	0	0	0	0	1	0	0	1	0.1
48	6	50	50	50	0	0	1	8.6	0	0	50	0	0	1	0.1	0	0	0	0	1	0	0.1
49	7	50	50	50	0	50	1	0	33	0	0	10	0	1	0	1	0	0	0	0	0	0
50	7	0	0	50	50	0	0	8.6	33	0	0	0	50	1	0	0	1	0	0	1	0	0
51	7	0	50	50	0	50	0	8.6	0	17	0	0	50	0	0.1	1	0	1	1	0	0	0.1
52	7	0	50	0	50	0	1	0	33	0	50	0	50	0	0.1	1	0	1	0	1	1	0
53	7	0	0	0	0	50	1	0	0	17	50	0	50	1	0	0	1	0	0	0	1	0.1
54	7	50	0	50	50	0	1	0	0	17	0	10	0	0	0.1	0	1	1	0	0	1	0.1
55	7	50	0	0	0	50	0	8.6	33	0	50	10	0	0	0.1	0	1	1	1	0	1	0
56	7	50	50	0	50	0	0	8.6	0	17	50	10	0	1	0	1	0	0	1	1	1	0.1
57	8	0	50	0	0	0	1	8.6	33	17	0	0	0	0	0	1	1	0	1	0	0	0.1
58	8	50	50	50	50	50	1	8.6	33	17	50	10	50	1	0.1	1	1	1	1	1	1	0.1
59	8	50	50	0	0	0	0	0	0	0	0	10	50	1	0.1	1	1	1	0	0	0	0
60	8	50	0	0	50	50	0	0	33	17	0	10	50	0	0	0	0	0	0	1	0	0.1
61	8	50	0	50	0	0	1	8.6	0	0	50	10	50	0	0	0	0	0	1	0	1	0
62	8	0	50	50	50	50	0	0	0	0	50	0	0	0	0	1	1	0	0	1	1	0
63	8	0	0	0	50	50	1	8.6	0	0	0	0	0	1	0.1	0	0	1	1	1	0	0
64	8	0	0	50	0	0	0	0	33	17	50	0	0	1	0.1	0	0	1	0	0	1	0.1

Table S4. First iteration of Design of Experiments for the development of a defined *Geobacillus* growth medium.

The 64 media formulations generated by the DoE Fractional Factorial design combining random combinations of the 21 factors identified from the literature search of defined media.