

Supplementary information

Isolation, identification and subsequent application of gram-positive bacterial strains for bioremediation of benzophenone

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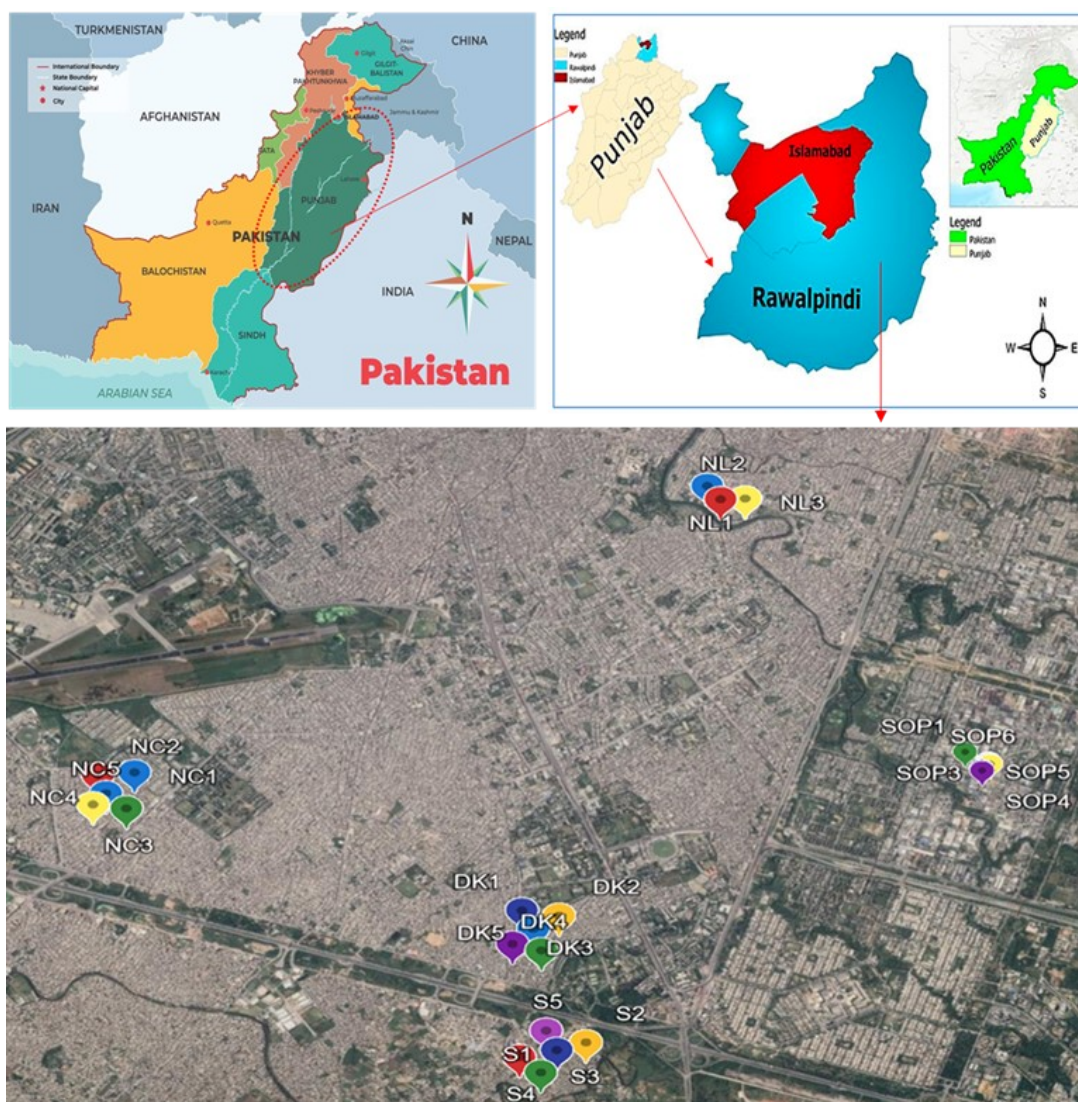


Figure S1. Map showing the wastewater sampling locations with different colors in Rawalpindi and Islamabad. Legend showing Rawalpindi sampling locations i.e., Noor Colony (NC1, NC2, NC3, NC4 & NC5), Sohan (S1, S2, S3, S4 & S5), Dhoke Kala Khan (DK1, DK2, DK3, DK 4 & DK5) and Islamabad sampling locations i.e., Nulla Lai (NL1, NL2 NL3), soap industries (SOP 1, SOP2, SOP3), Rawalpindi / Islamabad map was adopted from (Akmal and Jamil, 2021).

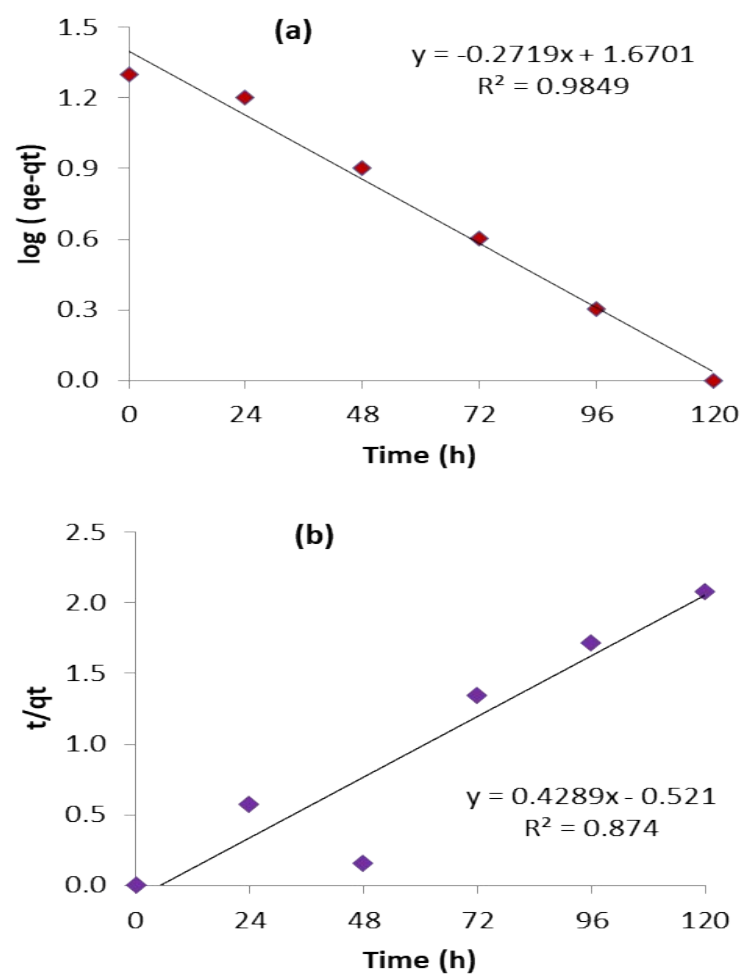


Figure S2. Plot of $\log (q_e - q_t)$ vs time of (a) *Bacillus pumilus* (S4) and (b) *Bacillus cereus* (DK2) and graph of $\log (t/q_t)$ vs time of (c) *Bacillus pumilus*(S4) and (d) *Bacillus cereus* (DK2) for determining the reaction order of biodegradation of BPZ.

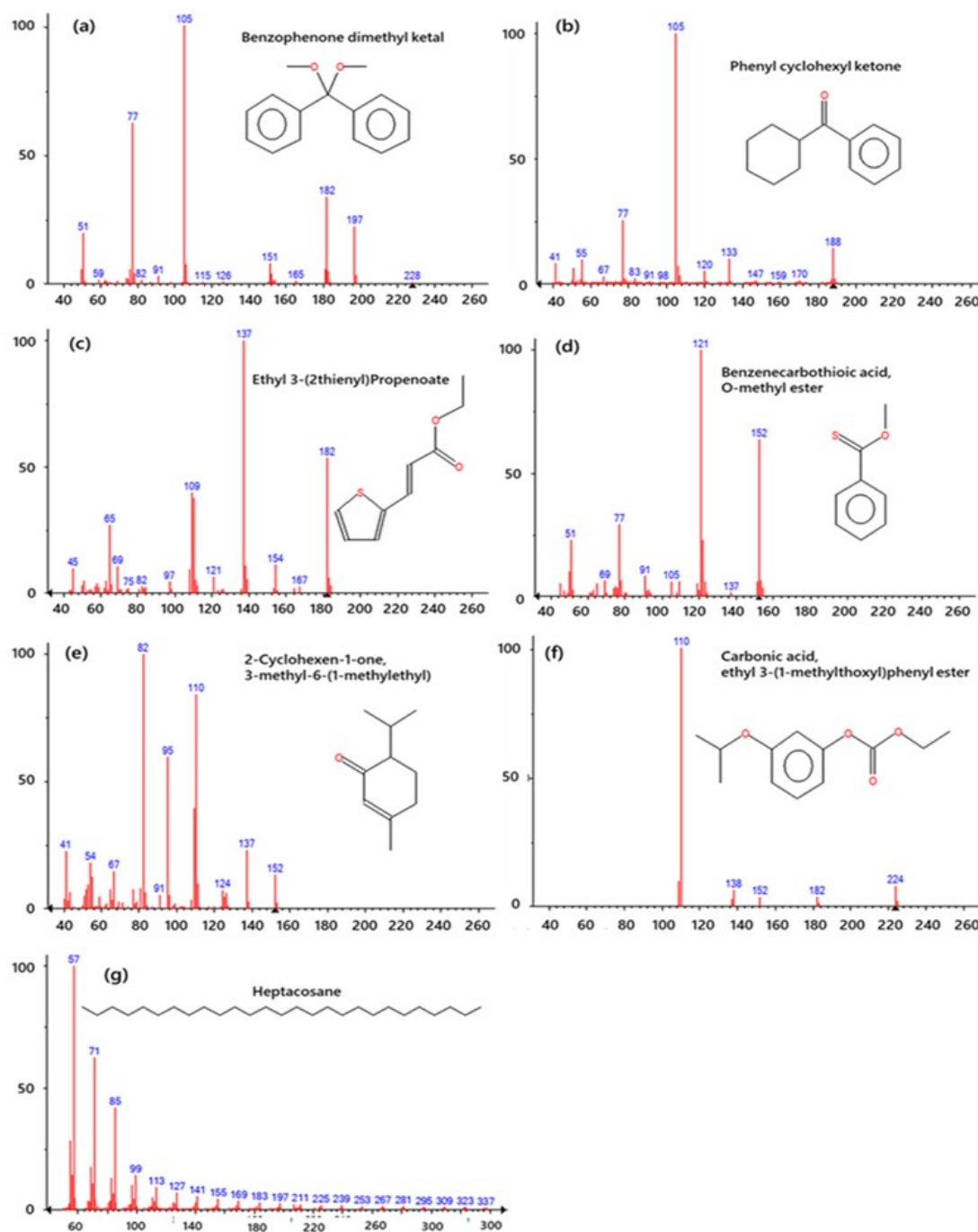


Figure S3. Intermediates of the biodegradation of BZP on the basis of m/z values and relative abundance using bacterial isolates DK2 and S4 (a) benzophenone dimethyl ketal and (b) phenyl cyclohexyl ketene, (c) Ethyl 3-(2thienyl)-propenoate, (d) Benzenecarbothioic acid, O-methyl ester, (e) 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl), (f) Carbonic acid, ethyl 3-(1-methylthoxyl)-phenyl ester, and (g) Heptacosane.



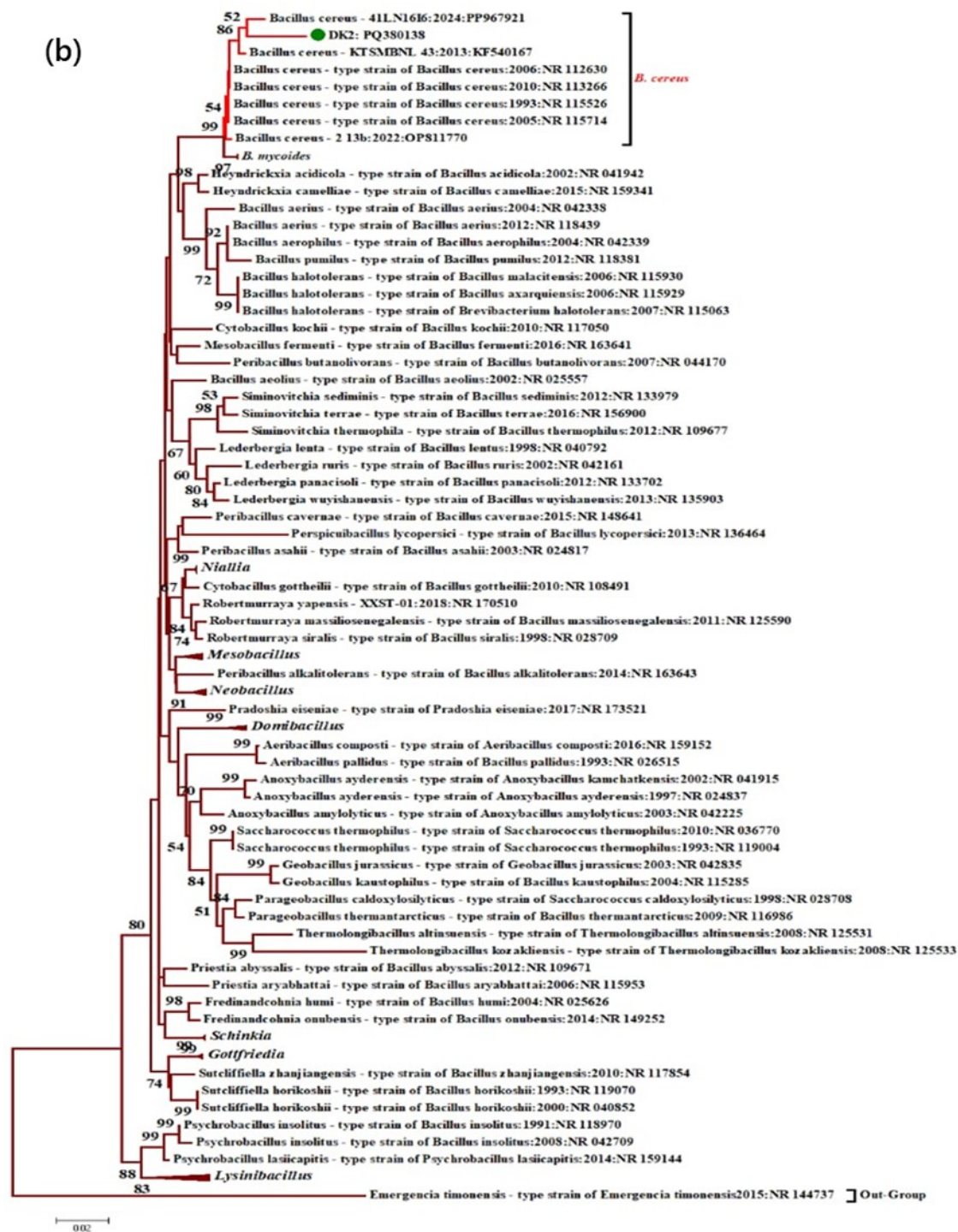


Figure S4. Phylogenetic tree of bacterial strains (a) DK2 (PQ380138) and (b) S4 (PQ380140) was created with closely resembled species of *Bacillus cereus* and *Bacillus pumilus* inferred from 16S rRNA gene sequences. Evolutionary analyses were conducted in MEGA7.

Table S1. Physicochemical characteristics of wastewater collected from different locations in Rawalpindi and soap industries in Islamabad, Pakistan.

S. No	Samples	Coordinates	pH	EC ($\mu\text{S cm}^{-1}$)	TDS (mg/L)	COD (mg/L)	BZP (mg/L)
1.	SOP1*	33°39'30"N 73°03'12"E	7.03 ± 0.43	153.97 ± 4.31	109.8 ± 8.13	153.3 ± 7.07	10
2.	SOP2*	33°39'36"N 73°03'10"E	6.91 ± 0.09	183.93 ± 11.9	183.93 ± 11.9	244 ± 19.09	08
3.	SOP3	33°39'37"N 73°03'13"E	6.98 ± 0.18	125.1 ± 11.0	160.3 ± 17.04	198.3 ± 10.07	04
4.	SOP4	33°39'37"N 73°03'13"E	8.81 ± 0.11	142.5 ± 22.2	215.8 ± 16.82	290.67 ± 12.2	ND
5.	SOP5	33°39'37"N 73°03'13"E	7.16 ± 0.16	200.43 ± 8.2	168.3 ± 17.04	352 ± 3.53	ND
6.	SOP6	33°39'37"N 73°03'13"E	9.01 ± 0.37	198.8 ± 10.6	241 ± 3.1	362.3 ± 6.9	10
7.	SOP7	33°39'37"N 73°03'10"E	9.8 ± 0.25	200.7 ± 30.0	168.2 ± 4.7	250.67 ± 5.6	ND
8.	NL1	33°37'55"N 73°03'08"E	7.1 ± 0.25	121.67 ± 11.0	181.2 ± 4.7	270.67 ± 25.6	ND
9.	NL2	33°38'00"N 73°03'08"E	6.96 ± 0.26	201.53 ± 0.63	162.23 ± 17.6	253.3 ± 4.94	ND
10.	NL3	33°38'03"N 73°03'03"E	6.71 ± 0.29	115.13 ± 4.7	121.78 ± 8.7	275.3 ± 2.8	ND
11.	NC1	33°37'37"N 73°06'11"E	7.02 ± 0.17	221 ± 1.131	145.2 ± 7.36	283 ± 19.33	06
12.	NC2	33°37'32"N 73°06'19"E	7.16 ± 0.22	174.88 ± 8.4	134.97 ± 13.9	261 ± 23.33	ND
13.	NC3	33°37'44"N 73°06'21"E	7.02 ± 0.26	211.1 ± 5.9	142.47 ± 10.7	359.3 ± 22.6	15
14.	NC4	33°37'38"N 73°06'22"E	7.12 ± 0.30	118.8 ± 10.9	296.3 ± 5.30	268 ± 26.7	12
15.	NC5	33°37'38"N 73°06'27"E	10.74 ± 0.190	121 ± 15.13	327.7 ± 37.7	482.67 ± 10.60	ND
16.	S1	33°39'40"N 73°05'46"E	7.22 ± 0.098	221.7 ± 19.6	245.7 ± 16.8	168.3 ± 12.0	18
17.	S2	33°39'43"N 73°05'38"E	9.98 ± 0.18	135.1 ± 18.0	168.3 ± 17.04	182.3 ± 7.07	ND
18.	S3	33°39'43"N 73°05'54"E	7.81 ± 0.11	142.5 ± 22.2	215.8 ± 16.82	290.67 ± 12.2	ND
19.	S4	33°39'37"N 73°05'55"E	7.16 ± 0.16	200.43 ± 8.2	168.3 ± 17.04	352 ± 3.53	ND
20.	S5	33°39'34"N 73°05'44"E	7.01 ± 0.37	146.13 ± 19.3	242.23 ± 23.1	382.3 ± 26.9	15
21.	DK1	33°39'04"N 73°05'22"E	7.06 ± 0.19	212.2 ± 19.5	239.13 ± 24.4	226.67 ± 23.4	ND
22.	DK2	33°39'10"N 73°05'15"E	7.25 ± 0.15	210.1 ± 19.4	159.7 ± 14.49	350.3 ± 8.49	ND
23.	DK3	33°39'10"N 73°05'31"E	7.16 ± 0.12	309.7 ± 15.6	208.87 ± 7.91	269.3 ± 18.39	16
24.	DK4	33°39'16"N 73°05'27"E	7.22 ± 0.24	340 ± 17.67	185.08 ± 1.13	118.3 ± 16.9	ND
25.	DK5	33°39'10"N 73°05'23"E	6.95 ± 0.39	243.8 ± 16.8	231.77 ± 11.80	144 ± 7.08	ND
NEQs permissible limit			6 – 9	400 – 600	>1000	<150	—
WHO/FAO permissible limit			6.5 – 9	400 – 600	>1000	<150	—

Samples collected from soap industries; the rest of the samples were municipal wastewater and were collected from different area in Rawalpindi DK: Dhoke Kala Khan, S: Sohan, NC: Noor Colony; NL: Nulla Lai; results were reported as mean ± Standard deviation (SD); ND: not detected

Table S2. Screening of bacterial isolates based on optical density (OD600) in MSM media spiked with different concentrations of BZP after 48 hours of incubation.

Sample location	No of bacterial isolates	No of BZP degrading isolates	MSM optimum conditions		Range of OD (600) at different BZP concentration (mg/L)				
			Temp. (°C)	pH	10	20	100	500	1000
S	40	01	30	7	0.2–0.5	0.2–0.5	0.3–0.4	0.3–0.6	0.3–0.6
DK	25	01	30	7	0.3–0.6	0.3–0.4	0.3–0.6	0.3–0.4	0.3–0.4
NL	35	01	30	7	0.2–0.6	0.3–0.5	0.3–0.4	0.2–0.6	0.2–0.4
NC	30	02	30	7	0.3–0.6	0.3–0.4	0.2–0.4	0.3–0.4	0.3–0.5
SOP	20	00	30	7	0.3–0.4	0.3–0.5	0.2–0.6	0.3–0.6	0.2–0.4
Total	150	05	—	—		—	—	—	—

Note: DK: Dhoke Kala Khan, S: Sohan, NC: Noor Colony; NL: Nulla Lai; SOP: soap industries

Table S3. Characterization of the bacterial isolates by measuring lag phase, doubling time, time to steady state and colony forming units using BZP as sole carbon source.

Bacterial activity parameters	Bacterial isolates	
	<i>Bacillus cereus</i> (DK2)	<i>Bacillus pumilus</i> (S4)
Estimated lag phase duration (h.)	4 ± 0.3	3.5 ± 0.2
Average doubling time (h)	1.7 ± 0.4	1.7 ± 0.5
Average growth rate (r/h)	0.48 ± 0.6	0.45 ± 0.9
Time to steady state (h)	72 ± 1.1	72 ± 1.3
CFU/mL Exponential phase	1.0 × 10 ⁵ ± 10 ³	1.0 × 10 ⁶ ± 10 ²

Table S4. Morphological characterization and physiological data of efficient bacterial strains.

Parameter	Bacterial strains	
	<i>Bacillus pumilus</i> (S4)	<i>Bacillus cereus</i> (DK2)
Form/ shape	Rod shaped	Rod shaped
Elevation	Raised	Raised
Margin	Coiled	square
Opacity	translucent	translucent
Pigmentation	White	Cream
Gram Stain	Gram-positive	Gram-positive
Growth temperature	5 — 55°C	4 — 55°C
Spore-Forming	endospore-forming	endospore-forming
Growth pH	4.0 — 9.0	6.0 — 7.0
NaCl tolerance	Ability to grow	Ability to grow

Table S5. Biochemical data of efficient bacterial strains.

<i>Bacillus pumilus</i> (S4)	Biochemical Properties	<i>Bacillus cereus</i> (DK2)	Biochemical Properties
Protease	+	Catalase	+
Lipase	+	Voges-Proskauer (VP)	+
xylanase,	+	Gelatin Hydrolysis	+
amylase	+	Lecithinase Activity	+
Gelatinase	-	Nitrate Reduction	+
Chitinase	-	Oxidase	-
Phosphatase	-	Indole	-

Table S6. Comparative analysis of Pseudo-first-order (PFO1) and Pseudo second-order (PSO2) kinetics of the degradation of BZP by *Bacillus pumilus* (S4) and *Bacillus cereus* (DK2).

Isolates	Pseudo first order model		Pseudo second order model	
	$K_1(h^{-1})$	R^2	$K_2(h^{-1})$	R^2
S4	0.8	0.98	0.57	0.87
DK2	0.6	0.95	0.94	0.99