

Supporting Information

**Influence of Thermophilic *Bacillus subtilis* YB7 on the Biodegradation of  
Long Chain Paraffinic Hydrocarbons ( $C_{16}H_{34}$  to  $C_{36}H_{74}$ )**

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**Table S1.** Purity mass fraction and supplier of chemicals.

S.No	Chemicals	Supplier	Purity mass fraction
1	n-hexadecane (C <sub>16</sub> H <sub>34</sub> )	Spectrochem pvt ltd	0.99
2	n-eicosane (C <sub>20</sub> H <sub>42</sub> )	Alfa Aesar	0.99
3	n-tetracosane (C <sub>24</sub> H <sub>50</sub> )	Alfa Aesar	0.99
4	n-octacosane (C <sub>28</sub> H <sub>58</sub> )	Alfa Aesar	0.99
5	n-dotriacontane (C <sub>32</sub> H <sub>66</sub> )	Alfa Aesar	0.99
6	n-hexatriacontane (C <sub>36</sub> H <sub>74</sub> )	Alfa Aesar	0.99
7	Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	Sisco research laboratories	0.99
8	Disodium hydrogen phosphate (Na <sub>2</sub> HPO <sub>4</sub> )	Merck specialities	0.995
9	Ammonium chloride (NH <sub>4</sub> Cl)	Sisco research laboratories	0.99
10	Sodium chloride (NaCl)	Merck specialities	0.995
11	Glucose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	Himedia	0.995
12	Magnesium sulfate hepta hydrate (MgSO <sub>4</sub> .7H <sub>2</sub> O)	Rankem	0.99
13	Acetone (C <sub>3</sub> H <sub>6</sub> O)	Sigma Aldrich	0.999
14	Ethyl acetate (C <sub>4</sub> H <sub>8</sub> O <sub>2</sub> )	Rankem	0.999
15	Toluene (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	Sigma Aldrich	0.999
16	Tris hydrochloric acid (Tris-HCl)	Himedia	0.99
17	Ferrous Sulfate (FeSO)	Merck specialties	0.99
18	Nicotinamide adenine dinucleotide phosphate, reduced tetrasodium salt (NADPH)	Sigma Aldrich	0.99
19	Nicotinamide adenine dinucleotide reduced form (NADH)	Sigma Aldrich	0.99
20	Polyethylene glycol tridecyl ether phosphate	Sigma Aldrich	0.99
21	Sodium hydroxide (NaOH)	Sigma Aldrich	0.99

**Table S2a.** Viscosity of hydrocarbons during degradation at 35°C as a function of incubation days\*.

S. No.	Incubation Days	C <sub>16</sub> H <sub>34</sub>	C <sub>20</sub> H <sub>42</sub>	C <sub>24</sub> H <sub>50</sub>	C <sub>28</sub> H <sub>58</sub>	C <sub>32</sub> H <sub>66</sub>	C <sub>36</sub> H <sub>74</sub>
1	0	5±0.1	7.3±0.6	17.1±0.5	19.2±0.7	24±0.4	27.2±0.5
2	1	3.4±0.5	5.3±0.2	14.5±0.2	15.8±0.4	20±0.1	24±0.3
3	5	3.2±0.2	5±0.1	13.8±0.1	15.3±0.2	19.5±0.3	18.9±0.6
4	10	3±0.3	4.8±0.6	12.2±0.1	14.4±0.1	18.1±0.2	21±0.6
5	15	2.9±0.4	4.4±0.4	12.5±0.0	13.1±0.1	15.5±0.4	16.1±0.4
6	20	2.8±0.1	4.1±0.1	12±0.0	11.4±0.4	14±0.3	15.3±0.3
7	25	2.4±0.0	3.9±0.1	11.6±0.0	10.4±0.1	13.5±0.2	14.9±0.8
8	30	2.1±0.1	3.5±0.0	10.1±0.6	9.3±0.1	12.9±0.2	14.1±0.4
9	35	2±0.1	3.4±0.0	9.3±0.9	8.8±0.3	12.8±0.1	13.7±0.3
10	40	2±0.0	3.4±0.1	9.1±0.1	8.7±0.1	12.8±0.5	13.5±0.1

\* Results represents mean ± standard deviation of three independent experiments.

**Table S2b.** Viscosity of hydrocarbons during degradation at 50°C as a function of incubation days\*.

S. No.	Incubation Days	C <sub>16</sub> H <sub>34</sub>	C <sub>20</sub> H <sub>42</sub>	C <sub>24</sub> H <sub>50</sub>	C <sub>28</sub> H <sub>58</sub>	C <sub>32</sub> H <sub>66</sub>	C <sub>36</sub> H <sub>74</sub>
1	0	5±0.4	7.3±0.2	17.1±0.3	19.2±0.3	24±0.5	27.2±0.1
2	1	3.2±0.3	4.8±.3	12±0.7	14±0.6	18±0.2	22±0.4
3	5	3±0.4	4.3±0.6	11.2±0.6	12.5±0.4	15±0.5	16.9±0.2
4	10	2.7±0.5	4±0.4	9.6±0.5	10.9±0.4	14.3±0.2	16.3±0.5
5	15	2.6±0.2	3.8±0.4	8.6±0.2	9.5±0.5	13.1±0.6	15.5±0.7
6	20	2.5±0.5	3.5±0.3	7.4±0.5	9.1±0.2	12.8±0.5	14±0.4
7	25	2.2±0.7	3.4±0.4	7±0.2	8.8±0.5	11.6±0.2	13.6±0.3
8	30	2±0.4	3.3±0.5	6.9±0.4	8.5±0.7	11±0.5	13±0.3
9	35	1.9±0.5	3±0.3	6.8±0.2	8.1±0.4	10.2±0.4	11.5±0.1
10	40	1.7±0.4	2.8±0.7	6.8±0.5	7.8±0.3	9.9±0.3	11.3±0.4

\* Results represents mean ± standard deviation of three independent experiments.

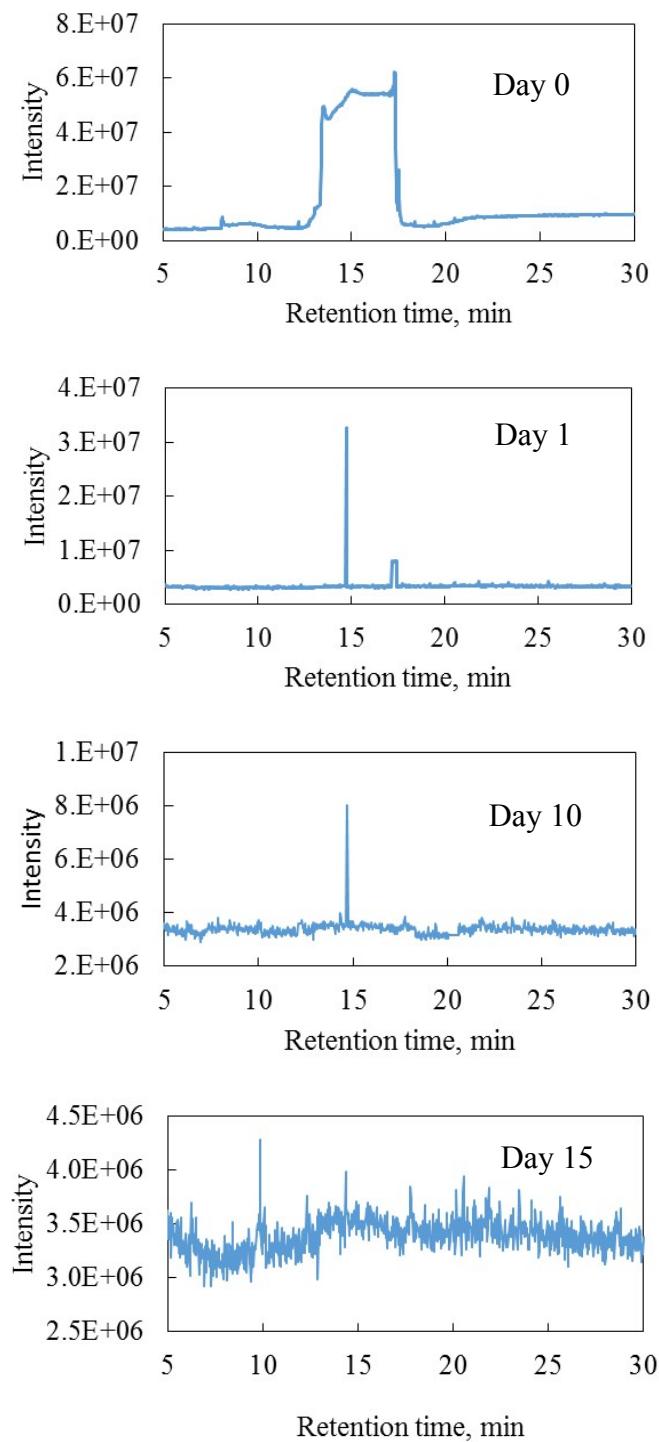
**Table S2c.** Viscosity of hydrocarbons during degradation at 75°C as a function of incubation days\*.

S. No.	Incubation Days	C <sub>16</sub> H <sub>34</sub>	C <sub>20</sub> H <sub>42</sub>	C <sub>24</sub> H <sub>50</sub>	C <sub>28</sub> H <sub>58</sub>	C <sub>32</sub> H <sub>66</sub>	C <sub>36</sub> H <sub>74</sub>
1	0	5±0.1	7.3±0.2	17.1±0.2	19.2±0.0	24±0.2	27.2±0.0
2	1	3.6±0.5	5.6±0.1	15.9±0.5	16.3±0.5	21±0.5	24.9±0.8
3	5	3.3±0.4	5.1±0.5	15.1±0.4	15.4±0.3	18±0.6	23.5±0.5
4	10	3.2±0.3	4.9±0.2	13±0.5	14.9±0.3	19±0.3	22±0.7
5	15	3±0.1	4.8±0.6	13.5±0.3	13.5±0.4	16.3±0.2	20.6±0.2
6	20	2.8±0.3	4.4±0.4	12.6±0.2	12.8±0.1	15.1±0.2	18±0.2
7	25	2.6±0.2	4.4±0.5	12.3±0.1	11.6±0.9	14.4±0.1	17.1±0.6
8	30	2.3±0.1	4.3±0.4	11.5±0.1	10.6±0.0	14±0.1	16.5±0.2
9	35	2.3±0.0	4.3±0.2	11.1±0.0	10.3±0.2	13.9±0.1	15.8±0.3
10	40	2.2±0.5	4.3±0.2	10.8±0.5	10.2±0.5	13.8±0.2	15.6±0.3

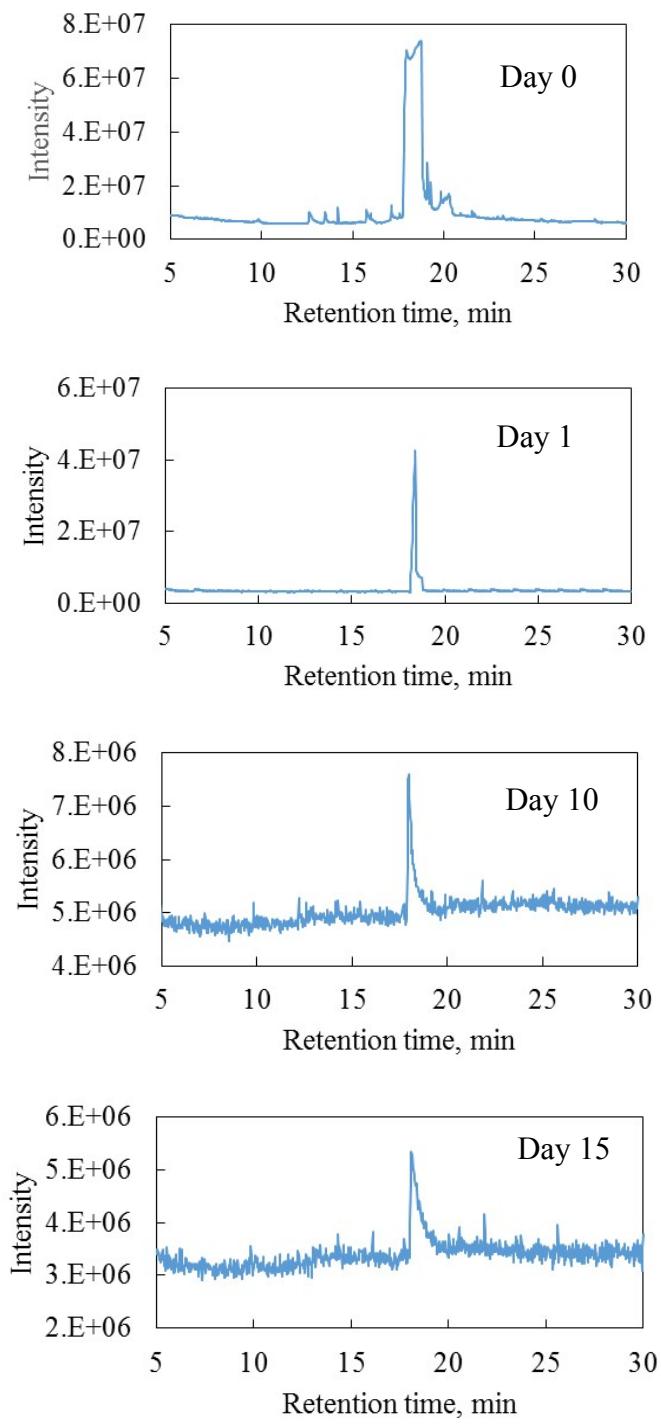
\* Results represents mean ± standard deviation of three independent experiments.

**Table S3.** Rate constant of a degradation process as a function of carbon number and temperature.

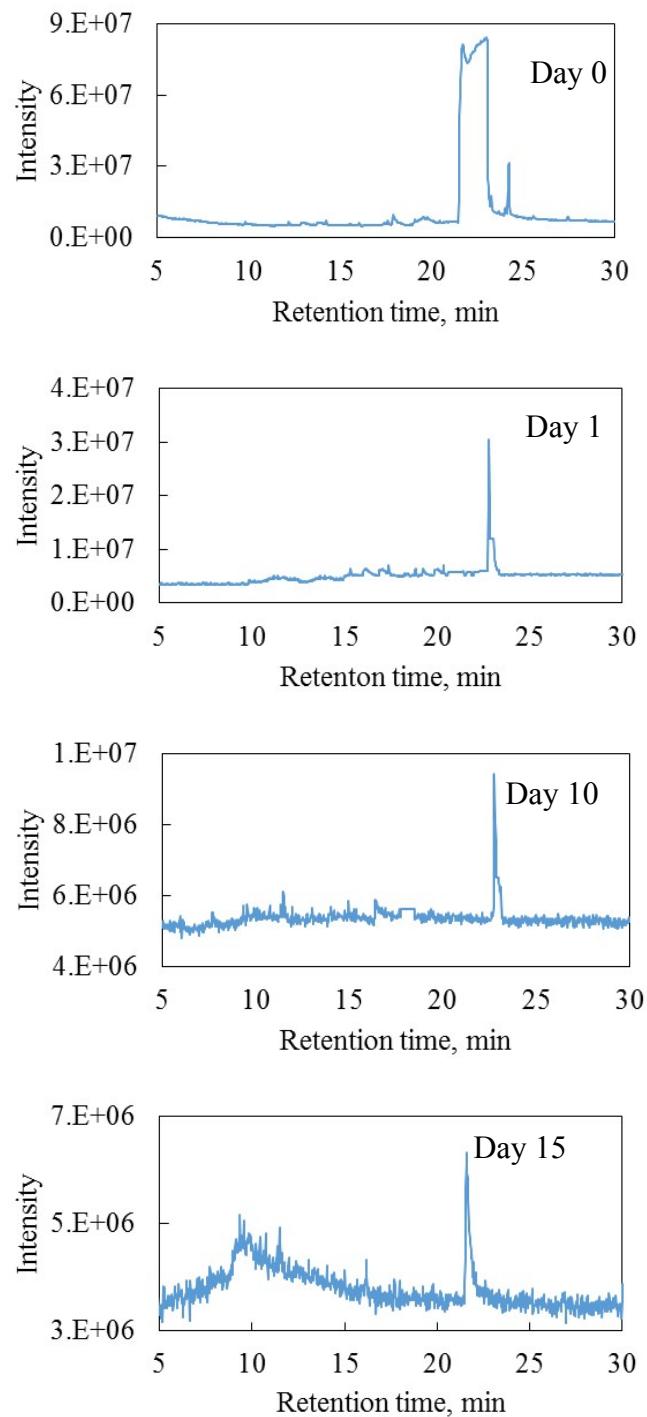
Temperature, °C	Carbon number	Rate constant, s <sup>-1</sup>		
		Day 1	Day 10	Day 20
35	16	1.18247E-05	3.25626E-06	1.73364E-06
	20	1.08982E-05	2.27559E-06	1.18068E-06
	24	1.08982E-05	2.05088E-06	1.02544E-06
	28	1.06052E-05	1.86278E-06	9.31388E-07
	32	8.73869E-06	1.75246E-06	8.76231E-07
	36	8.02254E-06	1.47334E-06	7.3667E-07
50	16	1.70101E-05	4.5278E-06	2.2639E-06
	20	1.43272E-05	3.25626E-06	1.62813E-06
	24	1.31879E-05	2.78697E-06	1.39349E-06
	28	1.18247E-05	2.19574E-06	1.1378E-06
	32	1.15075E-05	1.86278E-06	9.61071E-07
	36	1.06052E-05	1.75246E-06	8.76231E-07
75	16	1.06052E-05	2.45401E-06	1.227E-06
	20	1.00405E-05	1.92214E-06	9.61071E-07
	24	8.9876E-06	1.86278E-06	9.31388E-07
	28	8.49501E-06	1.65176E-06	8.25878E-07
	32	8.02254E-06	1.55911E-06	8.02254E-07
	36	7.56859E-06	1.35554E-06	6.96744E-07



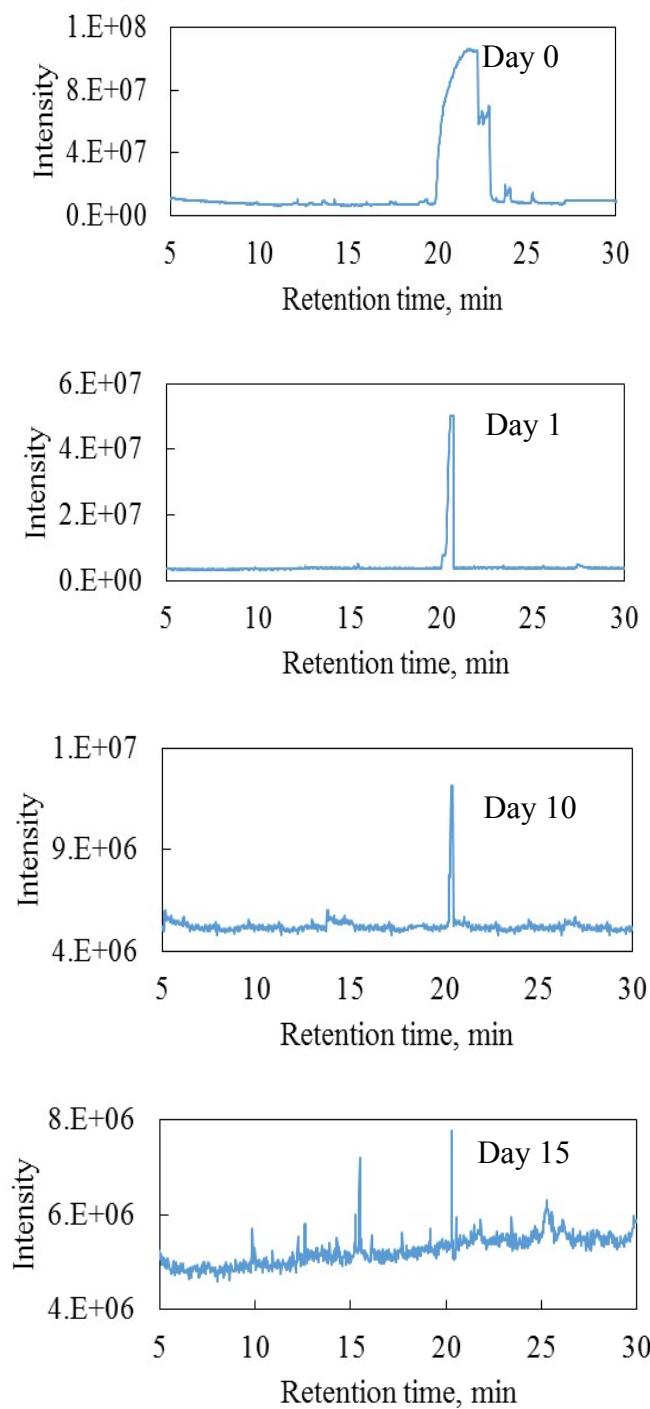
**Figure S1a.** Gas chromatogram of n-hexadecane (C<sub>16</sub>H<sub>34</sub>) before and after degradation using *Bacillus subtilis* incubated at 50°C.



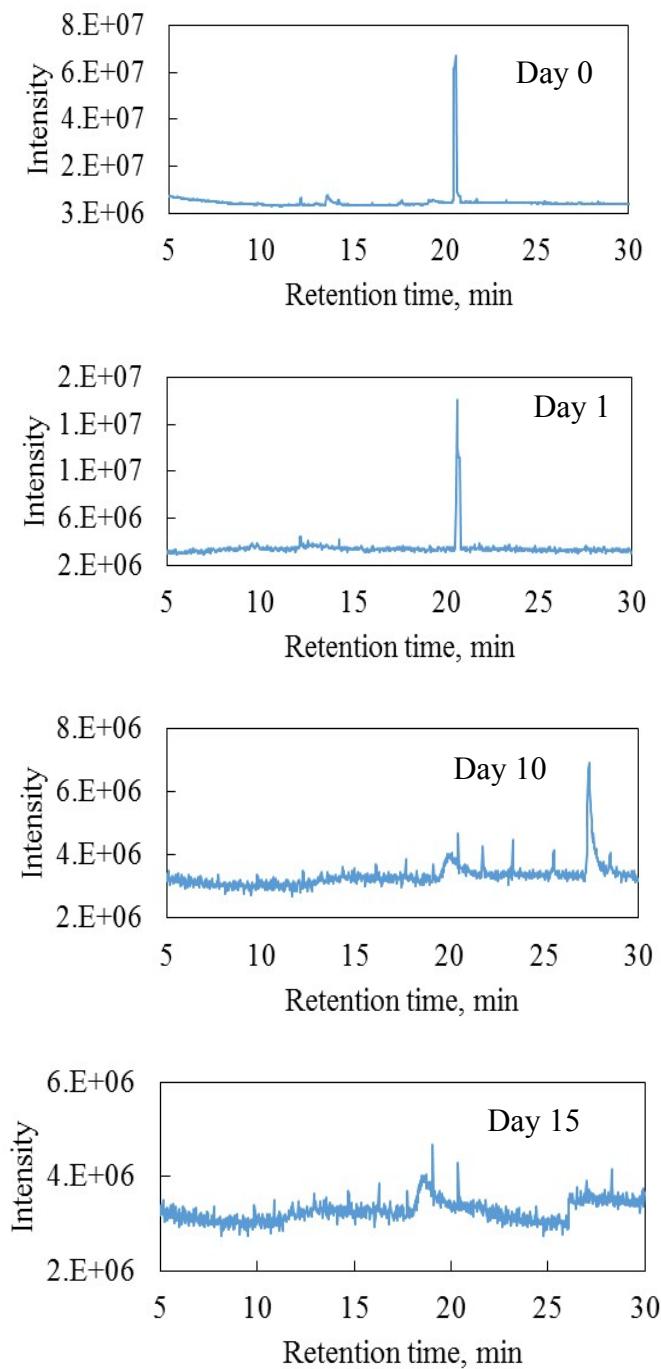
**Figure S1b.** Gas chromatogram of n-eicosane ( $C_{20}H_{42}$ ) before and after degradation using *Bacillus subtilis* incubated at 50°C.



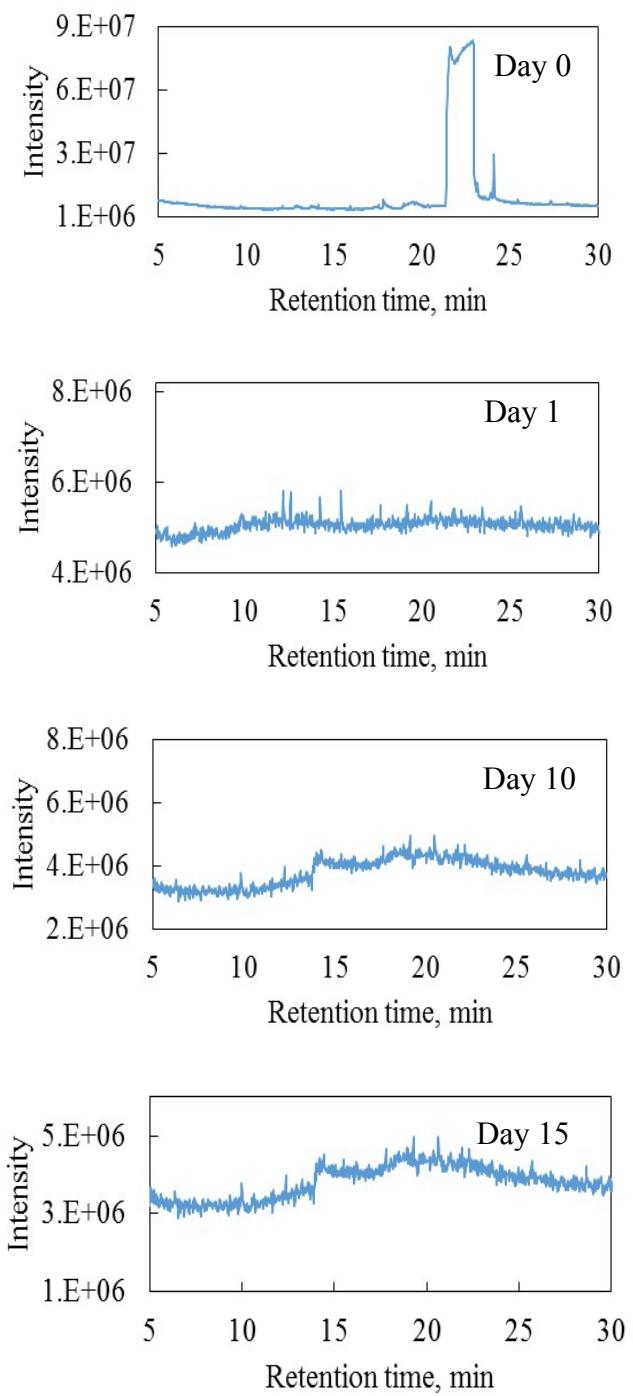
**Figure S1c.** Gas chromatogram of n-tetracosane ( $C_{24}H_{50}$ ) before and after degradation using *Bacillus subtilis* incubated at 50°C.



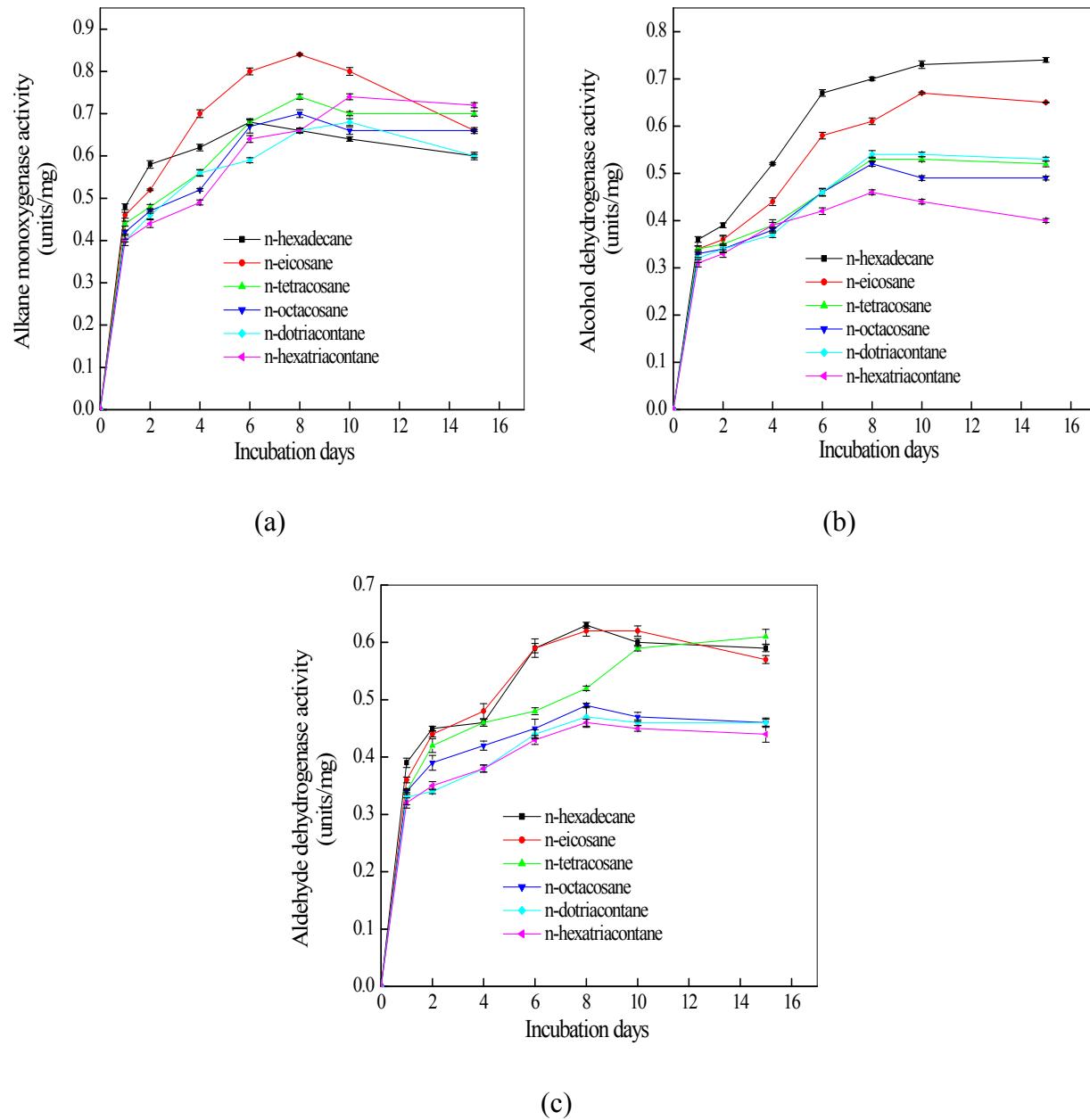
**Figure S1d.** Gas chromatogram of n-octacosane ( $C_{28}H_{58}$ ) before and after degradation using *Bacillus subtilis* incubated at 50°C.



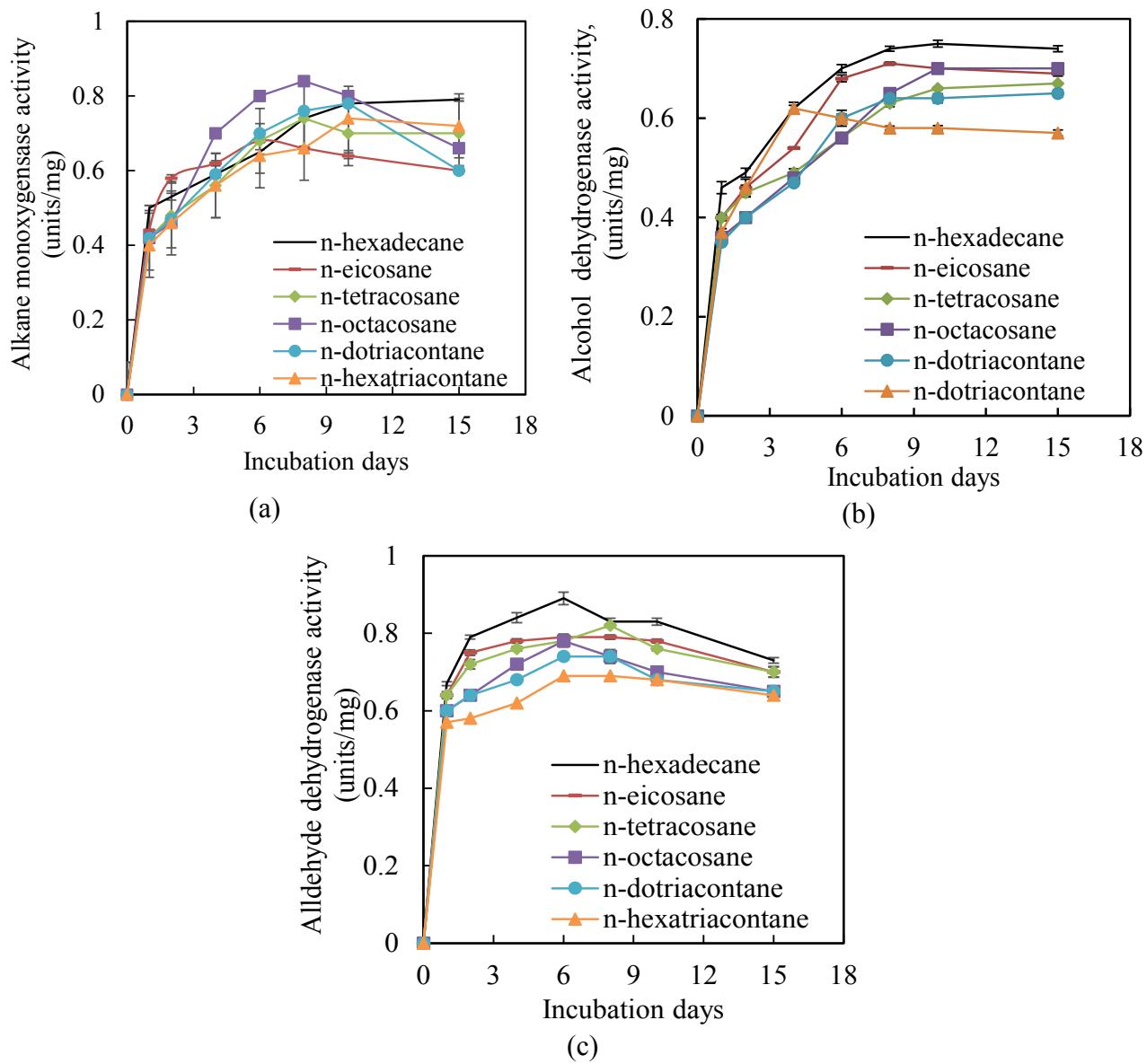
**Figure S1e.** Gas chromatogram of n-dotriacontane ( $C_{32}H_{66}$ ) before and after degradation using *Bacillus subtilis* incubated at 50°C.



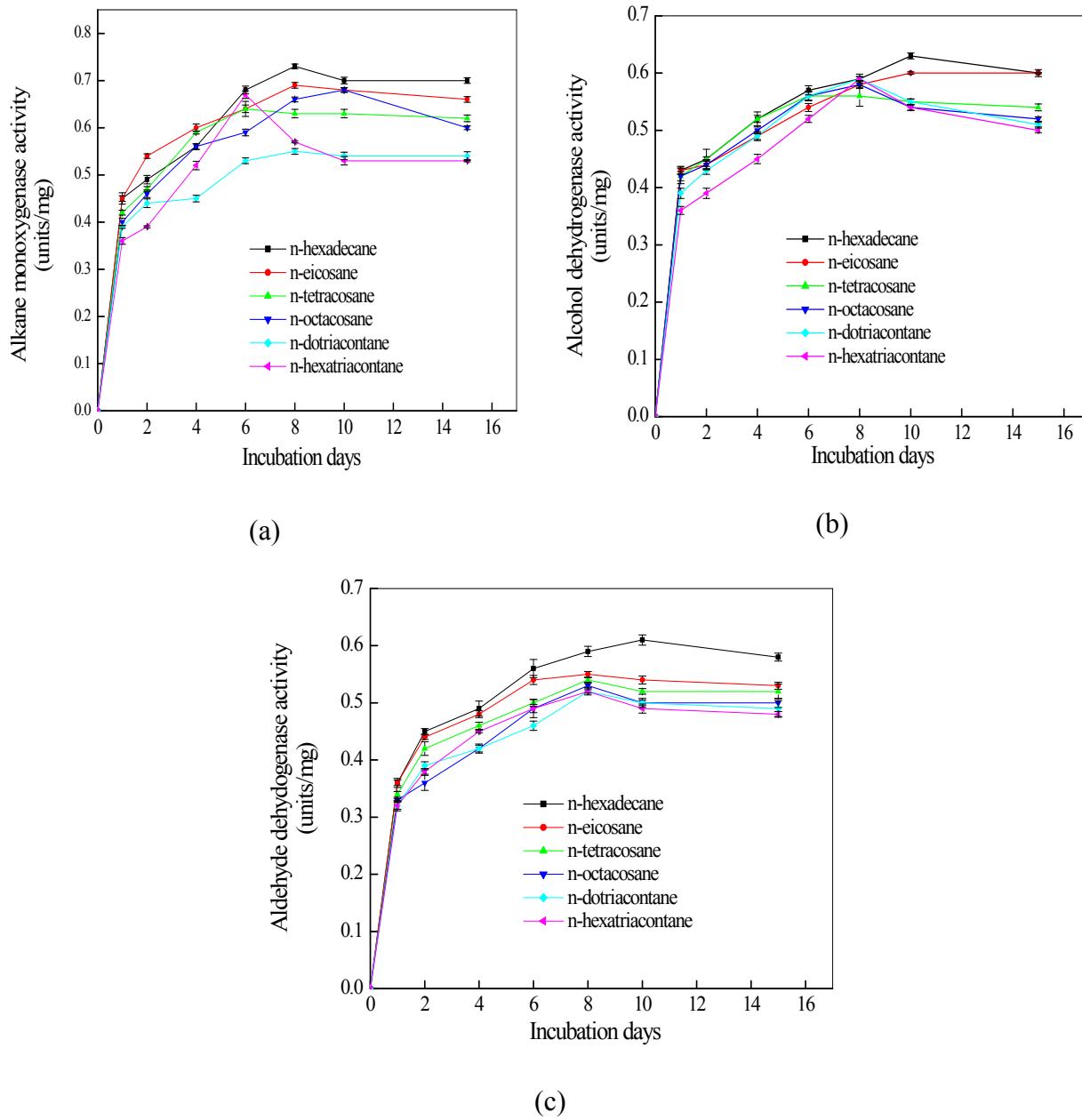
**Figure S1f.** Gas chromatogram of n-hexatriacontane ( $C_{36}H_{74}$ ) before and after degradation using *Bacillus subtilis* incubated at 50°C.



**Figure S2a.** Enzymatic activity of the hydrocarbon samples incubated at 35°C, (a) alkane monooxygenase; (b) alcohol dehydrogenase; (c) aldehyde dehydrogenase; results represent mean  $\pm$  standard deviation of three independent experiments.



**Figure S2b.** Enzymatic activity of the hydrocarbon samples incubated at 50°C, (a) alkane monooxygenase; (b) alcohol dehydrogenase; (c) aldehyde dehydrogenase; results represent mean  $\pm$  standard deviation of three independent experiments.



**Figure S2c.** Enzymatic activity of the hydrocarbon samples incubated at 75°C, (a) alkane monooxygenase; (b) alcohol dehydrogenase; (c) aldehyde dehydrogenase; results represent mean  $\pm$  standard deviation of three independent experiments.