

SUPPLEMENTAL FIGURES AND TABLES

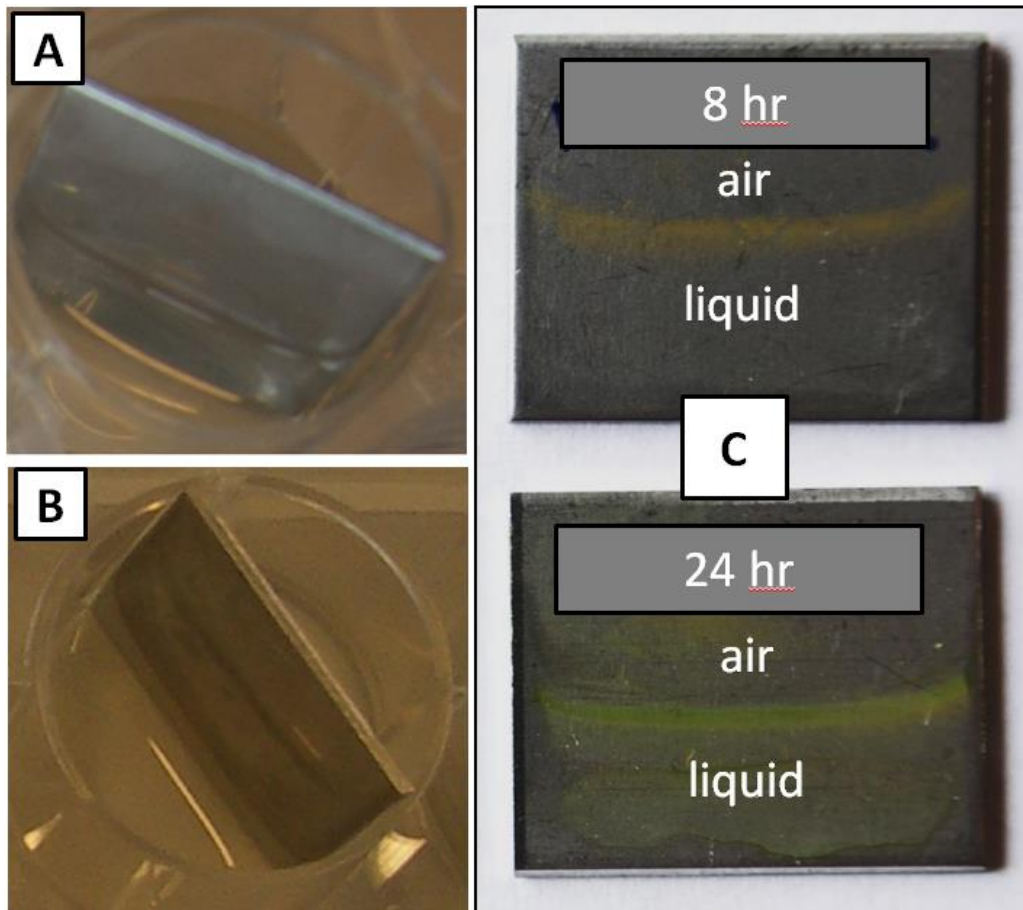
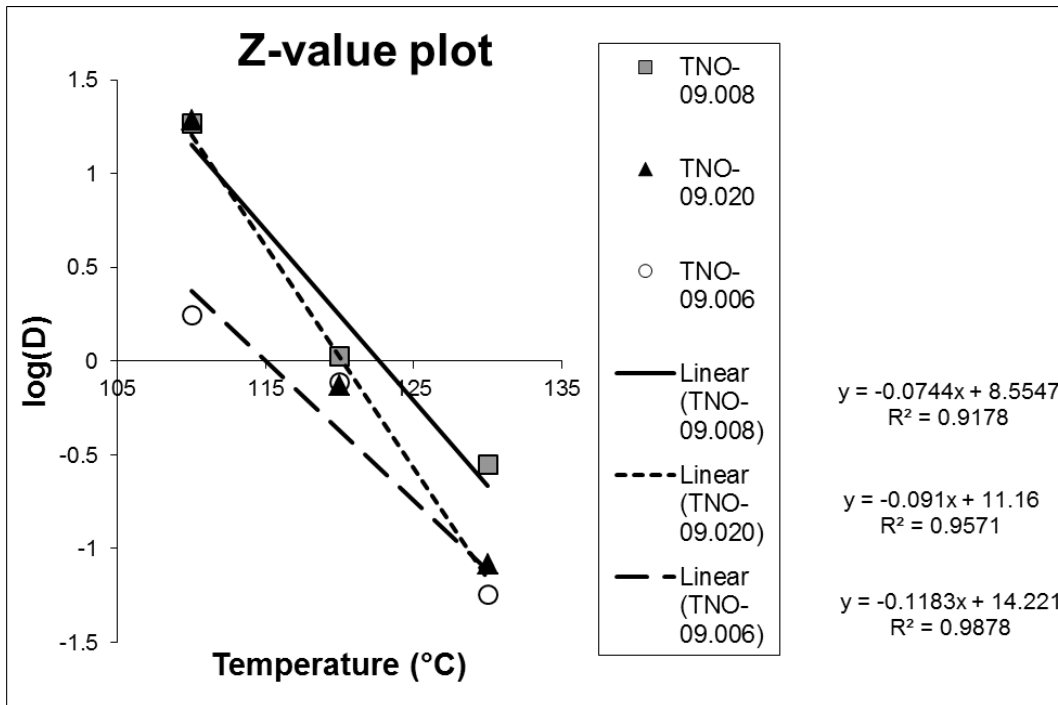


Figure S1. Standing steel biofilm model system. *Geobacillus thermoglucosidans* TNO-09.020 was cultured for 24h at 65°C. **(A)** Culture well with tryptone medium and stainless steel (SS) coupon. **(B)** Same well after 24h culturing: horizontal biofilm visible on washed SS coupon. **(C)** Auramine-stained coupons were harvested, washed and stained after 8 or 24 hours of culturing. The submerged and air-exposed parts of the coupons have been indicated (liquid air)

A)

Inactiv. Temp.	Strain	<i>Kmax</i>	<i>Nres</i>	<i>D</i>	<i>R square</i>
110 °C	TNO-09.006	1.25	3.11	2	0.9643
	TNO-09.008	0.13	1	18	0.9872
	TNO-09.020	0.12	1	19	0.8921
120 °C	TNO-09.006	3.01	2.92	0.8	0.9924
	TNO-09.008	2.19	4.03	1.1	0.9546
	TNO-09.020	3.09	5.01	0.8	0.9786
130 °C	TNO-09.006	40.34	2.08	0.06	0.9788
	TNO-09.008	8.24	1.49	0.28	0.9892
	TNO-09.020	27.88	2.22	0.08	0.9775

B)



C)

Strain	<i>slope</i>	<i>Z</i>	<i>R square</i>
TNO-09.006	-0.0744	13	0.9178
TNO-09.008	-0.091	11	0.9571
TNO-09.020	-0.1183	8	0.9878

Figure S2. Heat inactivation kinetics of spores from thermophilic strains

The heat inactivation kinetics of spores isolated in this study indicated by **(A)** D-values and other inactivation equation parameters, **(B)** linear logD/temperature equations, and **(C)** their slopes and Z-values. The D-values of the spore batches were derived from plots with log CFU versus incubation time by fitting the following log-linear model with tail to the data by using Excel add-in GInaFiT:

$$\log N_t = (\log N_0 - \log N_{res}) * \exp(-k_{max} * t) + \log N_{res}$$

In which $\log N_t$ is the spore count at a certain time point, $\log N_0$ is the spore count at $t=0$, $\log N_{res}$ is the number of spores in the tail and k_{max} is the inactivation constant. The D-value was derived by $D = \ln(10)/k_{max}$. In case of no tailing ($\log N_{res} = 0$), the model was reduced to a log-linear inactivation model. The z-values were calculated by plotting the logD value against the temperature and performing a linear regression. The z-value was calculated by $z = -1/\text{slope}$.

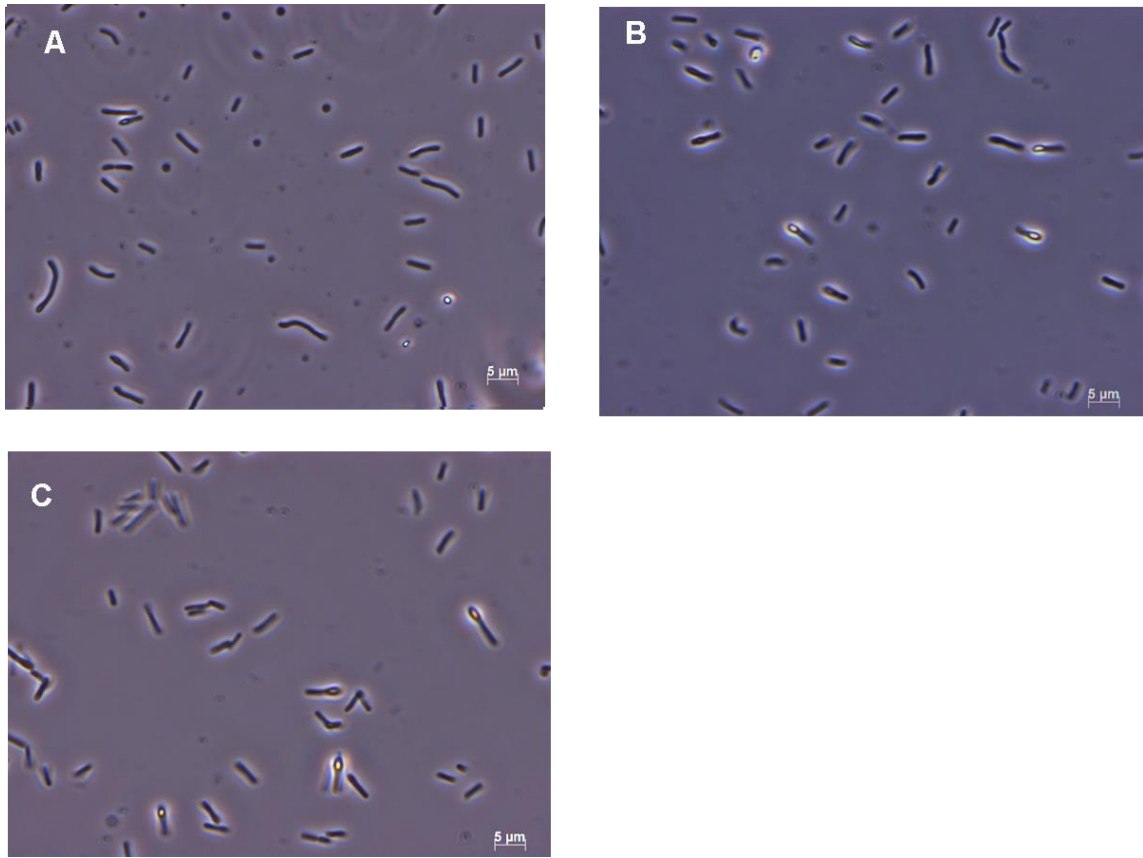


Figure S3. Phase-contrast micrographs of thermophilic sporeformers. Planktonic cells and spores of the 3 thermophilic spore forming isolates. **A)** *Anoxybacillus flavithermus* TNO-09.006; **B)** *Geobacillus stearothermophilus* TNO-09.008; **C)** *Geobacillus thermoglucosidans* TNO-09.020.

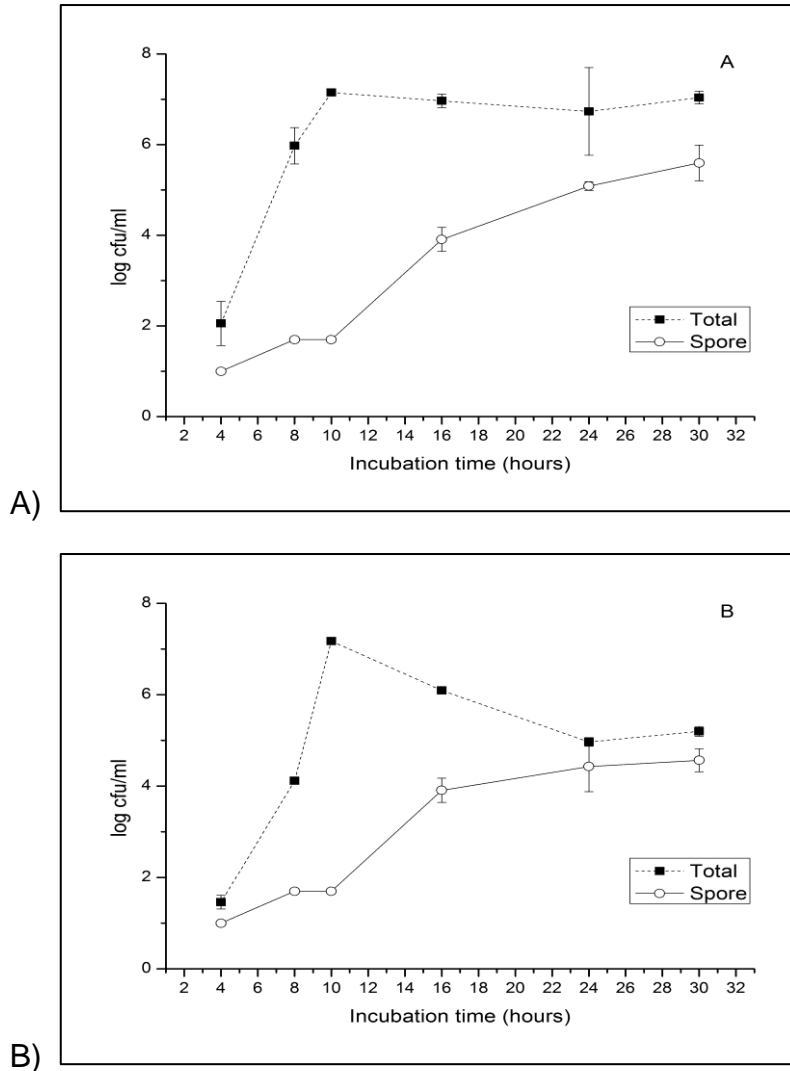


Figure S4. Colony forming units of *Geobacillus thermoglucosidans* TNO-09.020 in the standing steel biofilm system.

Graphical representation of bacterial counts development (CFU/ml) in the static standing steel biofilm system with 1% Tryptone at 60 °C. The 2 panels represent **(A)** the planktonic cells from the medium and **(B)** biofilm cells from the steel coupon. The total CFUs were determined, vegetative cells and spores (■), and for the same samples treated for 30 min at 100 °C, allowing spore counts (○). Means and standard deviations from 3 replicate static culture systems are shown.

Sample description	Id	Temperature in factory	Concentration or volume	Log(CFU/ml)		
				Total 30 °C	Total 55 °C	Spore 55 °C
M1 (Standard milk)	M1	4 °C	undiluted	7.0	3.0	2.0
M2 (Standard milk)	M2	4 °C	undiluted	4.5	3.3	1.1
Pipeline 1	i01	75 °C	0.07 g/ml	8.7	5.0	4.0
Pipeline 2	i02	75 °C	1.5 ml	8.7	4.0	4.0
Pipeline 3	i03	75 °C	0.33 g/ml	6.0	5.0	4.0
Pipeline 4	i05	75 °C	1.5 ml	2.0	4.0	3.0
Evaporator 1	i06	65 °C	1.0 g/ml	8.0	4.0	2.0
Evaporator 2	i07	65 °C	1.0 g/ml	6.1	3.4	3.0
Pasteur	i08	68 °C	0.90 g/ml	6.0	3.0	3.0
Tank	i09	45 °C	1.0 g/ml	8.0	4.0	3.0
Tankfilter	i10	37 - 45 °C	1.0 g/ml	7.0	3.0	2.0
Drying tower	i11	60 °C	0.15 g/ml	7.0	3.0	2.0
Dairy concentrates	i14	20 - 60 °C	0.10 g/ml	2.1	2.2	<1.8
Dairy concentrates	i15	20 - 60 °C	0.10 g/ml	<2.1	1.8	1.8

Table S1. Colony forming units of dairy factory samples used in this study.

Medium	Temperature	Fraction	Log(cfu) / Fraction
M2 (standard milk)	55°C	medium	5.7
		steel	5.4
		well	5.5
	65°C	medium	3.3
		steel	1.6
		well	2.1
M3 (Standard milk)	55°C	medium	5.4
		steel	5.5
		well	5.6
	65°C	medium	6.3
		steel	4.8
		well	5.8
Evaporator (i7)	55°C	medium	7.0
		steel	5.5
		well	5.4
	65°C	medium	5.7
		steel	4.2
		well	4.7

Table S2. Colony forming units of enrichments at 55°C and 65°C.

Standard milk (M1 and M2) or sterile milk inoculated with industrial fouling from a whey evaporator (i07) was cultured O/N at 55 or 65°C in plastic culture wells containing standing steel coupons (static biofilm model). The table shows the CFUs in the different fractions in the biofilm system: fraction in the medium (2 ml), on the stainless steel surface, and attached on the plastic well.