Supplementary Material for:

Implications of limited thermophilicity of reduction of nitrite for control of sulfide production in oil reservoirs

By:

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Table S1. Physico-chemical data for samples of injection water (14IW), produced water (18PW) or source water (also referred to as makeup water) 22SW, collected from the MHGC field. Data are averages for 6 samples.

Sample ID	**pH**	**NaCl (M)**	**Sulfide (mM)**	**Sulfate (mM)**	**Nitrate (mM)**	**NH_4^+ (mM)**	**Acetate (mM)**
MHGC_14IW	7.75	0.11	0.03	0.06	0.38	0.41	ND
MHGC_18PW	7.84	0.12	0.05	0.0	0.0	0.47	ND
MHGC_22SW	7.19	0	0.0	3.4	0.92	0.36	ND

Table S2. Comparison of the *nirK* or *nirS* sequence of pure isolates with nitrite reductase sequence from NCBI database. The sequences were obtained from the PCR product after amplification of *nirK* with GnirK2F primer and *nirS* with NirS2F primer.

Strain	**Genbank Accession #**	**Nucleotide blasted (bases)**	**Identity**	**E-value**	**Sequence identification**
G. stearothermophilus DSM22	KX139464	375	366/366(100%)	0	*G. stearothermophilus* partial *nirK*
G. thermodenitrificans DSM465	KX139465	375	366/366(100%)	0	*G. thermodenitrificans* partial *nirK*
G. toebii DSM14590	KX139466	375	366/366(100%)	0	*G. toebii* partial *nirK*
G. thermocatenulatus DSM730	KX139467	375	343/366(94%)	$1e^{-154}$	*G. thermodenitrificans* partial *nirK*
G. subterraneus DSM13552	KX139468	375	363/366(99%)	0	*G. thermodenitrificans* partial *nirK*
Thauera sp. TK001	Not issued*	96	22/24(92%)	$4e^{-6}$	*Thauera phenylacetica* *nirS* partial sequence

*Sequence was too short to be issued an accession number:
AAGGAAACCGGCATGGTGTATGTCGGTGGATTATCGCGACCTCGACAACCTCAAGCTCAAGATGATCGAGGCGGCACCCTTCCTGCACGA CGGCGG
Results are for tblastx search of translated protein sequence of 24 aa.

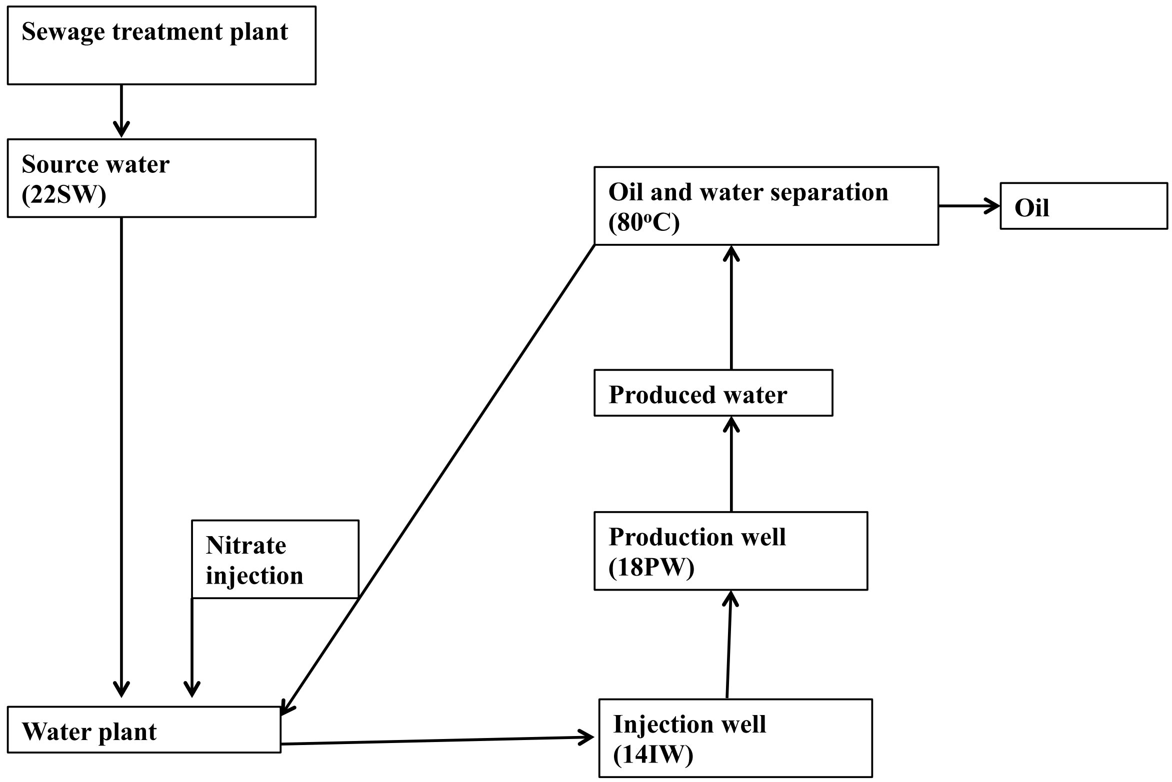


FIG. S1. Schematic of the MHGC field indicating source water, as well as injection and production wells, as explained in more detail elsewhere (Voordouw et al. 2009, *Environ. Sci. Technol.* 43 (24), pp 9512–9518, DOI: 10.1021/es902211j). Note that in the water plant source water (also referred to as makeup water) and produced water are combined to injection water, which is distributed to multiple injection wells throughout the field. Source water (makeup water) is needed to replace the volume of oil produced, i.e. without its the pressure differential in the field, which moves oil and water, would fall. Sampling sites for source water (22SW), injection water (14IW) and produced water (18PW) are indicated. Temperatures of these samples were between 10^{o}C and 30^{o}C at the time of collection.

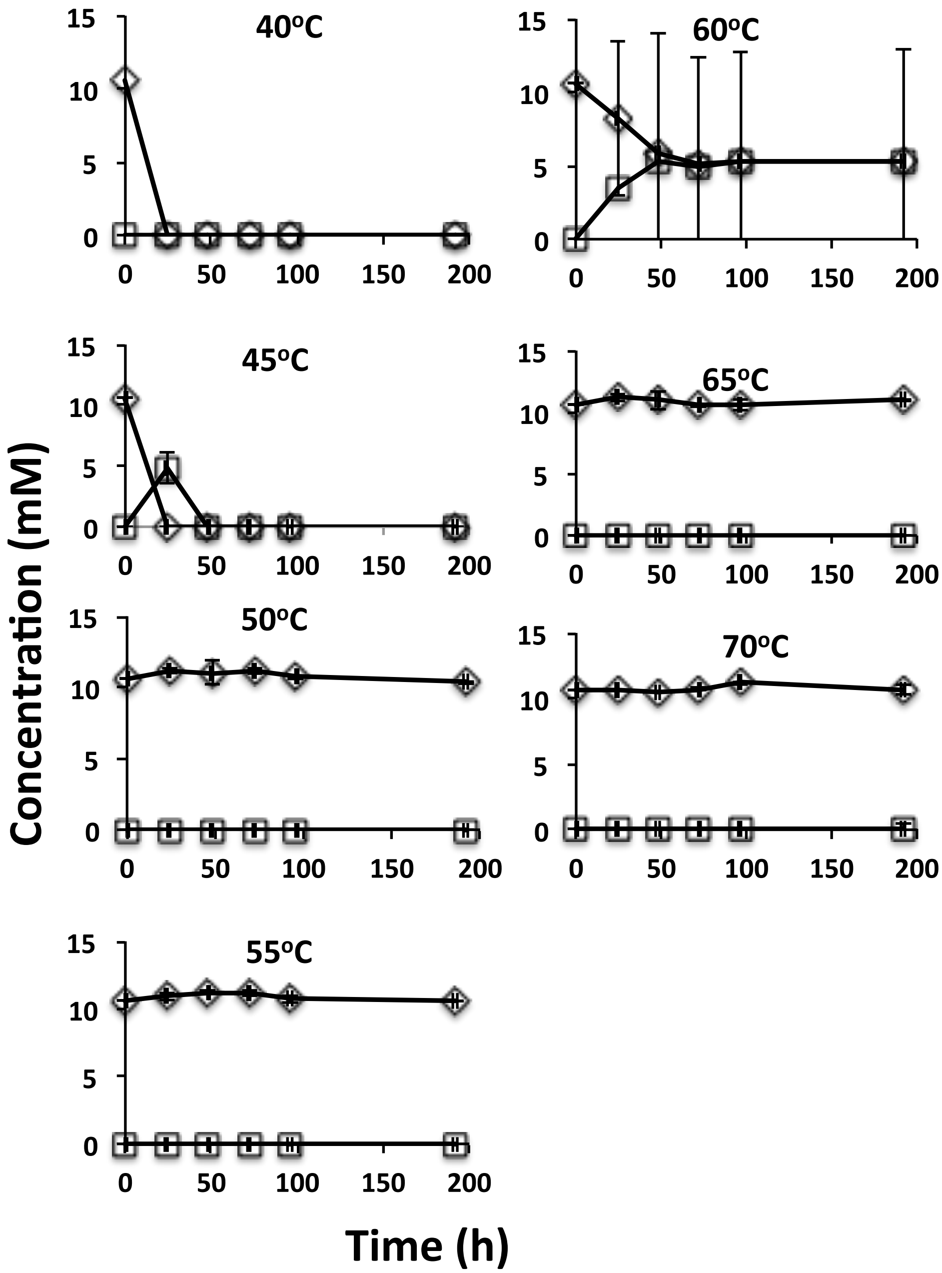


FIG. S2. Reduction of nitrate (◇) to nitrite (□) with VFA as electron donor by concentrated 14IW consortia at different temperatures. Data shown are averages of duplicate incubations. No reduction of nitrate was seen at 40 or 70°C in the absence of inoculation.

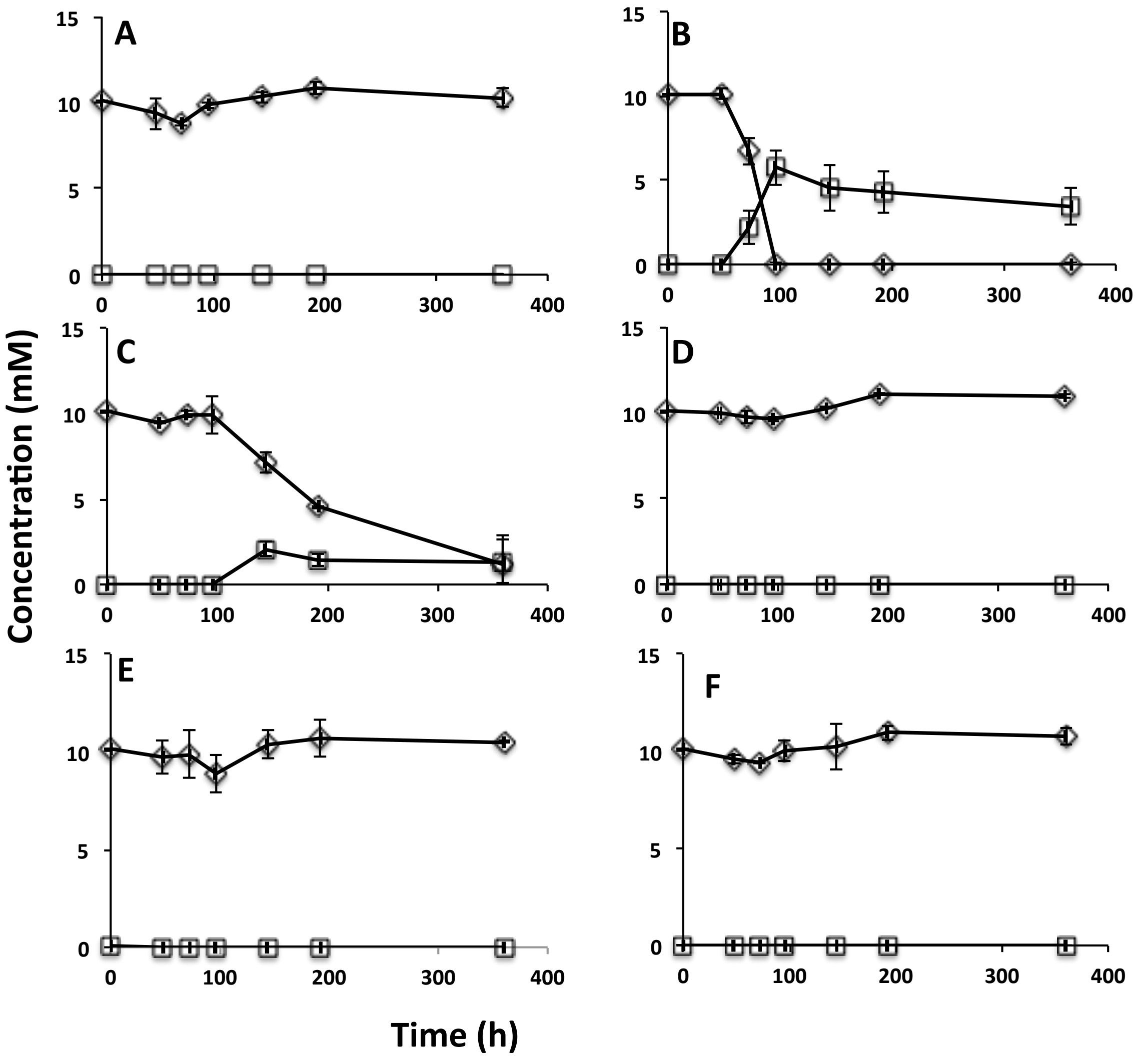


FIG. S3. Reduction of nitrate (◇) and formation of nitrite (□) by 18PW samples incubated at 40°C using BTEX compounds, dissolved in HMN, as electron donor and nitrate as the electron acceptor. The BTEX compounds were benzene (A), toluene (B), ethylbenzene (C), *m*-xylene (D), *o*-xylene (E) and *p*-xylene (F). In (B) reduction of 10 mM nitrate to 5 mM nitrite and 2.5 mM N_2 required 10 + 25 = 35 mM of electrons, which required oxidation of 0.97 mM toluene to CO_2. Since 28 mM toluene in 0.5 ml HMN was provided in a total volume (HMN plus aqueous phase) of 19.5 ml, the overall concentration of toluene was 28*0.5/19.5 = 0.72 mM. Hence, the partial reduction of nitrate is consistent with a limiting concentration of electron donor. In (C) reduction of 9 mM nitrate to 1 mM nitrite and 4 mM N_2 required 2 + 40 = 42 mM of electrons, which required oxidation of 1 mM mM ethylbenzene to CO_2. Since the overall concentration of ethylbenzene was 24*0.5/19.5 = 0.62 mM, the partial reduction of nitrate is consistent with a limiting concentration of electron donor. Note that instead of N_2, either NO or N_2O could also be products.

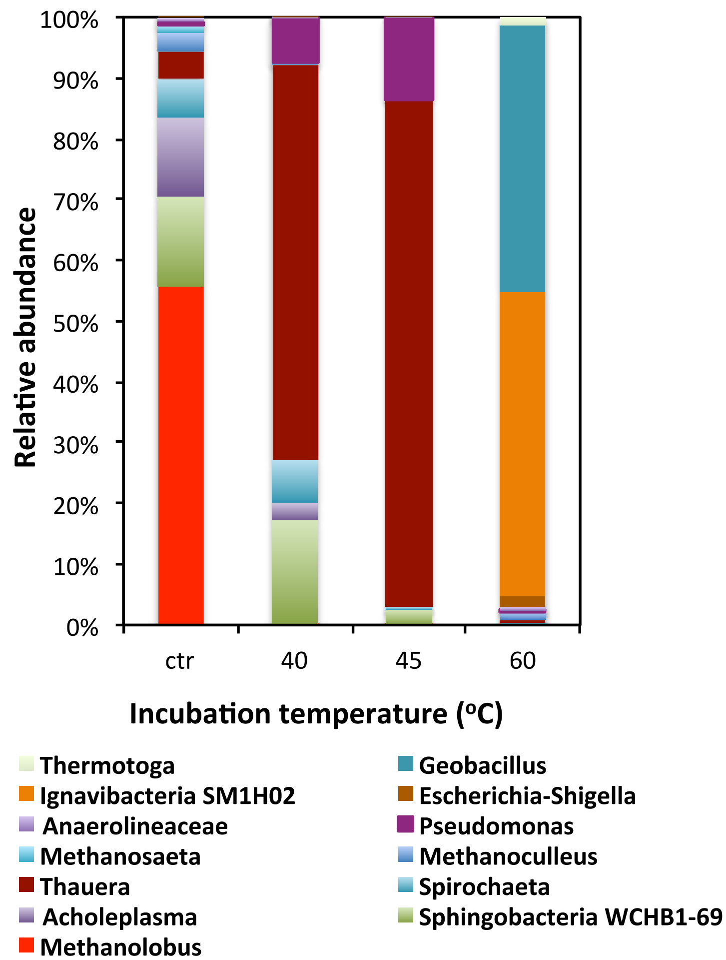


FIG. S4. Microbial community composition of 14IW incubated in CSBK medium with nitrate and VFA at different temperatures at the genus level. The community composition of 14IW prior to incubation is also shown (Ctr).

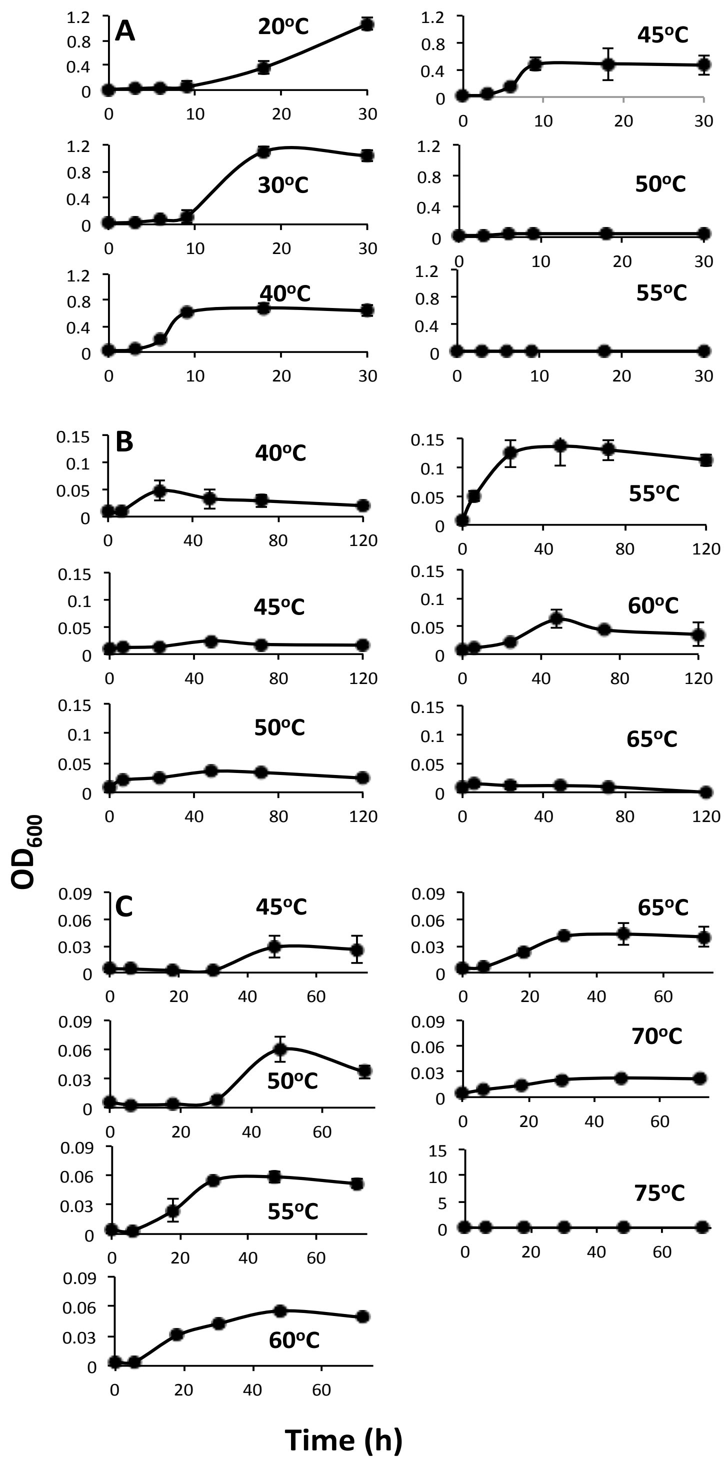
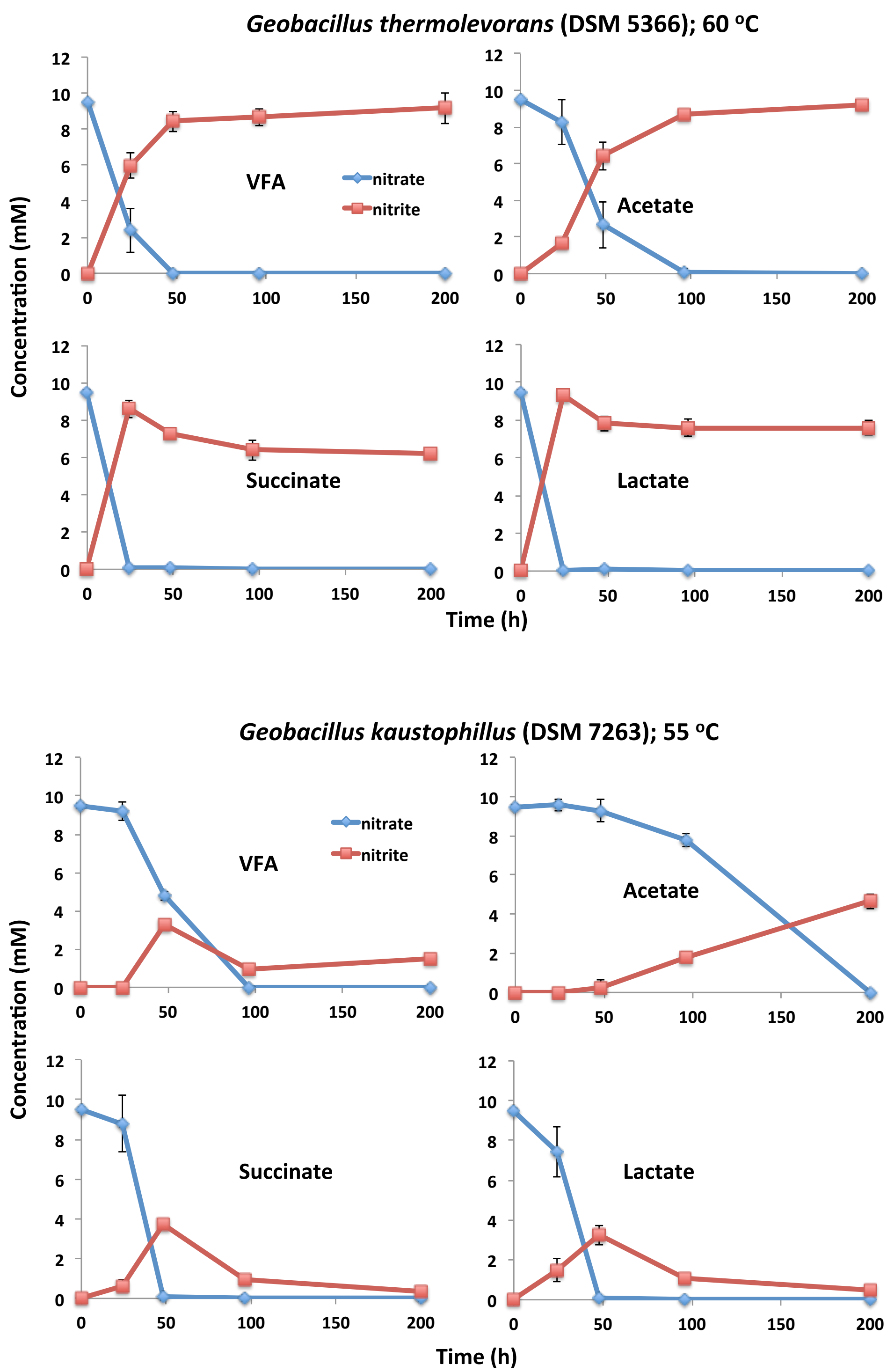
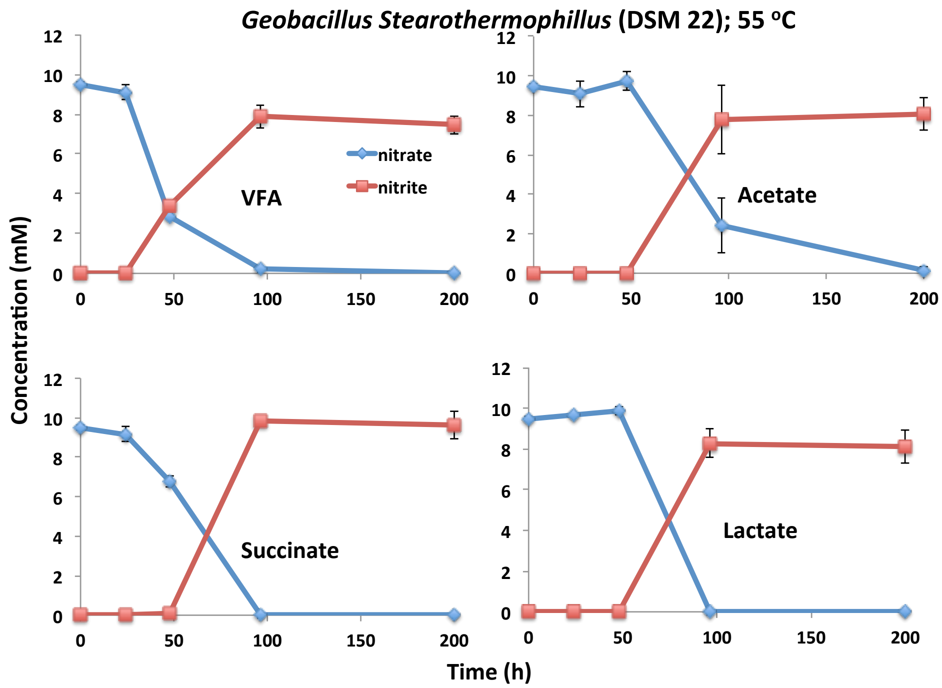


FIG. S5. Temperature dependence of growth (OD_{600}) of (A) *Thauera* sp. TK001, (B) *Petrobacter* sp. TK002 and (C) *Geobacillus* sp. TK003 in CSBK medium with 10 mM nitrate and 3 mM VFA. Data are averages of duplicate incubations. Data for reduction of nitrate to nitrite of these cultures are presented in Fig. 3.



Geobacillus thermolevorans (DSM 5366); 60 °C
Concentration (mM)
VFA
nitrate
nitrite
Acetate
Succinate
Lactate
Time (h)
Geobacillus kaustophillus (DSM 7263); 55 °C
Concentration (mM)
VFA
nitrate
nitrite
Acetate
Succinate
Lactate
Time (h)

FIG. S6.



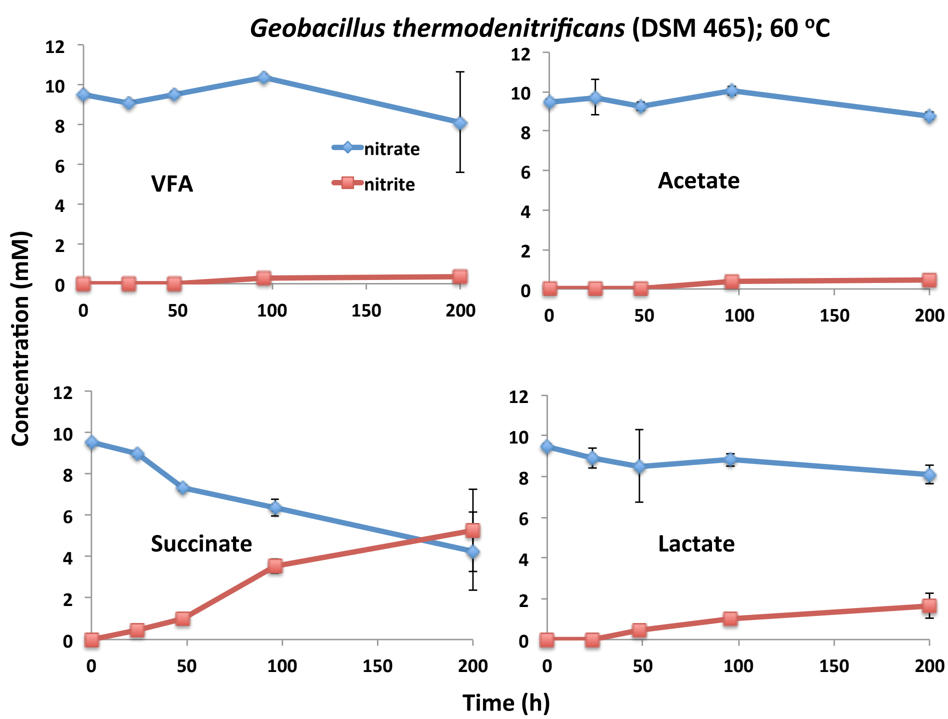
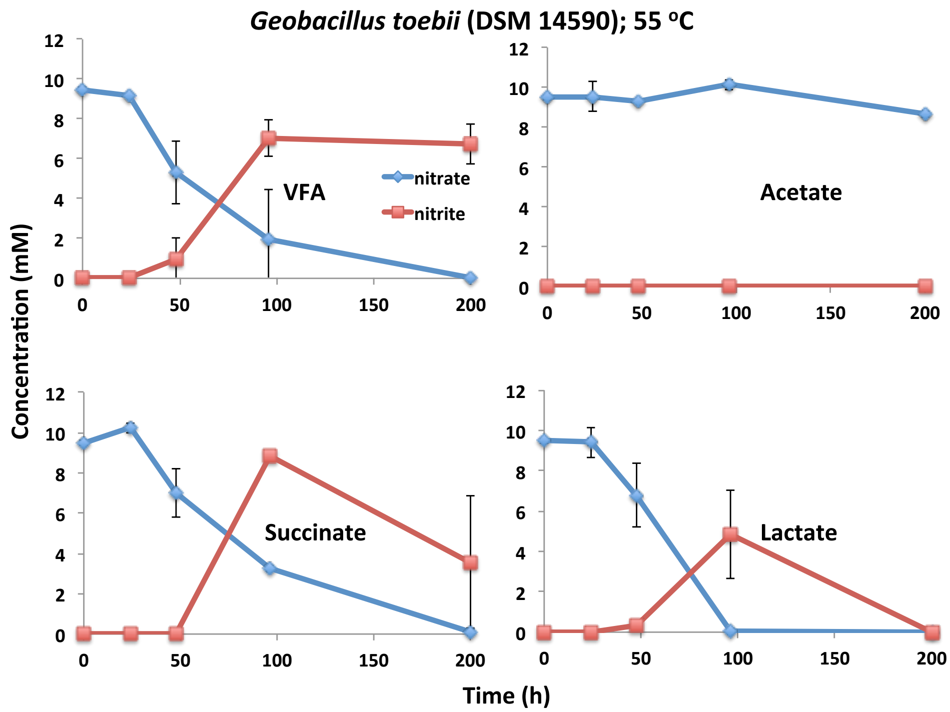


FIG. S6. **(continued)**



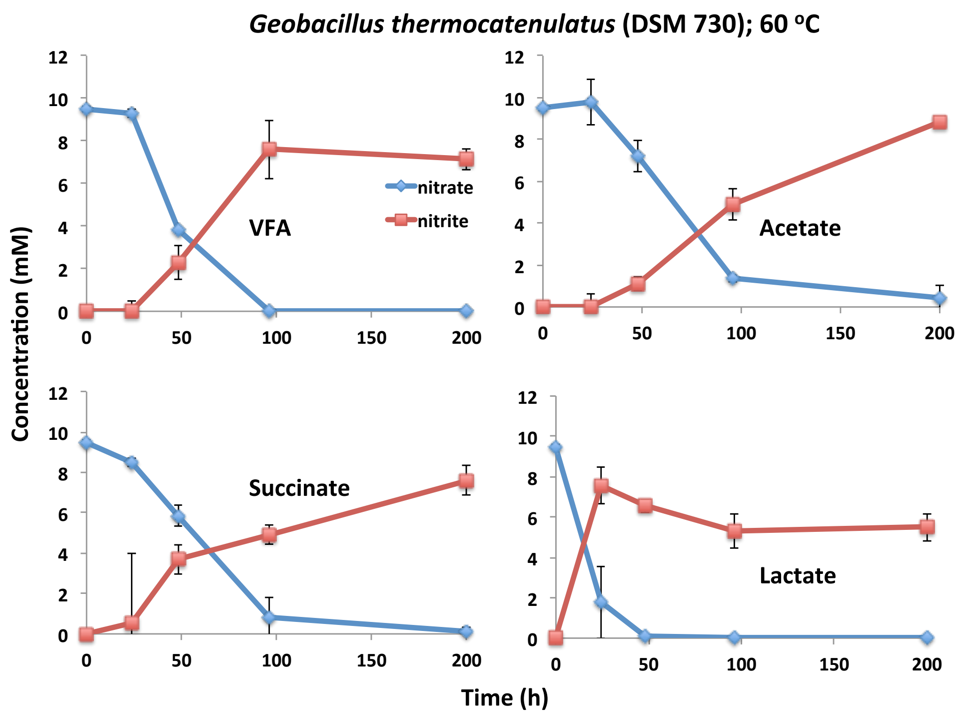


FIG. S6. **(continued)**

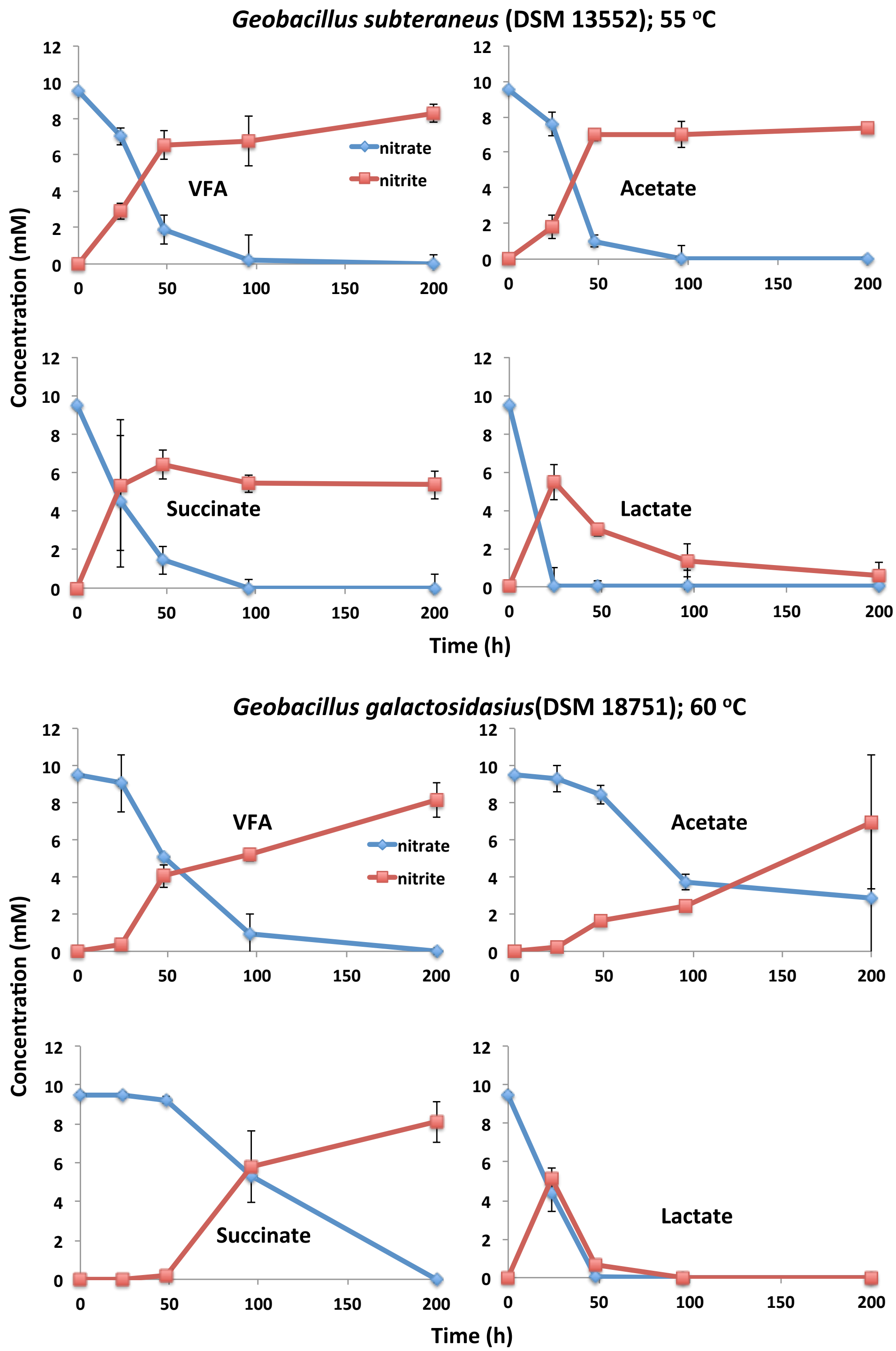


FIG. S6. **(continued)**

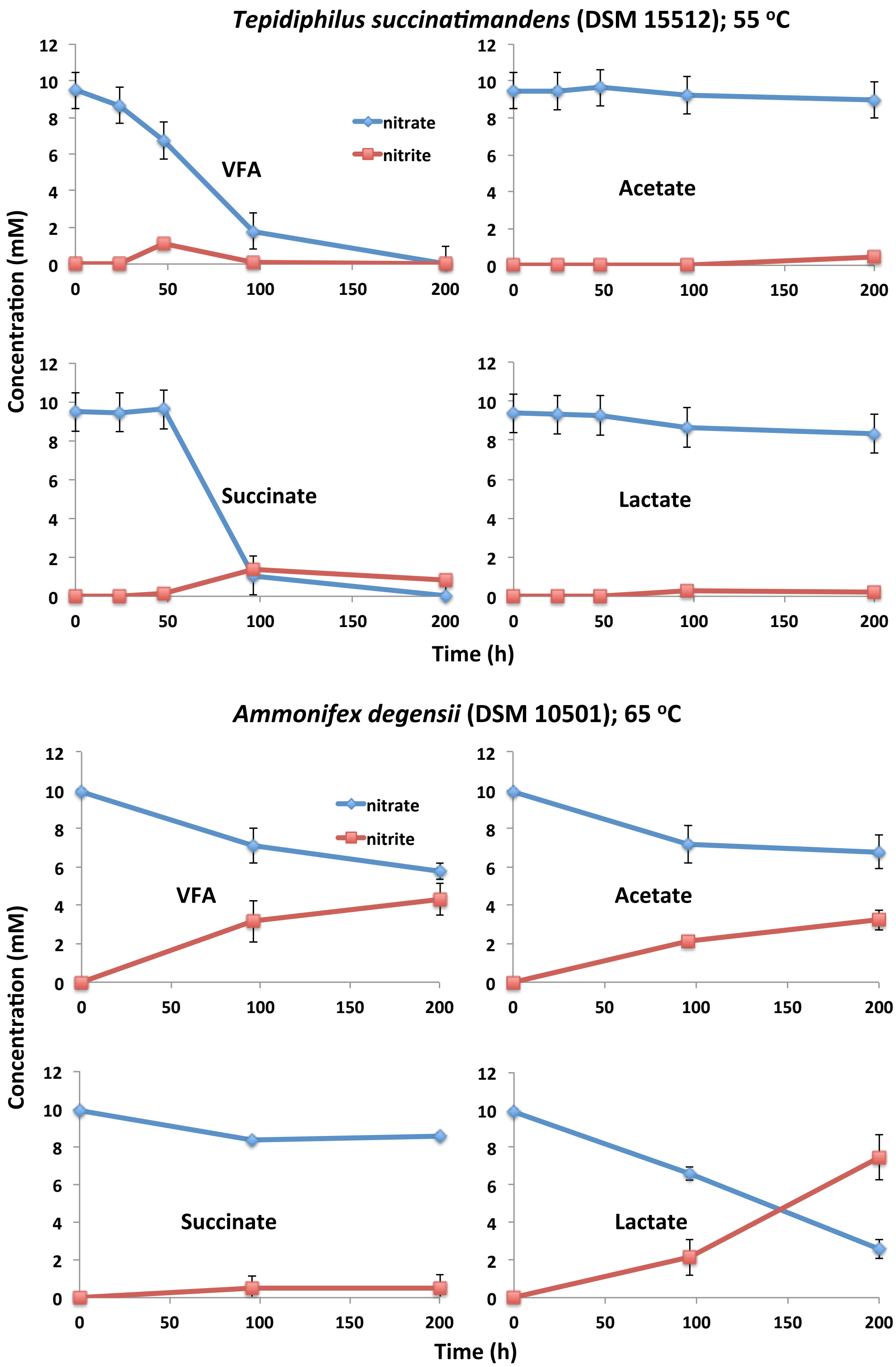


FIG. S6. **(continued)**

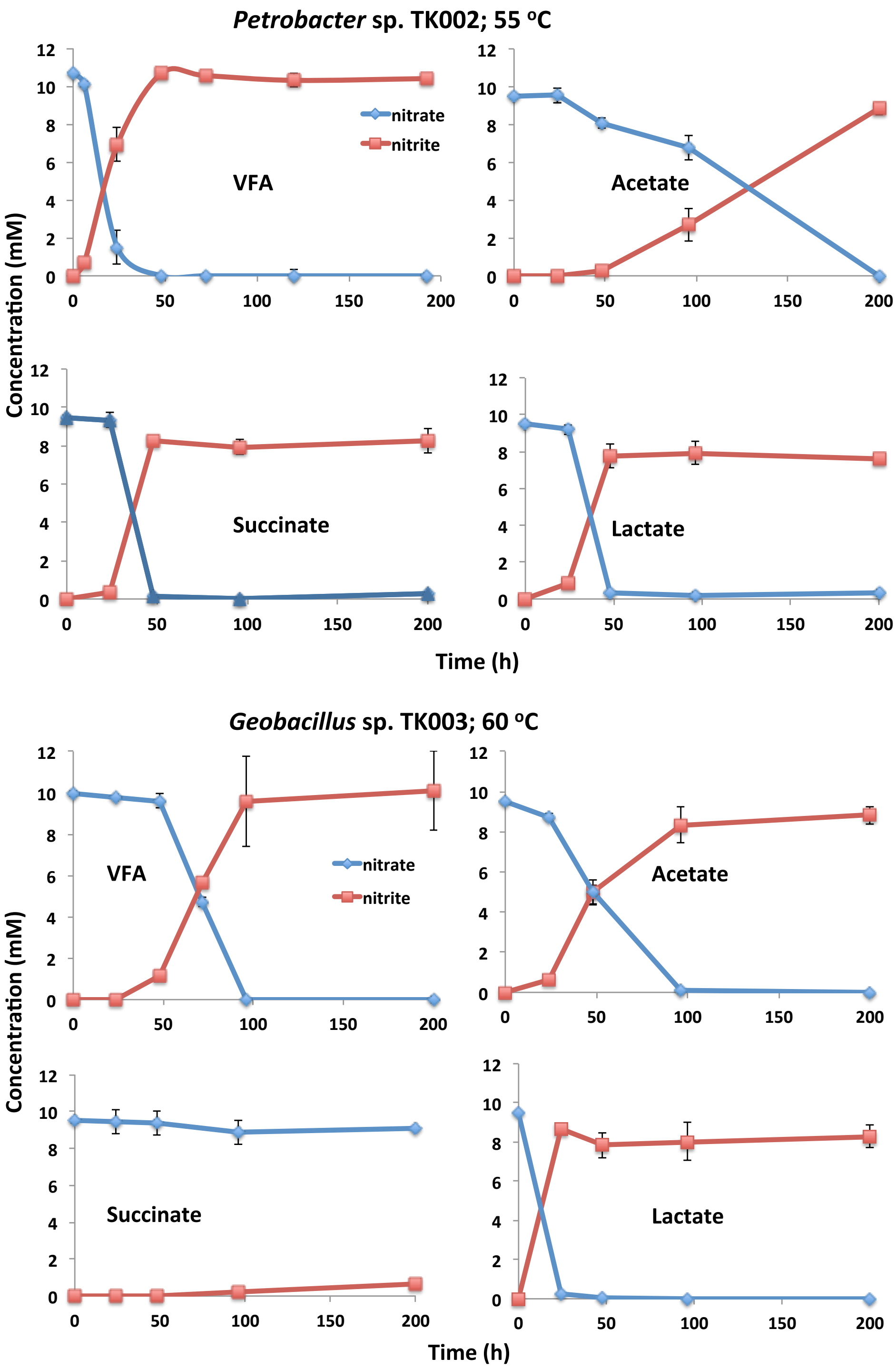


FIG. S6. Incubation of deposited tNRB cultures, obtained from the DSMZ, as well as of TK002 and TK003 in CSBK medium with nitrate and either VFA, acetate, succinate of lactate at the optimum growth temperature as indicated. Data shown are averages of duplicate incubations.