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Calorimetric studies of the growth of *Desulfovibrio desulfuricans* in the presence of nitrocellulose

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Abstract

Calorimetric results indicate that nitrocellulose (NC)-induced changes in the metabolism of *Desulfovibrio desulfuricans* 1388 are caused by both chemical (nitrate) and physical (biofilm formation) factors. Nitrate added to lactate-based culture medium with nitrocellulose competed for the electron flux from lactate and suppressed the bacterial sulfidogenesis and growth. The presence of an insoluble compound (carbon backbone of the polymer) induced the creation of a biofilm-like structure with its own metabolism.

Keywords: *Desulfovibrio desulfuricans*; Nitrocellulose; Heat production; Sulfate-reducing bacteria

1. Introduction

The stable synthetic polymer nitrocellulose (NC) (11.8% N) undergoes transformation by anaerobic *Desulfovibrio* cells in the late growth phase [1,2]. *Desulfovibrio desulfuricans* 1388 nitroetherase activity produces nitrate in culture medium. NMR and FTIR spectroscopy [3,4] revealed that unsubstituted glucopyranose fragments were present in the polymer molecules. It was also shown that nitrate was reduced to ammonium by nitrate- and nitrite reductases [3] and that NC in turn suppressed the growth of *D. desulfuricans* 1388 [2]. Freedman et al. [5] described the adverse effect of NC on sulfidogenesis of a sulfate-reducing mixed culture. It was important to examine the cause of this phenomenon, but it is difficult to measure the overall metabolic rate in anaerobic processes, except by calorimetry, which can be used to obtain the peak heat production (HP) rate as an index of the energetic state of cells. The overall aim of this study was to investigate the growth of *D. desulfuricans* 1388 with NC by calorimetry in combination with biochemical and enzymatic data to assess bacterial metabolism under various environmental conditions. Any changes in culture medium causing an alteration in the balance between catabolic and anabolic processes is reflected in HP [6,7,8]. The appropriate environmental conditions in the calorimeter, i.e. in this case, anaerobiosis and temperature regime, allows the direct measurement of HP during bacterial growth.

2. Materials and methods

D. desulfuricans 1388 strain (VKM 1388) was donated by the Russian Collection of Microorganisms (Pushchino, Russia). The composition of Postgate B culture medium containing 32 mM calcium lactate and protocol for anaerobic cultivation of the cells in batch culture were described earlier [1]. For the different sets of experiments, 10 g/l NC (11.8% N), 10 g/l cellulose or 0.12 mM nitrate was added to the culture medium. Bacterial growth in Postgate B medium with only lactate was used as the control.

Protein was determined according to a modified Lowry's method [9] using bovine serum albumin (BSA) as standard. The growth characteristics were evaluated according to Pirt [10]. Hydrogen sulfide was determined by the method of Truper and Schlegel [11]. Lactate was determined by a modified enzymatic method with lactate dehydrogenase [12].

The peak HP was estimated using an LKB batch, a heat conduction calorimeter (Biological Activity Monitor BAM; successors to LKB are Thermometric AB, Järfälla, Sweden) at 30 °C [13]. It was electrically calibrated at the beginning of each day. Three millilitre of inoculated culture medium (with NC or nitrate

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Table 1
Growth characteristics and sulfide production of *D. desulfuricans* 1388 in Postgate B medium in the presence of nitrate, NC or cellulose

Growth conditions	Growth rate (h^{-1})	Growth yield (g/mol)	Catabolic activity (mmol/g h)	Sulfide production (mM)
Control	0.074 ± 0.003	6.7 ± 0.2	11.0	11.2 ± 0.3
Nitrate	0.060 ± 0.001	5.9 ± 0.0	9.8	9.0 ± 0.2
NC	0.057 ± 0.002	5.7 ± 0.2	10.0	8.7 ± 0.2
Cellulose	0.071 ± 0.001	6.5 ± 0.1	10.7	–

or cellulose) was placed in sterile 3-ml glass vials and bubbled with argon that was sterilized by passage through 0.45 μm-pore filter. A control vial contained 3 ml of inoculated culture medium without any additions. The vials were hermetically sealed. The thermal equilibration time for the vials was 15–20 min.

HP in each treatment condition was evaluated in three independent experiments.

P-values were calculated using Student's *t*-test, $\alpha = 0.05$.

3. Results and discussion

The thermal power–time curves for *D. desulfuricans* 1388 grown in the absence/presence of NC are shown in Fig. 1. Three peaks (39 μW at 13.5 h, 71 μW at 18 h and 95 μW at 22 h) are in the curve for cells without NC. The curve with NC has only one peak (90 μW at 20 h). At the highest HP, the residual concentration of lactate were 10.5 ± 0.3 mM in control and 18.5 ± 0.57 mM with NC. Hence the decrease in HP following the peak was not caused by a limitation of the energy source.

After 22 h, the curve of the control returned to the baseline at the same time as the exhaustion of the lactate (Fig. 1). In the presence of NC, HP reached a constant level indicating that some metabolic activity remained despite lactate exhaustion.

The greater specific HP in the presence of NC (9.8 ± 0.08 J/mg protein as compared to the control value of 8.1 ± 0.05 J/mg protein) and the decrease of the growth yield (Table 1) probably reflect a change in the anabolic/catabolic balance in the cells. This is surprising because NC has always been considered to be biologically inert. It seems possible that either the appearance of nitrates in the culture medium or the carbon backbone of the polymer as an insoluble compound influenced cellular metabolism. Reasons for the observed changes in HP were studied in the following experiments.

Earlier it was shown that the growth and sulfidogenesis of some sulfate-reducing bacteria are inhibited by nitrate [14,15]. Free nitrate adsorbed by NC during chemical synthesis of polymer was injected into the culture medium. It was found that the addition of 10 g/l NC produced 0.12 mM free nitrate. Therefore, this concentration was chosen for studying the influence of nitrate on bacterial thermogenesis in the next series of experiments. The power–time curve of *D. desulfuricans* 1388 growing in Postgate B medium with 0.12 mM of nitrate is shown in Fig. 2. The shape of the curve after addition of nitrate was similar to the control except there was no peak at 13.5 h. Nitrate, like NC, increased the specific HP (10.1 ± 0.3 J/mg protein). The residual lactate concentration at the highest HP was 17.8 ± 0.37 mM and was close to the corresponding value from the experiments with NC. The growth characteristics of *D. desulfuricans* 1388 cultivated in different environmental conditions are shown in Table 1.

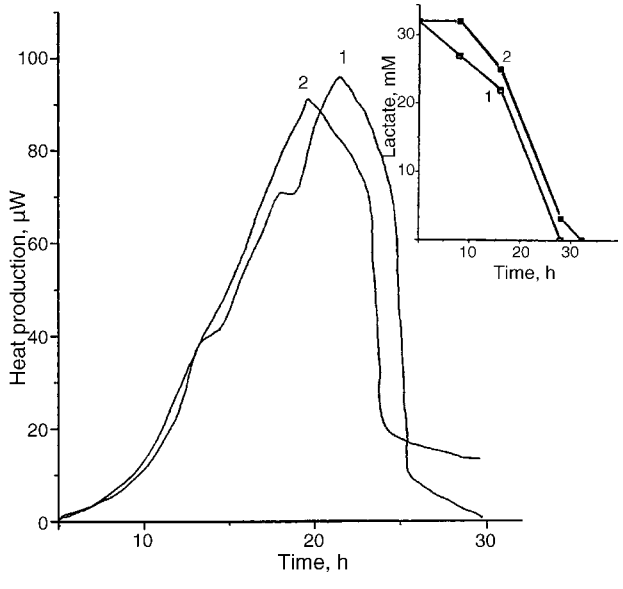

Fig. 1. Thermal growth profiles of *D. desulfuricans* 1388 cultured in Postgate B medium in the absence (1) or the presence (2) of nitrocellulose (10 g/l). *Inset*: lactate consumption by *D. desulfuricans* in the absence (1) or the presence (2) of nitrocellulose.

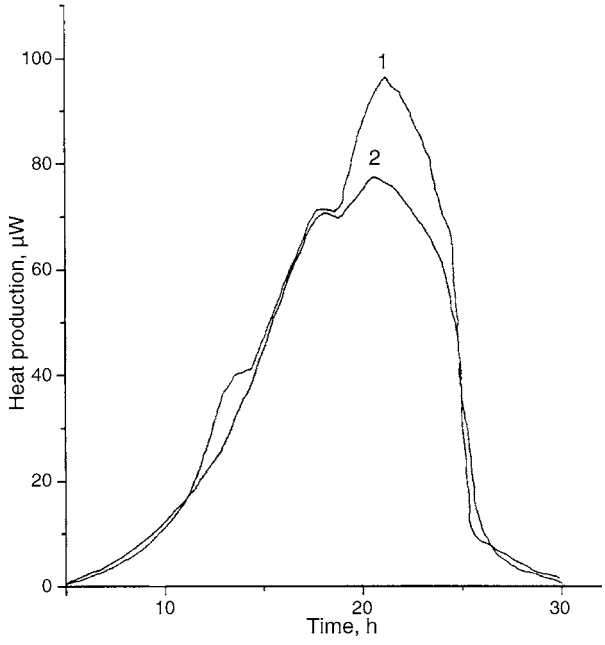

Fig. 2. Thermal growth profiles of *D. desulfuricans* 1388 cultured in Postgate B medium in the absence (1) or the presence (2) of nitrate (0.12 mM).

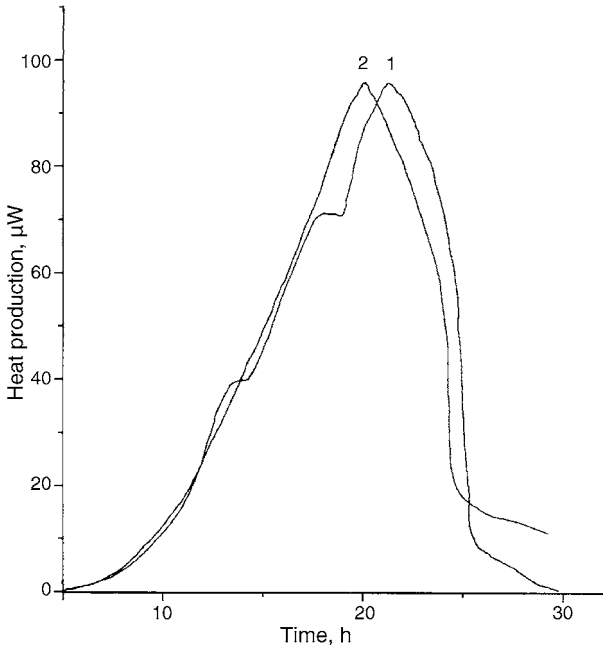

Fig. 3. Thermal growth profiles of *D. desulfuricans* 1388 cultured in Postgate B medium in the absence (1) or the presence (2) of cellulose (10 g/l).

From the fact that the growth characteristics in the experiments with NC and free nitrate in terms of growth rates; catabolic activities and growth yields are similar, it can be concluded that nitrate is one of the factors that caused the observed changes in bacterial HP in the presence of NC.

The mechanism of NC inhibition of bacterial growth is not known. The reduction of free nitrate by nitrate reductase leads to production of nitrite that is a competitive inhibitor for disulfite reductase in sulfate-reducing bacteria [16]. This leads to the suggestion that, in the presence of NC or nitrate, an electron flux from lactate was distributed between two electron acceptors—sulfate and nitrate. Although nitrate is considered as the more preferable electron acceptor for cell extracts of sulfate-reducing bacteria [17], its reduction in vivo is an antioxidant protective process rather than an energetic one. It is probable that the presence of free nitrate (or NC) in the culture medium led to insufficient energy being available for biosynthesis. This phenomenon is currently under investigation.

Nitrate is capable of affecting bacterial metabolism and consequently thermogenesis of *D. desulfuricans* 1388, but its presence does not account for all the changes in the thermal profiles, i.e. the absence of some peaks in the curves and the time shift of the curve with NC compared to the control. The insoluble cellulose backbone of the polymer could be another factor affecting the bacterial thermogenesis because the metabolism of microorganisms attached to a firm surface differs from that of planktonic microorganisms [18]. We established that *D. desulfuricans* 1388 excreted the polysccharides and formed the biofilm-type structure in the presence of NC [19]. Model experiments were performed in which cellulose was added to the culture medium. No effect was found on the overall HP (Fig. 3) but the shape of power–time curve was changed in that there were no secondary peaks in the thermal profile and the maximum of HP was 2 h earlier than in the control. It is suggested that the formation of the biofilm is manifested as the 15 μW constant level in the power–time curves with cellulose and NC.

In conclusion, although the high molecular mass NC-polymer does not penetrate the bacterial cells, it still appears to affect their metabolism. The calorimetric results suggest that in the presence of NC, the changes in *D. desulfuricans* 1388 metabolism are caused by both chemical (nitrate) and physical (biofilm formation) factors. Intracellular nitrate reduction competes for the electron flux from lactate and suppresses bacterial sulfidogenesis and growth. The presence of the insoluble carbon backbone of the polymer in culture medium induces the creation of a cellular biofilm-like structure with its own metabolism.

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