Alternative substrates of bacterial sulphate reduction suitable for the biological-chemical treatment of acid mine drainage

Alena Luptakova[1] *and* ***Eva Macingova***

The impacts of AMD pollution on biological systems are mostly severe and the problem may persist from many decades to thousands of years. Consequently AMD prior to being released into the environment must be treated to meet government standards for the amount of metal and non-metal ions contained in the water. One of the best available technologies for the removal of metals from AMD is precipitation as metal sulphides. SRB applications for AMD treatment involve a few principal stages. The first stage is the cultivation of SRB i.e. the bacterial sulphate reduction. At the laboratory conditions the sodium lactate is the energetic substrate for the growth of bacteria. Its price is not economic for the application in the practice and is needed investigate the alternative substitutes. The aim of this work was the cultivation of SRB using the selected energetic substrates such as: calcium lactate, ethanol, saccharose, glucose and whey. Experimental studies confirm that in the regard to the amount of reduced sulphates the calcium lactate and ethanol are the best alternative substrates for the bacterial sulphate-reduction.

Key words: *biological sulphate reduction, sulphate-reducing bacteria, calcium lactate, whey,*

Introduction

Acid mine drainage (AMD) represent one of the major environmental problems concerning of the mining and post-mining processing activities in the worldwide (Johnson et al., 2006). AMD generally contains dissolved heavy metals, iron precipitates and have very low pH value about 1.5-2.0 and moderate (0.35–0.55 $g.l^{-1}$) or high (1.5–7.2 $g.l^{-1}$) sulphate concentration. Metals of particular interest in AMD include Cu, Zn, Cd, As, Mn, Al, Pb, Ni, Ag, Hg, Cr and Fe (Kontopouls, 1988). Estimation of the impacts and the extent of the AMD problem on various water resources have been reported by researches for a number of continent and country. They have observed that the consequence of acidity and heavy metal contamination in aquatic and terrestrial ecosystems is a reduction in both species diversity and the total biomass composition of such systems (Lintnerova, 2002; Younger, 2004). The impacts of AMD pollution on biological systems are mostly severe and the problem may persist from many decades to thousands of years (Janova and Vrana, 2004; Kaduková and Štofko, 2006). Consequently AMD prior to being released into the environment must be treated to meet government standards for the amount of metal and non-metal ions in the water.

Various methods are used for remediation of AMD in the world, but any of them is universal. Generally are distinguished two variant strategies for treating AMD: active and passive treatment technologies (Skousen et al., 1995; Balintova and Kovalikova, 2008). Active treatment systems have been characterised as systems that make use of conventional wastewater treatment processes, require ongoing inputs of electrical energy and/or chemical reagents in a controlled process (Younger, 2004). Passive treatment has been defined as system which utilises naturally available energy sources such as topographical gradient, microbial metabolic energy, photosynthesis and chemical energy and requires regular but infrequent maintenance to operate successfully over its design life (Kalin et al., 2006). Both active and passive systems may be implemented using physicochemical or biological treatment technologies (Skousen et al., 1995; Johnson, 2000). Nowadays attention is pay to physical, chemical and biological methods for the selective recovery of metals from AMD. These methods have the potential to recover metals in a suitable form for commercial use. One of the best available technologies for the removal of metals from AMD is precipitation as metal sulphides. Furthermore, due to the different pH values of metal sulphide precipitation, selective recovery of valuable metals (Cu, Ni, Co, Zn etc.) from AMD can be achieved by hydrogen sulphide. However, chemical sulphide precipitation has not been generally used probably due to the high cost of reagents. Sulphates usually present in AMD should be removed. A good way to obtain it is to reduce sulphates to hydrogen sulphide by sulphate-reducing bacteria (SRB) and use the biologically generated hydrogen sulphide to precipitate the metals from AMD.

SRB may be one of the oldest forms of life on Earth. They can be found back billions of years in the geologic rock record to the Early Archean (3900 to 2900 million years ago) (Postgate, 1984). Ancient SRB left their first mark on their environment in pyrite minerals (FeS_2) as old as 3400 million years (Ohmoto et al., 1993). Today, these bacteria are widespread in marine and terrestrial aquatic environments. Their

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ability to adapt to extreme physical and chemical conditions enables them to play an important role in global geochemical cycles (Odom and Singleton, 1993; Johnson, 2000) predominantly in the sulphur cycle that constitutes one of the best examples of the impact exerted by living organisms on geochemical cycles. SRB is a wide term that is applied to diverse collection of obligate anaerobic bacteria that use sulphates as an electron acceptor in the anaerobic oxidation of inorganic (H_2+CO_2) or organic substrates (lactate, acetate etc.). They can produce a considerable amount of hydrogen sulphide, which reacts easily in aqueous solution with the available metal ions to form insoluble products, most commonly FeS_2, leading to the production and transformation of natural mineral deposits (Odom and Singleton, 1993). Sulphates are reduced by SRB according to the generalized reactions (1 and 2):

$$4\,H_2 + SO_4^{2-} \xrightarrow{SRB} H_2S + 2\,H_2O + 2\,OH^- \quad \text{Inorganic substrate} \quad (1)$$

$$\text{Organic matter} + SO_4^{2-} \xrightarrow{SRB} HS^- + HCO_3^- + H_2O \quad \text{Organic substrates} \quad (2)$$

SRB can use a large range of substrates as electron donors and carbon sources, which oxidize incompletely (to acetate) or completely (to CO_2). These substrates are usually organic compounds including sewage sludge, leaf mulch, wood chips, animal manure, vegetal compost, sawdust, mushroom compost, whey, and other agricultural waste. Various types of synthetic organic compounds have also been used, especially small molecular weight compounds, such as lactate, acetate, propionate, pyruvate, ethanol and other alcohols (Dvorak et al., 1992; Liamleam and Annachhatre, 2007).

Methods of SRB applications for AMD treatment involve a few principal stages. The first stage is the cultivation of SRB i.e. the bacterial sulphate reduction. In the laboratory conditions the sodium lactate is the energetic substrate for the growth of bacteria. Its price is not economic for the application in the practice and is needed investigate the alternative substitutes. Therefore the aim of this work was the cultivation of SRB using the selected energetic substrates such as: calcium lactate, ethanol, saccharose, glucose and whey.

Materials and methods

Microorganisms - for the experiments the cultures of SRB (genera Desulfovibrio) isolated from the potable mineral water (Gajdovka spring, Slovakia) were used. The SRB were selected from the mixed cultures by the modified dilution method (Postgate, 1984).

Cultivation of sulphate-reducing bacteria - cultivation conditions were following: temperature 30 °C, anaerobic conditions, 10% inoculum of SRB, the selective nutrient medium DSM-63 (the energetic substrate for the growth of SRB was sodium lactate (Lac-Na)) or its modifications. The basis of this modification was the Lac-Na substitution by the adequate amount of chosen substrates: calcium lactate (Lac-Ca), ethanol (Eta), saccharose (Sach), glucose (Glu), liquid whey (Whey-L) and solid whey (Whey-S). Sodium lactate, calcium lactate, ethanol, saccharose, glucose were used in the form of chemicals with the analytical grade. Solid whey was bought in the chemist. Liquid whey comes from dairy industry (cheese production).

Analytical procedures - a nefelometric method was used to measure the sulphate concentration using a Spectromom195 instrument. The absorbance of the sample was measured at a wavelength of 490 nm. A glass pH electrode combined with the reference Ag/AgCl electrode was used to measure pH. The acid solution of $CuSO_4$ was used for the monitoring of the hydrogen sulphide presence.

Results and discussion

Attributes of the positive SRB growth are the black precipitates occurrence and hydrogen sulphide formation. These evidences were observed in studied substrates except Whey-S and abiotic controls (Fig. 1). Black precipitates represent iron sulphides (salts of iron are present in the nutrient medium (Postgate, 1984)). Their creation can be described according aforementioned reactions (2) and following (3):

$$Fe^{2+} + H_2S \longrightarrow FeS + 2H^+ \quad (Fe^{2+} \text{ is standard compound of nutrient medium DSM-63}) \quad (3)$$

Figures 2 – 13 describe the changes of the sulphate concentration and pH values in the course of SRB cultivation using selected substrates and corresponding abiotic controls. In all cases Lac-Na was used as reference growth substrate. The decrease of the sulphates concentration was observed in all studied substrates except Whey-S and all abiotic controls.

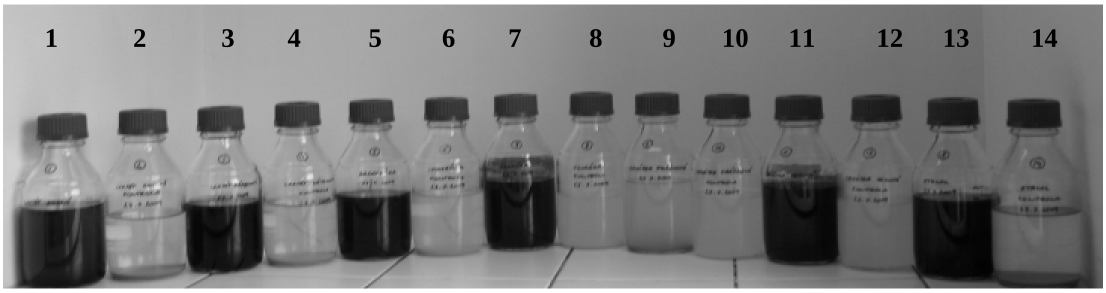


Fig. 1. Comparison of black precipitates formation during SRB cultivation using chosen substrates and abiotic controls (1) – sodium lactate; (2) – sodium lactate abiotic control; (3) – calcium lactate; (4) – calcium lactate abiotic control; (5) – saccharose; (6) – saccharose abiotic control; (7) – glucose; (8) – glucose abiotic control; (9) – solid whey; (10) – solid whey abiotic control; (11) – liquid whey; (12) – liquid whey abiotic control; (13) – ethanol; (14) – ethanol abiotic control.

One of the characteristics of all known SRB is their sensitivity to even mild acidity. Most of them are inhibited at pH<5.5 (Castro et al., 2000; Johnson, 2006). Therefore value of pH is very important for regular course of the bacterial sulphate reduction (i.e. in fact the bacterial sulphide-genesis). In the case of Lac-Ca pH value changes during SRB cultivation showed the similar profile in the compare with classical substrate Lac-Na (Fig. 3). After initial decreasing of pH from 7.7 to 6.7 minor increasing of pH values to 7.0 followed. Then it remained without strong changes and the average pH values were about 7.1 that is suitable for the bacterial sulphate reduction (Fig. 2). The lactate oxidation by SRB was realized without problems and lactate was converted to acetate at the simultaneously bicarbonate alkalinity production according to reaction (4).

$$2\ CH_3CHOHCOO^- + SO_4^{2-} \xrightarrow{SRB} 2\ CH_3COO^- + 2\ HCO_3^- + H_2S \quad (4)$$

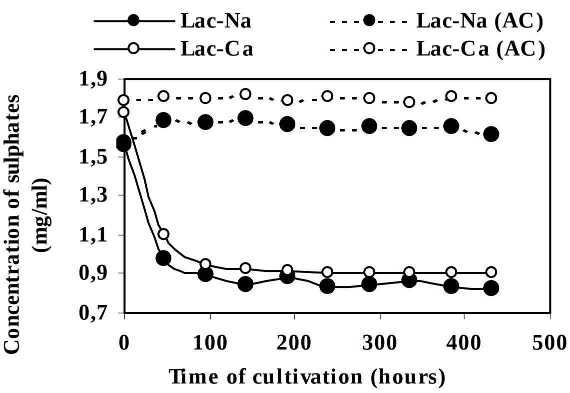


Fig. 2. Sulphate concentration changes during the SRB cultivation. Substrate: calcium lactate – Lac-Ca; abiotic control – Lac-Ca (AC)

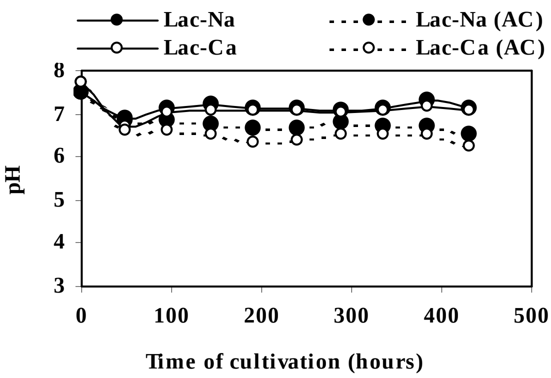


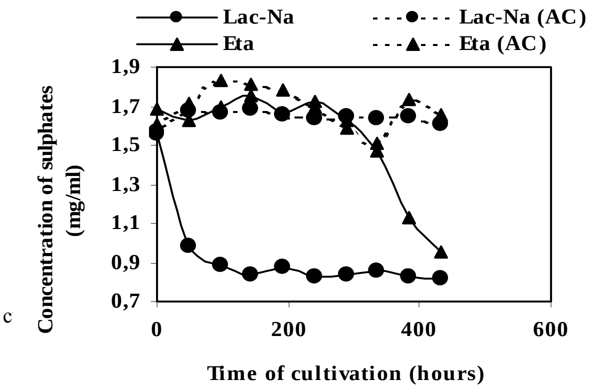
Fig. 3. pH values changes during the SRB cultivation. Substrate: calcium lactate – Lac-Ca; abiotic control – Lac-Ca (AC)

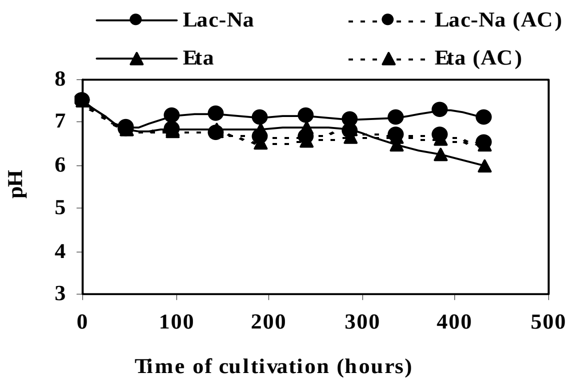
At the using Eta pH values after 48 hours decreased to 6.8. Until 300 hours it were almost identically (Fig. 5) and since came to gradually decreasing of pH to 6.0. It related with major sulphates decreasing (Fig. 4). The profile curves on the Fig. 4 and 5 can be explained to the next considerations. According to Liamleam and Annachhatre (2007) the ethanol oxidation by SRB illustrated reactions (5) and (6). Acetate is a major degradation intermediate and its oxidation (reactions 6) produces the bicarbonate alkalinity, which neutralizes solution.

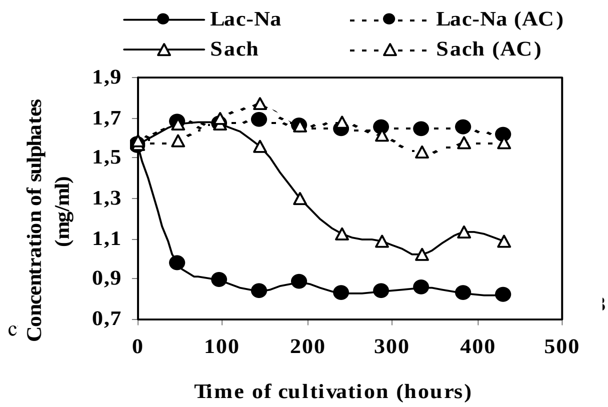
$$2\ CH_3CH_2OH + SO_4^{2-} \xrightarrow{SRB} 2\ CH_3COO^- + HS^- + H^+ + 2\ H_2O \quad (5)$$

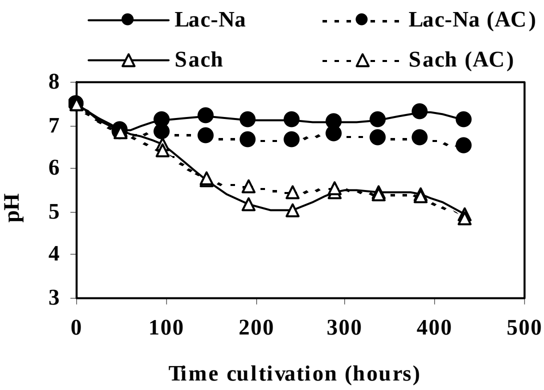
$$CH_3COO^- + SO_4^{2-} \xrightarrow{SRB} 2\ HCO_3^- + HS^- \quad (6)$$

Sugars are effective electron donors that are easily degraded under anaerobic conditions. Figures 6 – 9 show the results of SRB cultivation by using saccharose and glucose as substrates. The decrease of the sulphates concentration was observed only the SRB presence. But the acidity increasing was detected also in abiotic controls. It could be explained by the sugar degradation. Sulphate-reducing bacteria are very sensitive to the presence of even low concentrations of acetic acid (Reis et al., 1992). At an alkaline pH the effect of acetic acid is negligible but dramatically increases with a drop in the reaction of the medium. By using saccharose and glucose as the carbon sources it was achieved a pH value lower than 5.5 and inhibition effect of acetic acid to the growth of SRB was observed.









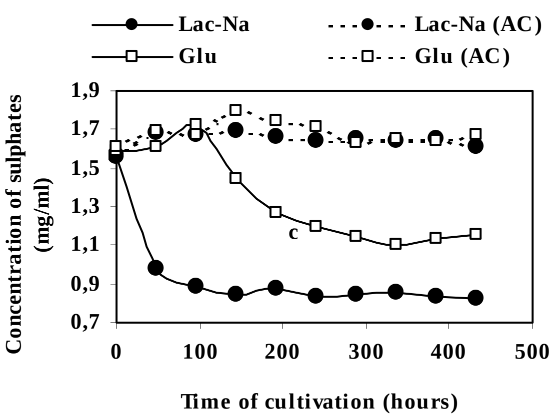


Fig. 8. Sulphate concentration changes during the SRB cultivation. Substrate: glucose – Glu; abiotic control – Glu (AC)

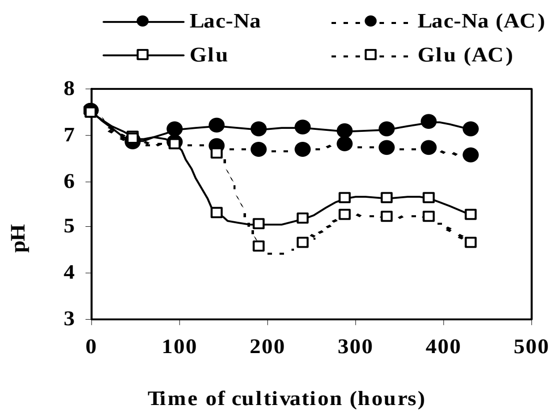


Fig. 9. pH values changes during the SRB cultivation. Substrate: glucose – Glu; abiotic control – Glu (AC)

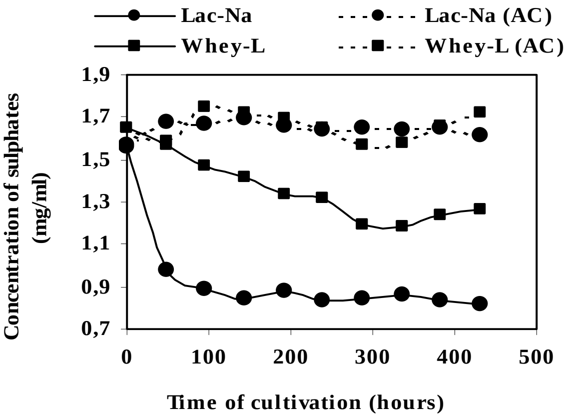


Fig. 10. Sulphate concentration changes during the SRB cultivation. Substrate: liquid whey – Whey-L; abiotic control – Whey-L (AC)

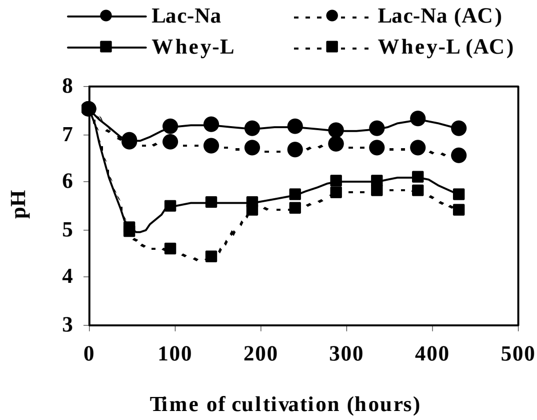


Fig. 11. pH values changes during the SRB cultivation. Substrate: liquid whey – Whey-L; abiotic control – Whey-L (AC)

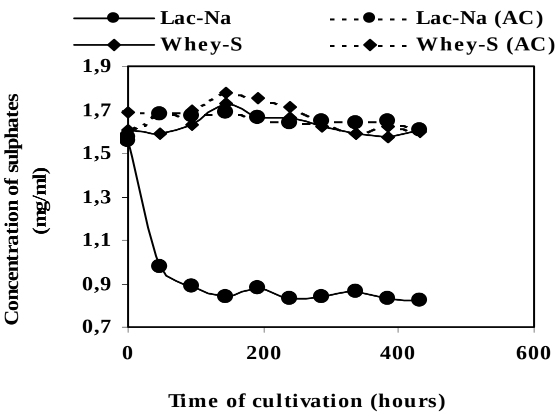


Fig. 12. Sulphate concentration changes during the SRB cultivation. Substrate: solid whey – Whey-S; abiotic control – Whey-S (AC)

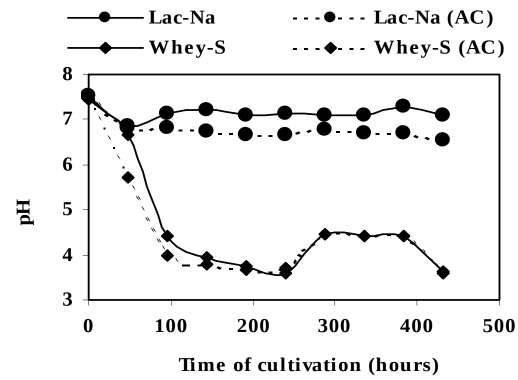


Fig. 13. pH values changes during the SRB cultivation. Substrate: solid whey – Whey-S; abiotic control – Whey-S (AC)

The similar course was observed in the case of liquid whey (Fig. 11), but the initial rapid decreasing of values pH (4.9 at the presence SRB) was later settled on the level over 5.5 and 6.0 and the bacterial sulphate reduction was stopped (Fig. 10).

Lactose is the major component of whey and can be metabolised into lactate, ethanol, acetate and carbon dioxide (Chartrain et al., 1987). The solid whey utilization was unsuccessful (Fig.12) because pH values decreased under suitable level (i.e. pH<5.5) for the SRB cultivation (Fig.13).

Figure 14 documented that chosen substrates except (Whey-S) are suitable alternative substitutes. In the regard to the amount of reduced sulphates the calcium lactate and ethanol are the best alternative substrates for the bacterial sulphate-reduction.

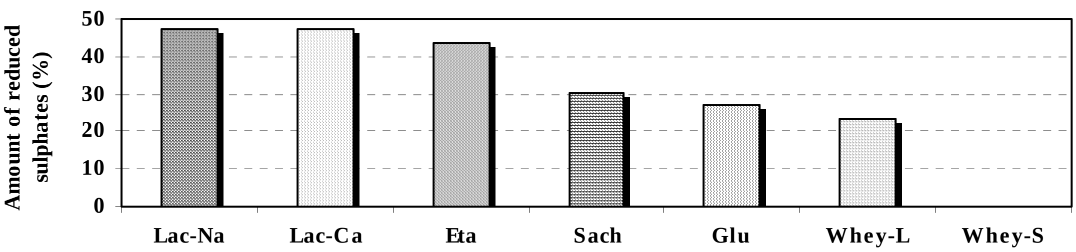


Fig. 14. *Comparison of reduced sulphates amount by SRB using substrates: sodium lactate (Lac-Na), calcium lactate (Lac-Ca), ethanol (Eta), saccharose (Sach), glucose (Glu), liquid whey (Whey-L) and solid whey (Whey-S).*

Conclusions

The first stage of SRB applications for AMD treatment is the cultivation of SRB i.e. the bacterial sulphate reduction. For the economically effective utilization is need investigate the alternative substitutes of sodium lactate that presents the standard growth substrate for SRB. The main aim of this work was the cultivation of SRB using the classical selective nutrient medium DSM-63 (the energetic substrate for the growth of SRB was sodium lactate (Lac-Na)) or its modifications. The basis of modification was the Lac-Na substitution by the adequate amount of chosen substrates: calcium lactate (Lac-Ca), ethanol (Eta), saccharose (Sach), glucose (Glu), liquid whey (Whey-L) and solid whey (Whey-S). Experimental studies confirm, that in the regard to the amount of reduced sulphates the calcium lactate and ethanol are the best alternative substrates for the bacterial sulphate-reduction.

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