

## BACTERIAL SULFATE REDUCTION AND pH: IMPLICATIONS FOR EARLY DIAGENESIS

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### ABSTRACT

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Decomposition of non-amine compounds, such as lactate, by the anaerobic bacterium *Desulfovibrio desulfuricans* produces a change in the chemistry of the surrounding fluids. Empirical studies demonstrate the following:

(1) *D. desulfuricans* are tolerant of alkaline pH-values (maximum pH = 9.2 for the strain used).

(2) Growth results in the lowering of pH to values near neutrality.

The factors which control this lowering in pH are release of CO<sub>2</sub> and the establishment of a proton gradient across the cell membrane. This energy-yielding metabolic process may make a significant contribution towards carbonate dissolution reactions and diagenetic reactions involving silica. These include silicification of Precambrian microorganisms, nucleation of chert nodules, and the replacement of sulfate minerals by silica.

### INTRODUCTION

The microbial decomposition of organic matter in sediment may influence the geochemical microenvironment in many ways. Significant effects of such decomposition include changes in pH, carbonate alkalinity, and Eh. In addition, bacteria may provide sites of nucleation for mineral growth.

The sediment, at any time, will be subjected to changing chemical conditions as nutrients suitable for the growth of one organism are replaced by those that promote the growth of a different organism. The sediment may alternately experience a pH increase due to the release of ammonia during protein decomposition (Abd-el-Malek and Rizk, 1963; Berner, 1969), followed by a pH decrease as the excess ammonia diffuses away and organic acids are produced by microbial fermentation; an aerobic environment frequently will be followed by one which is anaerobic. This paper describes *in vitro* experiments which describe the effects of bacterial sulfate reduction in a defined system and suggest how such metabolic activities will influence microenvironments such as pore spaces.

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Anaerobic sulfate-reducing bacteria, predominantly of the genus *Desulfovibrio*, are ubiquitous in oxygen-free environments. These bacteria have long been considered important agents in sedimentation and early chemical diagenesis, particularly with respect to changes in Eh and the precipitation of ferrous sulfide (cf. ZoBell, 1942, 1946; Emery and Rittenberg, 1952; Baas-Becking et al., 1960) and the destruction of sulfate deposits (Friedman, 1972). The influence of the sulfate-reducing bacteria upon pH changes during diagenesis and the importance of these changes to decomposition reactions has been discussed (Gardner, 1973; Aller, 1982). However, few descriptions of the metabolism of *Desulfovibrio* species appear in the geological literature, and those that exist are too general in the light of recent discoveries by microbiologists (Odom and Peck, 1981).

Controlled in vitro experiments reveal that growth of *Desulfovibrio desulfuricans* on lactate, their preferred carbon source, results in a significant pH decrease. Growth on other substrates, for example amine-containing compounds, could result in ammonia release and a pH increase. The observed metabolically-mediated pH reduction may significantly alter diagenetic reactions within sediments. Early diagenetic reactions which may be influenced by such pH reductions include: (1) base-exchange reactions with clays; (2) preservation of kaolinite in the marine environment (Millot, 1953); (3) crystallization of certain micas from clay minerals (Dapples, 1967); and (4) decomposition of certain silicate minerals due to hydrolysis reactions (Krumbein, 1972).

The magnitude of this effect can be seen in relation to the dissolution of calcium carbonate. Dissolution of mollusc shells has been found to begin immediately after the death of the organism with a loss of 10–20 wt. % per year (Hecht, 1933, reported by Fairbridge, 1967). Furthermore, in sediment with abundant organic material, only casts and molds remained after a similar time. In laboratory experiments, Hecht (1933) found shell weight diminution as great as 25% in two weeks. Gypsum crystals which formed on the fleshy parts of the bivalves indicate that bacterial sulfate reduction may be involved in the dissolution of the shell as a result of acid production and the associated pH decrease. Berner (1969) reported suppressed pH-values and dissolution of calcium carbonate during bacterial decomposition of two varieties of clam. These experiments had an inoculum of mud to insure the presence of sulfate-reducing bacteria [previous experiments on fish were conducted in the absence of sulfate reduction and a pH increase was reported by Berner (1969)]. In the absence of calcium carbonate, Berner found that a pH approaching 4 was attained during the decomposition of *Venus mercenaria*, indicating unknown acids, in addition to carbonic acid produced from microbially generated CO<sub>2</sub>, were evolved.

A second diagenetic reaction which may be mediated by the metabolic products of sulfate reduction is the nucleation and precipitation of silica. Diagenetic silica formation from saturated solutions is controlled by pH (Krauskopf, 1959). Mechanisms of altering pH conditions in local environ-

ments to produce a chert nodule, for example, may be explained by the microbial degradation of organic material within the host-sediment. The factors that influence this decrease in pH are presented.

## RESULTS

Cells of *Desulfovibrio desulfuricans* were cultured in Postgate medium c (Postgate, 1979) at varying pH-values. Growth rate was determined by measuring optical density at 540 nm, utilizing a Klett-Summerson<sup>®</sup> photoelectric colorimeter and relating this to a previously determined relationship between optical density and cell number (Czechowski and Rossmore, 1980). pH was measured using a standard combination electrode. Cultures were incubated at 35°C anoxically in the dark.

Fig. 1 shows the effect of pH of the media upon the growth of *D. desulfuricans*. Growth was determined by measuring the time required for the cells to reach an optical density of 120 KU ml<sup>-1</sup> ( $2.4 \cdot 10^8$  cells/ml; one Klett unit, KU, is equivalent to  $\sim 2 \cdot 10^6$  cells).

At pH-values above 9.2 no growth was observed in this particular strain (Fig. 1). Between pH-values of 9.2 and 8.5 growth appeared to be inhibited by pH, requiring up to five days to reach 120 KU ml<sup>-1</sup>. Optimum growth occurred at all pH-values below 8.5. The final pH for all the cultures was between 7.0 and 7.4, thus indicating that growth resulted in a pH decrease.

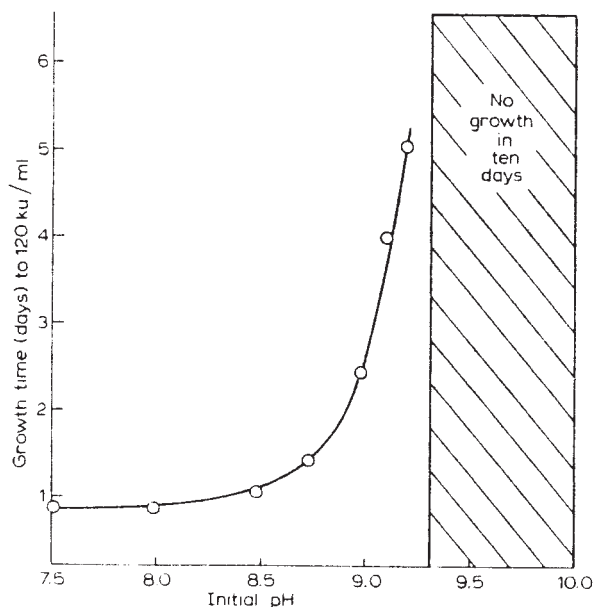


Fig. 1. Effect of initial pH upon growth of *Desulfovibrio desulfuricans*. No growth occurred at pH-values above 9.3; growth was inhibited at pH-values between 8.5 and 9.2. Growth measured as optical density at 540 nm, recorded as Klett units (KU ml<sup>-1</sup>).

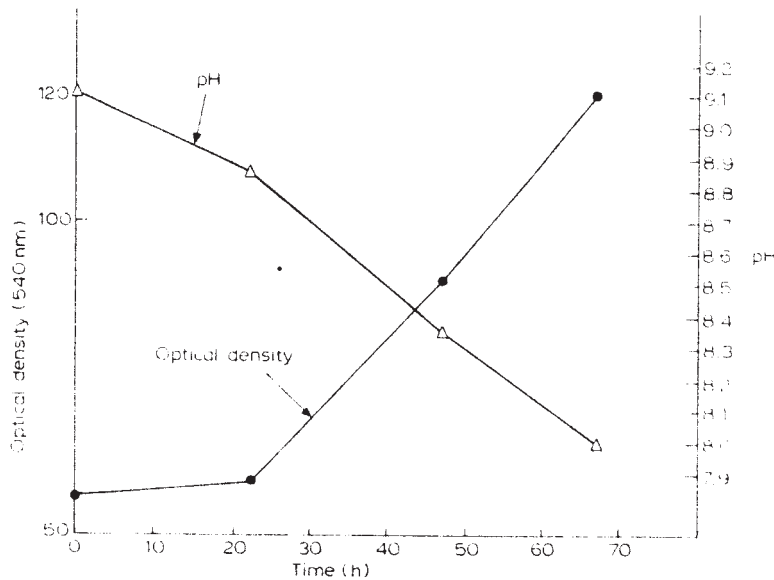


Fig. 2. Effect of growth upon pH. Growth measured as optical density at 540 nm. Continued growth results in a pH decrease.

Fig. 2 shows more specifically the effect of growth upon pH. Growth is initially slow, with little or no cell division occurring and metabolism restricted to a survival level (maintenance metabolism). This capability allows cells to survive for some period of time in environments inhospitable to normal growth. As maintenance metabolism continues metabolic products accumulate and the resultant pH decrease alters the environment to one more suitable for growth. As more rapid growth begins, the pH decreases further and conditions more conducive to growth prevail. After ~ 48 hr., the pH has reached a value of 8.5 and rapid growth begins. Therefore, even though the higher pH-values are unsuitable for growth, maintenance metabolism does occur and results in a pH decrease. The final pH-values agree well with those of Thorstenson (1970), Ben-Yaakov (1973), and Gardner (1973).

Fig. 3 represents the metabolic pathway of *D. desulfuricans*, using only lactate as a growth substrate. This occurs in two parts with the lactate initially oxidized to pyruvate and then converted to acetate and  $\text{CO}_2$  in an energy-yielding pathway. This pathway results in the removal of eight hydrogen atoms from two lactate molecules to produce four hydrogen molecules. These are passed through the cell wall via the specialized enzyme hydrogenase, where oxidation of hydrogen occurs, releasing protons outside the cell membrane. The electrons removed from hydrogen pass back into the cell via vectorial electron transport and are utilized in a second reaction, the reduction of sulfate to sulfide (Odom and Peck, 1981). This process uses the eight electrons derived from external hydrogen and also eight

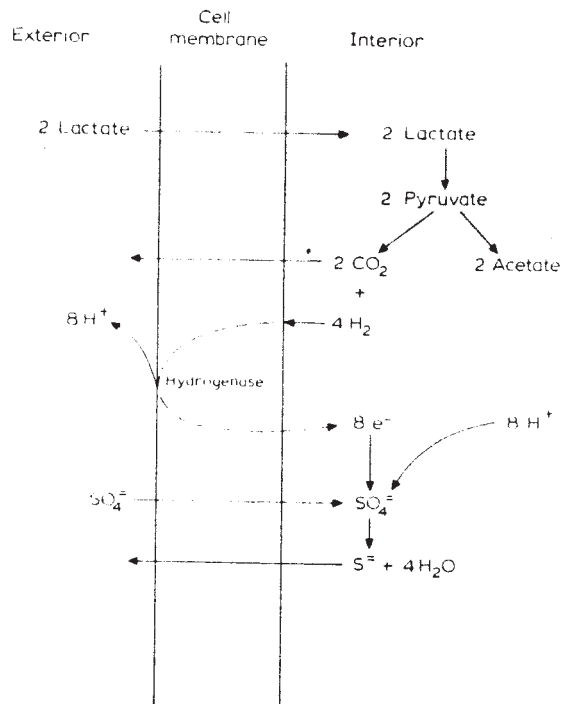
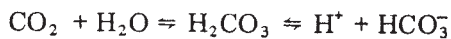


Fig. 3. Metabolism of *Desulfovibrio desulfuricans* utilizing a lactate substrate. Along with a proton gradient, metabolites include CO<sub>2</sub> and H<sub>2</sub>S (modified from Odom and Peck, 1981).

protons from inside the cell and therefore is equivalent to the net movement of protons from inside to outside the cell thus producing a proton gradient. The proton gradient is then used in the production of metabolic energy (ATP).

The metabolic activities of *Desulfovibrio* species thus result in several significant changes in the external growth environment. These include the production of CO<sub>2</sub>, the conversion of sulfate to sulfide ions, and the establishment of a proton gradient across the cell membrane.

The production of CO<sub>2</sub> and sulfide ions has been shown to significantly affect pH (Berner et al., 1970). There are two CO<sub>2</sub> molecules produced per sulfide ion and thus a net production of protons occurs at alkaline pH-values. The CO<sub>2</sub> forms bicarbonate ions:



at pH-values from ~ 7 to ~ 10. Proton production may be partially counteracted by the production of sulfide ion which reacts to form HS<sup>-</sup> at these pH-values. The net reaction may be written as:



This results in a net proton production and an increase in carbonate alkalinity. Therefore, even under conditions of high buffering capacity the metabolic activity of *Desulfovibrio* species should result in a significant pH decrease in alkaline environments.

The significance of the proton gradient remains to be determined. It is, however, likely that a net proton transfer across a cell membrane creates a microenvironment which differs radically from the adjacent pore fluids. This may, in part, explain the unexpectedly low pH reported by Berner (1969) during decomposition of *Venus mercenaria*.

#### CONCLUSIONS

Within naturally buffered systems such as marine water, the pH is found to be relatively constant. In microenvironments, such as pore spaces however, where microbially mediated decomposition reactions are occurring, the local chemical characteristics may be significantly altered, both at the microbial cell surface and within the surrounding pore space. This has implications for inorganic dissolution and precipitation reactions.

The decomposition of organic matter in aquatic and soil environments often results in the depletion of available oxygen and the production of a variety of carbon compounds that support the growth of *Desulfovibrio* species. The metabolic activities of *Desulfovibrio* species provide localized chemical environments which influence early diagenetic reactions. Vectorial electron transport and the production of a proton gradient across the cell membrane may make a significant contribution toward diagenetic reactions. The rapid silica precipitation required for the exquisite fossil preservation of microorganisms in the geologic record (cf. Barghoorn and Schopf, 1965, 1966) may well be attributable to a proton gradient. Soluble silica, in the form of monosilicic acid,  $H_4SiO_4$ , dissociates to  $H_3SiO_4^-$  at pH-values above 9.7. As pH decreases, especially adjacent to the cell surface, a localized precipitation of silica should occur, entombing the cell in silica. This mechanism of silica precipitation may explain the nucleation of silica to form chert, and the replacement of sulfate minerals by various forms of silica as reported by Friedman and Radke (1979), Friedman and Shukla (1980), Tucker (1976), and Chowns and Elkins (1974). Numerous other bacteria utilize a proton gradient and further studies are required to develop a model for microbially-induced silica precipitation.

#### REFERENCES

- Abd-el-Malek, Y. and Rizk, S.G., 1963. Bacterial sulphate reduction and the development of alkalinity, I. Experiments with synthetic media. *J. Appl. Bacteriol.*, 26: 7-13.
- Aller, R.C., 1982. Carbonate dissolution in nearshore terrigenous muds: the role of physical and biological reworking. *J. Geol.*, 90: 79-95.

- Baas-Becking, L.G.M., Kaplan, I.R. and Moore, D., 1960. Limits of the natural environment in terms of pH and oxidation-reduction potentials. *J. Geol.*, 68: 243-284.
- Barghoorn, E.S. and Schopf, J.W., 1965. Microorganisms from the late Precambrian of central Australia. *Science*, 150: 337-339.
- Barghoorn, E.S. and Schopf, J.W., 1966. Microorganisms, three billion years old, from the Precambrian of South Africa. *Science*, 152: 758-763.
- Ben-Yaakov, S., 1973. pH buffering of pore water of recent anoxic marine sediments. *Limnol. Oceanogr.*, 18: 86-94.
- Berner, R.A., 1969. Chemical changes affecting dissolved calcium during the bacterial decomposition of fish and clams in seawater. *Mar. Geol.*, 7: 253-274.
- Berner, R.A., Scott, M.R. and Thomlinson, C., 1970. Carbonate alkalinity in the pore waters of anoxic marine sediments. *Limnol. Oceanogr.*, 15: 544-549.
- Chowns, T.M. and Elkins, J.E., 1974. The origin of quartz geodes and cauliflower cherts through silicification of anhydrite nodules. *J. Sediment. Petrol.*, 44: 885-903.
- Czechowski, M.H. and Rossmore, H.W., 1980. Factors affecting *Desulfovibrio desulfuricans* lactate dehydrogenase. *Dev. Ind. Microbiol.*, 21: 349-356.
- Dapples, C.C., 1967. Diagenesis of sandstones. In: G. Larsen and G.V. Chilingar (Editors), *Diagenesis in Sediments*, Elsevier, Amsterdam, pp. 91-126.
- Emery, K.O. and Rittenberg, S.L., 1952. Early diagenesis of California basin sediments in relation to origin of oil. *Am. Assoc. Pet. Geol. Bull.*, 36: 735-806.
- Fairbridge, R.W., 1967. Phases of diagenesis and authigenesis. In: G. Larsen and G.V. Chilingar (Editors), *Diagenesis in Sediments*, Elsevier, Amsterdam, pp. 19-90.
- Friedman, G.M., 1972. Significance of Red Sea in problem of evaporites and basinal limestones. *Am. Assoc. Pet. Geol. Bull.*, 56: 1072-1086.
- Friedman, G.M. and Radke, B., 1979. Evidence of sabkha overprint and conditions of intermittent emergence in Cambrian-Ordovician carbonates of northeastern North America and Queensland, Australia. *Northeast. Geol.*, 1: 18-36.
- Friedman, G.M. and Shukla, V., 1980. Significance of authigenic quartz euhedra after sulfates; example from the Lockport Formation (Middle Silurian) of New York. *J. Sediment. Petrol.*, 50: 1299-1304.
- Gardner, L.R., 1973. Chemical models for sulfate reduction in closed anaerobic marine environments. *Geochim. Cosmochim. Acta*, 37: 53-68.
- Hecht, F., 1933. Der Verbleib der organische Substanz der Tiere bei meerischer Einbettung. *Senckenbergiana*, 15: 165-249.
- Krauskopf, K.B., 1959. The geochemistry of silica in sedimentary environments. In: H.A. Ireland (Editor), *Silica in Sediments*, Soc. Econ. Paleontol. Mineral., Spec. Publ. No. 7, Tulsa, Okla., pp. 4-19.
- Krumbein, W.E., 1972. Rôle des microorganismes dans la genèse, la diagenèse et la dégradation des roches en place. *Rev. Écol. Biol. Sol*, 9: 283-319.
- Millot, G., 1953. Héritage et néoformation dans la sédimentation argileuse. 19e Congr. Géol. Int., C. R., Alger, 1952, 18: 163-175.
- Odom, J.M. and Peck, Jr., H.D., 1981. Hydrogen cycling as a general mechanism for energy coupling in sulfate-reducing bacteria, *Desulfovibrio* sp. *F.E.M.S. Microbiol. Lett.*, 12: 47-50.
- Postgate, J.R., 1979. *The Sulphate-reducing Bacteria*. Cambridge University Press, Cambridge, 151 pp.
- Thorstenson, D.G., 1970. Equilibrium distribution of small organic molecules in natural waters. *Geochim. Cosmochim. Acta*, 34: 745-770.
- Tucker, M.E., 1976. Replaced evaporites from the late Precambrian Finnmark, Arctic Norway. *Sediment. Geol.*, 16: 193-204.
- ZoBell, C.E., 1942. Changes produced by microorganisms in sediments after deposition. *J. Sediment. Petrol.*, 12: 127-136.
- ZoBell, C.E., 1946. Studies on redox potential of marine sediments. *Am. Assoc. Pet. Geol. Bull.*, 30: 477-513.