ORGANIZATION AND ACTIVITY OF *IN SITU* SULPHOXIDIZING BACTERIAL COMMUNITIES - A POSSIBLE MODEL FOR THE EXPERIMENTAL STUDY OF BIOLOGICAL OXIDATION OF HYDROGEN SULPHIDE

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Abstract. The paper presents the basic elements of an experimental device for the cultivation and study of sulphoxidizing bacteria, under monitored conditions. The proposed experimental scheme takes over some aspects of organization and function of these bacterial communities in natural conditions and allows not only to outline their physiological characteristics but also to evaluate their capacity to biologically oxidize the hydrogen sulphide. The work suggests also the possibility to use some equipments with similar operating principles for controlling the hydrogen sulphide emissions.

Key words: hydrogen sulphide, sulphoxidizing bacteria, experimental device

1. INTRODUCTION

Along the Romanian seashore, between Mangalia and Venus there are numerous submarine mesothermal sulphurous springs, localized at a depth ranging from 0. 30-0. 50 m up to 10-12 m. Hydrogen sulphide occurrence leads to the accumulation of specialized bacterial communities-sulphoxidizing bacteria.

In the shallow depth zone, the existing substrate in sulphurous springs area consists of clusters of rocks. The rock surface bathed by sulphurous water is covered by a white-yellowish or grey-whitish bacterial mat with characteristic aspect.

The bacterial mat has a complex enough structure, being made up of bacterial cells and inert material of inorganic or organic nature. The inorganic matter is more often represented by precipitated mineral salts and sulphur particles whereas the organic fraction is constituted of exocellular material, resulted from bacterial metabolic activity with a role in fixing and retaining the cells on the substrate.

The bacterial populations identified by us within these areas belong mainly to colourless sulphurous bacteria group of *Thiothrix* and *Beggiatoa* types. Other groups have low densities and their presence and activities are inconsistent (*Thiocapsa*, *Chlorobium*).

Luxuriant growth of colourless sulphurous bacteria leads to the appearence of some filamentary agglomerations like fluffy cotton balls. The *Thiothrix* genus bacteria are aerobic, sulphoxidizing organisms, having the ability to fix themselves to the substrate with the aid of some formations similar to crampons.

In this area, the particularly high dynamics of water masses and the low hydrogen sulphide concentrations are facilitating the massive development of this group. Of particular interest is the presence of bacteria of *Beggiatoa* genus in the network made up by *Thiothrix* filaments. Bacteria of *Beggiatoa* genus are known in the literature as "gradient organisms", since they can move and populate the areas of optimal O_2 and H_2S values. Experimental modelling of behaviour in redox gradient can be carried out by their cultivation in a semisolid agar column, in the presence of O_2 and H_2S diffusing from opposite directions. At the growth disc level, O_2 and H_2S concentrations diminish to zero due to the consumption (Nelson *et al.*, 1986).

The *Thiothrix* agglomerations probably exhibit a multitude of microhabitats, different with regard to the optimal conditions while the *Beggiatoa* populations have a behaviour similar to those from sediments, occupying favourable redox horizons.

The functions of sulphoxidizing bacterial mat are of particular theoretical and practical importance. The interference zone of oxygenated and anaerobic media constitutes a particular habitat selecting organisms of specialized metabolic characters; these organisms represent the connecting ring through which the transfer of material and energy is carried out.

From a practical point of view, the sulphoxidizing bacteria ability to retain and detoxify H_2S - which is toxic for most beings - might be used by man to monitor this compound emissions. Understanding the factors conditioning these organisms *in situ* (O_2, H_2S, pH, t°C, requirements of vitamins or growth factors, relationship with other bacterial or invertebrate populations) implies the carrying out of some controlled culture systems.

Under natural conditions, bacterial growth occurs as a pellicle; that is why, in order to understand the functionality of these communities, the physiology of microbial biofilms should be taken into consideration.

To study the biofouling McCoy *et al.* (1981) use an experimental equipment consisting of a rotative disc placed in a cylindrical container fed with nutrients and bacterial inoculum. The microbial film is formed on a number of rods or on a detachable muff of the disc.

For the study of the primary biocide effects in areas contaminated with petroleum, Costerton and Lashen (1984) used an equipment (Robbin's mechanisms) and Hamilton *et al.*, (1985) tested the biocide substances effects on biofilm *in situ* or in laboratory simulating conditions, using a modified Robbin's mechanism.

Robinson *et al.*, (1984) and Nelson *et al.*, (1985)

used an annular reactor incorporating glass detachable sections (a device submitting the microbial film to high shearing forces) for studying the relationship between the adsorption process of baterial cells, polysaccharides production and growth rate.

Wimpenny *et al.*, (1987) have conceived and built a completely closed experimental system of aseptic manipulation in variable conditions of aeration and nutrient supply, allowing the development of a microbial film of constant thickness. The microbial film develops within the groove of a plate fixed on a disc that rotates slowly under a scraping bar. When the film size exceeds the calibrated depth, the excess will be removed by the scraping bar. In an improved variant, the system consists of a teflon disc on which there are fixed 15 surfaces, each of them having 15 wells where are placed plugs representing the substrates on which the microbial film develops. The plugs are delimitated within their walls by a gasket forming a groove representing the space where the biofilm develops. In this way the thickness of the microbial film may be controlled.

The outer surface of the rotative disc passes under a scraping bar. The main disc is operated by a synchronic motor. The system is completely closed within a glass container and can be sterilized in autoclave. The disc bearing the film is irrigated by a sterile medium also exhibiting entries and exits for gases. The samples can be taken by taking over the discs using a sample-holder mechanisms (Wimpenny *et al.*, 1987).

2. EXPERIMENTAL MODEL

In the area of the studied sulphurous springs, H_2S oxidation and bacterial growth occur in an open system in which H_2S and oxygen concentration vary significantly and the contribution of the chemical or biological factors to the hydrogen sulphide oxidation process is difficult to evaluate. The two substrates are intermittently accessible, even aleatory, their concentration at cell/medium interface, depending on the intensity of water mass movements and the two substrates mixture.

These factors correlate with the emergence of a bacterial biofilm mosaical structure due to the existing microhabitats which are different with regard to the nutrients and redox conditions.

Our experimental model implies a semi-enclosed system within which the two substrates are supplied in counter-current at an interface level representing bacterial growth area. Since the cell growth process depends on the simultaneous presence of the two reactants, bacteria multiplication will occur in the form of a pellicle along the diffusion surface. At the same time the biofilm functioning will prevent the mixing of solutions and chemical oxidation of H_2S.

As the solutions move along the series of growth units (surfaces), they are depleted, then they are re-circulated and re-enriched in substrates. The equipment diagram endows the use of electrodes for recording pH, H_2S, O_2 and T°C values, a device to determine the solutions' flow (Fig. 1).

The growth surfaces are detachable and can be periodically inspected in order to determine the cell biomass or for the quantitative evaluation of other parameters.

At their lower parts the growth surfaces are lined by a porous polymer basal layer allowing the nutrients' diffusion. Within each cell growth element the

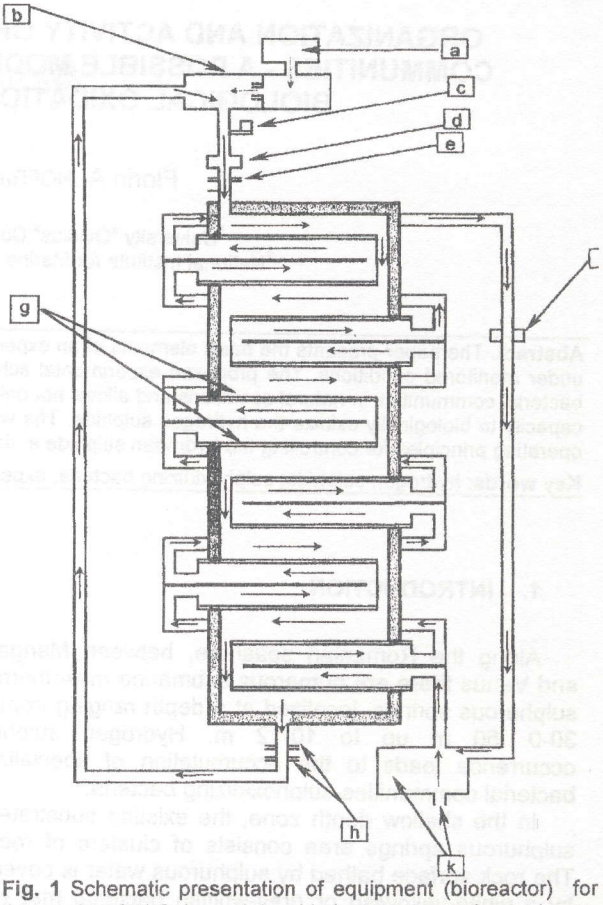

Fig. 1 Schematic presentation of equipment (bioreactor) for the study of H_2S bacterial oxidation: a. room for hydrogen sulphide production; b. reaction room (solubilisation of hydrogen sulphide); c. device for recording of liquid with H_2S flow capacity; d. pump; e. electrode system for recording and control of pH, H_2S, to values at the entry into bioreactor; f. pump; g. element (unity) of nutrition and development of biofilm; h. electrode system for recording pH, H_2S, 0_2, TOC values at the exit from bioreactor; k. room for mixing and re-enrichment of depleted solution.

oxygenated mineral solution (with 0,2% acetate) is directed in counter-flow with the H_2S solution by the aid of a device having also the role to produce a thin liquid layer. The lower part of the growth element is constituted of an impermeable material preventing the mixing of the two solutions (Fig. 2).

As both solutions move away from source, they lose reactants by diffusion; their concentration gradually diminishes towards the extremities of the culture recipient, so that the successive growth surfaces show gradual variations of the redox potential (Fig. 3).

In fact, the growth surfaces placed in close proximity of both sources show opposite redox conditions. Inoculation with a heterogeneous natural sample leads to micro-zoning of the existing populations, depending on the distance from source, and the optimal growth conditions for a certain organism can easily be determined. In the case when the inoculum is represented by a pure culture, one can define the conditions under which the organism produces a maximal biomass.

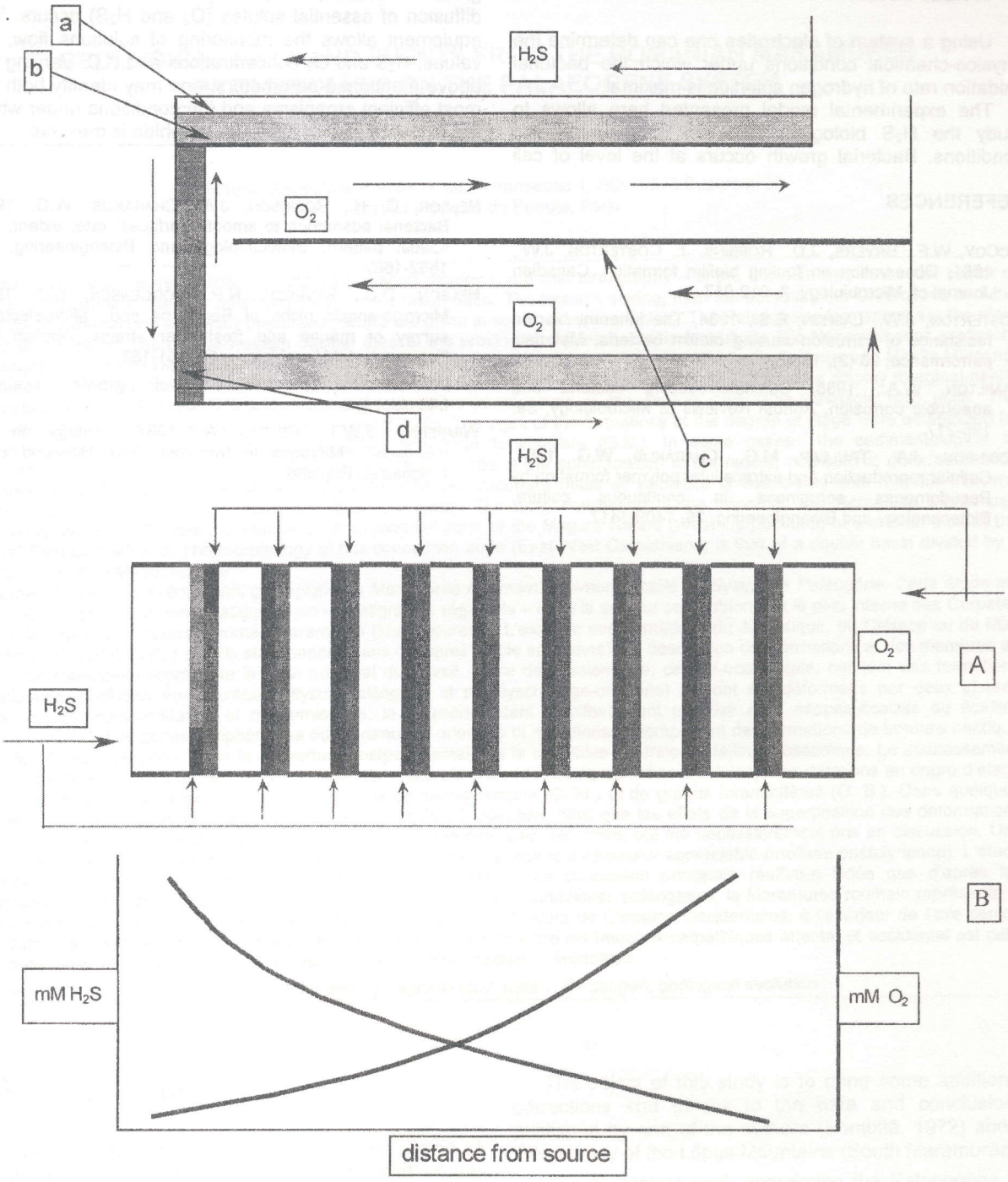

Fig. 2 The functional unity of bioreactor (a. detachable surface of cell attachment and growth; b.basal layer of permeable porous polymer; c. surface for directing the solution; d. impermeable surface.

Fig. 3 A, B. Schematic presentation of theoretical O_2 and H_2S consume at growth units (surfaces) level

3. REMARKS

Using a system of electrodes one can determine the physico-chemical conditions under which the bacterial oxidation rate of hydrogen sulphide is maximal.

The experimental model presented here allows to study the H_2S biological oxidation under monitored conditions. Bacterial growth occurs at the level of cell growth surfaces, where simultaneous counter-flow diffusion of essential solutes (O_2 and H_2S) occurs. The equipment allows the monitoring of solutions flow, pH values, H_2S and O_2 concentrations and t° C. Varying the above mentioned parameters, one may identify both the most efficient organisms and the conditions under which the oxidation rate of hydrogen sulphide is maximal.

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