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BIOCATALYTIC HYDROGEN SULPHIDE REMOVAL FROM GASEOUS STREAMS

G. TÓTH ✉, É. LÖVITUSZ, N. NEMESTÓTHY, K. BÉLAFI-BAKÓ

University of Pannonia, Research Institute on Bioengineering, Membrane Technologies and Energetics,
10 Egyetem Str., 8200 Veszprém, HUNGARY
✉E-mail: tothgabor2@gmail.com

Hydrogen sulphide is one of the most important substances responsible for unpleasant odour emissions in gas phase. It is often formed in higher concentration beyond other sulphur containing volatile compounds like methane thiol (MT), dimethyl sulphide (DMS) and dimethyl disulphide (DMDS). Removal of hydrogen sulphide is usually carried out by physical-chemical methods (e.g. adsorption), but nowadays some bio-processes may be considered as promising alternatives. Certain sulphur oxidising thiobacteria can be successfully applied for hydrogen sulphide conversion from gaseous streams like biogas. Various strains have been applied so far for degradation of hydrogen sulphide, they belong mostly to the group of Thiobacillus, which are autotrophic microorganisms. These autotrophic bacteria have the drawback in application that they grow slower than the heterotrophic ones and it is more difficult to control their growth. A number of chemotrophs are suitable for the biodegradation of H_2S. These bacteria grow and produce new cell material by using inorganic carbon (CO_2) as a carbon source and chemical energy from the oxidation of reduced inorganic compounds such as H_2S.The objective of the work described here was to study the ability of elimination of hydrogen sulphide by two chemotrophic microorganisms (*Thiomonas intermedia, Thiobacillus thioparus*) in a batch bioreactor. The other aim was the study of the immobilization of these bacteria to different supports.

Keywords: hydrogen sulphide, chemotrophic, biological oxidation *Thiomonas intermedia*, *Thiobacillus thioparus*, batch reactor, support

Introduction

Volatile sulphuric compounds like methane thiol (MT), dimethyl sulphide (DMS), dimethyl disulphide (DMDS) are harmful, corrosive components, and present in various gas streams, e.g. biogas [1, 2], among them hydrogen sulphide can be found in relatively high concentration.

The concentration of hydrogen sulphide varies between 0,1 és 2% and depends on the quality of feed substance [3]. Utilisation of biogas is mainly hindered by its hydrogen sulphide content, therefore its removal is essential. The current technologies based on chemical removal are quite expensive, thus usage of biogas in power plants is not supported [4].

Yet, in most cases physico-chemical methods are used. Recently biological processes received some attention due to their effectiveness and low operational and maintenance costs [2], [4]. Biological removal of hydrogen sulphide can be carried out by autotrophic and heterotrophic ways. From the operation point of view heterotrophic strains are more beneficial, but mos chemoautotrophic bacteria seem suitable for degradation of the compound. These bacteria can live using inorganic carbon (e.g. CO2) as carbon source, while energy is obtained by oxidation of reduced compounds (e.g. H_2S) [5].

The aim of this work was to study a suspended batch bioreactor, where chemoautotrophic aerob bacteria (*Thiomonas intermedia, Thiobacillus thioparus*) are able to use carbon dioxide present as carbon source, while hydrogen sulphide is converted into sulphur powder or oxidised sulphur compounds. The investigations were conducted in liquid phase by using free and immobilised cells.

Materials and methods

Microorganisms

Thiomonas intermedia is an aerobic, chemoautotrophic bacterium, purchased in lyophilised state from the National Collection of Agricultural and Industrial Microorganisms (NCAIM Budapest, Hungary). For its growing Thiomonas intermedia broth elaborated by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany) was used [6]. Its composition is (g/L): NH_4Cl 0.1, KH_2PO_4 3.0, $MgCl_2$*6 H_2O 0.1, $CaCl_2$ 0.1, $Na_2S_2O_3$*5 H_2O 5.0, yeast extract 1.0, 1000 ml distilled water. During the growing period pH was adjusted to 5.5–6.0, then it was incubated at 33°C temperature and 120 rpm.

The other, also chemoautotrophic aerobic bacterium is *Thiobacillus thioparus*, which was purchased from DSMZ (Germany) strain collection. Its growing parameters are the same as given in *Thiomonas intermedia*. The features of microorganisms are summarized in *Table 1*.

Table 1: The features of *Thiomonas intermedia* és a *Thiobacillus thioparus*

	Thiobacillus thioparus	*Thiomonas intermedia*
optimal pH	5–9	5–7.5
optimal temperature [°C]	33–35	30–35
cell type	Gram negative	Gram negative
Shape, size	rod, 0.9–1.8 µm	-
Trophity	obligate chemoautotrophic	facultative chemoautotroph
Energy source	Thiosulphate, sulphide	Thiosulphate, sulphide
Oxygen demand	aerob	aerob
Source	[7]	[8]

Analysis

To follow the concentration of hydrogen sulphide in the liquid phase a photometric method was applied. The principle of the measurement is that the sulphide in acidic environment gives a colourful reaction with N,N-dimethyl-p-phenilene diamin resulting in methylene blue, which can be measured by photometer in 670 nm after 20 min reaction time, using 5 cm cuvette (HachLange DR 3800) [9]. The advantages of the method are that easily applicable, having good reproducibility and the measuring range is wide: between 0.1 and 1.2 mg S^{2-}/L.

The composition of the gas phase was determined by Dräger X-am 7000, which is a mobile electrochemical device, able to detect hydrogen sulphide and carbon dioxide.

The experiments were carried out in 250 ml, jacketed, thermostated reactor *(Figure 1)*, where the total volume of the liquid phase was 200 ml. Its composition was: 10% inoculum, 5%, water containing 2500 mg/L hydrogen sulphide and 85% broth. The optical density of the samples were measured by a photometer (HachLange DR 3800), in 620 nm. The biochemical reactions in the reactor resulted in a decrease of sulphide content in the liquid phase.

The water vapour in the gas phase disturbs the optimal operation of the gas analysis equipment, therefore the vapour had to be condensed before. Samples were taken every half an hour during the exponential growing period of the bacterium, otherwise it was taken every hour, and dissolved sulphide concentration and optical density were determined.

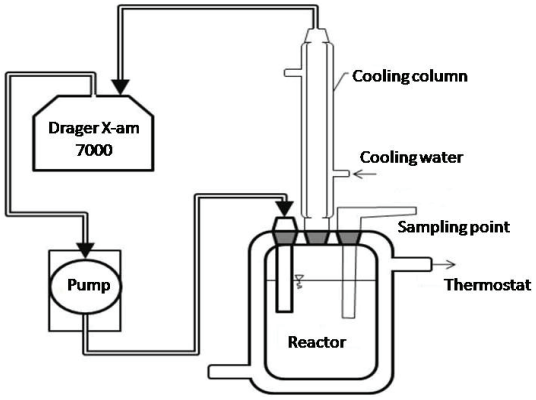


Figure 1: Set-up of the batch reactor

Immobilization

The strains were immobilised in three types of supports and the process was checked in a serial of measurements. MAVICELL-B cellulose beads, alginate beads and granulated activated carbon were used in the experiments.

MAVICELL-B *(Table 2)* is cellulose beads, widely used for immobilization of various microbes, having large adsorption surface area, thus a highly suitable support, moreover it can withstand to the corrosive effect of hydrogen sulphide. The cells can be bound on the surface of the support by adsorption.

Table 2: Features of MAVICELL-B

Feature	
Regenerated cellulose content	45–55%
Ash	35–40%
Particle size	2–3.5 mm
Aggregate thickness	250–300 g/dm^3
water uptake at 25ºC	150–200%
Special pore volume	1.5–2 cm^3/g
Special pore surface area	8–10 m^2/g
Swelling	
increase in diameter	1.5 fold
increase in volume	3 fold

The other support used was alginate beads, widely used in biotechnology for immobilization of cells and enzyme, *(Figure 2)* by the so called entrapment technique. During jellification small hollows are formed in alginate where biocatalysts (enzymes and cells) can be entrapped. The structure of the gel is compatible with the biocatalysts, thus no chemical modification is needed [10].

Finally the granulated activated carbon (GAC) support was purchased from Airwatec s.a. (Belgium).

Figure 2: Alginate beads containing cells

Due to its high surface area it seems also a promising support material for immobilisation of microorganisms. The features are listed in *Table 3*.

Table 3: Features of activated carbon

Parameter	Value
Total surface area (BET) (m^2/g)	1080
pH	7.0
Water content (%)	1,1
Ash content (%)	8.6
Granules Diameter (mm)	1.0

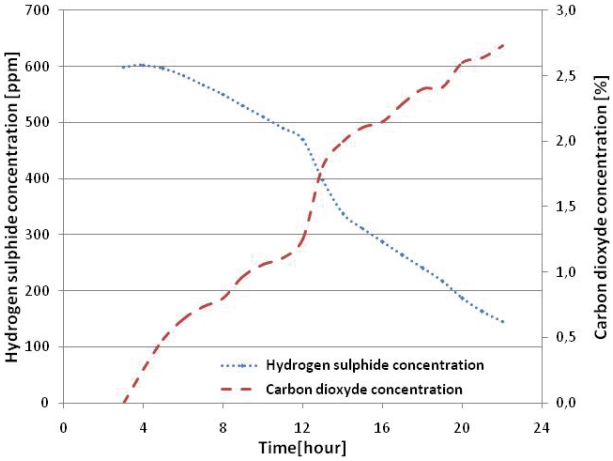
During the experiments immobilised strains were kept in two vessels (for the two strains) and a blind one was used as a control, for all the three types of immobilization. Samples were taken from all vessels regularly, and sulphide contents were determined. Then fresh broth was given to the strains.

In the end of the experiments the amount of immobilised cells (on the surface of MAVICELL and activated carbon particles, as well as in the alginate beads) – or more precisely the protein content – was measured by the modified Folin-method.

Results

Firstly 24 h long batch fermentations were carried out by free *Thiomonas intermedia* and *Thiobacillus thioparus* microbes. The results are shown in *Figure* ***3*** and *Figure* ***4***.

As it can be seen from *Figure* ***3*** and *Figure* ***4*** after a 8–12 h lag phase bacteria started to grow, the exponential growing period was found from around 10th h till 15-16th h, then a the rate of growing was slower. All the four parameters measured confirmed that the maximal growing rate of both bacteria was at the 12–13th h of the fermentations.



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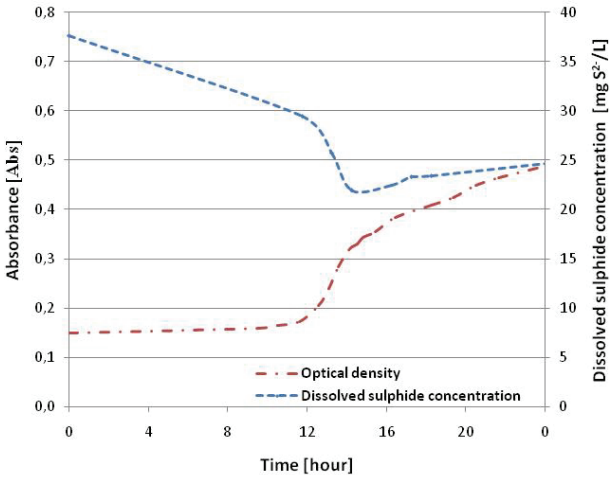
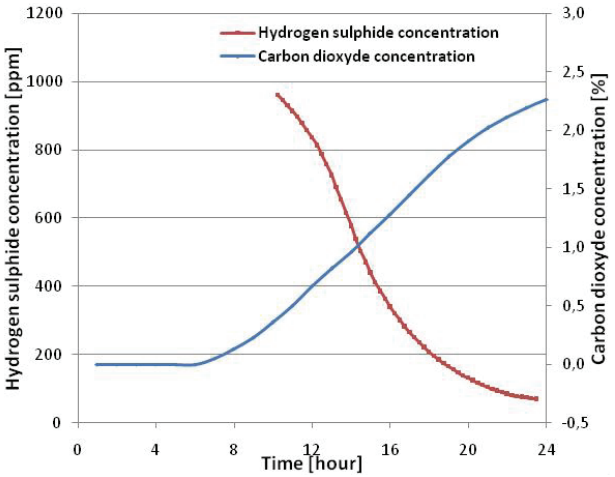


b

Figure 3: Concentration of hydrogen sulphide and carbon dioxide (a), dissolved sulphide and optical density of *Thiomonas intermedia* (b) as a function of time

Moreover it can be established that both *Thiomonas intermedia* and *Thiobacillus thioparus* reduced the concentration of hydrogen sulphide in the reaction mixture, i.e. they mostly obtained energy from degradation of hydrogen sulphide (dissolved sulphide) during the metabolisms.

In both *Figures 3/b* and *4/b* dissolved sulphide concentration as a function of time shows some discrepancy, which is difficult to explain. We think that the analytical method (by photometer) may be disturbed by some nutrient compound present in the liquid phase, therefore another method should be found in future. Another reason of the strange behaviour might be that certain metabolic products were formed during the fermentation which resulted it in the second half of the experiments.



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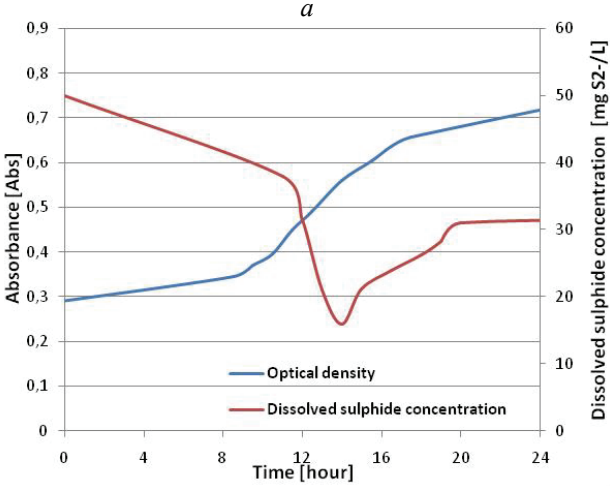


b

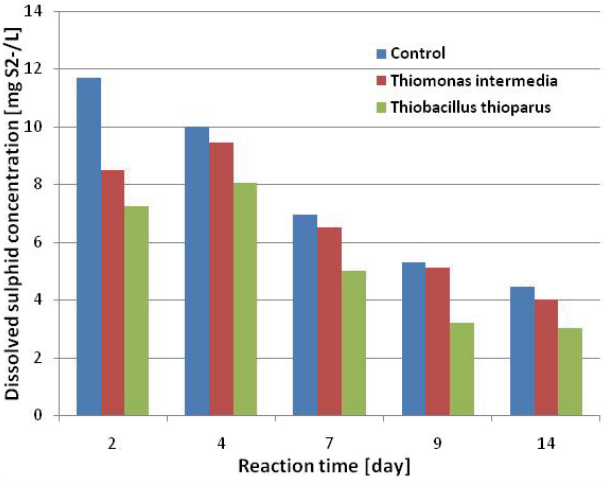
Figure 4: Concentration of hydrogen sulphide and carbon dioxide (a), dissolved sulphide and optical density of *Thiobacillus thioparus* (b) as a function of time

Operational stability in the liquid phase

The bacteria were immobilised in three different support: MAVICELL-B cellulose beads, alginate beads and granulated activated carbon. To check their work they were kept in reaction mixtures, containing nutrients and hydrogen sulphide, and samples were taken regularly. *Figure 5* presents the experimental results: the variation of hydrogen sulphide concentration [mg S^{2-}/L] compared to the control (bind) one as a function of reaction time in cases of MAVICELL-B cellulose beads *(Fig. 5/a)*, alginate beads *(5/b)* and granulated activated carbon *(5/c)*.

It can be observed that a decrease in hydrogen sulphide concentration compared to the blind was measured, due to the activity of the bacteria immobilised on the support.

To check this phenomenon protein content of the supports (measured in the end of the experiments by washing out the microbes from the surface of the support, or – in case of alginate – destroying the gel structure) was determined. The results are summarized in *Table 4*.



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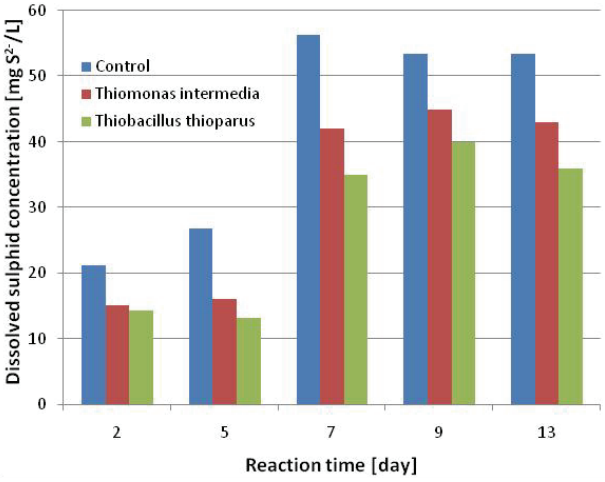

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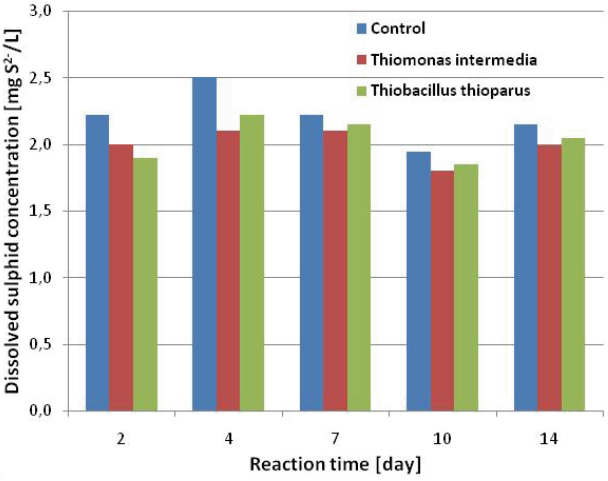

c

Figure 5: Operational stability of bacteria immobilised in MAVICELL-B (a), Ca-alginate (b) and activated carbon granulates (c)

From the *Table 4* it can be seen that the protein measurement confirmed our experimental results: considerable amount of microbes were immobilised on all of the supports. Since bacteria were entrapped in

alginate (not only adsorbed on the surface), much higher protein content was found in that case.

Table 4: The amount of protein (microbes) measured in the various support

	Mavicell-B	Ca-alginate	GAC
Thiomonas intermedia [mg DM/g carrier]	0.12	6.3	0.15
Thiobacillus thioparus [mg DM/g carrier]	1.83	7.1	2.05

It can be stated that the bioconversion processes can be followed by measuring the hydrogen sulphide concentration in the liquid phase, but the photometric determination of the total sulphide content can not be applied here. Based on the protein determination it is obvious that the microbes were able to immobilise in the supports and worked properly to reduce hydrogen sulphide concentration in the liquid phase.

Evaluation

As a summary it can be stated that our experiments proved: the two microbes investigated are suitable for the biological degradation, removal of hydrogen sulphide. However, the analytical method to follow the bioconversions should be improved and we should continue the experiments in gas phase. Based on the successful immobilisation and measurements in liquid phase, the bioreactor system should be redesigned to be able to operate in gas phase and to determine exactly the ability and effectiveness of the bacteria.

Acknowledgement

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