

Microbial hydrogen-sulphide elimination in continuous biotrickling reactor by immobilized *Thiobacillus thioparus*

Gábor Tóth, Éva Lövitusz, Nándor Nemestóthy, Katalin Bélafi-Bakó*

University of Pannonia, Research Institute on Bioengineering, Membrane

Technologies and Energetics

10. Egyetem Str., Veszprém, 8200 Hungary

Tel. +36 (88) 624726, Fax: +36 (88) 624292

E-mail: bako@almos.uni-pannon.hu

Abstract

Nowadays removal of hydrogen sulphide from gaseous streams by biological treatments is a promising alternative procedure, among them biotrickling reactor seems a reliable and efficient system. To maximize the performance, strains should have high hydrogen sulphide elimination efficiency; excellent carriers should be selected where the microbes can be immobilized. In this study various carriers were used as the support medium for the immobilization of *Thiobacillus thioparus* and a continuous biotrickling reactor was constructed and operated for H₂S removal. We found that our systems with Mavicell and Kaldnes supports are able to remove H₂S from the gas mixture with high efficiency (95-100 %), and the specific removal capacity was calculated as a high as 30-40 g S/m³h.

1. Introduction

Biological techniques for hydrogen sulphide elimination can be applied in wide range and seem quite promising in certain circumstances where the aim is to remove „smelly” compounds. Among these components hydrogen sulphide is one of the most important substances, since its smelling limit value is rather low, 0.5-2.0 ppb [1].

Nowadays the biological removal of air pollutants has been studied intensively [2]. One of the intensification methods of these biosystems is the immobilization of the microbes in a form of biofilm, which exploits the natural bounding capability of certain microorganisms on a given surface, thus the pollutants can be eliminated with higher effectiveness [3]. The performance of an immobilized film bioreactor can be enhanced by selection of a proper support material for the given microorganism [4]. The suitable supports provide optimal conditions for the microbes, having high specific surface area [5].

Currently natural support materials including soil, compost, peat ...etc. are often used as media for biofiltration [6]. Although these materials are considered as rather cost effective media, their practical application is still limited [7] mainly due to their aging which causes declining effectiveness. Therefore the novel research tendency leads towards the synthetic materials [8-10], including ceramic saddles polyethylene pall rings, synthetic foams, activated carbon, extruded diatomaceous earth pellets, glass beads and Ca-alginate.

In our experiments Mavicell-B cellulose beads, activated carbon, polyethylene rings (Kaldnes-K1) and alginate beads were used as support materials. All of these supports are synthetic materials, two of them (activated carbon and alginate) have already been studied, but no literature reports on application of Kaldnes-K1 and Mavicell-B have been found so far.

The microbes can be grown onto the surface of the activated carbon, Mavicell and Kaldnes, thus the affinity of the bacteria to the surface area influenced highly the quality and thickness of the biofilm. In case of alginate, however, microbes are entrapped into the alginate beads (known amount of bacteria), thus it does not depend on the surface of the support. Hence the two different immobilization methods can be compared.

Bacteria belonging to the *Thiobacillus* strains have higher hydrogen sulphide elimination efficiency than other sulphide oxidising microorganisms [9, 10]. In our preliminary experiments two colourless sulphur bacteria were studied in a batch system (*Thiomonas intermedia*, *Thiobacillus thioparus*). They were immobilized on three different supports and the operational stabilities were compared [12]. The results have shown that the degradation ability of immobilised *Thiobacillus thioparus* was higher both in soluble and immobilised forms. Therefore the experiments were continued with this bacteria aiming to construct a biotrickling reactor and accomplish a successful continuous system for hydrogen sulphide elimination from gas streams.

2. Materials and methods

2.1. Microorganism

The strain *Thiobacillus thioparus* was purchased from the strain collection of DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany). It was grown on a special *Thiomonas intermedia* broth, its composition is as follows (g/L): NH_4Cl 0.1, KH_2PO_4 3.0, $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ 0.1, CaCl_2 0.1, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5 \text{H}_2\text{O}$ 5.0, yeast extract 1.0, and 1000 ml distilled water [13]. 33 °C temperature and 120 rpm shaking were maintained for incubation under sterile condition. The bacterium used sulphide as an energy source in the multistep oxidation procedure, thus oxidised sulphur compounds are formed, causing acidification of the system [15][16]. To avoid it phosphate buffer was applied to maintain pH at 5.8 (adding 0,356 g K_2HPO_4 to every l broth).

2.2. Immobilisation of the bacteria

50 ml concentrated inocula (its total solid substance, TSS was 0.38 g/L) and 140 ml sterile broth were added to 70 ml sterilized support and it was incubated for 2-3 days. The immobilization was followed by protein determination. The bacteria immobilized on the support was filled into a glass column, thus the experiments were carried out from this point under non-sterile conditions. A “blind” column was used for comparison purposes, its infection was prevented by using 1.5 % sodium benzoate solution.

2.3. Supports used

The first support used was alginate beads, widely used in biotechnology for immobilization of cells and enzymes (Figure 1) 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 [16, 17].

The second support, the granulated activated carbon (GAC) was purchased from Airwatec s.a. (Belgium) (Figure 2). Due to its high surface area it seems also a promising support material for immobilisation of microorganisms [18]. The features are listed in Table 1.

MAVICELL-B (Table 2) is cellulose beads, purchased from Magyar Viscosagyár, (Nyergesújfalú, Hungary) 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.

Finally Kaldnes K1 polyethylene rings (Evolution Aqua, Lancashire, UK) were used as supports (Figure 3), which is often applied in waste water treatment technologies, where biofilms are needed. its length is 7 mm, diameter 10 mm. Since the diameter of the column planned to use as reactor is similar, the rings were splitted into two halves.

2.4. Designing the continuous reactor system

Experiments were carried out in two parallel, same volume columns (Table 3 and Figure 4). The columns can be divided into three parts: the first one is a thermostated (jacketed) reactor space containing the bacteria immobilized on the support; the second part is chilled by a cryostat to remove the water vapour by condensation, while the third part is a thermostated one again. The feed stream (model gas to be separated) is introduced in the bottom of the first part of the column. The hydrogen sulphide concentration was followed by a gas sensor, placed on the top of the column reactors. It is a sensitive sensor, thus it is important to maintain the temperature of the gas stream in the same level, moreover to remove moisture, that's why were the two upper parts built in the system.

Similar gas mixture (same inlet rate: 360 ml/min and H₂S concentration: 80-100 ppm) was introduced to both columns, while a broth was trickling (rate 0.7 ml/min) onto the support packed in the column, which was collected in the bottom of the column and recirculated by a peristaltic pump.

The column was characterized by numerous data, summarized in Table 3. The average reaction time was 200-220 hours.

2.5. Gas mixture used

In the experiments a model gas mixture was used containing 40-44% (v/v) CO₂, 1-2 % (v/v) O₂, 80-100 ppm H₂S and 54-58 % (v/v) N₂.

2.6. Analysis

2.6.1. Following the gas composition

The gas was introduced in the bottom of the column and went through the active support, thus its H₂S concentration decreased. This reduction was followed by a FIGARO TGS 825 sensor placed on the top of the column. These TGS (Taguchi Gas Sensor) type sensors are based on a metal oxide, which are contaminated by some noble metal. These metals – upon heating – can react with the de-oxidising gases present (e.g.: H₂S), which gives a signal in the resistance of the cell. The higher the gas concentration, the lower is the resistance [19]. The measuring range of the sensor is 0-100 ppm and it was calibrated by a Drager X-am 7000 type mobile gas analyzer.

2.6.2. Protein determination

Protein content was determined by the modified Folin method, which gives a blue colour reaction with proteins in alkali media. The reaction took 30 min and the solutions were measured at 720 nm. Calibration was carried out by using BSA protein.

3. Results

3.1. Alginate

As a result of the immobilization procedure, *Thiobacillus thioparus* bacteria were successfully entrapped in the alginate beads, and finally 6.5-7 mg protein / g support

was measured. Alginate beads with the bacteria were packed into the column and experiments were carried out to study the H₂S reduction in the gas stream. However, the H₂S level did not decline, though the protein level has not decreased, indicating that the microbes were still there. It seemed that the structure of the beads has changed, somehow they have lost their water content and the mechanical stability. The volume of the beads decreased and the support was shrinking due to its own weight, losing the majority of the surface area. That's why it did not work properly.

Chang and co-authors have carried out similar experiments [20] with alginate where *Thiobacillus thioparus* was entrapped. They saturated the gas stream with water vapour before introducing it, thus the support packed did not lose its water content and the stability was maintained during operation. In industrial applications, however, the aim is to prepare a stable, easy-to-handle and mechanically strong support and alginate does not seem sturdy enough here.

3.2. Activated carbon

The next support was activated carbon, where the bacteria were immobilized. The results of the experiments in the column are shown in Figure 5. As it can be seen the H₂S was not eliminated properly, the H₂S content in the gas fluctuated randomly (Figure 5a), the operation was not satisfactory, though the presence of the microbes were proven by checking the protein content (Figure 5b). It seemed again that the structure of the support caused the problem. The activated carbon granules were sticking together, they formed plugs (Figure 6) which hindered gas flowing in the

column. These plugs started to rise up slowly, up to the sensor. Thus we found that the support was not suitable for H₂S elimination from the gas.

3.3. Mavicell

Our next support was Mavicell where the bacteria were immobilized. The beads were packed to the reactor and experiments were carried out with circulating the model gas mixture. The results are presented in Figure 7. Starting the gas introduction into the column, the H₂S concentration in the gas stream leaving the trickling bioreactor was declining sharply, and finally it was stabilized at the level of 5 ppm. It means that in the reactor 90-95 % of the H₂S was removed compared to the control column where no microbes were present. The bacteria on the Mavicell support worked effectively, their amount was slightly increased according to the data on protein determination (Figure 7b).

During the steady state operation period the specific removal capacity (productivity) of the column (related to the reactor volume) was calculated as 30 g H₂S/m³h, which is a similar value as Oyarzu'n et al. reported [21] using *Thiobacillus thioparus* immobilized on peat, while Ramírez et al [22] achieved lower specific capacity (14.9 g S/m³h) using polyurethane foam as support, with similar removal efficiency (99,8 %).

3.4. Kaldnes K1 media

In case of Kaldnes K1 support the experimental results were similar to the Mavicell (Figure 8). H₂S concentration in the gas was reduced by the bacteria from the initial 90 ppm down to 0-5 ppm, which means 95-100% removal efficiency. The amount of microbes on the Kaldnes support was 20 % higher than in Mavicell beads (Figure 8b).

The specific removal capacity during the steady state operation was calculated as 35-40 g H₂S/m³h, which is slightly higher than it was in Mavicell. This value is even higher than the result of Eliasa et al. [23], who used a mixture of pig manure and sawdust as support, and reported 28.5 g H₂S/m³h specific capacity with >95% removal efficiency and 40.5 H₂S/m³h specific capacity with >90% removal efficiency.

4. Conclusions

A continuous biotrickling column reactor was designed and operated packed with *Thiobacillus thioparus* bacteria immobilized on various supports (Alginate, activated carbon, Mavicell and Kaldnes) for H₂S elimination from gaseous streams.

Application of Mavicell cellulose beads and Kaldnes K1 polyethylene rings as supports for this colourless sulphur oxidising bacteria has not been reported so far, hence the results obtained were compared to other supports

Our experiments have proven that *Thiobacillus thioparus* bacteria can be immobilized onto these supports, among them Kaldnes K1 was found the best. We believe that our systems with Mavicell and Kaldnes supports are able to remove H₂S from the gas mixture with high efficiency (95-100 %), and the specific removal

capacity was calculated 30-40 g S/m³h, which is similar to the literature data.

Therefore we think that these biotrickling systems are suitable for H₂S elimination from gases. Now the aim is to carry out long-term experiments (operation stability, reliability) using controlled oxygen concentration. Although oxygen should be present in these systems (since the bacteria need it), but its high level is not desirable (methane – oxygen mixture!), therefore in the next set of experiments we will try to lower its level to reach a minimum value where the systems still work properly.

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Figure 1: Alginate beads



Figure 2: Activated carbon granules used

Table 1: The parameters of activated carbon

Parameter	Value
Total surface area (BET) (m ² /g)	1080
pH	7
Water content (%)	1,1
Ash content (%)	8,6
Granules Diameter (mm)	1

Table 2: Features of MAVICELL-B

Feature	Value
Regenerated cellulose content (%)	45-55
Ash (%)	35-40
Particle size (mm)	2-3,5
Aggregate thickness (g/dm ³)	250-300
water uptake at 25 °C (%)	150-200
Special pore volume (cm ³ /g)	1.5-2
Special pore surface area (m ² /g)	8-10
Swelling	
increase in diameter	1,5 fold
increase in volume	3 fold

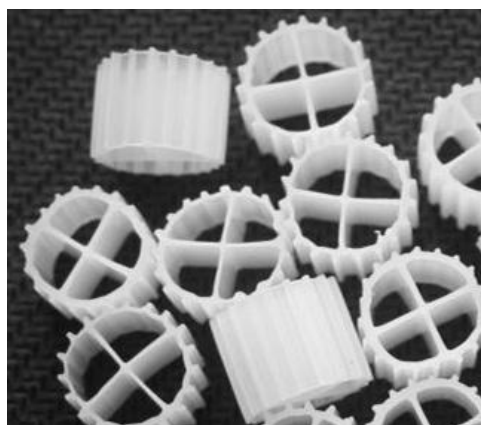


Figure 3: Kaldnes K1 polyethylene rings

Table 1: Features of the bioreactor

Feature	Value
Height of each column (mm)	250
Diameter of each column (mm)	20
Volume of each media (ml)	70
Porosity of Mavicell-B (ml)	25
Gas retention time in Mavicell-B (s)	4,1
Porosity of activated carbon (ml)	15
Gas retention time in activated carbon (s)	2,45
Porosity of beads of alginat (ml)	35
Gas retention time in beads of alginat (s)	5,7
Porosity of Kaldnes K1 media (ml)	36
Gas retention time in Kaldnes K1 media (s)	5,9
Gas flow rate (ml/min)	366
Recirculation of substrate (ml/hour)	36
Surface loading (m ³ /m ² h)	70

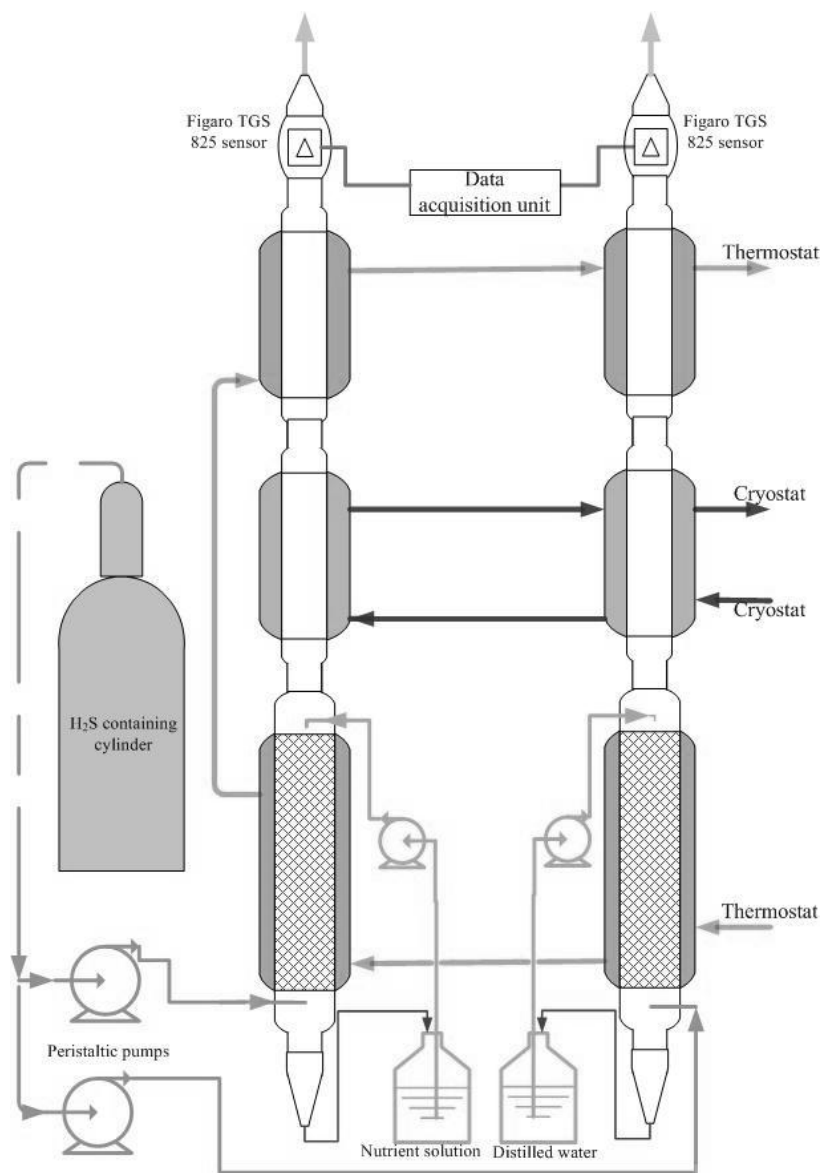
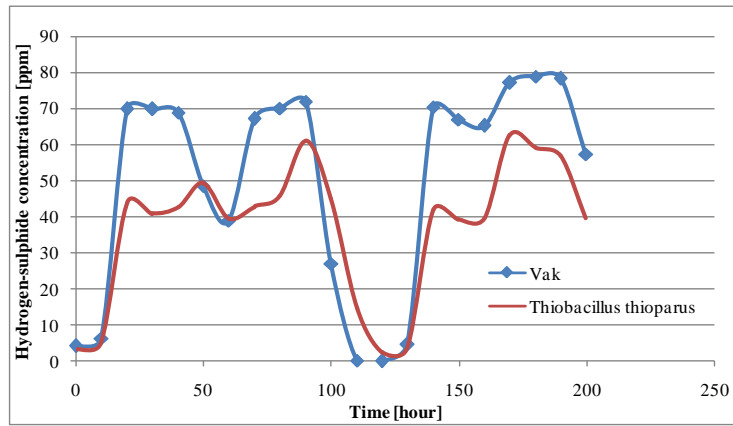
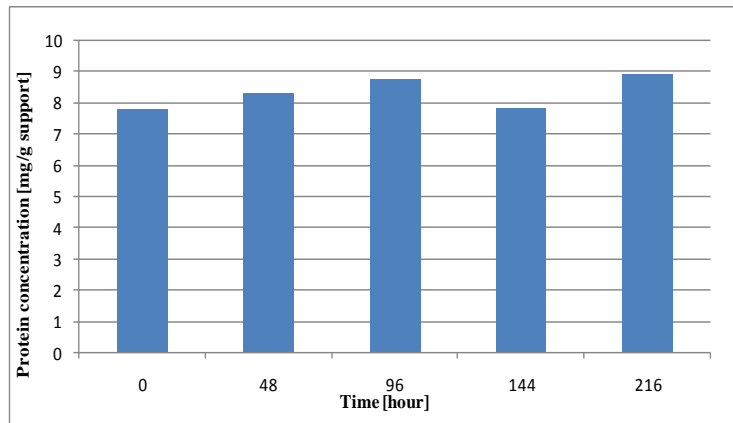


Figure 4: Set-up of the continuous biotrickling column reactor



(a)



(b)

Figure 5: H₂S elimination by *Thiobacillus thioparus* immobilized on activated carbon (a), amount of protein on the surface of the support (b)

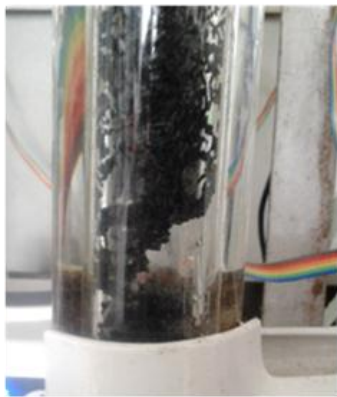
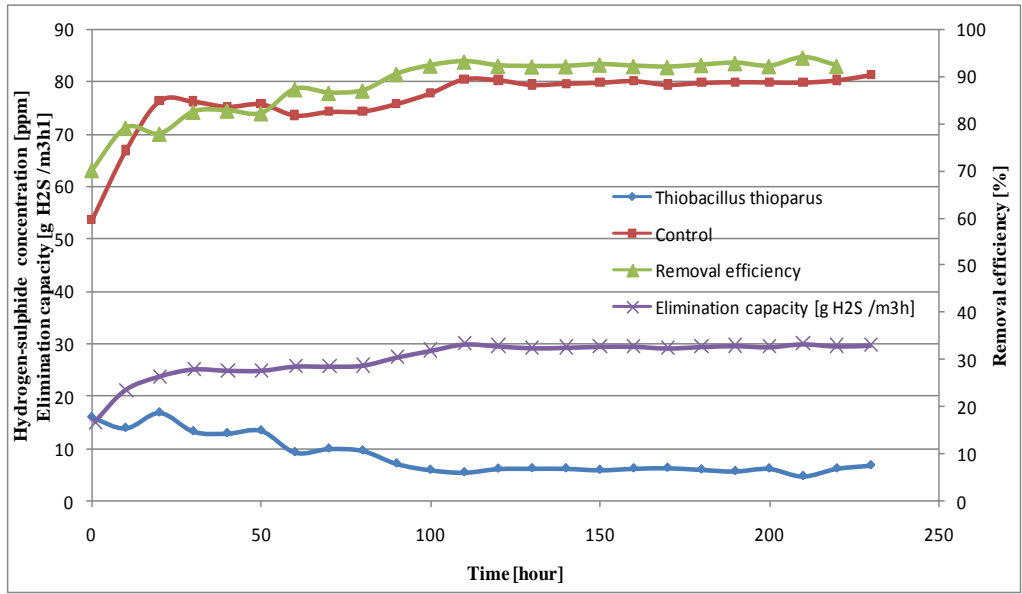
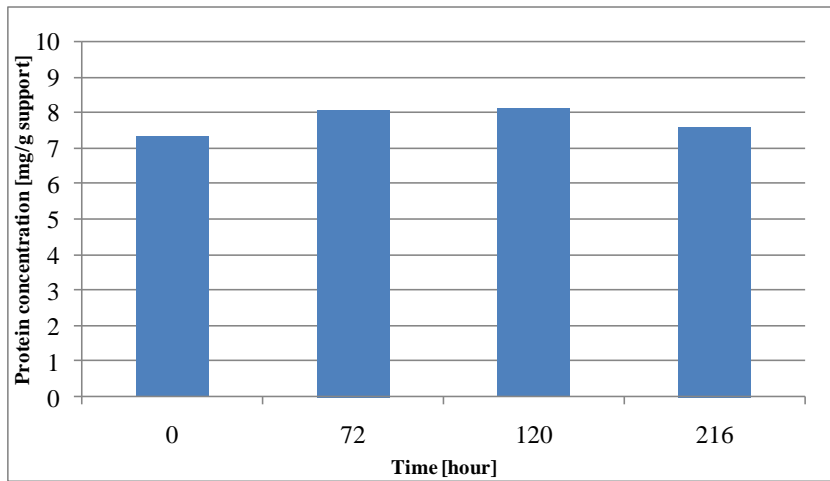


Figure 6: The picture of the activated carbon plug in the column

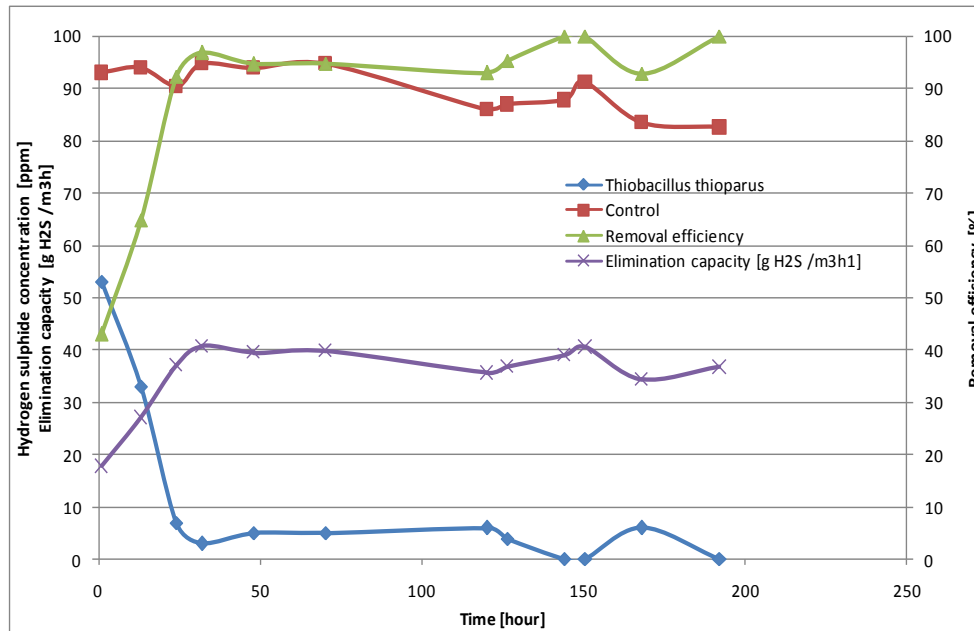


(a)

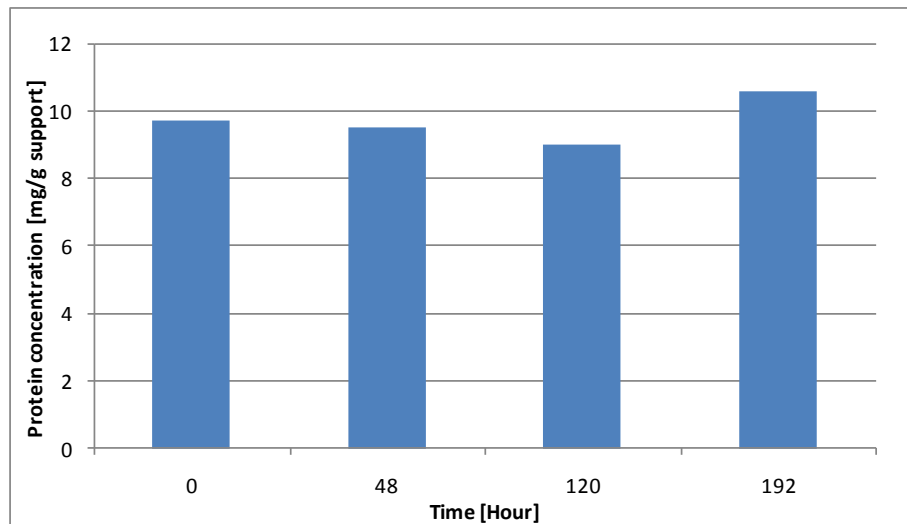


(b)

Figure 7: H₂S removal by the bacteria immobilized on Mavicell B (a), amount of protein on the surface of the support (b)



(a)



(b)

Figure 8: H₂S elimination by *Thiobacillus thioparus* immobilized on activated carbon (a), amount of protein on the surface of the support (b)