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MICROBIAL HYDROGEN SULFIDE ELIMINATION IN A CONTINUOUS BIOTRICKLING REACTOR BY IMMOBILIZED *THIOBACILLUS THIOPARUS*

Elimination of hydrogen sulfide 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 sulfide elimination efficiency; excellent carriers should be selected where the microbes can be immobilized. Various carriers were used as the support medium for the immobilization of *Thiobacillus thioparus* and a continuous biotrickling reactor was constructed and operated for H_2S elimination. It was found that such systems with Mavicell and Kaldnes supports are able to remove H_2S from gas mixtures with high efficiency (95–100%), and the elimination capacity was calculated as a high as 30–40 g S/(m^3·h).

1. INTRODUCTION

Biological techniques for elimination of hydrogen sulfide can be applied in a wide range and seem quite promising in removing malodorous compounds. Among these components hydrogen sulfide is one of the most important substances, since its smelling limit value is rather low, 0.5–2.0 ppb [1]. The biological elimination 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 a 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]. Suitable supports with a high specific surface area provide optimal conditions for the microbes [5].

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Although cost effective natural support materials including soil, compost, peat, etc., are often used as media for biofiltration [6], in practice they are not quite suitable for biotrickling reactors [7]. Synthetic materials [8–10] such as ceramic saddles, polyethylene pall rings, synthetic foams, activated carbon, extruded diatomaceous earth pellets, glass beads and Ca-alginate seem better supports for immobilization of microbes.

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

Microbes can be grown onto the surface of activated carbon, Mavicell B beads and Kaldnes K1 rings, thus the affinity of the bacteria to the surface area influenced highly the quality and thickness of the biofilm. In the 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 quite high hydrogen sulfide elimination efficiency [10–12]. 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 [13]. The results have shown that the elimination 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 sulfide elimination from gas streams.

2. MATERIALS AND METHODS

Microorganism. The strain *Thiobacillus thioparus* was purchased from the strain collection of Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany. It was grown on a special *Thiomonas intermedia* broth, its composition being as follows (g): NH_4Cl 0.1, KH_2PO_4 3.0, $MgCl_2 \cdot 6H_2O$ 0.1, $CaCl_2$ 0.1, $Na_2S_2O_3 \cdot 5H_2O$ 5.0, yeast extract 1.0, and 1000 dm^3 distilled water [14]. Incubation under sterile conditions was maintained at 33 °C and 120 rpm. The bacterium used sulfide as an energy source in the multistep oxidation procedure (where oxygen is needed), thus, causing. To avoid acidification of the system with oxidised sulphur compounds [16, 17], phosphate buffer (0.356 g K_2HPO_4 to every dm^3 of broth) was applied to maintain pH at 5.8.

Immobilization of the bacteria. 50 cm^3 of concentrated inocula (TSS 0.38 g/dm^3) and 140 cm^3 of sterile broth were added to 70 cm^3 of 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 blank column was used for comparison purposes, its infection was prevented by using 1.5 wt. % sodium benzoate solution.

Supports. Alginate beads, widely used in biotechnology, were first applied for immobilization of cells and enzymes (Fig. 1) by the entrapment technique. During jellification, small hollows were formed in alginate where biocatalysts (enzymes and cells) can be entrapped. The structure of the gel was compatible with the biocatalysts, thus no chemical modification were needed [17, 18].

Fig. 1. Alginate beads

Table 1

Parameters of activated carbon granules

Parameter	Value
Total surface area (BET), m^2/g	1080
pH	7
Water content, wt. %	1.1
Ash content, wt. %	8.6
Granule diameter, mm	1

Another support, granulated activated carbon (GAC), was purchased from the Airwatec, s.a. (Belgium) (Fig. 2). Due to its high surface area it seems also a promising support material for immobilization of microorganisms [19]. Its parameters are listed in Table 1. Porous cellulose beads of the particle size of 2–3.5 mm (Magyar Viscosagyár, Nyergesújfalu, Hungary, brand name Mavicell B) were used for immobilization of cells.

Their properties are given in Table 2. Finally, Kaldnes K1 polyethylene rings (Evolution Aqua, Lancashire, UK), often applied in waste water treatment technologies, were used as supports (Fig. 3). The length of the ring was 7 mm, diameter 10 mm. Each ring was split into two halves, since the diameter of the column used was similar.

Fig. 2. Activated carbon granules

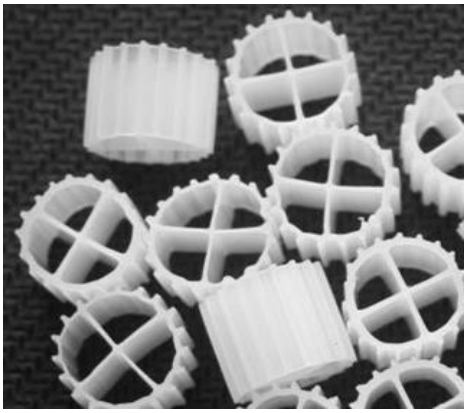

Fig. 3. Kaldnes K1 polyethylene rings

Table 2

Physical properties of Mavicell B beads

Parameter	Value
Regenerated cellulose content, wt. %	45–55
Ash content, wt. %	35–40
Particle size, mm	2–3.5
Aggregate density, g/dm^3	250–300
Water uptake at 25 °C, wt. %	150–200
Specific pore volume, cm^3/g	1.5–2
Specific pore surface area, m^2/g	8–10
Swelling	
increase in diameter	1.5 fold
increase in volume	3 fold

Experimental set-up and operational conditions. Experiments were carried out in two parallel, similar volume columns (Fig. 4, Table 3). Each column consisted of a thermostated (jacketed) reactor containing the bacteria immobilized on the support, a cryostat to remove water vapour by condensation, and another thermostated part.

The feed stream (model gas to be separated) was introduced in the bottom of the column. The hydrogen sulfide concentration was controlled by a gas sensor, placed on the top of the column reactors. Due to high sensitivity of the sensor, it was important to

maintain the temperature of the gas stream at a constant level, and to remove moisture, in two upper parts specially built in the system.

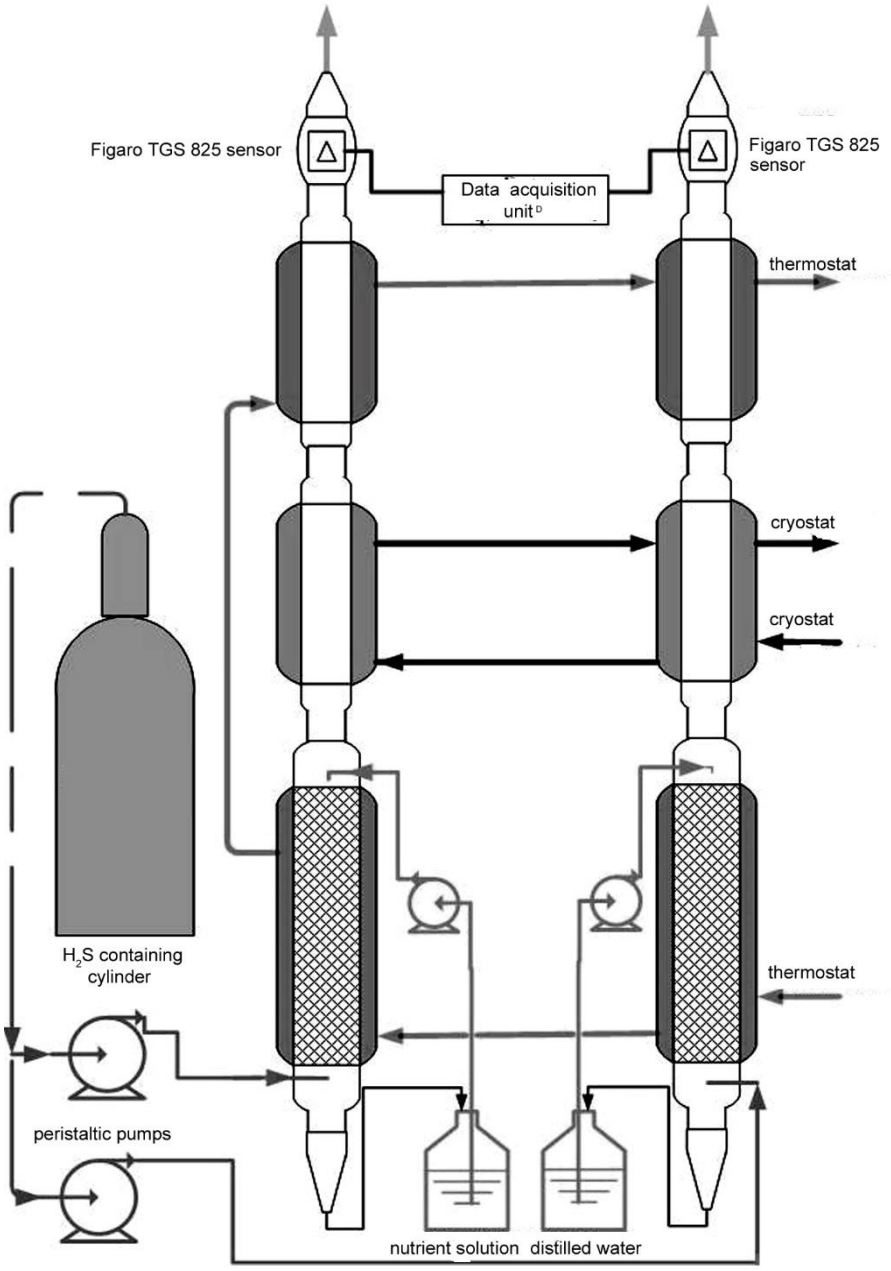

Fig. 4. Set-up of the continuous biotrickling column reactor

Table 3

Parameters of the bioreactor

Parameter	Value
Column height, mm	250
Column diameter, mm	20
Column volume, cm^3	70
Free volume in the packed column (Mavicell B), cm^3	25
Gas residential time in Mavicell B, s	4.1
Free volume in the packed column: activated carbon, cm^3	15
Gas residential time in activated carbon, s	2.45
Free volume in the packed column: beads of alginate, cm^3	35
Gas residential time in beads of alginate, s	5.7
Free volume in the packed column (Kaldnes K1), cm^3	36
Gas residential time in the Kaldnes K1 media, s	5.9
Gas flow rate, cm^3/min	366
Recirculation of substrate, cm^3/h	36
Surface loading, $m^3/(m^2 \cdot h)$	70
Volumetric mass loading rate of H_2S, $g/(m^3 \cdot h)$	48–50

Similar gas mixtures (H_2S concentration 80–100 ppm) were introduced to both columns at the same inlet rates of 360 cm^3/min, while the broth was trickling through the support packed in the column at the rate of 0.7 cm^3/min. The broth was collected in the bottom of the column and re-circulated with a peristaltic pump. The bioreactor operation time was 200–220 hours.

A model gas mixture containing 40–44 vol. % of CO_2, 80–100 ppm of H_2S, 56–60 vol. % of N_2, and 1–2 vol. % of O_2 was applied. In the model of low quality biogas, nitrogen was used instead of methane. The pressure in the container was 0.1 MPa and a constant flow rate was maintained by means of a peristaltic pump.

Inlet gas composition. The gas introduced at the bottom of the column flowed through the active support, thus H_2S concentration decreased. This reduction was monitored by a FIGARO TGS 825 sensor placed on the top of the column [20]. The measuring range of the sensor was 0–100 ppm. It was calibrated by a Drager X-am 7000 type mobile gas analyzer.

Protein determination. Protein content in the support was determined by the modified Folin method (all reagents were purchased from Reanal, Hungary) [21]. The protein was removed from the support by alkali washing pre-treatment. The reaction took 30 min and the solutions were analysed spectrophotometrically at 720 nm. Calibration was carried out by using BSA protein.

3. RESULTS

3.1. PERFORMANCE OF REACTOR FILLED WITH 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 obtained. Alginate beads with the bacteria were packed into the column to study the H_2S reduction in the gas stream. However, the H_2S level did not decrease, indicating that the microbes were still there. It seems that the structure of the beads has changed, somehow they have lost their water holding capacity and 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.

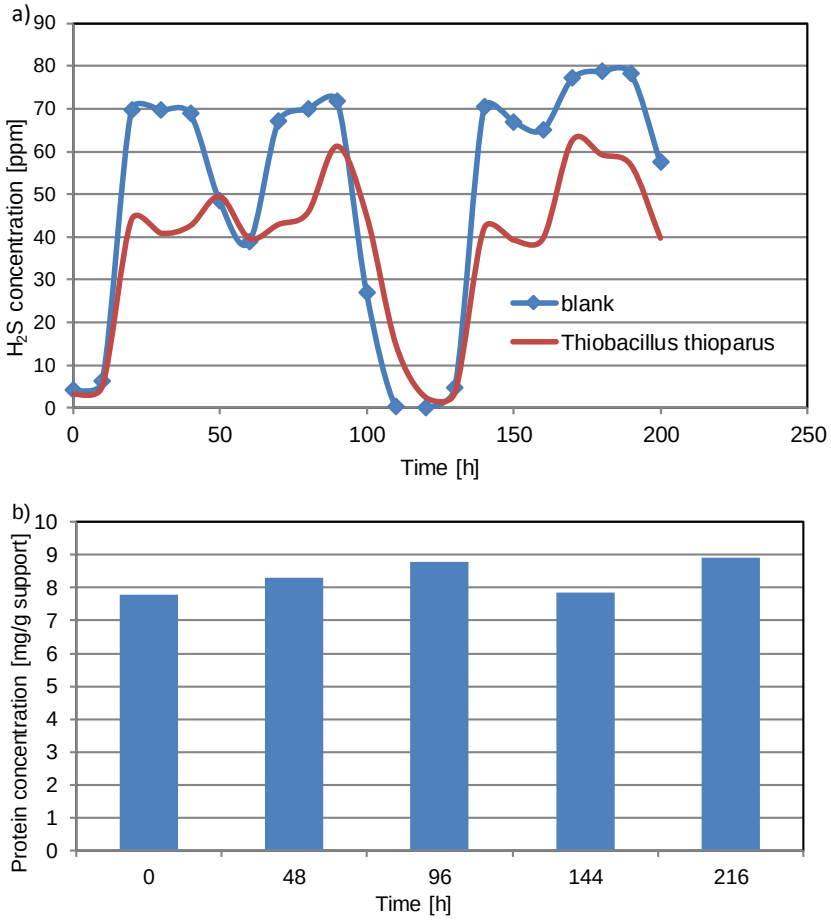

Fig. 5. H_2S elimination by *Thiobacillus thioparus* immobilized on activated carbon (a), and protein content inof the support (b)

Chung at al. carried out similar experiments [22] with alginate entrapping *Thiobacillus thioparus*. 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. PERFORMANCE OF A REACTOR FILLED WITH ACTIVATED CARBON

The results of the experiments with the use of activated carbon are shown in Fig. 5. As can be seen, H_2S was not eliminated properly. Its content in the gas fluctuated randomly (Fig. 5a), the operation was not satisfactory, though the presence of the microbes were proven by checking the protein content (Fig. 5b).

The structure of the support might be the source of the problem. The activated carbon granules were sticking together, they slowly formed plugs (Fig. 6) which hindered gas flowing through the column. These plugs started to increase, up to the sensor. Thus we found that the support was not suitable for elimination of H_2S from the gas.

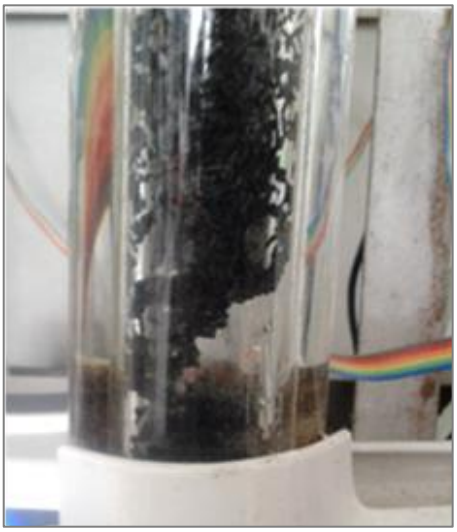

Fig. 6. Activated carbon plug in the column

3.3. PERFORMANCE OF A REACTOR FILLED WITH MAVICELL B BEADS

The results of the experiments using Mavicell B beads are presented in Fig. 7. After supplying the column with the gas, the H_2S concentration sharply decreased, and finally it was stabilized at the level of 5 ppm. This means that in the reactor 90–95% of H_2S was removed compared to the blank (control) column. The bacteria on the Mavicell B

support worked effectively. Their amount was slightly increased according to the data on protein content (Fig. 7b).

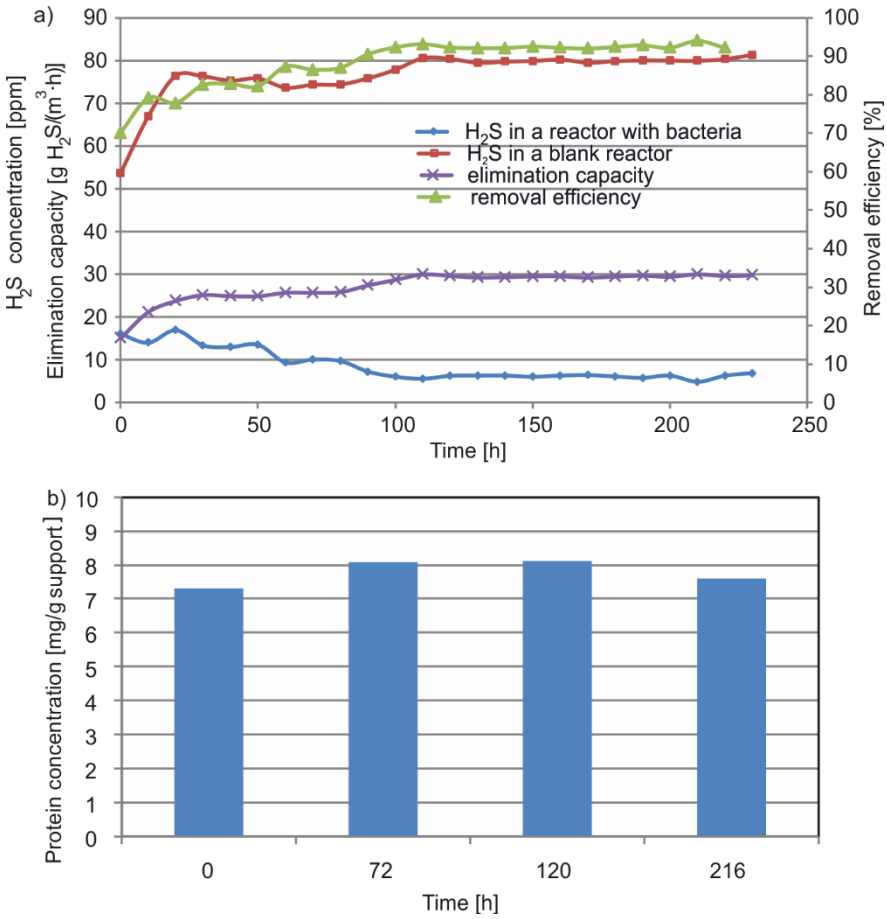

Fig. 7. H_2S elimination by the bacteria immobilized on Mavicell B beads (a), and protein content in the support (b)

During the steady state operation period, the elimination capacity of the column (related to the reactor volume) was calculated as 30 g $H_2S/(m^3 \cdot h)$, which is a similar value as Oyarzun et al. reported [23] using *Thiobacillus thioparus* immobilized on peat, while Ramírez et al. [24] achieved lower elimination capacity (14.9 g $H_2S/(m^3 \cdot h)$ using polyurethane foam as a support, with a similar elimination efficiency (99.8%).

3.4. PERFORMANCE OF A REACTOR FILLED WITH KALDNES K1 MEDIA

The results for Kaldens K1 support were similar to those for the Mavicell B beads (Fig. 8). H_2S concentration in the gas was reduced by the bacteria from the initial 90 pm down to 0–5 ppm, which corresponds to 95–100% elimination efficiency. The amount of microbes on the Kaldnes K1 rings support was by 20% higher than that in the Mavicell B beads (Fig. 8b).

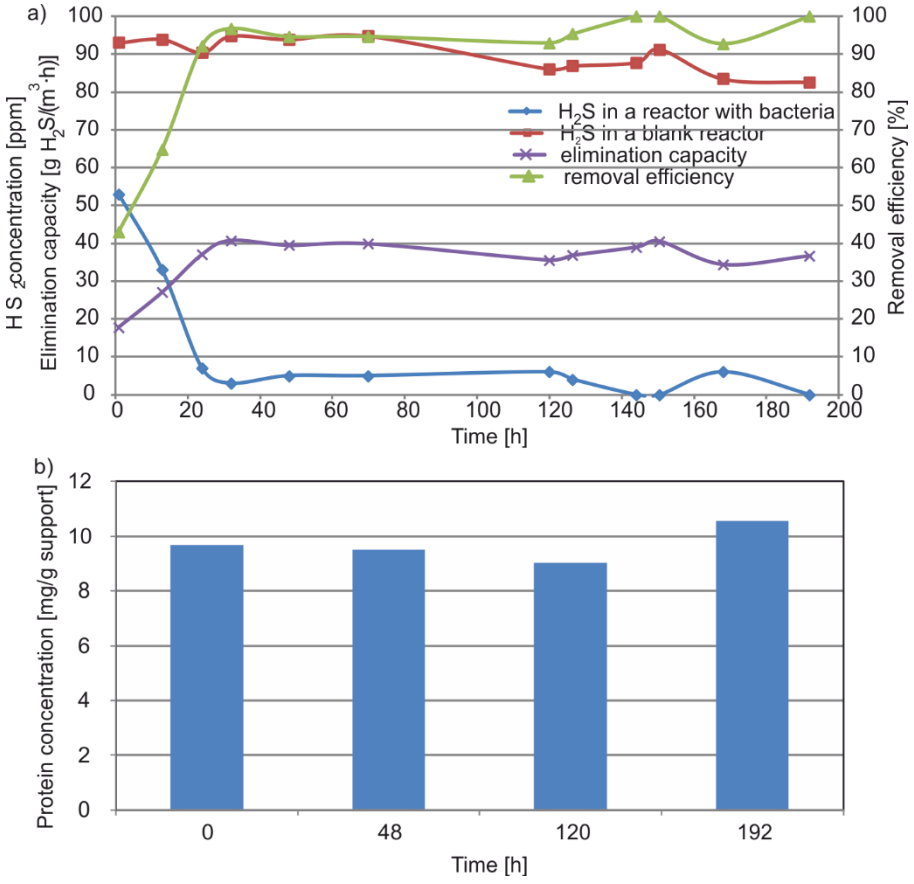

Fig. 8. H_2S elimination by *Thiobacillus thioparus* immobilized on Kaldnes K1 media (a), and protein content of the support (b)

The removing capacity during the steady state operation was calculated as 35–40 g $H_2S/(m^3 \cdot h)$, which is slightly higher than it was for Mavicell B beads. This value is even higher than the result of Eliasa et al. [5], who used a mixture of pig manure and sawdust as a support, and reported 28.5 g $H_2S/(m^3 \cdot h)$ elimination capacity with higher than 95%

elimination efficiency and 40.5 g $H_2S/(m^3 \cdot h)$ elimination capacity with higher than 90% elimination 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 B beads, and Kaldnes K1 rings) for H_2S elimination from gaseous streams. Application of Mavicell B cellulose beads and Kaldnes K1 polyethylene rings as supports for this colourless sulphur oxidising bacteria has not been reported so far.

The experiments have proven that *Thiobacillus thioparus* bacteria can be immobilized onto these supports, Kaldnes K1 rings being found the best. The presented systems with Mavicell B and Kaldnes K1 supports are able to remove H_2S from the gas mixture with high efficiency (95–100%), and the elimination capacity was calculated as 30–40 g $H_2S/(m^3 \cdot h)$, which is similar to the literature data. It can be concluded that these biotrickling systems are suitable for H_2S elimination from gases. To improve the system and to step towards industrial utilisation further study is needed, involving long-term experiments and optimization of oxygen concentration.

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