International Biodeterioration & Biodegradation 105 (2015) 185–191

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Degradation of hydrogen sulfide by immobilized *Thiobacillus thioparus* in continuous biotrickling reactor fed with synthetic gas mixture

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ARTICLE INFO

Article history:
Received 15 July 2015
Received in revised form
8 September 2015
Accepted 9 September 2015
Available online 21 September 2015

Keywords:
T. thioparus
Microaerobic
Packed bed reactor
H_2S elimination
Biogas

ABSTRACT

In this work, a sulfur-oxidizing bacteria, *Thiobacillus thioparus* (immobilized on Mavicell B support) was employed to develop a microaerobic, biotrickling filter reactor for the efficient elimination of H_2S from synthetic (bio)gas. To test the capability of this particular strain in oxygen-limited atmosphere, fixed bed reactor was operated under 0.25–5 vol.% O_2 concentrations and its H_2S decomposing ability was statistically evaluated. It was found that the system achieved 100% H_2S elimination efficiency when at least 2.5 vol.% oxygen was provided. Further decrease of O_2 levels to 0.25–1 vol.% cut the reliability and caused the loss of H_2S biodegradation performance. The results of this study contributed to understand the behavior of *T. thioparus* under microaerobic conditions and thus may help to design efficient gas purification processes for biogas technology.

1. Introduction

Anaerobic digestion (AD) is one of the biological methods to treat and valorize hazardous wastewaters, sludges, agricultural as well as industrial residues (e.g. expired food) in order to recover biogas, which is an important renewable energy carrier (Weiland, 2010) Hence, organic waste processing technologies attached with AD can meet the requirements of simultaneous waste degradation and energy generation, which contributes to sustainability.

In the course of AD, complex biochemical reactions take place inside the fermenter, which are catalyzed by diverse microbial communities fulfilling different functions such as hydrolysis, acidogenesis and methanogenesis. In the end, biogas is obtained as a gaseous phase end-product. Biogas is a multi-compound mixture consisting mainly of methane and carbon dioxide. Nevertheless, biogas may also contain several other, contaminating substances. Although these impurities are mostly found in trace quantities, their presence is a challenging factor for the effective biogas utilization. Among these disadvantageous compounds, malodorous hydrogen sulfide is a significant one (Wu et al., 2015).

H_2S is primarily formed via the anaerobic decomposition of sulfur-containing proteins and amino acids e.g. cysteine (Landaud et al., 2008). The composition of biogas, and hence the concentration of H_2S in it are dependent on the technological specifications (including the type of the feedstock and the fermentation conditions) and may vary even under quasistationary circumstances (Deublein and Steinhauser, 2010; Ryckebosch et al., 2011). However, in many cases, the amount of hydrogen sulfide present in the raw biogas (normally up to 1000–2000 ppm) is high enough to cause severe technological difficulties and failures over time. For example, the corrosion of the combustion engines can occur due to the generation of SO_2 and sulfuric acid, when biogas is utilized for energy valorization. Therefore, to ensure long-term operation and extended lifetime of devices, certain purification methods are recommended to get in advance rid of this hazardous and toxic gas component (Diaz et al., 2010; Ryckebosch et al., 2011).

For biogas upgrading, various end-of-pipe approaches have been widely employed (Bauer et al., 2013). In the line of opportunities, microbiological processes were proven attractive and promising for scaled up implementations because of their reliable performance and relatively low maintenance/operational expenditures (Tchobanoglous et al., 2003; Szentgyörgyi, 2010a). The H_2S can be eliminated by means of specific, sulfide-oxidizing microbes that catalyze the fundamental reaction of Eq. (1).

$$2H_2S + O_2 \rightarrow 2S + 2H_2O \quad (1)$$

The oxygen demand of the process is around 2–6 vol.% and can

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http://dx.doi.org/10.1016/j.ibiod.2015.09.006
0964-8305/

provide sufficiently high H_2S elimination capacity (Jensen and Webb, 1995; Ryckebosch et al., 2011). In reality, depending on the metabolism of microbes and the actual oxygen concentration, more oxidized forms of sulfur (e.g. thiosulfate, sulfite, sulfate) can be discharged besides elementary sulfur (Chung et al., 1997; Duan et al., 2005; Tang et al., 2009).

Although the aerobic, biogas-desulfurization tower reactors are considered quite advantageous, the N_2 content of the treated gas can be an issue since NO_x, as environmental pollutants can be formed when biogas is burnt in the engines. The appearance of molecular N_2 gas is attributed to the oxygen demand of the technology, which, in general, is satisfied by air supplementation. As it is well-known, air is composed of approximately 21 vol.% O_2, while the rest is almost exclusively nitrogen (~78 vol.%). Unfortunately, the inert content represented by nitrogen is not easy to remove and the separation of CH_4 and N_2 is still a challenging task (Lokhandwala et al., 2010). Thus, the presence of N_2 in considerable amounts limits the upgrading of biogas into biomethane and restricts the injection to the natural gas pipeline. Therefore, biological H_2S-elimination systems for biogas upgrading should be further improved to make them more viable and more competitive with the physico–chemical processes (Elias et al., 2002; Oyarzún et al., 2003). This can be done by retrofitting biological H_2S-eliminators that may involve (i) the reconsideration of bioreactor design and (ii) the careful selection of the whole cell biocatalysts.

As for better biocatalyst candidates, there are certain sulfur-oxidizing microbes that can work in oxygen-limited atmosphere or in other words, under microaerophilic conditions, when the O_2 concentration in the gaseous phase does not exceed 1 vol.% (Ramos et al., 2013, 2014). The apparent benefit of oxygen-lean biological desulfurization is that it could lead to lowered N_2 (inert) content in the off-gas of the reactor and thus, such process would be more viable for biomethane (a renewable natural gas substitute) production or for internal combustion engines to generate electricity from biogas.

In previous works, the upstream-side of AD processes has been thoroughly investigated to produce biogas (Szentgyörgyi et al., 2010a,b). In this study, it was aimed to focus on the downstream-side of the technology by designing and constructing a packed bed, efficient biogas-desulfurization reactor. For this purpose a colorless sulfur bacteria, *Thiobacillus thioparus* was applied in long-term experiments with model gas mixtures. This strain was already applied in previous investigations (Kuenen and Beudeker, 1982; Chung et al., 1996; Oyarzún et al., 2003; Ramírez et al., 2009). However, to the authors' knowledge, it has been hardly examined under strongly-oxygen limited, microaerobic conditions. Therefore, reporting on the experiences with such systems could facilitate the knowledge transfer from academic to industrial entities and consequently, may be useful to develop novel applications for future AD technologies.

2. Materials and methods

2.1. Microorganism

In this study, an obligately chemolithotrophic bacteria, *T. thioparus* (purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) was applied. It was maintained in 70% glycerol at −80 °C. The bacteria was periodically grown on Petri plates using the following medium under aerobic conditions (g l^{-1}): 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; agar 15.0; 1000 cm^3 distilled water. The dishes were incubated at 33 °C and the fresh colonies were picked up to prepare the inoculum. Obligate chemolithotroph microorganisms are dependent on reduced inorganic substances as energy sources, however, it has been demonstrated that a variety of organic compounds may be utilized during cell growth (Kuenen and Veldkamp, 1973). This is the reason why the medium for culture maintenance and inoculum preparation was supplemented with yeast extract.

2.2. Inoculum preparation

The inoculum was prepared aerobically in a 500 ml flask (working volume 300 ml) containing the medium described in Section 2.1 (except agar). The pH was set to 5.5–6 and subsequently the flask was placed in an agitator at 120 rpm and 33 °C for 48 h. Prior to immobilization and reactor start-up, the optical density (OD_{600}) in the obtained inoculum was determined (g dry cell weight l^{-1}) by a spectrophotometer (PG Intruments, model T80).

2.3. Biocatalyst immobilization

In this work, *T. thioparus* was immobilized on Mavicell B, which is a porous, cellulose-based support material (Zoltek Companies Inc., USA; formerly known as Viscosa Company in Nyergesújfalu, Hungary), having the characteristics listed in Table 1. The immobilization procedure was as follows: 60 cm^3 sterilized Mavicell B carrier, 140 cm^3 sterilized nutrient solution and 50 cm^3 (OD_{600}: 0.35 g dry cell weight l^{-1}) fresh inoculum was mixed under laminar air flow. Afterward, the flask was incubated at 33 °C for 2–3 days to achieve sufficient biofilm attachment on the bead.

2.4. The experimental procedures

The H_2S-elimination tests were performed in a continuously operated, microaerobic reactor (Fig. 1). To start the reactor, as a first step, certain amount of Mavicell B beads – pre-colonized by *T. thioparus* – was loaded to the column reactor (Table 2). From that point onwards, sterile conditions were no longer maintained. During the start-up period (roughly 10 days), firstly – before the long-term H_2S elimination tests – the reactor was operated to promote biofilm growth and to get the bacteria acclimatized to the experimental circumstances.

As it can be seen in Fig. 1, the reactor was coupled with a gas delivery system, employing separate pumps for each gas. It was responsible to mix H_2S (substrate), O_2 (substrate), CO_2 (carbon source), and N_2 (as CH_4 replacement for safety reasons) and ensure the synthetic feed (bio)gas with proper composition (Table 2). CO_2 (99.99 vol.%) and N_2 (99.99 vol.%) were supplied from individual gas storage tanks stored at ambient (20 °C) temperature, while O_2 came from air. H_2S was generated via reacting FeS (technical grade, Sigma–Aldrich) with HCl (37%, Sigma–Aldrich), diluted with N_2 and collected in a 30 L volume gas bag. From this balloon, high H_2S-content gas with appropriate flow rate was sucked by a pump (model: Masterflex L/S digital drive, Cole–Parmer®) to mix and dilute with the other gases and achieve target H_2S concentration in the feed. The overall gas feeding rate was checked by a flowmeter. Similarly to this work, synthetic biogas was used by other studies to

Table 1
The characteristics of Mavicell B carrier.

Cellulose content – (m/m)%	45–55
Moisture content – (m/m)%	10–15
Ash content – (m/m)%	35–40
Bead diameter – (mm)	2–3.5
Aggregate density – (g dm^{-3})	250–300
Specific pore volume – (cm^3 g^{-1})	1.5–2
Specific surface area – (m^2 g^{-1})	8–10