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Photoautotrophic removal of hydrogen sulfide from biogas using purple and green sulfur bacteria

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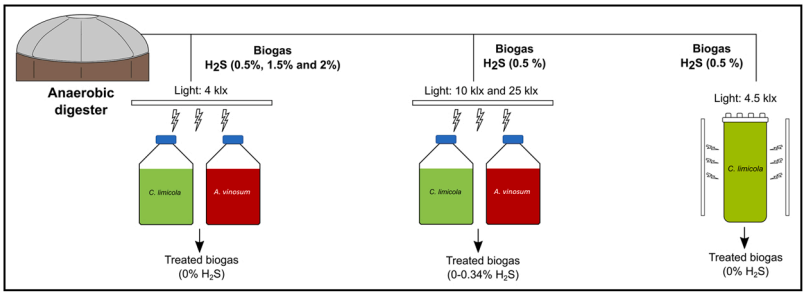
HIGHLIGHTS

- Purple and green sulfur bacteria were effective in removing H_2S.
- Tests under lower light intensity experienced higher growth and removal capacity.
- Increased H_2S content led to higher removal rates, but slower bacterial growth.
- H_2S removal efficiency of 100 % recorded during photobioreactor operation.

GRAPHICAL ABSTRACT



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ABSTRACT

Biogas desulfurization based on anoxygenic photosynthetic processes represents an alternative to physico-chemical technologies, decreasing the risk of O_2 and N_2 contamination. This work aimed at assessing the potential of *Allochromatium vinosum* and *Chlorobium limicola* for biogas desulfurization under different light intensities (10 and 25 klx) and H_2S concentrations (1 %, 1.5 % and 2 %) in batch photobioreactors. In addition, the influence of rising biogas flow rates (2.9, 5.8 and 11.5 L d^{-1} in stage I, II and III, respectively) on the desulfurization performance in a 2.3 L photobioreactor utilizing *C. limicola* under continuous mode was assessed. The light intensity of 25 klx negatively influenced the growth of *A. vinosum* and *C. limicola*, resulting in decreased H_2S removal capacity. An increase in H_2S concentrations resulted in higher volumetric H_2S removal rates in *C. limicola* (2.9–5.3 mg L^{-1} d^{-1}) tests compared to *A. vinosum* (2.4–4.6 mg L^{-1} d^{-1}) tests. The continuous photobioreactor completely removed H_2S from biogas in stage I and II. The highest flow rate in stage III induced a deterioration in the desulfurization activity of *C. limicola*. Overall, the high H_2S tolerance of *A. vinosum* and *C. limicola* supports their use in H_2S desulfurization from biogas.

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1. Introduction

Energy production from the burning of fossil fuels such as coal, oil and their derivatives represent limited energy resources worldwide. Biogas production has gained traction in recent years as a renewable energy source and easy production from numerous wastes by anaerobic digestion (Chynoweth et al., 2001). Biogas is composed mainly of CH_4 (40–75 %) and other gases such as CO_2 (15–60 %), H_2S (0.005–3 %), nitrogen and oxygen in a low proportion (less than 2 %) (Ángeles et al., 2020). According to the European Biogas Association, biogas production in the European Union (EU) could increase from 16.6 Mtoe in 2019 to 28.8 or even 40.2 Mtoe by 2030, which represents 2.7–3.7 % of the estimated energy consumption of the EU in 2030 (Scheuer et al., 2017; EurObserv'ER, 2020). Biogas upgrading is required to meet EU quality biomethane standards reducing the amount of CO_2 and H_2S present in the biogas for use as vehicle fuel or injection into the natural gas grid, or even for use in combined heat and power generation (Prussi et al., 2020). For gas grid injection, standards are strict, with concentrations of CH_4 > 80–96 %, CO_2 2–3 %, O_2 0.2–0.5 %, H_2S 5 mg m^{-3}, NH_3 3–20 mg m^{-3}, and siloxanes 5–10 mg m^{-3} (Muñoz et al., 2015). Hydrogen sulfide (H_2S) is one of the main biogas contaminants, which presence is undesirable due to its toxicity and corrosive nature. In presence of water, H_2S rapidly oxidizes to sulfuric acid, causing corrosion of metals, which damages reactors, storage tanks, compressors, engines and pipelines (Allegue and Hinge, 2014; Hurse et al., 2008). Biogas can be desulfurized using physicochemical and biological methods. However, physicochemical methods exhibit higher energy consumption and involve the generation of additional waste compared to their biological counterparts (Allegue and Hinge, 2014). Biological methods can overcome these drawbacks while bringing additional benefits from their environmental friendliness and potential sulfur recovery. Biological biogas desulfurization can be performed using chemolitotrophic and photosynthetic bacteria with high removal efficiencies (Struk et al., 2020).

Chemolitotrophic bacteria are a group of microorganisms which obtain energy from inorganic sulfur compounds such as sulfide, elemental sulfur, thiosulfate, or organic sulfur compounds. Oxygen is used as an electron acceptor by chemolitotrophic bacteria for the aerobic biodegradation of sulfide, while some species can use alternative electron acceptors (e.g., nitrate or nitrite) under anoxic conditions (Tang, Baskaran and Nemati, 2009). Chemolitotrophs are used in biofilters, biotrickling filters and bioscrubbers at full scale in conventional reactor configurations and in biogas desulfurization technologies (Syed et al., 2006). Despite the inherent merits of these biotechnologies for biogas desulfurization, oxygen dosing must be carefully controlled at optimal levels to prevent the formation of elemental sulfur (clogging the packed beds) and the creation of explosive oxygen/methane mixtures or to minimize O_2 levels in biomethane. The formation of biofilms and biofouling due to excessive biomass growth in packed bed bioreactors represents also an unsolved operational problem of conventional biotechnologies (Syed et al., 2006; Mudliar et al., 2010). Suspended biomass bioreactors (airlift, bubble column, or stirred tank bioreactors) can mitigate the traditional clogging issue of biofilters and biotrickling filters, as there is no support material for sulfur and biomass accumulation (González-Cortés et al., 2020). For example, Haosagul et al. (2020) proposed that the selective use of suitable microorganisms can improve the performance of bioscrubbers. González-Cortés et al. (2021) successfully tested a novel gas-lift bioreactor design. Moreover, Jiang et al. (2020) designed a bubble column reactor with reflux outlet, which greatly enhanced H_2S removal. However, suspended bacterial biomass reactors still present disadvantages such as the need for an external electron acceptor supply such as O_2 (entailing explosion risks) or NO_3/NO_2 (entailing higher operating costs) (Pokorna and Zabranska, 2015).

These limitations require research in alternative biological biogas desulfurization technologies.

Phototrophic sulfur bacteria are a diverse group of prokaryotic organisms composed of green sulfur bacteria (GSB) and purple sulfur bacteria (PSB) initially classified by their characteristic colours and high tolerance and use of sulfide (Imhoff, 2008). These microbial communities perform anoxygenic photosynthesis and utilize various sulfur compounds as electron donors (Janssen et al., 1999). Their metabolism is unique and provides advantages, such as a minimized risk of O_2 and N_2 contamination compared to microalgae or chemolitotrophs (Muñoz et al., 2015). Depending on the light intensity, sulfide is oxidized to sulfur under light-limiting conditions or to sulfate in excess of the light supply (Kushkevych et al., 2021a). The metabolism of GSB (mainly from the *Chlorobiaceae* family) is strictly anaerobic and use CO_2 as the only carbon source (Imhoff, 2014a). Hydrogen sulfide provides electrons and is oxidized to elemental sulfur, which is stored in the form of globules on the outer side of the cell membrane (Brune, 1995). Overall, GSB are more resistant to high sulfide concentrations than PSB (Frigaard and Dahl, 2008). The family *Chromatiaceae* or PSB (order *Chromatiales*) has a similar metabolism, but PSB store the produced sulfur globules inside their cells. Some PSB species are also capable of oxidizing thiosulfate and sulfite (Imhoff, 2014b) and of tolerating trace levels of oxygen (e.g *Allochromatium vinosum*) (Frigaard and Dahl, 2008).

Hydrogen sulfide removal has been investigated using green sulfur and purple sulfur bacteria due to their less restrictive growth requirements and slight tolerance to O_2 respectively (Pokorna and Zabranska, 2015). Fed-batch and continuous lab-scale suspended growth bioreactors inoculated with *Chlorobium* sp. (GSB) were investigated with high H_2S removal efficiency above 90 % (Basu et al., 1996; Kim and Chang, 1991; Lee and Kim, 1998). In the case of PSB, similar results were obtained by Borkenstein and Fischer (2006) in a photobioreactor inoculated with the mutant *A. vinosum* strain 21D (PSB) and using batch mode with mixed culture dominated by *Chromatiaceae* (Egger et al., 2020). Unfortunately, the number of studies assessing the influence of environmental and operational parameters on the biogas desulfurization performance of PSB and GSB is limited.

This study aimed to systematically compare the ability of GSB and PSB to remove H_2S from biogas using *A. vinosum* (GSB) and *Chlorobium limicola* (PSB) as model organisms. The influence of light intensity and H_2S concentration on biogas desulfurization in batch photobioreactors was investigated. In addition, the influence of the rising biogas loading rate in biogas desulfurization using *C. limicola* was investigated in a column photobioreactor operated in continuous mode.

2. Materials and methods

2.1. Inoculum and culture conditions

Phototrophic sulfur bacteria *A. vinosum* (DSM 180) and *C. limicola* (DSM 258) were purchased from the German Collection of Microorganisms and Cell Cultures. The used mineral salt medium (MSM) was prepared according to Malik (1983), with some modifications to exclude the resazurin, ferric citrate, sodium bicarbonate and ammonium acetate. The MSM used during the operation of the bioreactor also included 10 mL L^{-1} of phosphate buffer (82.4 g L^{-1} $Na_2HPO_4$0.12 H_2O and 26 g L^{-1} KH_2PO_4) to maintain the pH at 6.8. Synthetic biogas with a composition of CH_4 (70 %), CO_2 (29.5 %) and H_2S (0.5 %) and a H_2S:N_2 gas mixture (22:78 %) were purchased from ABELLO LINDE (Barcelona, Spain).

2.2. Batch GSB and PSB kinetic tests

Batch experiments were carried out in 1.2 L gastight glass bottles (Afora, Spain) in triplicate. Bottles were initially filled with 270 mL of MSM and headspace was flushed with helium for 40 s at high flow rate to displace the air atmosphere. Bottles were closed with a butyl septum and a plastic screw cap to maintain anaerobic conditions. After autoclaving, the pH was adjusted to 6.8 with sterile H_2SO_4 or NaOH in tests inoculated with GSB and to 7.2 in tests with PSB. The headspace of the bottles was then flushed with synthetic biogas for 20 min under sterile

conditions. pH was adjusted again after 2 h of stabilization under continuous magnetic agitation at 200 rpm. Bottles were inoculated under sterile conditions with 30 mL of the corresponding inoculum and incubated in a water bath at 30 ± 2 °C and 200 rpm of magnetic agitation. Aliquots of 5 mL of cultivation broth were drawn every two days under sterile conditions to monitor culture absorbance at 650 nm (used as a proxy of bacterial growth), pH and SO_4^{2-} concentration. Headspace sample of 100 μL was drawn periodically for gas concentration measurements (CH_4, CO_2, H_2S and O_2) using a gastight syringe (Hamilton, USA).

2.2.1. Test series 1: desulfurization of biogas using GSB and PSB

In test series 1 the growth of *A. vinosum* and *C. limicola* in desulfurization of biogas containing H_2S concentration of 0.5 % was evaluated. Bottles were incubated under an average light intensity of 4500 lux (TL-D36W/840, Phillips). An abiotic control without biomass was also prepared to evaluate H_2S losses because of dissolution in culture medium. When the H_2S concentration in the headspace was depleted, 40 mL of the H_2S:N_2 mixture was injected to reach a 1 % H_2S content in the headspace.

2.2.2. Test series 2: influence of H_2S concentration on GSB and PSB growth

The growth and ability to remove H_2S of *A. vinosum* and *C. limicola* was investigated under light intensity of 4500 lux in experimental set-up as described in Section 2.2. using biogas supplemented with H_2S at 1 %, 1.5 % and 2 %.

2.2.3. Test series 3: influence of light intensity on GSB and PSB growth

A. vinosum and *C. limicola* tests were prepared as previously described (Section 2.2.) under a synthetic biogas atmosphere and two light intensities (25 klx and 10 klx) using 150 W LED panels (Phillips, Spain). An abiotic control without biomass was also prepared to evaluate H_2S losses because of dissolution in culture medium. When the H_2S headspace concentration was depleted, 40 mL of 22 % H_2S was supplemented into the bottles to reach 1 % concentration.

2.3. Continuous biogas desulfurization with GSB in column photobioreactor

Photobioreactor under continuous mode (Fig. 1) consisted of an enclosed glass cylinder (9.5 cm inner diameter, 42 cm height) (Afora, Spain) with 2.3 L of working volume and stirred at 200 rpm. Four 21 W fluorescent lamps (T521W 865/D FSL, China) were vertically arranged to provide an average irradiation of 4000 lux on the photobioreactor surface. The photobioreactor (autoclaved at 121 ºC) was filled with sterilized MSM under sterile conditions and inoculated with 230 mL of fresh *C. limicola* inoculum. The headspace of the photobioreactor was flushed with biogas to remove O_2. The culture broth in the photobioreactor was maintained at 26 ± 1 °C and the pH was monitored periodically. The synthetic biogas mixture was fed into the reactor using a WM 120. S pump (Watson-Marlow, GB) using a metallic diffuser (pore size of 2 μm) located at the bottom of the photobioreactor with a flow rate of 2.9 L d^{-1} in stage I, 5.8 L d^{-1} in stage II and 11.5 L d^{-1} in stage III (Table 1). Culture samples (20 mL) were taken every 3 days in stage I and every 2 days in stages II and III to monitor culture absorbance, pH and sulfate and sulfide concentrations. Gas samples in the inlet and outlet of the photobioreactor were periodically taken to measure the gas composition. The cultivation medium was periodically replenished manually with sterile MSM through a sampling port equipped with a sterile 0.22 μm filter in order to compensate liquid sampling.

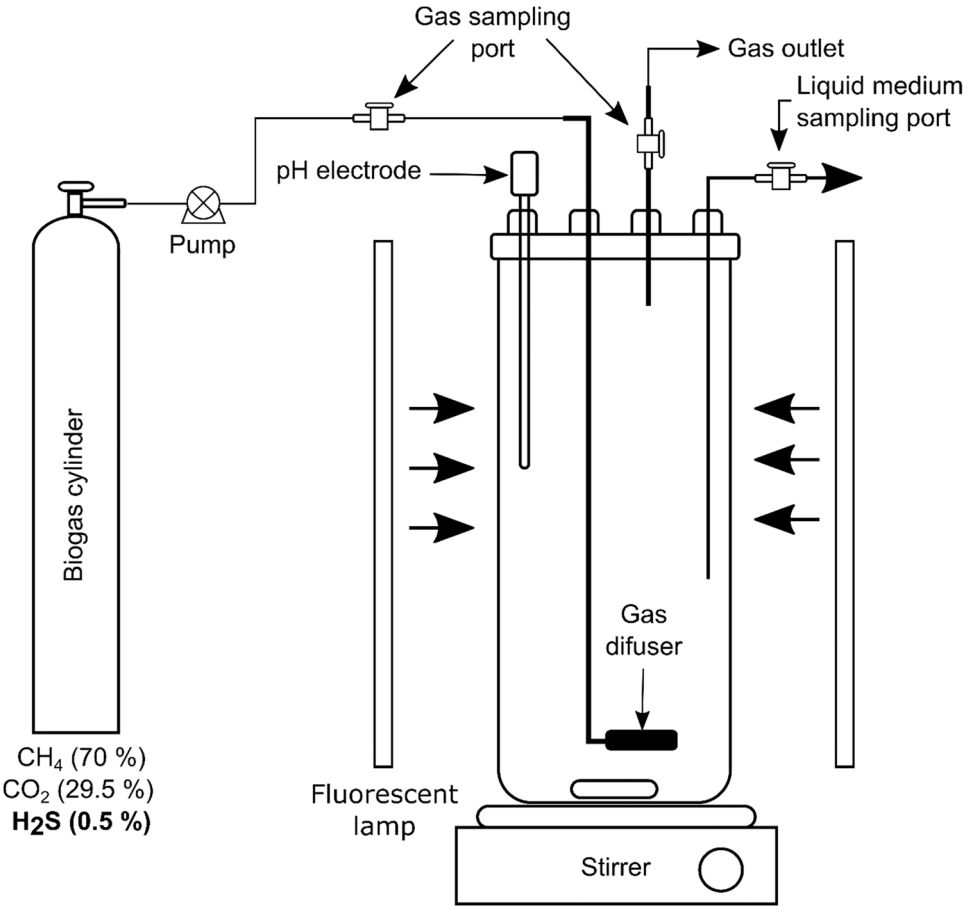

Fig. 1. Diagram of the photobioreactor used for biogas treatment desulfurization.

Table 1
Summary of the operational parameters set during continuous photobioreactor operation.

	Duration (days)	Vol (L)	Average light intensity (lux)	Biogas feed rate ($L\ d^{-1}$)	S_2 – loading rate ($mg\ d^{-1}L^{-1}$)
Stage I	41	2.3	4000	2.9	7.7
Stage II	7	2.3		5.8	15.3
Stage III	7	2.3		11.5	30.6

2.4. Analytical procedures

Culture absorbance at 650 nm (OD_{650}) was analysed on a UV-2550 spectrophotometer (Shimadzu, Japan). Biogas composition in the headspace was measured using a CP-3800 gas chromatograph equipped with a TCD detector (Varian, USA) according to García et al. (2019). Analysis of pH was carried out with a pH meter Sension+ PH3 (Hach, USA) coupled with a data logger (Consort, Belgium). SO_4^{2-} ions concentration was analysed by HPLC-IC. The light intensity was measured using a LX-105 luxmeter (Lutron, USA). Sulfide analysis was carried out using photometrical sulfide test kit (Spectroquant, Germany) according to manufacturer's instructions.

3. Results and discussion

3.1. Batch GSB and PSB kinetic tests

3.1.1. Test series 1: desulfurization of biogas using GSB and PSB

H_2S concentration in the headspace of the photobioreactors decreased in the first two days of experiment in both *A. vinosum* and *C. limicola* tests (Fig. 2A), due to the combination of H_2S dissolution in the MSM, similar to the results obtained in abiotic control test and subsequent metabolisation by both phototrophic bacteria, removing H_2S completely. In abiotic control only a partial removal of 41 % was detected. Additional supplementation of H_2S up to 1 % on the day 7 of experiment was added for stimulated bacterial growth, resulting in a rapid decrease of H_2S concentration in headspace. Thus, *A. vinosum* completely removed additional H_2S in two days, while *C. limicola* completely removed more than 90 % of the H_2S supplemented in 5 days. In the abiotic control test H_2S concentration decreased from 0.43 % to 0.26 % within the first two days due to gas solubilisation in the liquid medium and remained constant at 0.26 % from day 2 to day 7. Similarly, H_2S supplementation in the abiotic control resulted in concentrations of 1.04 %, which decreased to stable values of 0.65 %. The consistent H_2S removal in the tests containing phototrophic bacteria compared to the abiotic control supports the key role of bacteria in H_2S removal. Therefore, it can be hypothesized that most of H_2S was metabolized by bacteria in order to support photolithoautotrophic growth, where H_2S is used as an electron donor (Frigaard and Dahl, 2008). The lower H_2S removal rate recorded in *A. vinosum* confirmed the lower affinity of *A. vinosum* for sulfide compared to *C. limicola* (Van Gemerden, 1984). The higher affinity of GSB for H_2S compared to PSB in general has been empirically observed in previous studies (Brune, 1995; Van Gemerden, 1984) although the exact mechanism underlying this higher affinity is still unknown. It has been hypothesized that the location of the primary electron acceptor plays a key role in this affinity for H_2S and that since GSB store the produced sulfur extracellularly, the electron acceptor would be located at the outer side of the cell membrane. In the particular case of PSB, which store sulfur intracellularly, electron acceptor would be located at the inner side of the cell membrane. However, evidence to support this claim is still inconclusive (Veldhuis and van Gemerden, 1986; Van Gemerden and Mas, 1995).

Initial H_2S concentration present in tests supported only a moderate growth of *A. vinosum* from 0.311 to 0.334 (OD_{650}) and of *C. limicola* from 0.066 to 0.151 (OD_{650}) during the first 7 days of experiment (Fig. 2B). Indeed, H_2S was gradually dissolved in the liquid medium and used by bacteria, which can explain the limited growth of both phototrophic bacteria in absence of H_2S in the headspace. The supplementation of H_2S required for growth by day 7 caused a rapid increase in culture absorbance in *A. vinosum* tests. The creation of intracellular sulfur globules due to the high abundance of H_2S also contributed to this increase in culture absorbance (Kushkevych et al., 2021b). However, this rapid increase in OD_{650} also supported a fast H_2S removal, resulting in the use of stored sulfur as an electron donor (Cork et al., 1985). On the other hand, *C. limicola* exhibited a moderate increase in absorbance after the addition of H_2S, which continued until the end of the experiment due to a slow assimilation of H_2S. The lower OD_{650} in *C. limicola* tests entailed that the replenished H_2S was enough to cover the electron donor requirements. Finally, the absorbance of the liquid medium in the abiotic control remained below OD_{650} 0.03 during the first 7 days, but slightly

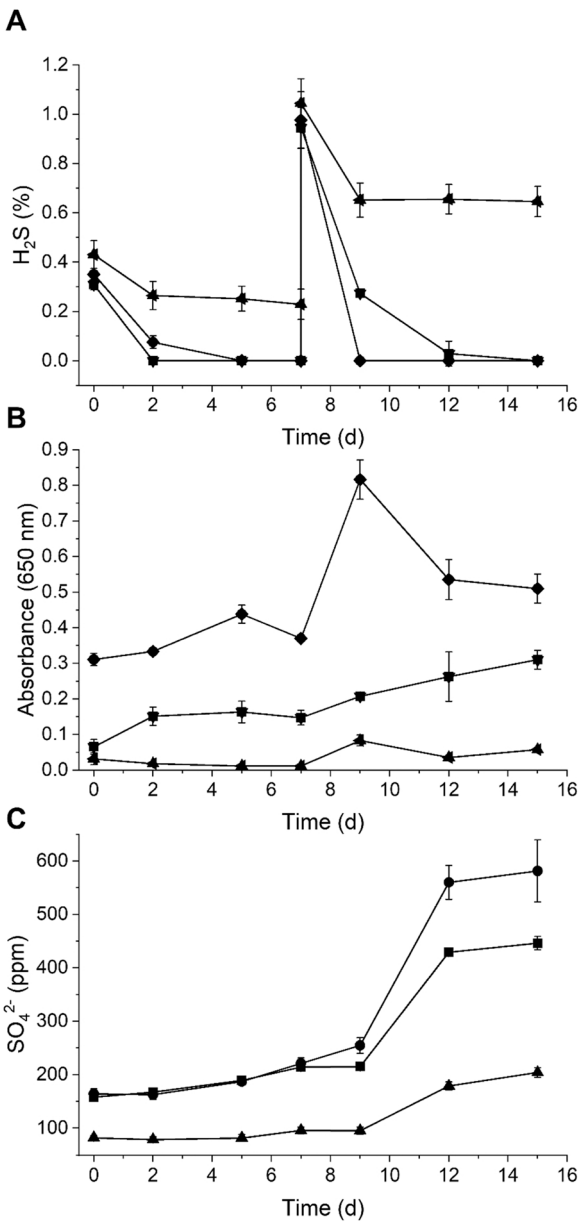

Fig. 2. Time course of the H_2S concentration (A), culture density (B) and sulfate concentration (C) in *A. vinosum* (●) and *C. limicola* (■) and abiotic control (▲) in tests under a biogas atmosphere.

increased from day 9 onwards as a resulting from sulfur precipitation due to photooxidation (Yang, 2021). Photooxidation does not necessarily require the presence of oxygen as it is simply a loss of electrons induced by light. However, despite authors precautions, such as thorough degassing of the cultivation medium and bottle headspace, there may have been some trace levels of O_2 in the bottle. Nonetheless, trace levels of O_2 alone are not enough to significantly influence the medium. Therefore, another mechanism must be in play such as light mediated reactions. H_2S in water exists as HS^- ions, which can form HS radicals as it absorbs the energy from the light source. In turn, HS radicals can create sulfur radicals, which can form polysulfides. These two mechanisms were together the likely cause of the slight rise of OD_{650}.

SO_4^{2-} concentration gradually increased from 162 to 581 mg L^{-1} in *A. vinosum* and from 157 to 446 mg L^{-1} in *C. limicola* tests (Fig. 2C). The correlation between H_2S removal in the headspace and sulfate formation supported that the main mechanism of desulfurization was via biological activity.

The green and purple sulfur bacteria use a complex mechanism to oxidize sulfur compounds, which is not yet fully understood (Frigaard and Dahl, 2008). GSB and PSB share various enzymes and pathways involved in the oxidation of reduced sulfur compounds (Fig. 3). These include enzymes such as flavocytochrome *c* sulfide dehydrogenase (Brune, 1995), sulfide:quinone oxidoreductase (Schütz et al., 1997) and the *Sox* system (Appia-Ayme et al., 2001). Photosystems of PSB and GSB are final acceptors of electrons yielded through metabolization of sulfur compounds. In process of anoxygenic photosynthesis, these electrons are then used to generate ATP and fuel other metabolic processes.

Flavocytochrome *c* represents a periplasmic enzyme that occurs in both PSB and GSB. It consists of two subunits. Large FccB is common for PSB and GSB, whereas small FccA exhibit some structural differences between groups (Frigaard and Dahl, 2008). Flavocytochrome *c* facilitates the transfer of electrons from reduced sulfur compounds such as sulfide to the reaction centre with the support of small c-type cytochromes as intermediaries (Brune, 1995). On the other hand, its role is still debated as in vivo experiments demonstrate that GSB and PSB can oxidize sulfide without flavocytochrome *c*, suggesting the presence of alternative pathways such as sulfide:quinone oxidoreductase (Frigaard and Dahl, 2008).

In the sulfide-quinone oxidoreductase (SQR) pathway, the electrons donated by the sulfide are transferred to quinone pool. Present in both GSB and PSB, SQR transfers the electrons to the electron transfer chain using quinol-oxidizing Rieske iron-sulfur protein and cytochrome *b* complex (Reinartz et al., 1998; Shahak et al., 1992).

The Sox enzyme system for the oxidation of thiosulfate is widely distributed in many microorganisms (Frigaard and Dahl, 2008). The basic *sox* genes (*sox* XABYZ) together with *dsr* genes present in *Chlorobium tepidum* allow for the oxidation of thiosulfate and sulfide to sulfur. In *Allochromatium vinosum* genes, *sox* AXB and *sox* YZ as well as the *dsr* gene cluster were also detected, although they may not be essential for sulfide oxidation as was proved by insertional mutagenesis (Friedrich et al., 2005).

During anoxygenic photosynthesis, they utilize sulfur compounds as electron donors. If there are not enough electrons available from first reaction (1), it progresses further producing sulfate (2) (Janssen et al., 1999):

$$2H_2S + CO_2 \rightarrow 2S^0 + (CH_2O) + H_2O \quad (1)$$

$$H_2S + \quad 2CO_2 + 2H_2O \rightarrow 2(CH_2O) + H_2SO_4 \quad (2)$$

The higher OD_{650} in *A. vinosum* tests likely explain the steady increase in SO_4^{2-} concentration compared to *C. limicola* tests, where SO_4^{2-} correlated with the removal of H_2S from the headspace. The major increase in SO_4^{2-} concentration was recorded in the final days of experimentation, when SO_4^{2-} concentration in *A. vinosum* and *C. limicola* tests reached up to 560 mg L^{-1} and 429 mg L^{-1}, respectively. No increase in SO_4^{2-} concentration was measured in the abiotic controls for the first 9 days of experiment. The gradual increase in sulfate concentration recorded in the final days of experimentation, reaching up to 179 mg L^{-1}, can be attributed to the chemical oxidation (catalysed by metals of the mineral salt medium) of dissolved H_2S with traces of oxygen present in headspace (Yang, 2021). The increase in SO_4^{2-} concentration in the tests inoculated with *A. vinosum* and *C. limicola* was probably mediated by sulfide underloading as described by the theory of the "van Niel curve" (Cork et al., 1985). This theory hypothesizes three potential sulfide

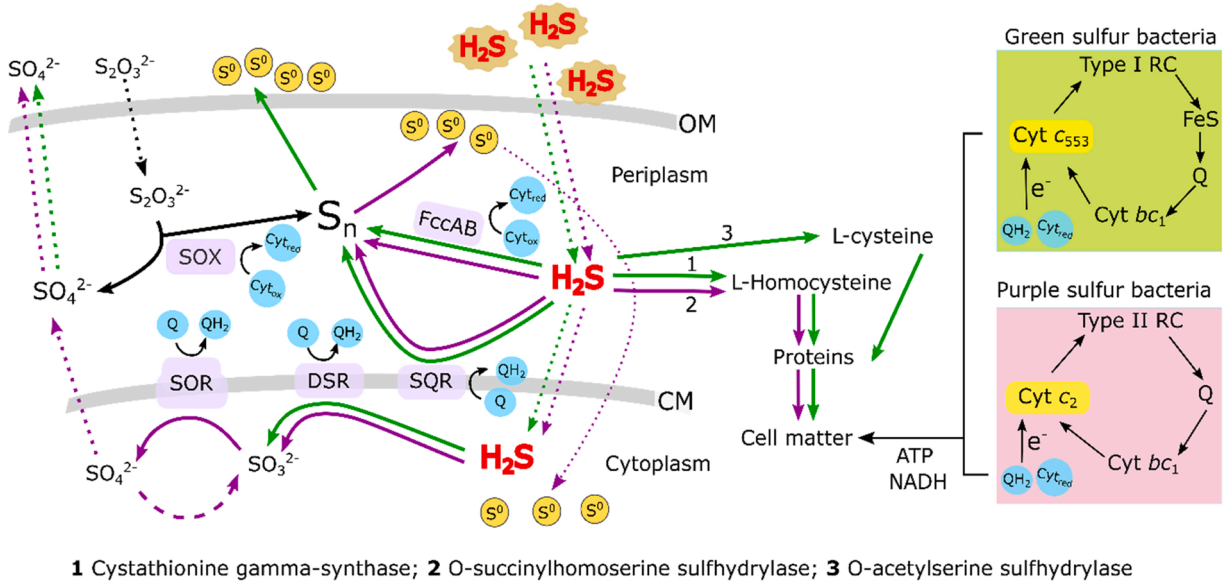

Fig. 3. Simplified overview of oxidative sulfur metabolism in PSB and GSB focused on H_2S metabolization. Green arrows indicate pathways of GSB, purple arrows of PSB and black arrows indicate pathways common in sulfur metabolism for both but not relevant to H_2S metabolization. Dotted lines represent transport of compounds. The photosystems of PSB and GSB are final acceptors of electrons yielded by metabolization of sulfur compounds. Quinones (Q), cytochromes (Cyt), sulfur compound oxidizing system (SOX), dissimilatory sulfite reductase (DSR), sulfide:quinone oxidoreductase (SQR), complex of several sulfite oxidoreductases (SOR), flavocytochrome *c* sulfide dehydrogenase (FccAB), reaction centre (RC), outer membrane (OM), cytoplasm membrane (CM). Based on the genome of *Allochromatium vinosum* and *Chlorobium limicola*, and modification of Frigaard (2016) and Frigaard and Dahl (2008).

metabolization scenarios in phototrophic sulfur bacteria based on the relationship between sulfide loading rate and light intensity: i) a balanced state where all sulfide is oxidized to sulfur without formation of SO_4^{2-}, ii) sulfide overloading, where sulfide accumulates in the medium due to an insufficient light energy supply, iii) sulfide underloading, where the demand for electron donor exceeds its supply (low sulfide loading) and consequently sulfate is formed from elemental sulfur according to Eq. (2).

CO_2 headspace concentration in *A. vinosum* and *C. limicola* tests, decreased from 34 % to 24 % and 26 % respectively, in the first two days, mainly due to use of CO_2 as carbon source by PSB and GSB. A decrease in the CO_2 concentration (by 35 %) was recorded in abiotic tests due to dissolution in the MSM. Consequently, the CH_4 concentration increased from 65 % to 75 %. The concentration of both gases remained constant till the end of experiment. Following bacterial acclimatization to the new environmental conditions and the beginning of their growth, substrate consumption by purple sulfur bacteria influenced the medium itself and the chemical equilibria. When CO_2 dissolves in the water phase, it forms carbonic acid (H_2CO_3), which further dissociates to H^+, HCO_3^-, and CO_3^{2-}. This leads to acidification of the media and pH control in the beginning of the assay is needed as described in the Materials and methods section. In our particular case, the consumption of CO_2 was probably responsible for the observed increase of pH in culture broth of *A. vinosum* test from 7.2 to 7.5 in the first days. A control of pH at values between 7.1 and 7.2 after day 2 of experimentation was necessary to avoid metabolic inhibition, similar to that described in other investigation using phototrophic bacteria. Alkaline condition can cause deactivation of cell enzymes, such as superoxide dismutase present in *A. vinosum* (Kanematsu and Asada, 1978), and inhibited the substrate metabolism together with electron transfer processes (Sepúlveda-Muñoz et al., 2020; Wang et al., 2011). On the other hand, the pH values in *C. limicola* and in the abiotic control test remained constant at 6.9–7.0 and 6.8–6.9, respectively, which contributed to limited growth and metabolism of *C. limicola*.

3.1.2. Test series 2: influence of H_2S concentration on GSB and PSB growth

All tests with *A. vinosum* exhibit H_2S removals over 90 % by day 7, along with H_2S headspace average volumetric removal rates of 2.4, 3.5 and 4.6 mg L^{-1} d^{-1} recorded in tests supplied with 1 %, 1.5 % and 2 % of H_2S, respectively (Fig. 4A). Interestingly, culture absorbance (OD_{650}) initially decreased in the tests conducted at 1 %, 1.5 % and 2 % from 0.221, 0.275 and 0.341–0.15 by day 2. This suggests possible interference of H_2S with MSM causing the initial high OD_{650} measured and the further metabolization of sulfur compounds is probably responsible for the observed decrease in OD_{650} on day 2. It was hypothesized that sulfur radicals and polysulfides can cause OD_{650} interference. The inoculum may have also played a role to some extent as it brought additional sulfur compounds created by bacterial metabolism (sulfur particles released from dead biomass or other metabolites). In this context, previous works have suggested potential interference of sulfur particles produced by bacteria with OD measurements (Kim et al., 1996; Lee and Kim, 1998). OD_{650} increased by day 4 and correlated with H_2S removal. From day 2 to day 4 (Fig. 4B), the higher the H_2S concentration the lower the OD_{650} growth rate, which may suggest a partial inhibitory effect of H_2S on *A. vinosum* metabolism (O'Flaherty et al., 1998). Hence, photobioreactors with a H_2S concentration of 2 % exhibited the slow growth (OD_{650} increase from 0.150 to 0.242), while tests with a H_2S concentration of 1 % exhibited the fastest growth (OD_{650} increase from 0.150 to 0.362). Interestingly, test with a H_2S concentration of 2 % was able to support the highest maximum absorbance at the end of the experiment (0.557 OD_{650}).

On the other hand, *C. limicola* (Fig. 5A) showed a faster H_2S removal compared to *A. vinosum*. H_2S headspace removal rates of 2.9, 3.9 and 5.3 mg L^{-1} d^{-1} were recorded in tests supplied with 1 %, 1.5 % and 2 % of H_2S, respectively. In four days, *C. limicola* completely removed H_2S in tests supplied at 1 % of H_2S, while removals of 93 % and 92 % were recorded in tests supplied with 1.5 % and 2 %. A complete removal was achieved by day 7 in all tests demonstrating the high potential of these photosynthetic organisms for the desulfurization of biogas with different H_2S concentrations.

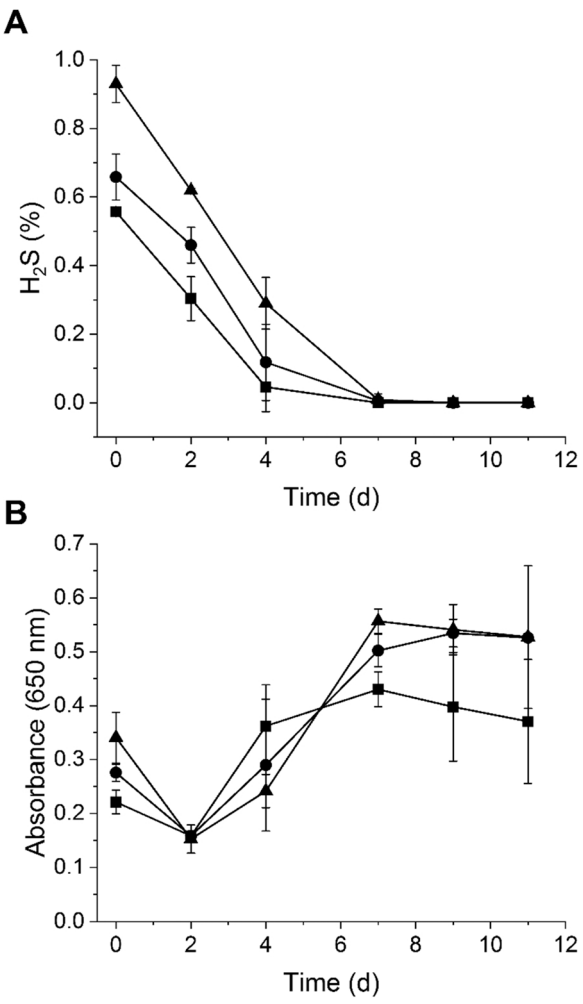

Fig. 4. Time course of the H_2S concentrations (A) and culture density (B) of *A. vinosum* under a biogas atmosphere containing H_2S concentrations of 1 % (■), 1.5 % (●) and 2 % (▲).

C. limicola experienced constant growth in the first days of experimentation (Fig. 5B). Test supplied with H_2S at 1 % exhibited the rapid growth of *C. limicola*, with OD_{650} increasing from 0.033 to 0.174 by day 2. Tests supplied with 1.5 % and 2 % H_2S concentration exhibited a 40 % lower growth rate compared to the test at 1%, reaching an OD_{650} of 0.110 and 0.103 by day 2, respectively. The maximum OD_{650} of 0.253 (1.5 %) and 0.255 (2 %) was achieved by day 7 in the tests supplied with the highest H_2S concentrations. From day 7, OD_{650} in the 2 % H_2S test remained constant, while a steady decrease in OD_{650} was observed in the 1.5 % H_2S test from 0.255 to 0.183 by day 9 and to 0.171 by day 11. The higher 2 % H_2S concentration likely provided a higher amount of electron donor (H_2S) to support the growth of bacterium for a longer period of time. Under a H_2S atmosphere with 1.5 %, the supply of electron donor was consumed quicker, which under continuous illumination led to inhibition of bacterial growth due to the starvation and damage by illumination (Imhoff, 2014b).

Both test series confirmed high H_2S removal efficiency using both GSB and PSB, demonstrating the high potential for their use in biogas desulfurization. Similar H_2S removal efficiency (100 %) was obtained with algal-bacterial consortia (Muñoz et al., 2015), however their use often entails N_2 and O_2 contamination of biogas (Ángeles et al., 2020).

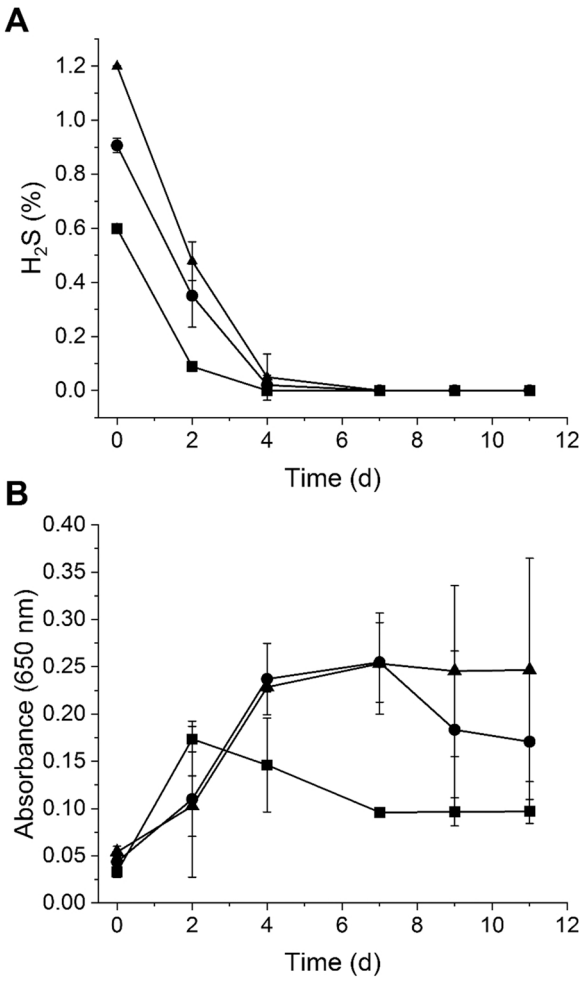

Fig. 5. Time course of the H_2S concentrations (A) and culture density (B) of *C. limicola* under a biogas atmosphere containing H_2S concentrations of 1 % (■), 1.5 % (●) and 2 % (▲).

Moreover, the high tolerance to sulfide (100–150 mg L^{-1}) favours GSB and PSB growth (Frigaard, 2016) as microalgae growth is inhibited at 16 mg L^{-1} (Ramírez-Rueda et al., 2020) because dissolved H_2S can inhibit oxygenic photosynthesis (Meier et al., 2018). It is safe to assume that GSB and PSB have evolved mechanisms that mitigate the inhibitory effects of hydrogen sulfide. They obtain energy through anoxygenic photosynthesis (Brune, 1995; Janssen et al., 1999) and use various pathways to oxidize hydrogen sulfide, thus reducing its concentration and decreasing the toxic effect of this molecule (Frigaard and Dahl, 2008). However, under high H_2S concentrations, even these protective mechanisms may not be sufficient. While the precise mechanism of inhibitory effect of H_2S on *A. vinosum* or *C. limicola* is not known, it can be assumed that it is not much different than in other bacteria. The negative effects of H_2S in bacteria in general include DNA damage, protein denaturation or inhibition of antioxidant proteins (Mendes et al., 2021). Same problems are also present, mainly sulfur clogging and O_2 contamination, when using chemolitotrophs such as *Thiobacillus*, *Acidithiobacillus* and *Thiothrix* (Tang, Baskaran and Nemati, 2009).

3.1.3. Test series 3: influence of light intensity on GSB and PSB growth

H_2S concentration in the photobioreactos inoculated with *A. vinosum* decreased from 0.50 % to 0.04 % and from 0.50 % to 0.01 % under 10 klx and 25 klx, respectively, in first two days of experiment, while a complete H_2S depletion was achieved on day 4 in both tests (Fig. 6A). Interestingly, the addition of H_2S up to 1 % by day 6 entailed a complete H_2S removal by day 8 under lowest light intensity, while H_2S concentration decreased from 0.54 % to 0.34 % by day 8 and remained constant afterwards at the highest light intensity tested. This suggested an inhibition of *A. vinosum* growth due to incubation at high light intensities. In this context, a high light intensity can produce bacteriochlorophyll triplet, which under aerobic conditions produces reactive oxygen species (ROS) leading to degradation of bacteriochlorophyll. Under anaerobic conditions, the triplet-state bacteriochlorophyll induces photodegradation of this pigment, producing different short-wavelength products (Granzhan et al., 2004). Although carotenoids serve as protection against ROS formation, they can be damaged by exposure to high light intensities and ROS, which results in their decomposition (Rajagopal et al., 2002). Because pigments are necessary for functioning of the photosynthetic apparatus in the bacterium, their degradation, and thus loss of function causes severe damage to the cell energy metabolism.

The OD_{650} in the tests inoculated with *A. vinosum* and incubated under 10 klx increases (OD_{650} increase from 0.123 to 0.255) in the first two days, while the OD_{650} in the 25 klx test was even higher (OD_{650} increase from 0.110 to 0.317) (Fig. 6B). The low H_2S concentrations prevailing in the headspace from day 2–6 resulted in a very limited bacterial growth regardless of the light intensity. The supplementation of H_2S by day 6 resulted in no change in OD_{650} in the 25 klx test as a result of the limited H_2S degradation recorded, while OD_{650} rapidly increased from 0.263 to 0.760 by day 9 in the test incubated at 10 klx. The latter growth was likely supported by the active H_2S degradation observed under the lowest light intensity tested.

No changes in SO_4^{2-} concentration were recorded in the first two days in *A. vinosum* test incubated at 10 klx, after gradual increase of SO_4^{2-} concentration (Fig. 6C) to 455 mg L^{-1} at the end of the experiment. The 25 klx test exhibited an increase in SO_4^{2-} concentration to 360 mg L^{-1} at the end of the experiment. The stabilization of sulfate concentration by day 6 correlated with the low metabolic activity (bacterial growth and H_2S degradation).

On the other hand, H_2S concentration in the tests inoculated with *C. limicola* and incubated at 10 klx decreased from 0.38 % to 0.11 % by day 2 and was completely removed by day 4 (Fig. 6D). On the contrary, a light intensity of 25 klx supported a gradual decrease in H_2S content from 0.31 % to 0.25 % in the first two days, and a complete removal by day 8. However, the addition of H_2S (up to 1 %) by day 6 (under 10 klx) and day 8 (under 25 klx) supported a rapid H_2S removal regardless of the light intensity used. Thus, H_2S concentration decreased by 84 % and was completely removed by day 11 under 10 klx, while incubation under 25 klx mediated a 78 % H_2S removal from day 8–11 and a complete H_2S depletion by day 13. Overall, higher light intensities seem to slow down *C. limicola* metabolism similarly to *A. vinosum* metabolism, but to a lower extent (Granzhan et al., 2004).

The OD_{650} of *C. limicola* concentration in the 10 klx test increased from 0.047 to 0.193 in the first 4 days (Fig. 6E). On the other hand, only a negligible *C. limicola* growth was recorded under 25 klx test (OD_{650} increase from 0.025 to 0.042 within the first four days). This *lag* phase likely caused the delay in H_2S degradation due to negative effect of high light intensities. Bacterial growth resumed in both tests after injection of H_2S, confirming that the H_2S is necessary for its growth. The 10 klx tests experienced a rapid increase in OD_{650} from 0.125 to 0.272 in two days, which in turn resulted in a depletion of H_2S and a subsequent decrease in culture absorbance to 0.177 in OD_{650}. *C. limicola* growth under 25 klx was slower, with OD_{650} increasing from 0.104 to only 0.166 by day 2 after injection.

SO_4^{2-} concentration in *C. limicola* tests (Fig. 6F) increased from 277 mg L^{-1} to a final concentration of 359 mg L^{-1} under 10 klx and from 241 mg L^{-1} to a final concentration of 323 mg L^{-1} for the 25 klx test. Both tests experienced negligible changes in SO_4^{2-}concentration within the first 4 days, followed by a gradual increase of SO_4^{2-} concentration. The higher maximum concentration of SO_4^{2-} in the 10 klx test correlated with the higher biomass and thus higher metabolic activity when compared to the 25 klx test.

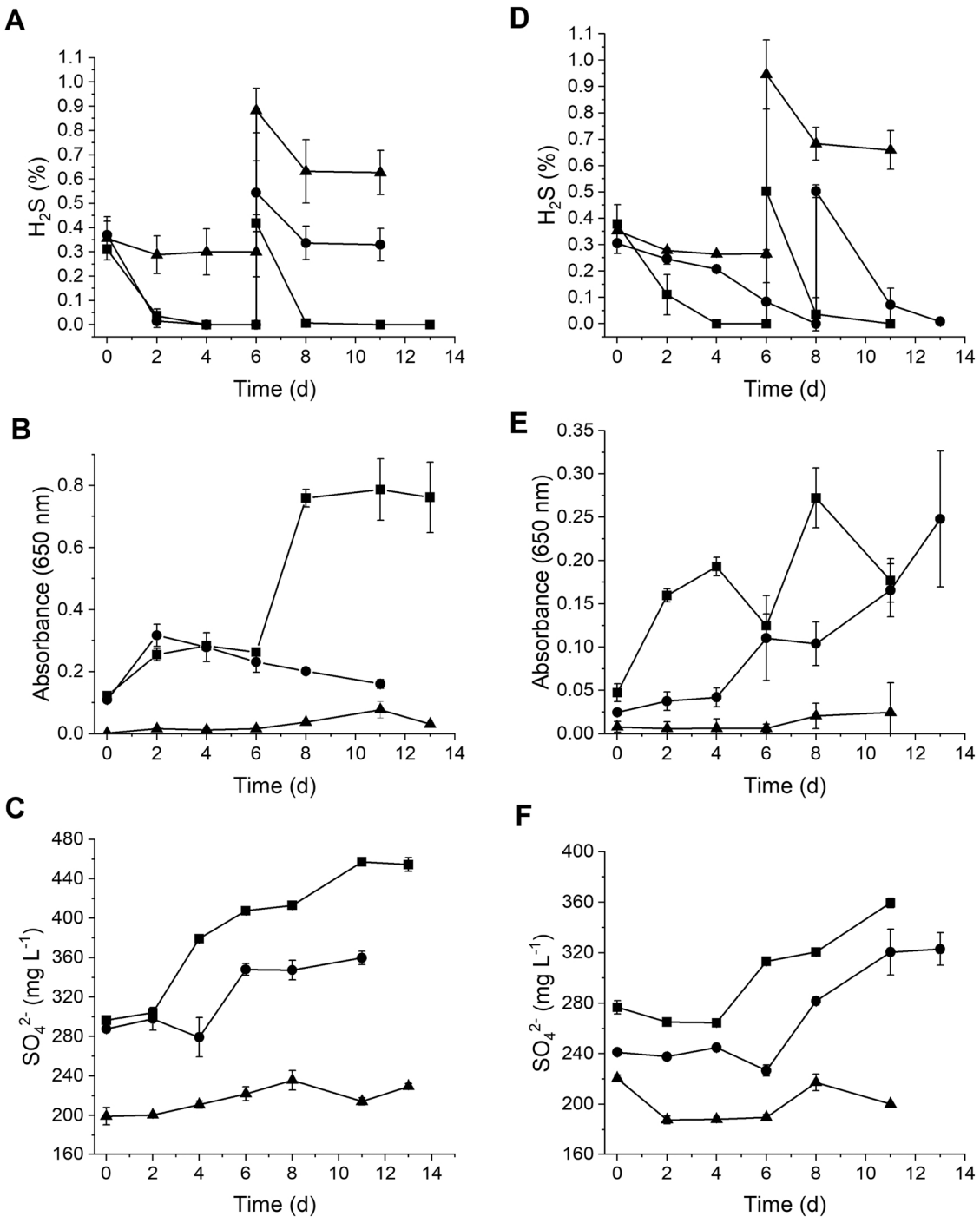

Fig. 6. Time course of the H_2S concentrations (A,D), density (B,E) and sulfate concentration (C,F) of *A. Vinosum* (A,B,C) and *C. limicola* (D,E,F) under a biogas atmosphere with at different light intensities 10 klx (■) and 25 klx (●) together with abiotic control (▲).

The pH values in the cultivation broth of *A. vinosum* under 10 klx remained constant at 6.90–6.99, while a slight increase within the first two days from 6.7 to 7.0 was observed in tests under 25 klx, stabilizing at this final value. On the contrary, the pH in *C. limicola* tests at 25 klx was stable (6.68–6.80), while incubation at 10 klx supported a larger range of pH values (6.56–6.93). After bacterial acclimatisation to the new environmental conditions and the beginning of their growth, substrate consumption by phototrophic sulfur bacteria influenced the medium itself and the chemical equilibria. In the particular case of *C. limicola*, the consumption of CO_2 was probably responsible for the observed increase in pH.

The trends present in the abiotic control were similar to those observed in Section 3.1.1. H_2S content in the abiotic tests decreased within the first two days of experiment and remained constant afterwards (Fig. 6A, D). The injection of fresh H_2S up to 1 % raised its concentration in abiotic control, which followed the same trend: initial decrease followed by stabilization. The absorbance of the liquid medium in the abiotic controls changed only slightly during the experiments (Fig. 6B, E). The sulfate content (Fig. 6C, F) varied in abiotic control during the experiments, but its values did not show a similar rising trend at the end of the experiment as in the inoculated bottles.

Indeed, light intensity plays an important role as it directly influences the cell growth and H_2S metabolization. Initially, the higher light intensity provided more energy, thus increasing the intensity of

anoxygenic photosynthesis and stimulating the growth of *A. vinosum* which led to initial higher OD_{650} on day 2 under 25 klx than 10 klx of light intensity. However, the higher abundance of light energy also caused a faster depletion of nutrients, such as H_2S. Without a suitable electron donor, light damages the cell (Imhoff, 2014b) causing the subsequent decrease in OD_{650} if H_2S is not replenished. The authors hypothesized that depletion of electron donor (H_2S) increased the inhibitory effect of high light intensity. This can explain why this phenomenon occurring in *A. vinosum* tests was not observed in the assays inoculated with *C. limicola*, as the H_2S consumption in the first two days was much slower compared to *A. vinosum*. Interestingly, it was observed that in both tested bacteria, 25 klx intensity slowed growth (*C. limicola*) or inhibited it completely (*A. vinosum*) with negative impact on H_2S removal as well. Similarly, Kim et al. (1992) reported light inhibition above 40 klx in GSB *Chlorobaculum thiosulfatiphilum* (previously *Chlorobium thiosulfatophilum*) while Takashima et al. (2000) observed gradual inhibition from 5 to 16 klx in *Prosthecochloris aestuarii* (GSB). On the other hand, increasing light intensity up to inhibitory value may by beneficial as it increases the cell growth (Sun et al., 2022; Ross and Pott, 2022) and H_2S removal (Egger et al., 2020).

3.2. Continuous biogas desulfurization with GSB in column photobioreactor

The adaptation of *C. limicola* to the new continuous conditions in the photobioreactor at the beginning of stage I, resulted in a *lag* phase lasting for first six days, with minimal changes in OD_{650} (Fig. 7A). *C. limicola* growth started by day 9, with an increase in OD_{650} from 0.116 to 0.288 by day 16 and up to 0.317 by day 34. At the end of stage I, *C. limicola* exhibited increased growth in OD_{650} from 0.317 at day 34–0.541 by day 40. Similarly, H_2S removal during the first 6 days of experiment was low due to the low OD_{650} in the photobioreactor at the beginning of stage I (Fig. 7A). The concentration of H_2S in the treated biogas gradually increased during the first days of experiment up to 0.25 % as a result of the gradual saturation of the cultivation broth with the H_2S dissolved from the 2.9 L d^{-1} of biogas supplied and the limited bacterial activity after inoculation. The gradual increase in biomass concentration from day 6 onwards resulted in a rapid increase in biogas desulfurization and a complete removal of H_2S was recorded from day 10 during stage I. The system supported average H_2S removal rates of 10.3 mg L^{-1} d^{-1} in this stage.

An increased growth of *C. limicola* was recorded during the first days of stage II and the cultivation broth reached a maximum OD_{650} of 0.797 by day 47. The increase in biogas flowrate up to 5.8 L d^{-1} during stage II did not impact in the biogas desulfurization efficiency between day 41 and 45 (average H_2S removal rates of 24.9 mg L^{-1} d^{-1}), although H_2S concentration in the treated biogas started to increase from day 47 onwards (H_2S removal rate of 22.7 mg L^{-1} d^{-1}). From day 47, the OD_{650} sharply decreased to 0.235 at the end of stage III. The increased biogas flow rate up to 11.5 L d^{-1} in stage III may have played an important role here, raising the H_2S concentration in the cultivation medium above inhibitory levels. Another factor that favours the rapid decrease in culture absorbance was the aggregation of biomass as visible flocks (photo available in Supplementary Material as Fig. S1), limiting the penetration of light and H_2S into cellular aggregations of GSB. Thus, stage III resulted in a deterioration of removal activity, from 37.2 mg L^{-1} d^{-1} on day 49–32.8 mg L^{-1} d^{-1} at the end of stage, which, together with an increase in biogas flow rate, supported an H_2S content of 0.08 % in the treated biogas. This problem could be mitigated under semi-continuous regiment mode of biomass harvesting.

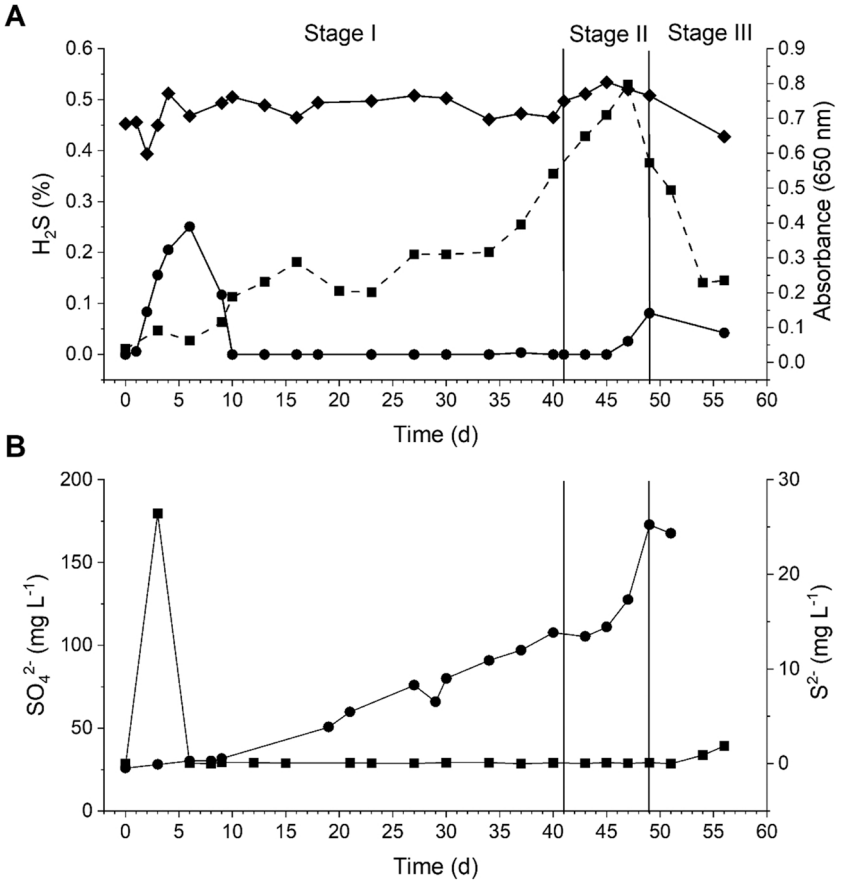

Fig. 7. Time course of biomass absorbance (■), H_2S concentration in the inlet (◆) and outlet (•) of the photobioreactor (A) inoculated with *C. limicola*. Time course of sulfate (•) and sulfide (■) concentration in cultivation broth in the continuous photobioreactor (B).

On the other hand, sulfide concentration in the cultivation broth peaked by day 3 (26.4 mg L^{-1}) as a result of the low bacterial activity and constant biogas supply during process start-up (Fig. 7B). Sulfide concentrations were negligible during the course of experimentation until day 54, when this analyte started to gradually increase along with the increase in H_2S concentration in the treated biogas. On the other hand, the SO_4^{2-} concentration steadily increased over time as a function of the H_2S loading rate (Fig. 7B). Therefore, the sulfate concentration increased from 26 to 108 mg L^{-1} during stage I. The increase in H_2S loading in stage II did not result in an increase in sulfate concentration from day 41–45 despite the complete H_2S abatement and negligible sulfide accumulation, suggesting accumulation of globular sulfur. Interestingly, a rapid increase in sulfate concentration from 111 to 173 mg L^{-1} was recorded between day 45 and 49.

The present results show that photobioreactor with *C. limicola* was able to reach removal efficiency of 100 % of H_2S in stages I and II with average removal rates 10.3 and 24.9 mg L^{-1} d^{-1} respectively. Compared to previous works, the removal efficiency is in line with what has been found in previous works: removal efficiency of 96–100 % when using microalgae (Toledo-Cervantes et al., 2017), 99 % utilizing sulfide oxidizing bacteria (Juntranapaporn et al., 2019), and 97–100 % using phototrophic bacteria (Basu, 1996; Kim et al., 1996). However, the negative effect of increased gas flow rate coupled with light attenuation and lowered accessibility of H_2S due to biomass aggregation prevented the bioreactor to maintain high removal efficiency in later stages of the test. This problem could be mitigated under semi-continuous regiment mode of biomass harvesting.

Some limitations should be also considered. First, the feasibility of maintaining sterile conditions under field application. The goal of the experiment was to assess the performance of green sulfur bacteria and purple sulfur bacteria for biogas desulfurization under laboratory conditions before any field trial. Under full scale applications, the operating conditions imposed (no external supply of O_2 or NO_3 and light) would favour the development of green sulfur bacteria and purple sulfur bacteria for biogas desulfurization in the photobioreactor and, therefore, the robust performance of the technology. Finally, it should be stressed that the biogas residence times tested in this proof-of-concept study (9.5–4.5 h) were significantly higher than those typically used in biotrickling filters devoted to biogas desulfurization (3–10 min). However, it must be stressed that this work represented one of the pioneer proof-of-concept studies of green sulfur bacteria and purple sulfur bacteria for biogas desulfurization (under batch and continuous mode) and it was not designed to maximize the biogas treatment capacity (i.e the biogas residence time). Future investigations should evaluate the scalability and optimization of the biogas residence time to maximize the biogas treatment using GSB and PSB.

4. Conclusions

This study confirmed the feasibility of biological biogas desulfurization using anoxygenic photosynthetic bacteria and elucidated the main factors that influence the desulfurization process. We have obtained encouraging results demonstrating complete H_2S removal in all *A. vinosum* and *C. limicola* tests with different concentrations of H_2S. The highest light intensity tested (25 klx) inhibited the growth and H_2S removal capacity of *A. vinosum*, while *C. limicola* supported complete removal of H_2S at 25 klx. The continuous photobioreactor operated with *C. limicola* operated with a 100 % H_2S removal efficiency during stage I and stage II. Biomass aggregation combined with an increased biogas loading in stage III resulted in a decreased desulfurization performance.

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CRediT authorship contribution statement

Martin Struk: Conceptualization, Investigation, Visualization, Writing - Original Draft. **Cristian A. Sepúlveda-Muñoz:** Writing - Review & Editing, Investigation. **Ivan Kushkevych:** Writing - Review & Editing. **Raúl Muñoz:** Conceptualization, Supervision, Methodology, Resources, Project administration, Funding acquisition, Writing - Review & Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.130337.

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