

Biogas biodesulfurization and simultaneous valorization into high-value bioproducts by *Rhodopseudomonas telluris*

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**Biogas biodesulfurization and simultaneous valorization into high-value bioproducts
by *Rhodopseudomonas telluris***

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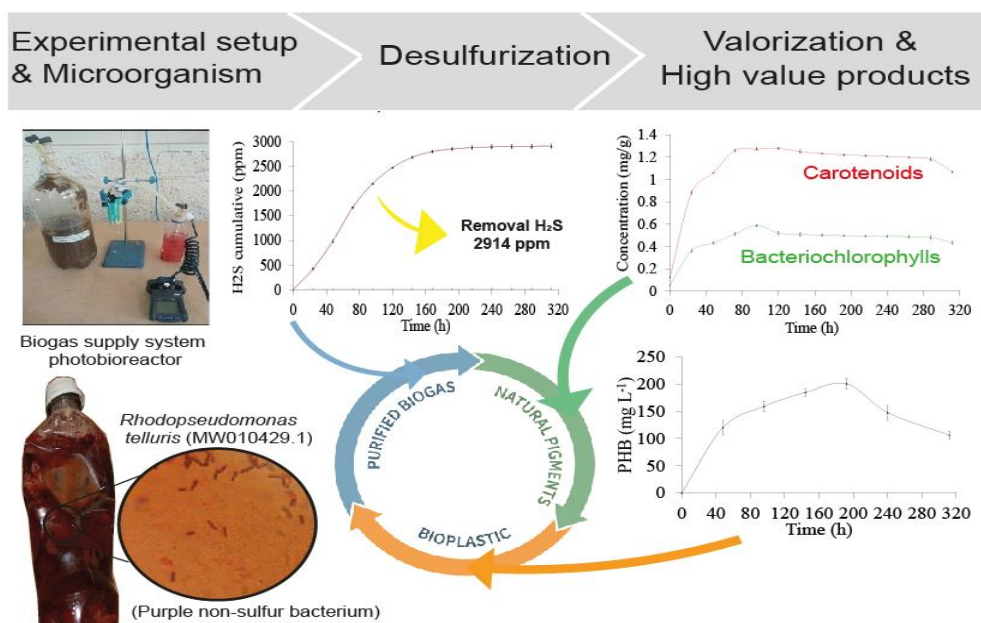
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32 Graphical Abstract



33 Abstract

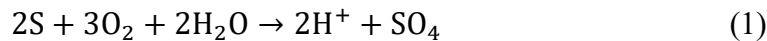
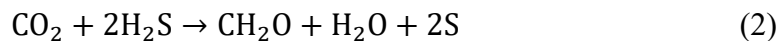
34 Hydrogen sulfide, characterized by being a toxic, corrosive, and unpleasant-smelling
 35 compound, is one of the anaerobic digestion products that forms part of the biogas
 36 composition. In this study, the metabolic capacity of *Rhodopseudomonas telluris* to oxidize
 37 hydrogen sulfide and produce high-value chemical compounds from biogas was determined.
 38 *Rhodopseudomonas telluris* demonstrated the metabolic capacity to grow autotrophically,
 39 achieving hydrogen sulfide removal values of 2917.36 ± 38.77 ppm at a maximum growth
 40 rate of 0.2 h^{-1} while maintaining the culture under conditions of pH between 6.0–7.0 and
 41 ORP between -162.6 to -132.3 mV. The detection of sulfate of $336.80 \pm 7.21 \text{ mg L}^{-1}$ indicated
 42 the metabolic capacity of *Rhodopseudomonas telluris* to perform the complete oxidation of
 43 hydrogen sulfide. During the process, *Rhodopseudomonas telluris* accumulated carotenoids,
 44 bacteriochlorophylls, and polyhydroxybutyrate at concentrations of $1.06 \pm 7.64 \times 10^{-2} \text{ mg g}^{-1}$,
 45 $0.43 \pm 1.29 \times 10^{-2} \text{ mg g}^{-1}$, and $200.69 \pm 9.78 \text{ mg L}^{-1}$, respectively. The ability of
 46 *Rhodopseudomonas telluris* to produce high-value-added chemical compounds confirms its
 47 potential use as an alternative in biogas purification bioprocesses under a circular economy
 48 framework.

50 Keywords

51 Biodesulfurization; Purple non-sulfur bacteria; Hydrogen sulfide; *Rhodopseudomonas*
 52 *telluris*; Sulfate; Pigments

Introduction

Hydrogen sulfide is a toxic, corrosive, and malodorous gas with a characteristic rotten-egg odor (Dada et al. 2025). Hydrogen sulfide is produced from the reduction of inorganic molecules during the anaerobic degradation of organic compounds in wastewater treatment or in domestic and industrial biogas production (Egger et al. 2023). In anaerobic bioreactors, hydrogen sulfide is generated by the proliferation of sulfate-reducing bacteria that compete with homoacetogenic bacteria and archaea for the substrate (Sarti and Zaiat 2011). Biogas produced from the anaerobic digestion of organic waste commonly has the following composition: methane (60–70% v/v), carbon dioxide (30–40% v/v), hydrogen sulfide (700–2000 ppm), and other impurities such as siloxanes (Egger et al. 2023). Prolonged exposure to hydrogen sulfide at concentrations near 5 ppm can cause eye damage and affect the respiratory tract, whereas concentrations of 1000–2000 ppm can cause loss of consciousness or even death (Dada et al. 2025). Furthermore, it causes corrosion in steel and concrete, severely damaging equipment and infrastructure used for biogas utilization (Sarti and Zaiat 2011); therefore, removing hydrogen sulfide from biogas is essential. Existing desulfurization technologies are classified into physicochemical methods, chemical oxidation/precipitation, and biological processes (Oh et al. 2026). Adsorption and chemical processes exhibit a high hydrogen sulfide removal capacity, but they require intensive consumption of chemicals, adsorbents, and energy, while generating polluting wastes (Oh et al. 2026; Krayzelova et al. 2014). Biological desulfurization is more favorable from an economic and environmental standpoint in the long term (Oh et al. 2026). Biological technologies, such as biodesulfurization, rely primarily on the use of microorganisms capable of growing in environments containing reduced inorganic sulfur compounds, such as hydrogen sulfide. In the biodesulfurization mechanism, microorganisms use hydrogen sulfide as an electron donor, carrying out the following oxidation reactions (Eqs. 1 and 2) (Pudi et al. 2022):



Among the main microorganisms capable of hydrogen sulfide oxidation are chemolithotrophic and photosynthetic bacteria (Struk et al. 2023). Purple non-sulfur bacteria (PNSB) possess oxidative enzymes (such as sulfide-quinone oxidoreductase,

flavocytochrome c-sulfide dehydrogenase, dissimilatory sulfite reductase, and heterodisulfide reductase-like proteins) that provide them with the metabolic capacity to grow photoautotrophically on various reduced sulfur compounds. PNSB utilize hydrogen sulfide for carbon dioxide fixation through their metabolic pathways, making this group promising candidates for biogas biodesulfurization and upgrading processes (Tec et al. 2023; Thi et al. 2024). Some PNSB genera, such as *Rhodopseudomonas*, have been reported to oxidize hydrogen sulfide. However, few studies have evaluated these microorganisms or consortia containing them in biodesulfurization processes (Frigaard and Dahl 2008). Another notable feature of the genus *Rhodopseudomonas* is its metabolic versatility in accumulating high-value bioproducts, such as pigments and biodegradable polymers (polyhydroxybutyrate). These traits highlight its relevance for sustainable and economically viable bioprocesses. Although biological methods can overcome energy consumption and waste generation drawbacks, current technical limitations still hinder their commercialization, making it necessary to explore alternatives that broaden their practical scope. Therefore, this study aimed to evaluate the potential of *Rhodopseudomonas telluris* for hydrogen sulfide removal and its metabolic capacity to generate high-value chemical products using biogas derived from the anaerobic digestion of organic waste.

Materials and methods

Microscopic characterization and molecular identification

A wet mount and Gram staining were performed from a Petri dish containing nutrient agar with the isolated *Rhodopseudomonas telluris* strain to observe its microscopic characteristics (Mehrabi et al. 2001). *Rhodopseudomonas telluris* was identified by LANGEBIO-CINVESTAV Genomic Services Unit (Guanajuato, Mexico), who performed PCR amplification and sequencing services. The resulting sequences were compared with other partial microbial sequences using BLAST (Basic Local Alignment Search Tool, National Center for Biotechnology Information). A phylogenetic tree was constructed using the Neighbor-Joining method with 1,000 bootstrap replicates in MEGA software version 11.0 to determine the evolutionary distances among PNSB groups (Tamura et al. 2021).

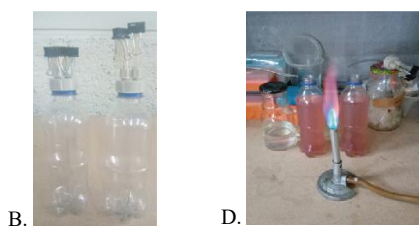
Growth and adaptation

For the reactivation and growth of *Rhodopseudomonas telluris*, a cryopreserved pure strain stored in Eppendorf tubes was used. Aliquots of 0.1 mL from each tube were inoculated onto

Petri dishes containing nutrient agar. The plates were placed in a transparent anaerobic jar and incubated for 168 h at 35 °C under a light intensity of 1500 lux provided by 100 W incandescent lamps. For adaptation, several loops of biomass were harvested from the Petri dish containing the pure *Rhodopseudomonas telluris* strain using a sterile inoculating loop. The biomass was transferred into pre-UV-sterilized plastic bottles containing 150 mL of modified mineral medium (pH 7.0), prepared according to van Gernerden and Beeftink (1978). The mineral medium composition (in g L⁻¹) was as follows: 0.31 Na₂S₂O₃, 0.25 NH₄Cl, 2 NaHCO₃, 0.2 CaCl, 0.6 K₂HPO₄, 0.5 KH₂PO₄, 0.2 MgCl₂·6H₂O, and 10 mL of trace element solution prepared according to Cisneros et al. (2024). The medium was supplemented with 1.5 g L⁻¹ sodium acetate and 0.1% yeast extract. The bottles were incubated in an orbital shaker at 150 rpm under the same incubation conditions described for reactivation and growth (Fig. 1A).

Experimental setup

Biodesulfurization assays were performed in duplicate under semi-batch mode using 300 mL plastic bottles with a working volume of 150 mL. Each bottle contained the modified mineral medium described by van Gernerden and Beeftink (1978), from which sodium acetate, yeast extract, and the salts Na₂S₂O₃ and ZnSO₄·7H₂O were omitted. Bottles were inoculated at 20% (v/v) with *Rhodopseudomonas telluris*, except for the abiotic control, which was used to determine the effect of non-biological factors on hydrogen sulfide removal. Biogas obtained from an anaerobic co-digestion process was supplied to each bottle every 24 h at a flow rate of 2 mL min⁻¹ for 3 min over a 312 h period (Fig. 1F). Immediately following gas supply, the hydrogen sulfide concentration in the headspace of each bottle was measured (Table 1). Throughout the experimental period, the bottles were maintained in an incubator at 35 °C with orbital shaking at 150 rpm and a light intensity of 1500 lux. All experiments were conducted in duplicate (Fig. 1).



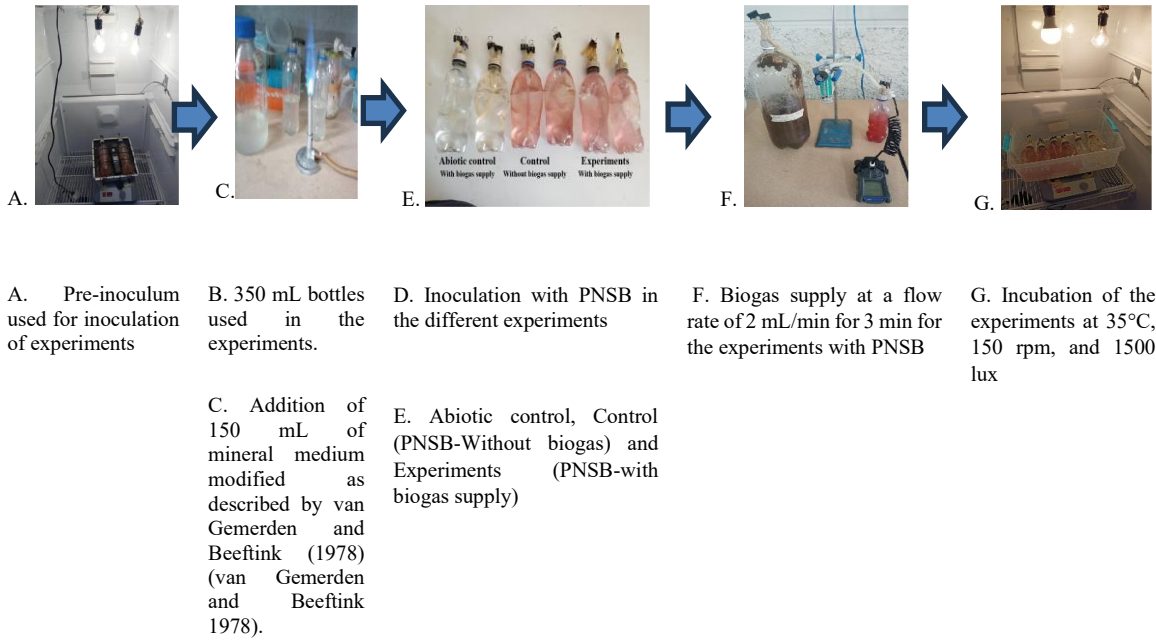


Fig. 1. Experimental setup to evaluate the hydrogen sulfide removal capacity of *Rhodopseudomonas telluris* in the experimental biogas biodesulfurization process

Estimation of kinetic parameters

Kinetic data for hydrogen sulfide removal were modeled using the modified Gompertz model (Eq. 3):

$$H = H_{\max} \exp \left\{ -\exp \left[\frac{R_{\max} e}{H_{\max}} (\lambda - t) + 1 \right] \right\} \quad (3)$$

Where $H_{\max}$, is the maximum removal value expressed in ppm; $R_{\max}$, is the maximum removal rate expressed in ppm h⁻¹; λ , is the lag phase duration in hours; t , is the cultivation time in hours; and e , is Euler's constant of 2.718282.

Microbial growth and mathematical modeling

Cell growth of *Rhodopseudomonas telluris* was determined spectrophotometrically by measuring the optical density (OD) of the culture at 630 nm every 24 h for 312 h. Biomass concentration was calculated from the absorbance values using a previously established calibration curve. Biomass concentration data (in g L⁻¹) were used to calculate the specific growth rate according to Eq. 4 (Muloiwa et al. 2020). Additionally, a modified Monod model (Eq. 5), as reported by Song et al. (2008), was applied to describe substrate-dependent cell growth and metabolic product formation.

$$\mu = \frac{\ln \left(\frac{X_2}{X_1} \right)}{(t_2 - t_1)} \quad (4)$$

$$\mu = \frac{\mu_{\max} S}{S + K_s + \left(\frac{S^2}{K_i}\right)} \times \left(1 - \frac{P}{P_{\text{CRIT}}}\right)^i \quad (5)$$

Where μ , is the specific growth rate (h^{-1}); X_2 and, X_1 are the biomass concentrations; t_1 and t_2 are the corresponding time points; $\mu_{\max}$, is the maximum specific growth rate; S , is the substrate concentration; K_s , is the substrate saturation constant (ppm); K_i , is the substrate inhibition constant (ppm); P_{CRIT} , is the critical product concentration (ppm); and i , is the degree of product inhibition (dimensionless). The parameters for both Eqs. 3 and 5 were estimated using the Generalized Reduced Gradient (GRG) algorithm coded in the Spyder environment (Anaconda, version 2025.06).

Analytical methods

Hydrogen sulfide concentration was determined using an MSA Altair 4XR® portable multi-gas detector, which was previously calibrated using an MSA® detector calibration kit. Sulfate concentration was measured using the turbidimetric method described by Kolmert et al. (2000).

The pH was measured using a HANNA Instruments® pH meter equipped with an HI 1131B electrode. Oxidation-reduction potential (ORP) was determined using a HANNA pH meter fitted with a platinum electrode and an Ag/AgCl reference half-cell.

Carotenoid and bacteriochlorophyll concentrations were determined following the methodology described by Lopez et al. (2020) with slight modifications. Culture samples 2 mL were centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. For extraction, 2 mL of an acetone/methanol solution 7:2 v/v was added to the pellet. The suspension was vortexed for 10 min and centrifuged again at 10,000 rpm for 10 min, after which the supernatant was collected. This extraction cycle was repeated until the biomass was completely decolorized. Both carotenoids and bacteriochlorophylls were determined spectrophotometrically and quantified using Eqs. 6 and 7 (Liu et al. 2019). The obtained values were converted to mg g^{-1} .

$$\text{Total carotenoids content} = A_{473} \times 1000 / (250 \times L \times W) \quad (6)$$

$$\text{Total bacteriochlorophylls content} = A_{771} \times 1000 / (76 \times L \times W) \quad (7)$$

Where A_{473} and A_{771} are the measured absorbances; L is the spectrophotometer cuvette path length; and W is the initial sample mass divided by the volume of extract obtained.

Polyhydroxybutyrate (PHB) extraction and quantification were performed as follows: 2 mL of culture medium was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded, and 1 mL of 2% NaClO solution was added to the pellet. The suspension was vortexed and incubated in an oven at 30°C for 1 h. After incubation, the sample was vortexed and centrifuged at 10,000 rpm for 10 min. The supernatant was removed, and 1 mL of absolute ethanol was added to the pellet. The mixture was again vortexed and centrifuged at 10,000 rpm for 10 min. After discarding the supernatant, 1 mL of acetone was added to the pellet, vortexed, and centrifuged under the same conditions (10,000 rpm, 10 min). To the resulting pellet, 1 mL of concentrated sulfuric acid was added, and the mixture was placed in a water bath at 90°C for 10 min to convert the intracellular PHB into crotonic acid. A blank was prepared using only concentrated sulfuric acid. Simultaneously, a calibration curve was constructed using crotonic acid standard solutions (0.1 µg crotonic acid/ 0.1 µl sulfuric acid) to prepare working standards of 5, 10, 15, 20, 25, 30, 40, and 50 µg/3 ml. Absorbance for samples, blanks, and calibration standards was measured at 235 nm using a Jenway Genova® spectrophotometer. A linear regression graph of concentration versus absorbance was plotted to obtain the quantification equation for PHB in the samples, with results expressed as mg L⁻¹ (Fathima S and Krishnaswamy 2017).

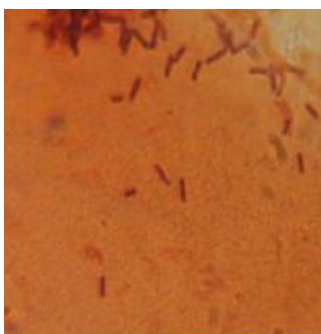
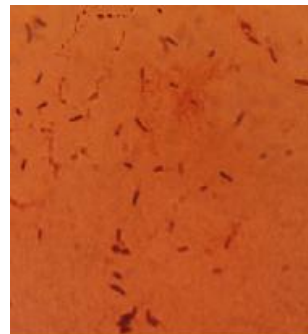
Results

Microscopic characterization, molecular identification, and phylogeny

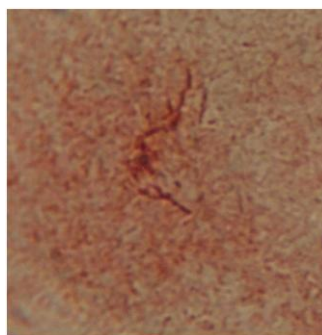
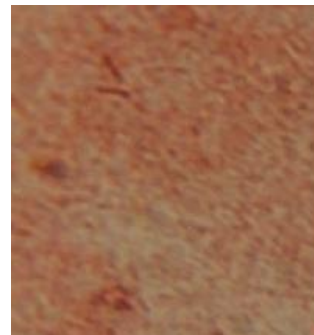
The bacterium used in this study was *Rhodopseudomonas telluris*, previously isolated from a wastewater lagoon in Gómez Palacio, Durango, Mexico, by Cisneros et al. (2024). In the preculture bottles, it exhibited an intense red color along with a strong rotten-egg odor. Microscopically, *Rhodopseudomonas telluris* proved to be a Gram-negative, motile, rod-shaped bacterium (Fig. 2).



A. Growth of *Rhodopseudomonas telluris* in mineral medium with sodium acetate



B. Images of microscopic morphology of wet mount (100x objective of the microscope)



C. Images of microscopic Gram staining (100x objective of the microscope)

Fig. 2. Visualization of the distinctive growth characteristics of *Rhodopseudomonas telluris* in liquid medium and microscopic characterization by wet mount and Gram staining

210 A phylogenetic tree was constructed to compare the isolate with 20 genera of purple non-
 211 sulfur bacteria within the *Rhodospirillaceae* group (Fig. 3). The isolate grouped within
 212 Alphaproteobacteria and showed its closest clustering relationship with *Rhodopseudomonas*
 213 *telluris* (MW010429.1). The reported evolutionary distance was 0.00167 for both
 214 *Rhodopseudomonas telluris* and *Afipia genosp* (U87769.1); however, the isolate and
 215 *Rhodopseudomonas telluris* clustered at the same node, providing the strongest tree-based
 216 support for the identification. Other Alphaproteobacteria in the analysis were more distant,
 217 with reported values from 0.13 to 0.22 (Supplementary material). These distances should be
 218 interpreted in the context of the sequence region analyzed and the tree-building method,
 219 rather than as a direct measure of physiological similarity.

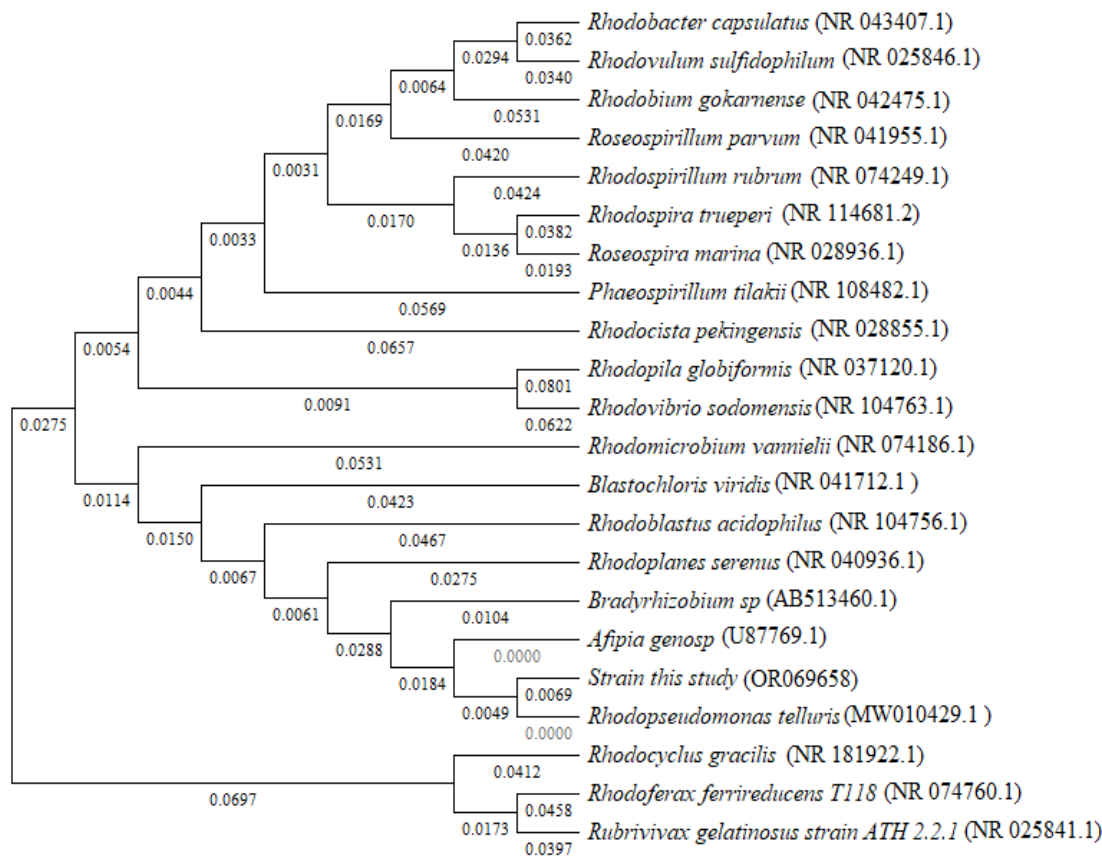


Fig. 3. Phylogenetic tree of species in the *Rhodospirillaceae* family of purple non-sulfur bacteria based on 16s RNA sequences. The neighbor-joining method was used to construct the tree with bootstrap values determined through 1000 iterations.

221

222 The strains belonging to the Betaproteobacteria class, *Rhodocyclus gracilis* (NR_181922.1),
223 *Rhodoferax ferrireducens* T118 (NR_074760.1), and *Rubrivivax gelatinosus* ATH 2.2.1
224 (NR_025841.1), exhibited evolutionary distance values of 0.23, 0.24, and 0.26, respectively,
225 and formed a branch separate from the main root grouping. This separation supports the broad
226 phylogenetic distinction represented in Fig. 3.

227

228 **Hydrogen sulfide removal**

229 The observed hydrogen sulfide removal profile and the values predicted by the modified
230 Gompertz model are shown in Fig. 4A. Removal increased progressively during the first 180
231 h, followed by a clear approach to asymptotic phase between approximately 200 and 312 h.

This pattern indicates an initial period of adaptation and increasing removal activity, followed by a phase in which the incremental removal became small. The kinetic parameters for the metabolic assimilation of biogas-derived hydrogen sulfide by *Rhodopseudomonas telluris* were: lag phase of 66.44 ± 1.11 h, estimated maximum cumulative removal of 2917.36 ± 38.77 ppm, and the maximum removal rate of 28.0 ± 0.28 ppm h⁻¹. The lag phase is consistent with an adaptation period before the culture expressed its highest apparent removal activity, whereas the asymptotic phase suggests that one or more operating conditions eventually constrained further removal.

Table 1. Hydrogen sulfide supply values and its removal over time in experiments with *Rhodopseudomonas telluris* and abiotic control

Time (h)	Hydrogen sulfide inlet (ppm)	Hydrogen sulfide inlet acumulative (ppm)	Hydrogen sulfide removal in experiment with <i>Rhodopseudomonas telluris</i> (%)	Hydrogen sulfide removal in abiotic control (%)
0	0	0	0.0±0	0.0±0.0
24	456	456	5.25±0.03	0.10±0.02
48	558	1014	11.86±0.07	0.15±0.00
72	785	1799	20.36±0.09	0.16±0.01
96	665	2464	26.10±0.03	0.19±0.01
120	558	3022	30.06±0.06	0.23±0.02
144	552	3574	32.72±0.19	0.25±0.03
168	682	4256	34.14±0.24	0.27±0.02
192	581	4837	34.74±0.31	0.28±0.02
216	609	5446	35.08±0.41	0.30±0.01
240	732	6178	35.22±0.49	0.32±0.00
264	569	6747	35.29±0.48	0.33±0.01
288	712	7459	35.32±0.48	0.36±0.01
312	756	8215	35.33±0.48	0.38±0.01

Table 1 shows the hydrogen sulfide removal percentages for the abiotic controls and the experiments with *Rhodopseudomonas telluris*. In the abiotic controls, the removal values obtained were $0.38 \pm 0.01\%$, indicating that of the total $35.33 \pm 0.48\%$ hydrogen sulfide removal, 35% was directly attributable to metabolic assimilation by *Rhodopseudomonas telluris* rather than abiotic factors.

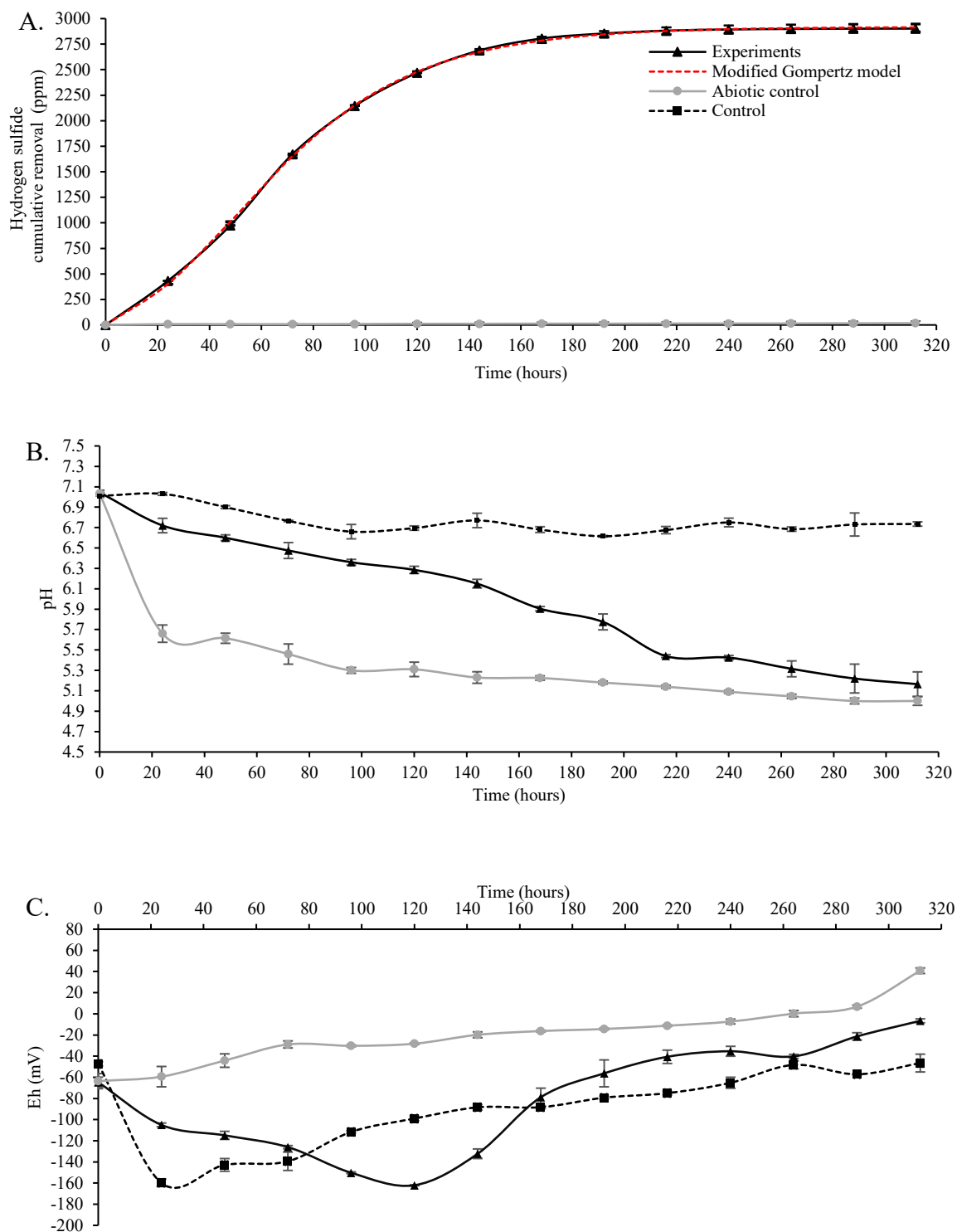


Fig. 4. A) Kinetic behavior of experimental data for hydrogen sulfide removal with curve estimation data modified Gompertz model B) pH values and C) Redox potential (Eh) obtained throughout the 312 hours that the experiment lasted and. The standard deviation for $n=2$ is shown in all graphs.

pH and redox potential

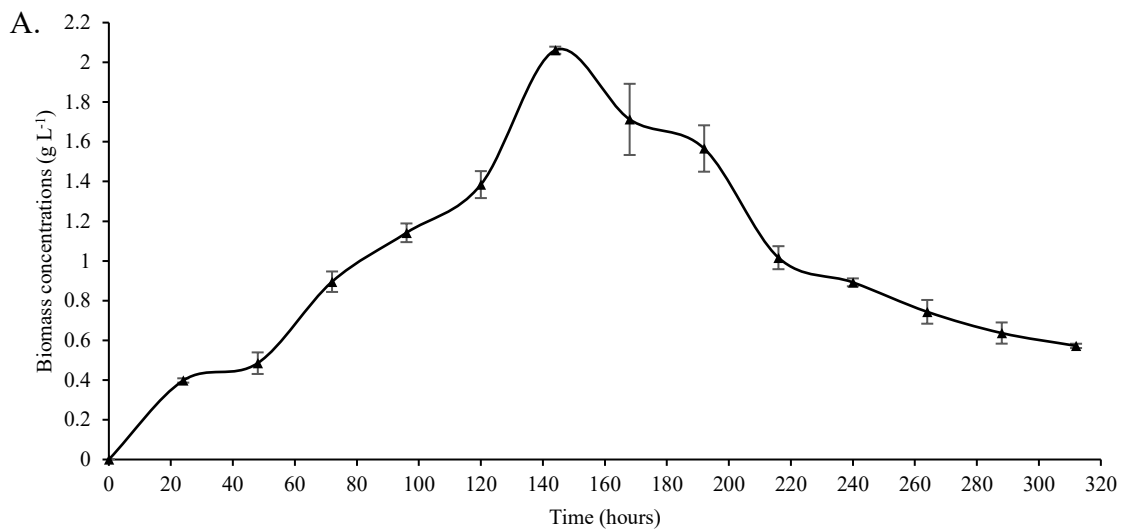
Initially, the pH was adjusted to 7.0 across all experiments; it was not controlled throughout the experimental period, but it was monitored, with pH variations over time presented in Fig. 4B. In the inoculated treatment, during the first 144 h, the pH gradually decreased to values of 6.15 ± 0.04 . However, starting at 168 h, the drop in pH became more pronounced, reaching values of 5.16 ± 0.12 at 312 h. In the abiotic controls, 24 h after the initial hydrogen sulfide supply, the pH values decreased to 5.66 ± 0.08 , and as incremental additions of hydrogen sulfide continued, the pH values further declined to 5.0 ± 0.04 . The abiotic control clearly demonstrates how the addition of hydrogen sulfide acidified the medium within the first few hours. Conversely, in the experiments with *Rhodopseudomonas telluris*, medium acidification occurred over a more extended period, providing evidence of hydrogen sulfide removal. Comparing the trend in pH values with the hydrogen sulfide removal efficiency by *Rhodopseudomonas telluris* reveals a correlation, as the highest hydrogen sulfide removal efficiency occurred during the first 168 h within a pH range of 7.0–6.0. In contrast, from 192 h onward, the steepest decline in pH was recorded (ranging from 5.9 to 5.3), accompanied by a significant reduction in hydrogen sulfide removal until complete cessation (Fig. 4A). According to these results, a pH range of 7.0–6.0 is optimal for cell growth and metabolic oxidation of hydrogen sulfide by *Rhodopseudomonas telluris*. Meanwhile, at pH values ranging from 5.7 to 5.0, growth was drastically impaired, and a decrease in biomass concentration was observed after 144 h (Fig. 5A).

The oxidation-reduction potential (ORP) is a parameter that broadly describes the physiological and metabolic state of bacteria during the oxidation of reduced sulfur compounds in the biodesulfurization process. In experiments inoculated with *Rhodopseudomonas telluris*, ORP values during the first 120 h were -162.1 mV, after which they gradually increased to -6.6 mV at 312 h (Fig. 4C). According to the kinetic behavior, the most negative ORP value coincided with the period of strongest biomass accumulation and hydrogen sulfide removal, while the later shift occurred alongside acidification and reduced removal activity. This temporal association is consistent with a change in the balance between reduced sulfur input, microbial electron transfer, and oxidized sulfur products. ORP

is, however, a composite measurement affected by pH and multiple redox couples; it should not be treated as a direct measurement of a particular intracellular pathway.

Biomass and microbial growth modeling

Rhodopseudomonas telluris showed an apparent exponential growth phase from 0 to 144 h, during which biomass reached $2.06 \pm 0.01 \text{ g L}^{-1}$ (Fig. 5A). After 168 h, biomass declined progressively and reached $0.57 \pm 0.01 \text{ g L}^{-1}$ at 312 h. The growth trajectory broadly followed the hydrogen sulfide removal profile: biomass increased during the period of increasing removal and declined as removal approached its asymptotic phase. Because the culture received repeated gas pulses without medium replacement, the later decline may reflect the combined effects of acidification, sulfide exposure, and accumulation of dissolved products rather than a single inhibitory factor.



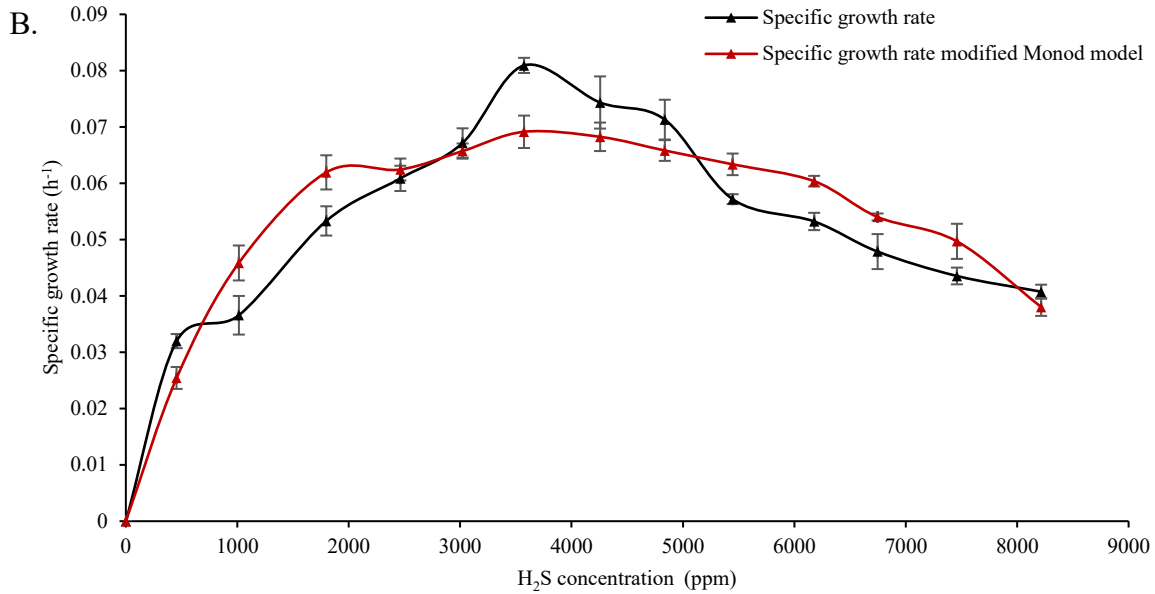


Fig. 5. Evaluation and modeling of *Rhodopseudomonas telluris* growth under the effect of supplied biogas concentration and produced sulfate. A) Biomass concentration with respect to time, B) Specific growth rates obtained from experimental data and those estimated by the modified Monod model

In fig. 5B, the observed specific growth rate μ values showed an increase with rising hydrogen sulfide concentrations up to 3574 ppm, above this concentration, μ values decreased significantly.

Estimation of the parameters $\mu_{\max}$, K_s , K_i , P_{CRIT} , and i with modified Monod model provided deeper insight into the kinetic behavior of *Rhodopseudomonas telluris* using biogas-derived hydrogen sulfide as a substrate and produced sulfate as an inhibitory metabolic product. Under optimal conditions, *Rhodopseudomonas telluris* reached a $\mu_{\max}$ of $0.20 \pm 0.01 \text{ h}^{-1}$. The K_s value was $3047.48 \pm 69.29 \text{ ppm}$, indicating that *Rhodopseudomonas telluris* can grow efficiently at these hydrogen sulfide concentration levels. The hydrogen sulfide concentration capable of inhibiting growth K_i was $4632.74 \pm 182.43 \text{ ppm}$. As well as P_{CRIT} value of $338.57 \pm 9.08 \text{ ppm}$ established the critical threshold concentration of produced sulfate that must not be exceeded to avoid cell growth inhibition.

Changes in sulfate concentration

Figure 6 shows a net increase in sulfate in the inoculated treatment over the 312 h experiment. The measured concentration was $71.27 \pm 7.75 \text{ ppm}$ after the first 24 h and reached $336.80 \pm 7.22 \text{ ppm}$ at the end. In the no-biogas control, the final concentration was $63.0 \pm 0.84 \text{ ppm}$,

suggesting a background contribution potentially associated with medium components, biomass turnover, or analytical background. The higher concentration in the treatment receiving biogas is consistent with conversion of reduced sulfur to sulfate.

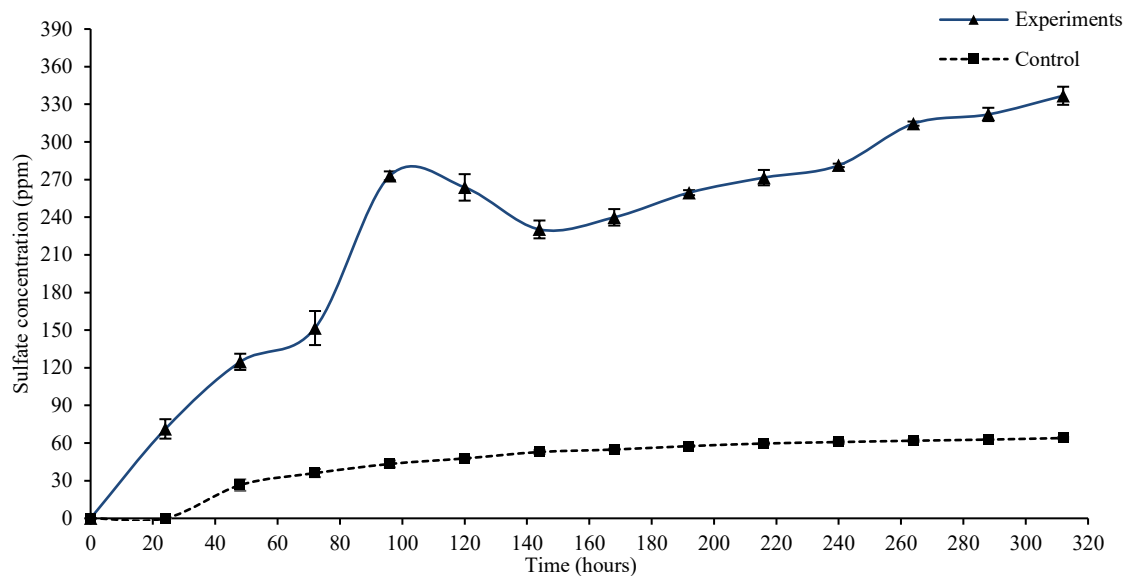


Fig. 6. Kinetic behavior of sulfate production in the experiments of *Rhodopseudomonas telluris* with biogas supply and controls

Pigments and PHB production

At the end of the experiment, the inoculated treatment showed a more intense reddish coloration than the control (Fig. 7), visually consistent with pigment accumulation. Carotenoids increased during the first 120 h and reached a maximum of $1.27 \pm 1.22 \times 10^{-2} \text{ mg g}^{-1}$, before declining to $1.06 \pm 7.64 \times 10^{-2} \text{ mg g}^{-1}$ at 312 h (Fig. 8A). This trajectory suggests that pigments accumulation was favored during active growth and early hydrogen sulfide removal, whereas the later decline coincided with reduced biomass and a more acidic medium. The endpoint concentration remained measurable, indicating that pigment-associated metabolism persisted through the end of the experimental period.

Time (0 h)

Time (312 h)

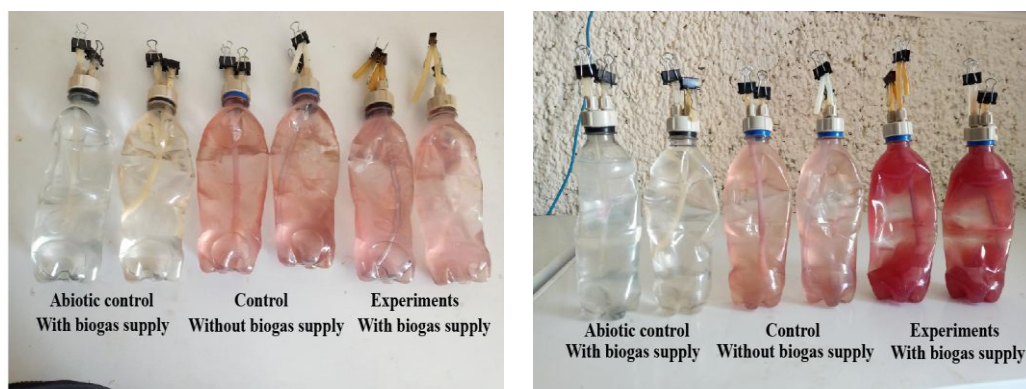


Fig. 7. Pigmentation of the experiment at the initial time (0 h) and final time of experimentation (312 h)

Bacteriochlorophyll concentration reached a maximum of $0.58 \pm 9.36 \times 10^{-3} \text{ mg g}^{-1}$ within the first 96 h and declined to $0.43 \pm 1.29 \times 10^{-2} \text{ mg g}^{-1}$ by 312 h. The earlier peak relative to the carotenoid maximum suggests that the two pigment groups did not respond identically over time (Fig. 8A). Because bacteriochlorophylls participate in light capture for anoxygenic photosynthesis, their early accumulation is consistent with establishment of photosynthetic activity. The later decrease may reflect physiological stress or changes in the amount and composition of biomass.

Under the tested hydrogen sulfide removal conditions, *Rhodopseudomonas telluris* accumulated polyhydroxybutyrate (PHB), reaching a maximum concentration of $200.69 \pm 9.78 \text{ mg L}^{-1}$ at 192 h (Fig. 8B). PHB accumulation peaked later than bacteriochlorophyll and close to the period when hydrogen sulfide removal began to approach a asymptotic phase. Its concentration subsequently declined, falling by approximately 50% by 312 h. This pattern is compatible with PHB functioning as a transient intracellular carbon and reducing equivalent reserve that can be mobilized when growth conditions deteriorate.

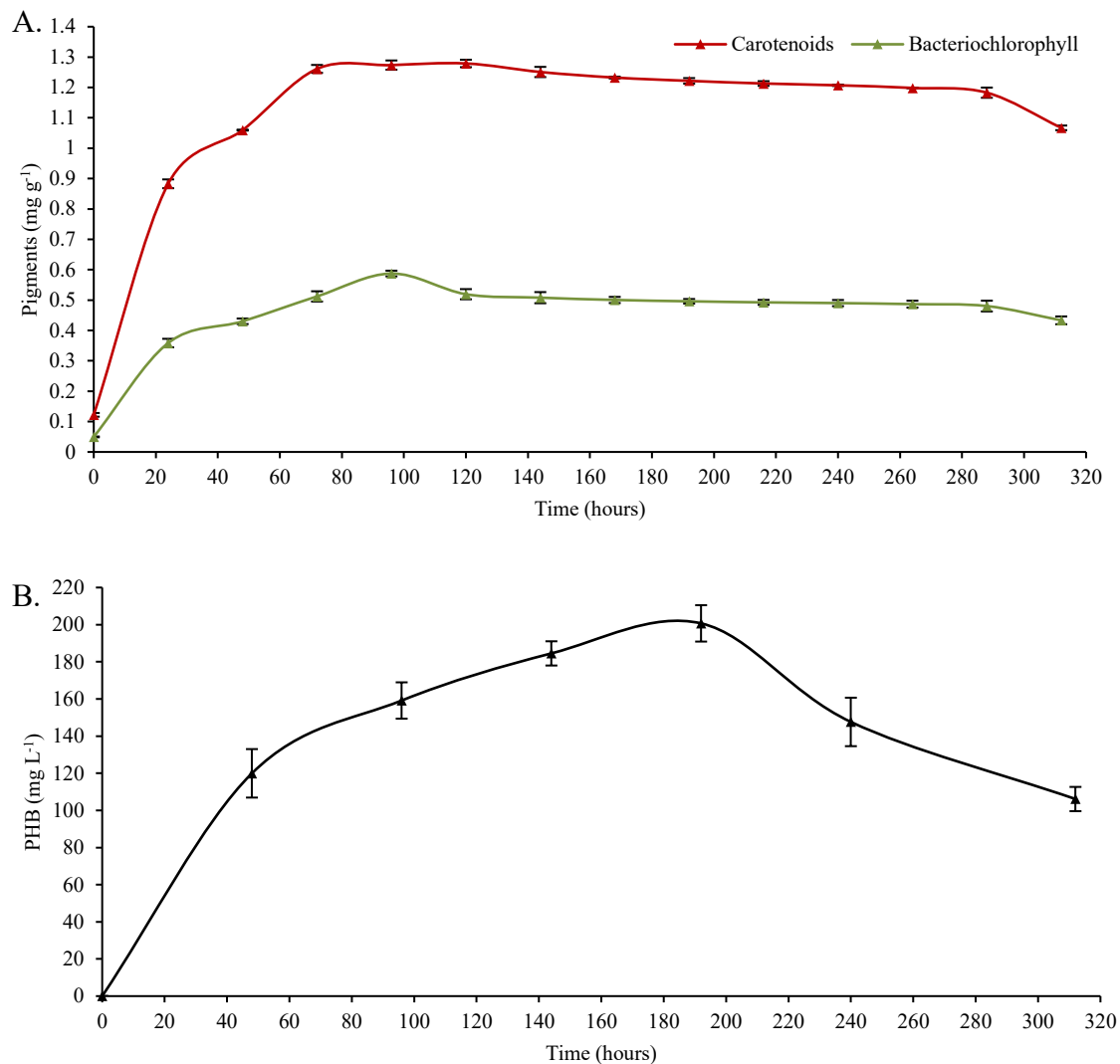


Fig. 8. Production of carotenoids, bacteriochlorophyll, and polyhydroxybutyrate (PHB) by *Rhodopseudomonas telluris* during biogas supply. The standard deviation for n=2 is shown in all graphs.

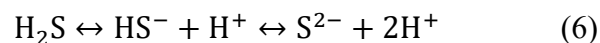
Discussion

Purple non-sulfur bacteria, also described more broadly as purple phototrophic bacteria in studies using mixed cultures, comprise metabolically versatile anoxygenic phototrophs with photoheterotrophic and, in selected conditions, photoautotrophic capabilities (Madigan and Jung 2009; Dhar et al. 2023). Their metabolic flexibility makes them promising candidates for coupling gas treatment with biomass and bioproduct generation.

Phylogenetically, purple non-sulfur bacteria represent a diverse group of anoxygenic photosynthetic microorganisms that belong to both the Alphaproteobacteria and

Betaproteobacteria classes (Madigan and Jung 2009). The 20 recognized genera in the family *Rhodospirillaceae* possess photoheterotrophic, photoautotrophic, and chemoheterotrophic capabilities (Rosenberg 2013). The present microscopy and Gram staining observations agree with descriptions of *Rhodopseudomonas* as Gram-negative, often motile rods (0.8–1.2 µm in width, 2.0–6.0 µm in length) that can form reddish to brownish cultures under illuminated, oxygen-limited conditions (Venkata et al. 2012, Dhar et al. 2023). Hydrogen sulfide removal after 200 h was low (Fig. 4A), which can be attributed to culture medium acidification, as pH values dropped to 5.42, likely affecting the metabolism of *Rhodopseudomonas telluris*. Upon solubilization in water, hydrogen sulfide acidifies the medium. The solubility of hydrogen sulfide is relatively high (up to 3200 mg/L at 30 °C), and concentrations ranging from 28 to 400 mg/L are inhibitory to various bacterial species (Shu et al. 2025). Although hydrogen sulfide was utilized by *Rhodopseudomonas telluris* as an electron donor during photoautotrophic growth, continuous hydrogen sulfide feeding without cell addition or medium replenishment favored an increase in hydrogen sulfide concentration in the liquid phase, thereby lowering the pH. Nevertheless, this provided insight into the assimilatory tolerance of *Rhodopseudomonas telluris* to hydrogen sulfide derived from anaerobic digestion biogas. Studies by Schütz et al. (1999) reported that hydrogen sulfide overload in the reactor impairs PNSB growth, mainly attributed to reduced ATP production resulting from the over-reduction of a quinone pool that disrupts photosystem electron flow. Furthermore, hydrogen sulfide disrupts disulfide bonds that maintain protein structures and reacts with essential nutrients in the medium (such as iron, nickel, or cobalt), precipitating them as insoluble crystals that bacteria cannot uptake (Shu et al. 2025). The high coefficient of determination $R^2 = 0.99$ obtained with the modified Gompertz model demonstrates its suitability for modeling hydrogen sulfide removal kinetics. The maximum removal rate achieved by *Rhodopseudomonas telluris* was substantially higher than the 10.77 ppm h⁻¹ reported by Egger et al. (2023) for purple phototrophic bacteria, emphasizing the high desulfurization potential of this strain.

Medium acidification caused by the addition of hydrogen sulfide occurs because its dissociation releases hydrogen ions (H⁺) (Eq. 6) (Siang et al. 2017; Borisov and Forte 2021).



It has been reported that in biodesulfurization systems, the decrease in pH values in addition to the accumulation of hydrogen sulfide in the mineral medium is caused by other hydrogen sulfide oxidation products, such as sulfate (Zhang et al. 2022). Other studies have indicated that a decrease in pH can inhibit microbial growth and retard the diffusion rate of hydrogen sulfide into the aqueous phase of the medium, ultimately impairing removal efficiency (Xu et al. 2016). The optimal pH range between 6.0 and 7.0 for hydrogen sulfide removal by *Rhodopseudomonas telluris* aligns with findings by Hidayat et al. (2017) and Giang et al. (2021), who reported that growth and hydrogen sulfide oxidation by sulfur-oxidizing bacteria occur under neutral conditions, with an optimal pH around 8.0. Janssen et al. (1997) and Xu et al. (2016) reported that a pH range of 7.5 to 8.0 was suitable for the oxidation of sulfide to sulfate.

The shift in ORP profile directly reflects the chemical transformation of sulfur provided further insight into the metabolic state of *Rhodopseudomonas telluris*. The most negative ORP value -162.1mV coincided with peak growth and maximum hydrogen sulfide removal. The above can be attributed to the fact that in photoautotrophic PNSB, reverse electron flow required to reduce NAD^+ using hydrogen sulfide as an electron donor operates optimally under reducing conditions 0-320 mV across the cytoplasmic membrane (Oktyabrskii and Smirnova 2012; Elbehti et al. 2000).

Hydrogen sulfide is a reducing molecule in which sulfur has an oxidation state of -2, whereas sulfate is more oxidizing with sulfur transitioning from an oxidation state of -2 to +6; thus, the increase in sulfate concentration (Fig. 6) partially explains the rise in ORP.

The lowest ORP values were recorded within a pH range of 6.0–7.0 (Figs. 8 and 9), indicating that pH directly influenced changes in ORP values. Oktyabrskii and Smirnova (2012) reported in the $\text{H}_2\text{S}/\text{SO}_4^{2-}$ system where hydrogen sulfide is converted to sulfate, the reaction involves both electrons and protons, accounting for the ORP shifts accompanied by pH changes. Growth modeling using the modified Monod equation R^2 of 0.90 effectively described substrate inhibition and product kinetics indicating the model's reliability and goodness of fit. The modified Monod model reported by Song et al. (2008) was applied to describe the observed growth rate kinetics of *Rhodopseudomonas telluris* regarding the effects of hydrogen sulfide concentration and sulfate formation. Previous studies have

reported that the modified Monod model is highly useful for describing the distinct kinetic phases of cell growth involving substrate inhibition and product formation (Song et al. 2010). The similarity between the values K_s and H_{max} , indicated that the growth of *Rhodopseudomonas telluris* was dependent on hydrogen sulfide concentration. Because final sulfate levels remained below P_{CRIT} value, sulfate accumulation was not a driving factor in the growth inhibition of *Rhodopseudomonas telluris*.

The metabolic capacity of *Rhodopseudomonas telluris* to oxidize hydrogen sulfide to sulfate can be attributed a process that may involve sulfide:quinone oxidoreductase and other sulfur-oxidation components as enzymatic complex known as Sox, which catalyzes oxidation reactions to produce sulfate (Frigaard and Dahl 2008).

Studies have reported that certain species of purple non-sulfur bacteria belonging to the families *Rhodopseudomonas*, *Rhodospirillum*, and *Rhodobacteraceae* utilize the SOX system for sulfate formation from sulfide (Appia et al. 2001).

In support of the indications provided by the P_{CRIT} values, it is worth noting that after 168 h when cell growth inhibition began sulfate production continued, rising from 240 ppm to 336 ppm. This phenomenon can be attributed to three factors: first, intracellular PHB reserves sustained cellular activity in *Rhodopseudomonas telluris* despite the absence of active CO_2 fixation; second, the structural stability of the membrane-anchored (periplasmic) sulfide-quinone reductase enzyme allowed it to maintain catalytic activity independently of bacterial cell viability (Schütz et al. 1999); and third, concomitant abiotic processes occurred, wherein the interaction of hydrogen sulfide with transition metals induced chemical oxidation (Hansen and van Gernerden 1972).

Rhodopseudomonas telluris demonstrated the ability to produce both carotenoids and bacteriochlorophylls under photoautotrophic conditions using biogas as the sole carbon and energy source.

The function of carotenoids in PNSB is to absorb light energy during photosynthesis and provide photoprotection against oxidative degradation (Sandmann 2023). Meanwhile, bacteriochlorophylls capture light energy and convert it into chemical energy (Canniffe and Hunter 2014).

The carotenoid production achieved in this study falls within the range reported for photoautotrophic conditions (between 1.5 and 2.5 mg g⁻¹) (Wang et al. 2019).

Recent studies on photoautotrophic bacteria indicate that hydrogen sulfide, at optimal concentrations, stimulates the metabolic pathways for carotenoid and bacteriochlorophyll synthesis. Acting as an electron donor, this compound regulates the photosynthetic apparatus and optimizes light absorption efficiency (Ángeles et al. 2026; Wei et al. 2025). The behaviors shown in Figs. 4C, 5A, and 8A demonstrate a correlation between pigment production, biomass, and hydrogen sulfide removal. Therefore, we can attribute the decrease in carotenoid and bacteriochlorophyll concentrations towards the end of the process to hydrogen sulfide toxicity. High concentrations of hydrogen sulfide in photoautotrophic bacteria have been reported to induce toxicity, degrading photosynthetic pigments and reducing photon absorption (Moskalenko and Makhneva 2012). Another relevant result of this research is the higher concentration of carotenoids relative to bacteriochlorophylls, which can be attributed to hydrogen sulfide-induced stress in *Rhodopseudomonas telluris*. Previous studies have also reported an increase in the carotenoid to bacteriochlorophyll ratio in *Rhodopseudomonas palustris* under stress conditions (Montiel-Corona and Buitrón 2025), as PNSB tend to increase carotenoid production due to their protective function for the cell (Sandmann 2023).

The production of PHB in this study was due to the fact that it has been reported that bacteria of the genus *Rhodopseudomonas* can fix CO₂ via the photoautotrophic pathway and convert it into acetyl-CoA, which is subsequently used for PHB biosynthesis (Mukhopadhyay et al. 2005). The PHB production phases correlated with the kinetic behavior of hydrogen sulfide removal. During the period of maximum hydrogen sulfide removal, the highest PHB concentration was detected, indicating that hydrogen sulfide served as an electron donor to fix CO₂, which was in turn transformed into PHB. In the decline phase of hydrogen sulfide removal when *Rhodopseudomonas telluris* no longer utilized hydrogen sulfide as an electron donor and, consequently, ceased fixing CO₂, the concentration of PHB began to decrease because the bacteria began mobilizing PHB as a carbon and energy source to maintain active metabolism. When carbon and energy are sufficient under stress conditions, bacteria of the genus *Rhodopseudomonas* accumulate PHB and subsequently consume it upon nutrient or carbon depletion, or when stress conditions become prolonged or severe (Touloupakis and Chatziathanasiou 2023; Montiel and Buitrón 2022; Brown et al. 2020).

Conclusions

In this study, it was demonstrated that the purple non-sulfur bacterium *Rhodopseudomonas telluris* (MW010429.1) is capable of removing and metabolically assimilating 2902.20 ± 39.59 ppm of hydrogen sulfide, corresponding to 35% of the total supplied in the biogas. Although continuous feeding over 312 h progressively reduced assimilation due to medium acidification, a pH range of 6.0–7.0 and negative ORP values of -162.1 mV were determined to represent the optimal conditions to favor the cellular growth of *Rhodopseudomonas telluris* and hydrogen sulfide removal. According to the parameters estimated by the modified Monod model, the affinity constant of 3047.48 ± 69.29 ppm indicated that *Rhodopseudomonas telluris* maintains efficient growth up to this concentration, reaching a maximum specific growth rate of $0.20 \pm 0.01 \text{ h}^{-1}$. Likewise, based on the inhibition constant, the critical limit to prevent the cessation of growth was established at 4632.74 ± 182.43 ppm. On the other hand, the critical product concentration revealed that the produced sulfate at 336.80 ppm exerted no inhibitory effect on bacterial development. The detection of carotenoids and bacteriochlorophylls confirmed the capacity of *Rhodopseudomonas telluris* to biosynthesize both pigments under photoautotrophic conditions using biogas, with a direct correlation observed between their production and the hydrogen sulfide concentration. Finally, the detection of polyhydroxybutyrate, a cellular storage polyester, demonstrated the strain's ability to fix CO_2 and channel it into the biosynthesis of this biopolymer. Overall, the remarkable metabolic flexibility of *Rhodopseudomonas telluris* positions this bacterium as a promising biocatalyst for biogas purification within the framework of bioprocesses aligned with the circular economy.

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