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Research article

Fate of H_2S during the cultivation of *Chlorella* sp. deployed for biogas upgrading

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ABSTRACT

The H_2S may play a key role in the sulfur cycle among the biogas production by the anaerobic digestion of wastes and the biogas upgrading by a microalgae based technology. The biogas is upgraded by contacting with slightly alkaline aqueous microalgae culture, then CO_2 and H_2S are absorbed. The dissolved H_2S could limit or inhibit the microalgae growth. This paper evaluated the role of dissolved H_2S and other sulfured byproducts under prevailing biogas upgrading conditions using a microalgal technology. At initial stages of batch cultivation the growth of *Chlorella* sp. was presumably inhibited by dissolved H_2S. After 2 days, the sulfides were oxidized mainly by oxic chemical reactions to sulfate, which was later rapidly assimilated by *Chlorella* sp., allowing high growing rates. The fate of H_2S during the microalgae cultivation at pH > 8.5 was assessed by a mathematical model where the pentasulfide, thiosulfate and sulfite were firstly produced and converted finally to sulfate for posterior assimilation.

1. Introduction

The anaerobic digestion of organic wastes produces biogas with a typical composition of CH_4 (40–60 %v/v), CO_2 (15–60 %v/v) and H_2S (0.005–2 %v/v) besides traces of volatile organic compounds, NH_3 and H_2O (Andriani et al., 2014). The removal of H_2S and CO_2 from biogas is a desirable process before its usage as sustainable fuel (Bahr et al., 2013). The biogas upgrading based on the deployment of microalgae for the CO_2 fixation has emerged as promising technology (Kumar et al., 2011; Bahr et al., 2013; Yan and Zheng, 2013). The biogas is upgraded in absorption systems (pH > 8), where CO_2 and H_2S are efficiently transported into the liquid phase (Bahr et al., 2013), and depending on pH they react to form predominantly bicarbonate HCO_3^- and hydrosulfide HS^- respectively (Equations (1) and (2)). HCO_3^- (Equation (3)) can be a source of CO_2 for supporting the microalgae growth, if the cells preserve adequate carbon fixation rates under surrounding alkaline and dissolved hydrogen sulfide ($H_2S_{(L)}$) environments (De los Cobos-Vasconcelos et al., 2016; Chang et al., 2016).

$$CO_2 + OH^- \leftarrow\rightarrow HCO_3^- \quad (1)$$

$$H_2S + OH^- \leftarrow\rightarrow HS^- + H_2O \quad (2)$$

$$HCO_3^- + H_2O + S \rightarrow CH_xO_yS_z + O_2 + OH^- \quad (3)$$

The H_2S may play an important role in the sulfur cycle among the biogas production by anaerobic digestion and the biogas upgrading by microalgae based technology. Both processes occurring together could generate biogas, a sustainable fuel, as its production and purification includes resources recovery, i.e. the H_2S can be a direct or indirect sulfur source for supporting the microalgae growth (Krauss and Schmidt, 1987) besides the nutrients recovered from digestate (Rachbauer et al., 2015) which is deficient in sulfate. H_2S could be either growth limiting or inhibiting substrate for some cyanobacteria strains (Ohki et al., 2012). The H_2S effect on eukaryotic cell of microalgae had been few reported, a relevant genera is *Chlorella* which present high biomass productivities and has showed robustness during its deploying in a biogas upgrading process (Kao et al., 2012). *Chlorella vulgaris* could use H_2S as substrate; its utilization is strongly pH dependent, reaching highest uptake rates at low pH (Spedding et al., 1980). However, Kao et al. (2012) reported the deployment of *Chlorella* sp. MM-2 for biogas upgrading, it was exposed to H_2S concentrations

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up to 150 ppm_v, stated that H_2S inhibited the growth of this microalgae instead of acids pH. These authors observed that H_2S was presumable converted by *Chlorella* sp. MM-2 to sulfate reducing the microalgae cell toxicity. Sulfate is easily assimilated by *Chlorella* and it can become a growth limiting substrate (Di Martino Rigano et al., 2000; Mera et al., 2016). The influence of other inorganic sulfur compounds on the microalgae growth (i.e. thiosulfate, tetrathionate, thiocyanate and elemental sulfur) is slightly less favorable as the reported effect for sulfate (Krauss and Schmidt, 1987). At pH > 8 the sulfides $H_2S_{(L)}$ and hydrosulfide (HS^-) are easily oxidized in presence of oxygen by both chemical and biological reactions, i.e. under oxygen rich aquatic systems like a fresh water lake in photic zone. Gun et al. (2000) found pentasulfide as main byproduct of sulfide oxidation. González-Sánchez and Revah (2007) have described the kinetics of sulfide oxidation in presence of an alkaliphilic sulfide-oxidizing bacteria consortium, where biotic and abiotic oxidation processes were evaluated. The aim of this work was to evaluate the growth kinetics of *Chlorella* sp. as a function of the likely prevailing concentrations of bicarbonate and dissolved H_2S reached during a biogas upgrading process at pH 8.5. A hybrid chemical-biological model was developed for describing the fate of H_2S, the kinetics of its consumption and the byproducts formation besides the sulfate uptake during the cultivation of the microalgae under slightly alkaline conditions.

2. Materials and methods

2.1. Microorganism

Chlorella sp. was growth in 500 ml Erlenmeyer flasks, with a useful volume of 200 ml of TP mineral medium (Andersen, 2005). All batch cultivations were performed by duplicate in a rotary shaker (100 rpm), illuminated with LED lamps at 200 μE m^{-2} s^{-1}, kept at 25 °C and with an initial pH around 8.5 (see Fig. S1). The irradiance on the bottom of the Erlenmeyer flasks was measured using a spherical quantum sensor (QLS-2101, Biospherical Instruments Inc.). For the kinetic assays the biomass concentration (X) was followed during the time, then specific growing rates (μ) corresponding to each assayed condition were computed from a semi-logarithmic linear regression time vs ln X, where the slope represents μ.

2.2. Growth batch cultures

The following assays were all executed by duplicate. In the first experimental stage, four batch cultures under different initial bicarbonate concentrations were assayed (0.07, 0.29, 0.45 and 0.6 M reached from the addition of respective amounts of sodium bicarbonate), these corresponded to gaseous CO_2 equilibrium concentrations of 1.1–19.7 %v/v at pH 8.5.

In the second experimental stage, the bicarbonate concentration of 0.29 M (5%vol of gaseous CO_2 under equilibrium at pH = 8.5) was selected as initial condition for all remaining batch cultures. To evaluate the fate H_2S as only sulfur source, $MgSO_4$ $7H_2O$ was replaced by $MgCl_2$ in the TP medium preserving the same Mg molar concentration. The addition of different amount of sodium sulfide (Na_2S) to each batch culture allowed to reach initial total sulfide ($H_2S_{(L)} + HS^-$) concentrations of 0.1, 0.5 and 1.0 mM (representing 40 to 400 ppm_v of gaseous H_2S under equilibrium at pH = 8.5). Abiotic and biotic assays were included in the evaluation as controls. The pH was maintained at 8.5 by gassing a stream of 5%v/v of CO_2 balanced with N_2 through the headspace of the Erlenmeyer flasks at a rate of 30 ml l^{-1} min^{-1} (see Fig. S3). The biomass and pH were measured every day by duplicate. Sulfate and phosphate concentrations were determined from a mixed sample including the duplicate. The sulfate/biomass yield ($Y_{X/SO4}$) was experimentally evaluated from the batch cultures.

2.3. Analytical determinations

2.3.1. Biomass dry weight concentration

Biomass dry weight was correlated with the optical density (OD) of homogeneous microalgal suspensions at a wavelength of 750 nm using demineralized water as blank, by means of a spectrophotometer (model T60, PG Instruments Ltd.). A correlation curve between OD and biomass dry concentration was assessed. Different biomass dry weight concentrations (X) were prepared by sequential dilution of a concentrated aliquot from the batch cultivations and then the OD was measured. Then 30 mL of the every different biomass density was centrifuged (15 min, 15000 g, 4 °C) obtaining a pellet which was washed twice with ultrapure water, centrifuged again and dried for 24 h, at 80 °C, in stainless steel centrifuge tubes. After drying, the weight of the pellet was determined on an analytical balance. Each measurement was realized in triplicate. A linear correlation resulted in $X = 0.164 \times OD$.

2.3.2. Ion chromatography

The sulfate and phosphate concentrations were measured in agreement with Lehr et al. (2012) by using an ion chromatography system (IC-System, model Compact IC Plus 882, Metrohm GmbH) equipped with an auto sampler unit (model Sample Processor 585) and inline dialysis cell for sample dilution, dialysis (0.2 μm cellulose acetate membrane) and chromatographic analysis. An aqueous sample of 1.5 mL from microalgal cultivations was diluted (1:10) with ultra-pure water and placed in the automated carrousel of the IC-System. Then 20 μL of the dialyzed sample were injected and analyzed using a Metrosep A Supp 5150/4.0 separation column (Metrohm GmbH) and a carbonate eluent (3.2 mM Na_2CO_3, 1.0 mM $NaHCO_3$, 12.5%v/v acetonitrile) with a flow rate of 0.7 ml min^{-1}.

2.4. Mathematical model of the chemical sulfide oxidation and the growth of Chlorella sp.

In order to explain the effect of $H_2S_{(L)}$ on the growth of microalgae cells under slightly alkaline conditions, a general mathematical model (Table 1) was developed, it was based on a series of sulfur-containing compounds mass balances i.e. hydrosulfide (HS^-), pentasulfide (S_5^{2-}), thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}) and sulfate (SO_4^{2-}) as well as for microalgae biomass, following the pathway showed in Fig. 1. $H_2S_{(L)}$ was considered in equilibrium with HS^- (see Equations (2) and (4)).

The proposed mechanism (see Fig. 1) involves direct $H_2S_{(L)}$ inhibition and oxic HS^-chemical oxidation reaction, where sulfides and sulfate are the most reduced and oxidized sulfur-containing compounds respectively. The mathematical model includes as main assumptions: the specific growth rate of *Chlorella* sp. is $H_2S_{(L)}$ inhibited, sulfate and light limited; the cell suspension in batch cultures was considered as perfectly mixed and operated under isothermal conditions. The microalgae cells acted as unique strain forming a homogeneous cell suspension, equivalent to a pseudo-solution. Stripping of H_2S to the headspace was considered in the model. The chemical and biokinetic parameters included in the model were either taken from literature or experimentally determined under similar conditions of the kinetic assays, a list of these parameters is showed in Table S1.

2.5. Model parameters estimation

At the total sulfide concentration of 1 mM, both biotic and abiotic assays were performed, and the experimental biomass and