sustainability

MDPI

Article

The Effect of Chemical Sulfide Oxidation on the Oxygenic Activity of an Alkaliphilic Microalgae Consortium Deployed for Biogas Upgrading

Arnold Ramírez-Rueda [1,†], Antonio Velasco [1,2,†] and Armando González-Sánchez [1,*]

[1] Instituto de Ingeniería, Universidad Nacional Autónoma de Mexico, Circuito Escolar, Ciudad Universitaria, Mexico City 04510, Mexico; aramirezru@outlook.com (A.R.-R.); jvelascotrejo@yahoo.com.mx (A.V.)

[2] Departamento de Biotecnología, Universidad Autónoma Metropolitana-Unidad Iztapalapa, Iztapalapa, Mexico City 09340, Mexico

* Correspondence: agonzalezs@iingen.unam.mx; Tel.: +52-55-5623-3600

† Co-first authors both contributed equally.

Received: 21 July 2020; Accepted: 13 August 2020; Published: 15 August 2020

Abstract: The oxygenic photosynthetic activity (OPA) of an alkaliphilic microalgae consortium was evaluated at different concentrations of dissolved sulfide under room temperature and well-defined conditions of irradiance and pH in a tubular closed photobioreactor. The kinetic assays showed that it was optimal at a sulfide concentration of 3.2 mg/L under an external photosynthetically active radiation of 50 and 120 μE/m^2 s together with a pH of 8.5 and 9.2. In contrast, the oxygenic photosynthetic activity was insignificant at 15 μE/m^2 s with a pH of 7.3, both in the absence and presence of sulfide. Consecutive pulse additions of dissolved sulfide evidenced that the accumulation rate of dissolved oxygen was decreased by the spontaneous chemical oxidation of sulfide with dissolved oxygen in alkaline culture media, mainly at high sulfide levels. At 3.2 mg/L of sulfide, the oxygenic photosynthetic activity was improved by around 60% compared to the treatment without sulfide at external irradiances of 120 μE/m^2 s, 30 °C, and pH of 8.5 and 9.2. Additionally, an even higher OPA enhancement (around 85%) was observed in the same previous conditions but using 16 mg/L of sulfide. Thiosulfate was the major end-product of sulfide by oxic chemical reaction, both in biotic and abiotic assays with yields of 0.80 and 0.68, respectively.

Keywords: microalgae; photosynthetic activity; hydrogen sulfide; dissolve oxygen

1. Introduction

Hydrogen sulfide (H_2S) is the main pollutant in gaseous fuels, such as natural gas and biogas. It produces corrosion, bad odors, and can be toxic under certain concentration levels [1]; therefore, H_2S removal from biogas is mandatory before its utilization to avoid affectations to human health and the environment. The biogas upgrading processes remove H_2S but also CO_2, H_2O, and O_2 to increase the biogas calorific value; this allows it to be used in cogeneration engines, gas turbines, fuel cells, steam boilers, etc. [2,3]. Currently, there are available commercial technologies for desulfurization and the removal of CO_2 from biogas. These include physicochemical processes, such as chemical precipitation, adsorption with activated carbon, cryogenic separation, and permeation by membranes; however, their high-energy consumption and waste management increase the operational costs [4,5]. In this context, biological treatments emerge as economical and viable options to remove gaseous H_2S: i.e., biofilters, biotrickling filters, bioscrubbers, and scrubbers coupled to oxidation bioreactors, whose usage depends mainly on the H_2S mass load and the removal efficiency required [6]. However, alternatives for biogas cleaning processes based on microalgae have been recently reported [7–9]. Bahr et al. [7] employed an absorption column (scrubber) coupled with an open photobioreactor (high-rate algal

pond) for the simultaneous removal of H_2S and CO_2 (upgrading) from biogas using an alkaliphilic microalgal-bacterial consortium. This configuration allows the removal of almost all H_2S contained in biogas to oxidize it to sulfate through sulfide-oxidizing bacteria by utilizing the molecular oxygen (O_2) produced by microalgae as an electron acceptor. The later phenomena could decrease the pH [10]. Recent studies [11,12] have reported H_2S removal efficiencies higher than 94% in photobioreactors deployed for biogas upgrading at inlet concentrations of around 1000 ppm_v for a semi-industrial scale and up to 5000 ppm_v at a bench scale. The latter suggests that the microalgae-based biogas upgrading process is a promising technology to remove H_2S, due mainly to its high mass transfer (absorption) fostered by chemical reaction under alkaline conditions [7]. Through photosynthesis, microalgae fix CO_2 to produce O_2 under the presence of light and essential macronutrients, such as nitrogen (N), phosphorus (P), and potassium (K) besides other micronutrients: i.e., sulfur (S). The S content in microbial biomass varies from 0.15% to 1.6% (*w*/*w*); it is a component of the lipid bilayer of the cell membrane and of regulatory compounds for some metabolites [13]. Microalgae can assimilate S in a preferred form of sulfate (SO_4^{2-}) instead of H_2S and its dissolved sulfide species [7,14]. H_2S can inhibit the oxygenic photosynthesis by decreasing the flow of electrons between photosystem II and photosystem I [15]; however, some cyanobacteria can performed anoxygenic photosynthesis using H_2S instead of H_2O as an electron source to transfer them to photosystem I through the enzyme sulfide:quinone oxidoreductase (SQR) [16].

In this context, Küster et al. [17] studied the toxicity of sulfide in the cultivation of *Scenedesmus* sp. and reported that at 2 mg/L, the growth rate decreased by 50%, whereas, González-Camejo et al. [18] found that a concentration of 5 mg/L reduced the oxygen production rate by 43% during the cultivation of *Scenedesmus* sp. when exposed to 300 $\mu E/m^2$ s at 24 °C. Moreover, González-Sánchez and Posten [14] noted that in the initial stages of batch cultivation, the growth of *Chlorella* sp. was completely inhibited by dissolved H_2S at 16 mg/L. However, they observed that after two days, the chemical oxidation of H_2S improved the growth of *Chlorella* sp. by the formation of sulfate, which was subsequently assimilated in the microalgae cells. At pH > 8, the sulfides $H_2S_{(L)}$ and hydrosulfide (HS^-) oxidized in the presence of oxygen by both chemical and biological reactions [1], where the kinetic of the chemical sulfide oxidation was assumed to follow a first order respect to sulfide and 0.2 respect to O_2 [19]. Therefore, H_2S absorption at pH > 8 would exponentially increase the dissolved sulfide concentration as the pH arises. On the other hand, under intensive oxygenic photosynthetic activities, the dissolved oxygen (DO) would accumulate in the photobioreactor culture broth above 20 mg/L [20]. The increased concentrations of dissolved H_2S and O_2 would enhance the chemical sulfide oxidation in agreement with Nielsen et al. [19], and then impact the metabolism of microalgae: i.e., the growing rate and oxygen production activity. Closed photobioreactors may perform kinetic tests in a less time demanding configuration, using the dissolved oxygen concentration as a useful parameter to characterize the photosynthetic activity of a microalgal culture besides the chemical sulfide oxidation under defined conditions [21,22].

The aim of the present work was to evaluate the oxygenic photosynthetic activity (OPA) of a microalgal consortium at different concentrations of dissolved sulfide in a tubular closed photobioreactor operated under different controlled conditions of pH and irradiance, elucidating the role of chemical sulfide oxidation.

2. Materials and Methods

2.1. Microorganisms and Culture Conditions

A native alkaliphilic microalgae consortium (AMC) was used as biological material. It was enriched from a soda lake (electrical conductivity of 12 mS/cm and pH between 9 and 11) located in Texcoco, Mexico [22]. For more than 5 years, the AMC was grown under a continuous regime in a 30 L bench scale high-rate algal pond (HRAP) photobioreactor [10], deploying a hydraulic retention time (HRT) of 15 days, feeding a modified Zarrouk mineral medium (pH 9) composed of (g/L): Na_2CO_3 (4.03),

$NaHCO_3$ (13.61), NaCl (1.0), K_2HPO_4 (1.0), K_2SO_4 (1.0), $CaCl_2{\cdot}H_2O$ (0.04), KNO_3 (2.52), $MgCl_2{\cdot}6H_2O$ (0.2), and trace elements (2 mL/L) [23]. The AMC was mainly composed of chlorophyte (*Picochlorum* sp.) and cyanobacteria (*Pseudoanabaena* sp.), which were previously identified by sequencing the gene 16S RNA [24]. During the continuous cultivation, the suspended cells of the AMC were collected under steady state performance [10] and centrifuged at 5000× *g* and 4 °C for 10 min.

The biomass was resuspended in 0.7 L of modified Zarrouk mineral medium under moderate mixing through a magnetic stirring plate at 350 rpm for twenty minutes. For kinetic assays, for the pH of 8.5 and 9.2, the mineral medium composition was adjusted by modifying the HCO_3^-/CO_3^{2-} molar ratio, while for pH 7.3, pure CO_2 was bubbled until reaching the desired pH. After each pH adjustment, the AMC was kept under the same previous moderate mixing regime; therefore, the AMC was added to a tubular closed photobioreactor (TCP) for the respective kinetic assay assessment. The biomass concentration of 0.28 ± 0.06 g/L was measured throughout the operation of the assayed pH values.

2.2. Experimental System

The kinetic experiments were carried out in a TCP shown in Figure 1; it was constituted by four U-shaped tubes connected to each other forming a loop, provided with four ports for attaching the pH, dissolved oxygen (DO), and temperature probes, as well as a glass bubble diffuser. The TCP was made of borosilicate glass with a height of 30 cm, an internal diameter of 2 cm, and a total length of 154 cm. Major details can be found elsewhere [22]. The highest U-shaped tube was operated as an airlift using a peristaltic pump (77962-20, Cole-Parmer, Mount Vernon, IL, USA) to recirculate the microalgae suspension culture at 0.72 L/min, which guaranteed homogeneous mixing through all closed circuits while avoiding biomass sedimentation. The effective aqueous and headspace volume were 520 and 30 mL, respectively, while the total surface/volume ratio was 200 m^2/m^3. The TCP was irradiated by cold light from LEDs (FSL-5050W300-N/W, Siled, Mexico City, Mexico) disposed in an internal cylindrical area of 0.6 m^2, which surrounded the TCP.

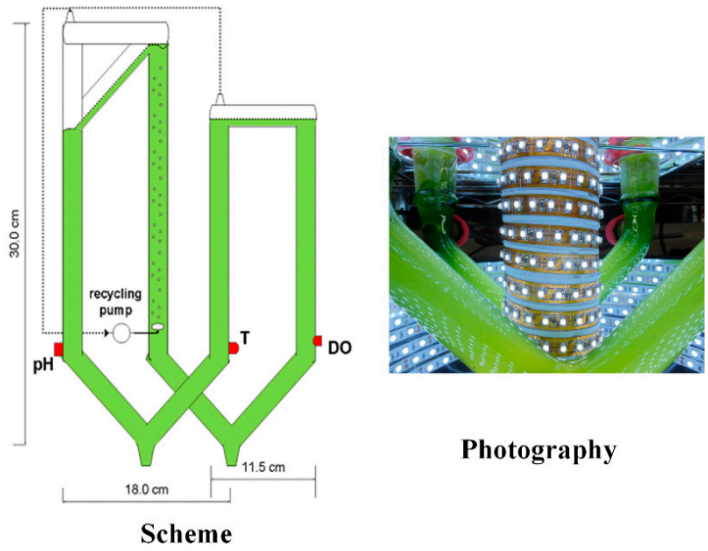

Figure 1. Tubular closed photobioreactor (TCP) used for the kinetic characterization of the chemical sulfide oxidation and the oxygenic photosynthetic activity, with a useful liquid volume of 520 mL and a headspace of 30 mL.

The photosynthetically active radiation (PAR), named here as irradiance, was controlled through a dimmer and measured by a light meter (LI250, LI-COR, Lincoln, NE, USA). An air heater (120193, Everheat, Atlanta, GA, USA) with analogous control (U3-LV, Labjack, Lakewood, CO, USA) allowed the temperature of the aqueous recycled culture to be controlled. When the microalgae suspension culture was introduced to the open TCP system, the peristaltic pump was immediately turned on for

equaling the temperature. This procedure lasted around 30 min, and, afterwards, the TCP system was closed. Consequently, the DO concentration, temperature, and pH were continuously measured. For the kinetic assays involving the presence of dissolved sulfide, a stock solution was added to the TCP a few minutes after the system was closed. The stock solution of dissolved sulfide (2 g/L of 98% purity $Na_2S{\cdot}9H_2O$) was prepared in deoxygenated ultrapure water. Corresponding volumes of stock sulfide solution (0.9 and 4.4 mL) were added from a top port for reaching 3.2 and 16 mg/L, respectively.

Kinetic Assays Approach

During the photosynthetic biogas upgrade under outdoor operation, the irradiance, temperature, pH, and H_2S content were considered as the most influential parameters on the oxygenic photosynthetic activity [20]. However, the number of kinetic assays were optimized by deploying an orthogonal L9(3^3) arrangement of three factors with three levels according to the Taguchi method [25]. Table 1 shows the optimized experimental design performed at room temperature of 31 °C, considering different irradiances (15, 50, 120 μE/m^2 s), pH (7.3, 8.5, 9.2), and sulfide concentrations (0, 3.2, 16 mg/L). Every combination of parameters represented one treatment, which was assayed in the TCP during a 90 min batch operation, by duplicate. The microalgal biomass, inorganic carbon, total nitrogen, sulfide, thiosulfate, and sulfate concentrations were assessed at the beginning and at the end of every single kinetic assay.

Table 1. Experimental design of kinetic assays.

Treatment No.	Variable Irradiance (μE/m^2 s); pH (-); Sulfide (mg/L)	Response Parameter
1	15; 7.3; 0	DO (mg/L)
2	15; 8.5; 3.2	
3	15; 9.2; 16	
4	50; 8.5; 16	
5	50; 9.2; 0	
6	50; 7.3; 3.2	
7	120; 9.2;3.2	
8	120; 7.3; 16	
9	120; 8.5; 0	

To evaluate the effect of chemical sulfide oxidation on the OPA without irradiance limitation, a second experimental design was carried out at a fixed irradiance of 120 μE/m^2 s and optimal temperature of 30 °C, while assaying consecutive additions of stock sulfide solution at pH of 8.5 and 9.2. The pulses of sulfide stock solution were injected to consecutively reach 3.2 and 16 mg/L in the aqueous phase inside the TCP. The duration of the batch cultures lasted 235 min; during the first 83 min, the experiment was carried out in the absence of sulfide, while from minute 96 to 153 and from minute 174 to 235, sulfide concentrations of 3.2 and 16 mg/L were reached, respectively. Before adding the first sulfide pulse, a short time passed in the opened TCP to stabilize the DO concentration around the value corresponding to 100% air saturation, and then the TCP system was closed. Therefore, three experimental stages were observed in a same batch assay (0, 3.2, and 16 mg/L of sulfide) (Table 2).

Table 2. Experimental design of consecutive additions of sulfide.

Sulfide Conc. (mg/L)	Experimental Stage (min)	Treatment No. 10	Treatment No. 11	Response Parameter
		pH	pH	
0	From 0 to 90	8.5	9.2	DO (mg/L)
3.2	From 91 to 170			
16	From 171 to 240			

To evaluate the role of the chemical sulfide oxidation on the OPA, abiotic treatments replicating the same above conditions shown in Table 2 were performed. The oxygen uptake rate due to chemical sulfide oxidation (CSO) both in the biotic and abiotic assays were quantified by Equation (1), considering the stoichiometric (Equation (2)) amount of O_2 needed to produce certain amounts of thiosulfate ($S_2O_3^{2-}$), of which concentrations were experimentally assessed at the beginning and at the end of every assay.

$$\mathrm{CSO} = \frac{2000 * \left(\left[S_2O_3^{2-}\right]end - \left[S_2O_3^{2-}\right]initial\right)}{112\ (Time\ of\ assay)} \quad (1)$$

$$2HS^- + 2O_2 \rightarrow S_2O_3^{2-} + H_2O \quad (2)$$

2.3. Oxygenic Photosynthetic Activity Evaluation

Figure 2 shows the total mass balance in the TCP (see Equation (3)). For the sulfide assays, the total experimental (dO_2/dt) reached was equal to the rate of oxygen production (OPA) minus the chemical sulfide oxidation rate, where the latter was computed independently from Equation (1). Equation (3) represents a coupled oxygen mass balance inside the TCP, including both volumes of aqueous (VL = 0.52 L) and gaseous phase (VG = 0.03 L), assuming that the gas/liquid equilibrium was attained (DO = O_{2gas}/He) at the duration of the kinetic assays. *He* represents the gas/liquid Henry constant of oxygen (37, dimensionless) at 1 atm and 30 °C.

$$\frac{dO_2}{dt} = \frac{d\mathrm{DO}}{dt}\left[1 + VG * \frac{\mathrm{He}}{VL}\right] = \mathrm{OPA} - \mathrm{CSO} \quad (3)$$

where t = 0; DO = $DO_{initial}$.

The specific oxygen production rate (rO_{2spec}) was computed by dividing the OPA by the averaged microalgal biomass concentration assessed at the end of each experimental assay.

2.4. Analytical Methods

The microalgal biomass was estimated by the direct measurement of the total suspended solid (TSS) concentration, which was determined by the gravimetric method of dry weight, as described in Standard Method [26]. The total dissolved sulfide concentration was determined photometrically at 665 nm by the methylene blue method 8131 [27] using a UV/VIS laboratory spectrophotometer (DR 5000, HACH, Loveland, CO, USA).

The concentration of dissolved sulfur compounds—i.e., sulfate, thiosulfate, and sulfite—besides phosphate were determined by ion chromatography (ICS-2000, USA) equipped with a separation column IonPac™, 4.0 mm × 250 mm (AS11-HC, Dionex, Sunnyvale, CA, USA). The water samples were filtered through a 0.45 µm syringe filter placed in 10 mL measuring container and placed in an autosampler. Double distilled water was used as mobile phase and 20 mM NaOH was automatically added from the eluent generator. A flow rate of 1 mL/min was selected and the data acquisition was performed using Chromeleon™ 6.8 software (Dionex, Sunnyvale, CA, USA).

Dissolved inorganic carbon and total nitrogen were determined using a TOC-L CSH analyzer coupled with a TNM-L chemiluminescence module with an autosampling tray (Shimadzu Corp., Kyoto, Japan). Dissolved oxygen concentrations (Orion 081010MD, Thermo Fischer Scientific, Waltham, MA, USA), pH (WD-358801-00, Oakton Instruments, Vernon Hills, IL, USA), and temperature (M-01, JAOW, Mexico City, Mexico) were monitored online in the TCP and logged in a PC every 5 s using Orion™ Star Com software. All the analyses were made by duplicate.

3. Results and Discussion

3.1. Kinetic Assays: Single Sulfide Addition

Figure 2 shows the DO concentration profiles induced under 0, 3.2, and 16 mg/L of sulfide at different irradiance and pH values. The DO concentrations in the assays without sulfide indicated that the OPA was negatively affected by low irradiance and neutral pH values, while the DO concentration rose when these values increased, as was expected. In this experimental set, the highest slope of DO (0.041 mg/L min) was observed at an irradiance of 120 μE/m^2 s and pH of 8.5 (Figure 2a), whereas Figure 2b shows that at a sulfide concentration of 3.2 mg/L and irradiance of 120 μE/m^2 s, the largest DO slope (0.055 mg/L min) and concentration (12 mg/L) were reached compared to the values observed at this irradiance, but they were under 0 and 16 mg/L of sulfide despite the differences of pH values evaluated.

These results indicated that a sulfide concentration of 3.2 mg/L enhanced the OPA and showed that an irradiance of 120 μE/m^2 s was the most important parameter for fostering OPA. In this respect, diverse studies [28–30] showed that irradiance played a very important role in photosynthetic CO_2 fixation, which rose linearly when the irradiance increased up to a critical irradiation depending on each species. Recently, Cheng et al. [31] reported that sulfide enhanced the cellular light absorption capacity by increasing the contents of photosynthetic pigments and light-harvesting proteins.

On the other hand, a very low OPA (DO slope of 0.009 mg/L min) was registered at irradiances of 50 and 15 μE/m^2 s (Figure 2b), while the lowest DO slope (0.005 mg/L min) was logged at 50 μE/m^2 s, probably due to the low pH evaluated (pH of 7.3), which could have promoted the endogenous respiration. The latter showed that pH also negatively affected the photosynthetic activity of the microalgae consortium more than a lower irradiance (15 μE/m^2 s; Figure 2b). This confirmed that the AMC was able to grow at alkaline and high pH conditions, where bicarbonate represented the main source of carbon, whereas photosynthetic activity was retarded at pH 7.3. This consequence is also observed in Figure 2c, where DO was negatively affected when an irradiance of 120 μE/m^2 s at a pH of 7.3 were deployed. During this experiment, a double negative effect was observed at a low pH and the highest sulfide concentration (16 mg/L), which induced a noticeable decrement of DO slope (−0.007 mg/L min) (Figure 2c) due to the large effect of the chemical oxidation reaction between sulfide and dissolved oxygen.

A similar effect on the DO concentration was observed at 15 μE/m^2 s and a pH of 9.2, where limited irradiance and a high sulfide level induced a decrease of DO concentration, which meant that OPA was lower than the CSO at the time evaluated. In contrast, Figure 2c shows that the DO slope increased (0.019 mg/L min) when irradiance and pH were 50 μE/m^2 s and 8.5, respectively. This suggests that OPA was slightly higher than CSO at a sulfide concentration of 16 mg/L. The ANOVA analysis, with a confidence interval of 95% (see Table S1), indicated that the most significant factors that affected DO concentration were—in order of importance—sulfide concentration, pH, and irradiance. The chemical oxidation reaction between sulfide and dissolved oxygen is an important occurrence phenomenon to consider when high levels of sulfide are present.

Another ANOVA analysis was made through hypothesis testing, with a confidence interval of 95%, which identified with the F distribution of previous experiments that there were no significant differences ($F < Fc$) between the initial and final values of biomass concentrations (0.28 ± 0.06 g/L), total nitrogen (375 ± 23 mg/L), phosphate (0.54 ± 0.11 g/L), inorganic carbon (2200 ± 283 mg/L), and sulfate (0.70 ± 0.04 g/L), while sulfite was not detected—maybe due to the short time tested (≤100 min).

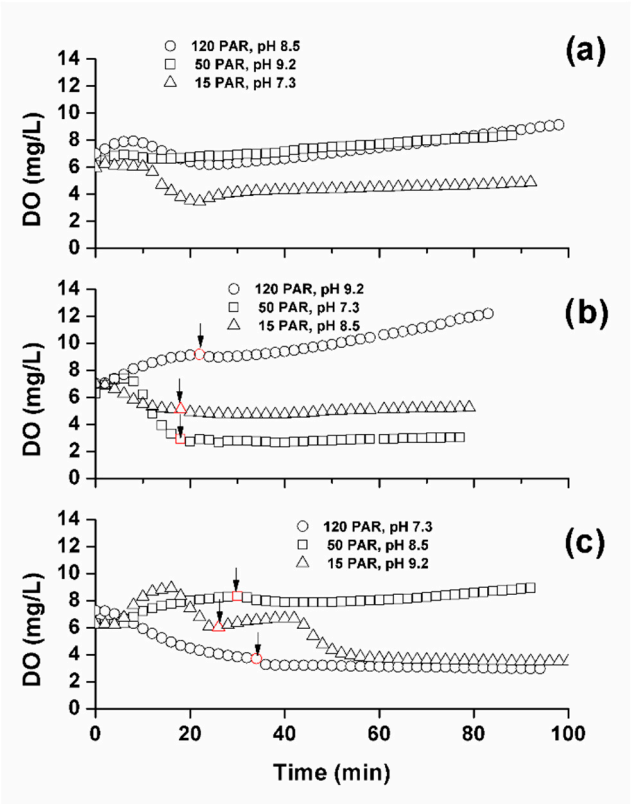

Figure 2. Dissolved oxygen concentration profile at (**a**) 0, (**b**) 3.2, and (**c**) 16 mg/L of sulfide vs. different values of irradiance and pH at room temperature for single sulfide addition assays. Arrows indicate sulfide addition. PAR is the irradiance as photosynthetically active radiation in $\mu E/m^2$ s.

3.2. *Consecutive Additions of Sulfide*

Figure 3 shows the profile of DO in TCP operated at 120 $\mu E/m^2$ s, 30 °C, and with pH values of 8.5 (Figure 3a) and 9.2 (Figure 3b), registered during three different stages of sulfide concentration (0, 3.2, and 16 mg/L). The performance of DO was observed for each stage (delimited by a dotted line) during the addition of progressive sulfide concentration. In the first stage, in absence of sulfide, a linear increase of DO was observed after the TCP was closed (from minute 15 to 75). The accumulation rates in these first stages were 4.6 and 5.05 $\mu molO_2/L$ min at pH of 8.5 and 9.2, respectively. In the second stage, with a sulfide concentration of 3.2 mg/L (initiated after desorption of dissolved O_2 in the photobioreactor), the DO concentration increased again with a rate of 5.66 and 6.59 $\mu molO_2/L$ min for pH of 8.5 (Figure 3a) and 9.2 (Figure 3b), respectively—both slightly higher than the first stage. These rate values confirmed that the sulfide addition of 3.2 mg/L improved the OPA of microalgae compared with the experiment without sulfide with a significant difference ($p = 0.039$) and 95% of confidence, which was previously observed in Figure 2a,b, when an irradiance value of 120 $\mu E/m^2$ s was tested. In the last stage (sulfide concentration of 16 mg/L), an increment of DO was also registered in both pH values tested (Figure 3); however, the accumulation rates were lower (3.90 and 3.60 $\mu molO_2/L$ min, respectively) than the previous two stages. The lower rate values indicated that the DO concentration was affected by the chemical oxidation of sulfide, as was previously observed (Figure 2c), although in this case (Figure 3), DO concentration was influenced in minor grade due to the best condition of irradiance and pH tested. The remaining dissolved sulfide registered after the treatment of 3.2 mg/L was 0.06 ± 0.02 mg/L for pH 8 and 0.04 ± 0.02 mg/L to pH 9.2, while values of 3.15 ± 3.21 mg/L for pH 8 and 0.33 ± 0.01 for pH 9.2 were logged after the treatment of 16 mg/L.

In this respect, Gun et al. [32] mentioned that dissolved sulfide species were more easily oxidized under alkaline conditions (pH ≥ 8) by chemical reactions: a circumstance that maybe favored the oxygenic photosynthetic activity, as the sulfides inhibitory effect diminished as chemical reactions proceeded. The pH values tested were 8.5 or 9.2 at the beginning of each experiment, and they slightly increased over time due to the release of OH^- ions during CO_2 fixation by photosynthesis. Such an increase was more pronounced in the experiment carried out with a pH of 8.5, with an increase of 0.3 units, compared to 0.05 for pH 9.2. Changes in pH can also serve as an indicator of the photosynthetic activity.

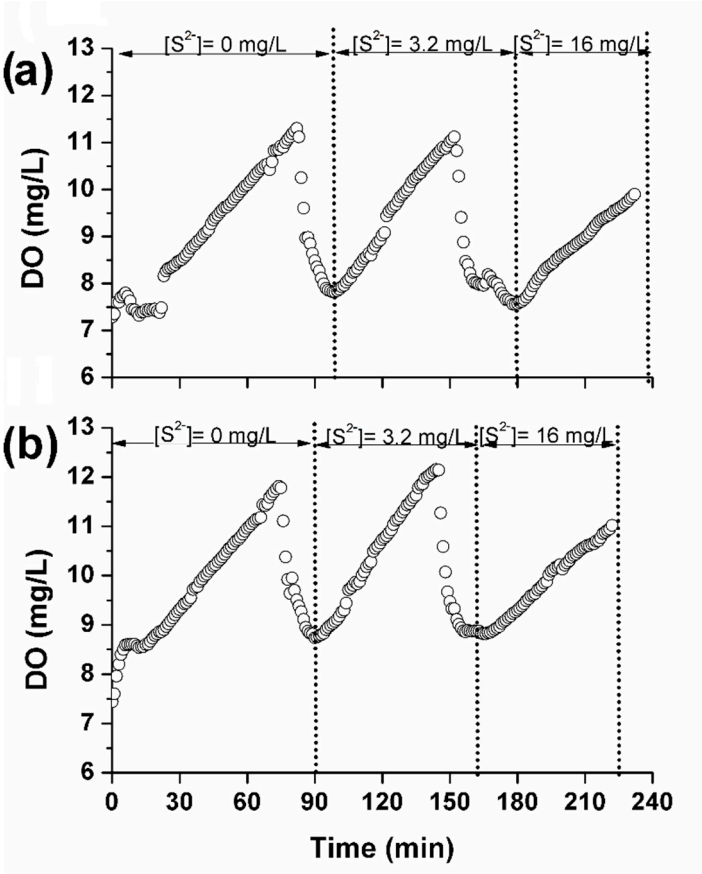

Figure 3. Dissolved oxygen concentration profile for biotic assays (○) at 120 μE/m^2 s, 30 °C, and two pH values: (**a**) 8.5 and (**b**) 9.2.

Among the pH values tested (Figure 4), no significant differences (p = 0.055) of OPA were observed (with a confidence of 95%), while for both sulfide levels of 3.2 and 16 mg/L, the OPAs were significantly different (p = 0.043), resulting around 60% and 85% higher than the control (without sulfide), respectively, which indicated that oxygenic photosynthetic activity was fostered in the presence of sulfide. These results agreed with Klatt et al. [33], who showed that the photosynthetic activity of cyanobacterium from sulfidic springs was improved when using a sulfide concentration and irradiance of 4.8 mg/L and 125 μE/m^2 s, respectively. They suggested that this was possible due to the regulatory effects of H_2S on photosystem I components and/or on the Calvin cycle, while Cheng et al. [31,34] mentioned that cell division, photosynthesis, and lipid accumulation in *Nannochloropsis oceanic* were improved in response to 0.5 mM of NaHS or 16 mg/L of sulfide. These results indicated that the dissolved sulfide species played an important role in the photobiological process of some microalgae species.

On the other hand, Miller and Bebout [15] indicated that dissolved sulfide could inhibit the electron transport during the photosystem II (PS II); however, it was also mentioned that the inhibition of PS II activity depended on the degree of sulfide tolerance of microalgae, which was a function of the dynamic

trait primarily shaped by sulfide levels in the environment. González-Camejo et al. [18] showed that the presence of sulfide had inhibitory effects at concentrations above 20 mg/L in the microalgae culture of *Chlorella* and *Scenedesmus*; the complete inhibition of microalgae growth was registered at a concentration of 50 mg/L of sulfide. In this context, our results showed that OPA was enhanced at the sulfide concentrations herein tested. It would be a relevant observation for biogas upgrading that 16 mg/L of remaining sulfide in the absorption column [18] corresponds under a physicochemical equilibrium to an outlet gaseous H_2S concentration of 50 and 0.1 ppm_v, when the column is operated at pH 8.5 and 9.2, respectively. Therefore, as OPA improved, the oxygen produced would promote higher CSO rates and then the latter would reduce the possible sulfide toxicity on microalgae due the continuous biogas feeding. Several papers [7,18] reported a complete H_2S removal from biogas under similar operational conditions (the inlet H_2S concentration of 5000 ppm_v and gas residence time of 23 min), observing no inhibition on the microbial population. However, the maximum H_2S elimination capacity of the microalgal-based biogas upgrading process, as reported by [20], would depend on the microalgae or microalgal-bacterial populations established there. In this context, the present study recommends—independently of the consortium used—to evaluate the CSO and OPA rates of the microalgal system to determine the convenient value of DO in culture media to treat the H_2S contained in biogas. An improved chemical sulfide oxidation reaction would be enough to remove H_2S from biogas and transform this to thiosulfate and sulfate in the HRAP, even in the absence of an alkaliphilic sulfoxidizing bacterial consortium.

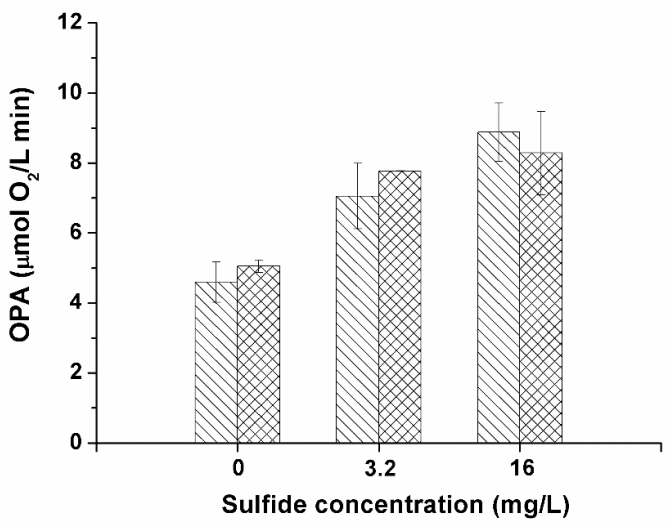

Figure 4. Oxygenic photosynthetic activity (OPA) at 120 µE/m^2 s, 30 °C, and two pH values of 8.5 (▨) and 9.2 (▩).

3.3. Thiosulfate Production and Oxygenic Photosynthetic Activity in Biotic and Abiotic Tests

Thiosulfate was the predominant specie registered in the chemical sulfide oxidation during the assays and no significant differences of thiosulfate production were observed among the pH of 8.5 and 9.2 in the couple experiment set tested (Table 3). Table 3 shows that higher $S_2O_3^{2-}$ concentrations were reached in the biotic experiment than in the abiotic test due to the OPA-induced accumulation of dissolved oxygen and, consequently, higher O_2/S^{2-} molar consumption ratios were attained (Table 3). The CSO rate was shown to be proportional to the available sulfide and dissolved oxygen concentrations [1], being, on average, the double for biotic tests with respect to the corresponding abiotic tests. Globally, the results shown in Table 3 suggest that the oxygen produced by photosynthesis was used to oxidize the sulfide to thiosulfate (see Equation (2)) by a chemical reaction. Meanwhile, in the abiotic tests, the dissolved oxygen was a limiting reagent for chemical sulfide oxidation because,

as the oxic reaction proceeded, the DO concentration was further decreased in the closed system down to around 4 mg/L (see Figures S1 and S2, Supplementary Materials).

On the other hand, the formation and accumulation of $S_2O_3^{2-}$ in abiotic and biotic assays indicated that the O_2/S^{2-} consumption molar ratio was always lower than 1.0. The latter indicated that sulfide was partially oxidized mainly to thiosulfate instead of sulfate under the timeframe of the kinetic assays (approx. 60 min), which did not mean that under continuous supply of O_2 and higher reaction time, the aqueous alkaline suspension would complete the total chemical sulfide oxidation to sulfate. The thiosulfate yields achieved at biotic and abiotic assays with a sulfide concentration of 3.2 mg/L were of 0.8 and 0.68, independently of the pH tested. Similar yields of around 0.65 were also recorded for biotic assays at a sulfide concentration of 16 mg/L; however, the abiotic test at this previous sulfide concentration registered the minor yield (0.37), reaching the lowest O_2/S^{2-} consumption molar ratio (Table 3) due to oxygen limitation. In respect to the other by products, neither sulfite nor sulfate were produced in the timeframe of the kinetic assays.

Some studies [35,36] mentioned that sulfate was preferable as the end-product for the growth of microalgal cultures because it was relatively inert and could be rapidly assimilated by the cells. González-Sánchez and Posten [14] showed that in a microalgae culture of *Chlorella* sp. under slightly alkaline conditions (pH 8.5), the chemical sulfide oxidation produced pentasulfide, sulfite, and thiosulfate in the first 24 h of batch cultivation, which were further oxidized to sulfate after two days of cultivation. In this context, under typical operation conditions of open high-rate algal ponds deployed for biogas upgrading, the hydraulic residence times were higher than 15 days [7], which could be enough to get sulfate. Then, under the continuous controlled supply of sour biogas to the upgrading system, the dissolved H_2S would enhance the OPA and probably the biomass productivity. The CO_2 removal from biogas and biomass productivity were directly linked, and optimizing the H_2S supply through the biogas load would foster biomethane production [10]. The specific oxygen production rates computed here were in the same order as those reported in other studies for non-extremophile microalgae: i.e., for *Chlorella vulgaris*, 78 μmol $O_2/g_{biomass}$ min [37], and 46 μmol $O_2/g_{biomass}$ min for *Scenedesmus almeriensis* [38]. Recently, for *C. vulgaris (AG 10,032)*, *S. quadricauda (AG 10,003)*, and *D. communis (AG 60,074)*, an optimal averaged value of 7 μmol $O_2/g_{biomass}$ min was reported [39].

Table 3. Chemical sulfide oxidation and oxygenic photosynthetic activity in biotic and abiotic tests.

Biotic Test						
Sulfide Conc. (mg/L)	**pH Value**	**$S_2O_3^{2-}$ (mg/L)**	**O_2/S Molar Consumption Ratio**	**dO_2/dt (μmolO_2/L min)**	**CSO (μmolO_2/L min)**	**OPA (μmolO_2/L min)**
3.2	8.5	4.7 ± 0.4	0.84 ± 0.02	5.66 ± 1.05	−1.39 ± 0.1	7.05 ± 0.94 (25.3) *
	9.2	4.0 ± 0.0	0.73 ± 0.07	6.59 ± 0.01	−1.18 ± 0.0	7.77 ± 0.01 (27.8) *
16	8.5	16.7 ± 2.6	0.60 ± 0.1	3.90 ± 0.05	−4.98 ± 0.8	8.88 ± 0.84 (31.8) *
	9.2	15.7 ± 2.8	0.56 ± 0.1	3.60 ± 0.35	−4.68 ± 0.8	8.28 ± 1.19 (29.7) *
Abiotic Test						
3.2	8.5	2.8 ± 0.4	0.50 ± 0.07	−0.38 ± 0.06	−0.83 ± 0.1	N.A.
	9.2	2.6 ± 0.2	0.47 ± 0.04	−0.65 ± 0.05	−0.77 ± 0.3	N.A.
16	8.5	6.5 ± 0.3	0.23 ± 0.1	−1.41 ± 0.4	−1.93 ± 0.4	N.A.
	9.2	6.2 ± 0.2	0.22 ± 0.1	−1.36 ± 0.1	−1.84 ± 0.5	N.A.

Negative value means that slope decreased, * rO_2spec (μmol$O_2/g_{biomass}$ min). N.A. Not applicable.

4. Conclusions

This study shows that CSO is an important phenomenon occurring during the microalgae-based biogas upgrading process, which can be regulated by OPA and vice versa under alkaline conditions. The CSO could reduce the possible sulfide toxicity on microalgae due the continuous biogas feeding. The oxygen produced by photosynthesis fosters the sulfide oxidation to thiosulfate by chemical reaction, while under abiotic conditions, the dissolved oxygen was shown as being a limiting reagent for chemical sulfide oxidation. At a sulfide concentration of 3.2 mg/L, the OPA was improved around

60% compared with the treatment without sulfide at external irradiances of 120 PAR and 30 °C for both pH tested, while an enhanced OPA of around 85% was computed in these same conditions, but using 16 mg/L of sulfide. The $O_2/S^{2-} < 1$ confirmed the partially sulfide oxidation route, which was not enough to produce sulfate. This study recommends evaluating the CSO and OPA rates of a microalgal system deployed to upgrade biogas to assess the convenient value of DO in culture media to efficiently remove the H_2S.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/12/16/6610/s1.

Author Contributions: Conceptualization, A.R.-R., A.G.-S., A.V.; data curation, A.V., A.R.-R.; methodology, A.R.-R., A.G.-S.; investigation, A.R.-R., A.G.-S., A.V.; resources, A.G.-S.; writing, A.V., A.R.-R.; writing—review and editing, A.G.-S., A.V.; supervision, A.G.-S.; project administration, A.G.-S.; funding acquisition, A.G.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financed by Energy Sustainability Fund (SENER-CONACYT 247006 Cluster Biocombustibles Gaseosos) and partially supported by DGAPA-IT-100317. This research work was performed in the IIUNAM-Environmental Engineering Laboratory (LIA), which holds an ISO 9001:2015 certificate.

Acknowledgments: We thank Roberto Sotero Briones Méndez, Daniel de los Cobos Vasconcelos, Margarita Elizabeth Cisneros Ortiz, and José Abdiel Olmedo Wooder for their technical support, besides to the members of the ALR group.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AMC	alkaliphilic microalgae consortium
TCP	tubular closed photobioreactor
HRAP	high-rate algal pond
HRT	hydraulic retention time
dO_2/dt	slope of experimental DO concentration profile ($\mu molO_2$/L min)
CSO	chemical oxidation reaction ($\mu molO_2$/L min)
OPA	oxygenic photosynthetic activity ($\mu molO_2$/L min)
DO	dissolved oxygen (mg/L)

References

1. González-Sánchez, A.; Revah, S. The effect of chemical oxidation on the biological sulfide oxidation by an alkaliphilic sulfoxidizing bacterial consortium. *Enzym. Microb. Technol.* **2007**, *40*, 292–298. [CrossRef]
2. Khan, I.U.; Othman, M.H.D.; Hashim, H.; Matsuura, T.; Ismail, A.F.; Rezaei-DashtArzhandi, M.; Azelee, I.W. Biogas as a renewable energy fuel—A review of biogas upgrading, utilisation and storage. *Energy Convers. Manag.* **2017**, *150*, 277–294. [CrossRef]
3. Adnan, A.I.; Ong, M.Y.; Nomanbhay, S.; Chew, K.W.; Show, P.L. Technologies for biogas upgrading to biomethane: A review. *Bioengineering* **2019**, *6*, 92. [CrossRef] [PubMed]
4. Muñoz, R.; Meier, L.; Diaz, I.; Jeison, D. A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading. *Rev. Environ. Sci. Biol.* **2015**, *14*, 727–759. [CrossRef]
5. Toledo-Cervantes, A.; Estrada, J.M.; Lebrero, R.; Muñoz, R. A comparative analysis of biogas upgrading technologies: Photosynthetic vs physical/chemical processes. *Algal. Res.* **2017**, *25*, 237–243. [CrossRef]
6. Cline, C.; Hoksberg, A.; Abry, R.; Janssen, A. Biological process for H_2S removal from gas streams: The shell-paques/thiopaq^{TM} gas desulfurization process. In Proceedings of the Laurance Reid Gas Conditioning Conference, Norman, OK, USA, 23–26 February 2003; pp. 1–18.
7. Bahr, M.; Díaz, I.; Dominguez, A.; González Sánchez, A.; Muñoz, R. Microalgal-biotechnology as a platform for an integral biogas upgrading and nutrient removal from anaerobic effluents. *Environ. Sci. Technol.* **2014**, *48*, 573–581. [CrossRef]
8. Bona, D.; Papurello, D.; Flaim, G.; Cerasino, L.; Biasioli, F.; Silvestri, S. Management of digestate and exhausts from solid oxide fuel cells produced in the dry anaerobic digestion pilot plant: Microalgae cultivation approach. *Waste Biomass Valori.* **2020**, 1–16. [CrossRef]

9. Santarelli, M.; Briesemeister, L.; Gandiglio, M.; Herrmann, S.; Kuczynski, P.; Kupecki, J.; Swiatkowski, B. Carbon recovery and re-utilization (CRR) from the exhaust of a solid oxide fuel cell (SOFC): Analysis through a proof-of-concept. *J. CO2 Util.* **2017**, *18*, 206–221. [CrossRef]
10. Franco-Morgado, M.; Toledo-Cervantes, A.; González-Sánchez, A.; Lebrero, R.; Muñoz, R. Integral (VOCs, CO_2, mercaptans and H_2S) photosynthetic biogas upgrading using innovative biogas and digestate supply strategies. *Chem. Eng. J.* **2018**, *354*, 363–369. [CrossRef]
11. Toledo-Cervantes, A.; Serejo, M.L.; Blanco, S.; Pérez, R.; Lebrero, R.; Muñoz, R. Photosynthetic biogas upgrading to bio-methane: Boosting nutrient recovery via biomass productivity control. *Algal. Res.* **2016**, *17*, 46–52. [CrossRef]
12. Del Rosario Rodero, M.; Lebrero, R.; Serrano, E.; Lara, E.; Arbib, Z.; García-Encina, P.A.; Muñoz, R. Technology validation of photosynthetic biogas upgrading in a semi-industrial scale algal-bacterial photobioreactor. *Bioresour. Technol.* **2019**, *279*, 43–49. [CrossRef] [PubMed]
13. Grobbelaar, J.U. Algal Nutrition. In *Handbook of Microalgal Culture: Biotechnology of Applied Phycology*, 1st ed.; Amos, R., Ed.; Blackwell Publishing Ltd.: Oxford, UK, 2003; pp. 95–115.
14. González-Sánchez, A.; Posten, C. Fate of H_2S during the cultivation of Chlorella sp. deployed for biogas upgrading. *J. Environ. Manag.* **2017**, *191*, 252–257. [CrossRef] [PubMed]
15. Miller, S.R.; Bebout, B.M. Variation in sulfide tolerance of photosystem II in phylogenetically diverse cyanobacteria from sulfidic habitats. *Appl. Environ. Microb.* **2004**, *70*, 736–744. [CrossRef]
16. Theissen, U.; Hoffmeister, M.; Grieshaber, M.; Martin, W. Single eubacterial origin of eukaryotic sulfide: Quinone oxidoreductase, a mitochondrial enzyme conserved from the early evolution of eukaryotes during anoxic and sulfidic times. *Mol. Bio. Evol.* **2003**, *20*, 1564–1574. [CrossRef] [PubMed]
17. Küster, E.; Dorusch, F.; Altenburger, R. Effects of hydrogen sulfide to Vibrio fischeri, Scenedesmus vacuolatus, and Daphnia magna. *Environ. Toxicol. Chem.* **2005**, *24*, 2621–2629. [CrossRef]
18. González-Camejo, J.; Serna-García, R.; Viruela, A.; Pachés, M.; Durán, F.; Robles, A.; Ruano, M.V.; Barat, R.; Seco, A. Short and long-term experiments on the effect of sulphide on microalgae cultivation in tertiary sewage treatment. *Bioresour. Technol.* **2017**, *244*, 15–22. [CrossRef]
19. Nielsen, A.H.; Vollertsen, J.; Hvitved-Jacobsen, T. Determination of kinetics and stoichiometry of chemical sulfide oxidation in wastewater of sewer networks. *Environ. Sci. Technol.* **2003**, *37*, 3853–3858. [CrossRef]
20. Toro-Huertas, E.I.; Franco-Morgado, M.; de Los Cobos Vasconcelos, D.; González-Sánchez, A. Photorespiration in an outdoor alkaline open-photobioreactor used for biogas upgrading. *Sci. Total Environ.* **2019**, *667*, 613–621. [CrossRef]
21. Cabello, J.; Toledo-Cervantes, A.; Sánchez, L.; Revah, S.; Morales, M. Effect of the temperature, pH and irradiance on the photosynthetic activity by Scenedesmus obtusiusculus under nitrogen replete and deplete conditions. *Bioresour. Technol.* **2015**, *181*, 128–135. [CrossRef]
22. De los Cobos-Vasconcelos, D.; García-Cruz, E.L.; Franco-Morgado, M.; González-Sánchez, A. Short-term evaluation of the photosynthetic activity of an alkaliphilic microalgae consortium in a novel tubular closed photobioreactor. *J. Appl. Phycol.* **2016**, *28*, 795–802. [CrossRef]
23. Sorokin, D.Y.; Lysenko, A.M.; Mityushina, L.L.; Tourova, T.P.; Jones, B.E.; Rainey, F.A.; Robertson, L.A.; Kuenen, G.J. *Thioalkalimicrobium aerophilum* gen. nov., sp. nov. and *Thioalkalimicrobium sibericum* sp. nov., and *Thioalkalivibrio versutus* gen. nov., sp. nov., *Thioalkalivibrio nitratis* sp.nov., novel and *Thioalkalivibrio denitrificancs* sp. nov., novel obligately alkaliphilic and obligately chemolithoautotrophic sulfur-oxidizing bacteria from soda lakes. *Int. J. Syst. Evol. Microbiol.* **2001**, *51*, 565–580. [CrossRef] [PubMed]
24. Granada-Moreno, C.I.; Aburto-Medina, A.; de los Cobos Vasconcelos, D.; González-Sánchez, A. Microalgae community shifts during the biogas upgrading in an alkaline open photobioreactor. *J. Appl. Microbiol.* **2017**, *123*, 903–915. [CrossRef] [PubMed]
25. Escalante-Vázquez, E.J. *Diseño y Análisis de Experimentos*, 1st ed.; Limusa: Distrito Federal, Mexico, 2014; p. 523.
26. APHA. *Standard Methods for the Examination of Water and Wastewater*, 22nd ed.; Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S., Eds.; American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF): Washington, DC, USA, 2012; p. 724.
27. HACH. Sulfide Methylene Blue Method: Water Analysis Handbook Procedures. Available online: https://www.hach.com/asset-get.download.jsa?id=11122402600 (accessed on 8 June 2020).

28. Torzillo, G.; Vonshak, A. Environmental stress physiology with reference to mass cultures. In *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*, 2nd ed.; Amos, R., Hu, Q., Eds.; Blackwell Publishing Ltd.: Hoboken, NJ, USA, 2013; pp. 90–113.
29. Benavente-Valdés, J.R.; Aguilar, C.; Contreras-Esquivel, J.C.; Méndez-Zavala, A.; Montañez, J. Strategies to enhance the production of photosynthetic pigments and lipids in chlorophycae species. *Biotechnol. Rep.* **2016**, *10*, 117–125. [CrossRef] [PubMed]
30. Dasgupta, C.N.; Nayaka, S. Comprehensive screening of micro-and macroalgal species for bioenergy. In *Algal Biofuels*, 1st ed.; Gupta, S.K., Malik, A., Bux, F., Eds.; Springer: Cham, Switzerland, 2017; pp. 39–56.
31. Cheng, J.; Wang, Z.; Lu, H.; Xu, J.; He, Y.; Cen, K. Hydrogen sulfide promotes cell division and photosynthesis of Nannochloropsis oceanica with 15% carbon dioxide. *ACS Sustain. Chem. Eng.* **2019**, *7*, 16344–16354. [CrossRef]
32. Gun, J.; Goifman, A.; Shkrob, I.; Kamyshny, A.; Ginzburg, B.; Hadas, O.; Dor, I.; Modestov, A.D.; Lev, O. Formation of polysulfides in an oxygen rich freshwater lake and their role in the production of volatile sulfur compounds in aquatic systems. *Environ. Sci. Technol.* **2000**, *34*, 4741–4746. [CrossRef]
33. Klatt, J.M.; Haas, S.; Yilmaz, P.; de Beer, D.; Polerecky, L. Hydrogen sulfide can inhibit and enhance oxygenic photosynthesis in a Cyanobacterium from sulfidic springs. *Environ. Microbial.* **2015**, *17*, 3301–3313. [CrossRef] [PubMed]
34. Cheng, J.; Wang, Z.; Lu, H.; Yang, W.; Fan, Z. Hydrogen sulfide improves lipid accumulation in Nannochloropsis oceanica through metabolic regulation of carbon allocation and energy supply. *ACS Sustain. Chem. Eng.* **2020**, *8*, 2481–2489. [CrossRef]
35. Krauss, F.; Schmidt, A. Sulphur sources for growth of Chlorella fusca and their influence on key enzymes of sulphur metabolism. *Microbiology* **1987**, *133*, 1209–1219. [CrossRef]
36. Pootakham, W.; Gonzalez-Ballester, D.; Grossman, A.R. Identification and regulation of plasma membrane sulfate transporters in Chlamydomonas. *Plant. Physiol.* **2010**, *153*, 1653–1668. [CrossRef]
37. Wang, S.K.; Stiles, A.R.; Guo, C.; Liu, C.Z. Microalgae cultivation in photobioreactors: An overview of light characteristics. *Eng. Life Sci.* **2014**, *14*, 550–559. [CrossRef]
38. Costache, T.A.; Acién Fernández, F.G.; Morales, M.M.; Fernández-Sevilla, J.M.; Stamatin, I.; Molina, E. Comprehensive model of microalgae photosynthesis rate as a function of culture conditions in photobioreactors. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 7627–7637. [CrossRef] [PubMed]
39. Kwon, G.; Le, L.T.; Jeon, J.; Noh, J.; Jang, Y.; Kang, D.; Jahng, D. Effects of light and mass ratio of microalgae and nitrifiers on the rates of ammonia oxidation and nitrate production. *Biochem. Eng. J.* **2020**, 107656. [CrossRef]