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Abstract

The effects of swine wastewater-derived biogas on microalgae productivity were determined. Experiments were conducted in a closed photobioreactor containing digestate effluent as culturing media and biogas in the headspace as source of CO_2. Experiments were carried out under mixothrophic and autothrophic conditions. Results showed that autotrophic growth rate (0.6 d^{-1}) was twofold faster than mixotrophic. Frequent reinjections of biogas containing up to 2,000 ppm of hydrogen sulfide was not inhibitory to microalgae growth. The rapid removal of H_2S in the system suggests photobioreactors can be an interesting alternative to biogas purification. A model to estimate microalgae productivity based on the amount of available CO_2, inorganic and organic carbon was developed and showed good data fit correlation ($r^2 = 0.99$).

Keywords

Biofiltration; CO_2; microalgae; H_2S; swine wastewater.

INTRODUCTION

Microalgae are photosynthetic organism's with high carbon dioxide uptake (10 to 50 times higher than terrestrial plants) (Ho et al., 2012). Despite of its efficiency as carbon sequester it is also being extensively studied worldwide as a promising alternative feedstock for biofuels, food, and other high-value products (Kumar et al., 2010). The use of microalgae as tertiary treatment process to remove nutrients from a variety of wastewaters has been demonstrated (Mezzari et al., 2013; Kumar et al., 2010) since earlier 50´s and has regained special attention lately due to intrinsic economic value of biomass.

The proliferation of microalgae in wastewaters during phycoremediation of nutrient-rich effluents offers a cost effective means of cultivation which can reduce overall infrastructure and operational costs. However, it seems that the productivity of biomass and the consumption rate of nutrients by microalgae is somewhat limited to the amount of atmospheric CO_2 (0.04% v/v) required for photosynthesis (Kumar et al., 2010; Abedini-Najafabadi et al., 2015; Cheng et al., 2015). In this regard, the use of external source of CO_2 can improve the efficiency of nutrient removal by phycoremediation while simultaneously boosting biomass productivity. In agricultural scenarios with large number of confined animals for example, CO_2 can be obtained directly from biodigesters located downstream of the wastewater treatment plant. Biogas is typically composed of 55-75% CH_4, 20-35% CO_2 and 1,000-5,000 ppm H_2S (Kao et al., 2012). The proximity of these biogas plants from tertiary treatment systems that are based on microalgae technology

can help minimize costs associated with transportation of CO_2 flue gases from distant locations (Kumar et al., 2010). However, little is known about the effects of biogas on microalgae growth. For instance, the potential inhibitory effects of high ammonia and hydrogen sulfide concentrations present in the biogas could pose limitations of biogas use on microalgae culturing systems (Kao et al., 2012). In this regard, the objective of this work was to investigate the effects of swine wastewater-derived biogas on the growth rate of microalgae *Chlorella* sp. and *Scenedesmus* sp. in a lab scale photobioreactor. A model to predict microalgae biomass yield from CO_2, total inorganic and organic carbon was developed. The usefulness of microalgae cultivation to remove CO_2 and H_2S from the biogas was also evaluated which can have implications as biofilters to increase biomethane value.

MATERIAL AND METHODS

The microalgae inoculum used in this work was obtained directly from a pilot scale facultative open pond lagoon treating nutrient-rich digestate from a biodigestor at the Brazilian Agricultural Research Corporation (EMBRAPA) wastewater treatment facility (Concordia SC, Brazil). The inoculum was composed by two dominant genera *Chlorella* sp. and *Scenedesmus* sp. as determined by optical microscopic analysis (E200 - Nikon).

Two interconnected 16.9 L glass photobioreactors (30 cm high x 20 cm diameter) were used. The reactors were hermetically closed to atmosphere using rubber stoppers. All lines and fitting were Teflon made to minimize losses through volatilization and diffusion. Each reactor was filled with 8.9L (8L headspace) of culturing media. The media was prepared by dilution of effluent from a field scale Up-flow anaerobic sludge blank reactor (UASB) treating swine wastewater in distilled water (1:20). The raw effluent physical-chemical characteristics was reported elsewhere (Mezzari et al., 2013)**.** Reactors were inoculated with 30% v/v (approximately 70 mg L^{-1} dry weight biomass) of a microalgae stock culture. One experiment was carried out under mixotrophic conditions (12h:12h; light:dark) and the other experiment was maintained under autotrophic conditions (24h light). The photobioreactor was kept at room temperature (22 ± 2°C) and exposed to red light emission diode light (PGL-RBC 2500, PARUS) at 630 nm and 148.5 µmol m^{-2} s^{-1} and under continuous mixing using a magnetic stirrer. Biogas was collected directly from an UASB treating swine wastewater at EMBRAPA using specific 10L polyethylene bags. Methane, CO_2 and H_2S concentrations in the biogas were: 65-73% (v/v), 20-25% (v/v); and 1800-3100 (ppmv), respectively. The photobioreactors were purged with biogas for 5 minutes prior to the beginning of the experiment. A negative photobioreactor control without microalgae and poisoned with sodium azide (1 g L^{-1}) to prevent bacteria growth was used to discern CO_2 losses by abiotic reactions.

CO_2, CH_4 e H_2S concentrations in the photobioreactor headspace were continuously monitored using a gas analyzer (GEM 5000-LANDTEC). 50-mL liquid samples were taken over time from the photobioreactor using gas tight syringe through a sampling valve installed in the bottom of reactor. P-PO_4; N-NH_3; N-NO_2; N-NO_3; were analyzed according to methods described in APHA et al., (2012). TOC and TIC were measured in a TOC analyzer (Multi C/N 2100, Analytik Jena). Microalgae biomass growth was determined in a spectrophotometer at 570 nm. Correlation between absorbance and dry weight biomass was $r^2 = 0.96$. A model to predict biomass was proposed:

$$\text{Biomass (as mg} - \text{C L}^{-1}) = \sum_{i=1}^{n} \frac{[(\text{CO2}_i - \text{CO2}_o) + (\text{TOC}_i - \text{TOC}_o) + (\text{TIC}_i - \text{TIC}_o)]/\text{FCM}}{WV}$$

Where: TOC and TIC are total organic and inorganic carbon content (mg), respectively, consumed between time t_0 and t_f (days). WV is the reactor total working volume (8.9 L). FCM is the fraction of carbon mass (0.5137) derived from a microalgae molar basis of $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti et al., 2007). The model reaches a plateau at the point where biomass growth cease [stationary phase, i.e., $\ln(X/X_O)/\Delta t$ (μ_X) ≤ 0,037]. This assumption was based on the fact that under stationary growth phase any additional carbon is used exclusively to maintain intracellular metabolic activities rather than increase cellular weight (Abedini-Najafabadi et al., 2015).

RESULTS AND DISCUSSION

The effects of biogas on microalgae production in a closed photobioreactor were determined. The maximum concentration of microalgae obtained under mixotrophic and autotrophic conditions were 1 g L^{-1} (at 14 days of experiment) and 0.9 g L^{-1} (at 9 days of experiment), respectively (Figure 1). Despite of the small increase in biomass concentration obtained in the mixotrophic photobioreactor, autotrophic conditions showed much faster biomass growth rate. The earlier stabilization of the growth curve observed in autotrophic conditions can be attributable to limiting nutrients availability and/or light deficiency due to shading effects exacerbated by the increased microalgae biomass (Cai et al., 2013). Microalgae exponential growth rate were 0.31 d^{-1} (84 mg L^{-1} d^{-1}) and 0.61 d^{-1} (120 mg L^{-1} d^{-1}) for mixotrophic (total daily light exposure of 6.4 mol m^{-2}) and autotrophic (total daily light exposure of 12.8 mol m^{-2}) conditions, respectively. According to Yan et al (2013) the daily photosynthetic flow in the autotrophic photobioreactor was closer to optimum (17.64 mol m^{-2}). Compared to negative experiment controls exposed to atmospheric CO_2 (50 mg L^{-1} d^{-1}) the effect of biogas on microalgae productivity was notably higher.

It is known that additional sources of CO_2 can enhance microalgae growth by twofold as compared to atmospheric CO_2 (Abedini-Najafabadi et al, 2015;. Cheng et al, 2015). CO_2 removal rate was higher in autotrophic than mixotrophic conditions corroborating with microalgae growth (Figure 1). CO_2 abiotic losses from negative control was 3.3 ± 0,9% per day (data not shown). A model to predict biomass production from CO_2, TIC and TOC concentration was developed. The model showed good correlation with the experimental data (r^2 = 0.99) independently of the experimental conditions tested (autotrophic or mixotrophic). The satisfactory model data fit indicated that headspace CO_2 was mainly incorporated into biomass. Overall, 70 to 93 % of CO_2 were removed from photobioreactor´s headspace over the course of 15 to 17 days. Methane concentrations decreased over time after each biogas reinjection (Figure 1). Methane losses ranged from 2 to 10% v/v. Whether methane removal was associated with dissolution and/ or aerobic degradation by methanotrophs remains unknown and requires further investigation.

The inhibitory effects of H_2S on microalgae growth was not observed in this study. This may be due to the lower amount of H_2S added into system (i.e., 1.1 mL H_2S/L/d) being much lower than the inhibitory threshold level (10.8mL H_2S/L/d) (Kao et al., 2012). H_2S was quickly and completely removed (100% removal efficiency) even after consecutives biogas reinjections (Figure 1). Removal of H_2S could be attributed to precipitation due to the very high oxidative characteristics of the culturing media (dissolved oxygen above 8 mg/L). Although not studied in this work, microbial mediated biological H_2S removal by chemiolitotrophic bacteria that oxidize H_2S to sulfate could also be possible. Regardless of the process however, the system was very efficient to remove H_2S from biogas which certainly calls the attention of entrepreneurs interested in the biomethane industry.

CONCLUSIONS

Photobioreactor containing headspace swine-derived biogas enhanced microalgae biomass rates up to 2.4 times as compared to atmospheric CO_2 concentrations. Microalgae exposed to autotrophic conditions showed twofold faster growth rate (0.6 d^{-1}) then mixotrophic conditions with consequently faster removal of CO_2 and nutrients (N and P). Hydrogen sulfide and CO_2 were efficiently (>99%) removed from headspace. Therefore, closed microalgae culturing systems can be a very attractive option to remove undesirable compounds from biogas stream, ultimately increasing biomethane value. The proposed model to estimate biomass yield based on the concentration of CO_2, TIC and TOC can be a simple and useful tool for determining biomass yields at scale up.

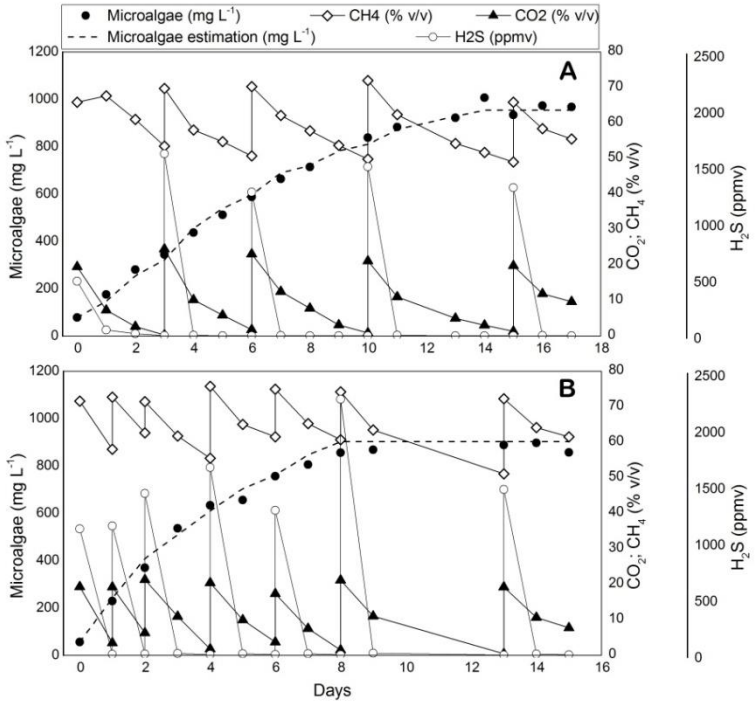

Figure 1. CO_2 (%v/v), CH_4 (%v/v), H_2S (ppmv) and microalgae biomass (mg/L) concentration profiles in the mixotrophic (A) and autotrophic (B) photobioreactors over time. Successive reinjections of biogas were performed over time. Dashed line shows the biomass model data fit.

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