Bioresource Technology 221 (2016) 385–393

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Nitrogen and phosphorus removal coupled with carbohydrate production by five microalgae cultures cultivated in biogas slurry

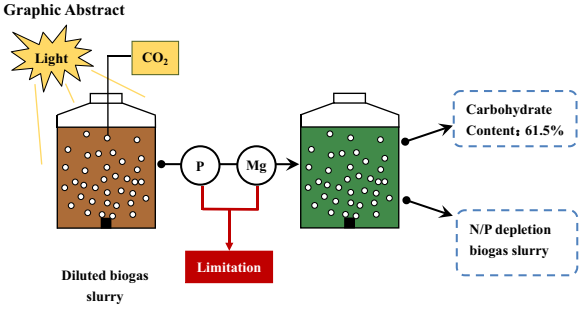
Fen Tan [a], Zhi Wang [a], Siyu Zhouyang [a], Heng Li [b], Youping Xie [a], Yuanpeng Wang [a], Yanmei Zheng [a], Qingbiao Li [a,b,*]

[a] Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, The Key Lab for Synthetic Biotechnology of Xiamen City, Xiamen University, Xiamen 361005, PR China
[b] College of the Environment and Ecology, Xiamen University, Xiamen 361005, PR China

HIGHLIGHTS

- Production of carbohydrate in microalgae from biogas slurry was proven feasible.
- High ammonia removal efficiency, rate and carbohydrate content have been obtained in biogas slurry.
- Phosphorus and magnesium starvation is proposed as a valid strategy for advancing the timing of carbohydrate accumulation in biogas slurry.
- Magnesium is proved firstly as the influence factor for carbohydrate accumulation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 July 2016
Received in revised form 6 September 2016
Accepted 7 September 2016
Available online 13 September 2016

Keywords:
Biogas slurry
Ammonia nitrogen uptake
Carbohydrate accumulation
Chlorella vulgaris

ABSTRACT

In this study, five microalgae strains were cultured for their ability to survive in biogas slurry, remove nitrogen resources and accumulate carbohydrates. It was proved that five microalgae strains adapted in biogas slurry well without ammonia inhibition. Among them, *Chlorella vulgaris* ESP-6 showed the best performance on carbohydrate accumulation, giving the highest carbohydrate content of 61.5% in biogas slurry and the highest ammonia removal efficiency and rate of 96.3% and 91.7 mg/L/d respectively in biogas slurry with phosphorus and magnesium added. Additionally, the absence of phosphorus and magnesium that can be adverse for biomass accumulation resulted in earlier timing of carbohydrate accumulation and magnesium was firstly recognized and proved as the influence factor for carbohydrate accumulation. Microalgae that cultured in biogas slurry accumulated more carbohydrate in cell, making biogas slurry more suitable medium for the improvement of carbohydrate content, thus can be regarded as a new strategy to accumulate carbohydrate.

1. Introduction

Anaerobic digestion is widely applied in clean energy production, solving the problem of waste contamination. Associate with the development of anaerobic digestion engineering, the increasing output of biogas slurries becomes inevitable and approximately 385 million tons of liquid waste was generated by over 30 million

* Corresponding author at: Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, No. 422, Siming South Road, Xiamen 361005, PR China.
E-mail address: kelqb@xmu.edu.cn (Q. Li).

http://dx.doi.org/10.1016/j.biortech.2016.09.030
0960-8524/

methane-generating tanks (Huang et al., 2014). With abundant nitrogen resources as a leading cause of eutrophication in the slurries, draining biogas slurries directly to the environment without sufficient pretreatment is considered to be at high risk of environmental hazards. Meanwhile, biogas slurry is particularly rich in nitrogen resources which are exactly needed for microalgae growth. Culturing microalgae to utilize nutrients in the bio-slurries and to produce high value-added products have been commonly realized as a potential strategy for emission reduction and renewable energy production (Lam and Lee, 2012). Among the microalgae-based high-value products, carbohydrate is one of the bio-products and potential feedstock for bioethanol production which has provoked heightened interest since energy crisis (Maity et al., 2014).

To improve microalgae-based carbohydrate production, researches have been designed and conducted. It is generally believed that carbohydrate generating process happened in Calvin cycle of photosynthesis and nitrogen starvation is one of the most effective way to trigger carbohydrate accumulation (Markou et al., 2015). Yet culturing microalgae in pure medium to produce carbohydrate as the feedstock of bio-ethanol is less competitive to traditional fossil energy and competed for freshwater (Ho et al., 2013).

Limited researches have been conducted with carbohydrate production as target in wastewater, among which *Spirulina* subsalsa, *Scenedesmus* obliquus and *Chlorella vulgaris* have been cultured in waste water of monosodium glutamate factory, food and municipal wastewater treatment plant respectively, achieving the highest carbohydrate content of 50% (He et al., 2013; Ji et al., 2015; Jiang et al., 2015), proving the feasibility of microalgae-based carbohydrate production in wastewater. However, distinguish from municipal wastewater and industrial wastewater, biogas slurry is recognized to have higher ammonia concentration and turbidity with low useable carbon resource and only quite a few studies using biogas slurry as culture media for biogas slurry treatment, neither did nutrients optimization employed to further improve carbohydrate productivity in biogas slurry. Manjinder consorted 3 microalgae strains in approximately 26-fold diluted poultry litter anaerobic digestion, achieving the highest carbohydrate content of 27.3% which is comparatively low (Singh et al., 2011). Yue Wang cultivated *C. vulgaris* JSC-6 with 5-fold diluted swine wastewater, achieving carbohydrate content reached up to 58% (per dry weight) and yet failed to declined ammonia to emission standard with above 150 mg/L ammonia left over after 12 days' cultivation (Wang et al., 2015). With these previous studies reported, it was hard to find a microalga that could accumulate high carbohydrate and consumed up ammonia quickly in biogas slurry at the same time.

Therefore, two microalgae species (five strains) were employed for their ability to survive in bio-slurry, remove nitrogen resources and accumulate carbohydrates simultaneously in this study. Meanwhile, it has been commonly realized that nitrogen depletion could enhance the carbohydrate content and nitrogen-starvation strategy has been widely employed to trigger carbohydrate accumulation, yet no efforts have been made to evaluate whether other components in the biogas slurry have a positive or negative influence on carbohydrate accumulation. Nutrients such as phosphorus and magnesium thus were added to further improve the cell density, ammonia removal performance and carbohydrate accumulation of three microalgae strains in diluted biogas slurry. By comparing the performance of five microalgae strains in diluted biogas slurry and further medium optimization, a high performance microalgae strain adapted to the biogas slurry with high carbohydrate content and high ammonia removal rate can be selected and utilized as the potential feedstock for bioethanol production.

2. Materials and methods

2.1. Source of biogas slurry

Biogas slurry used in this paper was obtained from the outlet of a 10 L semi-continuously fed swine anaerobic digester which has 400 mL daily import (TS = 10%) and output. Biogas slurry was the supernatant liquid of output which was centrifuged (universal 320R, Hettich, Germany) in 8000 rpm for 10 min. Biogas slurry collected daily and stored in a 4 °C refrigerator without autoclaving or adjusting of pH value. However, the initial concentration of ammonia in biogas slurry is too high for microalgae strains to bear (Tam and Wong, 1996). Meanwhile, the color of the biogas slurry is deep dark brown which would adversely affect light penetration and therefore prevent algal growth. Dilution of biogas slurry based on the ammonia concentration thus needed for the culture of microalgae and 8 times diluted biogas slurry was employed in this study (Table 1).

2.2. Microalgae strains and preculture conditions

C. sorokiniana FACHB-275, *C. vulgaris* and *S. dimorphus* FACHB-1266 were bought from algal-species database of Wuhan Institute of Hydrobiology, Chinese Academy of Sciences. *C. vulgaris* ESP-6 and *Desmodesmus* sp. F51 were obtained from the Department of Chemical Engineering, National Cheng Kung University, Taiwan. All the microalgae species used in this study have been tested in previous researches that conducted in waste water (Quiroz Arita et al., 2015), proving a good potential and feasibility of apply these strains to biogas slurry. Microalgae strains were precultured at 27 ± 1 °C in 1 L Modified Bold Basal 3N medium (Modified BBM) which consists of (g/L) $NaNO_3$ (0.075), K_2HPO_4 (0.0383), KH_2PO_4 (0.088), $MgSO_4{\cdot}7H_2O$ (0.075), $CaCl_2{\cdot}2H_2O$ (0.025), NaCl (0.025), $FeCl_3{\cdot}6H_2O$ (0.00177), EDTA (0.00244), trace metal solution consist of (mg/L) $ZnSO_4{\cdot}7H_2O$ (0.073), $CoSO_4{\cdot}7H_2O$ (0.016), $MnSO_4{\cdot}5H_2O$ (0.584), $Na_2MnO_4{\cdot}2H_2O$ (0.00148), $NiCl_2{\cdot}6H_2O$ (0.000149). The pre-culture medium was autoclaved at 121 °C for 20 min. The microalgae strain culture was illuminated 24 h a day with a light intensity of approximately 60 μmol/m^2/s. The pre-culture maintained about 3.5–5 days with a continuous supply of 2.5% CO_2 at an aeration rate of 0.2 vvm and an agitation rate of 300 rpm. The OD_{685} of microalgae inoculum was about 0.620 after 5-fold diluted with distilled water. Microalgae strains were maintained in a BG-11 plate for long time storage.

2.3. Experimental setup

Glass bottle (1000 ml) sealed with rubber plug was used as batch photo bioreactor with external light illuminations provided by fluorescent lamps (14W TWG114, Philips, China) that equipped along both of its sides (Fig. 1). Three different media were used for cultivation of microalga strains in the PBR: Modified Bold Basal 3N

Table 1
Biogas slurry nutritional liquid formula.

Component	Solution (mg/L)	Component	Solution (mg/L)
TN	146.64 ± 8.12	TP	2.18
NH_4-N	135.5∼143	PO_4^{3-}-P	2.01 ± 0.04
COD	120.77 ± 15.09	Mg	0.9429
Ca	0.5762	Na	6.8470
K	4.2381	Cu	$234.5 * 10^{-3}$
Cr	$4.444 * 10^{-3}$	As	$14.95 * 10^{-3}$
Zn	$642 * 10^{-3}$	Pb	$4.897 * 10^{-3}$
Cd	$1.093 * 10^{-3}$	N:P	73:1