Energy Conversion and Management 158 (2018) 201–209

Contents lists available at ScienceDirect

Energy Conversion and Management

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

Inhibition of thermochemical treatment on biological hydrogen and methane co-production from algae-derived glucose/glycine

Richen Lin[a,b,c], Jun Cheng[a,*], Jerry D. Murphy[b,c]

[a] State Key Laboratory of Clean Energy Utilization, Zhejiang University, Hangzhou 310027, China
[b] MaREI Centre, Environmental Research Institute, University College Cork, Cork, Ireland
[c] School of Engineering, University College Cork, Cork, Ireland

ARTICLE INFO

Keywords:
Glucose
Glycine
Maillard reaction
Biohydrogen
Biomethane

ABSTRACT

Algae have emerged as a sustainable feedstock for gaseous biofuel production (such as hydrogen and methane). Fermentative sugars and amino acids can be obtained after suitable pretreatment and hydrolysis of algae. However, binary interactions between the carbonyl group (−C═O) in sugars and the amino group ($-NH_2$) in amino acids possibly occur during thermal pretreatment, resulting in deficient hydrolysis and fermentation performance. In this study, algae-derived glucose and glycine as model substrates were subjected to thermochemical treatment (135 °C for 15 min) under neutral, acid and alkaline conditions to assess their decomposition routes and the associated implications on sequential biohydrogen and biomethane fermentation. Acid treatment mainly resulted in direct decomposition of glucose into 5-methylfurfural ($C_6H_6O_2$, 34.4% of peak area). While thermal treatment with deionized water and alkaline led to the formation of nitrogen-containing Maillard compounds, namely 1-azido-4-dimethylaminobenzene ($C_8H_{10}N_4$, 33.1%) and 2,3,5-trimethylpyrazine ($C_7H_{10}N_2$, 49.0%), respectively. Untreated glucose/glycine yielded a biohydrogen production of 171.9 mL/g, while alkaline treatment exhibited a biohydrogen yield of only 5.9 mL/g due to the great loss of fermentable substrate. The total energy conversion efficiency (ECE) of 71.1% was achieved through the second-stage biomethane fermentation of untreated glucose/glycine. Comparatively, alkaline treatment significantly inhibited the total energy recovery with an ECE of 31.9%. The findings of this study suggested that optimised pretreatment strategy for algae needs to be developed to avoid fermentable compounds loss and achieve a higher ECE.

1. Introduction

Gaseous biofuels, such as biohydrogen and biogas, exhibit significant potential for future renewable and sustainable energy supply [1]. The conversion of biomass into biofuels is achievable via two general technologies: thermochemical and biochemical conversion. Gasification is the heating of biomass at high temperatures (800–1000 °C) with low oxygen content to produce combustible gas mixtures. Syngas is the common product, which is a mixture of CO, H_2, CH_4 and CO_2. Syngas is a low calorific gas (typical 4–6 MJ/m^3) that can be burnt directly or used as a fuel for gas engines and gas turbines [2]. Biohydrogen and biomethane can be produced through dark fermentation and anaerobic digestion, which employ a variety of microorganisms to degrade organic biomass at low temperatures (35–65 °C). The mixture of hydrogen and methane (together termed hythane) has been recognized as a more efficient and cleaner high-value fuel [3]. Hythane fuel usually contains 20% of hydrogen and 80% of methane, which corresponds to a heating value of approximately 34.4 MJ/m^3. Given the high energy content and superior combustion characteristics, hythane can be used as an ideal transport fuel [3].

Biological hydrogen and methane production from renewable biomass waste via fermentation possesses the unique advantage of improved sustainability as compared to thermochemical processes [4–7]. Integrating two-stage dark hydrogen and subsequent biomethane fermentation for biohythane production has been proven to be an efficient process to maximize gaseous energy recovery from various feedstocks [8–11]. Algae including microalgae and macroalgae are seen as plentiful and sustainable feedstocks for future third generation biofuel [12,13]. Algae present superior advantages compared to lignocellulosic biomass [12] such as: (1) higher growth rate with high CO_2 fixation

Abbreviations: 5-HMF, 5-hydroxymethylfurfural; ECE, energy conversion efficiency (%); GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry; HPB, hydrogen-producing bacteria; HPLC, high performance liquid chromatography; MPA, methane-producing archaea; SMPs, soluble metabolic products; VS, volatile solid (g); H_m, maximum gas yield potential (mL/g); R_m, peak gas production rate (mL/g/h); λ, lag-phase time (h); T_m, gas production peak time (h)

* Corresponding author.
E-mail addresses: richen.lin@ucc.ie (R. Lin), juncheng@zju.edu.cn (J. Cheng), jerry.murphy@ucc.ie (J.D. Murphy).

https://doi.org/10.1016/j.enconman.2017.12.052
Received 5 October 2017; Received in revised form 14 December 2017; Accepted 15 December 2017

capacity; (2) lack of necessity of arable land to grow; (3) minimal content of lignin. The principal fermentative components of algae are carbohydrates and proteins. The contents of carbohydrates (6–64% of dry matter) and proteins (8–71% of dry matter) in algae are high, but species and season dependent [14,15]. The contents of carbohydrate and protein in a brow seaweed (*Laminaria digitata*) vary significantly with season [15]. In August, *Laminaria digitata* exhibits a C/N ratio of 32 (relatively high carbohydrate content but low protein content), and results in the highest biomethane yield of 327 mL/g VS. The carbohydrate-derived glucose and protein-derived glycine are found in high concentrations in many algae species such as *Arthrospira*, *Monostroma*, and *Ulva* [16,17]. Glucose is mainly derived from the hydrolysis of glycogen, trehalose, glucosyl–glycerol and laminarin in algae. The hydrolysate of *Ulva lactuca*, a species of green macroalgae has a sugar composition of 27% glucose [18]. The content of glycine derived from proteins in green seaweed varies from 6.1 to 10.1 g/100 g of total protein [17]. Therefore, the rich contents of fermentable carbohydrates and proteins make algae an ideal feedstock for biological fermentation.

However, the fermentation of algae biomass may not be straightforward due to several potential restraints including the recalcitrant substrate constituents, cell wall degradability, and unfavorable carbon to nitrogen ratio [12]. Pretreatment of algae to release reducing sugars (such as glucose and xylose) and amino acids (such as glycine and arginine) is a common strategy prior to fermentation for improving gaseous energy recovery. Of numerous pretreatment methods (such as acid, alkaline, ultrasonic, microwave, thermal, enzymatic and their combinations), thermochemical treatment employing acid and alkaline was found efficient to destroy cell structure and release fermentable components from algae [12,16,19,20]. The highest glucose yield from macroalgae *Gelidium amansii* was obtained by acid-catalyzed hydrothermal hydrolysis (161 °C, 2.0% H_2SO_4 for 40 min) [21]. Steam-heating acid pretreatment was demonstrated as effective for mixtures of microalgae (*Arthrospira platensis*) and macroalgae (*Laminaria digitata*), resulting in a reducing sugar yield of 0.268 g/g volatile solid (VS) [16].

Fermentable reducing sugars and amino acids can be effectively released during thermochemical treatment of algae. However, in addition to the occurrence of the hydrolysis reaction, the interactions between the carbonyl group (−C═O) in sugars and the amino group ($-NH_2$) in amino acids (namely the Maillard reaction) may possibly take place in thermochemical environment. The Maillard reaction could reduce the yield of sugars and amino acids and generate undesirable byproducts (such as nitrogen-containing compounds). These generated nitrogen-containing compounds are difficult to degrade under anaerobic conditions, and may cause considerable inhibition to fermentative bacteria, leading to decreased biofuel production [22–25]. Liu et al. demonstrated that thermal pretreatment at 175 °C for 60 min of biomass wastes (such as kitchen waste and vegetable/fruit residue) led to a decrease in biomethane production by 7.9–11.7% [26]. Zhang et al. also demonstrated the negative effects of melanoidins on anaerobic digestion of waste activated sludge by thermal pretreatment (200 °C) [24]. Posmanik et al. concluded that hydrothermal treatment at high temperature (350 °C) exhibited the highest energy return as oil but the lowest energy return as biomethane [27]. These researches indicated that thermal pretreatment under severe conditions might cause inhibitory effects on fermentation/anaerobic digestion for biofuel production, presumably due to the occurrence of the Maillard reaction during pretreatment. Therefore, understanding the way how the Maillard reaction influences hydrolysis reaction is fundamental for future pretreatment optimization and co-production of biohydrogen and biomethane from algal biomass. To the best of our knowledge, there is a research gap on the chemical interactions between algae-derived sugars and amino acids under various thermal treatments, and particularly on the implications to subsequent biohydrogen and biomethane fermentation.

The innovation of this study is the investigation of the reaction routes of glucose and glycine under various thermal treatment conditions (under deionized water, acid and alkaline), and assessment of the impacts on subsequent biohydrogen and biomethane fermentation. The use of the algae derived glucose and glycine as model substrates would be helpful for the understanding of the reactions in pretreating algae and beneficial for the further fermentation optimization. The detailed objectives of this study are to: (1) Evaluate the effects of thermochemical treatment on decomposition of algae-derived glucose and glycine; (2) Assess the performances of biohydrogen fermentation, soluble metabolic products formation, and subsequent biomethane fermentation from untreated and treated glucose and glycine; (3) Reveal the Maillard reaction mechanism of glucose and glycine under various thermochemical conditions.

2. Materials and methods

2.1. Inocula

Hydrogen-producing bacteria (HPB): HPB were originally sourced from activated sludge in a mesophilic digester treating swine slurry in Zhejiang Province, China. The collected sludge was heat-treated at 100 °C for 30 min to inactivate methanogens, and then was acclimatized three times to harvest mixed spore-forming HPB such as *Clostridium* species. The acclimatized HPB was used as inoculum for dark hydrogen fermentation.

Methane-producing archaea (MPA): MPA were sourced from the same mesophilic digester as HPB. Enriched MPA, mainly including *Methanosarcina* and *Methanothrix*, were used as the inoculum for methanogenesis. The inoculum was degassed at a temperature of 35 °C for 7 days prior to the biomethane fermentation.

2.2. Experimental design

2.2.1. Thermochemical treatment

The thermochemical treatment of the mixtures of glucose and glycine are detailed in Table 1. The treatment was conducted in an autoclave (Sanyo MLS-3780, Japan). A total amount of 2.5 g of glucose and glycine (mass ratio of glucose to glycine = 1:1) was mixed in conical flasks. Untreated mixture of glucose and glycine was used as control group (Group 1). For thermochemical treatments, a volume of 50 mL of deionized water (Group 2), dilute acid (1.0 v/v% sulfuric acid, Group 3), and dilute alkaline (1.0 wt% sodium hydroxide, Group 4) was added into the conical flasks, respectively. Then the conical flasks were transferred into autoclave and subjected to thermal treatment at 135 °C for 15 min. The treatment parameters were previously proven to be effective for various feedstocks (such as *Chlorella pyrenoidosa* and cassava starch) [28], and therefore were employed in this study.

2.2.2. Fermentation

Biohydrogen fermentation: Batch experiments of dark biohydrogen fermentation were performed in duplicate in 8 glass fermenters with a working volume of 300 mL, as schematized in Fig. 1 [29]. Four biohydrogen fermentation groups were assessed: Group 1 (untreated glucose/glycine); Group 2 (neutral treated glucose/glycine); Group 3 (acid treated glucose/glycine); and Group 4 (alkaline treated glucose/glycine). Each bottle contained 50 mL of the solution of glucose and glycine as feedstock and 25 mL of acclimatized HPB as inoculum. The total

Table 1
Thermochemical treatment of the mixtures of glucose and glycine.

Group	Substrate	Thermal treatment condition
1	Glucose (1.25 g)/glycine (1.25 g)	/
2	Glucose (1.25 g)/glycine (1.25 g)	135 °C for 15 min in deionized water
3	Glucose (1.25 g)/glycine (1.25 g)	135 °C for 15 min in acid
4	Glucose (1.25 g)/glycine (1.25 g)	135 °C for 15 min in alkaline