Algal Research 17 (2016) 253–260

Contents lists available at ScienceDirect

Algal Research

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

Multiple synergistic benefits of selective fermentation of *Scenedesmus* biomass for fuel recovery via wet-biomass extraction

YenJung Sean Lai [a,*], Allison McCaw [a], Aura Ontiveros-Valencia [a], Yue Shi [b], Prathap Parameswaran [c,*], Bruce E. Rittmann [a]

[a] Swette Center for Environmental Biotechnology, The Biodesign Institute at Arizona State University, P.O. Box 875701, Tempe, AZ 85287-5701, USA
[b] College of Power and Energy Engineering, Harbin Engineering University, Harbin, Hei Long-jiang Province, China
[c] 2118 Fiedler Hall, Department of Civil Engineering, Kansas State university, Manhattan, KS 66506, USA

ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form 27 April 2016
Accepted 2 May 2016
Available online 27 May 2016

Keywords:
Scenedesmus
Selective fermentation
FAME
Solid retention time
Wet biomass extraction
Biohydrogenation

ABSTRACT

Selective fermentation (SF), a novel alternative for microalgae-derived biofuel, ferments carbohydrates and proteins in microalgae biomass to volatile fatty acids (VFAs), which makes it easy to extract the lipid with low-toxicity solvents. A further advantage of SF is that the lipids are biohydrogenated to more valuable fuel precursors. This work evaluated SF using semi-continuous studies at pH values of 5 and 7 and with solids retention times (SRTs) of 2 to 15 days. SRT > 2 day enhanced VFA recovery from the non-lipid fractions by 4-fold (vs feed biomass), conserved the lipids, increased lipid wet-extraction (lipids assayed as fatty acid methyl esters, FAME) by at least 6900-fold (vs feed biomass), and shifted the FAME profile toward more saturated fatty acids through biohydrogenation. The performance benefits were accompanied by selective enrichment of biohydrogenating strains, e.g., *Prevotella* and *Porphyromonodaceae*, along with other fermenting bacteria that generate VFAs and H_2, e.g., *Veillonellaceae*. SF with a 5-day SRT had greater lipid wet extraction, compared to 2-day SRT, due to increased cell disruption and solvent permeability. By integrating multiple downstream processes into one step, SF offers important advantages for sustainable biofuel production from microalgae.

1. Introduction

Although microalgae-derived biofuel is a promising alternative to petroleum-based fuel [1,2], its application has been hampered by (1) the high capital cost of cultivation and harvest, (2) energy-intensive pretreatment of the biomass and lipid extraction with toxic solvents, and (3) the need to improve lipid quality by further refining. This study focuses on the second and third problems.

Microalgae-derived lipids are mainly triacylglycerols (TAGs) enclosed within intracellular compartments [3,4]. These TAGs are not readily accessible by solvents, and cell disruption is required for efficient solvent extraction [3,5–9]. Pretreatment includes acid or base, hydrothermal, enzymatic, osmotic, and pulsed electric field methods [3,5–11], all of which are energy and capital intensive. The gold standards for solvent extraction are based on using toxic chloroform and methanol solvents: 1:1 chloroform:methanol [12] and 2:1:0.8 chloroform:methanol:H_2O [13]. Hexane is a less-toxic solvent that is commonly used at industrial scale, but it does not achieve significant lipid yields without pretreatment [9,10]. Moreover, the dewatering and drying steps associated with conventional solvent extraction are costly in terms of energy and economics [5,14,15]. Recent research points to the promise of lipid wet extraction using environmentally safe surfactants, if the cells can be disrupted [16–18]. A low-energy means to achieve cell disruption before biomass wet extraction would be of high value.

Saturated fatty acids are more desirable than unsaturated fatty acids as biofuel precursors [1,19–21]. Microalgae that can contain a high lipid content per unit biomass – such as *Chlorella*, *Scenedesmus*, and *Nannochloropsis* – have fatty acids with a high degree of polyunsaturation: e.g., C16:1, C18:1, and C18:3. The fatty acid profile can be made more saturated and desirable through hydrogenation via chemical catalysis, but it adds significant cost for biofuel production [1,22]. An alternative to catalytic hydrogenation is microbial biohydrogenation, which Lai et al. [23] observed during selective fermentation of *Scenedesmus* biomass with anaerobic sludge as the inoculum at pH values of 5 and 7 (but not 9). Biohydrogenation was accompanied by enrichment of fermenting bacteria likely able to carry out biohydrogenation: *Prevotella*, *Porphyromonodaceae*, *Lachnospiraceae*, *Ruminococcaceae*, and unclassified *Bacteroidales* families [23–25]. Thus, managing the microbial ecology is essential for sustained biohydrogenation.

Fermentation is a mature biotechnology based on conversion of a variety of organic substrates, including wastes, to volatile fatty acids (VFA) and H_2 [26,27]. Carbohydrates, proteins, and lipids in organic wastes have distinctive degradation kinetics [23,28–30]. Important is that lipid fermentation could be relatively slow compared to carbohydrates

* Corresponding authors.

http://dx.doi.org/10.1016/j.algal.2016.05.001
2211-9264/

and proteins, leading to selective fermentation which keeps the lipids intact while the other components are hydrolyzed and fermented.

Due to the slow kinetics of lipid hydrolysis, bacteria specializing in lipid fermentation have slower growth rates that make them susceptible to washout in a fermentation reactor having a relatively short solids retention time (SRT). Because some non-lipid components are co-extracted along with microalgae lipids, downstream processing is needed to separate them from the valuable fuel precursors [5,23]. By fermenting non-lipid components to VFAs, selective fermentation (SF) minimizes the need to separate non-lipid components of algal biomass. An optimum SRT for SF would maximize the conversion of carbohydrates and proteins to VFAs, while conserving the lipids. The differential impact of SRT on the fermentation kinetics of the different components of algal biomass is unexplored.

In addition to SRT, pH has a strong impact on fermentation performance, including the distribution of VFAs and the resulting microbial communities and biohydrogenation [23,31–33]. Considerable studies have been carried out for batch fermentation of microalgae biomass, but it was focused on non-lipid components alone or on lipid-extracted algae (LEA) biomass [34–36]. The combined impacts of pH and SRT on SF, including on lipid wet extraction and biohydrogenation, is as yet unexplored.

In this study, we performed semi-continuous SF using anaerobic digester sludge as the inoculum, at pH 5 and 7, and operated at 15-, 10-, 5-, and 2-day (d) SRTs. The main goals of our study were to evaluate: (1) SRTs and pHs at which lipids (represented as FAME) from *Scenedesmus* biomass are conserved; (2) the effect of SRT and pH on fermentation of non-lipid biomass fractions to VFAs; (3) the relationship between microbial community structure and function (e.g., biohydrogenation during semi-continuous SF as a function of SRT and pH); and (4) the compatibility to do biomass wet extraction for the favorable SRTs (from items 1 and 2).

2. Materials and methods

2.1. Biomass

40 L of lipid-rich *Scenedesmus* biomass, freshly harvested from a pilot-scale algal open pond under consistently nutrient-depleted conditions, was obtained on a periodic basis from the Arizona Center for Algal Technology and Innovation (AzCATI), Mesa, AZ. The biomass was stored in a cold room at 4 °C located at the Biodesign Institute, Tempe campus of Arizona State University.

2.2. SF experiments at different SRTs and pHs

SF experiments were initiated as batch experiments according to the procedures of Lai et al. [23]. In brief, reactor set up began by mixing *Scenedesmus* biomass and an inoculum of anaerobic digested sludge obtained from Mesa Northwest Wastewater Reclamation Plant (MNWWRP) in the ratio of 350 mL algal biomass: 150 mL anaerobic digested sludge. Two pH conditions were established – 5 and 7 – by buffering with 40 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) and phosphate, respectively. Methanogenesis was inhibited with 10 mM 2-bromoethanesulfonic acid (BES). 15-μM sodium sulfide, a reducing agent, was added to ensure anaerobic conditions. The fermentation reactors were well mixed with an incubator shaker at 210 rpm (New Brunswick Scientific, Enfield, CT) and kept at 37 °C. We observed no evidence of cell disruption from the incubation conditions themselves [18].

Batch fermentations were carried out for a period of 32 days, at which time we began semi-continuous operation with a series of SRTs: 15, 10, 5, and 2 d. Appropriate volumes of biomass (i.e., 30, 45, 90, and 225 mL per d, respectively, for a total volume of 450 mL volume) were withdrawn and replaced with feeding biomass to achieve the desired SRT, and the pH was buffered to the same value (5 or 7) as in the batch experiments. Biomass and gas samples were taken at regular intervals to obtain measurements outlined in analytical methods. Three different batches of feed algal biomass were needed to cover the 4 sets of SRT studies. The feeding biomass was characterized on a regular basis to ensure that the biomass was not subject to pre-fermentation. If significant VFA changes in the biomass feed were detected, we switched to a fresh batch of feeding biomass. DNA pellets were collected from the anaerobic sludge inoculum, raw *Scenedesmus* biomass, and fermented samples at the end of steady state conditions for each SRT condition for two pH conditions for microbial community analysis.

2.3. Analytical methods

All analytical assays were adapted from Lai et al. [23]. Slurry samples for total suspended solids (TSS) and volatile suspended solids (VSS) were assayed directly by dry weight according to *Standard Methods* [37]. The volatile fatty acids (VFA) were assayed after 0.2-μm membrane filtration (Pall Science, NY, USA). Total COD was measured using a HACH COD kit (concentration range 10–1500 mg/L) and quantified by using a spectrophotometer (DR-2800, HACH, Loveland, CO). Volatile fatty acids were measured with an HPLC (Shimadzu, USA) equipped with an Aminex HPX-87H column [38]. Gas samples were analyzed for CH_4 and H_2 by gas chromatography [38]. Carbohydrate analysis was adapted from the previous study using a colorimetric method and quantified against glucose standards. Fatty acid methyl ethers (FAMEs) were quantified using a gas chromatograph (Shimadzu GC 2010, Japan) equipped with a Supelco SP-2380 capillary column (30 m × 0.25 mm × 0.20 μm) and flame ionization detector (FID). All FAME components were quantified against a 37-component FAME-mix standard (Supelco, PA, USA) and were identified by comparing the peak retention times to those of standard compounds.

2.4. Total FAME assays

Total FAME is distributed between the solid phase of slurry biomass and free fatty acids. Thus, 30-mL biomass samples were first separated into two fractions, biomass and liquid, by centrifugation (Eppendorf 5810R, NY, USA) for 15 min at 4000 rpm. These two fractions were freeze-dried using a FreeZone Benchtop instrument (Labconco, MO, USA). Freeze-dried samples were weighed and then spiked with 2 ml of 3-N methanolic HCl (Sigma-Aldrich, MO, USA) to initiate direct trans-esterification with incubation at 85 °C for 2.5 h. The FAMEs were quantified by GC-FID as described above. Total FAME is the sum of FAMEs from the two fractions. The net change of total FAME was estimated using the Chemical Oxygen Demand (COD) before and after fermentation to produce electron-equivalent mass balances, by computing the COD equivalents obtained from the measured individual FAMEs [23]. The equation for computing the net change in FAME is given as represented by Eq (1) and the degree of biohydrogenation between the initial sample and after SF was defined using the differential saturation degree with Eq. (2) adapted from Lai et al. [23] as described below.

$$\text{Net change of total FAME} = \left[\frac{\text{Total FAME}_{before} - \text{Total FAME}_{after}}{\text{Total FAME}_{before}}\right] \times 100(\%) \quad (1)$$

where total FAME (mg COD) was the sum of FAME in the slurry biomass and liquid phase before and after fermentation. FAME values were converted to COD unit (mg) according to their electron equivalences.

$$\text{Differential saturation degree} = \left[\frac{\text{Saturated FAME}_{after} - \text{Saturated FAME}_{before}}{\text{Total FAME}_{before}}\right] \times 100(\%) \quad (2)$$