Bioresource Technology Reports 9 (2020) 100344

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.journals.elsevier.com/bioresource-technology-reports

Upgrading brown grease for the production of biofuel intermediates

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ARTICLE INFO

Keywords:
Brown grease
Fermentation
Lipid extraction
Butyric acid
Technoeconomic analysis

ABSTRACT

Products from waste streams can reduce the amount of materials going to landfills or being incinerated. Brown grease captured from grease traps or wastewater facilities is produced at approximately 1,500,000 metric tons annually in the United States. Two brown grease samples were characterized from a local collector. Raw brown grease was fermented using *Clostridium butyricum* to produce butyric acid, achieving a yield of 0.55 butyric acid/g sugars indicating that glycerol and lactic acid were also converted. Hexane extraction of the fermentation broth gave an 81.3% recovery of lipids. Techno-economic analysis calculated a minimum fuel selling price of $1.81 per gallon gasoline equivalent (GGE) for a facility based on a city the size of Denver and a brown grease delivery price of $100 per dry metric ton. Exploiting waste streams as feedstocks can significantly reduce costs to produce fuels and can be brought to scale more rapidly due to their ready availability.

1. Introduction

Brown grease is generally collected from grease traps mainly found in restaurants and commercial food preparation facilities or in wastewater treatment facilities and approximately 1,500,000 metric tons are produced in the United States per annum (Milbrandt et al., 2018; Nelson and Searle, 2016). It is not usually considered a suitable feedstock for biofuel (namely biodiesel) production because of high water and free fatty acid content. Brown grease is often taken from the various collection sites and either landfilled or combusted, resulting in significant loss of potential feedstock and revenue opportunities. Some brown grease is converted to biogas via anaerobic digestion though it is not an ideal substrate with operational challenges including inhibition of complete conversion, sludge flotation, foaming, and system blockages (Long et al., 2012). There are reports that some portion of brown grease is processed by rendering plants for use in various industries, e.g. biofuels, lubricants, but the volume of material monetized in this fashion is not known, and not considered to be significant. Since the collection infrastructure is already in place in most communities, it would be straightforward to establish a system to deliver this feedstock to a centrally located biorefinery (perhaps at or near a landfill or wastewater treatment facility) for conversion to biofuels and bioproducts.

Although processes have been developed to convert brown grease to biodiesel, they require a two-stage conversion process to allow both the triglycerides (the typical feedstock for biodiesel) and free fatty acids to be converted. In our proposed process, the carbohydrate component of brown grease would not be sent back to wastewater treatment, but rather would be valorized through the same conversion processes envisioned for algal biomass with a potential to increase the biofuel yields by as much as 2-fold depending on the overall composition of the brown grease. The algal biomass conversion processes that we are replicating with brown grease are the Combined Algal Process (CAP) (Dong et al., 2016a) and the Parallel Algal Process (PAP) (Knoshaug et al., 2017). These approaches are based on a hybrid biochemical and thermochemical processing strategy for selective recovery and conversion of algal biomass components, namely carbohydrates to fuels or chemicals and lipids to renewable diesel blendstock. Both processes are similar, starting with pretreatment of the feedstock to release carbohydrates for fermentation into fuel derivatives, followed by extraction of lipids from the fermentation broth, and distillation of the final products. In the CAP process, the aqueous and solids fractions can be fermented together because the primary fermentation product (ethanol) can be recovered by distillation. The PAP process has an extra step of solid liquid separation to remove the solids from the liquor for fermentation facilitating recovery of fermentation products other than

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https://doi.org/10.1016/j.biteb.2019.100344
Received 21 August 2019; Received in revised form 31 October 2019; Accepted 1 November 2019
Available online 07 November 2019

ethanol that require more complex purification steps. The separated solids are subsequently extracted as in the CAP process. We have explored various options for these processes to build the concept of a flexible, multi-product algal biorefinery to mitigate the current reality that algal biomass feedstock is expensive and requires near-complete utilization of the cellular components to be competitive with petroleum. The use of brown grease as a feed stock can also benefit from this strategy and will also start with a much-reduced feedstock cost and thus offer a quicker route to biofuel production at a cost that can compete with petroleum. Brown grease can also be used to blend with algal biomass feedstocks to offset seasonal algal productivity variability (Davis et al., 2012).

Butyric acid is a valuable chemical in its own right and is also a versatile fuel precursor (Gaertner et al., 2009; Goulas and Toste, 2016; Renz, 2005; Saboe et al., 2018; Serrano-Ruiz et al., 2010; Sjoblom et al., 2016; Zhang et al., 2009) typically made by several *Clostridium* species (Jha et al., 2014) and from lignocellulosic hydrolysate (Nelson et al., 2017). *Clostridium butyricum,* a gram-positive, spore-forming, strictly anaerobic bacterium, can produce high amounts of butyric acid and also forms acetic acid as a byproduct during fermentation (Dwidar et al., 2012; He et al., 2005). High productivity and titers can be achieved using *C. butyricum* for butyric acid production (Jha et al., 2014; Zigova et al., 1999). Here we report the availability, fermentation, lipid extraction, and economics of raw brown grease as a feedstock to produce precursor molecules for fuels and chemicals.

2. Materials and methods

2.1. Brown grease logistics, availability, and samples

To understand the logistics of current brown grease collection and disposal, as well as identify locations that could serve as potential biorefineries and determine their scale, a survey was conducted in four urban areas around the country: New York, Los Angeles, Chicago, and Denver. These locations were chosen based on the study that identifies the first three locations as the largest brown grease generating areas in the country (Milbrandt et al., 2018). The Denver urban area represents a medium sized city in the Rocky Mountain region and a local data source. Requests for information on the volume collected and disposal destination were solicited from businesses that offer brown grease collection services, wastewater treatment plants (WWTPs), waste collection services, landfills, sanitation districts, and state environmental protection agencies. Two brown grease samples were received from a brown grease collector local to the Denver area obtained from different urban restaurant locations.

2.2. Small scale microwave pretreatment to determine optimal conditions for raw brown grease

The experimental design for lipid extraction from the raw brown grease samples (BG) was based on a central composite design (CCD) with 3 factors (temperature, sulfuric acid concentration, and time) and 3 levels. Previously we had found that the optimum pretreatment condition for algal biomass was approximately 145 °C and 2% sulfuric acid for 10 min (Dong et al., 2016a; Dong et al., 2016c). In this study, the experimental conditions were designed based on this optimum pretreatment condition (Table 1). A total of 20 runs were performed with the center point at 145 °C, 2% (*w*/w) sulfuric acid concentration, and 10 min. The center point conditions were performed as 6 replicates.

Lipid extraction was performed by adding raw brown grease (4 mL) and sulfuric acid sequentially into microwave tube reactors. Each tube was heated to the specified pretreatment temperature and held for a specified time. After pretreatment, the biomass was allowed to cool to ambient temperature. An equal volume of hexane was added and stirred on a multi-position stir plate (Velp, Bohemia, NY, US) for 1 h with 30 s vortex mixing every 15 min. The extraction mixture was then centrifuged in a bucket rotor at 2000*g* for 10 min to assist phase separation. The upper hexane phase was carefully transferred to a pre-weighed glass tube, the hexane was evaporated at 40 °C in a TurboVap Concentration Workstation (Caliper Life Sciences, East Lyme, CT), and the dry weight of the remaining oil was obtained. The fatty acid content was measured as fatty acid methyl esters (FAME) in the recovered oil and analyzed by the FAME analysis protocol as described in the analytical section.

Table 1
CCD experimental conditions to determine optimal pretreatment conditions for raw brown grease.

Experiment run order	Temp °C	H_2SO_4 % (w/w)	Time min
1	125	3.20	5
2	165	0.80	5
3	111	2.00	10
4	165	3.20	15
5	145	0.00	10
6	165	0.80	15
7	145	2.00	10
8	145	2.00	18.4
9	125	0.80	15
10	145	2.00	10
11	145	2.00	10
12	145	2.00	10
13	179	2.00	10
14	145	2.00	10
15	145	2.00	10
16	145	4.02	10
17	165	3.20	5
18	125	3.20	15
19	145	2.00	1.6
20	125	0.8	5

2.3. Fermentation

Clostridium butyricum (ATCC 19398) was used for fermentation. Frozen seed stocks were stored in 50% glycerol at −80 °C. One vial of seed stock was revived by growth in a 300 mL serum bottle with 50 mL of deoxygenated seed medium. The seed culture medium contained 38 g/L Reinforced Clostridial Medium and 20 g/L glucose and was sterilized by autoclaving at 121 °C for 15 min. The seed cultures were incubated anaerobically at 37 °C and 120 rpm for 22 h. The revived seed culture was then used to inoculate a fermenter at a starting OD_{600} of 0.5.

A 500 mL BioStat-Q Plus fermenter (Sartorius, Göttingen, Germany) was used to grow the inoculum in a 300 mL working volume. The medium recipe originated from ATCC Medium 2107 and was optimized for use with *C. butyricum*. The fermenter inoculum medium consisted of 5 g/L yeast extract, 10 g/L peptone, 3 g/L $(NH_4)_2SO_4$, 3.26 g/L KH_2PO_4, 0.3 g/L $MgSO_4$-7 H_2O, 0.02 g/L $CaCl_2$-2 H_2O, 0.02 g/L $MnSO_4$-H_2O, 0.03 g/L $FeSO_4$, 0.5 g/L L-cysteine, 0.2 g/L resazurin, and 5 g/L glucose. The fermenter was operated at 37 °C, 150 rpm agitation, 30 ccm nitrogen gas sparging (thus creating and maintaining a strictly anaerobic environment within the fermenter), and started at a pH of 7, allowing the fermentation to naturally decrease to a pH of 6 and controlled with 4 M sodium hydroxide. This seed culture was grown for six hours and reached an OD_{600} of 7.9 and had no measurable glucose left in the medium to avoid carryover.

The brown grease fermentation was performed in the same equipment and with the same conditions as for the inoculum. The medium for the raw brown grease fermentation consisted of BG#1 sample and contained added nutrients which diluted the brown grease to 86% of the original concentration. The added nutrients consisted of 3 g/L $(NH_4)_2SO_4$, 3.25 g/L KH_2PO_4, 0.3 g/L $MgSO_4$-7 H_2O, 0.02 g/L $CaCl_2$-2 H_2O, 0.02 g/L $MnSO_4$-H_2O, 0.03 g/L $FeSO_4$, and 0.5 g/L L-cysteine.

Each fermenter received 300 mL of diluted brown grease and nutrient medium and was inoculated with 19 mL of inoculum culture to give an initial OD_{600} of 0.5. Two control fermentations were performed. The control medium was identical to the medium used for the inoculum fermenter though with the addition of either 5 g/L of glucose or starch and 15 g/L of casamino acids. Antifoam 204 was added to all fermentations to reduce foaming during fermentation as needed.

2.4. Lipid extraction

Lipid was extracted from the raw, pretreated, or post-fermented samples using hexane. Brown grease (raw, pretreated, or post-fermented) and an equal volume of hexane (470 mL of each) were sequentially added into a flask. The extraction mixture was mixed on a magnetic stir plate overnight then centrifuged at 200 *g* for 10 min for phase separation. The hexane/oil layer was recovered, and the hexane removed by rotary evaporation leaving the isolated oil. The hexane extraction was repeated three times and the extracted oil from all three extractions was combined and the FAME content analyzed.

2.5. Analytical methods

2.5.1. FAME

FAME content was determined using an in-situ transesterification procedure as described (Van Wychen et al., 2015). Briefly, an internal standard consisting of tridecanoic acid methyl ester and the solvents 2:1 (*v*/v) chloroform:methanol and 0.6 M HCl:methanol were added to approximately 10 mg of lyophilized brown grease in pre-weighed GC vials. The samples were capped, vortexed, and placed on an 85 °C heating block for 1 h and cooled to room temperature. Hexane was added and incubated at room temperature for 1 h. The extracted lipids as fatty acid methyl esters (FAME) were measured by GC-FID (Agilent 7890B, Santa Clara, CA, USA) using a DB-Wax capillary column 30 m, 0.25 mm ID, and 0.25 µm FT with a 1 µl injection at 10:1 split ratio, inlet temperature of 250 °C with a constant flow of 1 mL/min helium. The oven temperature profile was as follows: 100 °C for 1 min, 25 °C/min up to 200 °C and hold for 1 min, 5 °C/min up to 250 °C and hold for 7 min. FID was performed at 280 °C with 450 mL/min zero air, 40 mL/min H_2, and 30 mL/min helium.

2.5.2. Lipid class determination

The total lipids in raw BG#1 were extracted by accelerated solvent extraction (ASE) as previously described (Dong et al., 2017). Briefly, 2 g of lyophilized raw brown grease was extracted in a Dionex ASE® 200 accelerated solvent extractor (ASE) system (Dionex, CA, USA) using a mixture of chloroform:methanol (2:1, *v*/v) at a pressure of 10.3 MPa and a temperature of 50 °C. The lipid fraction was dried in a vacuum oven overnight at 40 °C after the solvents in the extracted oil were evaporated using a TurboVap Concentration Workstation at 40 °C. Lipid classes were determined by solid phase extraction (SPE) using aminopropyl cartridges (Agilent Technologies) as previously described (Kruger et al., 2018). Approximately 25 mg of lipid was dissolved in 100 µL of 95:3:1 hexane:chloroform:methanol and loaded into a cartridge that had been pre-conditioned with 12 ml of hexane. The neutral lipids, fatty acids, and polar lipids were eluted using three successive washes (6 mL) with 2:1 chloroform:isopropyl alcohol, 98:2 diethyl ether:acetic acid, and 6:1 methanol:chloroform respectively. Each wash was collected in a pre-weighed test tube, dried down overnight in a vacuum oven, and weighed to obtain gravimetric recoveries.

2.5.3. Organic acids

Soluble carboxylic acids from the raw brown grease aqueous phase or after fermentation were recovered by filtering through a 0.2 µm nylon filter into an LC vial. The concentrations of carboxylic acids were measured via a HPLC (Agilent1100 series, Santa Clara, CA, USA) outfitted with a refractive index detector. Carboxylic acids were determined using an Aminex HPX-87H (300–7.8 mm) organic acid column and Cation H+ guard cartridge (Biorad Laboratories, Hercules, CA, USA) maintained at 65 °C with mobile phase consisting of 0.01 N sulfuric acid and a flow rate of 0.6 mL/min.

2.5.4. Carbohydrates

The carbohydrate content in the raw brown grease samples was analyzed as previously described (Van Wychen and Laurens, 2015). In short, approximately 50 mg of lyophilized brown grease was weighed into a glass pressure tube where a two-step hydrolysis took place. Samples were acidified with 500 ul of 72% sulfuric acid and placed in a 30 °C water bath for 1 h with vortexing every 10–15 min. Samples were then diluted with 14 mL of nanopure water and placed in an autoclave at 121 °C for 1 h. Samples were allowed to cool to room temperature, neutralized to pH 6–8 with calcium carbonate, and filtered through a 0.2 µm nylon filter into LC vials for analysis.

Total and monomeric sugar content in the raw and aqueous raw brown grease samples were analyzed as follows (Sluiter et al., 2006). Total sugars were performed on filtered samples which were prepared by acidifying each sample with a volume of 72% sulfuric acid resulting in a final acid concentration of 4%. After samples were capped and vortexed, they were placed in an autoclave at 121 °C for 1 h. Upon removal, samples were allowed to cool to room temperature, neutralized, and filtered as previously stated. Glucose and other sugars were measured via HPLC-RID (Agilent 1100 series, Santa Clara, CA, USA) using a Shodex Sugar SP0810 (300_8 mm) column (Phenomenex, Torrance, CA, USA) with Cation H+ and Anion CO_3– de-ashing guard cartridges (Biorad Laboratories, Hercules, CA, USA). Mobile phase consisted of nanopure water at a flow rate of 0.6 mL/min and a column temperature of 85 °C, both guard cartridges were placed outside the column compartment at room temperature.

2.5.5. Protein

Percent protein was determined on lyophilized brown grease by multiplying percent nitrogen by a conversion factor of 4.78 (Laurens, 2015). Nitrogen was determined by weighing approximately 5 mg of raw brown grease into a pre-weighed tin foil sheet that was folded and pressed into a packet. The sample was then analyzed on an Elementar Vario EL Cube CHN Analyzer (Ronkonkoma, NY, USA) where it was combusted at 950 °C in the presence of oxygen, passed through a GC column, and analyzed with a thermal conductivity detector. The resulting values, measured as singlet analyses for each sample, are reported as weight percent protein of the sample.

2.5.6. Solids and ash

Percent solids were determined (Sluiter et al., 2008a) on raw brown grease samples where approximately 1000 mg were weighed into an aluminum pan and dried at 40 °C under vacuum for 3 days and allowed to cool to room temperature before taking final weights. Total percent ash was determined on lyophilized brown grease (Sluiter et al., 2008b). Approximately 100 mg of sample was weighed into pre-weighed crucibles, dried at 105 °C overnight and then placed in a muffle furnace having an oven temperature program as follows; hold at 105 °C for 12 min, ramp to 250 °C at 10 °C/min, hold at 250 °C for 30 min, ramp to 575 °C at 20 °C/min, hold at 575 °C for 180 min, and finally cooled to 105 °C. Crucibles were then removed and allowed to cool to room temperature before taking final weights.

3. Results and discussion

3.1. Brown grease logistics and availability

Dedicated brown grease collection companies, also called haulers, obtain the material from restaurants and other food-processing establishments, and dispose of it through various methods including landfills, wastewater treatment plants (WWTPs), rendering plants,

incinerators, or anaerobic digesters. Rendering plants either collect brown grease themselves or receive it from haulers and/or WWTPs to further process the material into valuable products and chemicals. WWTPs either collect brown grease on site as part of routine operations (though this is becoming increasingly uncommon as brown grease can block sewage lines if it is not removed upstream at grease traps) or receive it from haulers. In some cases, the material is processed on-site via anaerobic digestion or incineration, or disposed of by delivering it to rendering plants, landfills, independent incinerators, or anaerobic digesters. The collection radius varies widely and depends largely on the size of the company and their fleet. The collection radius could be anywhere between 25 miles and less for small companies to 200–300 miles and more for larger companies. Three metro areas (New York, Los Angeles, and Chicago) are the largest sources of brown grease in the US (collectively producing about 15% of the total brown grease in the country) and thus establish the limit of economy of scale. However, these locations are also areas with more progressive thinking in terms of reuse and recycle of waste and businesses that use or recycle fats, oils, and grease have been established in those areas. Therefore, these cities may not be an appropriate place to start in terms of ready availability due to competition with existing commercial applications. Our current survey indicates that brown grease in these locations is being used by rendering companies, co-digested with sludge in anaerobic digesters, incinerated, and a tiny fraction is being used as a blendstock for biodiesel production. Brown grease in the Denver urban area and in other locations across Colorado is composted. Our survey also revealed that many other smaller cities in the country are sending brown grease to landfills and thus have inexpensive or "free" feedstock availability, however, further investigation into other geographic areas was beyond the scope of this study.

3.2. Compositional analysis of raw brown grease

The composition of the raw brown grease samples was characterized (Table 2). The total solids content in BG#1 is about 8%, while the BG#2 sample had < 1% total solids, indicating a large amount of water in both samples. Brown grease sample BG#1 was determined to contain a higher percentage of sugars and lipids than BG#2 and was thus used for fermentation and lipid extraction. The high free fatty acids content (as FAME) in both raw brown grease samples indicates a high energy potential for both samples.

The aqueous phase of the two samples were analyzed to quantify free monomeric sugar. The aqueous phase was then hydrolyzed with acid to quantify total sugar which includes oligomeric as well as monomeric sugar (Table 3). Surprisingly, most of the sugars in the aqueous phase of BG#1 sample were found to be in the monomeric form. There was no detectable sugar in BG#2, probably because the solid content in BG#2 is too low (0.82%).

3.3. Determination of optimal conditions for pretreatment and lipid extraction from raw brown grease

Pretreatment conditions with having a high severity (increased temperature and acid concentration) were needed to achieve FAME yields higher than 90% (Fig. 1) while, interestingly, a FAME yield of 92% was obtained without any pretreatment suggesting that lower severity pretreatment somehow interfered with lipid extraction. These pretreatment results are significantly different than our observations with algal biomass (Dong et al., 2016c), likely because there are no physical barriers, e.g. cell walls, to prevent the lipids from interacting with the solvent. In addition, we observed that pretreatment at lower severity caused emulsions during the extraction, indicating that partial hydrolysis released some emulsifiers that inhibited subsequent lipid extraction thus leading to low FAME yields. This situation has been observed before for wet extractions (Dong et al., 2016b). In addition, we hypothesized that a significant portion of the carbohydrates present in raw brown grease arose from starch present in the waste foods washed down the drain. As starch is more readily hydrolyzed by fermentative organisms than cellulose, hemicellulose, or algal gluco-mannans, we proposed that pretreatment (which was inhibitory for lipid extraction) would also be unnecessary for carbohydrate hydrolysis. Therefore, we concluded that there was no benefit to pretreating raw brown grease prior to lipid extraction.

Table 2
Composition of raw brown grease samples.

Sample	Solid	Ash	FAME	Protein	Total Sugars	Sum
ID	%	%	%	%	%	%
BG#1	8.2	1.5	76.3	10.6	5.3	93.6
BG#2	0.9	9.1	59.3	11.3	2.0	81.7

Total lipid in the raw BG#1 sample was recovered and analyzed by ASE followed by SPE. Lipid classes (%*w*/w) of the oil extracted from the BG#1 sample were found to be 28.7% ± 2.4% neutral lipids, 61.0% ± 4.1% free fatty acids, and 2.5% ± 0.2% polar lipids. Free fatty acid (FFA) is the major component in the total lipid, indicating a deep hydrolysis of triglycerides in the grease trap. Typically, highly efficient base catalysts are used in transesterification of triglyceride for biodiesel production. The high FFA content in raw brown grease makes it unfavorable for biodiesel production due to saponification of FFA using these typical catalysts. FFA can, however, be an acceptable feedstock for hydrotreating to produce renewable diesel blendstock (RDB) based on our results with lipids extracted from the biomass of the algal strain *Scenedesmus acutus* which also contains a high percentage of FFA (Dong et al., 2015; Kruger et al., 2017).

3.4. Fermentation

Raw BG#1 sample was used for fermentation due to its higher initial sugar concentrations and because of the determination that pretreatment offered no additional benefit to sugar hydrolysis and was detrimental to lipid extraction. The low concentration of monomeric glucose in the raw brown grease was completely utilized to below detection limits within 8 h of inoculation (Fig. 2). Total glucan utilization, which we had hypothesized was starch, in the brown grease sample was 59% from an initial value of 0.98 g/L to 0.46 g/L. Butyric acid showed minimal initial increase but then reached a final titer of > 4 g/L and was continuing to increase at the end of the fermentation. For comparison, fermentations of 5 g/L glucose or starch in lab media were run in parallel. Fermentation proceeded rapidly with all of the glucose consumed within 5 h with maximum butyric acid concentration being reached shortly thereafter at 1.8 g/L giving a yield of 0.36 g butyric acid/g glucose. Starch was 98% utilized by the end of the fermentation in the lab medium control with a final titer of 1.6 g/L butyric acid and a yield of 0.31 g butyric acid/g starch demonstrating that *C. butyricum* is proficient at hydrolyzing starch. Given the incomplete utilization of the brown grease glucan in BG#1 and the near complete utilization of starch in the lab medium control, it is likely that not all the glucose identified in the total sugar analysis in the brown grease comes from starch. Total sugar analysis of the BG#1 sample also shows low concentrations (< 0.3 g/L) of xylose, galactose, and arabinose which are typically associated with cellulosic biomass and suggest that a small proportion of the carbohydrate present in this sample comes from cellulosic biomass likely from vegetable matter that was washed down the drain and caught in the trap.

Given the butyric acid yield from starch in the lab medium control, the titer of butyric acid in the raw brown grease can only be attributed to the combined utilization of monomeric glucose (0.8 g/L), starch (0.5 g/L), glycerol (2.5 g/L), and lactic acid (3.0 g/L) (Figs. 2 and 3). The butyric acid yield for BG#1 fermentation is 0.55 g butyric acid/g

Table 3
Concentration (g/L) of soluble compounds in the raw brown grease aqueous phase.

Sample ID	Monomeric sugar		Total sugar		Lactic acid	Glycerol	Acetic acid
	Glucose	Total*	Glucose	Total*			
BG #1 Aqueous	3.05	3.38	3.19	3.66	5.88	2.9	2.05
BG #2 Aqueous	0.00	0.00	0.00	0.00	0.00	0.00	0.43

* inlcudes monomeric and oligomeric forms.

glucose, starch, glycerol, and lactic acid combined. The conversion of lactate and glycerol has been observed previously and is noted to be quite robust once typical carbon sources (e. g. glucose) are consumed (Abbad-Andaloussi et al., 1995; Abbad-Andaloussi et al., 1998; Colin et al., 2001; Detman et al., 2019; Hu et al., 2019). Yield for the glucose and starch lab media controls was 0.36 and 0.31 respectively. The titer of butyric acid was continuing to increase in the brown grease fermentation while lactic acid was decreasing at the end of the fermentation suggesting that, if continued longer, the titer of butyric acid would likely be higher. It is not clear, however, if the added expense of extended fermentation time is warranted. It is possible to use lactic acid along with butyric and acetic acid in an upgrading process for hydrocarbon production and will be explored in the future (Goulas and Toste, 2016). The titer from raw brown grease was low due to a low starting concentration of sugars. Typical titers observed from various plant biomass hydrolysate feedstocks containing much higher initial concentrations of glucose ranged from 7 to 70 g/L with yields ranging from 0.24 to 0.75 g butyric acid/g sugars, comparable to our yields from brown grease (Dwidar et al., 2012; He et al., 2005; Jha et al., 2014). Other fermentation products, acetic acid (~3 g/L) and formic acid (trace), were also produced in the fermentation of the raw BG#1 sample while only small amounts (< 1 g/L) were produced in the lab medium controls.

3.5. Lipid extraction

During the first two oil extractions of the fermented BG#1 stillage, a stable emulsion layer was formed. The pH of the fermentation broth was 8.3 which is conducive to soap formation from FFA at this pH leading to an emulsion. Thus 2.5% of sulfuric acid (*w*/w) was added to the fermentation broth to reduce the pH to 2 prior to extraction thereby preventing the FFA from forming an emulsion. The emulsion after the 3rd extraction was remarkably reduced and approximately 12% more FAME was recovered. A total FAME yield of 81.3% was obtained after three rounds of extraction. Small amounts of oil trapped in particulate matter likely adheres to the fermenter and is retained in the equipment during fermentation leading to lower FAME yield of fermentation stillage compared to extraction of un-fermented BG#1.

3.6. Residuals

Since most of the carbohydrates have been converted into butyric acid along with most of the lipids being extracted, the protein content from the original brown grease along with the remaining non-fermentable carbohydrates will be enriched in the remaining fermentation stillage. This residue can be processed by traditional anaerobic digestion to produce methane and nitrogen and phosphorous recovered for use as fertilizer.

3.7. Technical economic analysis

To understand the economic potential and feasibility of this technology, we developed a techno-economic analysis (TEA) for the process. The TEA includes a process model with mass and energy balances calculated in Aspen Plus (V7.2) (Fig. 4). Brown grease entering the facility undergoes large scale lipid extraction with the extracted lipids to be hydrotreated to fuel products. We determined that the low level of butyric acid produced by fermentation did not warrant the extra process steps and thus eliminated those steps for our model, leaving both carbohydrate and protein residuals from the extraction to enter anaerobic digestion (AD) and a combined heat and power (CHP) system. The TEA takes mass and energy balances from the process model and calculates equipment size and cost, operating expenses, and additional financial assumption for an nth plant large scale facility. In all, the TEA calculated the minimum fuel selling price (MFSP) needed to have the facility reach a net present value of 0 while also maintaining an internal rate of return of 10%.

The base facility was scaled to have one large brown grease conversion plant support a market the size of Denver estimated at 44.5 metric tons/day of brown grease. The resulting base case MFSP was

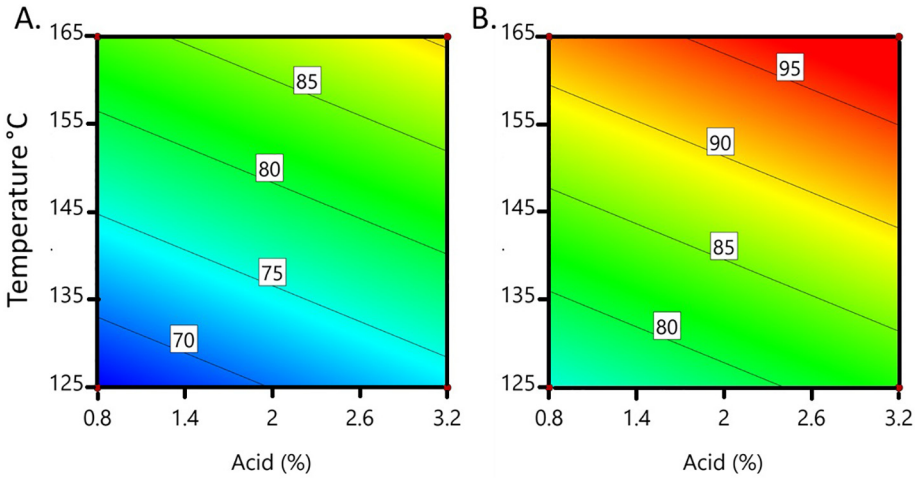

Fig. 1. Response plots of FAME yield for the raw BG#1 sample following a CCD of pretreatment conditions. Temperatures (111 °C – 179 °C) and sulfuric acid concentrations (0–4%) varied as per the CCD. A; FAME yield after a 5 min pretreatment. B; FAME yield after a 15 min pretreatment.

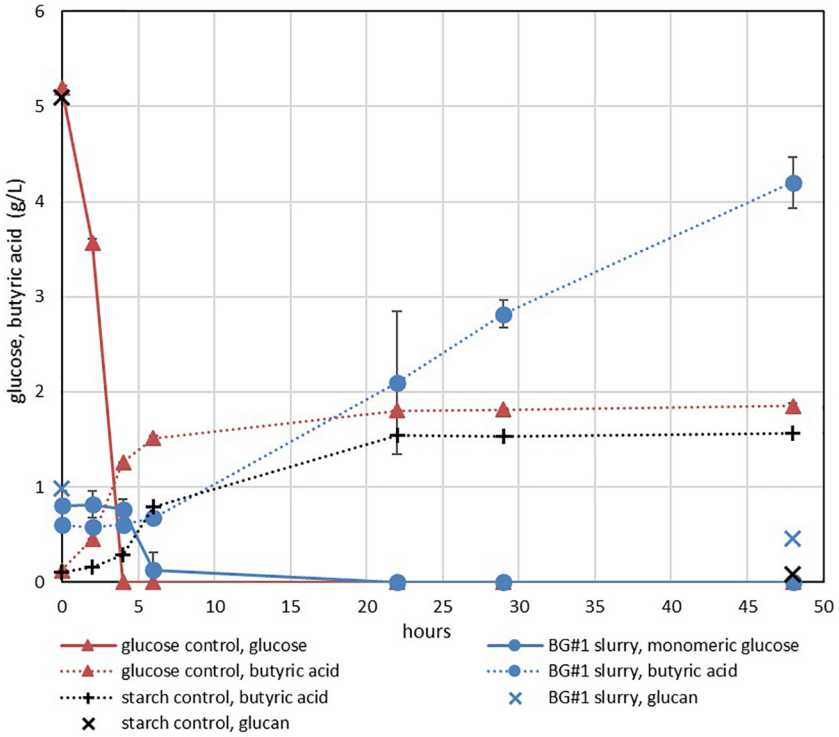

Fig. 2. Fermentation of BG#1 and lab media controls.

calculated at $1.81/GGE for a single facility handling brown grease based on a delivered cost of $100/ton. We chose to include a delivery cost for brown grease rather than assume that it would be free or even carry a negative cost as a conservative approach to help highlight the commercial potential of this process. As will be shown below, the economics can be even more compelling if brown grease can be delivered for less than $100/ton. The brown grease accounts for 29% of the total MFSP with a matching 29% contribution from hydrotreating. Lipid extraction accounts for 19% of the MFSP followed by AD (16%), and finally CHP (6%). When the feedstock price varies, the MFSP can range from $0.01/GGE (taking a credit of $240/ton by avoiding tipping fee) to $2.97/GGE based on a reported price for purified brown grease of $320/ton (Fig. 5). Again, holding the cost of brown grease constant at $11/ton, cities with a higher production of brown grease (e.g. New York) could achieve an MFSP of $1.11/GGE, whereas, in smaller cities (e.g. Virginia Beach) the MFSP will increase to $2.23/GGE in the base case.

4. Conclusion

Raw brown grease is a suitable and cost-effective feedstock for the production of a fuel intermediate. High yields of butyric acid (with small amounts of other carboxylic acids) via fermentation can be achieved. These additional carboxylic acids can be used in-situ with

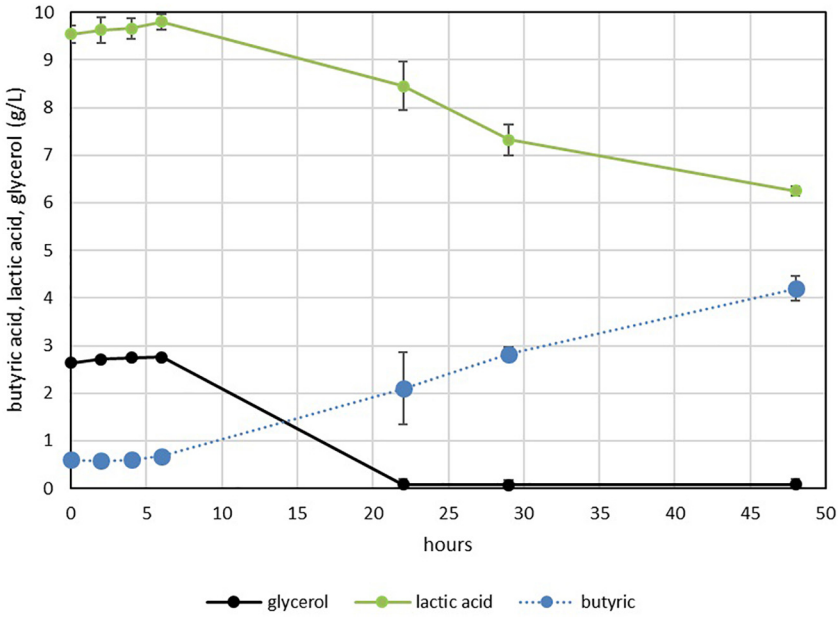

Fig. 3. Conversion of glycerol and lactic acid to butyric acid in un-pretreated BG#1.

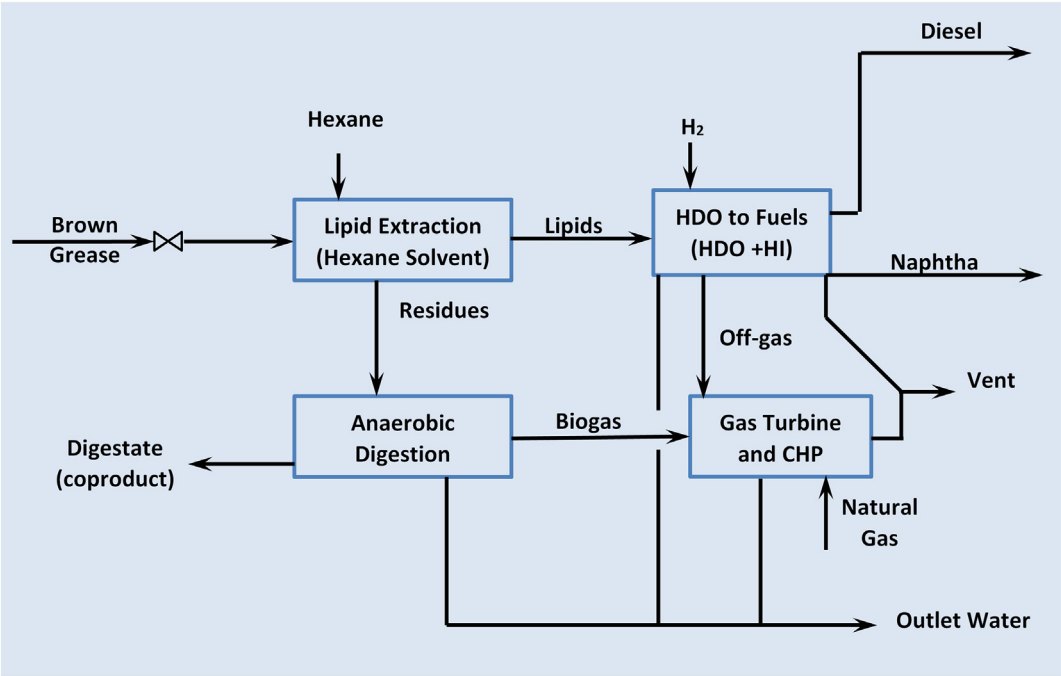

Fig. 4. ASPEN process model. Hydrodeoxygenation (HDO. Hydroisomerization (HI). Combined heat and power (CHP).

Fig. 5. Brown grease "At the Gate" MFSP sensitivity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

butyric acid for upgrading into fuels. Lipid extraction from the fermentation stillage resulted in > 80% recovery and without the fermentation step, the lipid extraction yield was 92% providing a valorization route for raw brown grease, having a modeled cost for biofuel production estimated to be $1.81/GGE for the average city producing brown grease.

Acknowledgements

The authors would like to thank David McCleskey, Transportation Manager, at Liquid Environmental Solutions, Denver, CO for the samples of raw brown grease and Madeline Lane at NREL for help in assembling the graphical abstract. This work was authored by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and Renewable Energy. The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for U.S. Government purposes.

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Tao Dong: Performed work on pretreatment of material and extraction of lipids. Wrote related sections.

Anelia Milbrandt: Performed work on logistics and availability. Wrote related sections.

Jennifer Clippinger: Performed work on TEA. Wrote related sections.

Darren Petersen: Performed work on analytical analysis.

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Declaration of competing interest

The authors declare that we have no competing interests for the work described in this manuscript.

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