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Combined algal processing: A novel integrated biorefinery process to produce algal biofuels and bioproducts

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ABSTRACT

The development of an integrated biorefinery process capable of producing multiple products is crucial for commercialization of microalgal biofuel production. Dilute acid pretreatment has been demonstrated as an efficient approach to utilize algal biomass more fully, by hydrolyzing microalgal carbohydrates into fermentable sugars, while making the lipids more extractable, and a protein fraction available for other products. Previously, we have shown that sugar-rich liquor could be separated from solid residue by solid–liquid separation (SLS) to produce ethanol via fermentation. However, process modeling has revealed that approximately 37% of the soluble sugars were lost in the solid cake after the SLS. Herein, a Combined Algal Processing (CAP) approach with a simplified configuration has been developed to improve the total energy yield. In CAP, whole algal slurry after acid pretreatment is directly used for ethanol fermentation. The ethanol and microalgal lipids can be sequentially recovered from the fermentation broth by thermal treatment and solvent extraction. Almost all the monomeric fermentable sugars can be utilized for ethanol production without compromising the lipid recovery. The techno-economic analysis (TEA) indicates that the CAP can reduce microalgal biofuel cost by $0.95 per gallon gasoline equivalent (GGE), which is a 9% reduction compared to the previous biorefinery scenario.

1. Introduction

Oleaginous microalgae are well known as promising candidates for renewable energy production mainly because of high biomass productivity and lipid content [1]. Microalgal biofuel research is generally centered on two distinct conversion pathways: algal lipid upgrading (ALU) and hydrothermal liquefaction (HTL). Both pathways target hydrocarbon fuel products [2,3,4,5,6]. It has been realized that the high cost of algal biomass is a major obstacle that impedes the commercialization of algal biofuel production [7]. Reducing the costs for algal biofuel production is a significant goal for the Department of Energy (DOE) that is outlined in the outlook presented in the multi-year program plan [8].

Currently, increases in lipid productivity, achieved primarily through improved growth rates or lipid content, are unlikely to reduce production costs to prices competitive with petroleum-based fuels. Improvements in cultivation capital expenditures (CAPEX) and in harvest costs can also help reduce costs, but further reductions in costs can be achieved by more complete utilization and valorization of all algal cellular components rather than relying solely on the lipid fraction [2,7]. Likewise efficient conversion and upgrading for all of the algal components into fuels and value-added chemicals can significantly reduce both production costs for algal biofuels and risks to stakeholders.

By applying mild processing conditions, microalgal biomass can be fractionated into three major streams: lipid, carbohydrate and protein, which can be converted into respective (co-)products with added-value. Previously, we successfully demonstrated an acid-based fractionation process for algal biomass in a Parallel Algal Processing (PAP) schematic [2] (and Fig. 1a). We previously demonstrated that dilute acid pretreatment can effectively hydrolyze algal structural and storage polysaccharides to release monomeric sugars (primarily glucose and mannose) into an aqueous stream, which was separated from solid residue (rich in lipids and protein) by solid/liquid separation (SLS). The sugars released in the liquor phase could be fermented to ethanol (or higher value co-products), while lipids could be recovered from the solid fraction using hexane extraction leaving a residue stream enriched in protein. The value of the sugar and lipid streams has been previously calculated based on sugar fermentation to ethanol and hydrodeoxygenation of lipids to a renewable diesel blendstock [2]. The value of amino acids in the protein stream can be valorized by conversion to branch-chained higher alcohols [9], anaerobic digestion [10], or other applications [11]. These cellular components can be upgraded into fit-for-use hydrocarbon based fuels and value-added co-products.

In the previous processing design approach [5], it has been indicated through process modeling that the liquor entrapped in the solid cake after SLS contains a considerable amount of sugars that cannot be

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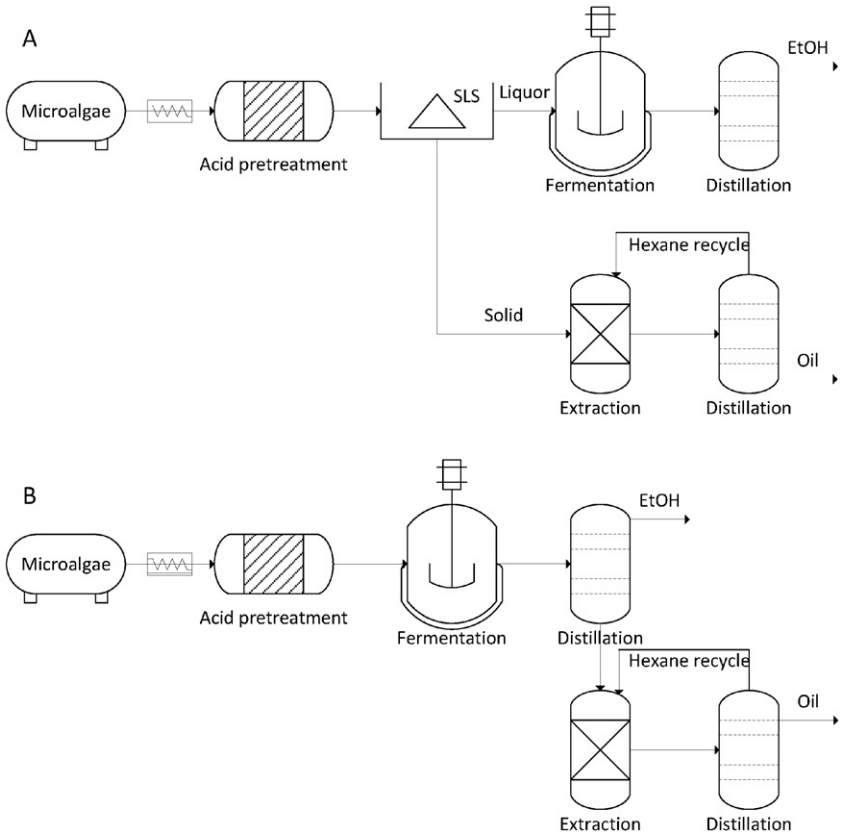

Fig. 1. Process flow diagrams for Parallel Algal Processing (PAP) and Combined Algal Processing (CAP) (A): PAP and (B): CAP.

used for fermentation without a costly washing step, resulting in a loss of overall fuel yield. To fully recover the hydrolyzed sugar for fermentation, a cost-effective approach is needed to improve the valorization of the carbohydrate stream to further reduce the cost of microalgal biofuel production.

The objective of this work is to investigate experimentally the process yields and cost impact of integrating a fermentation approach with subsequent lipid extraction using a Combined Algal Processing (CAP) configuration (Fig. 1b). In the dry-grind corn ethanol industry, whole grains are ground, starch is hydrolyzed enzymatically, and the resulting slurry is used as a feedstock for ethanol fermentation. Ethanol is distilled from the fermentation beer which contains oil and other nonvolatile residues. Then, oil can be recovered from the stillage [12,13]. This process was rapidly adopted by a significant number of corn biorefineries, providing inspiration for us to modify our benchmark PAP scheme. In the integrated CAP configuration, by skipping the SLS unit, the whole slurry will be directly used for fermentation, after which ethanol and lipids can be recovered sequentially from the post-fermented broth. Our hypothesis is that the ethanol yield could be significantly improved by simplifying the processing. In addition, the capital and operating costs for SLS can also be avoided to reduce overall fuel cost. Our goal is to demonstrate that the highly integrated CAP configuration will lead to a higher total energy yield to fuels and a lower cost for algal biofuel production.

2. Materials and methods

2.1. Materials

Scenedesmus acutus (LRB-AP 0401) biomass was provided by Dr. J. McGowen at the Arizona State University. In brief, biomass was obtained in a controlled fashion in outdoor flat panel (650 L) photobioreactors in nitrate deplete cultivation media. Cultivation time after reaching nutrient deplete conditions depended on final target biomass composition desired, which, depending on the season, typically was 6 to 9 days for lipid accumulation under nutrient deplete conditions to reach the targets of ~40% each of carbohydrate and lipid content (batch number B.0401_1102012PBR2, 4–8 and B.0401_10242012_PBR3, Harvest#75). Harvesting the biomass was accomplished using centrifugation (Alfa Laval, Lund, Sweden) and the material was frozen until needed [14].

2.2. Biomass pretreatment

Pretreatment of the microalgal biomass was performed in a batch-type reactor, a 4-L (2-L working volume) ZipperClave® (ZC) reactor (Autoclave Engineers) previously described [2,15,16]. Steam was directly injected into the bottom of the reactor through ports in a rotary-plow type agitator and constant temperature was achieved by controlling the steam pressure in the reactor. The ZC reactor is also equipped with an electrical heating blanket set at reaction temperature to lessen steam condensation due to heat losses through the reactor wall. The contents within the ZC reactor typically reached reaction temperature within 5 to 10 s of starting the steam flow as measured by two thermocouples, one inserted into the bottom and one near the middle of the reactor. At the end of pretreatment, the steam pressure was slowly released through a condenser over a period of 15 to 30 s to eliminate boil-over while still allowing for steam escape to reduce slurry dilution by condensate. The ZC is able to pretreat biomass at high solid concentrations using direct steam injection for rapid heating and mixing, which are all important process parameters for an economical commercial reactor.

A total solid content of starting biomass paste was determined at 105 °C using a Mettler-Toledo SP precision infrared balance (Columbus, OH). Wet algal paste (300 g), H_2SO_4 and water were sequentially added to the sample canister achieving a final solids loading of 25% (*w*/*w*) and

an acid concentration of 2% (*w*/*w*). All samples were pretreated at 155 °C for 15 min [2]. At the end of the reaction the sample canister was removed from the ZipperClave and cooled in ice water. A set of 10 identical pretreatment reactions was conducted to provide sufficient substrate for the fermentation experiments.

The pretreated algal slurry (PAS) was combined and refrigerated until needed. Glucose and mannose yields were determined by subtracting the weight of fraction insoluble solids (FIS) [17] from the slurry weight and converting mass of the remaining liquor into volume using a density conversion. Fermentable monosaccharides in the hydrolysate liquor were analyzed by HPLC. Oligomeric sugars were determined using a second acid hydrolysis as described previously [18]. Mass of sugar was determined by multiplying concentration by liquor volume, and normalized against the initial mass, as previously described [15].

The PAS was split into two fractions. The first fraction was centrifuged using a Q-20 Western Centrifuge (Ill.) equipped with a 30-micron basket inserted inside the centrifuge to separate hydrolysate liquor from pelleted solid fraction at 8437 *g* for 20 min. The liquor collected after centrifugation was neutralized to pH 5.2 using NaOH prior to fermentation via PAP (Fig. 1a). The second fraction of PAS without SLS representing the CAP was neutralized to pH 5.2 using NaOH prior to fermentation (Fig. 1b).

2.3. Fermentation

Fermentations were done in shake flasks or fermenters. In both cases, an overnight seed culture of *Saccharomyces cerevisiae* D5A [19] was grown in 200 mL YPD (1% yeast extract, 2% peptone, 20 g/L glucose) in a 500 mL baffled flask at 37 °C, 225 rpm from a 1:10 dilution from a fresh culture grown overnight the previous night. Cells were harvested and suspended at cell density of approximately 40 g/L. Shake flasks and fermenters were inoculated at an initial $OD_{600} = 1$.

For triplicate shake flask experiments, 46.5 mL of neutralized algal pretreated liquor or PAS was either supplemented with 0.25 g/L yeast extract and 0.5 g/L peptone (YP) termed as YP+, or with 2 of the same volume of water (YP−) in a 125 mL baffled flask. Flasks were inoculated with cells and water bringing the final volume to 50 mL, capped with a water trap, and incubated at 37 °C and 150 rpm. Samples were taken periodically for HPLC to track sugar consumption and ethanol formation.

For triplicate fermenter (Biostat Q+, 500 mL vessel, Sartorius) experiments, 300 mL of neutralized PAS supplemented with YP (YP+) or water(YP−), and a pure sugar control media, YPDM (1% yeast extract, 2% peptone, 40 g/L glucose, and 20 g/L mannose), were used. Fermenters were controlled at 37 °C and pH 5. Samples were taken for HPLC analysis for sugar consumption and ethanol formation during the fermentation. For both flask and fermenter experiments, the fermentation broth was recovered and ethanol was removed as described below.

2.4. Ethanol removal

Ethanol was removed by heating the fermentation broth using operating conditions established for the NREL biorefinery distillation column to ensure that this process step would not impact lipid recovery or quality. Conditions for ethanol removal were established to approximate the residence time based on the size and number of individual distillation trays and flow rate of beer, of 228 L/min into the column, which translated into 85 °C to 90 °C for 5 min. The fermentation broth containing the residual solids was heated in laboratory water bath to 85 °C and held for 5 min. The samples were cooled to room temperature and the weights were taken to ensure ethanol removal. The fermentation broth after ethanol removal is termed as stillage.

2.5. Lipid extraction

After ethanol removal, an additional 0.6% (*w*/*w*) H_2SO_4 was added to the stillage for better free fatty acid (FFA) extraction. Hexane (equal volume to the stillage at a solid content of 17%) was added in each flask for extraction on a multi position magnetic stir plate (Velp, Bohemia, NY, US) overnight. Then, the stillage & solvent mixture was transferred to centrifuge tubes for phase separation at 2000 *g* for 10 min. The hexane phase was collected in a pre-weighed glass tube and evaporated in a TurboVap concentration workstation (Caliper Life Sciences, East Lyme, CT) at 40 °C. Afterwards, the glass tubes containing crude lipid fraction were placed in a vacuum oven at 40 °C overnight to evaporate the residual volatile solvent. The stillage after lipid extraction is termed extracted stillage. The FAME content of the extracted lipid stream and extracted stillage was measured as described above. The FAME recovery (as a proxy for extractable lipids) was calculated based on the baseline-measured FAME content of the starting material.

2.6. Analytical

2.6.1. Moisture and ash analysis

Crucibles were preconditioned in the 575 °C muffle furnace overnight to remove any combustible contaminants. Once the crucibles came to room temperature, their weights were recorded. In each crucible 50 ± 2.5 mg of dried algae was added and the weight of each sample was recorded. Biomass samples were then placed in a 40 °C vacuum oven overnight to remove moisture and the oven dry weight of the sample was recorded the next day once the samples had come to room temperature. Finally the samples were placed in the ramping 575 °C oven overnight. The weight of the ash content was calculated from a final weight [20].

2.6.2. Carbohydrate analysis

Lyophilized biomass (25 mg) and 250 μL of 72% (*w*/*w*) sulfuric acid were added into a 10 mL glass tube. The first step hydrolysis was performed in 30 °C water bath for 1 h. Then, 7 mL of 18.2 MΩ water was added to the tube. The tube was sealed and autoclaved for 1 h at 121 °C. The tube was allowed to cool down to room temperature and an aliquot of sample was neutralized to pH 6–8 using calcium carbonate. The neutralized sample was filtered through a 0.2 μm nylon membrane filter for HPLC analysis [21]. The sugar analysis was performed on an Agilent high performance liquid chromatography (HPLC) equipped with Shodex SP8010 column and RID detector. The column was kept at 85 °C and the detector was set at 55 °C. Flow rate was 0.6 mL/min with water as mobile phase. Injection volume was 50 μL and the run time was 42 min. The carbohydrate composition was tested with a calibration range of 0.05 g/L to 6 g/L for cellobiose, glucose, xylose, galactose, arabinose, and mannose [21].

To quantify glucose and mannose at the start and end of fermentation, samples were filtered through a 0.2 μm nylon filter, diluted as necessary, and run by high performance anion exchange (HPAE) on a Thermo Scientific Dionex ICS 5000 system equipped with pulsed amperometric detection (PAD). A Dionex CarboPac PA20 column preceded by a Dionex AminoTrap was run at 0.5 mL/min and 35 °C for both column and detector compartments using the quadruple waveform recommended by Dionex for carbohydrate detection. Samples were injected at 10 μl and an eluent of 27.5 mM sodium hydroxide was used to separate the monosaccharides followed by a gradient from 2–17% of 1 M sodium acetate and 100 mM sodium hydroxide.

2.6.3. Ethanol analysis

To quantify ethanol produced throughout fermentation, an aliquot of each sample was filtered through a 0.2 μm nylon filter and run by high performance liquid chromatography (HPLC) on an Agilent HPLC equipped with a Biorad Aminex HPX-87H column (55–65 °C) and a

refractive index detector with a 0.01 N sulfuric acid mobile phase at 0.6 mL/min.

2.6.4. FAME analysis

Lipid content in biomass was measured as total fatty acid methyl ester (FAME) content after a whole biomass *in situ* transesterification procedure, optimized for microalgae [22]. A total of 7 to 10 mg of lyophilized biomass was transesterified with the presence of 0.2 mL of chloroform/methanol (2:1, *v*/*v*) and 0.3 mL of HCl/methanol (5%, *v*/*v*) for 1 h at 85 °C with a known amount of tridecanoic acid (C13) methyl ester as an internal standard. FAMEs were extracted with hexane (1 mL) at room temperature for 1 h and analyzed by gas chromatography–flame ionization detection (GC–FID) on an Agilent 7890 N; DBWax-MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Quantification of the FAMEs was based on integration of individual fatty acid peaks in the chromatograms and quantified using a 5-point calibration curve (0.5–2 mg mL^{-1}). The individual FAME concentrations were normalized against the internal standard tridecanoic acid methyl ester.

2.6.5. Protein analysis

Nitrogen content in dry biomass was determined by an external laboratory (Hazen Labs, Golden, CO) on a Flash EA 1112 Series Thermo Analyzer using the classical Dumas method, with thermal conductivity detection (TCD). The method is described in ASTM D5373 (coal) and ASTM D5291 (petroleum products). Weighed samples were combusted in oxygen at 1000 °C. The combustion products (including N and NOx) were swept by a helium carrier gas through combustion catalysts, scrubbers and through a tube filled with reduced copper. The copper removes excess oxygen and reduces the NO_x to N_2. The N_2 was then separated from other gases on a chromatography column and measured with a TCD. A nitrogen-to-protein conversion factor of 4.78 was used to estimate the protein content in microalgal biomass [23].

2.7. Calculation of theoretical conversion yields

Theoretical yields were calculated as described previously [2] assuming conversion of all fermentable sugars with a 51% theoretical ethanol fermentation yield (i.e. metabolic yield) from glucose [24] and conversion of total fatty acid content of the biomass to hydrocarbon-based renewable diesel at a 78 wt.% renewable diesel yield from total fatty acids (based on previously documented assumptions for lipid hydrotreating with high selectivity to diesel) [25].

Table 1
Composition and theoretical fuel potential of *S. acutus* biomass (all data shown as % DW) used for pretreatment and fermentation experiments.

Composition and calculated theoretical fuel equivalent (BTU and GGE/ton) in *S. acutus* biomass	
Total carbohydrates (% DW)	39
Glucose (% DW)	30
Mannose (% DW)	8
Galactose (% DW)	1
Ethanol (% DW)[a]	20
Ethanol (gallon/ton)	59
Gasoline equivalent (gallon/ton)[b]	39
Btu equivalent ($\times 10^3$)	4520
Fatty acids (FAME) (% DW)	41
Hydrocarbon (% DW)[c]	32
Diesel equivalent (gallon/ton)	99
Btu equivalent ($\times 10^3$)	12,180
Total fuel energy ($\times 10^3$ Btu)	16,700
Total gasoline gallon equivalent (GGE/short ton)	144
Protein (% DW)	8
Ash (% DW)	2

All the composition analyses were done in triplicate.
[a] 51% glucose-to-ethanol conversion (theoretical).
[b] 65.8% ethanol-to-gasoline conversion.
[c] 78% FAME-to-hydrocarbon conversion (theoretical).

Table 2
Glucose and mannose yields after acid pretreatment.

Sugar format	Glucose		Mannose	
	Yield %	Conc. g/g biomass	Yield %	Conc. g/g biomass
Monomeric	73.8 ± 0.9	0.22	80.0 ± 2.9	0.06
Total	78.9 ± 1.2	0.24	86.4 ± 2.4	0.07

Total = monomeric + oligomeric (n = 8 replicates).

2.8. Techno-economic analysis (TEA)

TEA modeling was conducted on the system, for both a process based on the PAP and CAP processing configurations. The TEA modeling methodologies and assumptions were based on details documented in prior work [5], which was focused on a CAP processing scheme for a hypothetical biorefinery facility processing an annual average feed rate of 1339 ton/day AFDW algal biomass at an incoming solid content of 20 wt.% (e.g. the model assumes a given algal biomass feedstock price which accounts for upstream cultivation and dewatering costs to harvest and concentrate the biomass up to 20 wt.% AFDW). Based on modeled material and energy flows for the integrated conversion system, capital and operating cost estimates are generated which are then utilized in a cash flow analysis to project the minimum fuel selling price (MFSP) required to achieve a 10% internal rate of return (IRR) for an "n^{th} plant" scenario, i.e. assuming a mature technology in which a sufficient number of biorefinery facilities have been built and operated to avoid cost over-runs, risk financing, unplanned downtime (beyond 35 days/year as assumed in the base case), etc. that would be typical for pioneer plant facilities.

The primary difference for the present TEA model considered here is that it makes use of current baseline experimental data presented in this paper, rather than future target projections as was the focus of the previously-cited work [5]. Additionally, it adds an operation for the PAP configuration, namely a centrifuge for solid–liquid separation, following the acid pretreatment step. The centrifuge is operated in a manner consistent with assumptions utilized in prior TEA modeling for the PAP approach, i.e. for modeling work presented in [2]; this includes a solid bowl centrifuge which recovers 99% of the insoluble solids (IS) at 30 wt.% IS concentration into the solid product phase, which incurs a loss of roughly 35% of the solubilized sugars, carried over into the solid phase. Aside from the inclusion of solid/liquid separation and the additional penalties incurred on yields (primarily sugar losses) and costs (capital expenses and power demand for the centrifuge), all other parameters were fixed in the PAP model consistent with the CAP scheme, including operating conditions and fractional conversions across each processing step (discussed in Section 3.6).

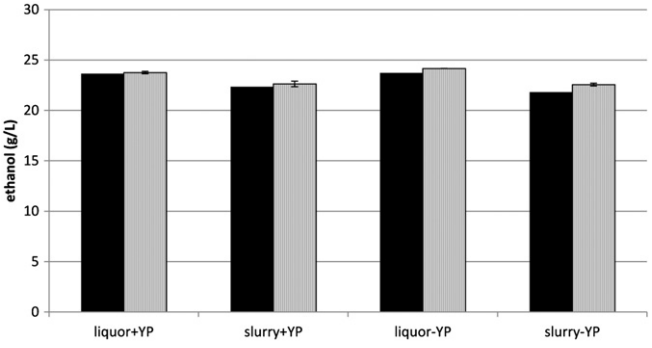

Fig. 2. Ethanol formation after 18 h (black bars) and 24 h (gray bars) in shake flasks by *S. cerevisiae* fermenting pretreated algal liquor or slurry with or without YP supplementation. Error bars are one sample standard deviation and all experiments were performed in triplicate.

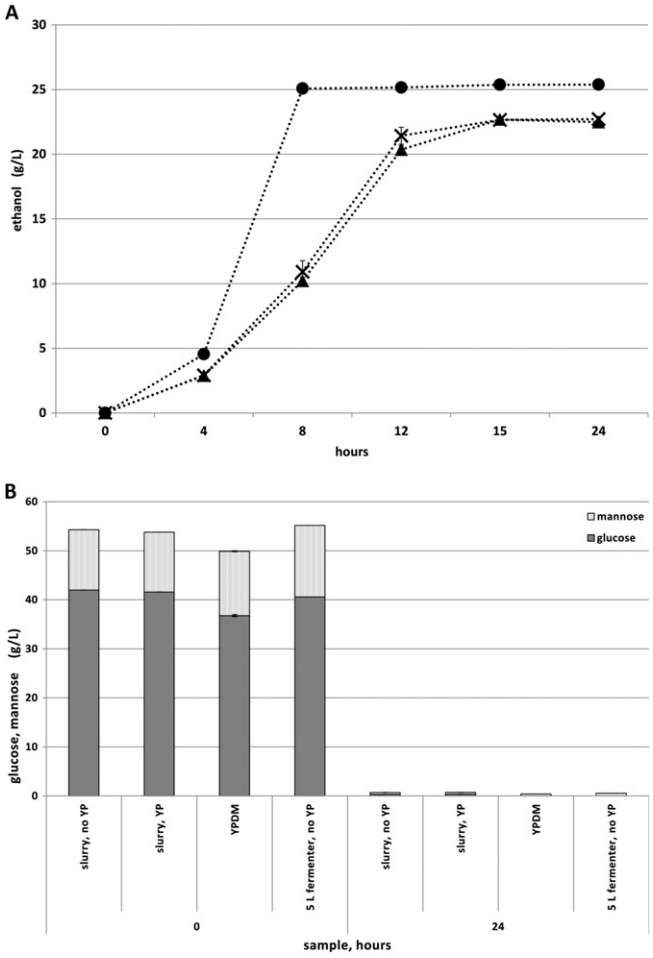

Fig. 3. Ethanol production and sugar utilization on PAS and YPDM (control) in fermenters. A. Ethanol production: PAS without YP addition, triangles. PAS with YP addition, X. YPDM, circles. B. Sugar utilization at 0 h and after 24 h of fermentation: Glucose, gray bar. Mannose vertical striped bar. Error bars are one sample standard deviation and all experiments were performed in triplicate.

3. Results and discussion

3.1. Compositional analysis of the biomass

Composition of the *S. acutus* biomass used in these experiments is shown in Table 1. The results reflect the typical compositional profile after an extended cultivation period under nitrogen-depleted conditions, resulting in the accumulation of both algal lipids and carbohydrates. The lipid content (measured as total FAME) was over 41% consistent with biomass of high lipid-based biofuel potential. In addition, the fermentable sugars, glucose and mannose made up over 38% of dry cell weight (DCW) of the biomass. The low protein content in this batch of biomass is typical of algal biomass cultivated under the nitrogen-deplete condition [2].

Theoretical conversion of carbohydrates and fatty acid to fuels was calculated based on the composition data. The fuel yields are presented on a BTU energy basis and then converted to gallon gasoline equivalent (GGE) per ton dry biomass, which can be considered a benchmark fuel yield unit [2,26]. The theoretical ethanol and hydrocarbon yields (Table 1) were calculated based on literature conversion factors of 51 wt.% (glucose-to-ethanol metabolic limit) and 78 wt.% (FAME-to-hydrocarbon), respectively [24,25]. A 100% extraction and conversion efficiency is assumed for the theoretical conversion projection. The *S. acutus* biomass was determined to have the potential to support 144 GGE per dry ton based on both sugar-derived ethanol and lipid-derived renewable diesel (Table 1). Notably, the fuel potential derived from fermentable sugar is 37% of that calculated from lipid, indicating a considerable amount of fuel energy can be recovered by utilizing the carbohydrate stream. Based on our earlier PAP scheme utilizing a solid–liquid separation (SLS) step to fractionate liquor from solid residue after pretreatment, about 35% of the hydrolyzed sugar would be carried over into the solid fraction given the feed and product solid concentrations around the centrifuge, leading to a reduction in achievable energy yield per unit biomass. Thus, a processing approach in which the total amount of sugar is utilized during fermentation is favored.

3.2. Sugar yield after dilute acid pretreatment

Results from the ZC pretreatment of *S. acutus* biomass are shown in Table 2. Samples were analyzed from 8 to 10 runs used to produce biomass for fermentation and lipid recovery. The average variation between samples was 0.9% and 1.2%, for monomeric and total (oligomeric + monomeric) sugars respectively. The concentration of degradation products and potential fermentation inhibitors HMF and furfural was measured as ranging from 1.15 to 1.24 g/L and 0 to 0.26 g/L, respectively. The concentration of total solids (insoluble and soluble) in the pretreated slurry was 16.1%, and the soluble solid content in the pretreated liquors was 11.4%.

The total sugar (oligomeric and monomeric) yield of both mannose and glucose approach 80%. Over 6.4% and 7.4% of the total glucose and mannose was in the oligomeric form representing a significant contribution of the overall sugar stream. The presence of oligomeric sugars suggests that the pretreatment severity, a combination of time and temperature, was not sufficient to completely hydrolyze the algal sugars into a monomeric form. On the other hand increasing the severity may also increase the concentration of degradation products, which are currently minimal. Oligomeric sugars would require additional hydrolysis using either enzymatic or thermochemical means to release monomeric sugars [27], though the increased yield would come at additional cost.

3.3. Fermentation

Shake flask fermentations of either the liquor or slurry proceeded rapidly. Glucose was not detected in the fermentation broth after 18 h (mannose was not followed) and ethanol production (Fig. 2) had

Table 3
FAME purity and recovery after fermentation with ethanol recovery by rotary evaporation or by using thermal treatment.

Process	Fermentation condition	EtOH removal	FAME purity[a] (%)	FAME recovery (%)
PAP	Lipid extraction prior to fermentation	–	97.8 ± 0.5	84.9 ± 1.6
CAP	PAS + YP (flask)	Rotovap	100.3 ± 0.8	84.3 ± 2.7
CAP	PAS + YP (flask)	Thermal treatment	95.1 ± 1.1	82.5 ± 0.7
CAP	PAS − YP (flask)	Thermal treatment	95.7 ± 0.5	82.2 ± 2.3
CAP	PAS + YP (fermenter)	Thermal treatment	98.8 ± 0.5	86.4 ± 2.6
CAP	PAS − YP (fermenter)	Thermal treatment	98.5 ± 1.2	87.0 ± 2.6

All experiments were performed in triplicate.
[a] Theoretical mass of fatty acids recovered in extract divided by measured mass of extract.

reached a maximum. The results with fermentations run without yeast extract and peptone supported the hypothesis that algal hydrolysate contains sufficient nutrients to support ethanol production.

Fermentation of neutralized PAS in fermenters proceeded rapidly and 97% and 98.5% of the glucose was gone in 12 h for the YP + and YP − slurry respectively, confirming our hypothesis that the algal hydrolysate can provide enough nutrients for effective fermentation (Fig. 3). Glucose was completely utilized in the clean sugar control, YPDM, within 8 h. The other major sugar present in the PAS, mannose, was also completely utilized (Fig. 3). Up to 22.7 g/L ethanol was produced during the fermentation in the pretreated slurry (Fig. 3). Overall, fermentation of the PAS is a rapid and robust process that does not require any additional nutrients for *S. cerevisiae* as it performed nearly as rapidly and with nearly the same ethanol productivity even in fermentations without added yeast extract and peptone.

3.4. Lipid yield after fermentation and ethanol removal

The lipid content in our *S. acutus* biomass was 41 wt.% DW, determined by FAME analysis (Table 1). The recovery of lipid shown in Table 3 represents the percentage of the initial lipid recovered from pretreated solids from the PAP scheme and from the stillage of the CAP scheme. Previously we reported lipid extractability of 77–90% for *S. acutus* having various compositional profiles under similar pretreatment conditions [2]. The recovery of lipids from CAP is in the range of 82–87% after fermentation and ethanol removal, indicating that the initial fermentation of soluble sugars in the pretreated slurry does not adversely affect lipid recovery. There is also no significant impact of the thermal treatment used for ethanol removal compared to rotovapping with respect to lipid recovery ($p > 0.05$). As shown in Table 3, the FAME concentration of extracted oil (though dark green in color) is very high, and therefore the extracted oil is expected to be an excellent feedstock for catalytic upgrading to produce hydrocarbon fuel or biodiesel.

According to previously documented TEA assumptions for extracted lipid processing, the extracted crude oil would be processed through a series of purification steps consisting of degumming, demetallization, and bleaching to remove phospholipids, metals, salts, and other impurities [5]. To determine the concentrations of impurities, the elemental analysis of the crude oil extracted from PAP and CAP approaches was conducted and the results are summarized in Table 4. The elements tested in this study are generally believed to be the most problematic for hydrodeoxygenation catalysts. It was found that most of these elements were under the detection limit, indicating that a demetallization process may be unnecessary. The phospholipid in the extracted oil is also very low as shown by the undetectably low level of phosphorus. This suggests that a degumming process can likely be skipped. It is speculated that the acid pretreatment could assist the hydrolysis of phospholipids to produce free fatty acids (FFAs) which are a preferred feedstock for hydrodeoxygenation. Alternatively, the acid pretreatment process is capable of removing phospholipids because this process is similar to the acidic degumming process applied in the vegetable oil industry [28].

Table 4
Elemental analysis of oil extracted using different approaches.

Elements (μg/g)	Oil extracted in PAP	Oil extracted in CAP
TX[a]	16	34
Calcium	1	2
Iron	<1	2
Magnesium	<1	<1
Phosphorus	2	6
Potassium	<1	<1
Sodium	9	2
Sulfur	47	63
Nitrogen	317	286

Nitrogen was detected by ASTM D4629. Other elements were detected by ICP.
[a] Total halogens (Cl + Br + I) as equivalent chlorine.

Table 5
FAME and sugar distributions in different fractions.

Original biomass		Liquor	Extracted oil	Extracted stillage		Mass balance	
FAME	Sugar[a]	Sugar[b]	FAME	FAME	Sugar[c]	FAME	Sugar
g/g biomass		g/g biomass	g/g biomass	g/g biomass		%	
0.411	0.382	0.280	0.358	0.024	0.092	92.8	97.5

FAME or sugar contents were expressed as g FAME or sugar/g original biomass DCW.
[a] Total glucose and mannose in original biomass.
[b] Monomeric glucose and mannose in the liquor after acid pretreatment.
[c] Total glucose and mannose in extracted stillage.

The sulfur and nitrogen content are relatively high probably due to carryover of these components from protein, chlorophyll [29], and other sulfur and nitrogen containing compounds native in the biomass or derived from the process. The need for additional cleanup procedures to remove nitrogen and sulfur from the crude extracted oil to facilitate catalytic upgrading is under investigation.

3.5. Composition of the extracted stillage

The extracted stillage residue was also analyzed to identify unutilized carbohydrates (Table 5) and unextracted lipid (Tables 5 and 6). Since no monomeric sugars were detectable in the fermentation beer at the end point of fermentation, the sugar identified in the extracted stillage must be either in an oligomeric form or associated with the solid residue. As shown in Table 5, about 22% of total glucose and 33% of total mannose were quantified in the post-extracted stillage residue. A total mass balance of 97.5% was obtained for fermentable sugars. This result is consistent with our finding (Table 2) that not all of the sugars were hydrolyzed under current pretreatment conditions. The optimization of pretreatment conditions is under investigation as is an evaluation of the costs and benefits of enzymatic hydrolysis.

About 5.8% of total FAME in the original biomass ended in the extracted stillage, leading to a total lipid mass balance of 92.8% for FAME (Table 5). After the acid pretreatment, a number of intercellular oil droplets are exposed and tend to adhere to the reactors and fermenters, resulting in oil loss during the operations. The oil loss is speculated to be reduced in a continuous operation, which will be a future target of process optimization.

As shown in Table 6, the fatty acid profile of lipids in the original biomass, extracted oil and extracted stillage residue is very similar. In a previous report, we speculated that the incomplete extraction of lipids might be ascribed to the polar lipids [2]. The current results indicate that this might not be the case due to the similar fatty acid profiles in extracted oil and the oil remaining in the extracted stillage. The physical entrapment and chemical interaction of lipids and cell wall residue might contribute to the incomplete extraction. These hypotheses are currently being investigated.

Table 6
Fatty acids profile in biomass, crude extracted oil and extracted stillage.

Fatty acid[a]	Biomass	Extracted crude oil		Extracted stillage	
		YP −	YP +	YP −	YP +
C16:0	12.5	12.6	12.6	13.1	13.2
C16:1n9	4.9	4.9	4.9	5.1	5.0
C16:2	3.2	3.2	3.2	3.1	3.1
C16:3	2.4	2.4	2.4	2.4	2.4
C16:4	2.1	2.2	2.1	2.1	2.1
C18:0	3.4	3.5	3.5	3.6	3.6
C18:1n9	52.2	51.9	51.9	52.1	51.9
C18:1n7	0.6	0.6	0.6	0.6	0.6
C18:2n6	6.4	6.5	6.5	6.2	6.2
C18:3n3	9.9	10.2	10.2	9.8	9.9
C20:1	1.0	1.0	1.0	1.0	1.0

[a] Fatty acids with relative content lower than 0.5% are not shown.

Table 7
Process conditions, conversion, and fuel yields obtained by PAP and CAP process models.

	PAP	CAP
Pretreatment		
Solids loading (wt.%)	20%[a]	
Acid loading (wt.% vs feed liquor)	2%	
Fermentable sugar release	74%	
Carbs to degradation products	1.5%	
Hydrolysate solid–liquid separation	Yes	No
Sugar loss (modeled)	37%	0%
Lipid loss (modeled)	1%	0%
Fermentation		
Fermentation batch time (hr)	18	
Sugar diversion to organism seed growth	6%[b]	
Total utilization of sugars for ethanol production	98.5%[c]	
Lipid extraction + upgrading		
Solvent loading (solvent/dry biomass ratio, wt)	5.9	
Total convertible lipid extraction yield	87%	
Polar lipid impurity partition to extract	<11.5%	
Hydrotreating RDB yield (wt.% of oil feed)	80%[b]	
Hydrotreating H_2 consumption (wt.% of oil feed)	1.7%[b]	
Fuel yields		
Renewable diesel blendstock (RDB, % of biomass DW)	29.0	29.2
Renewable diesel blendstock fuel yield (GGE/ton)	94.9	95.5
Ethanol (% of biomass DW)	9.4	14.9
Ethanol fuel yield (GGE/ton)	19.5	30.9
Total gasoline equivalent fuel yield (GGE/ton)	114.4	126.3

[a] Experimental work based on 25% solids, adjusted here to 20% solids for consistency with previously published modeling framework [5]; pretreatment performance is expected to remain unchanged at this value (prior unpublished data).
[b] Values were not determined here as part of the scope of experimental work; set consistent with previously documented models [5].
[c] Does not include sugar diversions to biomass growth and contamination by-products assumed in the model; after accounting for these additional factors, overall utilization of available sugars to ethanol is 89.5%.

In addition, as shown in Table 6, the polyunsaturated fatty acid (PUFA) content was not changed during the process, suggesting that value-added co-products like omega-3 fatty acids and epoxy products with high oxirane number [31,32] might be produced via the CAP. This is particularly important because a number of microalgae strains could accumulate considerable amounts of PUFA along with fuel range fatty acids [32–34]. The CAP preserves the PUFA and makes it possible to utilize these high-value PUFAs to further reduce the cost of biofuel production and replace petroleum products [35].

3.6. Techno-economic analysis

The experimental data presented above were incorporated into the TEA model for the CAP processing schematic, with the key parameters relevant to the model shown in Table 7 along with resulting fuel yields predicted from the model. For the PAP comparison, beyond the addition of the solid/liquid separation centrifuge and resulting penalty incurred on sugar losses (37% into the solid phase) and lipid losses (1% fixed in the biomass solids into the liquor phase), all other process assumptions pertaining to fractional conversions across each processing step were maintained consistently with the CAP process; this includes 2 wt.% acid loading in the pretreatment reactor, 74% solubilization of fermentable carbohydrates to sugars, 98% conversion of available sugars across the fermentation step in less than 18 h, and 87% extraction of FAME lipids at a total solvent loading of 5.9 kg hexane/kg dry biomass solids delivered to extraction (Table 7). While less sugar and marginally fewer lipids are available for fermentation and extraction respectively under the PAP schematic, it is not expected that conversion performance of those constituents that reach the respective fermentation/extraction operations would differ between the two approaches. This is partially supported for the extraction step based on the data presented in Table 3, which did not show lipid (FAME) recoveries being statistically different between the PAP basis and most CAP cases evaluated.

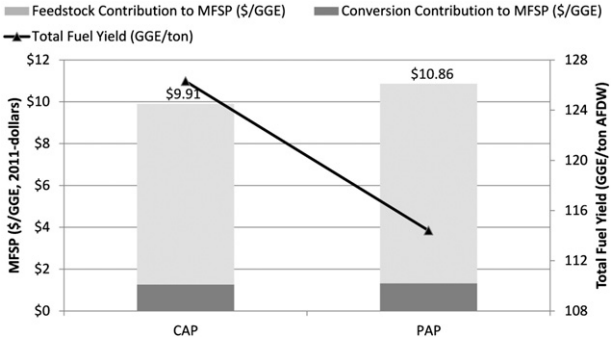

Fig. 4. Summary of yield and cost results from TEA modeling for CAP and PAP processes.

The resulting fuel yield for the integrated Aspen process model is estimated at 126 GGE/ton (AFDW) for the CAP process, roughly 88% of the theoretical maximum yield presented in Table 1. This is encouraging for a concept that may still be viewed in early stages of development, and is significantly higher than demonstrated yields from terrestrial feedstock pathways for hydrocarbon fuel production (ranging from 44 to 87 GGE/ton for biochemical and thermochemical conversion pathways from herbaceous and woody feedstocks [8]). It also exceeds currently demonstrated fuel yields via algal hydrothermal liquefaction (HTL) at 83 GGE/ton [8], although the associated composition and specifically lipid content for such a comparison needs to be based on a consistent feedstock basis for a meaningful comparison. In comparison, the PAP process configuration yields an estimated 114 GGE/ton, roughly 10% lower than the CAP combined fuel yield. This is driven primarily by a 37% reduction in ethanol yield (20 vs 31 GGE/ton) with a lesser 1% reduction in diesel blendstock yield (95 vs 96 GGE/ton), both in turn attributed to similar losses in sugars and lipids respectively associated with the solid/liquid separation step utilized for the PAP process. Following the trend in yields, the estimated MFSP is also 9% higher for the PAP configuration relative to the CAP process, at $10.86/GGE and $9.91/GGE for the PAP and CAP processes respectively, based on an assumed algal biomass feedstock cost of $1092/dry ton attributed to currently estimated projections for the cost of algal cultivation and dewatering up to 20 wt.% solids [8] (outside the scope of this analysis focused only on conversion performance). The resulting yields and MFSP estimates are shown in Fig. 4, with MFSP contributions broken down between feedstock cost and conversion cost allocations.

4. Conclusion

An integrated algal biorefinery process, termed CAP, is successfully demonstrated. The algal slurry after acid pretreatment is a sufficient medium for cultivating yeast to produce ethanol without any additional nutrients. Almost all the fermentable sugars were utilized in CAP. The utilization efficiency of carbohydrates is significantly improved (ethanol yield of 31 GGE/ton biomass) compared to previous refinery design cases (ethanol yield of 20 GGE/ton biomass). Lipid yield is not adversely affected by fermentation and ethanol removal, reaching 87% of FAME recovery. CAP can further reduce microalgal biofuel cost by 9% achieving a modeled energy yield of 126 GGE/ton of total fuel products with $9.91/GGE from *S. acutus* biomass. Removing an additional SLS reduced capital and operating cost resulting in a simplified and robust process. It is likely that a number of high-value co-products, such as PUFA and protein residue, may also be produced via the CAP processing concept, resulting from the process' relatively non-destructive nature of fractionating whole algal biomass to individual component constituents. High-value co-product opportunities possess potential to significantly reduce the high cost of algal biofuel production, especially from the extracted stillage fraction, which, in our current analysis, is relegated to anaerobic digestion for biogas production.

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