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ARTICLE

An alternative biorefinery approach to address microalgal seasonality: blending with spent coffee grounds

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An effective method for the production of fuels and chemicals from microalgae is to ferment the carbohydrate fraction, extract the lipids and convert the resulting solids through hydrothermal liquefaction (HTL). In this process, known as Combined Algal Processing (CAP), multiple fuel precursors are produced effectively. However, one of the key challenges associated with a microalgae-based biorefinery is the reduced productivity of algae in the colder seasons. In this investigation, it was evaluated the potential for spent coffee grounds (SCG), a potentially valuable waste stream, to be blended with biomass from the microalgae *Scenedesmus acutus* (HCSD) to make up for the productivty shortfalls in periods of lower microalgae productivity to maximize the capacity for downstream equipment throughout the year. Two different blend ratios were compared to only microalgae biomass or SCG, one representing winter season (40% microalgae and 60% SCG – blend 1) and another representing autumn and early spring (60% microalgae and 40% SCG – blend 2). Pretreatment of the blends showed higher monosaccharide release yields compared to microalgae alone, with an increase in mannose and galactose specifically. In the fermentation of the pretreated slurries, all the monosaccharides were consumed, resulting in ethanol titers of up to 23 g/L for the SCG blend, compared to 14 g/L ethanol for the algae alone. The lipid extraction from the blends resulted in yields of 95.5-99.7% (which translates to 173.8-193.5 kg/tonne of dry biomass processed in this biorefinery scenario) compared to 92.2% in HCSD (216.2 kg/tonne of dry biomass) and 68.1% in SCG (90.8 kg/tonne of dry biomass) alone. The residual solids left after fermentation and lipid extraction were converted via hydrothermal liquefaction (HTL) to produce bio-crude. The bio-crude yield was higher for microalgae (24.6%) than for the two blend cases (blend 1 - 17.5% and blend 2 - 19.7%). Theoretical energy calculations showed that the addition of SCG gave similar yields of fuel (gallon of gasoline equivalent) from the blends when compared to microalgae alone (94.7 - 96.5% depending on the blend of SCG). This work demonstrates that SCG can be easily incorporated with microalgae into a combined processing methodology and can therefore be used effectively during periods of lower availability of microalgae maintaining maximum operating levels of the conversion process equipment year-round. Moreover, co-processing algae with SCG not only leads to increased ethanol titers in the fermentation but also improves the lipid extraction yields.

1. Introduction

Microalgae have been widely demonstrated to be a highly promising candidate for alternative fuel production (1,2). While the majority of research has focused on lipid based fuels, microalgae also contains substantial protein and carbohydrate fractions (3–5). Recently, it was demonstrated that by combining processing stages together, termed Combined Algal Processing (CAP), the cost of biofuel production could be reduced substantially compared to a focus on lipids alone (6). The range of products is not limited to fuel products as a range of other bulk chemical precursors have been demonstrated (6,7). The CAP configuration includes an acid pretreatment to depolymerize the carbohydrates into monosaccharides to be fermented to ethanol. The fermented slurry is then submitted to lipid extraction with hexane as the extracting solvent. The solvent phase is separated from the solids and fed into a distillation column to recover the solvents for reuse in extraction and the lipids for upgrading to renewable diesel blendstock. Because a significant portion of algal biomass remains after the fermentation and extraction, the option of maximizing biofuel yields by carrying out hydrothermal liquefaction (HTL) to produce bio-crude was explored (8).

While microalgae can be grown year round, a key challenge associated with future microalgal biorefineries is the lower productivity and consequently, the lower availability of

microalgal biomass during winter, autumn and spring seasons (9–12). This low productivity is due to seasonal temperature and insolation fluctuations throughout the year. Therefore, it is challenging to optimize and scale the downstream equipment to maximize conversion to products throughout the year.. Wendt *et al.* studied the possibility to store microalgae in periods of higher supply (spring and summer) to be used in the lower supply seasons (autumn and winter) (13). However, this would require the conversion processes to be scaled at for biomass throughput below peak summer levels, as well as risking loss of biomass quantity and quality through less than optimal storage, making the overall yearly biofuel production rates lower (14).

In this paper, an alternative solution to this challenge is presented, blending microalgae with spent coffee grounds (SCG) in periods of lower productivity (autumn, winter and early spring). With this approach the biorefinery can run at design specification operating levels throughout the entire year. During periods of higher microalgae supply, the operating levels would be in accordance with this availability, while during the periods of lower supply, SCG would be added to make up and keep the biorefinery running at high operating rates.

The United States Department of Agriculture estimates a yearly consumption of 9.8 million tonnes of coffee beans worldwide (15). Depending on the coffee bean origin and on the brewing process, SCG composition includes carbohydrates (42-55 % w/w), triglycerides (10-24 % w/w), protein (10-18 % w/w), lignin (0-25 % w/w), chlorogenic acids (1-3 % w/w), caffeine (0-0.4 % w/w) and ash (1-2 % w/w) (16–21). The high percentages of carbohydrates and triglycerides suggest that this can be a suitable feedstock to be blended with microalgae in the biorefinery concept. In addition, there is the possibility to obtain this feedstock for little or no costs (the only costs to be considered are the collection and transportation costs) and the presence of similar components in both SCG and lignocellulosic biomass. To this end, this study aims to demonstrate the suitability of SCG as a blending feedstock with microalgae in a modified CAP design (**Error! Reference source not found.**).
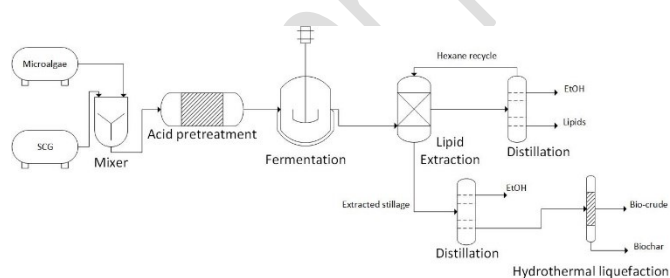

Figure 1. Modified CAP biorefinery process configuration employed in this investigation. This process includes an acid pretreatment, a fermentation to produce ethanol, a lipid extraction and a hydrothermal liquefaction to produce bio-crude and biochar.

2. Materials and methods

2.1. Material acquisition

Scenedesmus acutus 0401 (HCSD) was grown outdoors in flat panel photobioreactors under nitrogen deplete conditions to increase the concentration of lipids and carbohydrates in the biomass. The seed was grown in outdoor reactors using nitrate as the nitrogen source during scale up. A total of 8 reactors, at 660 L/ reactor, were used to produce the biomass. Harvesting was accomplished using Alfa Laval centrifuge (Warren, MI). The harvested biomass was shipped frozen to NREL and stored frozen till needed. Spent coffee grounds, provided by the NREL café, were homogenized by thorough stirring where big agglomerates were broken down to smaller particles. The original composition of these two feedstocks is present in Table 1. Both the algae and spent coffee grounds feedstock samples were prepared as follows: 250 g of sample were weighted into a pre-weighted cannister; followed by the addition of deionized water to make up a solution of 25% solids (considering the moisture content of both feedstocks – 35.4% for microalgae and 35.9% for spent coffee grounds); sulphuric acid was added to obtain a final solution of 2% H_2SO_4.The blends were then prepared by mixing the two feedstocks in the below mentioned percentages and the same procedure used in the preparation of the pure streams was then followed. The composition of the four slurries prepared are as follows:

- HCSD – *Scenedesmus acutus*
- SCG – spent coffee grounds
- Blend 1 – 40 % HCSD and 60% SCG (w/w) – representing winter
- Blend 2 – 60 % HCSD and 40% SCG (w/w) – representing autumn and early spring

The percentages of microalgae and SCG in blend 1 and 2 representing winter and autumn seasons were based on results obtained from a model previously developed at NREL (22,23).

Table 1 – Original feedstock and blends composition

%	*Scenedesmus acutus*	Spent Coffee Grounds
FAME	23.5	16.3
Carbohydrates	38.1	50
Glucose	27.5	9.7
Galactose	1.9	11.2
Mannose	8.7	28.7
Protein	14.7	11
Ash	2.3	2.2
Total	78.6	79.5

2.2. Acid pretreatment

Pretreatment experiments were carried out in a bath-type ZipperClave® reactor, previously described (6,24,25). 250 g of wet biomass were loaded into the reactor. Water and sulfuric acid were added achieving a 25% (*w/w*) total solids and 2% (*w/w*) H_2SO_4 solution (considering the biomass moisture). The reactor was heated up to 155 °C with the aid of steam injection at the bottom of the reactor, increasing the pressure inside the reactor to approximately 5 bar. After 15 minutes the cannister containing the pretreated slurry was removed and cooled in ice water. A set of three replicates for each of the feedstocks studied was conducted to provide enough substrate for fermentation.

A Mettler-Toledo SP precision infrared balance (Columbus, OH) was used to determine the total solid content of biomass at 105 °C. Additional pretreatment determinations were previously described (6).

2.3. Fermentation

A seed culture of *S. cerevisiae* D5A was grown in YPD at 37 °C in a shake flask at 225 rpm overnight.

The triplicates obtained in the pretreatment experiments were combined and neutralized to an approximate pH of 5. 270 mL of pretreated slurry were added to the fermenters and supplemented with 30 mL of 10x yeast extract-peptone (100 and 200 g/L, respectively) for a total volume of 300 mL. Fermenters were inoculated to an initial OD_{600} of 0.7. Fermentations were run for 48 hours while the fermenters were maintained at pH 5.5 (with 5 M NaOH), 37 °C and stirred at 250 rpm. Samples were taken for HPLC analysis to determine sugar consumption and ethanol production during fermentation.

Control media to replicate the sugars content of either the pretreated algae or SCG contained a base of yeast extract (10 g/L) and peptone (20 g/L). In addition, the algae control media contained approximately 23.9 g/L glucose, 2.9 g/L galactose and 9.5 g/L mannose, while the spent coffee grounds control media contained 2.7 g/L glucose, 29 g/L galactose and 54 g/L mannose. Periodic fermentation samples were taken for HPLC analysis.

2.4. Lipid extraction

The fermented slurry was put in contact with hexanes (1:1 ratio, w/w) in Erlenmeyer flasks with overnight agitation on a multi position magnetic stirrer plate (Velp, Bohemia, NY, USA). It was recently learned that ethanol can act as an effective co-solvent with hexanes for higher lipid yields, and so the extraction was performed before ethanol recovery in contrast to an earlier published procedure (6). The samples were then transferred to conical centrifuge tubes and centrifuged for 10 minutes at 2000 g. The organic phase (containing both the hexanes and lipids that migrated from the fermented slurry) was separated and collected in pre-weighted glass vials and subsequently evaporated in a TurboVap concentration workstation (Caliper Life Sciences, East Lyme, CT, USA) at 40 °C. The glass vials were then left overnight in a vacuum oven at 40 °C for further residual solvent evaporation. The glass vials were weighed to determine the total lipids obtained. FAME extraction yields were calculated based on the FAME content of the original feedstock.

2.5. Hydrothermal liquefaction and analysis

The extracted slurry obtained from the lipid extraction was initially vacuum dried and then freeze dried to remove water, ethanol and any remaining hexanes. 1 g of these solids and 4 g of water were added to the HTL reactors and heated to 300 °C (26). After 30 minutes of reaction time, the reactors were cooled in cold water. The contents of the reactor were then transferred to a separatory funnel. Dichloromethane (DCM) was used to help in the removal of any residual components left in the reactor and transferred to the separatory funnel. This was shaken and left to rest for phase separation. Once the two phases were clearly separated, both were removed and collected in separate pre-weighed vials. The bio-crude phase was submitted to solvent evaporation in a TurboVap concentration workstation at 40 °C followed by overnight evaporation in a vacuum oven at 40 °C. Vials were weighed and the dry bio-crude ash-free yields were determined considering the initial load of solids including ash. Biochar was obtained through filtration of both the organic and aqueous phases when collecting them from the separatory funnel.

2.6 Analysis

2.6.1 Carbohydrate analysis

Carbohydrate analysis followed the NREL laboratory analytical procedure developed by Van Wychen et al. (XX). This analysis consists on a two-step hydrolysis performed on lyophilized material (original feedstocks and intermediate solids). Approximately 25 mg of each sample was weighted into a pressure tube, followed by the addition of 250 µL of 72% sulfuric acid (Ricca Chemical Company, Arlington, TX) with constant vortexing. Pressure tubes were then placed in a water bath at 30 °C with vortexing 10 to 15 minutes. After 1 hour, samples were diluted with 7 mL of 18.2 mega-ohm water, vortexed and placed in an autoclave for 1 hour at 121 °C. Samples were then cooled down, neutralized to a pH of 6-8 using calcium carbonate and filtered using 0.2 µm nylon filters to HPLC vials.

All liquid fraction samples were analyzed for total and monomeric sugars using the laboratory analytical procedure devoleped by Sluiter et al. (XX). Monomeric sugar analysis on pretreated liquor was performed by dilution of the sample followed by neutralization to a pH between 6-8 using calcium carbonate and filtered using 0.2 µm nylon filters into LC vials. Total sugars were determined by one-step hydrolysis where the samples were diluted and 72% sulfuric acid (Ricca Chemical Company, Arlington, TX) was added to make a solution with 4% acid concentration. Samples were autoclaved at 121 °C for 1 hour, let to cool down at room temperature, neutralized with calcium carbonated to pH 6-8 and filtered to an HPLC vial using 0.2 µm nylon filters.

HPLC analysis on carbohydrates on the original feedstocks, total and monomeric sugars was done using a HPLC-RID (Agilent 1100 series, Santa Clara, CA, USA) equipped with a Shodex Sugar SP0810 (300 mm x 8 mm) column (Phenomenex, Torrance, CA, USA) Cation H+ and Anion CO3- de-ashing guard cartridges (Biorad Laboratories, Hercules, CA, USA). Mobile phase was 18.2 mega-ohm water at a flow of 0.6 mL/min. Column temperature was 85 °C and guard columns were left outside at room temperature.

Monomeric sugars in the fermented samples were analyzed using an HPAEC-PAD system due to the same elution time of mannose and ethanol. The monomeric sugar content of these samples was obtained by dilution and filtration of these samples. The HPAEC-DAD system(Dionex ICS-5000+, Sunnyvale, CA, USA) using a PA-1 column guard. Mobile phase was 14 mM of sodium hydroxide prepared in house from 50% (w/w) sodium hydroxide solution (Fisher Chemical, Hampton, NH, USA) with a flow rate of 1.0 mL/min. Both the column and guard were heated up to 35 °C.

2.6.2 Ethanol analysis

Fermentation samples were filtered and analyzed for ethanol content using an HPLC-RID (Agilent 1100 series, Santa Clara, CA, USA) equipped with an Aminex HPX-87H (300 mm x 7.88 mm) organic acids column and a Cation H+ guard column (Biorad Laboratoris, Hercules, CA, USA). 0.01 N sulfuric acid was used as mobile phase at a flow of 0.6 mL/min. Column was heated up to 55 °C. Mobile phase prepared in house using 10 N sulfuric acid (Ricca Chemical Company, Arlington, TX, USA).

2.6.3 FAME analysis

FAME analysis on the raw biomass and extracted FAME was performed following the laboratory analytical procedure developed by Van Wychen et al. where 7 to 10 g of sample were weighed in GC vials followed by drying in a vacuum oven 40 °C for two days (XX). 25 μL of internal standard consisting of tridecanoic acid methyl ester, 200 μL of 2:1 (v/v) chloroform:methanol and 300 μL of 0.6 M HCL: methanol were added to the samples using gas-tight syringes. Vials were then sealed and vortexed before being placed in a preheated block at 85 °C. After 1 hour, vials were removed from digital dry block and left to cool down at room temperature for no longer than 1 hour. 1 mL of HPLC grade hexane was added to the samples. Samples were vortexed and left undisturbed for 1 hour. A fraction of the upper phase of the samples (FAME in hexane) was removed from the vials, transferred to a new set of GC vials and diluted in hexane depending on the biomass nature. A GC-FID (Agilent 7890B, Santa Clara, CA, USA) system equipped with a DB-Wax capillary column 30 m, 0.25 mm ID and 0.25 μm FT, a 1 μL injection at 10:1 split ratio, a constant flow rate of 1 mL/min of helium, inlet temperature of 250 °C and an oven temperature at 100 °C for 1 minute, 25 °C/min up to 200 °C, hold for 1 minute, 5 °C/min up to 250 °C, hold for 7 minutes, FID at 280 °C with 450 mL/min zero air, 40 mL/min of hydrogen and 30 mL/min of helium.

2.6.4 Protein analysis

Protein analysis was performed by determining the nitrogen percentage in the samples (slurry, liquor or lyophilized solid material) and then using 4.78 as a conversion factor to obtain final protein percentage (XX). For original biomass or intermediate solid samples, approximately 5 to 10 mg (depending if it is original biomass or intermediate solid, respectively) of lyophilized material was weighted on a small tin foil sheet, which was then folded and pressed into a packet. For liquid and slurry samples, 10 or 20 mg of sample, respectively, was weighted into a small tin foil capsule. Nitrogen analysis was performed in an Elementar Vario El Cube CHN Analyzer (Ronkonkoma, NY, USA). Samples were combusted at a 950 °C in an oxygen rich environment, where the produced gas was run through a GC column and detected via a thermal conductivity detector.

2.6.5 Moisture and ash analysis.

The laboratory analytical procedure developed by Van Wychen et al. (XX) was used to determine the moisture and ash content in the samples. Approximately 25 mg of biomass was weighted into pre-weighed crucibles, which were placed in an oven at 40 °C for two days. Crucibles were removed from the oven, cooled at room temperature and weighted to determine moisture content. Same crucibles were then placed in a muffled furnace

increasing the temperature as follows: 12 min at 105 °C, followed by an increase to 250 °C at 10 °C/min, 30 min at 250 °C, followed by an increase at 20 °C/min until 575 °C, 180 min at 575 °C, then a temperature decrease and held at 105 °C. The crucibles were then cooled at room temperature and weighed to determine the ash content.

2.7 Theoretical conversion yields calculations

Theoretical conversion yields were calculated assuming that all fermentable sugars are being converted to ethanol with a 51% theoretical yield and the fatty acid are converted to renewable diesel with a 78 wt.% theoretical yield (29,30). The HTL bio-oil calculation was made using the Demirbas equation (eq. 1) to determine the energy content of the bio-oil produced (31).

$$HHV\ [MJ/kg] = 33.5(C) + 142.3(H) - 15.4(O) \qquad (1)$$

Where C, H and O are the percentages of carbon, hydrogen and oxygen, respectively, in the bio-oils obtained. All the results were converted to MJ equivalent for comparison reasons and then to gasoline equivalents considering 1 gasoline gallon equivalent is 122.48 MJ (32). Conversion from bio-oil to fuel was assumed to be 100% (33). This is then converted to metric units (L/tonne).

3. Results and discussion

3.1 Acid pretreatment

The aim of pretreatment is to depolymerize the carbohydrates present in the feedstock into fermentable sugars. The pretreatment of HCSD gave a high level of glucose, up to 26.2 g/L (**Error! Reference source not found.**), approximately 44% of the available glucose in the algal biomass is therefore released as monomeric glucose at this stage. These yields are calculated based on the sugar concentration before and after pretreatment. The monomeric yield is obtained by the ratio of the monomeric glucose after pretreatment divided by the total (monomeric and oligomeric) glucose in solution before pretreatment. Additionally, lower quantities of galactose and mannose are also released with a substantial proportion being present as oligosaccharides. Alternatively, SCG does not contain substantial levels of glucose, and only 12% of the original glucose present in the SCG is released during pretreatment to monomeric glucose. This suggests that at least some of the glucan present in SCG is in a recalcitrant form, possibly cellulose. It does not appear that the low glucose yields were due to degradation of the glucose to hydroxymethyl furfural (HMF) during pretreatment because HMF levels did not exceed 1.1 g/l in the liquor phase suggesting that the pretreatment severity was not excessively high. However, high levels of galactose and mannose are recovered from the pre-treatment, demonstrating that both algal and SCG hydrolysates would be suitable for further fermentation. A blend of both the HCSD and SCG released approximately the sugar profile that would be expected from the proportion of SCG added. In total the sum of sugars available for fermentation is therefore higher with the blends than the algal biomass alone.
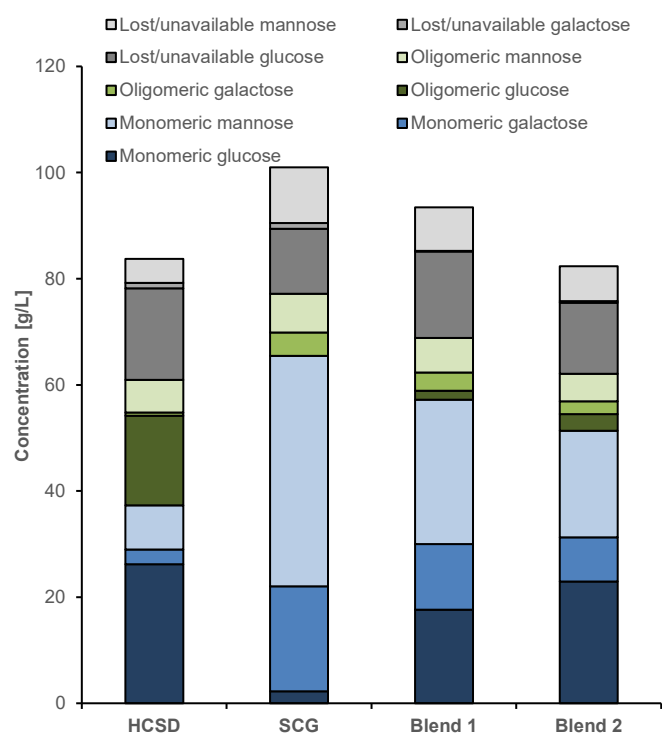
Figure 2 - Major fermentable sugars released from the pre-treatment, as monosaccharides (blue), oligosaccharides (green) and sugars either converted to inhibitors or unavailable for fermentation (grey)

3.2 Fermentation

The resulting slurries from pretreatment were pH adjusted and fermented (Figure 3). In order to determine if the slurries contained inhibitory compounds, control fermentations having the same sugar profiles as the HCSD and SCG slurries were also investigated. The monomeric sugars were consumed at approximately the same rate in the controls as compared to the slurries and is suggestive that there are no nutrient limitations or inhibitory compounds in either the HCSD or SCG slurries (Figure 3 a-d). *S. cerevisiae* is well suited for these fermentations because it can metabolize all three of the major sugars present in both algae and SCG. All sugars were consumed within 24 hours, with some diauxic behavior observed with glucose being consumed preferentially.

The blends of SCG and HCSD behaved similarly with ethanol concentrations of 20.3 g/L achieved for blend 1 and 18.6 g/L for blend 2 falling between the concentrations obtained for microalgae (14.0 g/L) and SCG (22.7 g/L) (Figure 3).

The ethanol produced from the fermentation was compared to the theoretical maximum, based on the monomeric sugar content of the pretreated slurry assuming a 51% theoretical ethanol fermentation yield (24,29). The yields ranged from 77-94% (table 2) demonstrating the suitability of fermentation after pretreatment.
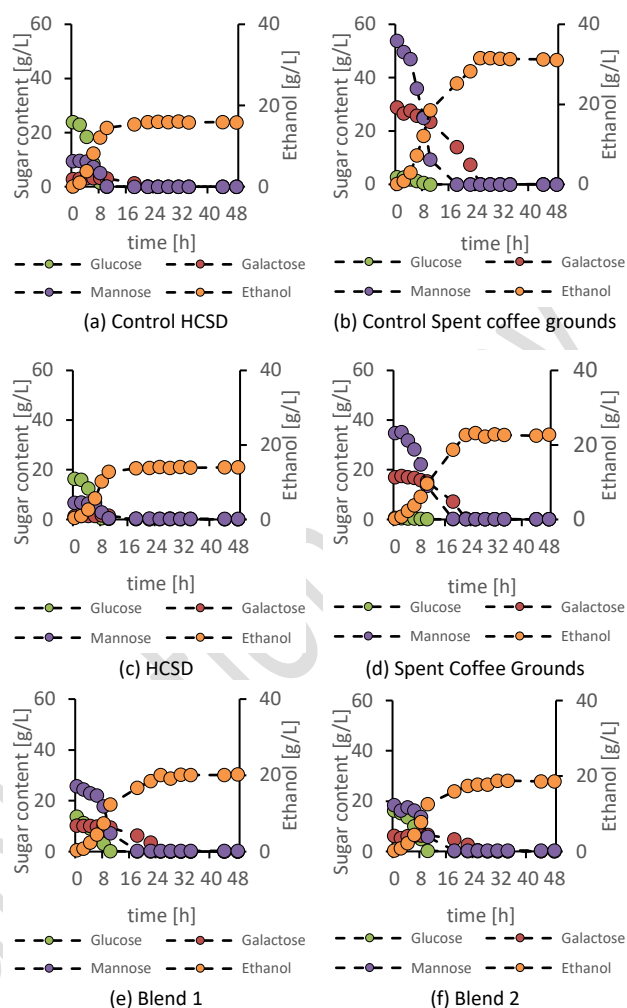

Figure 3 – Sugar consumption and ethanol production during fermentation of the various feedstocks.

Table 2 – Ethanol titers and yields in the fermentations for the four pretreated slurries

	Titer [g/L]	Yield (%)
Control HCSD	15.8 ± 0.0	85.6 ± 2.6
Control SCG	31.1 ± 0.0	71.3 ± 4.6
HCSD	14.0 ± 0.2	93.6 ± 0.3
SCG	22.7 ± 0.0	80.3 ± 2.7
Blend 1	20.3 ± 0.2	76.8 ± 2.6
Blend 2	18.6 ± 0.1	86.7 ± 4.9

3.3 Lipid extraction

After fermentation, the lipids were recovered from the fermented slurry using three successive rounds of hexane extraction (Figure 4), with a combined yield of >92% from the algae and blend materials. This demonstrates that lipid is not being consumed or degraded by the yeast during fermentation and the high yield is due to the presence of ethanol produced by fermentation acting as a co-solvent. However, the lipid yields obtained on the fermented SCG alone, were considerably different from the other fermented slurries. In the first extraction the yield was considerably lower than the other samples, and overall only 68% of the original lipid was

recovered. This was presumably due to the formation of a double layer observed during the agitation of the SCG fermented slurry with the extraction solvent, while this double layer was not observed for microalgae and both blends. The surface of the SCG might be more hydrophilic and resistant to hexane mass transfer. There are small amounts of surface active compounds (e.g. peptide, protein, polar lipids) in algal biomass and these surfactant might help reduce the surface tension between the hexane and biomass, increased hexane mass transfer for a better extraction (34). On the other hand, stable emulsion caused by surfactant is not favored for phase separation after the extraction, but it was noticed that emulsion was not stable after the extraction and could easily be broken by gravimetric settling or a centrifugation. The higher lipid yield in a blend system indicates that lipid yield was improved by the presence of algal biomass. This problem could be solved by increasing the agitation of extraction or adding a fourth extraction step. However, as this was not observed with the blends, it is unlikely to be a problem in the biorefinery system.
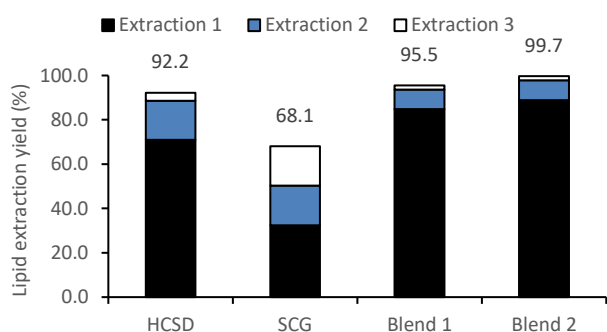

Figure 4 – 1st, 2nd and 3rd extraction yields. These yields are calculated based on the amount of lipids obtained in extraction and the lipids in original feedstock.

Applied to a biorefinery scenario and given the increased lipid extraction efficiency of the blends, an increase in total lipids extracted is realized from the blends over algae or SCG alone (Table 3).

Table 3 – Percentage of lipids extracted and total mass of lipids extracted per tonne of dry biomass extrapolated to a biorefinery scenario. The percentage of lipids is calculated by dividing the lipids extracted by the amount of lipids present in the fermented slurry. HCSD and SCG have a solid content of 37% and 38%, respectively.

	% lipids extracted	Total lipids extracted [$kg_{lipids}/tonne_{biomass, dry}$]
HCSD	92.2	216.9 ± 3.2
SCG	68.1	90.8 ± 1.2
Blend 1	95.5	173.8 ± 3.5
Blend 2	99.7	193.5 ± 0.1

The extracted lipids were converted into fatty acid methyl esters (FAME) to assess the lipid profile (Figure 5). The fatty acid extracted from HCSD was predominantly oleic acid, whereas from the SCG, was linoleic and palmitic. This is in keeping with the typical fatty acid profile of both feedstocks (16,35). The lipid extracted from the blends was a direct mixture of the two profiles.
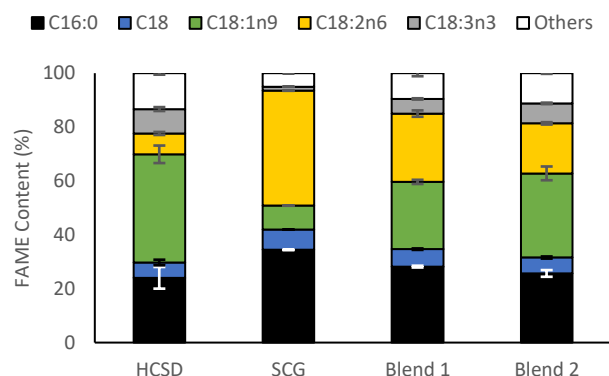

Figure 5 – Lipid composition (FAME composition as an average between the three extractions)

The composition of residual solids remaining after fermentation, extraction of the lipids, and drying is given in Table . This material is rich in unfermented, complex, polymeric carbohydrates and protein. The sum of all the percentages of all the components specified is approximately 70%. Such low mass closure can be explained by the presence of unquantified compounds in algal biomass (e.g. nucleic acids, algaenan, moieties from polar lipids, etc) and unquantified compounds from SCG (e.g. lignin, caffeine and chlorogenic acids). After the consumption of monomeric carbohydrates during fermentation and the extraction of lipids, these unquantified compounds make-up a larger proportion in the residual solids.

Table 4 – Residual solids composition

	Carbohydrates (%)	Lipid (%)	Protein (%)	Ash (%)
HCSD	22.3 ± 0.7	1.9 ± 0.2	30.7 ± 0.2	14.4 ± 0.5
SCG	21.3 ± 1.3	5.1 ± 0.8	25.2 ± 0.1	16.7 ± 0.8
Blend 1	23.3 ± 1.5	2.8 ± 0.6	28.3 ± 0.2	14.7 ± 0.6
Blend 2	20.1 ± 0.1	1.9 ± 0.2	29.8 ± 0.3	14.8 ± 0.1

3.4 Hydrothermal liquefaction

The residual solids still have large amounts of organic carbon which has the potential to be valorized into further useful components (8). To this end they were converted via hydrothermal liquefaction. The dried solids were reconstituted with water and subjected to HTL, yielding bio-oil to further improve the biofuel yield of the biorefinery. The bio-crude yields are given in table 5.

Table 5 – HTL bio-crude gravimetric yields (dry ash free basis)

	Bio-crude gravimetric yield (%)	Bio-char gravimetric yield (%)
HCSD	24.6 ± 0.3	11.0 ± 2.5
SCG	20.0 ± 6.0	6.4 ± 3.5
Blend 1	17.5 ± 5.1	9.4 ± 3.4
Blend 2	19.7 ± 6.3	13.6 ± 0.9

The yields were reasonably similar, ranging from 18 to 25%, being the highest for microalgae and the lowest for the blend 1, which is the blend with higher percentages of SCG. Unlike the

other conversion processes employed, the bio-crude results for the blends do not fall between the results for SCG and microalgae, which correlates with the reduced lipid content in the blends when compared to the residual produced from the SCG.

Table 6 – CHN analysis of bio-crude and respective HHV calculated using Eq. 1

	Elemental Analysis				
	C (%)	H (%)	N (%)	O (%)[a]	HHV [MJ]
HCSD	74.1 ± 0.3	8.6 ± 0.1	9.2 ± 0.0	8.1 ± 0.3	35.8
SCG	72.8 ± 0.7	8.2 ± 0.1	9.2 ± 1.0	9.8 ± 0.2	34.6
Blend 1	74.0 ± 0.5	7.9 ± 0.1	9.0 ± 0.2	9.0 ±0.5	34.7
Blend 2	75.0 ± 1.9	8.2 ± 0.4	9.1 ± 0.3	7.8 ± 2.5	35.6

[a] Determined by the difference between the totals and the sum of carbon, hydrogen and nitrogen percentages.

3.5 Biofuel precursor yields in a biorefinery scenario

The CAP scheme, as applied to microalgae and typical seasonal blends with SCG in a biorefinery scenario, produces three biofuel precursors: bioethanol, lipids and bio-crude. To directly compare the overall output of each fractionation and recovery step, the energy content of each fraction was converted into gasoline equivalents (Table 7). The HTL bio-crude energy content was calculated based on its elemental composition (as described in the methods section). All the values were then converted to the same units (MJ equivalent) and finally to gasoline equivalents in metric units (L/tonne).

Table 7 – Fuel yields in the four feedstocks in a potential biorefinery scenario.

Theoretical calculations	HCSD	SCG	Blend 1	Blend 2
Total Carbohydrates (% DW)	38	50	45	43
Ethanol (% DW)[a]	19	25	23	22
Gasoline equivalent (L/Tonne)[b]	162	211	191	182
MJ equivalent	4754	6191	5616	5329
Fatty Acids (FAME) (% DW)	24	16	19	21
Hydrocarbon (% DW)[c]	18	13	15	16
Diesel equivalent (L/Tonne)	216	150	176	189
MJ equivalent	6519	4530	5325	5723
HTL bio-oil (% DW)[d]	5	4	4	4
bio-oil MJ equivalent[e]	1558	1123	1212	1332
Total Gasoline Equivalent (L/Tonne)	437	404	414	422

[a] 51% glucose-to-ethanol theoretical conversion; [b] 65.8% ethanol-to-gasoline conversion; [c] 78% FAME-to-hydrocarbon theoretical conversion; [d] HTL experimental results; [e] based on experimental results and equation 1

Excitingly, the seasonal inclusion of SCG blended with algae into the CAP process produces similar levels of fuel energy (measured in gasoline equivalents). While HCSD produced the highest gasoline equivalent value (437 L/tonne), the blends with SCG were comparable producing between 414-422 gasoline equivalent L/tonne. This is a reduction of between 3.5 - 5.3% respectively, in the energy produced compared to when HCSD is used. This is mainly due to the higher carbohydrate content, leading to more ethanol which is less energy dense than renewable diesel from lipids. And these numbers could be higher if the pretreatment resulted in higher monomeric sugar

yields, because carbohydrates are a better feedstock for fermentation to ethanol than for HTL where they primarily contribute to biochar production (36). Further development of this process to better match the feedstock would be warranted to maximize total biofuel yields and reduce overall production costs. Such results suggest that these blends can be effective to mitigate periods of lower microalgae supply to maintain biofuel precursor production with minimal overall impact.

4. Conclusion

In this investigation SCG were assessed to evaluate whether they could be used to make up the shortfall in microalgae production in colder seasons of the year (winter and autumn). To this end, blends of *Scenedesmus acutus* and SCG were co-processed in the CAP process, previously demonstrated to have higher economical potential than alternative algal platforms. The aim of the acid pretreatment step was to depolymerize the macromolecules in the feedstock into fermentable sugars. The pretreatment results were satisfactory as the blends yielded higher concentrations of fermentable sugars (glucose, galactose and mannose) than the microalgae feedstock. These sugars were then all consumed in the fermentation leading to higher quantities of ethanol produced (20.3 g/L for blend 1 and 18.6 g/L for blend 2) compared to the 14.0 g/L produced in HCSD. The lipids extracted from the fermented slurries of the blends resulted in higher overall yields, though this represented a slight reduction in the total amount of lipids extracted in the blends (62-68 kg/tonne of wet biomass) compared to the lipids extracted from HCSD alone (76.8 kg/tonne of wet biomass) because SCG had a lower lipid content than algae. Finally, the residual solids left after the lipid extraction were used as feedstock in an HTL process to produce bio-crude. The gravimetric yields obtained in this process for the different feedstocks were relatively similar, ranging from 18 to 25% (AFDW).

To assess the potential of these blends compared to pure microalgae, the energy content of the three fuel products was compared. Although the blends led to a lower total gasoline equivalent than HCSD, the differences registered are relatively small (5.3% for the blend representing winter and 3.5% for the blend representing autumn). This work demonstrates that SCG can be effectively used as a blend in microalgae-based biorefineries using the CAP configuration during periods of lower supply of this feedstock.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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