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Anaerobic bioprocessing of wastewater-derived duckweed: Maximizing product yields in a biorefinery value cascade

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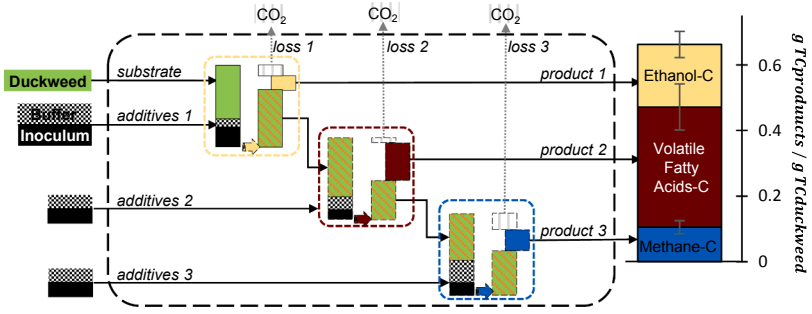
GRAPHICAL ABSTRACT



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ABSTRACT

This study integrated the sugar and carboxylate platforms to enhance duckweed processing in biorefineries. Two or three bioprocesses (ethanol fermentation, acidogenic digestion, and methanogenic digestion) were sequentially integrated to maximize the carbon-to-carbon conversion of wastewater-derived duckweed into bioproducts, through a series of laboratory-scale experiments. Reactors were fed either raw (dried), liquid-hot-water-pretreated, or enzymatically-saccharified duckweed. Subsequently, the target bioproduct was separated from the reactor liquor and the residues further processed. The total bioproduct carbon yield of 0.69 ± 0.07 g per gram of duckweed-C was obtained by sequential acidogenic and methanogenic digestion. Three sequential bioprocesses revealed nearly as high yields (0.66 ± 0.08 g of bioproduct-C per duckweed-C), but caused more gaseous carbon (dioxide) loss. For this three-stage value cascade, yields of each process in conventional units were: 0.186 ± 0.001 g ethanol/g duckweed; 611 ± 64 mg volatile fatty acids as acetic acid/g VS; and 434 ± 0.2 ml methane/g VS.

1. Introduction

Modern economies utilize renewable resources to fulfill only a minor fraction of their total energy and chemical demands, and rely instead on nonrenewable resources such as coal, crude oil, and gas. However, the economic and environmental disadvantages of fossil fuels have led to increased efforts to find alternative resources to fulfill energy and chemical needs (Jung et al., 2016). Among the alternatives, biomass is the only renewable resource for chemicals. In order to utilize biomass as an alternative to fossil-based raw materials, it must be processed in integrated, complex biorefineries, analogous to petroleum refineries, by targeting an array of end products with different market

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values, chemical properties, and quantities (Biddy et al., 2016). In a biorefinery, this portfolio of products can be achieved through multiple pathways, some of which may be in parallel and others in sequence (Cherubini et al., 2009). In this study we investigate a sequential case, producing ethanol, mixed carboxylic acids, and methane in a value cascade (Keegan et al., 2013; Kehili et al., 2016; Liu et al., 2019).

Biomass composition and availability is of particular importance for providing a reliable feedstock for biorefining, along with its social acceptance and environmental performance for long term sustainability (Mertens et al., 2019). Given that renewable alternatives should ideally be abundant, inexpensive, and complement rather than compete with food production, there is a preference for non-edible plant-based raw materials (biomass) as feedstocks for biorefineries (Cherubini, 2010). One alternative feedstock which fulfills these criteria is duckweed (*Lemnaceae*), a family of fast-growing, simple, floating aquatic plants, consisting of 38 species in five genera (Les et al., 2002). Duckweeds can accumulate high amounts of starch (up to 46% of dry mass) under nutrient starvation (Zhao et al., 2015). In addition, due to their relatively low lignin content (1%-3%), duckweeds do not require harsh chemical pretreatments prior to processing. Because they float, duckweeds are easy to harvest, and their small dimension (0.1 cm to 1 cm) eliminates the need for size reduction (Cui and Cheng, 2015). Furthermore, duckweeds are resilient to a broad range of nutrient concentrations; therefore, they can be grown on wastewater steams (Cheng and Stomp, 2009) and require minimal agricultural inputs. In wastewater treatment applications, the costs of a duckweed biorefinery could potentially be subsidized by reduced disposal fees, as is commonly the case for products such as methane and compost produced from wastewater sludges. The advantages duckweed possesses as a feedstock has encouraged several prior research studies, focusing on three platforms of biorefineries: (1) thermochemical conversion into syngas, as well as gasoline, diesel, and jet fuel (Baliban et al., 2013); (2) sugar platform conversion into alcohols (Ge et al., 2012; Zhao et al., 2014; Su et al., 2014); and (3) carboxylate platform conversion into VFAs (Calicioglu et al., 2018).

It is known that valorizing process residues from fermentation effluents is technically feasible for duckweed (Calicioglu and Brennan, 2018). However, an integrated biorefinery value cascade has not previously been investigated for this renewable feedstock. The integration of various anaerobic bioprocesses involving sugar and carboxylate platforms might be particularly advantageous for nutrient rich feedstocks like duckweed. A feedstock high in nutrients reduces the need to import and supplement nutrients for various fermentation processes, and any excess nutrients remaining after anaerobic bioprocesses are complete could also be valorized as one of the end products.

Although current biorefineries generally target ethanol or other liquid biofuels as the primary end product, methanogenic (anaerobic) digestion (MAD) of fermentation residues is a common practice in order to improve both environmental and economic performance of ethanol production processes (Bondesson et al., 2013; Dererie et al., 2011). However, these residues could also be processed into higher-value compounds. One alternative pathway suitable for establishing such a product value cascade is the carboxylate platform, which utilizes mixed cultures for acidogenic anaerobic digestion (AAD) of organic matter with carboxylic acids (i.e. volatile fatty acids, VFAs) as products and/or precursors of higher-value chemicals and biofuels such as esters, alcohols, and alkanes (Holtzapple et al., 1999). Although chemical inhibition of methanogenic activity is often used to ensure the stability of the carboxylate platform, another inhibition method is to operate acidogenic digestion process at high pH (9–10) values, which in turn gives higher VFA yields (Calicioglu et al., 2018) at short residence times of up to ten days. This inhibition technique also allows remaining residues to be bioprocessed into methane further, if the alkaline pH control is stopped to allow the pH to drop to neutral. Under this scenario, a sequential biorefinery process train with a value cascade of end products, integrating the sugar, carboxylate, and biogas platforms, would sequentially produce ethanol, VFAs, and methane.

The overall yield of biomass-to-co-products has been reported in the literature as the cumulative energy content of the co-products (Bondesson, 2008; Wu et al., 2015). However, for a biochemical biorefinery that includes co-products sold into other market segments, a mass approach for calculating the actual process yields as a function of theoretical potential might be more suitable. In this study, the carbon-to-carbon conversion of a feedstock into platform intermediates and/or bioproducts was considered, which not only provides a common set of units for system input and outputs, but also reveals how the atmospheric carbon sequestered in the biomass "fractionates" among the platform chemicals and bioproducts in the output portfolio.

In large-scale applications, pH is often controlled with inorganic chemicals such as sodium hydroxide and hydrochloric acid, but in this case, buffer was used in simpler reactor systems that allowed for low-cost replication of multiple treatments. The conversion of buffer added in the upstream or midstream of a particular anaerobic bioprocess to desired products (e.g. conversion of citrate buffer added during a fermentation process into VFAs during abiogenic digestion) is possible in laboratory-scale reactors. To control for this effect, the carbon-to-carbon yield calculation framework introduced in this study includes a correction for the buffer assimilation effect. This approach enables estimation of the actual yields from the substrate, and therefore allows for better estimates of commercial yields for practicing engineers.

This study utilizes wastewater-derived duckweed to investigate the potential for sequencing anaerobic bioprocesses (i.e., ethanol fermentation, acidogenic digestion, and methanogenic digestion) in an integrated biorefinery system. The aim of the study is to determine the most suitable combination of three candidate bioprocesses to produce ethanol, carboxylic acids, and methane, optimized for the maximum carbon-to-carbon conversion of duckweed to these products while producing fertilizers as a side product.

2. Materials and methods

2.1. Analytical methods

The moisture, total solids (TS), and volatile solids (VS) contents were determined according to the National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedure (LAP) for biomass and total dissolved solids of liquid process samples (Sluiter et al., 2008). Ash content was measured according to NREL LAP for determination of ash in biomass (Sluiter et al., 2004).

Glucose and ethanol quantification were performed using a Waters high performance liquid chromatograph (HPLC) equipped with a refractive index detector (Waters, Milford, MA) and a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm; Bio-Rad, Richmond, CA) with 0.8 ml/min of 0.012 N sulfuric acid as the mobile phase. The detector and column temperatures were constant at 35 °C and 65 °C, respectively. Prior to HPLC analysis, samples were centrifuged at 4 °C for 20 min at 5200 × *g* and the supernatant filtered through 0.2 µm nylon syringe filters.

VFAs were quantified using gas chromatography (GC) (SHIMADZU, GC-2010 Plus, Japan) with a flame ionization detector. The final total VFA yields were calculated in terms of acetic acid equivalents per gram of duckweed volatile solids added (HAc_{eq} g $VS_{duckweed}^{-1}$) (Siedlecka et al., 2008), and as grams of carbon in VFAs per gram of total carbon in duckweed added (g VFA-C g $TC_{duckweed}^{-1}$).

Carbon quantification of liquid and solid samples was performed using a total carbon (TC) analyzer (SHIMADZU, TOC-V CSN, Kyoto, Japan) equipped with solid sample module (SHIMADZU, 5000A, Kyoto, Japan).

Headspace gas pressures in acidogenic and methanogenic reactors were measured using a pressure gauge (Grainger, DPGA-05, USA). If pressures were positive, volumes of gas production from the acidogenic and methanogenic reactors were measured at ambient temperature

using a water displacement device. The device was filled with 0.01 M hydrochloric acid solution to prevent microbial growth. Carbon dioxide (CO_2) gas production from the ethanol fermentation reactors was also measured to complete the carbon balance. The headspace temperature was assumed to be constant and equal to 35 °C during the measurement, due to the rapid sampling process (El-Mashad, 2013; Theodorou et al., 1994). Volume readings were reported at standard temperature and pressure. Volumetric methane (CH_4) and hydrogen (H_2) concentrations were determined by collecting headspace gaseous samples using a 250 µl airtight syringe (Hamilton, Reno, NV, USA) and injecting onto a GC (SRI Instruments, SRI310C, Torrance, CA, USA) equipped with 6-foot molecular sieve column (SRI 8600-PK2B, USA), operated in continuous mode at 80 °C with argon as the carrier gas. Volumetric CO_2 concentrations were quantified using an identical GC (SRI Instruments SRI310C) equipped with 3-foot silica gel packed column (SRI, 8600-PK1A, USA) in continuous mode at 60 °C with helium as the carrier gas. Carbon loss in reactors in the form of CO_2 was expressed as grams of carbon in CO_2 per gram of TC in duckweed added (g CO_2-C g $TC_{duckweed}^{-1}$).

The raw and processed duckweed were analyzed at the Penn State Agricultural Analytical Services Laboratory for fertilizer potential. Total ammonia nitrogen (TAN) was determined by ion specific electrode method. Total nitrogen was quantified by combustion. Total phosphorus and total potassium were quantified by microwave-assisted acid digestion method (Peters, 2003). All fertilizer tests were performed in duplicate.

2.2. Plant material, cultivation, and pre-processing

Duckweed (*Lemna obscura*, 100% sequence identity to accession number GU454331.1, in the NCBI database (Calicioglu and Brennan, 2018)) was collected on September 20, 2016, from an open pond within the effluent spray fields of the Penn State Wastewater Treatment Plant, also known as the "Living-Filter". In August and September, the pond received on average (n = 9): 1.7 ± 0.5 mg L^{-1} carbonaceous biological oxygen demand; 2.2 ± 0.2 mg L^{-1} phosphorus; 0.2 ± 0.0 mg L^{-1} TAN; 15.1 ± 1.5 mg L^{-1} nitrate; 1.2 ± 0.1 mg L^{-1} nitrite; and 1.3 ± 0.4 mg L^{-1} total Kjeldahl nitrogen. The average water quality of three grab samples obtained from the surface of the pond at the harvest day was reported as: 35.1 ± 1.3 mg L^{-1} total chemical oxygen demand, 17.5 ± 0.7 mg L^{-1} soluble chemical oxygen demand, 2.0 ± 0.3 mg L^{-1} TAN, 2.5 ± 1.3 mg L^{-1} nitrate and 1.5 ± 0.6 mg L^{-1} phosphate. After harvest, the duckweed was wet sieved with tap water to remove smaller and coarser impurities, dried at 45 ± 3 °C to a constant weight over two days, and analyzed for its moisture (6.9 ± 1.3% wet basis), VS (85.8 ± 1.2% of TS), and TC (40.5 ± 0.3%) contents. The composition of duckweed was determined by wet chemistry analyses as (all values on a % dry weight basis): cellulose (12.6 ± 0.2); hemicellulose (21.0 ± 0.5); starch (10.8 ± 0.1); lignin (0.8 ± 0.2); water soluble carbohydrates (20.1 ± 0.1); and crude protein (18.3 ± 0.3) (Dairy One Wet Chemistry Laboratory, Ithaca, NY). The carbon-to-nitrogen ratio of the duckweed was 14.3:1.

Enzymatic liquefaction and saccharification of the duckweed was performed in four 2-L flasks with 1 L total working volume. Prior to liquefaction, 50 g duckweed (dry weight basis), equivalent to 20.3 ± 0.15 g TC, was sterilized by autoclaving with 945 ml water for 30 min at 121 °C. Then the pH was adjusted to 7.0 ± 0.1 with 2 M hydrochloric acid. Once the slurry was cooled to 90 °C, α-amylase (Sigma Aldrich, A3403, USA) was added at a loading of 5000 units g $starch^{-1}$. The flasks were incubated for one hour at 90 °C for liquefaction. Following liquefaction, the pH was adjusted to 5.2 ± 0.1 with sodium citrate buffer, yielding 25 mM in the total working volume. After pH adjustment, 334 units of glucoamylase g $starch^{-1}$ (Sigma Aldrich, 10115, USA) and cellulase (Novozymes, Cellic® CTec2, Denmark) with 60 filter paper unit g $cellulose^{-1}$ loadings were added to each flask, and then sealed with rubber stoppers. Saccharification was then performed at 50 °C, while mixing at 120 rpm for 24 h. All experiments and sampling were conducted under sterile conditions. Glucose and ethanol concentrations were quantified before and after liquefaction, and after saccharification. The theoretical maximum glucose concentrations of glucose and starch components of duckweed was calculated according to Gulati et al. (1996), and the water soluble sugar content of the duckweed was considered as glucose (i.e. fermentable by *Saccharomyces cerevisiae*) for a conservative estimate of the maximum theoretical glucose yield. Saccharified duckweed was utilized in individual ethanol fermentation, acidogenic digestion, and methanogenic digestion processes, or in the first stage of sequential processes of the value cascade.

Liquid hot water pretreatment was carried out in a 500 ml stainless steel Parr reactor (Parr Instrument Company, model 4575, Moline, IL), with a pressure limit of 345 bar. The vessel was filled with 30 g duckweed (dry weight) and 270 g distilled water. The temperature was ramped up to 150 °C within 15 min, followed by pressurization with nitrogen gas for 5 min which was monitored using a digital pressure transducer (Tasker et al., 2016).

2.3. Inocula

2.3.1. Yeast strain

The yeast, *Saccharomyces cerevisiae* (ATCC 24859), was enriched in basal medium containing (g L^{-1}): glucose (20); yeast extract (Difco, Sparks, MD) (6); $CaCl_2{\cdot}2H_2O$ (0.3); $(NH_4)_2SO_2$ (4); $MgSO_4{\cdot}7H_2O$ (1); and KH_2PO_4 (1.5). The culture was grown at 30 °C for 24 h, centrifuged at 2880 relative centrifugal force (rcf) for 20 min (Eppendorf, 5804 R, Germany), and the pellet refrigerated for less than two hours before being used to inoculate the ethanol fermentation reactors.

2.3.2. Acidogenic anaerobic seed

A mixture of silage, rumen fluid, anaerobic wastewater sludge, and compost was used as acidogenic seed. Silage and rumen fluid were obtained from the Pennsylvania State University Dairy Farm (University Park, PA). Anaerobic wastewater sludge was obtained from the Pennsylvania State University Wastewater Treatment Plant's secondary anaerobic digester. Compost was obtained from the Pennsylvania State University composting facility. All sources were mixed and acclimated to basic conditions (pH 9.2) as described in detail previously (Calicioglu et al., 2018). The VS content of the final acidogenic inoculum was 52.2 ± 1.1% of the TS, and the moisture content was 84.2 ± 0.5%.

2.3.3. Methanogenic anaerobic seed

Methanogenic seed was obtained from the Penn State Wastewater Treatment Plant secondary anaerobic digester. The inoculum was starved for two days prior to use in the biochemical methane potential (BMP) assays. The final composition of the starved methanogenic seed was: 98.0 ± 0.0% moisture, and 75.1 ± 3.2% VS of TS.

2.4. Anaerobic bioprocessing scenarios in a biorefinery system

Raw, pretreated, and saccharified duckweed were anaerobically processed into a value cascade of end products (i.e. ethanol, VFAs, and methane, respectively) through two or three sequential anaerobic bioprocesses. The single end product yields of individual processes were also quantified. The potential of producing fertilizer as a side product from the final residuals was evaluated. After each step, the desired end product was recovered from the process liquids, and the residues were further processed. Total carbon content, rather than VS, was used as a basis of reactor dosing for various substrates in anaerobic bioprocesses due to the following advantages: (1) duckweed sequesters atmospheric carbon, and therefore determining the fate of the carbon through bioprocesses is important; (2) VS determination for process residues high

in ethanol and VFAs (i.e. stillage and acidogenic digestate) can be inaccurate since these volatile compounds are underestimated during the determination of solids content (Vahlberg et al., 2013); (3) calculating VS equivalence of methane as an end product is not practical while constructing material balances, and therefore TC provides a uniformly applicable platform for comparison; (4) inorganic carbon can also be consumed and converted to other forms during acidogenic and methanogenic digestion. The carbon to VS ratio for the raw duckweed and other substrates was calculated and used to dose the same amount of carbon in the feedstock for each unit operation; namely, pre-processed duckweed for single processes at the initial stage of a cascade, or the residues of the upstream anaerobic bioprocesses for subsequent stages of a cascade. The solid and liquid residues of each process were carried to the next process, keeping the same ratio of solids to liquids for subsequent stages. Details on the substrates used (i.e. the type of pre-processed duckweed), operation of the bioreactors, end product separation for each anaerobic bioprocess, as well as the overall product yield calculations for the value cascades, are provided in the following sections.

2.4.1. Ethanol fermentation and distillation

Only saccharified duckweed was subjected to ethanol fermentation; the raw and pretreated duckweed were excluded from the assay, since they had low levels of the monosaccharides that are fermentable by standard yeast. Following saccharification, a 0.8 g yeast pellet (dry weight) was added to each fermentation flask, which was then incubated at 32 °C while mixing at 120 rpm for 24 h. The produced gas was vented out from an outlet through the rubber stopper, and its carbon dioxide was captured in 10 M sodium hydroxide solution. Glucose and ethanol concentrations at 0 h, 12 h, and 24 h were quantified. Ethanol yields were expressed as g ethanol g glucose recovered^{-1}, and g ethanol g $TS_{duckweed}{}^{-1}$. In order to compare the ethanol yields to those of other products, grams of carbon in ethanol per gram of TC in duckweed added (g ethanol-C g $TC_{duckweed}{}^{-1}$) was also calculated.

The constituents of the fermentation flasks were then combined and transferred to a vacuum evaporation setup. In order to keep the VFAs in the stillage, the pH was increased to 7.8 ± 0.1 by 5 M sodium hydroxide addition. The ethanol distillation was performed by keeping the slurry temperature at 80 °C. After distillation, a portion of the stillage was tested for fertilizer potential. The remaining stillage was subjected to acidogenic digestion or BMP assays.

2.4.2. Acidogenic digestion and membrane separation

Batch reactors (300 ml working volume) were fed with raw, pretreated, or saccharified duckweed, or with ethanol fermentation residues to achieve a total substrate carbon loading of 10.1 ± 0.1 g L^{-1}, which is equivalent to a carbon loading of 25 g L^{-1} raw duckweed. The VS variation between reactors was less than 18%. The inoculum was added at a substrate-to-inoculum ratio of 10:1 on a VS basis calculated for raw duckweed. Initial pH values were adjusted to 9.2 after the reactors were supplemented with 4.0 g L^{-1} sodium carbonate as a buffer, which is equivalent to about 5% of the duckweed carbon input and was quantified in the carbon balance accordingly. All reactors were purged with nitrogen gas and sealed with rubber stoppers and aluminum crimp tops. Reactors were operated under mesophilic (35 °C) conditions for 10 days. Once every two days, headspace gas volume was quantified, liquid samples were collected, and the pH was adjusted to 9.2. Test reactors were run in triplicate, and controls (with no substrate) were run in duplicate. The VFA production in the control reactors was found to be negligible compared to that achieved in the active reactors; therefore, were not subtracted.

Following acidogenic digestion, the digestates were centrifuged at 2880 rgf for 30 min (Eppendorf, 5804 R, Germany). The supernatants were filtered through a 0.2 μm nylon filter and their pH values were adjusted to 4.0 using 5 M and 1 M hydrochloric acid prior to membrane separation of the VFAs. Pellets were saved to be combined with the reactor liquids following membrane separation.

Nanofiltration of the digestates was performed as described by Xiong et al. (2015) in a 200-ml dead-end nanofiltration vessel (Amicons, Stirred Cell 8200, USA) at ambient temperature, using a thin film membrane (GE Osmotics, DL, USA) with an effective filtration area of 28.7 cm^2. The vessel was pressurized to 0.5 MPa using nitrogen gas. At the beginning of each filtration process, membranes were flushed with deionized water for 30 min. Approximately 70 ml of each digestate was added to the continuously-stirred vessel. Once 70% of the original digestate volume was collected as permeate, the same amount of deionized water was added to the vessel and re-collected, again equaling 70% of the original volume. The recovery efficiency was calculated through a VFA balance over retentate, first permeate, and second permeate, on a VFA carbon basis. The retentate volume was made up to its original value of approximately 70 ml by deionized water, and was mixed back with the pellets, to be used for BMP and fertilizer assays.

2.4.3. Biochemical methane potential (BMP) assays

The BMP assays with duckweed were carried out based on the protocol proposed for bioenergy crops and organic wastes (Angelidaki et al., 2009) with slight modifications. Batch reactors (160 ml total volume, 64 ml working volume) were filled with 18 ml inoculum, equivalent to a substrate-to-inoculum ratio of 2.0 for raw duckweed on a VS basis. All test reactors were provided with substrate yielding 4.1 ± 0.03 g L^{-1} TC, which is equivalent to the value for 10 g L^{-1} raw duckweed. Sodium bicarbonate (4 g L^{-1}) was provided to the reactors as a buffer. After the initial pH was adjusted to 7.2 ± 0.3 by adding 2 M solutions of hydrochloric acid and sodium hydroxide, the bottles were purged with a 80/20 (by volume) mixture of N_2/CO_2 gas for 3 min prior to sealing with butyl rubber septa and aluminum crimp tops. Reactors were incubated at 35 ± 0.5 °C for 42 days, until the incremental gas production was less than 5% of the cumulative value. Test reactors were run in triplicate, and the controls (without substrate) were run in duplicate. Biogas volumes in control bottles were subtracted from those of the tests before reporting the biomethane yields. However, the absolute biogas values were used for carbon balances as these balances explicitly included the inorganic carbon inputs (e.g. from buffer solutions) in the controls. Biomethane yields were expressed as ml per gram of VS duckweed added (ml CH g $VS_{duckweed}{}^{-1}$, and as grams of carbon in CH_4 per gram of TC in duckweed added (g CH_4-C g $TC_{duckweed}{}^{-1}$).

2.5. Overall duckweed-to-bioproduct conversion yields and carbon balances

2.5.1. Duckweed-to-bioproduct conversion yields and carbon balances in individual reactors

In all bioprocesses, liquid, solid, and gaseous TC were quantified. The losses associated with sampling events were estimated by taking into account the sampling volumes. The VFA losses during solids drying were estimated as 55% for basic reactors (Vahlberg et al., 2013). The mass closure has been calculated as the ratio of the final to initial total carbon values.

Initial and final fractionation of TC among individual triplicate reactors were reported. Initial TC consisted of substrate (raw, pretreated, or saccharified duckweed, or the residues of the previous bioprocess), inoculum (yeast, acidogenic seed, or methanogenic seed) and buffer (sodium citrate, sodium carbonate, or sodium bicarbonate) for each bioprocess. Final TC consisted of the target bioproduct (ethanol, VFAs, or methane), slurry excluding the target chemical, and the losses in the gaseous form (i.e. carbon dioxide for ethanol fermentation and methanogenic digestion, methane and carbon dioxide for acidogenic digestion).

2.5.2. Duckweed-to-bioproduct conversion yields and carbon balances of sequential processes

Overall product yields were calculated by taking the recovery efficiencies of the products after separation into account. The fraction of the TC recovered in the form of a target product was calculated by multiplying the TC fraction of the target chemical with the recovery efficiency. The remaining (i.e. non-recovered) TC of the target product in the reactor was added to the TC value of the slurry, and accounted for in the fraction of the residue for a given bioprocess. This adjustment was done since unrecovered product could be the substrate in the next process. Carbon-to-carbon conversion yields of the sequential processes were calculated using Eqs. (1)–(3).

$$\frac{TC_{products}}{TC_{duckweed}} = \sum_{i=1}^{n} \left(\frac{\left(\frac{TC_{product}}{TC_{duckweed}}\right)_i}{1 + \beta_i \left(\frac{TC_{buffer}}{TC_{duckweed}}\right)_i} \right) \quad (1)$$

$$\left(\frac{TC_{product}}{TC_{duckweed}}\right)_i = f_{recovered\_product,i} \left(1 + \frac{f_{additives_i}}{f_{substrate_i}}\right) \prod_{j=0}^{i-1} f_{residue_j} \left(1 + \frac{f_{additives_j}}{f_{substrate_j}}\right) \quad (2)$$

$$\left(\frac{TC_{buffer}}{TC_{duckweed}}\right)_i = \frac{f_{buffer_0}}{f_{substrate_0}} + \sum_{i=1}^{n} \left[\left(\frac{f_{buffer_i}}{f_{substrate_i}}\right) \prod_{j=0}^{i-1} f_{residue_j} \left(1 + \frac{f_{additives_j}}{f_{substrate_j}}\right) \right] \quad (3)$$

where

$TC_{products}$ = TC recovered in the products (ethanol VFA and/or methane);

$TC_{duckweed}$ = TC in initial substrate (raw, pretreated, or saccharified duckweed) added;

TC_{buffer} = buffer carbon introduced in a given bioprocess;

$f_{inoculum}$ = fraction of TC in the inoculum;

f_{buffer} = fraction of TC in the buffer;

$f_{additives}$ = fraction of TC in the additives (i.e. sum of the inoculum and buffer fractions);

$f_{substrate}$ = fraction of TC in the substrate (i.e. raw, pretreated, saccharified duckweed, or the residues of the previous bioprocess) initially fed to a given bioprocess;

$f_{recovered\_product}$ = fraction of the reactor TC recovered in the form of a particular product (ethanol, VFAs or methane) after the bioprocess; and

$f_{residue}$ = fraction TC remaining in a given bioprocess, to be subjected to sequential processing.

Since inocula have negligible product yields, their influence on the calculations was neglected. However, buffers used in anaerobic processes were often a significant part of the carbon mass and could be converted into bioproduct. This effect has been taken into account as a correction (Eq. (1)), by introducing the term β_i, the buffer assimilation potential of a given conversion process, utilizing the accumulated buffer in the reactor, which takes the value of zero for ethanol fermentation and one for acidogenic digestion and methanogenic digestion.

2.6. Fertilizer potential assessment

Raw, pretreated, and anaerobically processed duckweed samples were subjected to fertilizer tests, as described in Section 2.1. The bioprocess residues involving saccharified duckweed that in bioprocessing sequences did not include ethanol as end products were excluded from this fertilizer assessment, as this route was not considered economically viable for VFA or methane production due to enzyme costs.

2.7. Statistical analysis

Data are presented as the mean ± standard deviation of triplicate samples unless specified otherwise. Significant differences between means were tested using one-way analysis of variance (ANOVA) and least significant difference (LSD) tests at a significance level of $p < 0.05$ using Minitab statistical package (Version 3.1, Minitab Inc., USA).

3. Results and discussion

3.1. Ethanol fermentation and distillation

Total maximum theoretical glucose yield in the reactors was calculated as 0.46 ± 0.0 g glucose g $TS_{duckweed}^{-1}$. After saccharification, actual glucose yield reached 0.38 ± 0.1 g glucose g $TS_{duckweed}^{-1}$ (18 ± 0.1 g L^{-1} in final reactor volume), which corresponds to 83.4 ± 0.2% glucose recovery efficiency. This value is lower than the sugar recovery reported by Xu et al. (2011), which was 96.8% of the theoretical glucose saccharification of *S. polyrrhiza* starch. The slightly low efficiency observed in the present study could be due to the assumption that all water soluble carbohydrates in the duckweed biomass were glucose.

The ethanol concentration observed in the fermentation reactor after 24 h was 8.7 ± 0.1 g L^{-1}, which corresponds to an ethanol yield of 186 ± 1.0 g ethanol kg $TS_{duckweed}^{-1}$. This result is comparable to the average value reported by Soda et al. (2015), who achieved an ethanol yield of 170 g kg^{-1} of dry *Wolffia globosa* biomass after simultaneous saccharification and fermentation (SSF) using α-amylase, amyloglucosidase, and dry yeast. Our results on a glucose basis were found to be higher than those reported by Yu et al. (2014) as 0.44 g g^{-1} (as glucose) for duckweed grown on Schenk & Hildebrandt medium and sewage wastewater, after 94% sugar recovery.

3.2. Acidogenic digestion

All reactors produced VFAs (Fig. 1), and approximately 80% of the final VFA values were achieved by day 5. Acetic acid was found to be the predominant VFA in all reactors (> 73%), as was also observed in another acidogenic digestion study of duckweed at high pH values (Calicioglu et al., 2018). High production of acetic acid can be attributed in part to the release of acetyl groups from hemicellulose under these conditions (Dahiya et al., 2015).

The final VFA concentrations ranged from 5.9 ± 0.7 to 12.3 ± 1.6 mg L^{-1} (Fig. 1). The highest VFA concentration was observed in reactors fed with saccharified duckweed (Fig. 1C), which produced a maximum rate of 4.8 g HAc_{eq} L^{-1} d^{-1} and an average rate of 1.23 g HAc_{eq} L^{-1} d^{-1}. The average final VFA composition in saccharified duckweed reactors consisted of 78.3% acetic, 16.3% propionic, 1.5% isobutyric, 2.0% n-butyric, and 1.9% isovaleric acids. These results correspond to a total of 620 ± 82 mg VFA as HAc_{eq} g VS_{added}^{-1}, under saccharified conditions, which is 2.2 times higher than that observed with raw duckweed. Since hydrolysis is the rate limiting step under anaerobic conditions (Ariunbaatar et al., 2014), the higher conversion efficiencies observed with saccharified (i.e. enzymatically hydrolyzed) duckweed is reasonable. The highest yields achieved were comparable to another acidogenic digestion study performed on a 1:1 mixture of primary and secondary wastewater treatment sludge, which achieved the highest VFA concentrations at pH 10 as 0.62 g VFA g VS_{added}^{-1} (Jankowska et al., 2015). The yield observed in our raw duckweed reactor, 288 ± 38 as HAc_{eq} g VS_{added}^{-1}, is comparable to the findings of a study conducted by Yuan et al. (2006) on acidogenic digestion of activated wastewater sludge at pH 10 and ambient temperature (233 mg VFA as HAc_{eq} g VS_{added}^{-1}). The fermented duckweed also produced similar amounts of VFAs (12.0 ± 1.3 g L^{-1}). Although fermented substrate is also previously saccharified, it is possible that the yeast cells present might not be as readily biodegradable. The yield on a VS basis (611 ± 64 mg VFA as HAc_{eq} g VS_{added}^{-1}), however, was not statistically different than that of saccharified duckweed, since the volatile solids content for the same

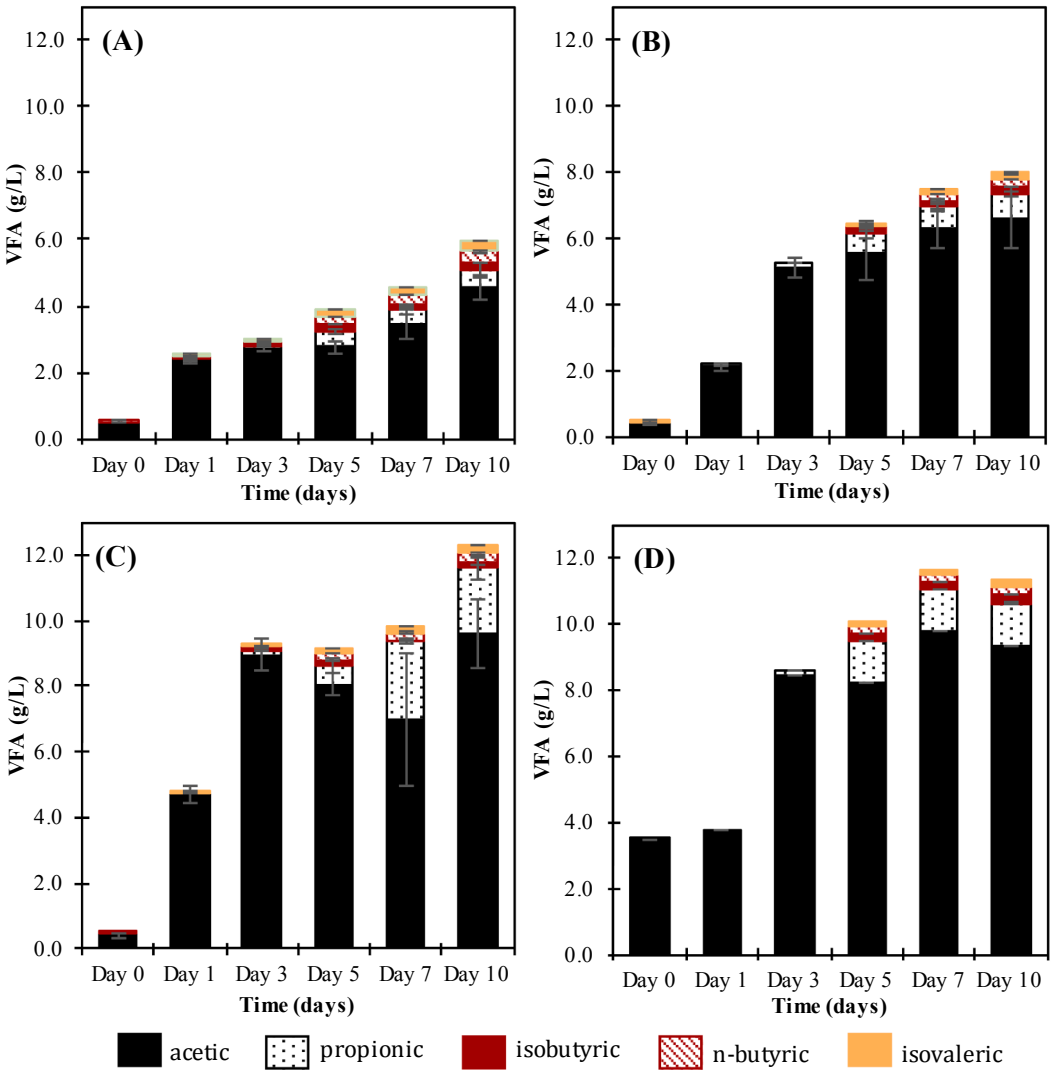

Fig. 1. Volatile fatty acid (VFA) profiles of the acidogenic duckweed reactors over ten days. Reactors were fed with: (A) raw; (B) pretreated; (C) saccharified; (D) saccharified and fermented duckweed.

amount of substrate carbon provided was lower in fermented duckweed residues.

Pretreatment also had a positive effect on VFA production, increasing the concentration by 44% to 8.5 ± 1.0 g L^{-1} and increasing the yield by 46% to 419 ± 51 mg VFA as HAc_{eq} g VS_{added}^{-1}, compared to raw duckweed.

Biogas recovery was minimal (< 30 ml g VS_{added}^{-1}) as expected under alkaline conditions in acidogenic digesters (Garcia-Aguirre et al., 2017), and predominantly consisted of CO_2. Over time, the final headspace gas compositions in the reactors changed, and the final contents were found to be: 8.6 ± 4.0% CO_2 and 0.0 ± 0.0% CH_4 for raw duckweed; 3.0 ± 0.0% CO_2 and 0.7 ± 0.1% CH_4 for pretreated duckweed; 5.7 ± 0.3% CO_2 and 0.0 ± 0.0% CH_4 for saccharified duckweed; and 3.4 ± 0.3% CO_2 and 1.6 ± 0.4% CH_4 for fermented duckweed. Hydrogen was not observed in the final headspace gas mixture of any reactor.

3.3. Biochemical methane potentials

In all reactors, approximately 90% of the total biogas production was observed in the first 21 days (Fig. 2). The biomethane yields ranged between 227 and 434 ml CH_4 g VS_{added}^{-1} at the end of 42 days (Fig. 2A–B), which is higher than the 114 ml CH_4 g VS_{added}^{-1} and reported for the anaerobic digestion of raw duckweed (Ren et al., 2018) and comparable to the yield reported by Toyama et al. (2018) as 343 ml CH_4 g VS_{added}^{-1} for *L. punctata* grown on secondary effluent of municipal wastewater.

Overall, substrates subjected to acidogenic digestion and membrane separation (Fig. 2B) yielded higher biomethane per VS than their counterparts subjected to less (or no) pre-processing (i.e. raw, pretreated, saccharified, or fermented, Fig. 2A), after their acidogenic digestion and recovery of VFAs. This general trend is due to lower VS contents per TC of the anaerobically processed substrates, although the

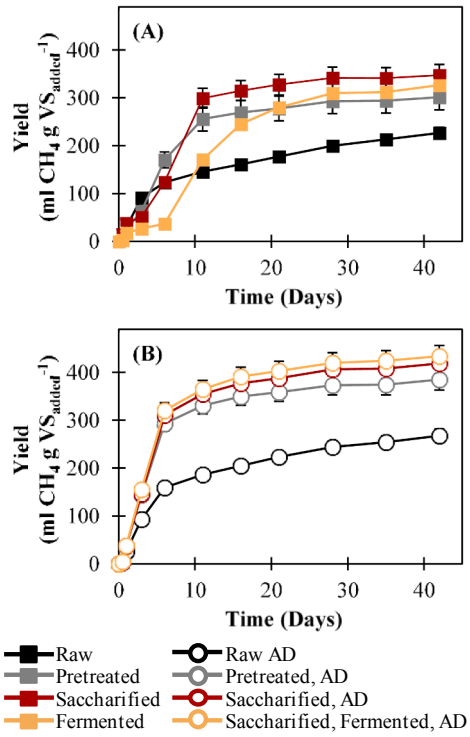

Fig. 2. Cumulative methane yields of the methanogenic duckweed reactors over 42 days. Reactors were fed with raw, pretreated, saccharified, and saccharified and fermented duckweed: (A) not subjected to acidogenic digestion; (B) subjected to acidogenic digestion and membrane separation. Control biomethane yields were subtracted from each case.

same initial TC concentration was provided to each reactor. The highest biomethane yield among all reactors was 434 ± 0.2 ml CH_4 g VS_{added}^{-1}, in the reactor with saccharified, fermented, and acidogenically-digested duckweed. This value was 62% higher than the corresponding acidogenically-digested raw duckweed reactor (268 ± 0.1 ml CH_4 g VS_{added}^{-1}), and 91% higher than the lowest observed value in the reactor fed with raw duckweed (227 ± 0.1 ml CH_4 g VS_{added}^{-1}). Considering that the VS content would also be low after two sequential bioprocesses and bioproduct recoveries, this result is reasonable. This conclusion is further supported by the methane yields as reported on the basis of added TC, which was higher in reactors fed with substrate prior to acidogenic digestion (ranging between 368 and 475 ml CH_4 g TC_{added}^{-1}) compared to those of the reactors fed with substrate after acidogenic digestion and membrane separation (ranging between 304 and 315 ml CH_4 g TC_{added}^{-1}). This comparison on the basis of added TC offers a more generalizable baseline for evaluating reactor performance and is reported in Section 3.4 on a carbon-to-carbon basis.

The highest biomethane yield observed for substrates without prior anaerobic bioprocessing was 348 ± 0.3 ml CH_4 g VS_{added}^{-1} for saccharified duckweed. The biomethane value observed by the saccharified and fermented duckweed was 327 ± 0.3 ml CH_4 g VS_{added}^{-1} which is higher than that reported for the anaerobic digestion of food waste fermentation residues of 248 ml CH_4 g VS_{added}^{-1} (Wu et al., 2015). However, the observed value is slightly lower than previous findings from a sequential ethanol fermentation and anaerobic digestion study on various duckweed sources, which reported 390 ml CH_4 g VS_{added}^{-1} (Calicioglu and Brennan, 2018). In that prior study the ethanol yield was lower, which potentially left more readily biodegradable materials for the downstream methane production. In addition, reactors fed pretreated duckweed provided the next highest yields (301 ± 0.3 ml CH_4 g VS_{added}^{-1}) to those of reactors fed fermented duckweed, with a 33% increase compared to raw duckweed biomethane yields (227 ± 0.1 ml CH_4 g VS_{added}^{-1}). However, all the results obtained in the present study are lower than the yield of 468 ml CH_4 g VS_{added}^{-1} previously reported for co-digestion of *Lemna gibba* biomass with excess sludge at a 50:20 mass ratio (Gaur et al., 2017). This might be due to the varying carbon to nitrogen ratio in these studies, as this parameter can have significant effects on the biomethane yields obtained from nitrogen-rich substrates, and can improve anaerobic digestibility if balanced with a co-substrate (Calicioglu and Demirer, 2017).

3.4. Overall duckweed-to-bioproduct conversion yields and material balances

3.4.1. Duckweed-to-bioproduct conversion yields and carbon balances in individual reactors

The fractional distribution of bioproduct yields on a carbon basis for all bioprocesses treated as individual unit operations is illustrated in Fig. 3. The fractional carbon distribution presented as initial and final %TC for each component associated with the reactors. The mass closure differences between initial and final TC values in the reactors were calculated as 4.3 ± 0.2% for ethanol fermentation, and ranged between 4.3% and 18.0% for acidogenic digestion, and between 3.5% and 8.0% for methanogenic digestion.

Saccharified duckweed produced the highest carbon-to-carbon conversion, for both acidogenic digestion (53.5 ± 0.04%) and methanogenic digestion (22.6 ± 0.6%) reactors. Ethanol fermentation and methanogenic digestion resulted in similar yields on a carbon-to-carbon basis.

Note that the yields reported in this section are for the individual unit operations; i.e., they do not take into account the reduced availability of substrate from one process to the next in a sequential application, or the recovery efficiencies of the target bioproducts. The actual yields, taking into account these essential aspects for a biorefinery, are provided in the following Section 3.4.2.

3.4.2. Duckweed-to-bioproduct conversion yields of sequential processes

The duckweed-to-bioproduct conversion yields of sequential processes were calculated by using Eq. (1) for one, two, and three processes (Fig. 4). The recovery efficiencies were reported as 83.0 ± 0.7% for ethanol fermentation, and ranged between 94.7% and 98.3% for acidogenic digestion. The recovery efficiencies were assumed as 100 ± 5% for methanogenic digestion, since only the gaseous (i.e. already separated) methane was used for the yield calculations, and dissolved methane was not taken into account. All values used in the calculations (Eqs. (1)–(3)) for individual reactors are given on, Table A1.

When individual processes are compared, the highest conversion efficiency (on a TC basis) was observed for acidogenic digestion of saccharified duckweed, as 0.57 g $TC_{products}$ g $TC_{duckweed}^{-1}$. This value was followed by acidogenic digestion of pretreated duckweed (0.39 g $TC_{products}$ g $TC_{duckweed}^{-1}$), which corresponds to a 56% increase compared to its untreated (raw) counterpart. The lowest carbon conversion value was achieved by ethanol fermentation (0.19 g $TC_{products}$ g $TC_{duckweed}^{-1}$), which could be increased by improving the separation efficiency.

In all sequential scenarios, the residuals of upstream bioprocesses were successfully valorized. The highest overall conversion yield among

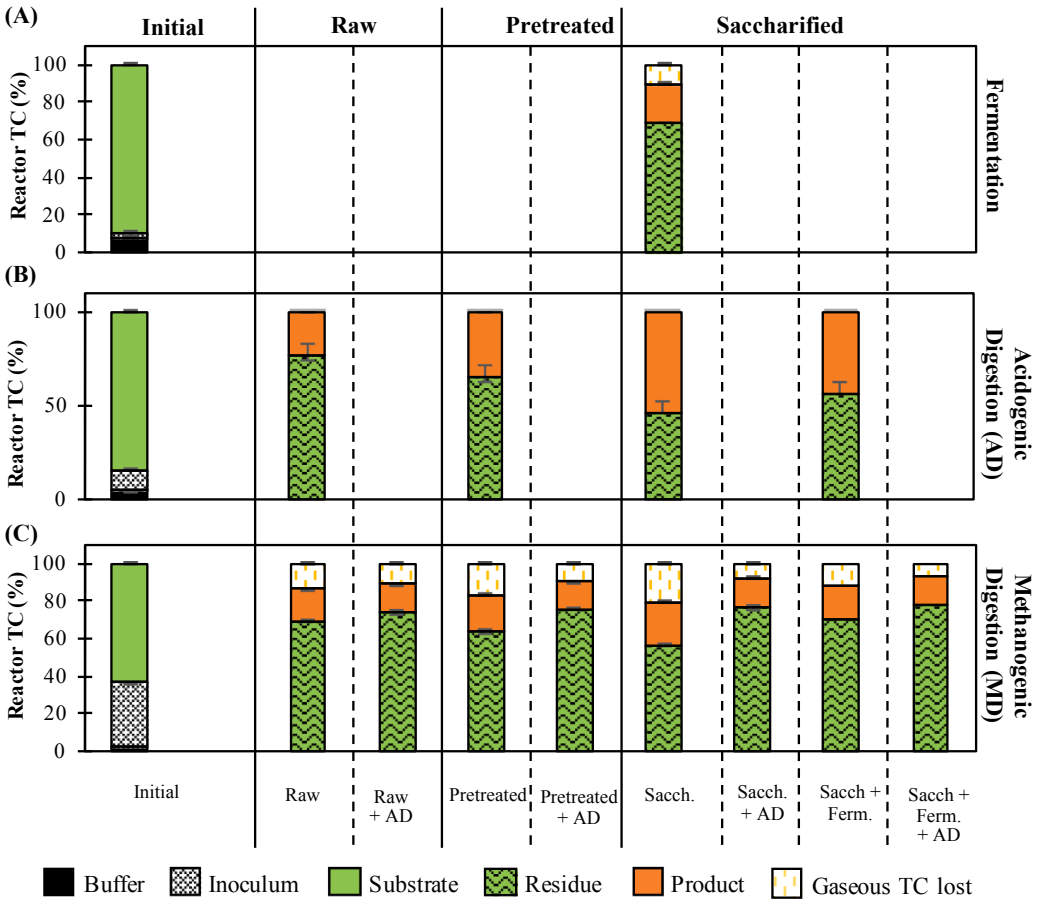

Fig. 3. Percent initial and final carbon contents of the bioreactors fed with raw, pretreated, and saccharified (sacch.) duckweed and subjected to: (A) ethanol fermentation (ferm.); (B) acidogenic digestion (AD); (C) methanogenic digestion (MD). The desired product in each process was ethanol (A), VFAs (B), or methane (C).

all scenarios was 0.69 g $TC_{products}$ g TC_{added}^{-1}, which was achieved by subjecting duckweed sequentially to acidogenic digestion and then methanogenic digestion. This scenario was very closely followed by another sequential scenario involving all three anaerobic bioprocesses investigated, cascading in the order of ethanol fermentation, acidogenic digestion, and methanogenic digestion (0.66 g $TC_{products}$ g $TC_{duckweed}^{-1}$). The slightly lower yield observed in these three sequential processes might be due to the carbon losses occurring in the upstream ethanol fermentation process. Since one mole of CO_2 is released per mole of ethanol produced, less carbon for the downstream processes might remain, whereas the carbon losses in acidogenic digestion were observed to be minimal in this study (Fig. 3B). This carbon-to-carbon conversion yield was much higher than the 0.22 g $TC_{products}$ g $TC_{duckweed}^{-1}$ previously reported for *L. punctata* grown on secondary wastewater effluents, when ethanol was targeted individually (Toyama et al., 2018). This best C conversion yield in the present study was similar to that reported by Kaur et al. (2019), who achieved 70% conversion of the organic carbon in duckweed (*L. minor*) to methane through a two-stage anaerobic digestion process with separated acidogenesis and methanogenesis.

The bioprocess sequence of ethanol fermentation followed by anaerobic digestion (i.e without acidogenic digestion) had a significantly lower carbon product yield (Fig. 4). The total yield of ethanol and methane from this sequence was 0.41 ± 0.06 g $TC_{products}$ g $TC_{duckweed}^{-1}$, corresponding to a bioenergy yield of 13.9 kJ g $TS_{duckweed}^{-1}$ when calculated as reported previously (Calicioglu and Brennan, 2018). This energy yield is comparable to the bioenergy yield obtained from sequential ethanol fermentation and anaerobic digestion of pretreated corn stover (15 kJ g^{-1}) (Bondesson et al., 2013). However, it must be noted that the moisture content of the duckweed is substantially higher than that of terrestrial biomass, and the costs of handling that liquid and concentrating solids to loading rates required for commercial systems would increase biorefinery system costs. Therefore, targeting higher-value co-products such as carboxylic acids may be essential for the profitability of duckweed biorefineries.

4. Fertilizer potential

The values for total nitrogen (TN), total ammonia nitrogen (TAN), total phosphorus, and total potassium of reactor effluents from individual and sequential processes are provided in Fig. 5. Overall, the nutrient concentrations on a mg kg^{-1} basis increased proportionally with the number of sequential processes. This was an expected result since the carbon in the biomass has been recovered in the form of bioproducts and the total mass was therefore reduced. Two exceptions to this pattern were observed for downstream sequences associated

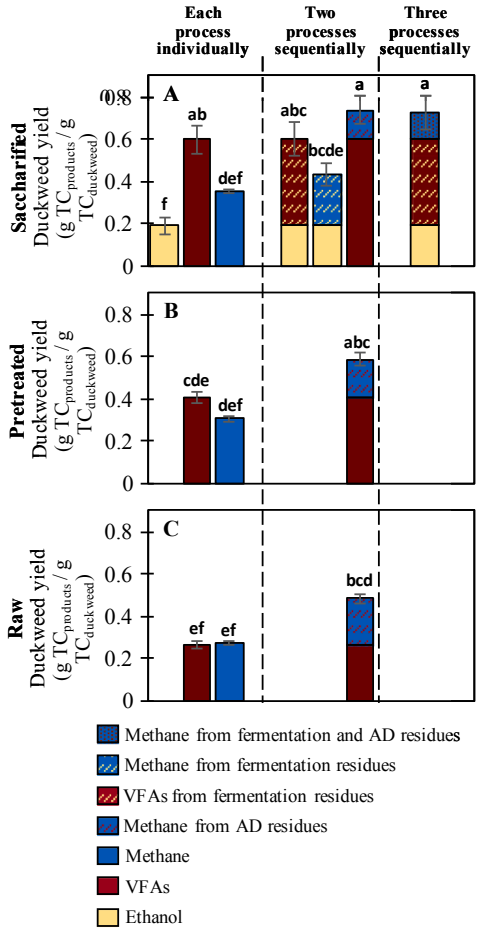

Fig. 4. Carbon-to-carbon conversion yields as a result of individual bioprocesses, two sequential bioprocesses, and three sequential bioprocesses for: (A) saccharified; (B) pretreated; (C) raw duckweed. Statistically insignificantly different stacked columns share a letter.

with pretreated duckweed and ethanol fermentation residue, as their TN concentrations were higher after acidogenic digestion, compared to sequential acidogenic and methanogenic digestion. The TN associated with the high microbial biomass as well as the high VFA recoveries observed during acidogenic digestion under these two conditions would both have been increased TN concentrations, while the subsequent methanogenic digestion involved both dilution of the nutrient concentrations with seed sludge and conversion of TN to TAN through endogenous respiration during the transition to the much lower microbial biomass concentrations in methanogenic digestion.

As expected, the TAN concentrations also increased with the degree of bioprocessing (Möller and Müller, 2012). However, the TAN content of the acidogenic digesters was relatively low, which might be due to volatilization of ammonia at operating pH values of 9.2, and thus would result in a loss of fertilizer capacity.

In general, the nitrogen, phosphorus, and potassium concentrations observed in the study were in alignment with the literature. For instance, Mulbry et al. (2005) reported N, P, and K concentrations of 45, 7.3, and 9.1 g kg^{-1}, respectively, for algal turf scrubber biomass grown on anaerobically digested dairy manure, and Wilkie and Mulbry (2002) reported 79.2, 15.4, 11.3 g kg^{-1}, respectively, for dried benthic freshwater algal biomass grown on digested dairy manure.

5. Conclusions

In this study it was shown that up to approximately 70% of the biomass carbon could be valorized by sequential anaerobic bioprocessing of wastewater-derived duckweed biomass, targeting VFAs and biomethane as end products. A series of three sequential processes, targeting ethanol, VFAs, and biomethane, did nearly as well resulting in a combined yield of 66% on a carbon basis. Saccharified duckweed showed the highest performance both for individual unit operations and sequential processes in terms of carbon-to-carbon conversion. While these technical conversion rates appear promising, it will be important to compare the economic feasibility of two and three sequential processes for a commercial biorefinery configuration. E-Supplementary data of this work can be found in the online version of the paper.

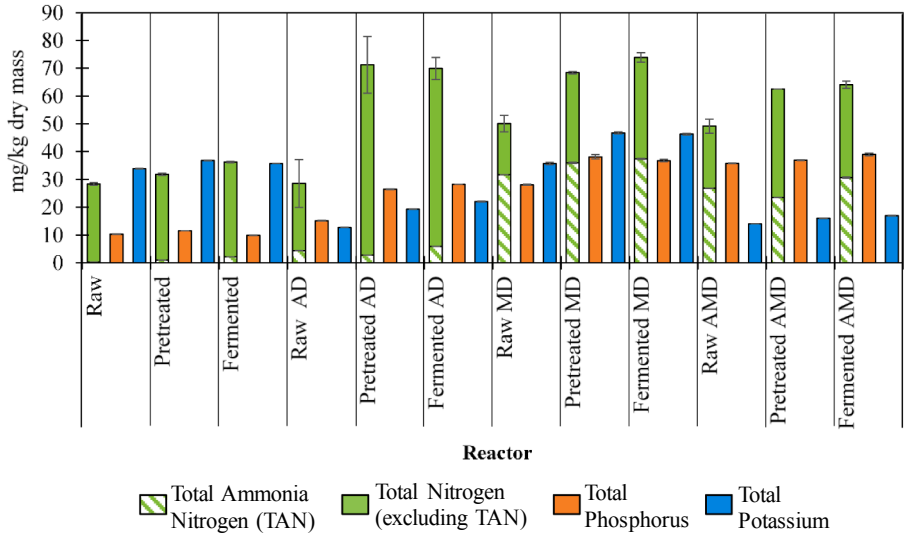

Fig. 5. Fertilizer potentials of raw and pretreated duckweed and reactor residuals following individual or sequential ethanol fermentation, acidogenic digestion (AD) and methanogenic digestion (MAD) in terms of total nitrogen (TN as N), total phosphorus, and potassium concentrations on a dry basis. Stacked bars represent total ammonifiable nitrogen (TAN) and other nitrogen species.

Acknowledgements

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Appendix A

Table A1
Descriptive table for values in Eqs. (1)–(3).

Variables	Processes								
	Saccharified Duckweed			Pretreated Duckweed			Raw Duckweed		
	Ethanol Fermentation	Acidogenic Digestion	Methanogenic Digestion*	Ethanol Fermentation	Acidogenic Digestion	Methanogenic Digestion	Ethanol Fermentation	Acidogenic Digestion	Methanogenic Digestion
f residue,0	1.00	1.00	1.00	n.a.	1.00	1.00	n.a.	1.00	1.00
f redisue, 1	0.73	0.49	0.63	n.a.	0.65	0.70	n.a.	0.77	0.75
f residue,2	na	0.57	0.76; 0.83	n.a.	na	0.81	n.a.	na	0.79
f residue, 3	n.a.	n.a.	0.84	n.a.	n.a.	na	n.a.	n.a.	na
f recovered_product, 1	0.17	0.51	0.23	n.a.	0.34	0.19	n.a.	0.22	0.17
f recovered_product, 2	na	0.43	0.19, .15	n.a.	na	0.15	n.a.	na	0.15
f recovered_product, 3	na	na	0.14	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
f additives, 0	0.00	0.08	0.08	n.a.	0.00	0.00	n.a.	0.00	0.00
f additives, 1	0.10	0.15	0.37	n.a.	0.15	0.37	n.a.	0.15	0.37
f additives, 2	na	0.15	0.37; 0.37	n.a.	0.15	0.37	n.a.	0.15	0.37
f additives, 3	na	na	0.37	n.a.	na	0.37	n.a.	na	0.37
f substrate 0	1.00	0.92	0.92	n.a.	1.00	1.00	n.a.	1.00	1.00
f substrate, 1	0.90	0.85	0.63	n.a.	0.85	0.63	n.a.	0.85	0.63
f substrate, 2	na	0.85	0.63; 0.63	n.a.	na	0.63	n.a.	na	0.63
f substrate, 3	na	na	0.63	n.a.	na	na	n.a.	na	na
f buffer, 0	0.00	0.08	0.08	n.a.	0.00	0.00	n.a.	0.00	0.00
f buffer, 1	0.08	0.04	0.03	n.a.	0.04	0.03	n.a.	0.04	0.03
f buffer, , 2	na	0.04	0.03	n.a.	0.04	0.03	n.a.	0.04	0.03
f buffer, 3	na	na	0.03	n.a.	na	0.03	n.a.	na	0.03
beta1	0.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	1.00
beta2	na	1.00	1.00	na	1.00	1.00	na	1.00	1.00
beta3	na	na	1.00	na	na	1.00	na	na	1.00

*If two values presented in one column, first is for sequential ethanol fermentation and methanogenic digestion, and the second value s for sequential acidogenic digestion and methanogenic digestion processes.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.121716.

References

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927–934. https://doi.org/10.2166/wst.2009.040.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L., 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Appl. Energy 123, 143–156.

Baliban, R.C., Elia, J.A., Floudas, C.A., Xiao, X., Zhang, Z., Li, J., Cao, H., Ma, J., Qiao, Y., Hu, X., 2013. Thermochemical conversion of duckweed biomass to gasoline, diesel, and jet fuel: process synthesis and global optimization BT – Industrial & engineering chemistry research. Ind. Chem. Res.

Biddy, M.J., Davis, R., Humbird, D., Tao, L., Dowe, N., Guarnieri, M.T., Linger, J.G., Karp, E.M., Salvachúa, D., Vardon, D.R., Beckham, G.T., 2016. The techno-economic basis for coproduct manufacturing to enable hydrocarbon fuel production from lignocellulosic biomass. ACS Sustain. Chem. Eng. 4, 3196–3211.

Bondesson, P.-M., Galbe, M., Zacchi, G., 2013. Ethanol and biogas production after steam pretreatment of corn stover with or without the addition of sulphuric acid. Biotechnol. Biofuels 6, 11. https://doi.org/10.1186/1754-6834-6-11.

Bondesson, P.M., 2008. Combined production of bioethanol and biogas from wheat straw. J. Environ. Manage. 86, 481–497.

Calicioglu, O., Brennan, R.A., 2018. Sequential ethanol fermentation and anaerobic digestion increases bioenergy yields from duckweed. Bioresour. Technol. 257, 344–348.

Calicioglu, O., Demirer, G.N., 2017. Carbon-to-nitrogen and substrate-to-inoculum ratio adjustments can improve co-digestion performance of microalgal biomass obtained from domestic wastewater treatment. Environ. Technol. (United Kingdom).

Calicioglu, O., Shreve, M.J., Richard, T.L., Brennan, R.A., 2018. Effect of pH and temperature on microbial community structure and carboxylic acid yield during the acidogenic digestion of duckweed. Biotechnol. Biofuels 1–19.

Cheng, J.J., Stomp, A.M., 2009. Growing Duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. Clean Soil Air Water 37, 17–26.

Cherubini, F., 2010. The biorefinery concept: using biomass instead of oil for producing energy and chemicals. Energy Convers. Manage. 51, 1412–1421.

Cherubini, F., Jungmeier, G., Wellisch, M., Willke, T., Skiadas, I., van Ree, R., de Jong, E., 2009. Toward a common classification approach for biorefinery systems. Biofuels. Bioprod. Biorefining 3, 534–546. https://doi.org/10.1002/bbb.172.

Cui, W., Cheng, J.J., 2015. Growing duckweed for biofuel production: a review. Plant Biol. 17, 16–23. https://doi.org/10.1111/plb.12216.

Dahiya, S., Sarkar, O., Swamy, Y.V., Venkata Mohan, S., 2015. Acidogenic fermentation of

food waste for volatile fatty acid production with co-generation of biohydrogen. Bioresour. Technol. 182, 103–113.
Dererie, D.Y., Trobro, S., Momeni, M.H., Hansson, H., Blomqvist, J., Passoth, V., Schnürer, A., Sandgren, M., Ståhlberg, J., 2011. Improved bio-energy yields via sequential ethanol fermentation and biogas digestion of steam exploded oat straw. Bioresour. Technol. 102, 4449–4455. https://doi.org/10.1016/j.biortech.2010.12.096.
Siedlecka, E.M., Kumirska, J., Ossowski, T., Glamowski, P., Gołębiowsk, M., Gajdus, J., Kaczyński, Z., Stepnowski, P., 2008. Determination of volatile fatty acids in environmental aqueous samples. Polish J. Environ. Stud. 17, 351–356.
El-Mashad, H.M., 2013. Kinetics of methane production from the codigestion of switchgrass and Spirulina platensis algae. Bioresour. Technol. 132, 305–312.
Garcia-Aguirre, J., Aymerich, E., González-Mtnez de Goñi, J., Esteban-Gutiérrez, M., 2017. Selective VFA production potential from organic waste streams: assessing temperature and pH influence. Bioresour. Technol. 244, 1081–1088.
Gaur, R.Z., Khan, A.A., Suthar, S., 2017. Effect of thermal pre-treatment on co-digestion of duckweed (Lemna gibba) and waste activated sludge on biogas production. Chemosphere 174, 754–763. https://doi.org/10.1016/j.chemosphere.2017.01.133.
Ge, X., Zhang, N., Phillips, G.C., Xu, J., 2012. Growing Lemna minor in agricultural wastewater and converting the duckweed biomass to ethanol. Bioresour. Technol. 124, 485–488.
Gulati, M., Kohlmann, K., Ladisch, M.R., Hespell, R., Bothast, R.J., 1996. Assessment of ethanol production options for corn products. Bioresour. Technol. 58, 253–264.
Holtzapple, M.T., Davison, R.R., Ross, M.K., Albrett-Lee, S., Nagwani, M., Lee, C.M., Lee, C., Adelson, S., Kaar, W., Gaskin, D., Shirage, H., Chang, N.S., Chang, V.S., Loescher, M.E., 1999. Biomass conversion to mixed alcohol fuels using the MixAlco process. Appl. Biochem. Biotechnol. 77–79, 609–631. https://doi.org/10.1385/ABAB:79:1-3:609.
Jankowska, E., Chwiałkowska, J., Stodolny, M., Oleskowicz-Popiel, P., 2015. Effect of pH and retention time on volatile fatty acids production during mixed culture fermentation. Bioresour. Technol. 190, 274–280. https://doi.org/10.1016/j.biortech.2015.04.096.
Jung, H., Baek, G., Kim, J., Shin, S.G., Lee, C., 2016. Mild-temperature thermochemical pretreatment of green macroalgal biomass: effects on solubilization, methanation, and microbial community structure. Bioresour. Technol. 199, 326–335.
Kaur, M., Srikanth, S., Kumar, M., Sachdeva, S., Puri, S.K., 2019. An integrated approach for efficient conversion of Lemna minor to biogas. Energy Convers. Manage. 180, 25–35.
Keegan, D., Kretschmer, B., Elbersen, B., Panoutsou, C., 2013. Cascading use: a systematic approach to biomass beyond the energy sector. Biofuels Bioprod. Biorefining.
Kehili, M., Schmidt, L.M., Reynolds, W., Zammel, A., Zetzl, C., Smirnova, I., Allouche, N., Sayadi, S., 2016. Biorefinery cascade processing for creating added value on tomato industrial by-products from Tunisia. Biotechnol. Biofuels 9.
Les, D.H., Crawford, D.J., Landolt, E., Gabel, J.D., Kimball, R.T., Rettig, J.H., 2002. Phylogeny and systematics of Lemnaceae, the Duckweed family. Syst. Bot. 27, 221–240.
Liu, Y., Nie, Y., Lu, X., Zhang, X., He, H., Pan, F., Zhou, L., Liu, X., Ji, X., Zhang, S., 2019. Cascade utilization of lignocellulosic biomass to high-value products. Green Chem.
Mertens, A., Van Lancker, J., Buysse, J., Lauwers, L., Van Meensel, J., 2019. Overcoming non-technical challenges in bioeconomy value-chain development: learning from practice. J. Clean. Prod. 231, 10–20. https://doi.org/10.1016/j.jclepro.2019.05.147.
Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. Eng. Life Sci. 12, 242–257.
Mulbry, W., Westhead, E.K., Pizarro, C., Sikora, L., 2005. Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresour. Technol. 96, 451–458. https://doi.org/10.1016/j.biortech.2004.05.026.
Peters, J.B., 2003. Recommended Methods of Manure Analysis. Soils. Wisc. Edu. https://doi.org/Recommended Methods of Manure Analysis (A3769).
Ren, H., Jiang, N., Wang, T., Mubashar Omar, M., Ruan, W., Ghafoor, A., 2018. Enhanced biogas production in the duckweed anaerobic digestion process. J. Energy Resour. Technol. 140, 41805. https://doi.org/10.1115/1.4039782.
Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Nrel, J.W., 2008. Determination of total solids in biomass and total dissolved solids in liquid process samples. Natl. Renew. Energy Lab. 9 https://doi.org/NREL/TP-510-42621.
Sluiter, A., Hames, B., Ruiz, R.O., Scarlata, C., Sluiter, J., Templeton, D., Energy, D. of, Dötsch, A., Severin, J., Alt, W., Galinski, E.A, Kreft, J.-U., 2004. Determination of Ash in Biomass. Microbiology 154, 2956–69. https://doi.org/TP-510-42622.
Soda, S., Ohchi, T., Piradee, J., Takai, Y., Ike, M., 2015. Duckweed biomass as a renewable biorefinery feedstock: ethanol and succinate production from Wolffia globosa. Biomass Bioenergy 81, 364–368. https://doi.org/10.1016/j.biombioe.2015.07.020.
Su, H., Zhao, Y., Jiang, J., Lu, Q., Li, Q., Luo, Y., Zhao, H., Wang, M., 2014. Use of duckweed (Landoltia punctata) as a fermentation substrate for the production of higher alcohols as biofuels. Energy Fuels 28, 3206–3216. https://doi.org/10.1021/ef500335h.
Tasker, T.L., Piotrowski, P.K., Dorman, F.L., Burgos, W.D., 2016. Metal associations in marcellus shale and fate of synthetic hydraulic fracturing fluids reacted at high pressure and temperature. Environ. Eng. Sci. 33, 753–765.
Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185–197.
Toyama, T., Hanaoka, T., Tanaka, Y., Morikawa, M., Mori, K., 2018. Comprehensive evaluation of nitrogen removal rate and biomass, ethanol, and methane production yields by combination of four major duckweeds and three types of wastewater effluent. Bioresour. Technol. 250, 464–473. https://doi.org/10.1016/j.biortech.2017.11.054.
Vahlberg, C., Nordell, E., Wiberg, L., 2013. Method for correction of VFA loss in determination of dry matter in biomass. Malmö.
Wilkie, A.C., Mulbry, W.W., 2002. Recovery of dairy manure nutrients by benthic freshwater algae recovery of dairy manure nutrients by benthic freshwater algae. Bioresour. Technol. 8524, 81–91. https://doi.org/10.1016/S0960-8524(02)00003-2.
Wu, C., Wang, Q., Xiang, J., Yu, M., Chang, Q., Gao, M., Sonomoto, K., 2015. Enhanced productions and recoveries of ethanol and methane from food waste by a three-stage process. Energy Fuels 29, 6494–6500. https://doi.org/10.1021/acs.energyfuels.5b01507.
Xiong, B., Richard, T.L., Kumar, M., 2015. Integrated acidogenic digestion and carboxylic acid separation by nanofiltration membranes for the lignocellulosic carboxylate platform. J. Membr. Sci. 489, 275–283. https://doi.org/10.1016/j.memsci.2015.04.022.
Xu, J., Cui, W., Cheng, J.J., Stomp, A.M., 2011. Production of high-starch duckweed and its conversion to bioethanol. Biosyst. Eng. 110, 67–72.
Yu, C., Sun, C., Yu, L., Zhu, M., Xu, H., Zhao, J., Ma, Y., Zhou, G., 2014. Comparative analysis of duckweed cultivation with sewage water and SH media for production of fuel ethanol. PLoS One 9, 1–15. https://doi.org/10.1371/journal.pone.0115023.
Yuan, H., Chen, Y., Zhang, H., Jiang, S., Zhou, Q., Gu, G., 2006. Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. Environ. Sci. Technol. 40, 2025–2029. https://doi.org/10.1021/es052252b.
Zhao, X., Elliston, A., Collins, S.R.A., Moates, G.K., Coleman, M.J., Waldron, K.W., 2014. Enzymatic saccharification of duckweed (Lemna minor) biomass without thermo-physical pretreatment. Biomass Bioenergy 47, 354–361.
Zhao, Y., Fang, Y., Jin, Y., Huang, J., Bao, S., Fu, T., He, Z., Wang, F., Wang, M., Zhao, H., 2015. Pilot-scale comparison of four duckweed strains from different genera for potential application in nutrient recovery from wastewater and valuable biomass production. Plant Biol. 17, 82–90. https://doi.org/10.1111/plb.12204.