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A synergistic combination of algal wastewater treatment and hydrothermal biofuel production maximized by nutrient and carbon recycling†

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This study introduces and analyzes a novel system for algal biofuel production that synergistically integrates algal wastewater treatment with hydrothermal liquefaction (HTL) of wastewater biosolids and algae into bio-crude oil. This system maximizes the biofuel potential of wastewater inputs by internally capturing and recycling carbon and nutrients – a powerful concept referred to as multi-cycle nutrient reuse, which amplifies waste nutrients into multiple cycles of algal biomass and bioenergy production. We call this system "Environment-Enhancing Energy" (E^2-Energy) because it can simultaneously improve conventional wastewater treatment by nutrient removal and production of a large amount of biofuel co-products. Moreover, E^2-Energy resolves several key bottlenecks commonly associated with large-scale algal biofuel production including: contamination of target high-oil algal cultures, high nutrient costs/usage, unsustainable fresh water usage, and large energy inputs for dewatering/extraction. A series of algal cultivation and HTL experiments were conducted to confirm the primary steps and performance characteristics of the E^2-Energy system. These experiments showed: (1) low-oil, mixed algal–bacterial biomass can be successfully cultured with the recycled HTL aqueous product; (2) both organics and nutrients are removed from wastewater during algal–bacterial biomass production (63–95% reduction); (3) this low-oil, algal–bacterial biomass can be converted into bio-crude oil *via* HTL with a high yield (~50%) and a net positive energy balance; and (4) the HTL step re-releases nutrients to an aqueous phase product that can be recycled back to step (1). This repeating loop of steps 1–4 facilitates multi-cycle reuse of nutrients and thus provides biomass amplification.‡ A mathematical model was also developed using STELLA® to simulate mass balances for long-term E^2-Energy operations with internal recycling of nutrients and carbon. The model results showed that E^2-Energy can amplify the biomass and biofuel production from wastewater by up to 10 times, which gives it the potential to replace all US petroleum imports using only current wastewater feedstocks and carbon dioxide from the atmosphere or point sources. Thus, E^2-Energy represents a major paradigm shift—where wastewater treatment systems become optimized biofuel producers with enhanced effluent quality, which provides a viable and advantageous pathway to sustainable, carbon-neutral energy independence.

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1. Introduction

Current fossil fuel usage is widely recognized as unsustainable because of diminishing supplies and a significant contribution to greenhouse gas (GHG) emissions. Renewable, carbon-neutral fuels are necessary for environmental and economic sustainability, and biofuels from biomass are one of the most feasible alternatives to petroleum. Among biomass materials, algae are one of the most promising feedstocks for next generation biofuels because many algal species have higher biomass productivity than terrestrial crops, and algae can grow on marginal land and water bodies, which results in less competition with food production for arable land.[1] Algae also have an important environmental advantage because they can be grown in wastewater and improve water quality. In contrast, most biofuel paradigms increase the competition for fresh water resources.[2]

Despite the apparent advantages of algal biofuels, significant economic, technical and environmental challenges still remain to be solved for scaling-up of algal biofuel production. Most of the current algal biofuel technologies have focused on cultivating relatively pure, high-oil algal species and extracting the oil for conversion to biodiesel *via* transesterification. Maintaining a pure culture of high-oil algae is difficult, but attempts

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are made through species selection, genetic modification and/or strict control of cultivation conditions. However, high-oil algae tend to grow slower than most low-oil algal strains and bacteria.[3–5] Thus, the need for selective production of high-oil algae often leads to significant contamination problems.[2] Another key practical limitation for the algal biofuel industry is obtaining a net positive cost and energy balance for the extraction of biofuel from wet algal biomass.[6] Multiple recent life cycle analyses showed that drying algal biomass sufficiently for conventional oilseed extraction techniques consumes more than 90% of the energy content of the algal oils.[7,8] Thus, future algal bio-energy schemes should avoid the need for dry algal biomass, and instead must be able to use wet biomass or algal species that excrete their oils from live cells.

Finally, several authors have raised concerns about the large amount of freshwater and fertilizer needed for full-scale algal biofuel production.[1,9] It has been estimated that meeting the US Energy Independence and Security Act (EISA) federal mandates for producing one billion gallons of biomass-based diesel with algal feedstocks would require 1238 billion gallons per year of fresh water (10% of US public water usage) and 564 million kg N (5% of US usage).[10] If purchased, the water and nutrient inputs significantly raise the cost of algal biofuels. Lundquist *et al.*[11] showed that algal biofuels produced with purchased inputs would exceed $400 per barrel, but integration with wastewater inputs and infrastructure could lower the net costs of algal biofuel production below $30 per barrel. Therefore, it is important to develop algal biofuel paradigms that integrate with wastewater treatment.

Hydrothermal liquefaction (HTL) is a thermochemical process that is promising for the conversion of wet algal feedstocks into biofuels.[12] HTL uses elevated temperatures (200–400 °C) and pressures (10–15 MPa) to convert organic solids in the feedstock into four products: (1) bio-crude oil, (2) solid residue, (3) gas rich in carbon dioxide, and (4) wastewater with high soluble concentrations of both organics and nutrients. HTL does not just extract oil, but also converts proteins and carbohydrates into oil, so the oil yield is much higher than the lipid content of the algal feedstock.[12,13] Therefore, a variety of feedstocks including bacteria, wastewater sludges,[14–16] and fast-growing, low-lipid algae,[17,18] have all been successfully converted into bio-crude oil *via* HTL. Thus, HTL resolves the contamination problems associated with current algal biodiesel paradigms. Additionally, HTL resolves the energy balance issues because water serves as the reaction medium for HTL, which avoids the need for biomass drying. After HTL, the bio-crude oil product is self-separating from the aqueous wastewater product. Thus, wet algal biomass feedstocks (20–30% solid content) are acceptable for HTL, which minimizes the energy used for dewatering algae and greatly improves both the net economic and energy returns for algal biofuels.

Post-hydrothermal liquefaction wastewater (PHWW) is a high-strength wastewater that can accumulate most of the feedstock nutrients (approximately 80%) and some of the organics (up to 40%),[17] which provides a significant opportunity for nutrient and carbon recycling. PHWW recycled back to the algae culturing system can allow for multiple cycles of algae growth on each aliquot of incoming nutrients, which maximizes bioenergy production per unit of nutrient input. This approach has been investigated in recent studies using HTL wastewater[19–21] and an earlier study suggested a similar approach but used a recondensed wastewater from gasification.[22] These studies show that nutrients in wastewaters from thermochemical conversion processes can be used for algae cultivation, but that significant dilution was required (50–500 times). These studies did not however identify a viable and sustainable source of dilution water and raised other important questions about how this nutrient recycling can be incorporated into an algae biofuel production system. This study addresses these issues in pursuit of an optimized system integrating algal wastewater treatment and bioenergy production including original process modeling to quantify the specific benefits of nutrient recycling and analyze the national implications for sustainable biofuels.

Specifically, this study investigates a novel integrated waste-to-energy system that we refer to as Environment-Enhancing Energy (E^2-Energy) that synergistically combines algal wastewater treatment with large-scale bioenergy production *via* hydrothermal liquefaction as shown in Fig. 1. The objectives of this study are to experimentally confirm the feasibility of the "Four Key Steps" of E^2-Energy as noted below, and then analyze overall mass balances and system impacts using a mathematical model that can simulate various application scenarios.

In the proposed E^2-Energy system, wastewaters from a variety of sources (*e.g.*, municipal, livestock, food processing) can be initially separated into a concentrated biosolids fraction and a dilute liquid fraction by common physicochemical processes (*e.g.*, sedimentation, filtration). Mixed cultures of low-lipid, fast-growing algae and bacteria are then cultivated in a combination of the dilute liquid wastewater fraction and recycled PHWW (Key Step 1). As the algae and bacteria grow symbiotically, the wastewater is treated by providing removal of organics and nutrients (Key Step 2). Note that the energy input for aerobic breakdown of wastewater contaminants is reduced by the oxygen provided during algal photosynthesis. Subsequently, the mixed culture biomass is harvested, and combined with the concentrated biosolids separated from the initial wastewater. This mixture is then fed into a HTL reactor for biofuel production (Key Step 3). The HTL process also generates a CO_2-rich gaseous product and strong wastewater

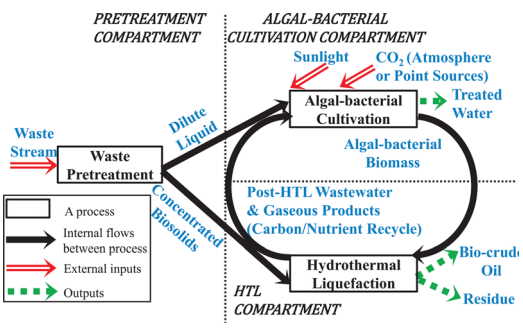

Fig. 1 Simplified schematic of the Environment-Enhancing Energy process for integrated wastewater treatment and biofuel production.

(PHWW) with re-released organics/nutrients (Key Step 4), which are recycled back to the algal–bacterial cultivation system for reuse. This recycling can be repeated again and again over multiple cycles of algal growth, harvesting and biofuel conversion that leverages the nutrient content of wastewater into maximum bioenergy quantities, which can be many times the original wastewater energy content.

The E^2-Energy system elegantly resolves several key bottlenecks in other current approaches to algal biofuel production, and provides significant environmental benefits, including carbon capture and wastewater nutrient removal. This novel approach embodies a significant paradigm change and brings together two important goals of modern society – "energy production" and "environmental protection" – into a complementary relationship, whereas historically these goals have most often been antagonistic. This harmonious combination is critically important for addressing the major challenges of a growing world population including energy security, climate change, quality of water resources and sustainable development.

2. Material and methods

2.1. Experiments

2.1.1. Key Step 1: algae cultivation in different concentrations of post-hydrothermal liquefaction wastewater (PHWW)

Growth medium. The cultivation medium was prepared from a combination of filtered municipal wastewater (WW) and PHWW from liquefaction of *Spirulina* (PHWW-Spirulina). The municipal WW was collected from the primary clarifier at a local WW treatment plant (Urbana-Champaign Sanitary District, UCSD) and filtered through a 1.5 μm pore size glass fiber filter (Whatman 934-AH) before usage. PHWW-Spirulina was recovered from a pilot-scale continuous HTL test using the blue-green alga *Spirulina* as the feedstock.[23] The characteristics of PHWW-Spirulina and the filtered municipal WW are provided in Table 1, which summarizes all the sources of PHWWs used in this study.

Algal inoculum. The inoculum of algae used in this study was a mixed algae culture that has been intermittently exposed to PHWW. The original seed was collected from the clarifier outlets at a local WW treatment plant (UCSD). Microscopic inspection of the original seed showed that it was mainly composed of single cell green algae including *Chlorella* spp., *Scenedesmus obliquus* and cyanobacteria (blue green algae). Several other algal species were also bio-augmented into the culture including *Chlorella protothecoides*, *Chlorella vulgaris*, *Botryococcus braunii*, *Nannochloropsis oculata*, *Spirulina platensis*, *Scenedesmus dimorphus*, and *Chlamydomonas reinhardtii*. Heterotrophic bacteria also existed and no algae purification or isolation procedure was conducted to exclude bacteria from this culture. In order to obtain a PHWW-adapted culture, the mixed algal–bacterial culture was exposed to slowly increasing amounts of PHWW. After several rounds of adaptation, microscopic observation showed that *Chlorella* spp. were the dominant species and this "PHWW-adapted culture" was maintained in municipal wastewater enriched with 0.5–1% PHWW-Spirulina. The adaptation procedure and culture maintenance were both performed in 250 mL Pyrex flasks on a shaker table rotating at 150 rpm, 25 °C and a light intensity of 50 μmol photon m^{-2} s^{-1}.

Inhibition test. PHWW-adapted algal culture (2 mL) samples were inoculated into 150 mL filtered municipal wastewater spiked with 0.5%, 1%, 2%, 5% and 10% PHWW-Spirulina in a 250 mL Pyrex flask. Flasks were placed on a shaker table rotating at a speed of 150 rpm with a light intensity of 50 μmol photon m^{-2} s^{-1}. Chlorophyll concentration was measured each day and dry cell weight was measured at the end of the experiment (day 10). Triplicate cultures for each condition were cultivated and the average values with standard deviations are reported.

Biomass quantification. Biomass was quantified by both dry cell weight and chlorophyll *a* content. Chlorophyll *a* concentration is an indication of autotrophic biomass (algae and cyanobacteria) while dry cell weight is the summation of the autotrophic and heterotrophic biomass. Dry cell weight was measured as total suspended solids (TSS) according to standard methods.[24] Chlorophyll *a* was extracted using methanol and the concentration was measured according to the approach of Porra *et al.*[25]

2.1.2. Key Step 2: pollutant degradation when algae was cultivated in 1% PHWW-PBR

Pollutant degradation batch tests. The removal of pollutants by PHWW-adapted algal–bacterial culture was determined using a mixture of 99% filtered municipal WW and 1% PHWW-PBR (see

Table 1 Chemical characteristics of post-hydrothermal liquefaction wastewater (PHWW) and filtered municipal wastewater[a]

Parameter	PHWW-Spirulina	PHWW-PBR	PHWW-Pond	PHWW-Carboy	PHWW-Manure[b]	Filtered municipal wastewater
Chemical oxygen demand (mg L^{-1})	128 000	120 897 ± 518	85 253 ± 854	99 372 ± 380	52 030	54
Total organic carbon (mg L^{-1})	41 136	39 432	23 208	34 964	NA	NA
Ammonia nitrogen (mg L^{-1})	3230	11 407	NA	NA	NA	NA
Total nitrogen (mg L^{-1})	5482	20 342	10 288 ± 167	17 307	5355	33
Total phosphorus (mg L^{-1})	1865	1558 ± 62	NA	686 ± 94	1499	8
pH	7.5	7.89	7.89	7.89	5.6	NA
Usage of this water source in this study	Step 1 and 3	Step 2	Step 4	Step 4	Step 3	Steps 1–3

[a] NA indicates values not measured. [b] This is a mixture of many PHWW samples from HTL using swine manure as feedstock under various HTL process conditions (pressure and reaction time). Additional information about the PHWW from HTL of manure feedstocks is available in a previous publication.[26]

Table 1 for water quality characteristics). The PHWW-PBR was produced in a batch HTL process using mixed algal–bacterial biomass from a photobioreactor (PBR) that was fed by 1% PHWW-Spirulina.[23] 10 mL of PHWW-adapted culture was seeded into a 2 L wide-base Pyrex flask containing 500 mL of medium that consisted of filtered municipal WW and 1% PHWW-PBR. The flasks were placed on a shaker table rotating at a speed of 150 rpm under a light intensity of 200 μmol photon m^{-2} s^{-1}. The headspace was filled with air enriched with 10% carbon dioxide and the headspace gas was refreshed every day during the experiment. Controls were cultivated under dark conditions to prevent photosynthetic growth and pollutant removal. Both the lighted batch reactors and dark controls were conducted in triplicate with average values and standard deviations reported.

Water quality analysis. Water samples were first filtered using 0.45 μm pore size syringe filters (Whatman puradisc-25 mm) to remove cells and particles. Then, soluble chemical oxygen demand (SCOD) was determined by visible light absorbance after dichromate digestion according to standard methods[24] with a HACH Model DR/2010 spectrophotometer. Total soluble nitrogen was measured using the HACH TNT Persulfate Digestion Method no. 10072. Ammonia nitrogen was determined according to the HACH Nessler Method no. 8038. Total soluble phosphorus was measured using the HACH PhoVer 3 Method no. 8190 with acid-persulfate digestion. Total soluble organic carbon (TOC) was measured as non-purgeable organic carbon according to standard methods.[24]

2.1.3. Key Step 3: HTL test using algal biomass harvested from three different cultivation systems

Mass algae cultivation as HTL feedstock. In order to simulate different types of algal cultivation systems, several pilot-scale algal bioreactors were used to produce biomass for subsequent HTL testing. The three cultivation systems used were carboy batch jars, an outdoor "open pond" batch, and a continuous-flow membrane photobioreactor (PBR). Each system used PHWW-adapted algae culture as an inoculum. The carboys (three 18 L jars) and continuous membrane PBR cultivation systems were fed by filtered municipal WW spiked with 1% PHWW-Spirulina, the outdoor open pond was grown using F/2 medium spiked with 0.01% PHWW-Manure.[23]

Algal biomass composition analysis. The crude protein of algal biomass was measured according to the methods of the Association of Official Analytical Chemists (AOAC 4.2.03). The lipid content was measured according to the Folch method.[27] The carbon, hydrogen and nitrogen contents of the algae were measured using a CE-440 CHN analyzer (Exeter Analytical, Inc., North Chelmsford, MA). The phosphorus content was measured by inductively coupled plasma mass spectroscopy (ICP-MS) (Sciex Elan DRC-e, Perkin Elmer, Norwalk, CT). Table 2 summarizes the characteristics of the algal biomass used.

Hydrothermal liquefaction (HTL) test. Algal biomass harvested from open pond cultivation, carboys and PBR were adjusted to a moisture content of 80% and then subjected to HTL conditions (300 °C, 10–12 MPa) with a reaction time of 30 min to test the feasibility of converting them into bio-crude oil. The HTL experiments were performed according to previously reported methods[17] using a 100 mL completely mixed stainless steel reactor with a 70 mL operating volume. The product mixture was separated using a vacuum filter (Whatman no. 4 Filter Paper) into a water insoluble product and PHWW. The moisture content of the water insoluble product was determined by distillation according to ASTM Standard D95-99.[28] Raw oil was defined as the water insoluble product after moisture removal and includes both oil and residual solids. The residual solid fraction in the raw oil product was measured as the toluene insoluble portion after a Soxhlet extraction according to ASTM Standards D473-02 (ref. 29) and D4072-98.[30] The toluene soluble fraction is referred to as bio-crude oil. The mass of the gas product was calculated from the ideal gas law using the residual pressure after cooling down the reactor and assuming 100% CO_2 as the HTL gaseous product. The PHWW yield was calculated by difference assuming the summation of all product yields to be 100%.

Table 2 Characteristics of harvested algal–bacterial biomass (dry matter basis)

	Cultivation systems		
Parameter (%)	Outdoor open pond	Carboy batch jar	PBR (photo-bioreactor)
Volatile solid content	83.3	93.4	95.5
Ash content	16.7	6.6	4.5
Crude protein	36.7	NA	66.1
Acid detergent fiber	5.8	NA	<0.5
Lipid content	14.9 ± 1.4	14.7 ± 1.1	20.1 ± 2.5
Carbon	46.2	49.8	51.1
Hydrogen	6.5	7.0	7.2
Nitrogen	6.2	9.8	10.1
Phosphorus	0.66	1.5	1.2

The energy consumption ratio (ECR) has been used by several past researchers to compare the energy input for the HTL process with the energy output in the oil produced by the process (E_{in}/E_{out}). In order to better assess the overall energy balance of the entire E^2-Energy process, we added energy input terms for algal cultivation (E_{cul}), algal harvesting (E_{har}), and hydrotreatment upgrading of HTL oil (E_{upg}) to make it a refinery-ready petroleum replacement. Since heating the reaction mixture is the predominant energy input for HTL, we neglected other minor HTL process energy inputs. Thus, the ECR was calculated according to the following equation, which was adapted from methods reported elsewhere.[31,32]

$$\mathrm{ECR} = \frac{E_{in}}{E_{out}} = \frac{E_{cul} + E_{har} + E_{HTL} + E_{upg}}{E_{out}} = \frac{E_{cul} + E_{har} + [W_i C_{pw} T + (1 - W_i) C_{ps} T](1 - R_h) + E_{upg}}{Y_{OIL}(\mathrm{HHV})(1 - W_i) R_c}$$

where W_i is the initial feedstock water content prior to conversion (20% in our case); T is the temperature increase required to reach conversion conditions (275 °C in our case); C_{pw} and C_{ps} are the specific heats of water and biomass, respectively (4.18 kJ kg^{-1} $°C^{-1}$ and 1.25 kJ kg^{-1} $°C^{-1}$); R_h and R_c represent the efficiencies of heat recovery and combustion energy, respectively, which were both assumed to have moderate values of 0.7.[33] Y_{OIL}

is the bio-crude oil yield fraction, and HHV (kJ kg^{-1}) is the higher heating value of the bio-crude oil that is calculated according to the Dulong formula.[34,35]

$$\mathrm{HHV}(\mathrm{MJ\ kg^{-1}}) = 0.3383C + 1.422\left(\mathrm{H} - \frac{\mathrm{O}}{8}\right)$$

where C, H and O are the mass percentages of carbon, hydrogen and oxygen, respectively. The energy consumption for cultivating algae in an open pond system and then harvesting it was estimated to be 1.5 MJ kg^{-1} based on the algal biofuel life-cycle data provided by Lardon *et al.*[7] The harvesting includes flocculation followed by rotary press dewatering to produce an algal paste with a solid content of 20%. The energy consumption for cultivating algae in a photobioreactor (PBR) was estimated by measuring the energy input for aeration in the gas-lift PBR we used in this study to produce the algae for subsequent HTL tests. The power consumption for our PBR aerator was 2 watts, and the biomass productivity averaged 1.5 g per day, resulting in an estimated cultivation energy consumption of 115 MJ kg^{-1}. Note that the energy for lighting our PBR was not included in the energy input because artificial lighting was only used for experimental convenience, and ultimately, solar lighting is envisioned as the only practical light source for full-scale algal biofuel applications. Xue *et al.*[8] estimated the energy consumption for harvesting and dewatering algae (to reach a solid content of 16% to 30%) to be 0.3–0.5 MJ kg^{-1} for flocculation followed by centrifugation. In this study, 0.5 MJ kg^{-1} was chosen as a conservative estimate for harvesting and concentrating PBR biomass at a solid content of 20% for HTL. The Carboy algal culture from this study is also a kind of photobioreactor and was assumed to use the same energy inputs for cultivating and harvesting algal biomass.

ECR can be calculated with or without the upgrading energy term. Upgrading is most likely needed to obtain drop-in transportation fuels in current petroleum refineries, but may not be needed for heating oil, asphalt, or future refineries built specifically for HTL oil feedstocks. The energy input for hydrotreatment upgrading includes energy input for steam heating and electricity, which has been estimated to be 0.42 MJ kg^{-1}.[36] Several recent studies have reported that the hydrogen for upgrading can be supplied by reforming the off-gas from the upgrading process without additional net energy input.[6,37,38] Thus, no additional energy input was included in our ECR calculations for the hydrogen used to upgrade HTL oil. Our ECR calculations assumed that the product yield of upgrading is 85% and the HHV of upgraded oil is 40 MJ kg^{-1}.[39]

2.1.4. Key Step 4: elemental analysis of HTL products. The elemental distribution of HTL products was measured to calculate how much of the organics and nutrients were re-released into different HTL products. The carbon, hydrogen and nitrogen contents of HTL raw oil and solid residue were analyzed using a CHN analyzer (CE-440, Exeter Analytical, Inc., North Chelmsford, Mass.); the oxygen content was calculated by difference. Therefore, the calculated oxygen content is slightly larger than the true value because it accounts for the mass of the other minor elements. The elemental composition of the bio-crude oil product was calculated by difference between raw oil and solid residue. HTL gas samples were analyzed using a Varian CP-3800 Gas Chromatograph as described previously.[40]

2.2. Model development

2.2.1. Modeling approach. To better understand the long-term, steady-state impacts of recycling nutrients and carbon multiple times in the E^2-Energy system, a mathematical mass balance model was developed to simulate a continuously operating system in terms of energy and material flows and key environmental impacts (wastewater treatment performance and greenhouse gas capture).

This multi-component mass balance model was developed using the STELLA® modelling platform and was applied to two scenarios of the E^2-Energy system for a specific amount of influent municipal wastewater (10^6 L per day or 10^3 m^3 per day). One scenario uses "baseline" parameters that were obtained from the experimental results of this study, whereas the second scenario uses some "improved" parameters that were identified through sensitivity analysis (described later) and adjusted based on data reported elsewhere in the literature. The later scenario was used to investigate the potential to improve the overall system performance *via* optimization of individual system components. The final outputs of the model include mass flows, energy production, pollutant treatment efficiency and GHG emissions, which are then compared between the two scenarios and with the performance of conventional municipal wastewater systems and typical algal cultivation systems.

2.2.2. Model description. The E^2-Energy model consists of three sub-models for mass, carbon and nitrogen, which are interrelated. Mass flow tracks the biosolids, which can be harvested for biofuel production. Carbon is tracked as both organic carbon and inorganic carbon (CO_2). Organic carbon serves as both a target pollutant and a substrate for heterotrophic growth. Inorganic carbon is the sole carbon source for autotrophic growth and an important GHG. Nitrogen flow is also simulated to quantify the multi-cycle nutrient reuse feature of E^2-Energy, which amplifies biomass production.

Each sub-model contains three major compartments, waste pretreatment (PT), algal–bacterial cultivation (AC) and hydrothermal liquefaction (HTL) as shown in Fig. 1. Table 3 provides definitions of the model parameters and the values used in the two modeling scenarios (baseline and improved). The specific processes and equations governing transformations in the nitrogen sub-model are shown in Table 4 as an example. The other two sub-models for mass and carbon are very similar and are not presented here to avoid unnecessary repetition. However, additional description of the model assumptions, formulations, and the transformation processes occurring for all three sub-models are provided in the ESI† for reference.

The nitrogen leveraging ratio (R_N), carbon leveraging ratio (R_C), and solids leveraging efficiency (R_S) were defined and used to evaluate the performance and energy production capacity of

Table 3 Nomenclature, process parameter definitions and values used in the E^2-Energy model

Nomenclature	
Parameters[a]	Symbols
Flow rate (L per day)	Q
Pretreatment	PT
Algal-bacterial cultivation	AC
Hydrothermal liquefaction	HTL
Influent/Effluent	INF/EFF
Soluble substrate of carbon/nitrogen, as TOC/TN (mg L^{-1})	S_C/S_N
Total soluble substrate of carbon/nitrogen (kg)	TS_C/TS_N
Suspended solids (mg L^{-1})	SS
Total nitrogen/carbon/ suspended solids (kg)	$TC/TN/TSS$
Primary sludge harvested in PT (kg)	PS
Biomass production (kg day^{-1})	X
Heterotrophic/Autotrophic microbes	$HETE/AUTO$
Observed biomass yield (g SS/g Substrate)	Y^{OBS}
Biosolids (kg)	BS
HTL yield (g product per g dry feedstock)	Y
Weight of HTL products (kg)	M
Removal efficiency	RR
The gravimetric ratio of element i in subject j *	f_i^j
Biodegradability of component i in subject j *	α_i^j
The partitioning ratio of element i into HTL product j *	y_i^j

[a] Except for variables marked with an *, superscripts indicate the process compartment (PT, AC or HTL), and subscripts describe the variable type. For example, TN_{INF}^{PT} means total nitrogen content in the influent of the pretreatment compartment, and RR_N^{AC} means the removal efficiency of nitrogen in the algal-bacterial cultivation compartment. Variables marked with an * are ratios or efficiencies where the superscript (j) indicates the fraction/component of interest and subscript (i) indicates the element or other variable type. For example, f_N^{SS} is the mass fraction of nitrogen in the suspended solids, whereas y_N^{OIL} is the partitioning ratio of nitrogen into the HTL oil product. [b] Values are obtained from experiments reported in this study. [c] Values are averages of three HTL testing results. [d] Parameters used were from results with processing *Chlorella pyrenoidosa* at 280°C and a 60 min reaction time. [e] Values were assumed based on experience or convenience. [f] Rationale for these values is provided in the Results and discussion section.

Table 3 (*Contd.*)

Process parameters and values	
Parameters (Symbol)	Value
Pretreatment Compartment (PT)	
Overall flow rate (Q)	10^6 L per day[e]
Suspended solids in influent of PT (SS_{INF}^{PT})	210 mg L^{-1} (ref. 41)
Soluble carbon in influent of PT (S_C)	50 mg L^{-1} (ref. 41)
Soluble nitrogen in influent of PT (S_N)	35 mg L^{-1} (ref. 41)
Suspended solids removal in PT (RR_{SS}^{PT})	0.7 (ref. 42)
Fraction of solids in primary sludge (f_{SPS})	0.2[b]
Fraction of carbon in primary sludge (f_C^{PS})	0.39 (ref. 43)
Fraction of nitrogen in primary sludge (f_N^{PS})	0.025 (ref. 42)
Specific gravity of primary sludge (G_{PS})	1.08 (ref. 44)
Biodegradability of carbon in raw waste (α_C^{RW})	0.9[e]
Biodegradability of nitrogen in raw waste (α_N^{RW})	0.9[e]
Algal-bacterial Cultivation Compartment (AC)	
Solid content of biosolids sent to HTL (f_{SBS})	0.2[b]
Observed heterotrophic biomass yield (kg biomass/kg TOC consumed) (Y_{HETE}^{OBS})	0.85[b]
Observed autotrophic biomass yield (kg biomass/kg TN consumed) (Y_{AUTO}^{OBS})	12.5[b]
Fraction of carbon in autotroph (f_C^{AUTO})	0.5[b]
Fraction of carbon in heterotroph (f_C^{HETE})	0.5[b]
Fraction of nitrogen in autotroph (f_N^{AUTO})	0.087[b]
Fraction of nitrogen in heterotroph (f_N^{HETE})	0.04 (ref. 44)
Suspended solids harvesting in AC (RR_{SS}^{AC})	0.99 (ref. 44)
Hydrothermal Liquefaction Compartment (HTL)	
Product yield of oil, PHWW, gas, residue (Y_{OIL}, Y_{PHWW},Y_{RES},Y_{GAS})	0.47, 0.30,0.18,0.05[b,c]
Carbon partitioning ratio into bio-crude oil, PHWW, gas and residue (y_C^{OIL}, y_C^{PHWW},y_C^{RES},y_C^{GAS})	0.64, 0.22,0.12,0.03[b,c]
Nitrogen partitioning ratio into bio-crude oil, PHWW, gas and residue (y_N^{OIL},y_N^{PHWW},y_N^{RES},y_N^{GAS})	Baseline: 0.31, 0.60,0.09, 0[b,c] Improved: 0.155, 0.80, 0.045, 0 (ref. 40)[d]
Biodegradability of carbon in PHWW (α_C^{PHWW})	Baseline: 0.69[b] Improved: 0.91[f]
Biodegradability of nitrogen in PHWW (α_N^{PHWW})	Baseline: 0.86[b] Improved: 0.96[f]

the E^2-Energy system. They were defined as the ratio of total nitrogen/carbon/suspended solids in the feedstocks flowing into the HTL compartment at steady state divided by the total nitrogen/carbon/suspended solids in the incoming waste flowing into the pretreatment compartment.

$$R_N = \frac{TN_{FEED}^{HTL}}{TN_{INF}^{PT}},\quad R_C = \frac{TC_{FEED}^{HTL}}{TC_{INF}^{PT}},\ R_S = \frac{TSS_{FEED}^{HTL}}{TSS_{INF}^{PT}}$$

Note that the calculation of R_S focuses on suspended solids because that is the form of organic material that can be most readily harvested for bio-energy production, which makes R_S the best measure of bioenergy amplification.

Sensitivity analysis was conducted to understand how parameter variations affect system performance in terms of the nitrogen, carbon and solids leveraging ratios (R_N, R_C, R_S) and to identify the parameters that are most critical for improving bioenergy production. Tested parameters included removal of suspended solids in PT (RR_{SS}^{PT}), biodegradability of nitrogen and carbon in PHWW (α_N^{PHWW} and α_C^{PHWW}), product yield of PHWW (Y_{PHWW}), nitrogen and carbon partitioning ratio in PHWW (y_N^{PHWW} and y_C^{PHWW}), which were selected because of their potential for improvement, based on performance data reported by others. Note that the latter three parameters would not change independently, because the summation of yields for all four HTL products must be equal to one in order to maintain conservation of mass. Thus, in the sensitivity analysis, when we increased or decreased the value of Y_{PHWW}, y_N^{PHWW}, y_C^{PHWW} by a certain percentage (*e.g.* 10%), we held the ratio of the other three HTL product yields constant, and the sum of all four product yields equal to one.

Table 4 Governing equations used for the nitrogen sub-model of the E^2-Energy system model

Pretreatment (PT)	
Inputs	From incoming waste stream: $TN_{INF}^{PT}, Q,\ TN_{INF}^{PT} = (SS_{INF}^{PT} \times f_N^{SS} + S_{N\,INF}^{PT}) \times Q$
Processes	• Nitrogen removal through primary sludge settling (assuming no soluble nitrogen removal) $SS_{EFF}^{PT} = SS_{INF}^{PT} \times (1 - RR_{SS}^{PT})$ $TSS_{PS}^{PT} = (SS_{INF}^{PT} - SS_{EFF}^{PT}) \times Q$ $TN_{PS}^{PT} = TSS_{PS}^{PT} \times f_N^{PS}$
Outputs	To AC: TN_{EFF}^{PT}, Q_L To HTL: TN_{PS}^{PT}, Q_s
Algal–bacterial Cultivation (AC)	
Inputs	From PT: TN_{EFF}^{PT}, Q_L From HTL: TN_{PHWW}, Q_{PHWW} $TN_{INF}^{AC} = TN_{EFF}^{PT} + TN_{PHWW}$
Processes	• Hydrolysis of residual suspended solids from PT $TS_{N\,INF}^{AC} = TN_{EFF}^{PT} + TN_{PHWW}$ • Soluble nitrogen removal by microbial uptake $TN_{BS}^{AC} = TS_{N\,INF}^{AC} \times RR_N^{AC}$ -Removal efficiency depends on nitrogen biodegradability of two incoming waste streams $RR_N^{AC} = \frac{TN_{EFF}^{PT} \times \alpha_N^{RW} + TN_{PHWW} \times \alpha_N^{PHWW}}{TN_{EFF}^{PT} + TN_{PHWW}}$ -Nitrogen utilization to support heterotrophic growth (heterotrophs have priority to use nitrogen) $X_{HETE}^{AC} = Y_{HETE}^{OBS} \times TC_{INF}^{AC} \times RR_C^{AC}$ $TN_{HETE}^{AC} = X_{HETE}^{AC} \times f_N^{HETE}$ -Nitrogen utilization to support autotrophic growth $X_{AUTO}^{AC} = Y_{AUTO}^{OBS} \times TN_{AUTO}^{AC}$ $TN_{AUTO}^{AC} = TN_{BS}^{AC} - TN_{HETE}^{AC}$ • Biomass harvesting $TN_{Harv}^{AC} = TN_{BS}^{AC} \times RR_{SS}^{AC}$
Outputs	To treated water: TN_{EFF}^{AC}, Q_{AC} To HTL: TN_{Harv}^{AC}
Hydrothermal liquefaction (HTL)	
Inputs	From PT: TN_{PS}^{PT}, Q_s From AC: TN_{Harv}^{AC} $TN_{FEED}^{HTL} = TN_{Harv}^{AC} + TN_{PS}^{PT}$
Processes	• Nitrogen release into the HTL product $TN_{OIL,PHWW,GAS\ or\ RES} = TN_{FEED}^{HTL} \times y_N^{OIL,PHWW,GAS\ or\ RES}$
Outputs	To biocrude oil: TN_{OIL} To residue: TN_{RES} To PHWW: TN_{PHWW}, Q_{PHWW}

3. Results and discussion

3.1. Key Step 1: biomass production in PHWW

Fig. 2 shows the experimental results from batch cultivations of mixed algal–bacterial biomass in various dilutions of post-HTL wastewater (PHWW) mixed with the filtered municipal wastewater. We observed two different primary modes of biomass growth depending on the PHWW dosage: heterotroph dominant mode (cultures spiked with 2%, 5% and 10% PHWW) and autotroph dominant mode (cultures spiked with 0%, 0.5% and 1% PHWW). In the heterotroph dominant mode, the dry cell weight of cultures increased with the PHWW dosage from 2% to 5% to 10% (see Fig. 2a). Actually, a linear relationship between the total organic content and biomass production was observed, where biomass production (mg L^{-1} dry wt) = 0.047 × COD (mg L^{-1}), R^2 = 0.98. This makes sense because most of the organics in the medium (>98%) came from PHWW, and heterotrophic growth generally depends on the available organic substrate. The biomass in heterotroph dominant mode was predominantly heterotrophic microbes as shown by very low chlorophyll *a* concentrations (see Fig. 2b).

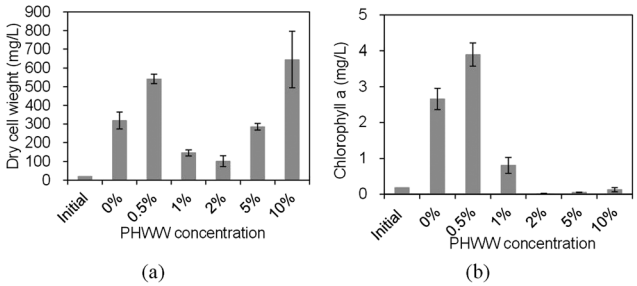

Fig. 2 Biomass production in filtered municipal wastewater spiked with various doses (0–10%) of post-HTL wastewater (PHWW-Spirulina) after 10 days of cultivation. (a) Total biomass production presented as dry cell weight. (b) Autotrophic biomass production presented as chlorophyll *a* concentration. Error bars represent the standard deviation (n = 3).

In contrast, cultures spiked with 0%, 0.5% and 1% PHWW were dominated by autotrophic microbes like algae and cyanobacteria. As shown in Fig. 2a, the final dry cell weight for these three cultures reached 318 mg L^{-1}, 541 mg L^{-1} and 146 mg L^{-1}, respectively. If we assume the same linear relationship between organic substrate and heterotrophic biomass production as noted above, then heterotrophic biomass would only account for 3 mg L^{-1}, 31 mg L^{-1} and 62 mg L^{-1} in cultures spiked with 0%, 0.5% and 1% PHWW, respectively. Thus, the autotrophic biomass production in these three cultures would account for 99%, 94% and 51% of total biomass production. Also, the higher chlorophyll *a* concentration at low PHWW dosages (see Fig. 2b) confirms more active autotrophic growth at low PHWW dosages.

The reason for the stark differences between the two biomass production modes can be explained by the idea that autotrophic and heterotrophic microbes in this system have a different response to PHWW. For autotrophic microbes like algae, there is a threshold concentration of PHWW, below which algal growth is enhanced; but above this threshold, algal growth is inhibited. As shown in Fig. 2b, the final chlorophyll *a* content of the cultures with 0.5% PHWW is 47% higher than the culture without any PHWW spike. This increase in autotrophic production is likely due to additional nutrients provided by PHWW. Nitrogen and phosphorus concentrations in PHWW are more than 150 times greater than the filtered municipal wastewater (see Table 1). However, when the PHWW concentration is above a certain threshold, the algal production is partially inhibited (*i.e.*, culture with 1% PHWW in Fig. 2b) or completely inhibited (cultures with 2%, 5% and 10%). In cultures spiked with 2%, 5% and 10% PHWW, algal cells were

observed microscopically and found to be lysed open, which could be explained by toxic effects associated with high PHWW dosages.

Many compounds identified in PHWW could be inhibitory or toxic to algae, including ammonia and various organic compounds. For instance, 2% PHWW would contain 64 mg L^{-1} NH_4^+-N (Table 1), which is a level reported as toxic for several algae species.[45–47] Organic compounds such as phenol, toluene, propenal, allyl alcohol and benzene have been identified in PHWW and are known to be toxic to algal growth.[26,48–50] However, concentrations of these organics in PHWW were not previously reported, and more research is needed to elucidate potential toxic effects on algae. In contrast to the autotrophs, heterotrophic microbes showed much less sensitivity to PHWW. If there are inhibitory effects of PHWW on heterotrophic microbes, they are outweighed by the positive effects of increasing organic substrate levels. As a result, no sudden decrease of heterotrophic biomass production was observed as the PHWW dosage increased.

Although both of the biomass production modes can effectively produce biomass, the autotrophic dominant mode is more favorable for the E^2-Energy system. This is because only the autotrophic microbial growth can amplify the energy harvest from wastewater by growing new photosynthetic biomass, which brings extra solar energy (and CO_2) into the wastewater biosolids. In contrast, the heterotrophic microbial growth is only able to recover a portion of the existing energy in wastewater. Therefore, it is preferable to keep the PHWW concentration low enough that it does not inhibit autotrophic microbial growth.

There is potential to increase the threshold PHWW concentration for autotrophic microbes *via* adaptation and species selection. These adaptive phenomena have already been observed in our PHWW-adapted mixed algal species, which, after long-term adaptation, can now grow well in medium spiked with 1% PHWW-Spirulina, but initially could not survive when the medium was spiked with 0.5% PHWW-Spirulina. Additionally, augmentation of cultures with algal species that have better tolerance to PHWW toxicity could also improve biomass production. For example, a wide range of ammonia toxicity has been reported for different algal species ranging from 0.35 mg L^{-1} to 250 mg L^{-1}.[51,52] Therefore, we expect that algal species with better tolerance of PHWW likely exist.

3.2. Key Step 2: pollutant degradation in PHWW

A second batch culture experiment was conducted in filtered municipal wastewater spiked with 1% PHWW-PBR to monitor the pollutant removal during biomass production. As shown in Fig. 3b, phototrophic algae dominated the lighted cultures with chlorophyll *a* increasing from 1 mg L^{-1} to 25 mg L^{-1} *versus* a negligible increase in the controls grown without light. Total biomass production also increased significantly, from 22 mg L^{-1} to 2500 mg L^{-1}. Assuming the heterotrophic bacteria had similar growth in lighted and dark cultures, the autotrophic algal biomass was about 14 times greater than the heterotrophic biomass production. This confirms the idea that encouraging autotrophic growth can increase overall biomass (and bioenergy) production.

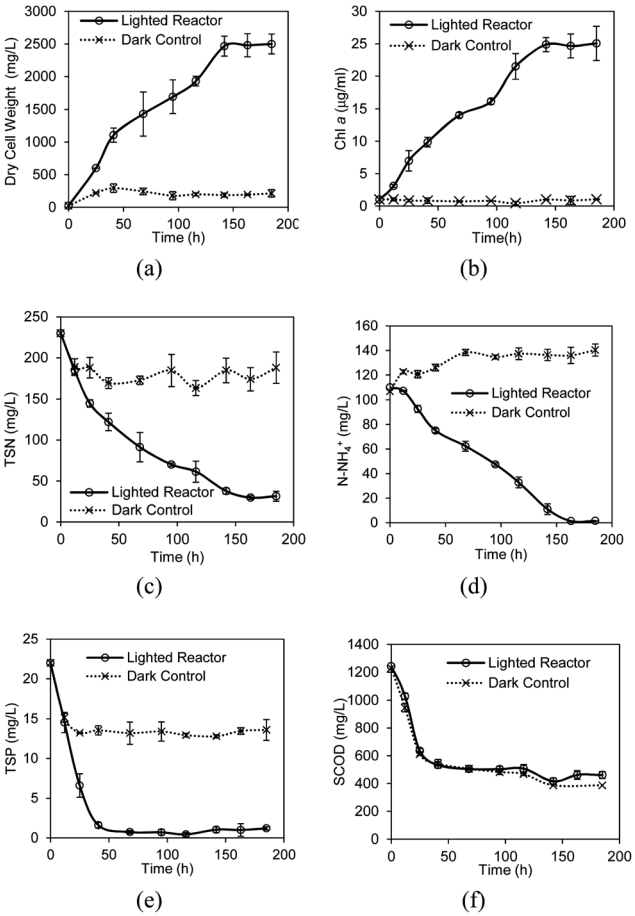

Fig. 3 Biomass production and pollutant degradation when algae were cultivated in municipal wastewater spiked with 1% PHWW-PBR. (a) Total biomass production presented as dry cell weight. (b) Autotrophic biomass production presented as chlorophyll *a* concentration. (c) Total soluble nitrogen removal. (d) Ammonia nitrogen removal. (e) Total soluble phosphorus removal. (f) Soluble chemical oxygen demand (SCOD) removal. Error bars represent the standard deviation ($n = 3$).

The consortium of algae and bacteria growing in our wastewater medium effectively removed both organics and nutrients. The lighted cultures with algal growth showed enhanced nutrient removal, but the removal of organics was essentially equal in lighted and dark cultures. As shown in Fig. 3c–e, the lighted cultures had good removal of total soluble nitrogen (TSN), ammonium nitrogen (NH_4^+-N), and total soluble phosphorus (TSP) – 86%, 100% and 95%, respectively. The dark controls had much lower nutrient removal efficiencies – 18% for TSN and 38% for TSP, whereas NH_4^+-N actually increased 31%. The increase in ammonia likely resulted from the breakdown of organic nitrogen into ammonia.

Removal of organics was similar in lighted cultures (63%) and dark controls (69%) (see Fig. 3f). There was slightly over 30% residual SCOD even after a relatively long cultivation time (185 hours), which indicates the presence of recalcitrant or slowly degrading organics. The residual TSN is likely to be nitrogen heteroatoms contained in these organic compounds.

Our initial analysis of these compounds found that many of the recalcitrant or slow-degrading compounds contain ring structures and nitrogen heteroatoms (see Table S2 in the ESI† for identified recalcitrant compounds). The formation of these compounds was likely related to cross-reactions with proteins and carbohydrates.[53] Recent studies have identified nitrogenous organics resulting from HTL treatment of algal feedstocks, such as amino-phenol, 2-piperidione, 2-pyrrolidinone, pyridiena and its derivatives, and piperidinone and its derivatives.[53–56] Further study is needed to characterize these residual compounds and develop effective removal methods. Potential methods include adsorption (with activated carbon or resins), ozone, and/or second-stage biological treatment with adapted or specialized microbes.

Bacterial activity is important for both pollutant degradation and biomass production with the E^2-Energy approach, even though they are typically considered a contamination problem in most other algal production systems. Bacteria can degrade organic pollutants that algae cannot utilize because only some algal species can conduct mixotrophic or heterotrophic metabolism, and the range of organics algae can digest is much narrower than for heterotrophic bacteria.[57] In addition, the consumption rate of organics by algae is generally slower than bacteria.[58,59] Microscopic observation of cultures grown in the dark showed that algal cell density was stagnant, indicating that active heterotrophic algae were scarce. In addition, since algal growth under lighted conditions did not enhance organic removal (Fig. 3f), mixotrophic algae also were apparently rare. Thus, almost all of the organic removal in both lighted and dark cultures can be attributed to heterotrophic bacteria.

A second benefit of the bacteria is their ability to transform nutrients to a form that algae are capable of utilizing. As shown in Table 1, approximately half of the nitrogen contained in PHWW-PBR was NH_4^+-N, and the other half was organic nitrogen, which autotrophic algae are unable to utilize directly. However, only 14% of TSN remained in the lighted culture and virtually zero NH_4^+-N (see Fig. 3c and d). This could be explained by transformation of organic nitrogen into usable forms (*e.g.* NH_4^+-N) during bacterial degradation of nitrogenous organics. As shown in Fig. 3d, the NH_4^+-N concentration in dark controls had a relatively fast increase in the first 68 hours, the same period when bacterial growth and SCOD removal occurred. Since bacterial growth also uses nitrogen and some could be converted into nitrite and nitrate, the total nitrogen release from degraded organics by bacteria was greater than the net increase of NH_4^+-N shown in Fig. 3d. After bacterial growth became limited by the available organic substrates, unused ammonium was left in the dark cultures. In contrast, autotrophic algae in the lighted cultures continued to consume any released NH_4^+-N until it was gone at around 160 hours. Thus, autotrophic algal biomass production and total nutrient removal was enhanced by bacterial activity. This highlights the importance of algal–bacterial consortiums for E^2-Energy systems and suggests that the balance of bacteria and algae could be further optimized to maximize pollutant removal and biomass production.

3.3. Key Step 3: HTL conversion of algal–bacterial biomass grown in wastewater

Algal–bacterial biomass harvested from three wastewater bioreactor systems as described earlier were successfully converted into a self-separating bio-crude oil product *via* HTL at 300 °C and 30 minutes of reaction time. As shown in Fig. 4, the biomass from the carboy had the highest bio-crude oil yield (52.2%), followed by biomass from PBR (51.3%), and biomass from open pond had the lowest yield (37.9%). The initial lipid content of these feedstocks varied from 15–20%, highlighting that high-lipid algae are not necessary for biofuel production with HTL. Others have reported successful conversion of low-lipid algae species such as *Chlorella pyrenoidosa* (<0.1% crude fat)[60] and *Spirulina* (5% crude fat)[18] with bio-crude oil yields ranging from 30%–50%. These studies suggested that protein was the primary source material for bio-crude oil production because it accounted for most of the feedstock mass. Considering the exceptionally high growth rates of some low-lipid algae, HTL of these algae into bio-crude oil is quite promising for large-scale algal biofuels. HTL can also convert bacteria and other types of biosolids into bio-crude oil. Several other studies have found that various wastewater sludges (primary, secondary and anaerobically digested) can all be converted into bio-crude oil by HTL with yields ranging from 25–45%.[14,16,61,62] Thus, in contrast to other algal biofuel production systems, E^2-Energy can elegantly handle any bacterial contamination by also converting bacterial biomass into usable biofuels.

In our tests, there was a conspicuous difference in the appearance of the raw oil product from HTL of the open pond biomass, which was a highly viscous, bitumen-like product that settled to the bottom of the aqueous phase. HTL oil from the carboy and PBR biomass had lower viscosity, density and was flowable at room temperature like crude petroleum. This difference may have resulted from the relatively high ash content (16.7%) of the open pond biomass, which can become mixed with the oil product. This kind of raw oil product could be fed to a biomass boiler, pyrolysis or gasification unit that can handle relatively high ash content feedstocks, or used as asphaltic binder.

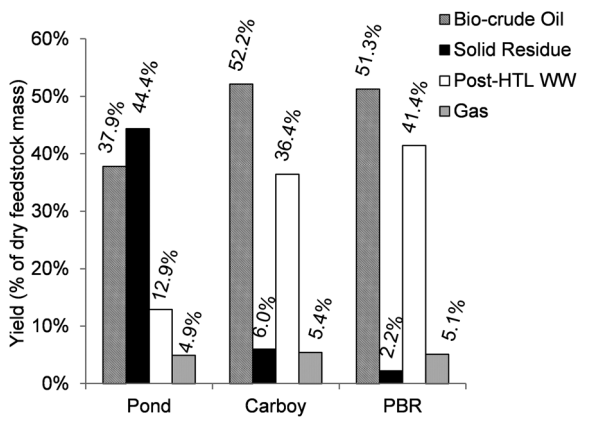

Fig. 4 HTL product yield using three kinds of algae feedstock from wastewater.

Despite the differences in raw oil products described above, the elemental composition of the bio-crude oil product (*i.e.*, the toluene soluble portion of raw oil) showed very similar properties for all three cultivation types (see Table 5). It is quite noteworthy that the HTL energy recovery into bio-crude oil was very high in all three cases-ranging from 82–88% of the energy content in the original feedstock. This is much higher than other conventional bioenergy processes such as anaerobic digestion of wastewater biosolids, which typically recovers only 25–60% of incoming energy content.[63] It is also better than algal biofuel schemes based on extracting lipids, which typically achieve 20–50% oil content in the algae.[64]

The high heating values (HHVs) shown in Table 5 range from 32–38 MJ kg^{-1}, which are comparable to previously reported HHVs from HTL of microalgae.[18,31,34,40] However, these values are lower than algal biodiesel (41 MJ kg^{-1})[65] and petroleum crude oil (45 MJ kg^{-1}),[66] which is a result of higher nitrogen content (5–7%) and oxygen content (8–15%) in HTL bio-crude oil. Petroleum typically has less than 2% oxygen[67] and less than 1% nitrogen,[68] while biodiesel from vegetable oil usually contains essentially no nitrogen and around 11% oxygen.[69] These HTL bio-oil products are heavy crudes that could be used for direct boiler firing and some types of turbines. If drop-in transportation fuels are desired, then HTL bio-crude oil will likely require upgrading to remove nitrogen and oxygen through hydrotreating, which requires some additional energy input. This application of hydroprocessing is envisioned as being similar to hydrotreating of petroleum with similar system requirements, and some sources even suggest it will probably be easier to develop refining techniques for heavy crudes.[70,71]

The Energy Consumption Ratio (ECR = E_{in}/E_{out}) was used in this study to quantify the net energy balance of the entire E^2-Energy process. An ECR below one indicates a positive energy balance because the energy output exceeds energy input. Note that only open pond cultivation systems are recommended for E^2-Energy because their lower energy inputs make them the most practical choice in applications targeting bioenergy production. Photobioreactor (PBR) systems are not recommended for large-scale algal biomass cultivation of E^2-Energy because they generally require a larger energy input and are more expensive to build and operate. For instance, the energy input for PBR algal cultivation alone could be 17 MJ kg^{-1} for a flat plate bioreactor, and up to 386 MJ kg^{-1} for a tubular PBR.[72] Therefore, PBR systems are more often used for high-value algal products rather than relatively low-value biofuel. Nevertheless, the ECR values for PBR and Carboy biomass (also a kind of PBR) were calculated for comparison purposes. As shown in Table 5, the ECR for the various cultivation systems ranges between 0.30 and 9.75 without upgrading and between 0.35 and 9.60 with upgrading assuming a moderate heat recovery efficiency (70%[33]). For open pond cultivation as recommended for large-scale E^2-Energy systems, the ECR is between 0.30 and 0.35. This is much better than many other current and proposed biofuel production processes. For instance, the corresponding ECR (E_{in}/E_{out}) for corn ethanol is about 0.57 based on literature reported data.[73] Algal biodiesel production *via* lipid extraction followed by either transesterification or hydrotreatment has been found to have an ECR value in the range of 0.75–4.0 depending on the cultivation and extraction methods used.[6,7] The majority of the energy savings for using the HTL conversion route in the E^2-Energy process rather than the algal biodiesel route comes from reduced energy inputs for drying and extraction. The analysis here provides a preliminary estimation of the energy balance in the E^2-Energy process that highlights the potential advantages of this novel approach. However, a more detailed LCA is recommended in future studies as more process-specific and scalable operating data become available.

Although economic analysis is very important, the process development for HTL and algal biofuels is still very immature and economic data are very limited. Data collection and validation of technical and economic system performance for an industry that has yet to be commercially realized is one of the biggest challenges for techno-economic analysis. A wide range of cost for microalgal biofuel has been reported, from $0.17 per L (ref. 11) to $10.6 per L (ref. 74) depending on the production scenario and assumptions used. A very recent publication by Delure *et al.* estimated that the cost for HTL bio-crude oil production using algae as feedstock is about $3.8 per L (including feedstock production, conversion and upgrading through hydrotreating).[75] This cost could potentially be significantly reduced if a credit for the value of wastewater treatment

Table 5 Bio-crude oil characteristics and energy balance of the E^2-Energy system for processing 1 kg dry algae

		Pond	Carboy	PBR
Elemental composition of bio-crude oil	C (%)	77.4	70.4	75.5
	H (%)	9.4	8.2	9.4
	N (%)	5.2	6.6	7.1
	O (%)	8.1	14.7	8.0
Higher heating value (MJ kg^{-1})	Feedstock	17.6	20.8	21.9
	Raw Oil	22.5	30.4	36.6
	Bio-crude Oil	38.1	32.9	37.5
Energy input for algal cultivation and harvesting (MJ)		1.50[a]	115.5[b]	115.5[b]
Energy input for HTL (MJ)		1.48	1.48	1.48
Energy output (MJ)[c]		10.09	12.00	13.47
ECR[d]		0.30	9.75	8.68
Energy Recovery of HTL		82%	82%	88%
Energy input for upgrading (MJ)[e]		0.16	0.22	0.22
Energy output after upgrading (MJ)[f]		9.01	12.42	12.21
ECR with upgrading		0.35	9.44	9.60

[a] For the E^2-Energy system, open pond cultivation is recommended, which has an estimated energy consumption of 1.5 MJ kg^{-1} (ref. 7) for cultivation and harvesting as discussed previously in Section 2.1.3. [b] Although PBR systems are not recommended for full-scale E^2-Energy systems, the ECR was still calculated for comparison. The energy consumption for cultivating algae in the PBR (and Carboy) was estimated by measurement of the PBR aeration energy input, and harvesting energy consumption was estimated according to Xu *et al.*[8] as discussed previously in Section 2.1.3. [c] 70% Heat recovery from HTL products was assumed in this analysis. [d] 70% Combustion efficiency was assumed in this analysis. [e] The energy consumption for upgrading is assumed to be 0.42 MJ kg^{-1} oil processed.[36] [f] The product yield of upgrading is assumed to be 85% and HHV of upgraded oil to be 40 MJ kg^{-1}.[39]

is included. For instance, Lundquist[11] reported that the overall cost for algal biofuel production was reduced 93% (from \$2.6 to \$0.17 per L) when including a wastewater treatment credit. Based on these two studies, we estimate that when wastewater treatment is synergistically integrated with HTL biofuel production, the cost has the potential to drop below \$0.40 per L. This preliminary economic evaluation highlights the strong potential of the E^2-Energy process, but also highlights the need for more comprehensive techno-economic analysis as more refined, relevant and scalable performance data become available.

3.4. Key Step 4: nutrient re-release from the HTL process

Fig. 5 shows the elemental distribution of carbon and nitrogen in the four different products of HTL. Bio-crude oil and PHWW are the two biggest pools for carbon and nitrogen among the HTL products. Most of the nitrogen was released into the PHWW product (51% for open pond biomass and 64% for both carboy and PBR biomass). This confirms that there is a significant opportunity for nutrient recycling with HTL, because PHWW would be recycled back to algal cultivation in the E^2-Energy process. Bio-crude oil was the second largest recipient of nitrogen, which was 28%, 32% and 35% for open pond biomass, carboy biomass and PBR biomass, respectively. Increasing the percentage of nitrogen released into PHWW increases the potential for biomass/biofuel amplification, and this percentage could be optimized *via* HTL reaction conditions, catalysts and/or feedstock pretreatment. For instance, earlier studies have reported that the ratio of nitrogen distributed into PHWW was a function of reaction temperature and could achieve 70–90% when treating municipal sewage sludge, algae, and high protein feedstocks.[40,67,76] Catalysts like sodium carbonate have also been shown to reduce the distribution of nitrogen to HTL oils.[31,76] Pretreatment could also potentially decrease the nitrogen partitioning into the oil product by cleaving amine groups from proteins and removing them prior to HTL.[31,67]

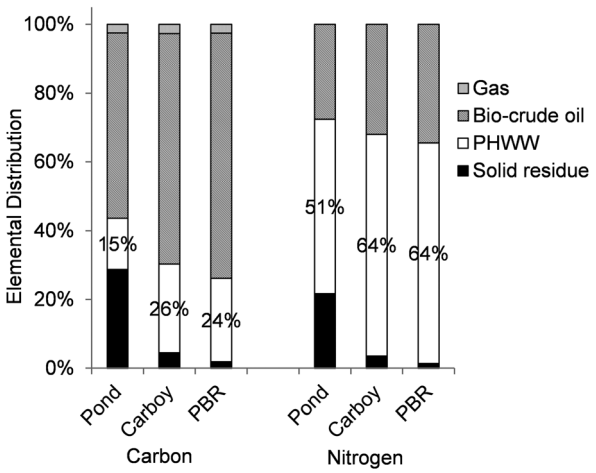

Fig. 5 Elemental distribution of carbon and nitrogen in HTL products.

The biggest pool for carbon was the bio-crude oil product of HTL, which accounted for 54%, 67% and 71% of feedstock carbon for open pond, carboy and PBR cases, respectively. Gas was the smallest pool for carbon in all three cases, and it consisted of over 98% CO_2 with very small amounts of CO and CH_4. This gas would also be recycled back into the algae cultivation unit in the E^2-Energy scheme. In this study, PHWW contained 15–24% of the carbon (as shown in Fig. 5), which is lower than previously reported values that ranged from 35%–55%.[31,40]

3.5. Model simulation of E^2-Energy

The experimental results provided above have confirmed the feasibility of the four main process steps in the proposed E^2-Energy system, and the ability to reuse nutrients for multiple cycles of algae growth. Specifically, the experiments for Step 2 and Step 3 as described above completed two rounds of HTL conversion of algal biomass to crude oil followed by cultivation of algae in the HTL wastewater (note the PHWW used for pollutant degradation experiments in Step 2 was from HTL of algal biomass grown on PHWW-Spirulina described in Step 3). However, these stepwise results do not fully represent the effects of long-term internal recycling on the steady-state mass balances, bioenergy production, and other important process characteristics. Thus, we developed a mathematical process model of the integrated E^2-Energy system using STELLA® modelling software created to investigate steady-state performance, provide sensitivity analysis, evaluate process improvements, and provide strategic guidance for life-cycle analysis and other sustainability measures. The list of model parameters and values is presented in Table 3. Most of the parameter values came from the average values measured in the experiments of this study, but some were taken from the literature or assumed values as noted by the reference numbers.

A sensitivity analysis was conducted to understand how parameter variations affect system performance in terms of the nitrogen, carbon and solids leveraging ratios (R_N, R_C, R_S) and to identify the parameters that are most critical for improving bioenergy production. This analysis revealed that the nitrogen biodegradable fraction for PHWW (α_N^{PHWW}) was the most sensitive parameter. Specifically, a 10% increase in α_N^{PHWW} caused increases of 11.8% in R_N, 10.6% in R_C and 10.3% in R_S. The other high-impact factor for the system was the partitioning ratio of nitrogen into PHWW, y_N^{PHWW}, which caused 11.3%, 10.6%, and 10.1% increases in R_N, R_C and R_S respectively when y_N^{PHWW} was increased 10%. Although the biodegradable fraction of carbon in PHWW, α_C^{PHWW}, was not as sensitive as the two other parameters discussed above, it would likely be changed at the same time as α_N^{PHWW} and has significant room for improvement.

As discussed earlier, optimized HTL process conditions, catalysts, and feedstock pretreatment could all potentially improve y_N^{PHWW}. Other previous studies have reported optimized values of y_N^{PHWW} ranging from 70–90%, indicating significant potential for improving y_N^{PHWW} from our baseline value (0.6). Therefore, we choose a value of 0.8 for y_N^{PHWW} in our

"improved" modelling scenario. Both α_N^{PHWW} and α_C^{PHWW} can potentially be improved by assembling specialized algal–bacterial consortia that can better breakdown recalcitrant compounds so that a higher percentage of carbon and nitrogen in PHWW can be converted into usable substrate for algae and bacterial growth. Also, pretreatment by ozonation is a widely used method expected to make recalcitrant PHWW compounds more biodegradable. It has been reported that ozone treatment could convert more than 95% of the non-biodegradable organic carbon and more than 75% of non-biodegradable organic nitrogen into biodegradable compounds.[77,78] Thus, in modeling the "improved" condition, we assumed that 70% of non-biodegradable carbon and nitrogen could have been converted into biodegradable material, resulting in "improved" values for α_N^{PHWW} and α_C^{PHWW} of 0.96 and 0.91, *versus* baseline values of 0.86 and 0.69, respectively.

The model results confirm that the E^2-Energy system can indeed leverage the nitrogen content in the incoming waste to support multiple cycles of algae and bacteria growth. The model mass balance results for the baseline and improved scenarios are shown in Fig. 6a and b. The nitrogen leveraging ratio (R_N) is 1.9 in the baseline scenario and 3.8 in the improved scenario under steady state conditions. R_N reflects the nutrient recycling efficiency of the system. It would be less than one in a typical algal cultivation system without nutrient recycling because only part of the soluble nitrogen would be used for biomass production. The US Department of Energy (DOE) has identified multi-cycle nutrient reuse as a goal for algal biofuels because US wastewater nutrient flows are insufficient to fully support the national need for renewable fuels with algae on the basis of single-use nutrients.[1] However, DOE did not identify the means of achieving multi-cycle nutrient reuse, and thus, the E^2-Energy system fulfills a recognized need in providing a viable pathway to achieve this goal. Multi-cycle nutrient reuse is a powerful concept that amplifies carbon capture and bio-energy production. As shown in Fig. 6, the carbon leveraging ratio (R_C) would range from 4.0–8.0 for an E^2-Energy system with baseline and improved scenario parameters, respectively. This occurs because photosynthesis would bring an extra 314 kg (baseline scenario) to 686 kg (improved scenario) of carbon into this system in the form of CO_2 to produce algal biomass. In contrast, R_C would be less than one in a typical conventional wastewater bioenergy system without photosynthetic biomass production because only part of the incoming carbon will end up in the final biofuel product. The combined result of multi-cycle nutrient reuse and carbon fixation (by photosynthesis) is increased biomass production, which is reflected in the value of R_S showing that the total biosolids harvest would be 5.2–10.1 times of the original incoming wastewater biosolids for the baseline and improved scenarios, respectively. The amplification of wastewater derived biosolids as represented by R_S provides the most important measure of the E^2-Energy system, because it shows how much more bioenergy can be produced *via* the concept of multi-cycle nutrient reuse.

The model results also showed that the toxic and inhibitory compounds in PHWW would not likely be a problem in real applications when treating municipal wastewater. The percentage of PHWW flowing into the algae cultivation process at steady-state will only be 0.48% and 0.90% in the baseline scenario and improved scenario, respectively. This level of PHWW was shown to support robust growth of mixed algal–bacterial biomass in our batch tests. We have also successfully operated a continuous PBR fed by 1% PHWW-Spirulina for more than one year (some data published elsewhere[23]) and this operating condition did not exert any obvious negative effect on algae growth. Therefore, the need for dilution does not impose an additional water demand and can be cost-effectively accommodated at full-scale.

We estimate there is potential to replace most if not all of the US petroleum imports with HTL bio-crude oil produced from just wastewater inputs by using E^2-Energy. To demonstrate this potential, we begin by noting that the domestic wastewater, animal manure, and landfilled food wastes in the United States contain more than 153 million dry tons collectable solids as shown in Table 6, which could potentially be used to produce bioenergy. By applying the E^2-Energy system to these various waste streams, the amount of biosolids produced in the baseline and improved scenarios would be between 0.80 and 1.55 billion dry tons. Subsequently, these biosolilds can be converted using HTL into 0.38 to 0.73 billion tons of bio-crude oil per year. By way of comparison, the total US oil demand was about 1.1 billion tons in 2011 of which about 0.5 billion tons was imported.[79]

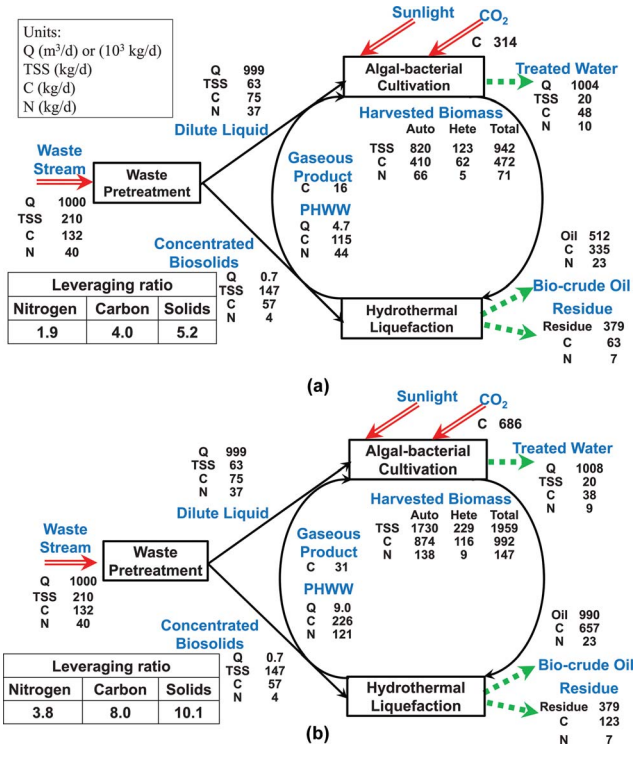

Fig. 6 Multi-component mass balance for two scenarios of E^2-Energy. (a) Baseline scenario-based on model parameters from this study. (b) Improved scenario based on model parameters reported in the literature that would improve the net energy yield.

Table 6 Estimation of collectable biosolids in the US

Sources	Quantity (million dry tons)
Human waste[80]	8[a]
Food scraps in municipal solid waste[81]	10[b]
Animal manure[82–84]	135[c]
Total	153

[a] The 8 million tons here only includes outgoing biosolids from the wastewater treatment plant (after aerobic and anaerobic digestion) rather than total initial incoming human waste. More biosolids than this quantity from human waste should be potentially available. [b] Assuming the moisture content of food scraps to be 70%. [c] Current total animal manure production is estimated to be 235 dry tons per year according to the total US livestock herds[82] and the average manure production rate[83] of different animals. The readily collectable portion of manure was estimated according to a USDA methodolgy.[84]

E^2-Energy also presents an advantageous paradigm shift for the wastewater treatment industry to become a significant net energy producer and achieve a net negative carbon footprint. In the past, wastewater technology has focused on cost-effective removal of aqueous pollutants rather than on energy efficiency. As a result, the wastewater industry commonly uses energy-intensive processes that account for approximately 3% of the total US electrical demand.[85] 50–60% of this energy is used for mechanical aeration in activated sludge processes.[86] However, in algal based wastewater treatment systems, photosynthetic aeration by algae can greatly reduce the mechanical aeration demand. One company that provides algal wastewater treatment systems, Algae Wheel (OneWater, Inc.), has reported a 50% energy reduction for aeration of their algal wastewater treatment plants.[87] In addition, E^2-Energy amplifies the energy harvest from wastewater by growing new biomass photosynthetically, which brings in extra solar energy (and CO_2) to the wastewater biosolids. Considering both the reduced energy inputs and increased energy outputs, E^2-Energy can help the wastewater industry to surpass the goals of carbon neutrality and net-zero energy wastewater treatment and instead become a significant net producer of renewable energy. In addition, algal-based treatment facilities can be less expensive to build and operate than conventional mechanical aeration facilities. For example, high-productivity algae ponds have a total cost that is estimated to cost up to 70% less than conventional activated sludge wastewater plants.[11,85] Enhanced effluent quality would also be achieved during algal wastewater treatment, especially for nutrient removal.[1,3,10]

There are many changes needed to realize this paradigm shift, and one of the most significant is that the wastewater treatment enterprise would become more like an agricultural operation that optimizes growth of photosynthetic biomass using wastewater nutrients. This would require significantly more land than current wastewater treatment systems. However, it would reduce the total land requirements for biofuels as both DOE[1] and National Research Council[6] estimate that the areal productivity for algal biofuels can potentially be more than 10 times greater than current terrestrial crop based biofuels. For example, if the current land area of corn for ethanol production (1.3% of the total US land area[79,82]) is used for algal cultivation, the algal biofuel produced could be up to 55% of current US petroleum consumption for transportation fuel usage (calculation based on values obtained from the literature[6]). Current corn ethanol production is providing less than 7% of the current US transportation fuel demand and needs arable land for corn production. In contrast, algae cultivation could be conducted on non-arable land areas like wastewater lagoons and stabilization ponds.

4. Conclusions

This study investigated the novel E^2-Energy system for algal biofuel production, which integrates algal biomass production during wastewater treatment and hydrothermal liquefaction of that biomass into bio-crude oil. A series of algal cultivation and hydrothermal liquefaction experiments were conducted to confirm the feasibility of the key process steps in the proposed system. This experimental work confirmed the ability to reuse nutrients multiple times for growing multiple rounds of algal biomass, thus amplifying the bioenergy that can be derived from waste sources. A mathematical model was then constructed to simulate the steady-state mass flows in this system and to guide further system optimization. The E^2-Energy process resolves the current major limitations to the economic and energetic feasibility of algal biofuels including the contamination of target high-oil algal cultures, high nutrient cost inputs, as well as the significant energy inputs for dewatering/extraction. The bioenergy potential of E^2-Energy is tremendous because of the multi-cycle nutrient reuse feature, which amplifies the biomass and bioenergy potential of wastewater. We have shown that using waste organic biosolids from only three major sources (municipal wastewater, food waste, and livestock manure) could potentially support the production of enough bio-crude oil to completely replace the current US demand for petroleum imports.

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