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Assessing the energy and environmental performance of algae-mediated tertiary treatment of estrogenic compounds†

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This study uses a systems-level modeling approach to illustrate a novel synergy between municipal wastewater treatment and large-scale algaculture for production of bio-energy, whereby algae-mediated tertiary treatment provides efficient removal of unregulated, strongly estrogenic steroid hormones from the secondary effluent. Laboratory results from previously published studies suggested that algae-mediated treatment could deliver roughly 75–85% removal of a model estrogen (17β-estradiol) within typical algae pond residence times. As such, experimental results are integrated into a comprehensive life cycle assessment (LCA) framework, to assess the environmental performance of an algae-based tertiary treatment system relative to three conventional tertiary treatments: ozonation, UV irradiation, and adsorption onto granular activated carbon. Results indicate that the algae-mediated tertiary treatment is superior to the selected benchmarks on the basis of raw energy return on investment (EROI) and normalized energy use per mass of estrogenic toxicity removed. It is the only tertiary treatment system that creates more energy than it consumes, and it delivers acceptable effluent quality for nutrient and coliform concentrations while rendering a significant reduction in estrogenic toxicity. These results highlight the dual water and energy sustainability benefits that accrue from the integration of municipal wastewater treatment and large-scale algae farming.

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Environmental impact

Integration of algaculture at a municipal wastewater treatment plant (WWTP) has been shown to deliver improved energy performance and enhanced nutrient removal. This study demonstrates that efficient removal of unregulated estrogenic chemicals is yet another sustainability benefit of this system. Compared to three conventional tertiary treatments (ozonation, UV irradiation, and adsorption onto granular activated carbon), the algae-mediated treatment is the only system that improves the energy efficiency of the WWTP, and it exhibits the lowest net energy consumption per mass of estrogen removed. These results are significant because they offer a means of removing unregulated contaminants that more than "pays" for itself on an energy basis, in stark contrast to conventional treatments, which are notoriously energy-consuming.

1. Introduction

There is growing emphasis on converting wastewater treatment systems into net-energy producing entities without compromising their ability to remove environmentally noxious substances such as nutrients, metals, and organic contaminants.[1,2] Simultaneously, algae-derived energy sources are considered increasingly promising alternatives to fossil fuels, because they are renewable, domestically available, and potentially less carbon-intensive. Algaculture systems can also be configured to make use of industrial wastes, such as carbon dioxide (CO_2) and wastewater effluents, which enables them to convert pollution into usable energy.[2,3]

It has been well documented that integration of municipal wastewater treatment and algae cultivation, whereby nutrient-rich partially treated wastewaters are recycled into algae cultivation ponds, could deliver significant sustainability benefits compared to the two standalone entities.[3–7] The wastewater treatment plant (WWTP) could leave higher concentrations of nitrogen (N) and phosphorus (P) in the secondary effluent for uptake during algaculture operations, without risking downstream eutrophication, thereby reducing energy consumption for nutrient removal, which otherwise accounts for roughly 30% of the total energy demand in an advanced treatment WWTP (with biological nitrification).[8] In turn, the use of recycled effluent N and P reduces the consumption of energy-intensive virgin fertilizers during algae farming. We previously reported that these offsets decrease the overall energy consumption and

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global warming potential (GWP) by roughly 20% and 40%, respectively, for an integrated algae system compared to the standalone WWTP and algae farm.[3] Sturm and Lamer[7] reported that replacement of biological nutrient removal (BNR) at an advanced WWTP with an algae-based system for N and P removal could reduce the daily energy consumption by nearly 60%. As such, nutrient recycling is one key synergy between municipal wastewater treatment and algae cultivation.

There have also been several recent studies assessing anaerobic digestion for conversion of algae into a usable energy carrier. Sialve *et al.*[9] concluded that digestion is the optimal conversion process for algae with lipid contents less than 40%. Life cycle assessment (LCA) results from Clarens *et al.* (2011) indicate that digestion of bulk algae biomass delivers *energy return on investment* (EROI) values of 1.06–1.72, for hypothetical systems with and without nutrient recycling.[10] These values are greater than the 1.0 favorability cutoff and they are also greater than corresponding EROI estimates for conventional algae-to-biodiesel systems without anaerobic digestion, 0.65–1.13 (depending on the extent of nutrient recycling). Menger-Krug *et al.*[4] found a dramatic disparity in energy performance for hypothetical WWTPs with and without algae cultivation and digestion systems in place. WWTPs without algae-mediated wastewater treatment exhibited EROI on the order of 0.38, which is much lower than net energy self-sufficiency. With algaculture and digestion of the harvested algae biomass, the same system achieves an EROI of 2.1–2.4 (for various efficiencies of CO_2 recycle). Most recently, Peng and Colosi[11] estimated a similar, though smaller, increase in EROI (from 0.53 to 0.66) when algae cultivation is implemented at a municipal WWTP and the resulting biomass is synergistically co-digested with the biosolids. Therefore, the wastewater treatment industry's good expertise and long familiarity with anaerobic digestion, in conjunction with growing emphasis on maximizing the energy recovery at WWTPs,[1] constitutes a second key synergy between municipal wastewater treatment and algae cultivation.

A third possible synergy between large-scale algaculture and municipal wastewater treatment pertains to removal of currently unregulated, estrogenic steroid hormones. These chemicals interfere with reproduction and development in humans and other species at concentrations down to 1–10 ng L^{-1}, and they are known to be released from municipal WWTPs following incomplete removal during treatment.[12] Toxicological studies reviewed by Faramarzi *et al.*[13] indicate that some green microalgae can mediate transformation of steroid hormones. Pflugmacher *et al.*[14] reported that several macroalgae mediate the transformation of weakly estrogenic contaminants, indicating that these reactions proceed *via* pathways similar to those used by mammalian livers. Regarding possible applications to water treatment systems, Lai *et al.*[15] observed photolysis, biosorption, and biotransformation of several estrogens in batch cultures of *Chlorella vulgaris*. Ge *et al.*[16] reported greatly accelerated photolysis of two steroid hormones in the presence of *Chlorella*, *Anabaena*, or *Microcystis*. Shi *et al.*[17] observed fair removal of estradiol (40% in 180 h) and ethinylestradiol (20% in 180 h) in a mixed algae culture containing *Anabaena*, *Chlorococcus*, *Spirulina*, *Chlorella*, and *Scenedesmus*. Finally, Zhang *et al.*[18] observed 85–95% removal of 17α-estradiol, 17β-estradiol, estriol, and estrone in batch cultures of laboratory-grown *Scenedesmus dimorphus* over 8 days, with the bulk of the removal occurring *via* algae-mediated biotransformation. Based on these studies, removal of unregulated estrogens from WWTP discharges is a novel third synergy between wastewater treatment and algae cultivation; however, this has not been previously accounted for using an LCA framework.

This study uses LCA to investigate the energy and environmental performance of algae-mediated estrogen removal, as compared to three conventional tertiary treatments. Literature data for bench-scale algae-mediated estrogen removal experiments are used as a basis for these assessments. This work is a logical extension of previous research into algae-mediated tertiary wastewater treatment, for enhanced removal of nutrients, pathogens, and industrial pollutants;[19,20] however, our study is the first to account for unregulated emerging contaminants within an LCA framework, as a means to articulate the dual water and energy sustainability benefits of the proposed system.

2. Methods

Spreadsheet LCA models were created for an advanced municipal WWTP without tertiary treatment or with one of four tertiary treatments: an algaculture system (algae), ozonation (OZ), UV irradiation (UV) in the presence of hydrogen peroxide (H_2O_2), or adsorption onto granular activated carbon (GAC) (Fig. 1A and B). All models assumed the same basic WWTP configuration, consisting of primary treatment, secondary treatment with biological nutrient removal (*e.g.*, Ludzack–Ettinger, Bardenpho, *etc.*), subsequent second-stage denitrification with methanol as a carbon source and alum-based phosphorus precipitation as needed to meet stringent TN and TP nutrient limits, solid handling and anaerobic digestion, and chlorination. Net energy consumption for each of these unit processes and each conventional tertiary treatment was taken from published literature studies.[8,21,22] The functional unit (FU) was defined as treatment of 10 million gallons per day (10 MGD) of secondary effluent containing typical concentrations of pertinent wastewater constituents, including several relevant estrogens: 17β-estradiol (E2), 17α-ethynylestradiol (EE2), and estrone (E1). The composition of the secondary effluent is presented in Table 1 (with additional details and citations in Table S2 of the ESI†). It was assumed that all final discharged effluents are required to achieve stringent permissible levels for biochemical oxygen demand (BOD), total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP), and fecal coliform concentrations, regardless of which (if any) tertiary treatment is used for estrogen control. The ESI† contains detailed documentation for LCA modeling of WWTP unit operations and representative effluent qualities.

The estrogenic steroid hormones selected for inclusion in this study are widely present in municipal WWTPs. E1 and E2 are widely detected in effluents and receiving waters. EE2 was included because it is one of the most strongly estrogenic substances in municipal wastewaters.[12] All estrogens (including

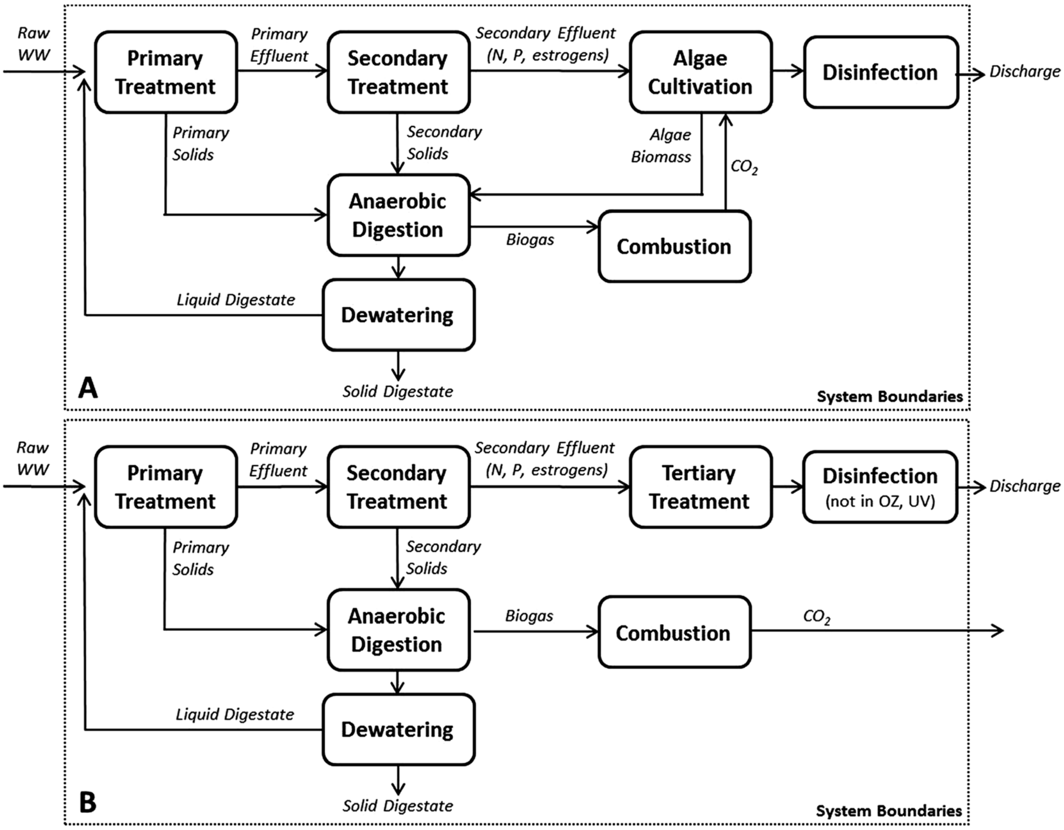

Fig. 1 A (top): process flow for a municipal WWTP incorporating algae cultivation for enhanced bio-energy production and polishing of emerging contaminants and/or dissolved nutrients, as adapted from Menger-Krug *et al.* (2012)[4] and the "tertiary algal nutrient recovery system" (TANR) from Steele *et al.* (2014).[2] B (bottom): process flow for a municipal WWTP incorporating a conventional tertiary treatment for polishing of emerging contaminants and/or dissolved nutrients. In panel B, chemical disinfection is not required for the OZ and UV systems, though it is required in the GAC system.

Table 1 Selected characteristics of the secondary effluent flowing into each modeled tertiary treatment system

Constituent	Concentration range[a]
BOD	6–12 mg L^{-1}
TSS	13–17 mg L^{-1}
TN	3–8 mg L^{-1}
TP	1–2 mg L^{-1}
Fecal coliform (MPN)	3.3×10^4 to 4.9×10^6 MPN per 100 mL
pH	6.4–72
Alkalinity	50–100 mg L^{-1} as $CaCO_3$
17α-Ethynylestradiol (EE2)	0.2–7.5 ng L^{-1}
17β-Estradiol (E2)	0.2–17 ng L^{-1}
Estrone (E1)	0.75–49.1 ng L^{-1}
Estrogenicity	~26 ng L^{-1} EEQ

[a] Concentration ranges were assigned to triangular or uniform distribution for use in Monte Carlo modeling. Table S2 of the ESI presents more detailed information on these distributions.

these) have some structural similarity to the prototypical feminine hormone, 17β-estradiol (E2), though they vary dramatically in their ability to exert estrogenic effects and their susceptibility to various wastewater treatments. To overcome these differences in estrogenic potency, previously published values of "relative estrogenic effects" (REE) were used to compute the aggregate toxicity based on individual concentrations in secondary and tertiary effluents. Consistent with common practice, relative estrogenicities of EE2 and E1 are computed using E2 as a reference, in units of "estradiol equivalents" (EEQ). The resulting average estrogenicity of the secondary effluent was 26.3 ng EEQ per L. Removal efficiencies for each individual estrogen were taken from the literature and used to compute expected reduction during each type of modeled tertiary treatment. The concentrations of E1, E2, and EE2 in each tertiary effluent were then aggregated together to compute the residual estrogenicity for each treatment, using the same REE values. Thus, overall estrogenicity removal could be computed for each tertiary treatment, by comparing the aggregate influent and effluent estrogenicities arising from various combinations of all three estrogens. Section 4 of the ESI† presents the molecular structures of each estrogen and also summarizes relative estrogenicity calculations and assumed estrogen removal efficiencies for each tertiary treatment of interest, with corresponding literature references.

The algae cultivation model was based largely on our previously published models for algae production in open ponds.[23] Selection of the hydraulic retention time (HRT) in the cultivation ponds was based on the results from experiments described in previously published literature studies.[3,10,15–18] The algae conversion model was adapted from our previously published model for production of algae-derived transportation fuels,[10] specifically "Case A", in which it is assumed that the bulk algae biomass is anaerobically digested to produce methane-derived bioelectricity. Synergistic effects, as reported by Peng and Colosi,[11] were not accounted for during anaerobic digestion. LCA models for the conventional tertiary treatments were sized to deliver "typical" HRTs for the desired FU flow rate. Design parameters were taken from previously published literature studies. The ESI† provides full documentation for all models.

The Crystal Ball add-on for Microsoft Excel was used to facilitate Monte Carlo analysis in all LCA models to capture uncertainty about model inputs and parameters taken from literature data and/or based on first-principles engineering calculations. LCA outputs included: direct land use per FU, energy return on investment (EROI), normalized energy use per mass of estrogenicity removed, and residual concentrations of selected wastewater constituents in the tertiary effluents. EROI accounts for energy production (E_{OUT}) divided by energy consumption (E_{IN}), such that values greater than 1 are considered desirable.

3. Results and discussion

3.1 Assessing algae-mediated estrogen removal

As a first step towards understanding the viability of algae-mediated estrogen removal during integrated algaculture and wastewater treatment, we collected literature data related to algae-mediated removal of estrogenic chemicals. E2 was selected as the target contaminant for this analysis because the steroid hormones are known to be the primary causative agent for feminization of aquatic wildlife, and E2 is the archetypal structure of the steroid hormone family. All other estrogenic steroid hormones elicit an estrogenic response by virtue of their structural similarity to E2.[12] Additionally, E2 has been widely detected at appreciable concentrations in WWTP discharges and agricultural runoffs.[12] It was hypothesized that reasonable consistency in previously reported removal of E2 would indicate that literature data for steroid hormones would be sufficient for a preliminary LCA-based assessment of algae-based estrogenicity removal.

Fig. 2 depicts normalized E2 concentrations over time during reactions with several types of active algae biomass, as collected from previously published studies. Interestingly, these datasets tend to exhibit a similarly shaped curve for normalized E2 removal over time, showing mostly good agreement with the pseudo first-order rate model: $R^2 = 0.98$ (ref. 15) and $R^2 = 0.92$ (ref. 16) for *C. vulgaris*; $R^2 = 0.96$ for *A. cylindrica*;[16] $R^2 = 0.98$ for *M. aeruginosa*;[16] and $R^2 = 0.99$ for *S. dimorphus*.[18] However, the Zhang *et al.*[18] results show more rapid initial removal of E2 than most of the other previously published studies; *i.e.*, >80% removal within the first 24 h, compared to 60% removal by *C. vulgaris*[15] and mixed algae species[17] for nearly the same time interval. Despite this, Zhang *et al.*[18] observed approximately the same overall extent of E2 removal as Lai *et al.*[15] for experiments with *C. vulgaris* and Shi *et al.* for experiments with a mixed algae culture.[17] All three datasets exhibit a somewhat horizontal asymptote at 75–85% removal, even though there was significant variability in experimental conditions used by each set of others. For example, the initial algae concentrations used by each author were widely different: Shi *et al.*[17] used 100 mg L^{-1}, Lai *et al.*[15] used 0.8–2.3 g L^{-1}, and Zhang *et al.*[18] used 40 mg L^{-1}. Cell concentrations from Ge *et al.*[16] could not be directly compared with the other studies due to the incompatible units. There was also significant disparity among initial E2 concentrations used in the previously published studies. The initial estrogen concentrations used by Lai[15] (0.5 μg L^{-1}) and Shi[17] (1 μg L^{-1}) were somewhat less than that used by Zhang *et al.*[18] (5 μg L^{-1}); however, Ge[16] used 4–16 mg L^{-1}, which is several orders of magnitude larger. We interpreted the generally good similarity in overall extent of removal, despite key differences in experimental setup among authors, as indication that the observed results are fairly robust across algae species, culture conditions, *etc.*

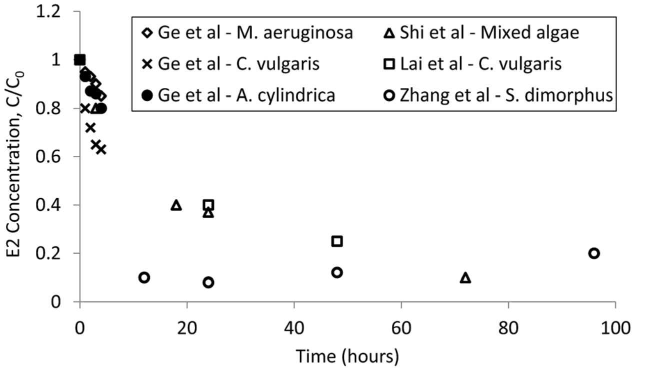

Fig. 2 Normalized E2 measurements as a function of time in previously published studies.

Another observation from Fig. 2 is that several genera that are of significant interest for bioenergy production (*e.g.*, *Chlorella*, *Spirulina*, and *Scenedesmus*) are also capable of appreciable estrogen removal.[10,23] However, it should be noted that previously published pilot-scale research using actual wastewater effluents suggests that there will be highly diverse mixed communities in the algae cultivation ponds.[7] Because most of the data in Fig. 2 correspond to individual experiments with single algal species, future work should focus on characterizing how mixtures of WWTP-relevant species remove E2 and mixtures of other relevant estrogenic chemicals.

Finally, with respect to the LCA objective of this study, an especially critical observation from Fig. 2 is that selection of the hydraulic retention time (HRT) is of utmost importance in the design of algae-mediated tertiary treatment systems. Longer HRTs in the algae cultivation ponds give rise to better estrogen removal and higher densities of harvestable algae (up to some threshold), but also require larger physical footprints to deliver the target FU. Thus, it is critical to select an HRT that optimally satisfies these three criteria: estrogen removal, algae biomass accumulation, and physical footprints. From Fig. 2 (and Fig. S3 and S4†), the most efficient estrogen removal occurs during the first 30 h. From previously published algae growth curves (*e.g.*, Fig. S2† of Zhang *et al.*[18]), the algae concentration increases most dramatically for this same period; therefore; ~15–48 h is a range of appropriate HRTs. The HRT for the algae cultivation pond was thus assigned to a triangular distribution over the range 0.5–2 days for LCA calculations pertaining to the algae-based tertiary treatment system. In this way, data arising from previously published research were used to inform LCA-based analysis of algae-mediated estrogen removal systems.

3.2 Evaluating the environmental favorability of algae-mediated estrogen removal

All modeled tertiary treatment systems were required to achieve predetermined effluent limits for BOD, TSS, TP, TN, and fecal coliform concentrations. In contrast, we did not require that all modeled tertiary treatment systems achieve the same removal efficiency for estrogenicity. Instead, we assessed what estrogenicity removal could be achieved for a "typical" installation of each technology sized to deliver permissible levels of the previously mentioned regulated contaminants. Because the energy and material inputs required for operation of each tertiary treatment are dramatically different among the four evaluated systems, we computed both the environmental *costs* (land use, energy use) and *benefits* (estrogen removal, BOD and TSS removal) for a typical installation of each treatment. We then used these results to formulate several key metrics, to assist in the evaluation of how algae-mediated tertiary treatment compares to its conventional alternatives.

3.2.1 Land use. Reiterating from Section 3.1, the HRT was the key design parameter used to determine the algae pond size that is required to deliver efficient estrogen removal. For HRT values spanning the range 0.5–2 days and the selected FU (10 MGD), expected removal efficiencies for individual steroid hormones are $\sim$70% for E2, $\sim$40% for E1, and $\sim$75% for EE2 (Table S9†). This corresponds to an overall estrogenicity removal on the order of 60%, based on the relative estrogenic potencies (in ng EEQ per L) of these three compounds. The corresponding pond area required for algae tertiary treatment is $\sim$7.6 ha (19 acres). This is somewhat large, but the Moore's Creek WWTP in Charlottesville, VA (USA) facility currently owns 45 acres,[22] and a recent geographic analysis found that more than 1.4 million suitable acres are available within a 1.5 mi radius of municipal WWTPs in the state of Kansas.[4] Thus, land use may not be an insurmountable obstacle.

The selected conventional tertiary treatment systems were also sized to accommodate the FU. The likeliest HRT values and their corresponding average estrogenicity reductions are as follows: 1–30 min and 84% EEQ reduction for OZ, <10 s and 7% EEQ reduction for UV, and 5–30 min and 81% EEQ reduction for GAC. Because these HRTs are so much shorter than for the algaculture system, the physical footprint required for each conventional tertiary treatment is much smaller than for the algae system.

3.2.2 Energy use. We were also interested to see how the algae-mediated tertiary treatment compares to the conventional tertiary treatments on the basis of key environmental impacts. Two energy metrics were computed for each impact: EROI for the 10-MGD system, and also a "normalized" net energy use parameter that accounts for estrogenicity removal during treatment. EROI is relevant because WWTPs will seek information about the energy efficiency of optional treatments intended to remove currently unregulated contaminants. On the other hand, the normalized net energy metric is a better illustration of the environmental cost/benefit ratio for each system, which will be important for making comparisons between technology options if these pollutants are regulated in the future. Table 2 presents computed values of both metrics for each of the conventional treatment systems, based on LCA modeling for each evaluated system. Relevant effluent concentrations are also presented, to show that the waste treatment efficacy is not significantly impaired in the proposed integrated algaculture system.

The most compelling observation from Table 2 is that the algae system delivers the highest EROI out of all four evaluated tertiary treatments. It also delivers the only increase in energy efficiency relative to the baseline WWTP scenario. Each other tertiary treatment mediates a slight to dramatic decrease in energy efficiency. The EROI of the UV system is roughly the same as that of just WWTP, because UV disinfection eliminates the need for chlorination and dechlorination, both of which require chemicals that are very energy-intensive to produce. (The tertiary effluent from the OZ system also does not require chemical disinfection, though the effluents from the algae and GAC systems do.) Still, the UV system evaluated in this study offers very slight reduction in estrogenic toxicity. This could be improved by substantially increasing the UV retention time and/or the dose of H_2O_2; however, this would also increase energy consumption in this system. All three other systems offer much better estrogenicity removal. For OZ and GAC, these performance enhancements are offset by marked decreases in EROI, which reflect significant electricity consumption during both processes. In contrast, the algae system offers good estrogenicity removal (56%) while actually increasing the EROI.

Our observation of improved energy performance following integration of algaculture with wastewater treatment is qualitatively similar to previously published results from other groups;[2,4,6,7,11] though differences in system boundaries, assumed process flow configurations, and reported metrics preclude direct quantitative comparisons among these data. The process configurations used by Peng and Colosi[11] and Sturm and Lamer[7] are most similar to what we used in our study, in so far as their algae ponds also receive secondary effluent. Peng and Colosi[11] reported quantitatively similar increases in EROI for addition of algae cultivation and digestion to a traditional WWTP, even though estrogen removal was not evaluated in their study at all and their assumptions about individual unit operations (most notably anaerobic co-digestion) and final effluent characteristics were different than what was used in the current study. Sturm and Lamer[7] also reported significant energy offsets for avoidance of BNR when algae are used to remove TN and TP in the final WWTP effluent. They did not present EROI, but their best results for production of a biofuel (rather than direct combustion of the algae biomass)

Table 2 Summary of key LCA results for a reference "WWTP" case (without tertiary treatment) and four evaluated tertiary treatment systems: algae cultivation, ozonation (OZ), UV irradiation (UV), and adsorption on granular activated carbon (GAC). All outputs represent computed average values from Monte Carlo simulations. See Table S12 for confidence intervals of selected parameters

Metric	WWTP	Algae	OZ	UV	GAC
EROI (MJ $\text{energy}_{\text{OUT}}$/MJ $\text{energy}_{\text{IN}}$)	0.50	0.65	0.24	0.47	0.35
Normalized net energy use[a] (MJ g^{-1} EEQ removed)	NA	6.5×10^4	3.1×10^5	2.3×10^6	1.8×10^5
Residual estrogenicity[b] (ng EEQ per L)	26.3	11.6	4.2	24.5	5.1
Residual BOD concentration[b] (mg L^{-1})	8.3	10.2	5.5	8.3	2.6
Residual TSS concentration[b] (mg L^{-1})	14.7	16.7	9.1	14.7	11.7

[a] Positive net values indicate that more energy is consumed than produced in the system. [b] Refers to discharged effluent: secondary for WWTP, tertiary for algae, OZ, UV, and GAC.

correspond to roughly 60% reduction in daily energy consumption, after accounting for energy gains and losses associated with adding the algaculture system. The process flow configuration used by Menger-Krug *et al.*[4] is similar to that of Lamer and Sturm[7] and also ours, except that their primary effluent and digestate liquid are fed into the algae cultivation pond, with secondary effluent, to supply nutrients. They then assumed that mixotrophic algae are able to consume BOD, which differs from our assumption of strictly phototrophic growth in the algae ponds. They found that the addition of an algaculture system decreases a WWTPs' net energy balance by 41–102%, depending on the extent to which CO_2 can be recycled. Correspondingly, EROI improves from 0.38 to 0.62–1.01. Finally, Beal *et al.*[6] and Steele *et al.*[2] reported significant improvements in energy consumption when algaculture is used instead of traditional secondary treatment (*i.e.*, primary effluent flows into the algae ponds). Neither study assumed that the algae biomass would be anaerobically digested with WWTP sludge to produce bio-electricity. Still, Beal *et al.*[6] reported an increase in EROI from 0.37 to 1.44 when algaculture is added to WWTP operations.

Steele *et al.*[2] did not report EROI metrics, but the results in their Fig. 6 suggest that using algae to treat primary effluent reduces WWTP energy use by roughly 60–80% compared to the WWTP baseline. In contrast, the results for their "tertiary algae nutrient removal" (TANR) case do not reflect any significant change in energy consumption. They did not explicitly account for energy production from the harvested algae, though they do make reference to anaerobic digestion at the WWTP as a possible means for further improving the energy efficiency of the integrated system.

The increase in EROI for the algae system that we analyzed in our study reflects both increased energy production and decreased energy consumption in the integrated algaculture system compared to the WWTP baseline. Again, this is qualitatively consistent with conclusions from Peng and Colosi[11] and Sturm and Lamer.[7] With respect to energy production, harvested algae biomass accounts for roughly 13% more digestible biomass compared to the WWTP baseline. This then gives rise to roughly 10% more methane and methane-derived electricity. Regarding energy consumption, algae-mediated uptake of nitrogen (as nitrate) and phosphorus from the secondary effluent reduces the need for subsequent denitrification and chemical precipitation. This means that smaller quantities of methanol and alum are required to achieve the same TN and TP limits, and less electricity is required for associated operations (*e.g.*, filter feeding, filtration, *etc.*). The latter effect is analogous to BNR offset articulated by Sturm and Lamer.[7]

From Table 2, the UV, GAC, and algae systems are all capable of reducing estrogenicity to varying extents with some considerable variability in the corresponding impact on EROI. Thus, it is difficult to say which offers the "best" performance overall. For better quantification of this tradeoff, Table 2 also presents each system's energy use as normalized by its estrogen removal. Based on these data, the algae system is by far the most energetically favorable for the evaluated tertiary treatments, because it offers the best ratio of environmental benefit (*i.e.*, estrogen removal) per energy cost. Of the conventional tertiary treatments, GAC offers the best performance in this category, closely followed by OZ. UV's performance in this metric is one order of magnitude worse than either GAC or OZ, despite its relatively good EROI performance. With respect to the algae system, it is encouraging that this tertiary treatment exhibits the best performance on a cost-benefit basis. This could motivate WWTPs to implement algae-based tertiary treatment as a means to produce energy while also removing currently unregulated emerging contaminants.

Finally, Table 2 presents BOD and TSS data for the effluents arising from each tertiary treatment. These values were computed based on mass flows for the algae-based tertiary treatment system shown in Fig. 1. In this system, the algae biomass is harvested directly out of the tertiary effluent, which is then disinfected *via* chlorination (if needed) and immediately discharged. Because there are no additional treatment steps following algae cultivation, BOD and TSS concentrations in the final effluent are very sensitive to the assumed capture efficiency for algae harvesting. We assumed that this value ranges from 90–100%; however, this will need to be verified in future work. Additionally, we did not attempt to account for the release of soluble organic material by the algae, even though this would increase the BOD concentration of the final effluent. Thus, this will also have to be evaluated in future studies. Under the current set of modeling assumptions, all four evaluated tertiary treatment systems produce effluents that meet relevant water quality standards for the selected parameters (Table S1†), including not only BOD and TSS but also TN, TP, and fecal coliform concentrations. Thus, the algae-based tertiary treatment, which is intended to improve energy efficiency (EROI) and estrogen removal, does not compromise a WWTP's ability to achieve its primary objective of protecting water quality.

3.2.3 Sensitivity. The results in Table 2 are compelling because they demonstrate that algae-mediated treatment could deliver simultaneous water quality and energy improvements at municipal WWTPs. But, even with the algae treatment, the whole-plant EROI is still less than 1, which means that the WWTP is still net energy-consuming. The United States Environmental Protection Agency (EPA) estimates that water and wastewater treatment operations account for roughly 3–4% of the nation's annual electricity consumption and up to 35% of the energy budget for a typical US city;[24] therefore, it would be valuable to convert WWTPs from energy consumers into energy producers.[1] We conducted a sensitivity analysis of our algae system results, in order to determine the input parameters that most strongly drive the overall EROI and net energy balance results. These parameters are important areas of future research if we wish to improve the overall efficiency of the integrated wastewater treatment and algae cultivation system. The results of the sensitivity analysis are presented as a tornado chart in Fig. 3.

Tornado charts present sensitivity information in a visual manner, showing to what extent an output parameter (*i.e.*, EROI for a WWTP incorporating algae-mediated tertiary treatment) changes in response to ±10% change in each input. This process is repeated systematically for each input parameter, one at a time. From Fig. 3, each of the top-five most impactful model inputs is directly correlated with EROI, such that an increase in each input yields an increase in EROI compared to the baseline estimate (black on right), or a decrease in each input yields a decrease in EROI (white on left). Fig. 3 also indicates that the top three most impactful model inputs pertain to co-digestion of the WWTP biosolids (primary and secondary) with the harvested algae biomass. This is interesting and meaningful because the field of algae co-digestion is quite immature in comparison to digestion of just WWTP biosolids or algae. Therefore, it is conceivable that additional research could improve the value of digestion-related inputs, including: methane yield for WWTP biosolids in the presence of algae, or percent digestibility (removal) of WWTP biosolids in the presence of algae. Improvement in either of these would increase the overall EROI. Thus, it is timely that there has been a recent increase in published research regarding digestion and co-digestion of algae biomass with WWTP biosolids (*e.g.* ref. 11 and 25). In contrast, it seems unlikely that additional research will change the influent BOD concentration.

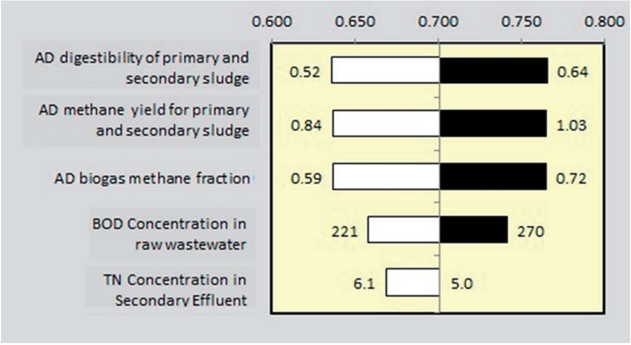

Fig. 3 Tornado plot to illustrate the impact of ±10% changes in each input parameter on the output value for EROI in the WWTP + algae treatment system. Only the top five most impactful inputs are shown. Black bars indicate increases in input parameters. White bars indicate decreases in input parameters.

It is impossible to use the results in Fig. 3 to predict the overall EROI that could be achieved if many or all of the co-digestion parameters were individually improved. Still, the axis at the top of Fig. 3 shows that improvement in just the most impactful parameter alone (methane yield for biosolids co-digested with algae) could increase the EROI from 0.70 to 0.76 (roughly 17% increase). If the combined effects of several improved parameters could increase the EROI above 1.0, it may motivate municipal WWTPs to implement algae-based tertiary treatments as a means to improve the water quality and energy efficiency at their plants. Given these encouraging preliminary results, future research should focus on characterizing removal of other steroid hormones under more realistic simulated culture conditions (*e.g.*, mixed algae communities, continuous flow conditions, ng L^{-1} concentrations of E2 and other estrogens in real secondary effluent, *etc.*), so that the LCA models can be enhanced and expanded. This will enable better understanding of whether this technology is workable under real-world conditions.

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