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Cultivation of microalgae in adjusted wastewater to enhance biofuel production and reduce environmental impact: Pyrolysis performances and life cycle assessment

Gang Li [a], Ruichen Hu [a], Nan Wang [b], Tenglun Yang [a], Fuzhuo Xu [a], Jiale Li [b], Jiahui Wu [a], Zhigang Huang [a], Minmin Pan [c,*], Tao Lyu [d,**]

[a] School of Artificial Intelligence, Beijing Technology and Business University, Beijing, 100048, China
[b] School of Art and Communication, Beijing Technology and Business University, Beijing, 100048, China
[c] Department for Solar Materials, Helmholtz Centre for Environmental Research GmbH-UFZ, Permoserstraβe 15, 04318, Leipzig, Germany
[d] Cranfield Water Science Institute, Cranfield University, College Road, Cranfield, Bedfordshire, MK43 0AL, United Kingdom

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ABSTRACT

The interest in microalgae as a renewable and sustainable feedstock for biofuels production has inspired a new focus in biorefinery. Current innovations in microalgae technology include the use of wastewater as the cultivation medium towards nutrients recovery, renewable energy generation, as well as wastewater treatment. Though recent studies have favoured the competitiveness of such an approach, how to maintain a high-quality microalgae-derived biofuel production in real wastewater with fluctuations in nutrient contents is still a challenge. This study investigated a strategy of adjusting the nutrient composition of the feeding wastewater (i.e. anaerobic digestion effluent) for microalgae cultivation (*Desmodesmus* sp.) and biofuel production. The addition of an appropriate amount of nutrients, including magnesium, iron and phosphorus, significantly enhanced the microalgal biomass production (0.78 g L^{-1}) compared with the original wastewater (0.35 g L^{-1}) and the standard BG11 microalgae cultivation medium (0.54 g L^{-1}). In terms of the potential biofuel quality, the use of adjusted wastewater led to a higher content of valuable products (aliphatic hydrocarbon and fatty acids were accounted for 23.98% and 42.33% of the whole biomass, respectively) along with a reduction in potentially toxic substances (nitrogen-containing compounds and polycyclic aromatic hydrocarbons were accounted for 7.96% and 7.09% of the whole biomass, respectively) compared with the other cultivation groups. Moreover, the lower optimal temperature of pyrolysis engendered by the adjusted wastewater was significant for reduction of process energy consumption, which in turn led to overall lowered environmental impacts (838.52 mPE_{T2000}, applying life cycle assessment) than did the original wastewater (1165.67 mPE_{T2000}) and standard cultivation medium (1347.63 mPE_{T2000}). This study demonstrated that the adjustment of wastewater can act as a potential approach for the improvement in the quality of microalgal biofuel production, with consequent reduced environmental impacts.

1. Introduction

To meet the demands of the upcoming energy shortage and to address the issue of climate change, the concepts of the circular economy and sustainable development have gained momentum for the exploration of renewables, e.g., through the sources of solar, wind, biomass, and geothermal energies (Bach and Chen, 2017). Among them, biomass acts as an indispensable role due to its multiple characters, e.g., renewability, closed CO_2 cycle, and enormous total potential energy capacity worldwide (Tursi, 2019). Advanced development in microbiological technology has favoured biofuel production from microalgal biomass as the third generation of bioenergy (Chowdhury and Loganathan, 2019; Stephens et al., 2010). In view of compositions, microalgal carbohydrates, lipids and proteins are ideal as feedstock for bio-oil production, as well incite the potential of fossil fuels replacement by microalgal biofuel (Almarashi et al., 2020). In addition to the contribution on carbon sequestration via rapid growth and photosynthetic functionality (Li et al., 2015), raw wastewaters can be reused to

* Corresponding author.
** Corresponding author.
E-mail addresses: minmin.pan@ufz.de (M. Pan), T.Lyu@cranfield.ac.uk (T. Lyu).

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cultivate microalgae, enhancing the growth of microalgal biomass and accumulation of lipid content (Behl et al., 2019; Zhou et al., 2011). Remarkable advantages from the application of wastewater as cultivation medium lies not only in reduction of operational costs, but also in further benefits in wastewater treatment (Li et al., 2019).

The nutrient content of raw wastewater normally exhibits high variability and, furthermore, some key elements for microalgal growth may be entirely absent, which could affect both the generation of desirable biofuel compositions and also nutrient removal from this constituent (Chen et al., 2015). In order to address these issues, strategies for the adjustment of raw wastewater, by amelioration of observed elemental deficiencies, have been explored to produce an effective cultivation medium. Among all elements, phosphorus is essential for DNA, RNA, and molecular structure building. Moreover, iron is indispensable for metabolic processes and magnesium plays a central role for chlorophyll formation in microalgal (Ermis et al., 2020). For instance (Shen et al., 2019), added extra phosphorus into swine wastewaters which resulted in an increase (27.9%) of biomass growth of *Chlamydomonas* sp. Furthermore, addition of trace metals, i.e., Mo, Mn, and Fe, has been observed with an increase of lipid productivity of *Scenedesmus obliquus* by 18.63%, 19.21%, and 27.95%, respectively (Han et al., 2019). Nevertheless, increases in algal biomass and lipid content could not always be translated to production of better quality biofuel (Hanifzadeh et al., 2018; Ruangsomboon et al., 2013). Thus, further investigation is needed for the evaluation of the effects of microalgal cultivation in adjusted wastewater on the final quantity and quality of biofuel production.

Considering energy conversion from microalgal biomass, techniques of thermochemical conversion, including gasification, combustion, hydrothermal conversion, and pyrolysis, are widely adapted regarding the properties of high efficiency, multiple products, and easy operation (Perera et al., 2021). Pyrolysis stands uniquely out among all thermochemical techniques as it is easily adjustable (according to targeted products) and is regarded as the most efficient approach (Ma et al., 2019; Perera et al., 2021). Appropriate pyrolysis conditions (e.g. pyrolysis temperature) and variations in composition of microalgal biomass are critical for extracting the most valuable products without generating potential toxic by-products (Li et al., 2020). For example, inappropriate pyrolysis temperature could lead to both wanted products (e.g. bio-oil, biochar) and unexpected substances (e.g. polycyclic aromatic hydrocarbons (PAHs) and N-containing compounds) (Brindhadevi et al., 2021). Furthermore, previous studies have demonstrated that, when the nitrogen content of the microalgae exceeded 5%wt, biofuels of unpredictable quality were generated, resulting in reduced calorific values and elevated production of nitrous oxide (NO_x) (Mehrabadi et al., 2015). Therefore, investigation into optimal approach for microalgae biomass growth using wastewater, together with the appropriate pyrolysis conditions, could benefit the quality of final biofuel.

Though promising, the current studies are usually in one-sided view towards increasing final production (biofuel), ignoring the impacts along with the whole life cycle. Indeed, potential negative environmental impacts could still be created along the entire process of microalgal cultivation in wastewater and then for biomass pyrolysis, for example, through emission of NO_x, which is a potent greenhouse gas (GHG) (Finkbeiner et al., 2006). Life cycle assessment (LCA) is a technique for simplifying the complexity of information from diverse environmental aspects caused by a process or product, for comparison and decision-making (Finnveden and Moberg, 2005). Thus far, LCA has been widely applied for the assessment of wastewater-based (e.g. municipal wastewater, and swine manure) algal biofuel production through pyrolysis (Diniz et al., 2017; Mu et al., 2014). Utilization of LCA across the entire process, from microalgal growth in adjusted wastewater, until final pyrolysis and product refinement, is of particular significance in order to explore and enable the application of novel technologies with reduced environmental impact (Grierson et al., 2013). However, assessment of the metrics of net energy gains and GHG emissions has, hitherto, been insufficiently investigated.

The present study designed an accurate approach of wastewater adjustment (nutrients addition according to specific water quality) towards enhancing microalgal biofuel production. Coupled with LCA, the environmental impacts of the entire process were assessed, contributing to the confirmation of sustainability for the proposed approach. To achieve this goal, anaerobically-digested (AD) effluent and *Desmodesmus* sp. were selected as model wastewater and process microalgae, respectively. The rates of generation of microalgal biomass and differences in composition were compared with samples cultivated in BG11 medium, raw AD effluent, and AD effluent adjusted by the addition of nutrients and trace metals. Microalgal biomass from these three groups were further employed as feedstocks for pyrolysis over the temperature range 300–800 °C in order to identify the optimal temperatures through evaluating the pyrolysis products. Moreover, LCA was conducted in order to evaluate the environmental impacts of biofuel production with the three different cultivation media. The results are of great significance for further development of such strategies for wastewater modification and process control, with the aim towards high quality microalgal biofuel production.

2. Materials and methods

2.1. Microalgal species and cultivation conditions

The microalgal species, *Desmodesmus* sp. EJ 8–10, was isolated from a local fresh water body and pre-cultivated in BG11 medium. The accelerated growth of *Desmodesmus* sp. in wastewater and balanced composition of pyrolysis products have been proved (Pourkarimi et al., 2019), thus, it was selected as the model microalgae in this study. Optimal cultivation conditions (illumination intensity: 6000 ± 100 lux; illumination duration 14 h: 10 h, light: dark; temperature 25 ± 1 °C, pH 7.5) were provided with constant stirring (magnetic stirrer, 200 rpm, 4 min per hour) (Li et al., 2021). All cultivation process was conducted aseptically. Anaerobic digestion effluents (applying two mesophilic up-flow solid reactors and one psychrophilic plug-flow reactor, from a rural pig farm, Beijing, China) were pre-centrifuged for 15 min (10000 rpm) and the supernatants then stored at 4 °C prior to use. The concentrations of NH_4^+-N, PO_4^{3-}, COD, Fe, Mg, total solids (TS), and volatile solids (VS) in the AD effluent were determined to be 1056 mg L^{-1}, 37 mg L^{-1}, 2950 mg L^{-1}, 2.7 mg L^{-1}, and 33.1 mg L^{-1}, 24.9 ± 1.8 g kg^{-1}, and 13.1 ± 0.9 g kg^{-1}, respectively.

Due to the high COD level (up to 2.95 g L^{-1}) and turbidity of the AD effluents, a dilution rate of 10% (named as ADE, pH 7.3) was adopted for the cultivation of *Desmodesmus*. sp (Luo et al., 2019). Notably, the medium ADE was found still to be insufficient in those nutrients essential for microalgal growth, *e.g.*, Mg, Fe, P. By extra additions of ammonium ferric citrate, K_2HPO_4, and magnesium sulfate, $MgSO_4{\cdot}7H_2O$, final concentrations of 7.4 mgL^{-1} Fe, 28.2 mgL^{-1} P, and 47.7 mgL^{-1} Mg were achieved (Li et al., 2013). For comparison purposes, the standard growing medium of BG11 was selected as a control group. All three media (ADE, adjusted ADE, and BG11) were utilised as feedstocks in batch (utilizing 100 mL flasks) for microalgal cultivation. An initial microalgal biomass of 0.1 OD_{680} (to provide same weight of biomass to each group) (Luo et al., 2019) was separately inoculated into each medium and cultivated for 14 days under the same conditions as previously noted.

2.2. Determination of elemental composition and thermal analysis

The major elemental compositions of each sample of the cultivated microalgal biomass (C, H, N and S, in triplicates) were measured by the Elemental Analyser (EA; Flash EA-1112, Thermo Scientific, Waltham, MA, USA). Lipid contents were determined according to the method of Bligh and Dyer (1959). Protein contents were calculated using Eq. (1) (Mulder, 1839; Salo-väänänen and Koivistoinen, 1996):

$$\text{Concentration of protein} = \text{Nitrogen content} \times 6.25 \quad (1)$$

Moreover, the higher heating values (HHV) of biomass samples were calculated according to equations (Eqs. (2) and (3)) (Friedl et al., 2005; Mahinpey et al., 2009):

$$\text{HHV(OLS)} = 1.87C^2 - 144C - 2082H + 63.8C \times H + 129N + 20147 \quad (2)$$

$$\text{HHV(PLS)} = 5.22C^2 - 319C - 1674H + 38.6C \times H + 133N + 21028 \quad (3)$$

C, H and N represent the contents of elemental carbon, hydrogen and nitrogen in the sample, respectively. Equations (2) and (3) were obtained through regression analysis of ordinary least squares (OLS) and partial least squares (PLS) calculations of the higher heating values for samples, following the proposals of Friedl et al. (2005). The average value of HHV (MJ kg^{-1}) was calculated according to Equation (4) (Friedl et al., 2005):

$$\text{HHV} = [\text{HHV(OLS)} + \text{HHV(PLS)}]/2 = (3.55C^2 - 232C - 2230H + 51.2C \times H + 131N + 20600) \times 10^{-3} \quad (4)$$

Thermal decomposition behaviours of microalgal biomass were characterised using thermogravimetric analysis (TGA; SDT-Q600, TA Instruments, New Castle, DE, USA). Specifically, 2–4 mg of samples were pyrolyzed from 50 °C to 800 °C with a heating rate of 20 °C min^{-1} (Castelli et al., 2000) with nitrogen (99.999% purity, 100 mL min^{-1}) as shielding gas.

2.3. Analysis of pyrolysis products

To investigate the pyrolysis products from harvested microalgal biomass, samples were pyrolyzed and analysed using a pyrolysis-gas chromatograph-mass spectrometer (Py-GC-MS), consisting of a rapid pyrolyser (Frontier Labs 3030i, Koriyama, Fukushima, Japan) coupled with a GC-MS (Agilent 7890A/5975C, Santa Clara, CA, USA). Different temperatures of pyrolysis (300 °C, 400 °C, 500 °C, 600 °C, 700 °C and 800 °C) were studied in order to determine optimal pyrolysis conditions. The operational conditions of the GC-MS were as follows: a HP-5 (30m × 0.25 mm × 0.25 μm) capillary column was employed with 1.0 mL min^{-1} He gas as carrier gas (in constant flow mode). A split ratio of 1:10 was applied to the sample injector. The initial oven temperature was kept at 40 °C for 3 min, and then increased at a rate of 5 °C min^{-1} to 200 °C and maintained for 5 min. Finally, the temperature was increased to 250 °C at 10 °C min^{-1} and further maintained for 5 min. Pyrolysis products were identified by searching their resultant mass spectra through the NIST11 database (National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.4. Life cycle assessment

To better evaluate the sustainability of biofuel production through the proposed processes, life cycle assessment (LCA) was applied in this study to estimate energy consumption and any environmental impacts of products of pyrolysis for microalgal biomass cultivated in different media and processed at the corresponding optimal temperature. The selected goals and scope followed in the present study are in accord with the ISO 14040 (Marsmann, 2000).

2.4.1. LCA goals and system boundary

The goals of applying LCA in this study are: Firstly, to assess the environmental impact of microalgal products through the entire microalgal cultivation and pyrolysis processes; Secondly, to evaluate and compare the differences of microalgal products processed through the various culture media; Finally, to provide prioritisation for the selection of cultivation medium for the production of optimum pyrolysis products. To achieve these goals, three major stages were included in this LCA model, i.e., microalgal cultivation, microalgal recapture, and microalgal pyrolysis. In this study, consumption of energy, water and nutrients during the process operation were regarded as system inputs. However, the depreciation of equipment (e.g., hydroextractor, pyrolyzer), the energy consumption of manufacturing nutrients added in the ADE, and the environmental impacts through microalgal growth were excluded. The capture of CO_2 through microalgal growth was ignored. Besides, no further treatment of pollutants during the process was conducted. As presented in Fig. 1, a schema describes the specific processing and system boundaries.

2.4.2. Assessment of environmental impact

Main pollutants generated from the proposed process were diverse gas waste and eutrophic pollutant from energy consumption. Thus, four categories of environmental impact were highlighted in this assessment, including: global warming (kg CO_2, CH_4, NO_X, CO-eq), acidification (kg SO_2, NO_X-eq), eutrophication (kg PO_4, NO_X-eq), and photochemical ozone synthesis (kg VOC, CO, CH_4-eq) (Li et al., 2020). MJ and kg were applied as the corresponding functional units. *Desmodesmus* sp. (EJ 8–10) of 1 kg biomass dry weight was used for the assessment of energy consumption and environmental impact.

2.4.3. Life cycle stages and inventory analysis

As three stages (microalgal cultivation, harvest, and pyrolysis) were included in this assessment, each stage contributed differently to the energy consumption and air pollution emission. In the cultivation stage, sterilization of the culture medium, inoculation in clean bench, stirring and illumination during cultivation were mainly considered; During the harvesting stage, centrifugation and freeze drying both contributed greatly to energy consumption; In the pyrolysis process, energy consumption through heating was mainly counted. The selection of processes included into each stage was based on previous study (Li et al., 2020). All energy consumption was firstly calculated by electricity consumption (kW•h) and then converted to energy consumption (MJ). The emission of air pollutants was calculated according to a previous study (Li et al., 2020).

In the microalgal cultivation stage, autoclave with operation condition of 121 °C (20 min, max. 8L) and inoculation in clean bench (max. 10 of 5 L flasks, 10 min) were conducted. In this study, 5 L flasks (working volume 3 L) were used for all cultivation. 4 plant lights (36 W) were applied for illumination with a light: dark ratio of 14 h: 10 h (14 days, 28.2 W). Thus, to achieve 1 kg biomass from different media (varying biomass production yields in different media), times of using autoclave and clean bench were calculated, and directly converted into energy consumption.

In the harvest stage, let flasks stand for 24 h (sedimentation). Supernatant was removed until 1 L liquid left. Centrifuge (max. 1.5 L, 6000 r/min, 10 min) was applied. Thus, times of centrifugation to get 1 kg biomass were calculated. To freeze-dry 1 kg biomass (48h), 91.2 kW h energy was consumed.

In the stage of pyrolysis, energy consumption was mainly contributed by the pyrolyzer and real-time analysis. During this process, the energy transfer inside the pyrolyzer was neglected. The energy consumption from the on-line analysis only considered when the pyrolysis products enter the GC/MS, and consumed equally 2.2 kW h for each analysis. Accordingly, the energy consumption during pyrolysis, which was to increase the temperature from 25.6 (room temperature) to the optimal condition for each group (700, 600, and 300 °C for biomass from BG11, ADE and adjusted ADE, respectively), was calculated (Table 1).

2.4.4. LCA model

Environmental impact was calculated by the sum of impacts from all emissions, referred to by (Equation (5)):

$$EI = \sum[Q_i \times F_i] \quad (5)$$

where EI represents the environmental impact; Qi is the amount of the ith emission; Fi refers to the equivalent to the environmental impact by

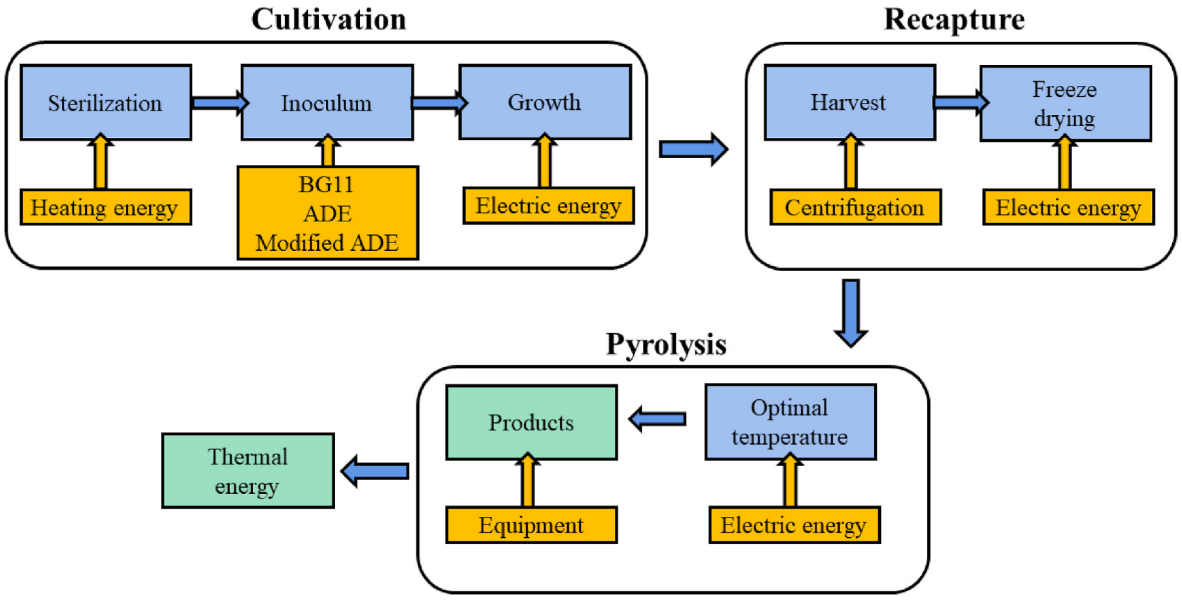


Fig. 1. System boundaries of LCA model for microalgal pyrolysis products.

Table 1
Inventory analysis of microalgal biofuel production.

	BG11 (kW•h)	ADE (kW•h)	adjusted ADE (kW•h)
Autoclave	210.8	200.5	166.4
Clean bench	3.7	3.5	2.9
Illumination	28.2	28.2	28.2
Centrifugation	1199	1032	944.9
Freeze-dry	91.2	91.2	91.2
Real-time analysis	2.2	2.2	2.2
Pyrolysis	1000	720	333.3

the ith emission (Li et al., 2020).

For the comparable evaluation of different types of impacts, the EI was further standardized according to Equation (6):

$$S_{EI} = EI \times R^{-1} \quad (6)$$

where, S_{EI} represents the standardized environmental impact; R refers to the standard benchmark (Li et al., 2020). Notably, different categories contribute to the total environmental impact at varying levels. Thus, weighted assessment (by applying weighting factor) was conducted in this study according to previous study (Li et al., 2020). Briefly, the method of target distance was applied to determine the weighting factor using following equation (Equation (7)):

$$W = E \times EN^{-1} \quad (7)$$

where, W is the weighting factor for each specific parameter, E is the total regional environmental impact potential in 1990, while, EN is the regional environmental impact potential in 2000. The unit of standardized environmental potential impact was represented by the standard person equivalent (mPE_{T2000}) (Li et al., 2020). Origin 8.5 (originLab, Northampton, USA) and SPSS 19.0 (IBM Corporation, Armonk, USA) were applied for data analysis and plotting. The data were checked for normality and homogeneity of variance prior to statistical analysis. If variables were not normally distributed, nonparametric test methods were used for the analysis. Therefore, one way analysis of variance (ANOVA) followed by Tukey's HSD test or/Kruskal-Wallis test followed by Dunn post hoc test were used to identify significant differences of pyrolysis products from different groups cultivated with different media at the 0.05 significance level ($p < 0.05$).

3. Results and discussion

3.1. Properties of microalgal biomass

After 14 days' cultivation, microalgae cultivated in the original AD effluents (ADE) achieved lower biomass production (0.35 ± 0.02 g L^{-1}) than those from BG11 media (0.54 ± 0.03 g L^{-1}) and similar investigation (0.41 g L^{-1}) (Ji et al., 2014). Nevertheless, the adjusted ADE generated significantly higher biomass production of *Desmodesmus*. sp (0.78 ± 0.01 g L^{-1}) when compared with all treatment groups in this study (BG11 and ADE) as well as similar investigation. Direct utilization of effluents from anaerobic digestion showed inhibition of *Desmodesmus. sp* growth when compared with the standard cultivation medium. However, this was significantly improved by the proposed modification approach on the ADE. Phosphorus and iron are closely involved in the cellular metabolic processes of microalgae (Ruangsomboon et al., 2013). The additions of ammonium ferric citrate and K_2HPO_4 to the adjusted ADE groups could therefore have significantly promoted microalgal metabolic rates. Meanwhile, the extra addition of magnesium could also have benefitted microalgal photosynthesis (Hanifzadeh et al., 2018). Consequently, enhanced metabolic and photosynthetic processes in the adjusted ADE groups contributed to the overall enhanced microalgal biomass growth.

Overall, reduced proportions of C, H, N, protein and HHV were observed from microalgal samples cultivated in ADE, than those in the BG11 (Table 2). In contrast, the adjusted ADE groups significantly yielded overall higher contents of C, H, N, S, HHV, and protein, indicating better elemental upcycling and growth of *Desmodesmus. sp*. Higher N contents of microalgal biomass (9.79% in adjusted ADE) was closely related to a potentially higher intensity of photosynthesis, which is reflected by higher contents of chlorophyll in microalgae, and may

Table 2
Compositional analysis of feedstock for pyrolysis.

Feedstock	Elemental analysis (%)				HHV (MJ kg^{-1})	Chemical analysis (%)	
	C	H	N	S		lipid content	Protein content
adjusted ADE	51.2	8.0	9.8	0.8	22.5	14.2	61.2
ADE	25.8	5.4	6.2	0.6	12.9	21.8	38.8
BG11	38.1	6.3	7.7	0.6	16.1	16.6	47.9

further directly lead to a higher accumulation of intercellular protein (Wang and Brown, 2013). Notably, due the higher contents of C, H and N, microalgal biomass cultivated in adjusted ADE, achieved higher HHV than did that from standard cultivation medium (BG11) and ADE. Higher HHV indicates higher energy density, and thus may promote further pyrolysis products in addition (Crombie and Mašek, 2014). Such phenomenon of higher biomass production with wastewater (adjusted) is well explained due to its abundant nutrients in high levels (Li et al., 2019).

3.2. Thermogravimetric analysis

Thermogravimetry (TG) and derivative thermogravimetry (DTG), as widely applied parameters for the study of pyrolysis process, were employed to characterise the thermal decomposition rates during pyrolysis of microalgal biomass (Chen et al., 2014). Overall, microalgal biomass cultivated in adjusted ADE effluents exhibited a slower rate of weight loss during pyrolysis than did those cultivated in BG11 and the original ADE effluents. Typically, three stages of algal pyrolysis could be observed (Fig. 2). In all groups, a loss of moisture occurred at temperatures less than 150 °C, during which weight losses of 5.36–10.31 wt% were observed. A further increase of pyrolysis temperature (up to 550 °C) led to rapid weight loss, which was attributed mainly to the degradation of carbohydrate, protein, and lipid (Chen et al., 2014). Above 550 °C, a slower decrease of TG was measured due to the continuing decompositions and carbonizations of microalgal residues (Bach and Chen, 2017). Meanwhile, the maximum decomposition rates also suggested similar results, of which microalgal biomass from adjusted effluents resulted in the lowest decomposition rates (Fig. 2b) and higher thermal residue (41.31%) than that cultivated from the BG11 and anaerobic effluents. The higher C contents in adjusted-ADE-derived biomass may due to higher amount of hemicellulose (cell wall composition) and other carbon-containing components accumulated in the microalgae, which could contribute to the thermal resistance of the microalgal biomass (Kebelmann et al., 2013).

3.3. Valuable products via microalgal pyrolysis

Products obtained from pyrolysis of microalgae are largely decided by both varying the chemical state of cultivation medium and the temperature of pyrolysis. Consequently, the present study focused on the comparison of pyrolysis products from different microalgal cultivation media (BG11, ADE and adjusted ADE) and by variation of pyrolysis temperatures (over the range 300–800 °C). The products from pyrolysis of biomass of *Desmodesmus. sp*. EJ 8–10 mainly consisted of aliphatic hydrocarbons (alkanes and olefins), aromatic hydrocarbons (benzenes, indenes and its derivatives), fatty acids (C10–C19), nitrogen-containing compounds (amides, nitriles, and pyridines), phenols, polycyclic aromatic hydrocarbons (PAHs), and other trace components, *e.g.*, ketones, alcohols, aldehydes, and furans. These findings are consistent with other relevant studies (Du et al., 2011; Li et al., 2019; Wang and Brown, 2013).

Among all pyrolysis products, aliphatic hydrocarbons, aromatic hydrocarbons, fatty acids, and some trace compounds, *e.g.*, phenol, are regarded as products of economic value. As valuable components in bio-oil, aliphatic hydrocarbons are significant in the production of biofuel. With increasing pyrolysis temperature from 300 to 800 °C, the relative content of aliphatic hydrocarbons, in products from *Desmodesmus. sp*. EJ 8–10 cultivated in adjusted ADE, exhibited a single peak, the maximum relative content of which, appeared at 400 °C (23.98%, Fig. 3a). Further increase of pyrolysis temperature (500–700 °C) generated no significant increases in aliphatic hydrocarbon content. This observation has also been revealed by previous studies (Li et al., 2013). In comparison with results from microalgae cultivated in BG11 and ADE media, biomass from adjusted ADE had significantly reduced the optimal pyrolysis temperature (700 and 600 °C for BG11-and ADE-cultivated microalgae, respectively), as well as increasing the relative contents of aliphatic hydrocarbons (17.84% and 22.91% of BG11 and ADE, respectively, Fig. 3a) in pyrolysis products. The higher relative content of aliphatic hydrocarbons from algae cultivated in adjusted ADE than in the other two medium might mainly result from the larger content of lipids, which could be pyrolyzed into aliphatic and aromatic hydrocarbons, and also compared favorably with pyrolysis products obtained from other algal species and biomass materials (Wang et al., 2013).

Meanwhile, the presence of aromatic hydrocarbon compounds in the pyrolysis products, which could in turn elevate octane number and thus promote the efficiency of combustion (Li et al., 2019), is also of high interest. In an industrial context, aromatic hydrocarbons also function as important chemical feedstocks (e.g., transportation fuel additives). Results indicated that aromatic hydrocarbons constituted the most abundant component in all pyrolysis products. With increasing pyrolysis temperature (300–800 °C), aromatic hydrocarbon content increased to a maximum of 45.21% at 800 °C from microalgae cultivated in the adjusted ADE. Although exhibiting a similar trend, pyrolysis of microalgae cultivated in adjusted ADE generated relatively lower contents of aromatic hydrocarbons than did the biomass in the BG11 and ADE groups (53.09% and 47.62%, respectively).

As the most significant precursor, decomposition of fatty acids under high temperatures may lead to the production of hydrocarbons. This was further proven in this study, where higher contents of fatty acids, from pyrolysis of microalgae cultivated in adjusted ADE, were achieved at lower temperatures (max. 42.33% at 300 °C). Notably, the adjusted ADE growth medium promoted significantly higher contents of fatty acids than those found in the BG11 (1.32%) and ADE (14.81%) groups. Pyrolysis products of microalgae usually contain lipid content less than 22%. The higher fatty acids achieved from the adjusted ADE (in the range of 27–55%) indicates a potential heterotrophical growth of microalgae in the medium (Yang et al., 2019). Observation of the distribution of fatty acids in the adjusted AD group further indicated

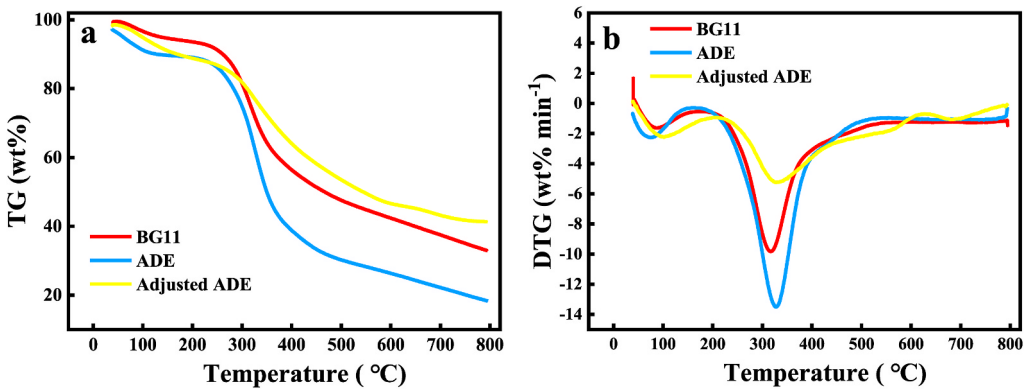


Fig. 2. Thermogravimetry and derivative thermogravimetry analysis of pyrolysis process.

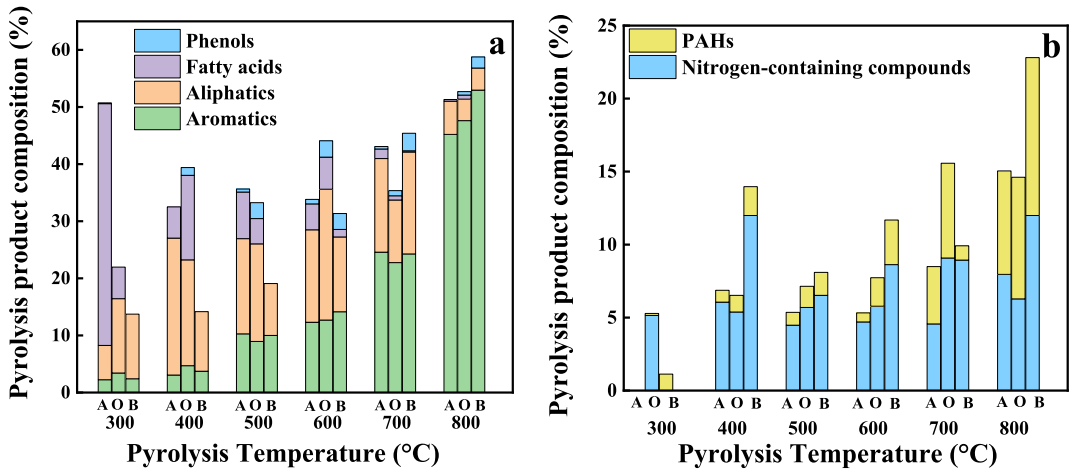


Fig. 3. Pyrolysis products of microalgal biomass cultivated from BG11 medium, anaerobic digestion effluents, and adjusted anaerobic digestion effluents, a) valuable compounds, and b) toxic compounds (A: adjusted AD effluents; O: original AD effluents; B: BG11).

long-chain fatty acids (mainly C16 to C18) as major components, which contains 16.04% of the total fatty acids, showing higher contents than those from BG11- (12.48%) and ADE-derived microalgal biomass (13.81%).

Such long-chain fatty acids are of high value when used as feedstocks for the production of biofuel through transesterification (Chen et al., 2015). Except for the components (occurring in large amounts) mentioned above, some products detected in trace amounts, e.g., phenols, are also of high commercial value (Du et al., 2011). However, the adjusted ADE medium produced lower contents of phenols (up to 0.78%) than pyrolysis of microalgal biomass from other media (3.07% from BG11 and 2.85% from AD).

3.4. Toxic pyrolysis products

Apart from valuable compounds, toxic products such as nitrogen-containing compounds and polycyclic aromatic hydrocarbons (PAHs), could be generated due to the compounds of protein in microalgal biomass, and insufficient degradation during pyrolysis (Lu et al., 2019), thus, affecting the quality of products, as well as causing environmental pollution. Thus, this aspect of the entire process required closer attention. During decomposition of protein, nitrogen-containing compounds are generated, the residues of which, when contained in bio-oil, may lead to excessive emission of nitrogen oxides (Thangalazhy-Gopakumar et al., 2012), and reduced efficiency during combustion. No significant correlation was found between pyrolysis temperature and the relative contents of nitrogen-containing compounds in products studied in this investigation. However, cultivation in adjusted ADE generated lower levels of nitrogen-containing compounds (up to 7.96%, 800 °C) than those derived from products cultivated in BG11 (up to 11.99%, 400 °C) and ADE (up to 9.08%, 700 °C) media, which corresponded well with relevant investigations (around 3.1–10.6%) (Yang et al., 2019). Additionally, polycyclic aromatic hydrocarbons (PAHs), generated during the process of insufficient combustion, may cause cancer in humans (Lu et al., 2019), and are always of the most concern. Results indicated production of higher relative contents of PAHs with increasing pyrolysis temperature for all three media, and this observation has been proven by relevant studies (Li et al., 2013). Nevertheless, pyrolysis of microalgal biomass from adjusted ADE led to lower contents of PAHs (7.09%) than did the samples cultivated in BG11 (10.82%) and ADE (8.33%) media.

In general, adjusted ADE promoted higher levels of valuable compounds (e.g., aliphatic hydrocarbons, fatty acids) and lowered those of toxic substrates (e.g., nitrogen-containing compounds, PAHs) when compared to standard BG11 and original ADE media. Composition changes of microalgal biomass during cultivation in varying media mainly accounted for such phenomenon. Moreover, the reduced temperature of optimal pyrolysis for biomass cultivated in adjusted ADE is economically highly significant for the reduction in energy consumption, and thus could additionally benefit industrial applications. However, the complexity of pyrolysis products, along with associated upgrading treatments, *e.g.*, deoxygenation, demineralization (Niu et al., 2022), and denitrification, should be further studied and could benefit utilization of such products as biofuels (Du et al., 2011). As lower temperatures (300–400 °C) may lead to highest production of valuable compounds, *e.g.*, aliphatic hydrocarbon and fatty acids, meanwhile, higher temperatures (up to 800 °C) lead to higher production of both valuable (aromatic hydrocarbon) and toxic compounds (PAHs and N-containing compounds), the optimal temperature for microalgal pyrolysis is always a trade-off decision among valuable products, toxic compounds, and energy consumption. The present results suggest the optimal pyrolysis temperature as a targeted-products orientated decision.

3.5. Life cycle assessment of products from pyrolysis of microalgal biomass

As the boundary for the investigation, our LCA has taken into consideration the processes of microalgal cultivation, harvesting, and biomass pyrolysis. Due to the various levels of microalgal biomass production in different media, amount of media (to achieve microalgal biomass production of 1 kg) that needed to be autoclaved, inoculated, and cultivated differed significantly, leading to divers levels of energy consumption and emission. For all three treatments, the harvesting process consumed the largest proportion of energy (50.89–65.29%, Fig. 4). Direct use of wastewaters (ADE) reduced total energy consumption from 20331.5 MJ (BG11) to 17489.9 MJ. Further adjustment of ADE (MADE) further significantly reduced total energy consumption to 12314.2 MJ. This was mainly attributed to by the increasing biomass production in adjusted wastewater, which contained more abundant and balanced nutrients for microalgal growth.

In order to assess the environmental burdens for pyrolysis products, the most influential impact categories, including global warming potential, acidification, eutrophication, and photochemical oxidation synthesis (POS) (Stephens et al., 2010), were chosen for the study. By employing normalization and weighting of parameters (Finnveden et al., 2009), more comparable results from different groups were achieved. The pyrolysis products from 1 kg microalgal biomass from adjusted ADE wastewater generated overall lower environmental impacts of all four parameters selected, and thus, led to a significantly lower total environmental impact (838.52 mPE_{T2000}) than did the processes using

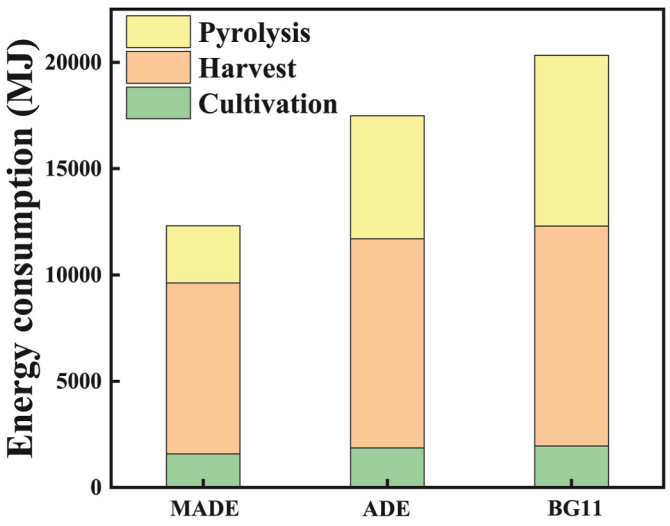


Fig. 4. Energy consumption distribution.

biomass cultivated in ADE (total, 1165.67 mPE_{T2000}) and BG11 (1347.63 mPE_{T2000}, Fig. 5a) media. During the entire production process, the relative distribution of each environmental categories was comparable among different groups (Fig. 5b). All groups contributed the most to eutrophication (777, 669, and 481 mPE_{T2000} by BG11, ADE, and MADE, respectively), which is mainly caused by the emission of NO_x (Nikkhah et al., 2019). Meanwhile, the contribution to POS and acidification problems using each individual medium exhibited comparable levels of impact. Notably, the formation of pyrolysis products from microalgal biomass showed the least impacts on global warming (36, 32, and 23 mPE_{T2000} by BG11, ADE, and MADE, respectively), which could mainly be due to the photosynthesis potential of microalgae.

Considering both products and sustainability, adjusted ADE could significantly reduce environmental impacts, while additionally demonstrating the potential of achieving higher value products along with a reduction in toxic compounds in the final materials. Mostly, the lower optimal temperature for pyrolysis of biomass cultivated in MADE is critical for further practical applications. Notably, the results of analysis of pyrolysis products and from the environmental impacts assessment (LCA) in this study were evaluated for a specific wastewater type (effluents of anaerobic digestion from rural pig farm) with fixed additives of substrate (P, Fe, and Mg). Therefore, variations in wastewater types and in modification additives may not be always consistent with these results. Special attention should be paid to those wastewater types requiring complex additives.

3.6. Implementation potential of the proposed strategy

In view of practical implementation, the water quality of discharged wastewater, including wastewater treatment plant effluent and anaerobic digested slurry, invariably fluctuates. Thus, challenges in stabilizing and improving water quality, for the purposes of producing a cultivation medium, is always critical. Nevertheless, biomass-derived energy, due to its various attracting properties, e.g., renewability, economy, and significance for mitigating environmental crises, is continuously providing a strong drive-force towards overcoming such obstacles and being a promising replacement of fossil energy carriers in the future (Tursi, 2019). This study proposes a feasible approach to address such problems, with a first step being the detection of water quality-related parameters, followed by further addition of necessary nutrients/elements, accordingly. These additives could be either specific nutrients or other types of wastewater, e.g., adding phosphorus-rich water into swine wastewater (Shen et al., 2019), or combining municipal wastewater with cattle manure (Luo et al., 2019). The adjusted wastewater is then likely to act as an optimum microalgae cultivation medium. Along with microalgal growth, nutrients could be upcycled to achieve both water remediation and production of microalgal biomass (Chen et al., 2015). In the real applications, the pre-treatment (e.g., settlement process) has been commonly deployed to reduce the turbidity of wastewater before using for microalgae cultivation, which in turn could improve the microalgal biomass growth. On the other hand, once wastewater contains extreme concentrations of toxic materials or requires large amounts of additives, the harvested microalgal biomass would be of low quality and produce a low quality of biofuel, and thus would be uneconomical for biofuel production. In this view, an alternative utilization of the microalgae, *e.g.*, as fertilizer, could be considered. Tough with advantages mentioned above, the present study is still facing obstacles for practical implementation. For instance, feasibility and economy of applying autoclaving and centrifugation for microalgal cultivation and harvest, which are general obstacles of microalgal technology in industrial application. Besides, for a more comprehensive evaluation, the energy consumption of manufacturing nutrients which added into the medium, should also be further calculated into the LCA process. This study provides a new approach to better achieve the concept of the circular economy, which could benefit the sustainable development of both microalgal products and of wastewater remediation.

4. Conclusions

The adjustment of anaerobic digestion effluents (adjusted ADE), through the addition of small amounts of Mg, Fe, and P, could significantly promote the growth of microalgal biomass and improve its composition towards enhancing biofuel production. The changes of

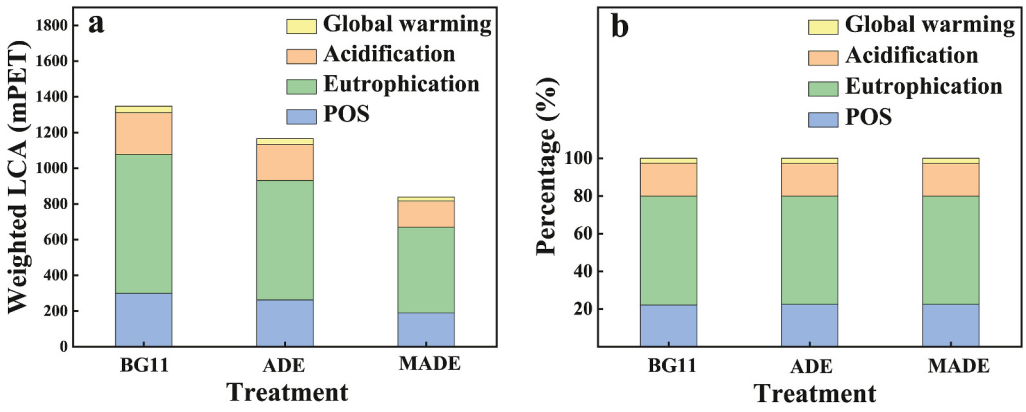


Fig. 5. Weighted life cycle assessment of varying media, a) weighted LCA index, and b) percentage distribution.

compounds in biomass resulted in higher thermal resistance when compared to that from original ADE and the standard BG11 cultivation medium. Both valuable aliphatic hydrocarbons and fatty acids were improved up to 23.98% and 42.33%, respectively, along with decreases in toxic compounds (nitrogen-containing compounds and PAH) in products from the adjusted ADE. Moreover, this approach also contributed to significantly reduced environmental impacts, as indicated by the LCA analysis. This study proposes a feasible process of upcycling nutrients from wastewater for valuable microalgal pyrolysis products.

CRediT authorship contribution statement

Gang Li: Methodology, Investigation, Writing – original draft. **Ruichen Hu:** Investigation. **Nan Wang:** Investigation. **Tenglun Yang:** Investigation. **Fuzhuo Xu:** Investigation. **Jiale Li:** Investigation. **Jiahui Wu:** Investigation. **Zhigang Huang:** Investigation. **Minmin Pan:** Writing – review & editing, Supervision. **Tao Lyu:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2022.131768.

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Cultivation of microalgae in adjusted wastewater to enhance biofuel production and reduce environmental impact: pyrolysis performances and life cycle assessment

Li, Gang; Hu, Ruichen; Wang, Nan; Yang, Tenglun; Xu, Fuzhuo ...

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