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Single-cell sorting of microalgae and identification of optimal conditions by using response surface methodology coupled with life-cycle approaches

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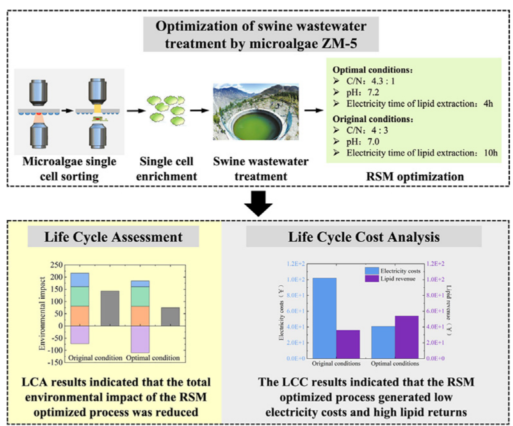
HIGHLIGHTS

- A single cell of microalgae ZM-5 was sorted and achieved its enrichment.
- RSM optimization achieved maximum pollutant degradation and lipid accumulation.
- LCA and LCC were jointly used as supporting tools for RSM design.
- Cultural optimization of ZM-5 was used as a case for the integration of RSM-LCA-LCC.

GRAPHICAL ABSTRACT



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ABSTRACT

Response surface methodology (RSM) has been widely used to identify optimal conditions for environmental microorganisms to maximize degrading pollutants and accumulating biomass. However, to date, environmental impact and economic cost have rarely been considered. In this study, a single cell of microalgae *Chlorella sorokiniana* ZM-5 was sorted, and its enrichment was carried out for the first time. The optimized conditions by RSM for achieving the highest COD, TN, TP removal and 352.61 mg/g lipid production were 24 h light time, 4.3:1C/N, 7.2 pH, and 30 °C temperature, respectively. Life-cycle approaches were then carried out upon this illustrative case, and the results indicated that the implementation of the above optimal conditions could reduce the total environmental impact by 48.0% and the total economic impact by 10.2%. This study showed the feasibility of applying life-cycle approaches to examine the optimal conditions of a biological process in terms of minimizing environmental impact and economic costs.

1. Introduction

Swine wastewater is considered one of the most polluting agricultural drainages due to its high contents of carbon and nitrogen, which might cause serious environmental pollution (Huang et al., 2017; Vanotti et al., 2017). The demand for eco-friendly practices has been rising, especially for appropriate treatments before swine wastewater discharge. As relatively inexpensive alternatives, biological technologies such as membrane bioreactors, sequencing batch reactors, constructed wetlands, and high-rate algal ponds have been more attractive in recent years (Christenson and Sims, 2011; Espinosa et al., 2021; Liu et al., 2013). Among these techniques, microalgae-based biotechnology could not only accumulate biomass components (lipids, etc.) but also has the capacity for wastewater

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bioremediation (Hu et al., 2013). Based on the above characteristics, people could simultaneously realize the purification and resource utilization of wastewater. For this purpose, it is important to identify microalgae sources that could maintain high metabolic potential in swine wastewater.

Conventional methods for microalgae isolation depend on cell cultivation by plating transformed cells on plates (Chen et al., 2015). However, this method was labor intensive and time consuming. Moreover, this technique could not isolate over 99% of uncultured microorganisms (Huang et al., 2015). For instance, from complex environmental samples with a large abundance of *Oscillatoria* or filamentous fungi, isolation of *Chlorella* with low abundance was not applicable and had great limitations. Therefore, there is an urgent need for an efficient sorting method for further research. At present, some studies use fluorescence-activated cell sorting (FACS) for cell screening and isolation. However, Hugo Lee et al. discovered that the separation and isolation of single-cell processes destroyed the structures of cells that are vulnerable or stressed (Lee and Engin, 2020). In addition, the disadvantage of FACS makes it difficult to cultivate single cells on a large scale after sorting (Lee and Engin, 2020). Single-cell sorting based on a laser-induced forward transfer (LIFT) system could precisely locate target cells and directly select target single cells from complex samples without externally tagging molecules. The system could ensure the purity of cells and prevent the contamination of miscellaneous bacteria and then minimize the negative impact on target isolated cells (Li et al., 2020; Wang et al., 2020b).

In wastewater treatment, many factors influence the growth of isolated microalgae, such as temperature, pH, nutrients, and light intensity. To improve the degradation rate of pollutants or increase biomass productivity, it is necessary to determine the optimal conditions for the growth of microalgae. In this regard, response surface methodology (RSM) provides more effective approaches based on statistical principles (Guo et al., 2015). Moreover, how to balance wastewater degradation and resource recovery is likely a complex trade-off to be solved. Guštin et al. discovered that the concentration of NH_4^+-N in wastewater could be reduced to a lower level and that ammonia toxicity was mitigated after ammonia stripping (Guštin and Marinšek-Logar, 2011). Ammonia stripping would lead to a loss and waste of 80% NH_4^+-N in wastewater. To solve this problem, Lu et al. proposed that swine wastewater could be diluted with wastewater, such as food drainage, for resource recycling and utilization (Lu et al., 2018). However, some organic carbon in wastewater could not be directly utilized by algal cells, which led to a poor COD degradation rate of only 16.44–46.51%. As such, multiobjective optimization will generate critical strategies to improve the wastewater treatment capacity by using microalgae while finding solutions for increasing the output of biomass.

Additionally, if the degradation rate and biomass accumulation were the only factors considered, the optimized conditions from RSM design would be appropriate. However, the implementation of the optimized conditions would require extra resource consumption, elevated emissions and financial investment. Due to the extra inputs and outputs, the environmental impacts and economic costs would probably increase. That is, when comprehensively considering the degradation rate, biomass production capacity, environment and economy, the conditions that were optimal for increasing the degradation rate and biomass accumulation could probably not be optimal for reducing environmental impacts and controlling economic costs. Thus, it is necessary to minimize the environmental impact and control the economic cost while maximizing the degradation rate of pollutants and biomass accumulation from the perspective of sustainability. In most studies using RSM to optimize the cultural conditions of environmental microorganisms, seemingly, the degradation rate or biomass productivity was the only concern (Chai et al., 2021; Yuan et al., 2020). For instance, *N. Nirmala* found that an increased lipid production of 15%–22% was achieved from microalgae after RSM optimization (Nirmala and S, 2020). However, large numbers of organic reagents used after optimization caused a waste of resources and a large environmental load. To the best of our knowledge, the environmental impact and economic cost of implementing the optimized conditions have rarely been discussed. With respect to this issue, one possible solution is to employ certain scientific approaches as supporting tools for RSM design to assess the environmental impact and economic costs. Thus, the combined use of life cycle assessment (LCA) and life cycle costing (LCC) presents a proper approach to achieve the objective because the combination of implementing both LCA and LCC can allow for a holistic assessment of environmental impacts and economic costs associated with the whole process of designing products, services or technologies (Gasia et al., 2021; Islam et al., 2015; Zhao et al., 2022).

In this study, single-cell sorting based on a LIFT system was used to isolate microalgae from swine wastewater. The microalgal growth, physicochemical properties, biomass accumulation, and removal efficiencies of pollutants (COD, N, and P) were studied by single-factor experiments. On this basis, three objectives were addressed by RSM-LCA-LCC analysis: (1) reaching a high degradation efficiency of swine wastewater, (2) achieving high biomass accumulation (resource recovery efficiency), and (3) examining whether LCA and LCC techniques can be employed to evaluate the optimized conditions obtained from the RSM design.

2. Methods and materials

2.1. Single-cell sorting

Swine wastewater and soil samples used as microalgae sources were collected from a constructed wetland in Harbin, China. Laser induced forward transfer (LIFT), a kind of laser-assisted bioprinting method based on microscopic imaging, was used as a novel and efficient method to isolate microalgae visually (Liang et al., 2022). For the laser spot is 0.5 μm, LIFT is a pinpointing technology which can be utilized to isolate microalgae at single cell level. Besides, the energy of the laser pulse is tiny (usually tens to hundreds of nanojoules), which result in the thermal energy transported to the microalgae can be neglected. Thus, the sorted individual microalgae cell can keep viability and can be cultured. Specifically, the samples were added to a sorting chip. The target single-cell microalgae were isolated from the sorting chip using PRECI SCS (HOOKE Instruments Ltd., China), a pulse laser ejection system. The sorted cells were then directly collected into a receiver containing sterilized BG − 11 culture medium and 3 g/L glucose under a constant illumination intensity of 6000 lx. The BG − 11 medium contained 1.5 g/L $NaNO_3$, 0.04 g/L $K_2HPO_4{\cdot}3H_2O$, 0.075 g/L $MgSO_4{\cdot}7H_2O$, 0.036 g/L $CaCl_2{\cdot}2H_2O$, 0.006 g/L $C_6H_8O_7$, 0.006 g/L $C_6H_8FeNO_7$, 0.001 g/L EDTA, 0.02 g/L Na_2CO_3, 0.00286 g/L H_3BO_3, 0.00181 g/L $MnCl_2{\cdot}H_2O$, 0.000222 g/L $ZnSO_4{\cdot}7H_2O$, 0.000079 g/L $CuSO_4{\cdot}5H_2O$, 0.00039 g/L $Na_2MoO_4{\cdot}2H_2O$, and 0.000049 g/L $Co(NO_3)_2{\cdot}6H_2O$. After seven days, the single-cell enrichment was transferred to new medium for another seven days. The final enrichment obtained at culture concentrations of 10^7 CFU/mL was deemed inoculum.

2.2. Identification

One isolated microalgae was identified according to its morphological characteristics by microscopy (CX23LEDRFS1C; OLYMPUS) as previously reported (Wang et al., 2020a). Additionally, the genomic DNA of isolated microalgae was extracted using a bacterial genome extraction kit (Tiangen, China) following the manufacturer's instructions. Polymerase chain reaction (PCR) amplification of the microalgal 18S rRNA gene was performed using primers 5’-CCTGGTTGATCCTGCCAG-3 and 5’-TTGATCCTTCTGCAGGTTCA-3. The PCR conditions consisted of an initial denaturation at 96 °C for 5 min followed by 30 cycles of denaturation at 96 °C for 20 s, annealing at 56 °C for 20 s, and extension at 72 °C for 20 s followed by a final 10 min extension at 72 °C. PCR products were sequenced by the BGI Gene Technology Company (Beijing, China). The sequence was compared with those available in the NCBI database using the BLAST program.

2.3. Biomass determination

The culture medium with isolated microalgae was filtered through a 0.45 μm fiber filter (Whatman, USA). The harvested cells were dried at

80 °C for 24 h and then utilized for further analysis. Carbohydrate analysis was performed based on previous studies with glucose as the standard solution (Yu et al., 2019). The carbohydrate content was calculated by a spectrophotometer (PERSEE, China) at a wavelength of 620 nm. Protein analysis was carried out by using a protein quantitative detection kit (Sangon, China), and protein concentrations were determined according to the Bradford method (Field and Field, 2010). Additionally, lipid productivity analysis for each microalga sample was measured according to previous studies (Hidalgo et al., 2016).

2.4. Single-factor experiments

Single-factor experiments were conducted to determine the environmental limits to pollutant removal and biomass productivity. Here, four levels of the respective six variable factors were carried out as follows: light time (0 h, 8 h, 16 h, and 24 h), light intensity (0 lx, 2000 lx, 4000 lx, and 6000 lx), pH (5, 6, 7, and 8), temperature (20 °C, 25 °C, 30 °C, and 35 °C), C/N (1:1, 4:3, 8:1, and 15:1) and wastewater concentration (200 mg/L COD, 400 mg/L COD, 600 mg/L COD, and 800 mg/L). In each experiment, one factor was changed while the other factors were kept constant (light time, 24 h; light intensity, 4000 lx; pH, 7; temperature, 30 °C; C/N, 8:1; concentration of wastewater, 400 mg/L COD). Periodically, COD degradation, TN degradation, TP degradation, and lipid production were evaluated every 12 h, and the detailed procedures for wastewater analysis were measured as previously reported (Chen et al., 2021; Hidalgo et al., 2016; Pereira et al., 2018; Wen et al., 2021; Sun et al., 2022). The experiments were performed in piggery wastewater systems in triplicate. The system was conducted with the stirring speed of 400 rpm/min and inoculum size of 10%. The sewage per liter contained 0.062 g of NaCl, 0.235 g of $CaCl_2{\cdot}2H_2O$, 0.152 g of $MgSO_4$, 1.019 g of NH_4Cl, 0.052 g of K_2HPO_4, 0.160 g of $NaNO_3$, and 0.377 g of $C_6H_{12}O_6$ with an average COD concentration of 400 mg/L.

2.5. RSM design applied to microalgae chlorella sp. ZM-5

To simultaneously maximize the pollutant removal and lipid production capacity, central composite design (CCD) based on RSM was employed to optimize the factors (temperature, pH, light time, and C/N) identified from the above single-factor experiments (Li et al., 2022a, 2022b). Experimental runs with three replications at a center point were designed for the identified factors. The ranges of these four factors were assigned specific values (Table 1). Minitab 17 (Minitab Inc., State College, PA, USA) was employed for statistical analysis of the CCD. Code levels and experimental values of COD degradation, TN degradation, TP degradation, and lipid production are shown in Table 2.

In addition, the experimental data were further analyzed by a second-order model with linear, quadratic, and interaction effects between the dependent and independent variables:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{j=1}^{n} b_{jj} X_j^2 + \sum_{i<j}^{n} b_{ij} X_i X_j \tag{1}$$

where Y refers to the response; X refers to the input variables; b_0 refers to the constant; and b_i, b_{ii} and b_{ij} represent the linear, quadratic and interactive effects, respectively.

Table 1
Levels of four factors for central composite design.

No.	Factors	Unit	+1 level	−1 level	0 level
X_1	Light time	h	24	16	20
X_2	C/N	–	8	1	4.5
X_3	pH	–	8	6	7
X_4	Temperature	°C	30	25	27.5

2.6. LCA and LCC analysis

Environmental impacts and economic costs were evaluated using LCA and LCC for the comparison of original culture conditions and optimal conditions (Fig. 1). The functional unit was defined as 1000 L of swine wastewater. System boundaries included the inputs of chemical substances and energy consumption, the outputs of substance emissions and biomass conversion. The analysis of the inventory included the carbon and nitrogen requirements for the culture medium, the chemical substance used to adjust the alkalinity of the culture medium, and electricity consumption for lipid extraction (see supplementary data Table S1 and Table S2). The *Ecoinvent* 3.4 database coupled with open LCA software was used to convert the inventory data into assessment results (Finkbeiner et al., 2006; Li et al., 2022a, 2022b).The ReCipe endpoint methods were used for the total environmental impact assessment (Nguyen et al., 2021). In addition, the system included four steps: (1) microalgae cultivation, (2) wastewater treatment, and (3) lipid extraction. For LCC, a steady-state cost model was used (Pretel et al., 2015). Market values of the chemical substance and electricity consumption were derived from the Chinese market for cost analysis according to the above three steps (see supplementary data Table S3).

Moreover, considering that the choice of some factors and methodologies could influence the predictive capability of results, uncertainty analysis was conducted to quantitatively assess their influence (Zhao et al., 2021a). The considered scenarios were the following: scenario 1 (electricity was produced by natural gas), scenario 2 (electricity was produced by biological gas), scenario 3 (electricity was produced by nuclear energy), scenario 4 (nitrogen source was provided with $(NH_4)_2SO_4$), and scenario 5 (lipid price was real-time price). For ease of comparison, the scenario that was used for LCA-LCC analysis was treated as a baseline scenario (coal as electricity source and NH_4Cl as nitrogen source), and the uncertainty analysis was conducted by comparing the five scenarios with the baseline scenario.

2.7. Analytical methods

In this study, IBM SPSS Statistics 23 was used to analyze the significance of the single-factor results. Experimental data were evaluated using one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Isolation and identification

One ZM-5 cell was isolated from the enriched swine wastewater sample based on a LIFT system (Fig. 2a). Single cells were successfully cultivated and recognized by microscopy (Fig. 2b). Although the isolation of single microalgae cell has been reported, our results presented evidence that ZM-5 could be cultivated on a large scale after sorting (Cabanelas et al., 2016). The 18S rRNA gene sequence analysis of ZM-5 confirmed that the microalgae showed similarity to *Chlorella sorokiniana* (GenBank accession number MZ557824), and the phylogenetic tree was constructed using MEGA software (Fig. 2c).

In addition, the lipid content, carbohydrate content and protein content of isolated *C. sorokiniana* ZM-5 biomass were tested. The results showed that the contents of lipid, carbohydrate and protein were 40.29%, 37.66% and 22.05%, respectively, indicating that ZM-5 was a lipid-producing microalgae.

3.2. Single-factor experiments

Environmental factors and nutrient stress have direct influences on the degradation ability and lipid content of microalgae (Dai et al., 2021; Iasimone et al., 2018; Seyed Hosseini et al., 2018). In this study, the effects of pH, temperature, initial concentration of swine wastewater, C/N, light time and light intensity were investigated.

As shown in Fig. 3, the highest nutrient degradation ability and lipid accumulation were obtained when the C/N ratio was 8:1. However, a marked

Table 2
Central composite design and results for pollutant removal and lipid productivity.

Run NO.	Light time(X_1)	C/N (X_2)	pH(X_3)	Temperature (X_4)	Observed values			
					COD degradation(%)	TN degradation(%)	TP degradation(%)	Lipid production (mg/g)
1	0	0	1	−1	51.38	46.15	37.60	275.37
2	0	1	−1	0	36.62	27.48	10.86	138.05
3	0	1	0	−1	41.82	35.86	17.35	159.34
4	1	0	−1	0	70.18	55.87	45.93	289.61
5	1	1	0	0	56.18	45.61	27.89	200.53
6	0	−1	0	−1	60.37	35.07	30.56	205.66
7	1	−1	0	0	75.58	50.34	44.17	245.72
8	0	0	0	0	70.24	58.72	48.65	315.52
9	−1	0	0	−1	56.57	43.01	35.59	275.22
10	0	−1	−1	0	55.06	27.64	24.51	159.44
11	0	0	0	0	72.37	57.47	49.59	316.25
12	–1	0	0	1	65.52	53.12	43.09	295.53
13	1	0	1	0	68.22	56.90	42.82	313.42
14	0	1	1	0	41.83	28.67	10.54	204.43
15	0	–1	0	1	71.01	45.07	40.40	225.61
16	–1	1	0	0	36.71	30.70	12.01	139.15
17	–1	–1	0	0	55.96	30.87	25.82	185.71
18	0	1	0	1	52.22	40.57	20.93	186.35
19	1	0	0	1	86.66	73.85	64.65	357.91
20	1	0	0	–1	76.66	63.02	54.28	334.14
21	0	0	0	0	71.62	58.23	49.55	321.82
22	0	0	–1	–1	60.53	40.42	30.75	249.84
23	0	0	–1	1	65.74	50.43	40.22	269.28
24	0	0	1	1	61.26	51.70	47.44	293.78
25	0	–1	1	0	60.59	33.78	28.71	174.97
26	–1	0	–1	0	65.65	35.93	25.90	224.36
27	–1	0	1	0	56.02	41.28	32.43	253.76

reduction in degradation efficiencies and lipid content could be observed when the C/N level decreased or increased. The reasons might be that PSI activity and dark respiration were negatively affected by ammonia, as a previous study reported (Markou et al., 2016). Additionally, the effect of pH revealed that pH had a significant effect ($p < 0.05$) on pollutant degradation and lipid accumulation. A maximum removal efficiencies of 43.68% COD, 33.01% TN, 37.87% TP, and 357.68 mg/g lipid accumulation were obtained when the pH was 7 or 8. The results suggested that microalgae absorbed carbon dioxide and released hydroxide, making the environment slightly alkaline and thus promoting ammonia removal and total phosphorus metabolism (Zhu et al., 2013). In Fig. 3, the optimal temperature ranged from 25 °C to 35 °C, with a higher degradation ability and lipid accumulation being found at 30 °C. At lower temperatures, the degradation and lipid production of ZM-5 were low, as the metabolic process did not obtain enough activation energy. When the temperature reached 30 °C, it favored higher degradation efficiencies of COD, TN, TP, and lipid accumulation due to sufficient activation energy. Furthermore, increasing the temperature above 30 °C might lead to cell disruption and decreased intracellular enzyme activity, which in turn decreased the pollutant removal and lipid content of ZM-5 (Singh and Singh, 2015). The light time and light intensity to which ZM-5 was exposed affected the degradation ability and lipid accumulation. The results demonstrated that the highest level was observed under 24 h and 6000 lx, indicating that a relatively high nutrient degradation capacity and lipid content could be achieved during relatively long light exposure. The data obtained were in agreement with those of previous studies that showed a linear increase in biomass production with an increase in the duration of the light period (Mueller et al., 2016; Singh and Singh, 2015).

Among these factors, four (pH, light time, C/N and temperature) that had a significant influence on the degradation ability and lipid accumulation were chosen for further optimization with RSM design.

3.3. Multiobjective optimization

The RSM design was employed for further multiobjective optimization for pollution removal and lipid accumulation. The experimental data were further analyzed by assuming second-order polynomials with linear, quadratic and interaction effects, as shown in Eqs. (2)–(5):

$$Y_1 = -430 - 4.59X_1 + 8.53X_2 + 96.6X_3 + 11.3X_4 + 0.0641{X_1}^2 - 1.202{X_2}^2 - 8.58{X_3}^2 - 0.24{X_4}^2 - 0.003X_1X_2 + 0.479X_1X_3 + 0.026X_1X_4 - 0.023X_2X_3 - 0.007X_2X_4 + 0.467X_3X_4 \quad (2)$$

$$Y_2 = -113.09 + 0.786X_1 + 3.353X_2 + 25.624X_3 + 0.947X_4 - 0.00031{X_1}^2 - 0.2317{X_2}^2 - 1.5892{X_3}^2 - 0.00095{X_4}^2 - 0.01199X_1X_2 - 0.0448X_1X_3 - 0.0023X_1X_4 - 0.0615X_2X_3 - 0.02364X_2X_4 - 0.0699X_3X_4 \quad (3)$$

$$Y_3 = -145.5 + 1.675X_1 + 4.503X_2 + 36.88X_3 - 0.44X_4 - 0.0118{X_1}^2 - 0.4{X_2}^2 - 2.374{X_3}^2 + 0.0148{X_4}^2 + 0.0029X_1X_2 - 0.1382X_1X_3 + 0.0088X_1X_4 - 0.0812X_2X_3 - 0.03537X_2X_4 - 0.0039X_3X_4 \quad (4)$$

$$Y_4 = -2592 + 20.8X_1 + 50.1X_2 + 553X_3 + 34.4X_4 - 0.329{X_1}^2 - 9.512{X_2}^2 - 38.86{X_3}^2 - 0.583{X_4}^2 + 0.024X_1X_2 - 0.35X_1X_3 + 0.087X_1X_4 + 3.63X_2X_3 + 0.202X_2X_4 - 0.1X_3X_4 \quad (5)$$

where Y_1 is the COD degradation rate, Y_2 is the TN degradation rate, Y_3 is the TP degradation rate, Y_4 is the lipid yield, X_1, X_2, X_3 and X_4 are the light time, C/N, pH and temperature, respectively.

For RSM design, the optimal culture conditions were 24 h of light time, 4.3:1 of C/N, 7.2 of pH, and 30 °C of temperature. Under the optimal conditions, the degradation rates of COD, TN and TP reached 84.38%, 72.77% and 64.06%, and the lipid yield reached 352.61 mg/g, respectively. The values after optimization were higher than those under the conditions before optimization. To verify the accuracy of the quadratic model, three replicate runs at optimal conditions were carried out. Under optimal conditions, the degradation rates of COD, TN, and TP and lipid yield were 83.61%, 71.38%, 65.89%, and 363.18 mg/g, respectively. This result coincided with the predicted values, and the recommended optimum conditions by RSM were validated.

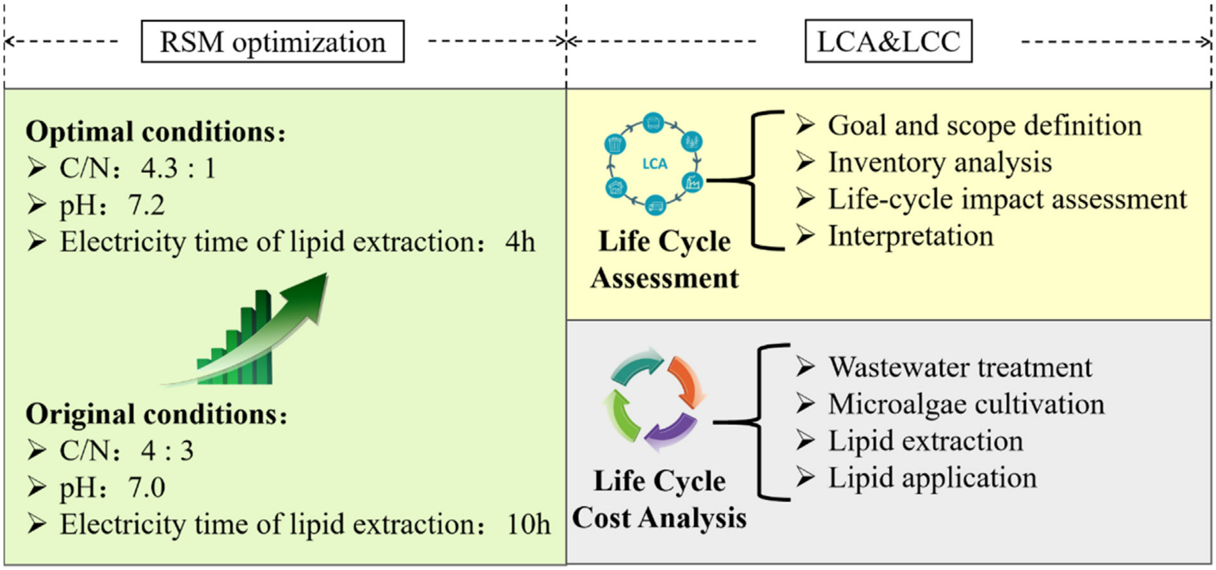

Fig. 1. Schematic diagram of combining life-cycle approaches with RSM for optimization analysis.

In addition, to achieve optimal conditions, it was necessary to change the original conditions as follows: adjusting the pH from 7.0 to 7.2, changing the C/N from 1 to 4.3 of the swine wastewater, and controlling the reaction temperature and light time from 10 h to 4 h.

3.4. Environmental and economic evaluation of implementing optimal conditions

For the degradation and lipid production process of swine wastewater with microalgae ZM-5, the design parameters used in this study were determined according to the above optimal conditions. This process consists of microalgae cultivation, wastewater treatment, lipid extraction and lipid application (Fig. 4a). As shown in Fig. 4b, the LCA results showed that the net negative environmental impact was reduced by approximately 48% and the environmental benefits of energy generation increased by approximately 49% after optimization. Evaluation of the contribution of each process to the environmental impact score revealed that the contributions of microalgae cultivation and wastewater treatment were over 75% of the final score. The changes in negative environmental impacts on microalgae cultivation and wastewater treatment were associated with the chemical substances used to adjust the pH and C/N of swine wastewater. However, although the contribution of lipid extraction was low, the reduction in environmental negative effects after optimization mainly depended on this process. The impacts on lipid extraction were associated with electricity consumption for the evaporation procedure. The decrease could be attributed to the reduced duration time for the evaporating procedure under the optimized conditions (four hours) compared to the original conditions (ten hours).

The total cost of the process after optimization was 6.66E+02￥, which was 10.24% lower than that of the cost before optimization (Fig. 5). On the one hand, electricity consumption made up more than half of the total cost, and the electricity cost was reduced by 14.88% after optimization. On the other hand, the economic benefit was 5.41E+01￥, which was 50.28% more than the cost after optimization. Thus, the increase in economic benefits was also an important factor for the reduction of total cost.

Based on the environmental impacts and economic costs of implementing the optimal conditions, this study demonstrated that LCA and LCC can play two important roles in supporting the RSM design. First, the

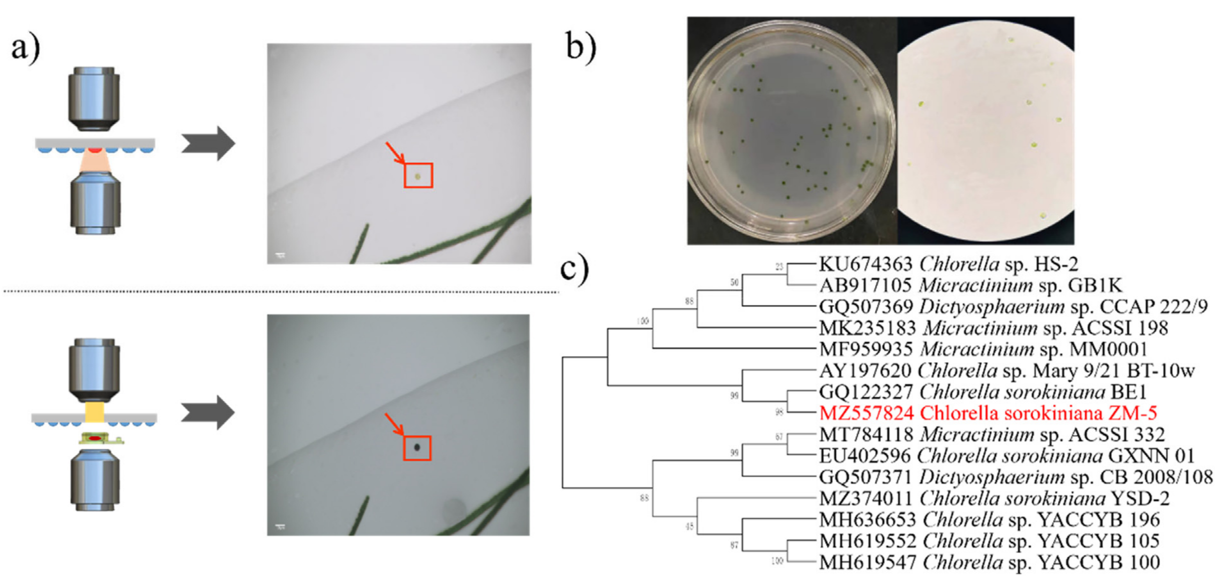

Fig. 2. Isolation and identification of *C. sorokiniana* ZM-5 a) single-cell sorting operation; b) morphology of *C. sorokiniana* ZM-5; and c) phylogenetic trees of *C. sorokiniana* ZM-5.

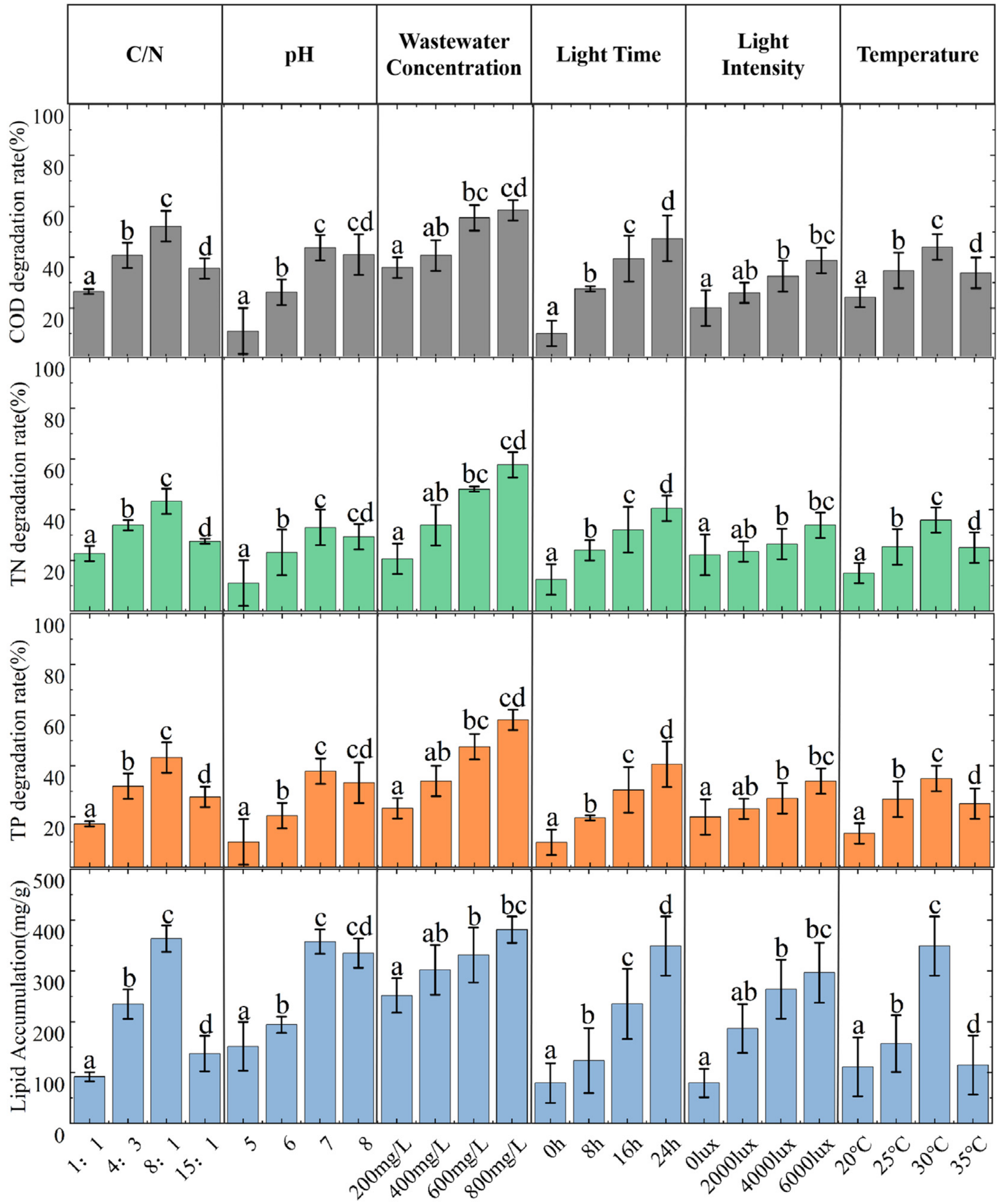

Fig. 3. Effects of environmental factors on nutrient removal efficiencies and lipid production by ZM-5. Data are presented as the mean ± standard deviation, $n = 3$. Different letters show significant differences from the control at $P < 0.05$ according to one-way ANOVA.

application of LCA and LCC could examine whether the optimal conditions for maximizing the degradation rate and biomass accumulation were also appropriate in terms of minimizing the environmental impact and economic costs. As shown in this study, the results of LCA and LCC demonstrated that the total environmental impact and the total economic costs were reduced by 48% and 10.24% for the optimized conditions (Figs. 4b, 5). The results indicated that the conditions obtained from the RSM design were appropriate not only for increasing the rate of swine wastewater degradation and biomass accumulation by microalgae ZM-5 but also for lowering the environmental impacts and economic costs. Second, using LCA and LCC could identify the major factors that influenced the environmental impact and economic cost resulting from implementing the optimized conditions. In this study, the influential factor for environmental impact was the electricity consumption associated with lipid extraction (Fig. 4b), and the main source for economic cost was electricity consumption (Fig. 5). Additionally, another likely situation is that the optimal conditions that lead to the maximum degradation rate and lipid production bring greater environmental impact or economic costs than the original conditions, indicating that decision-making should make trade-offs between pollutant removal, biomass accumulation, and the environment or economy. Under this situation, the identification of the major factors influencing the environment or costs could refer to some specific aspects, and based on the identified aspects, trade-offs could be carried out.

3.5. Improvement analysis

Several factors were selected to conduct the uncertainty analysis, including natural gas as the electricity source (scenario 1), biological gas as the electricity source (scenario 2), nuclear energy as the electricity source (scenario 3), $(NH_4)_2SO_4$ as the nitrogen source (scenario 4) and with the real-time price of lipids (scenario 5). Environmental impacts were

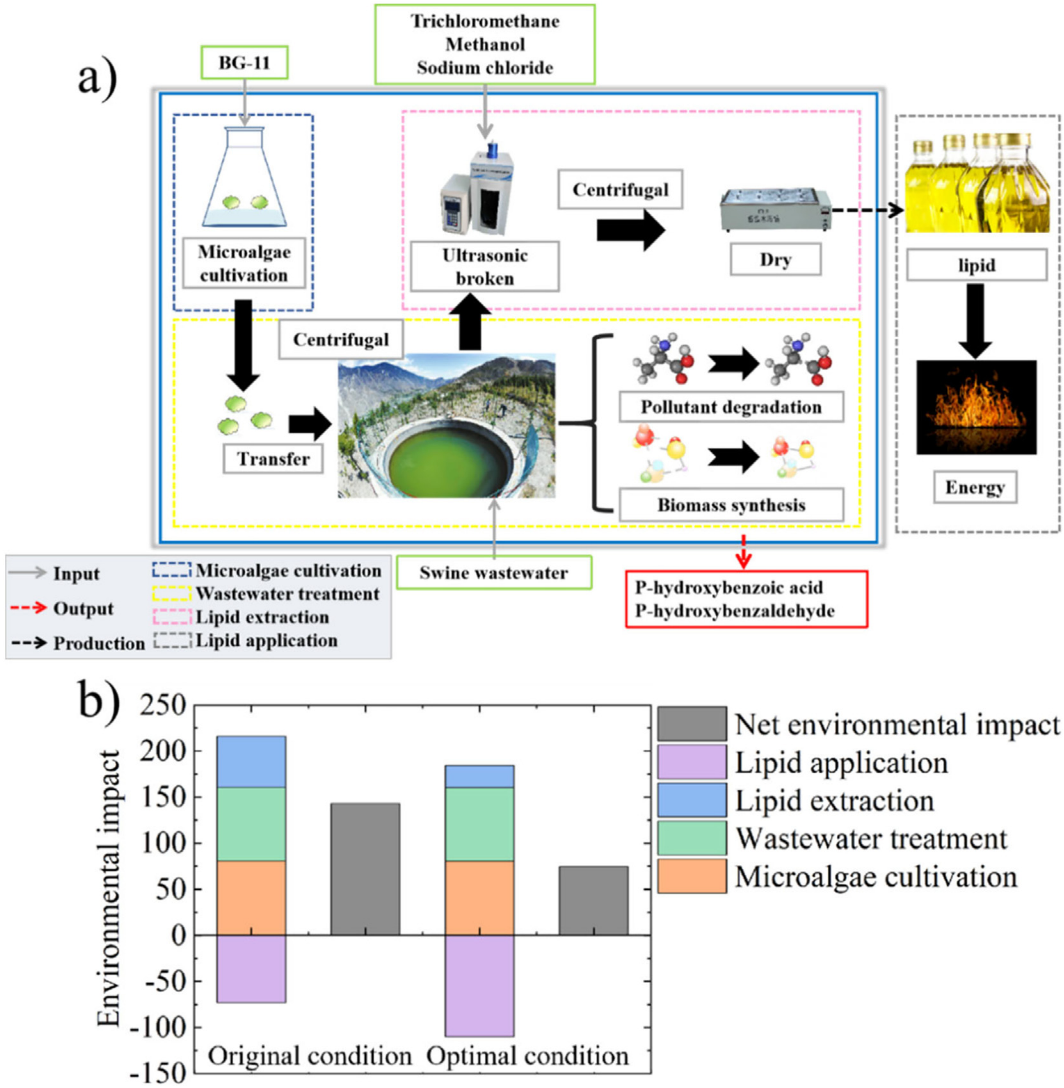

Fig. 4. a) The system boundary and b) total environmental impacts before and after optimization.

considered under scenarios 1, 2, 3 and 4, and economic impacts were considered under scenario 5 (Fig. 6).

The main source of the negative impacts on the environment has been pinpointed to be the power generation unit (Tables S1, S2). The demand for electricity is relatively higher than that of other energy forms because of its use in microalgae cultivation, wastewater treatment, and lipid extraction processes. Employing an energy optimization strategy will facilitate lipid production with a lower environmental impact. For instance, renewable energy such as natural gas, biological gas and nuclear energy seem appropriate choices that dramatically promoted their applications to reduce the total environmental impacts (Dinca et al., 2007; Ghannadzadeh, 2018; Wang et al., 2019). More specifically, with respect to the comparison of scenario 1 scenario 2 scenario 3 with scenario base, the total environmental impacts decreased significantly by 74.5%, 85.1%, and 88.4%, respectively (Fig. 6a). This suggested the importance of employing clean energy sources to minimize the negative environmental impacts in the process of wastewater treatment and biomass transformation by microalgae. With respect to scenario 4, another method that could potentially decrease negative environmental impacts was considered. However, the changes in environmental impact were negligible when comparing scenario 4 ($(NH_4)_2SO_4$ as the nitrogen source) with the baseline scenario.

The specification of the aforementioned scenarios was confined to the general checklist of improvement analysis for life-cycle approaches (Zhao et al., 2021b). However, the fluctuations in the price of biological oil resulted in different total economic costs (Fig. 6b). With respect to the comparison of scenario 5 with the baseline scenario, the total cost of scenario 5 is reduced by 5%. This is because average biological oil prices in 2020 were higher than average in previous years (Zhao et al., 2021a). However, biological oil prices are subject to many factors, such as international bio-oil prices collapse due to COVID − 19 (Jia et al., 2021).

In this study, LCA and LCC models were carried out on a microalgae-based piggery wastewater treatment and biomass transformation case that was performed at the laboratory scale. It is worth noting that most multiple objective optimization processes were executed at the laboratory scale (Pokorska-Silva et al., 2019). Hence, a viewpoint was proposed that the implications from LCA and LCC evaluation at the laboratory scale could be seen as a reference point for further field-scale evaluation.

4. Conclusions

A single cell of microalgae ZM-5 was sorted, and the maximum pollutant degradation and lipid accumulation were achieved by RSM design. This study also demonstrated that using life-cycle approaches could help to evaluate the environmental impact and economic cost of implementing the optimal conditions obtained from RSM design. By means of cultural optimization of ZM-5 as a case study, the application of life-cycle approaches presented quantified evaluation results and

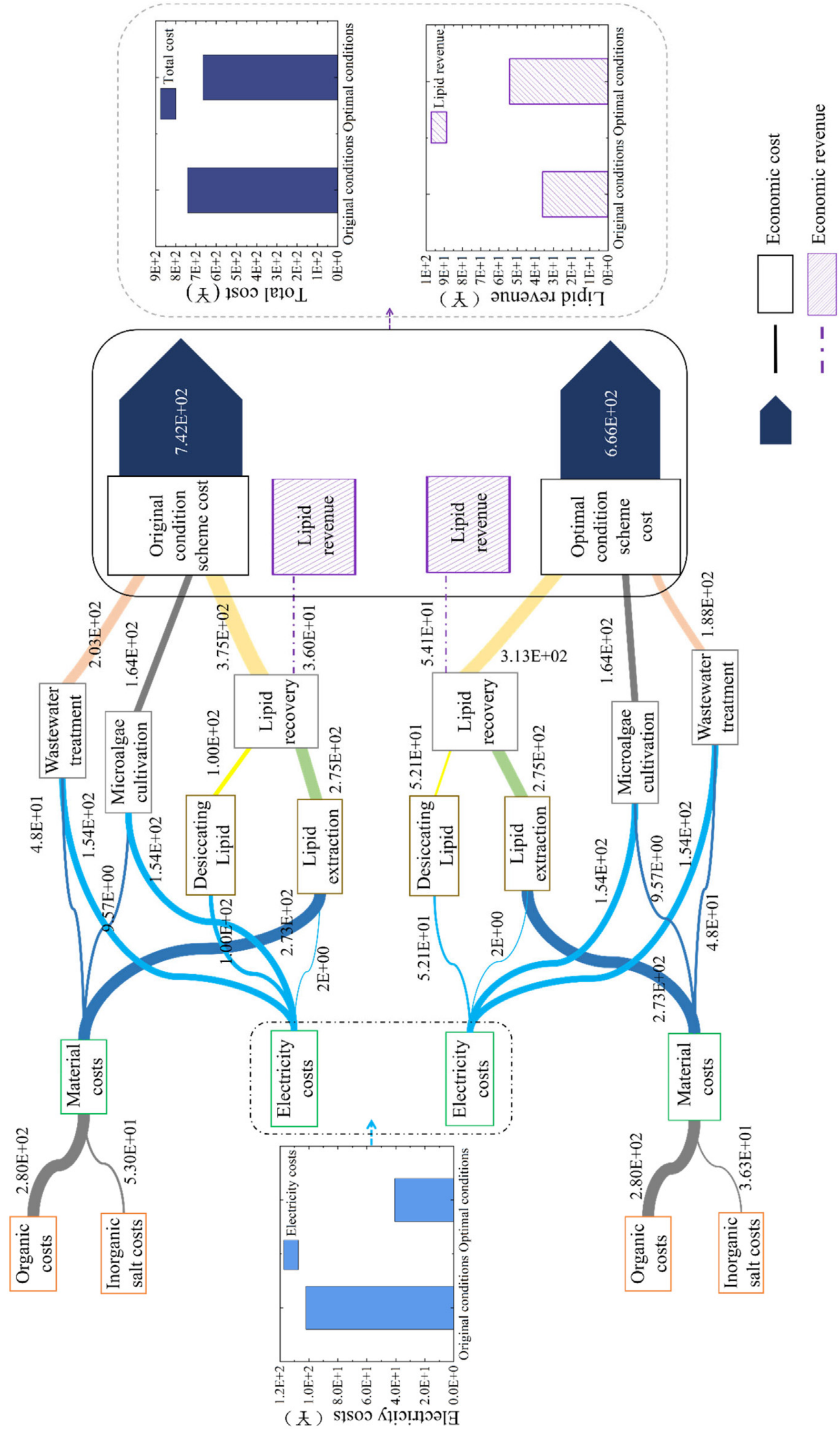

Fig. 5. Total economic costs before and after optimization.

identified the main contributing factors to the environmental impact and economic cost. Conducting uncertainty analysis could help to avoid the recommendations being made based only on one hypothesis or one methodology.

CRediT authorship contribution statement

All the authors have made key contributions to finishing and improving this paper. Xinyue Zhao, Xiangwei Meng and Yan Liu finished writing and investigation. Shunwen Bai, Bei Li, Hang Li, and Ning Hou performed the software and investigation. Chunyan Li helped conduct the conceptualization and 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.

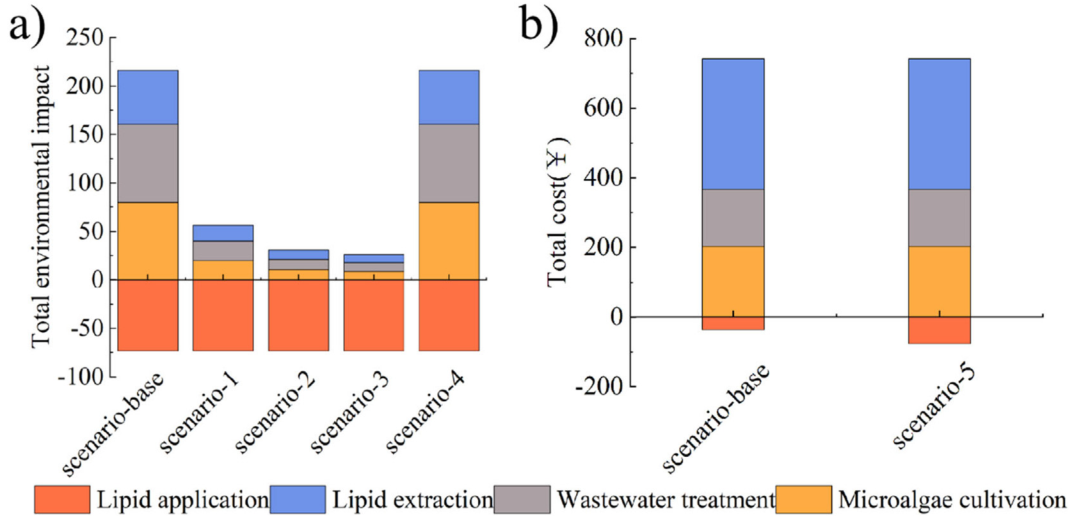

Fig. 6. Improvement analysis based on the baseline and five scenarios before and after optimization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155061.

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