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From waste-activated sludge to algae: a self-reliant cultivation process in photoreactors using saline conditions

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ABSTRACT

In this study, microalgae–bacteria (MB) systems using saline conditions (3 and 5% salinity) were built in order to use waste-activated sludge (AS) as raw material for cultivating lipid-rich microalgae. Algae were observed to be flourishing in 60 days of operation, which totally used the N and P released from the sludge biomass. A prominent improvement of lipid content in MB consortia was obtained under algae growth and salinity stimulation, which occupied 119–136 mg/g-SS rather than a low content of 12.1 mg/g-SS in AS. Lipid enrichment also brought a 3.1–3.3 times total heat release (THR) in the MB biomass. The marine spherical algae *Porphyridium*, as well as filamentous *Geitlerinema, Nodularia, Leptolyngbya* were found to be the main lipid producers and self-flocculated to 23.0% (R1) and 33.5% (R2) volume under the effect of residue EPS. This study had a big meaning in not only waste sludge reduction but also in manufacturing useful bioenergy products.

Key words: lipid content, microalgae, microalgae–bacteria system, total heat release, waste-activated sludge

HIGHLIGHTS

- Self-reliant MB consortia systems were built with 3 and 5% of saline water.
- MB consortia achieved 119–136 mg/g-SS lipid content.
- MB consortia had 3.1–3.3 times the total heat release than AS.
- MB consortia could self-flocculate to 23.0–33.5% volume.
- Spherical *Porphyridium* and filamentous *Cyanobacteria* used N and P from AS.

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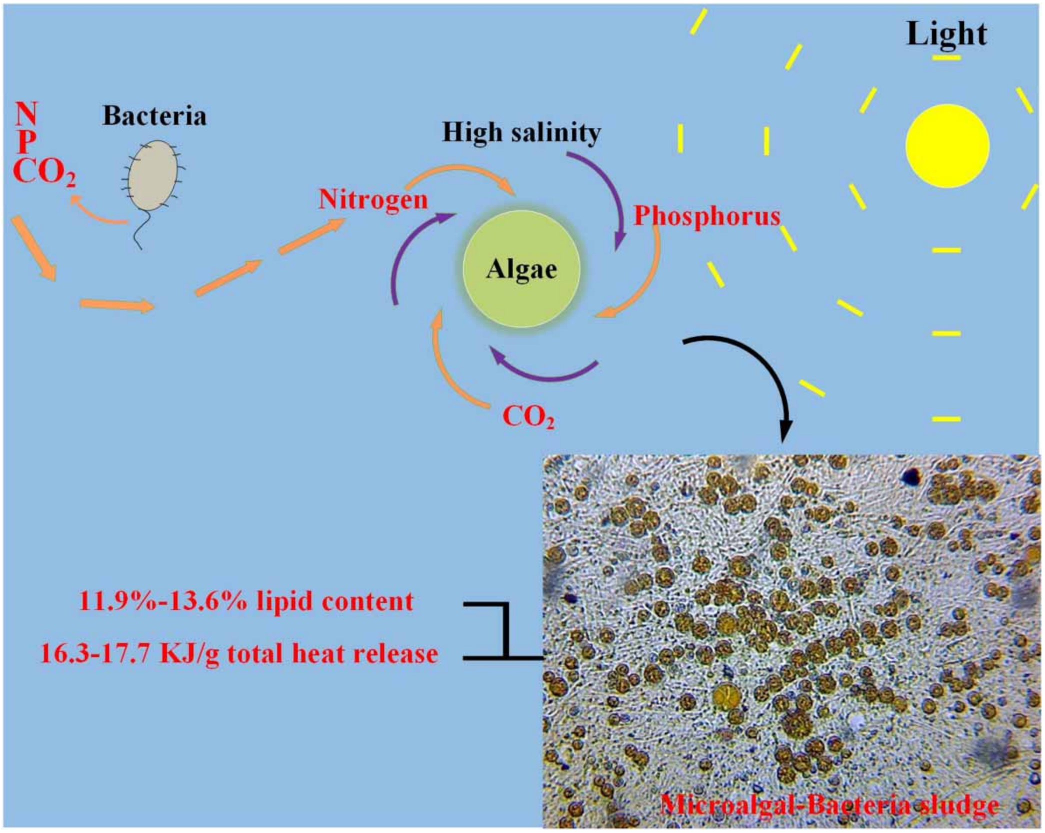
GRAPHICAL ABSTRACT



1. INTRODUCTION

Over the last century, it has been found that a huge quantity of waste sludge can be generated from the biological wastewater treatment processes, which was mainly produced in activated sludge (AS) systems (Sánchez-Zurano *et al.* 2022; Sun *et al.* 2022; Zhu *et al.* 2022). In China, nearly 60 million tons of waste sludge (water content > 80%) can be generated in 1 year, causing a big risk of contaminating the local environment (MHURD 2021; Cheng *et al.* 2022; Kang *et al.* 2022). There are major environmental, technical, regulatory, and financial challenges in the existing methods of treating, handling, disposing, and utilizing waste sludge, with the treatment process occupying up to 40–50% of the total capital and operation cost (Seiple *et al.* 2017; Li *et al.* 2021b). New utilization technology with more environment and economic profit is thereby needed.

Waste sludge contains large amounts of carbon organic and inorganic substances (nitrogen and phosphorus), which can be used as nutrients for cultivating microalgae to produce biodiesel (Tan *et al.* 2020a). The extraction of the organic and inorganic nutrients could be costly and laborious, meanwhile generating most of the sludge mass as waste residues (Li *et al.* 2021b; Pap *et al.* 2021). On the other hand, the cultivation of pure microalgae from sludge was claimed to be inefficient in algae biomass harvesting and lipid productivity (Oladoja *et al.* 2020; Tan *et al.* 2020b).

Recently, a new microalgae–bacteria (MB) consortia was investigated by many researchers in photo-sequencing batch reactor (PSBR) through adding or no-adding some specific algae spices into the AS under light illumination conditions (Chen *et al.* 2019; Meng *et al.* 2019; Li *et al.* 2021a). MB consortia has been proposed as a sustainable biotechnology that exploits renewable sunlight, consumes atmospheric CO_2, and allows for N and P removal and recovery, while generating valuable bioproducts from the algal biomass. The lipid content of the MB consortia system could reach to 16.3% in a symbiotic system with inoculated microalgae *Scenedesmus* sp. versus sludge at 1:1 (Chen *et al.* 2019). However, there are two aspects of limitations for the current MB consortia biotechnology. Firstly, the MB method was primarily used in wastewater treatment processes, which have abundant nutrients from the consistent influent, but the information about its application in low-nutrient (self-reliant) systems was unclear. Secondly, in practice, large-scale addition of external algae was unlikely due to high cost

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of algae cultivation. In more practical self-transformed MB systems with no large scale of external algae, the lipid contents were reported to be relatively low, between 1.3 and 6.6%, still uneconomical for biodiesel extraction, especially for a low-strength wastewater system with 0% saline condition (1.3% lipid content) (Meng *et al.* 2019; Cao *et al.* 2022; Purba *et al.* 2023).

High salinity is reported to be a stimulus for lipid production in microalgae because lipids can be liberally synthesized under a saline environment to counteract the osmotic pressure, which preserved the algae membrane from damage (Cheng *et al.* 2014). For instance, lipid productivity of *Chlorella sorokiniana* could reach to 19.66 mg/L·day^{-1} when salinity increased to 2%, 2.16 times than 0 salinity condition (Zhang *et al.* 2018a). It has also been proved that 1–5% high salinity condition can be used to stimulate lipid accumulation during the MB consortia cultivation in the wastewater treatment process, and higher 3 and 5% salinity was more profitable for lipid synthesis (Meng *et al.* 2019; Cao *et al.* 2022). Therefore, it could be concluded that 3–5% salinity can also be implied in the self-reliant cultivation process for obtaining lipid-enriched MB consortia.

The main purpose of this study is to convert waste-activated sludge into lipid-rich microalgae in an MB consortia system. This MB system was fed by its own released nutrients from AS flocs, and employed 3 and 5% saline conditions as lipid simulation factors. The authors want to explore (i) the change of the sludge characteristics, (ii) the lipid accumulation and potential sludge flammability, and (iii) the biological community of microalgae in the MB system. Results of this experiment can provide scientific data for the transformation from bacteria to algae in waste sludge, and give new meaningful insights into not only reducing the sludge content, but also producing high value-added lipids.

2. MATERIALS AND METHODS

2.1. Reactor design and incubation conditions

Two identical photoreactors (R1 and R2, $D \times H = 5$ cm $\times$ 60 cm) with a total volume of 1.2 L and an effective working volume of 0.8 L (Supplemental material, Figure S1) were operated for 60 days. Aerobic AS from a local municipal sewage treatment plant was used as the original sludge. The sludge (water content > 99%) was collected from the secondary sedimentation tank sited after an anaerobic–anoxic–aerobic (A^2/O) wastewater treatment tank in this plant, which had a normal aerobic AS property. The original sludge was inoculated into the reactors (MLSS 5 g/L, MLVSS 3.1 g/L, SVI_{30} 106 mL/g), the supernatant of which was washed and replaced three times by tap water to remove the dissolved nitrogen (N) and phosphorus (P). 24 and 40 g of NaCl were added to R1 (3% salinity) and R2 (5% salinity), respectively. The reactors were operated without any excess substrate, and no water was added as an influent. Sludge was sampled every 15 days for tests from the beginning, and supernatant water was taken every 8 days since day 12. The working volume was kept at 0.8 L by replenishing high salinity water (3 and 5%) when water level drops due to sampling or loss.

The two reactors were maintained at a temperature of 21–25 °C. Aeration was provided 24 h/day by an air stone at the bottom, which was connected with an air pump (3 L/S) for each reactor. Two aspects of reasons were considered for the aeration condition: first, aeration was used for decomposing the bacteria biomass into inorganic nutrients (N, P, and CO_2) through aerobic respiration to support the algae assimilation (Wang *et al.* 2022a). Second, aeration was used for stirring the sludge in order to promote the nutrients (N, P, CO_2) and O_2 exchanging between bacteria and algae, meanwhile to more evenly receive light illumination.

White light (5,700 K) was produced by 60-cm LED tubes (Opple, 7W, China) from 9:00 am to 21:00 pm (12 h) every day. Four tubes were evenly placed in two sides at a 5 cm distance from the reactors, and the light illuminance was measured to be 180 μmol·m^{-2} s^{-1} by a photosynthetically effective radiometer (SM206-PAR, Xinbao Keyi, China).

2.2. Analytical methods

ML(V)SS, SVI_{30}, PO_4^-P, and three kinds of nitrogen (NH_4^+-N, NO_2^--N, and NO_3^--N) were determined with standard methods (APHA 2012). DO and PH values were monitored by a DO/PH meter (WTW Multi 3430, Germany). Chlorophyll *a* in sludge was determined by referring to the method of Zhang *et al.* (2018a). Lipid content was measured using Marella's method (Marella *et al.* 2019). EPS was detected using the method of heat extraction by Zhang *et al.* (2016).

The morphology of the two types of sludge as well as their algae species were pictured using a microscope (H1605, YUEREN, China). The particle size/distribution of the MB consortia was obtained by a laser particle size analyzer (MS2000, Malvern, UK). The fatty acid methyl esters (FAMEs) of sludge lipid were identified using a gas chromatograph-mass spectrometer (GC-MS, 7890A-5975C, Agilent, USA) to analyze lipid composition (Meng *et al.* 2019). The microalgae

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community was analyzed by SHUANGYU Inc. (Tianjin, China) using a metagenomic sequencing method according to the description by Zhang *et al.* (2022).

Microscale combustion calorimetry (MCC) tests including specific heat release rates (HRR), peak heat release rates (pHRR), total heat release (THR), and max peak temperature ($T_{\max}$) were carried out by a microscale combustion calorimeter (FTT0001, FTT, UK). Five milligrams (5 mg) of powdered sludge sample was heated to 750 °C at a heating rate of 1 °C/s. N_2 and O_2 flow rates were set at 80.0 and 20.0 cc/min, respectively.

Water and sludge sample tests were triply conducted, and all data were statistically analyzed in Excel 2020 (Microsoft, USA).

3. RESULTS AND DISCUSSION

3.1. Sludge characteristics

3.1.1. Formation of an MB consortia

After about 10 days of operation since the system started, the optical microscopy of sludge demonstrated that lots of free algae (like *Chlorella*) appeared in R1 and R2, and the reactor color turned from yellow-brown to green (Supplemental material, Figures S2 and S3), indicating the successful formation of MB consortia sludge. Thereafter, the green color of R1 and R2 became yellow at 30 days, then R1 turned green again (45–60 days), but R2 remained yellow (45–60 days).

From the microscopic images of the MB sludge of 45 days (Figure 1(b)–1(e)), it could be found green filamentous algae and a brown spherical algae species were dominant in R1, while the green filamentous algae seemed to be less abundant in R2, thus the reactor had a much lighter color (Figure 1(a); Supplemental material, Figure S3). Meanwhile, beige/light yellow color bacteria such as *Hoeflea* and *Brevundimonas* were found to be abundant in R2 (Supplemental material, Table S1), which might also cause yellowing of the biomass in this reactor (Scotta *et al.* 2011; Jung *et al.* 2013). These indicated the microbial community in the two reactors had some distinction due to the different salinity levels (3 and 5% salinity). On the other hand, the algae could tightly adhere to the sludge flocs (Figure 1(b)–1(e)), forming larger MB consortia aggregates. This implied the microalgae could precipitate with the sludge rather than float in the water when the aeration stopped.

The current MB consortia were successfully formed in a self-reliant system, and the algae growth was entirely dependent on the nutrients from the sludge. It was approved that the AS can be directly used for the cultivation of microalgae.

3.1.2. Biomass content

MLSS in R1 and R2 both largely decreased from initial values of 4.5, 4.7 g/L (0 day) to 1.5, 1.4 g/L (60 days) ($p = 0.83 > 0.05$), respectively (Figure 2(a)). The big sludge reduction illustrated the severe degradation of the AS in famine conditions. On the contrary, chlorophyll *a* content in R1 and R2 significantly increased to 8.6 and 4.1 mg/g-SS ($p = 0.16 > 0.05$),

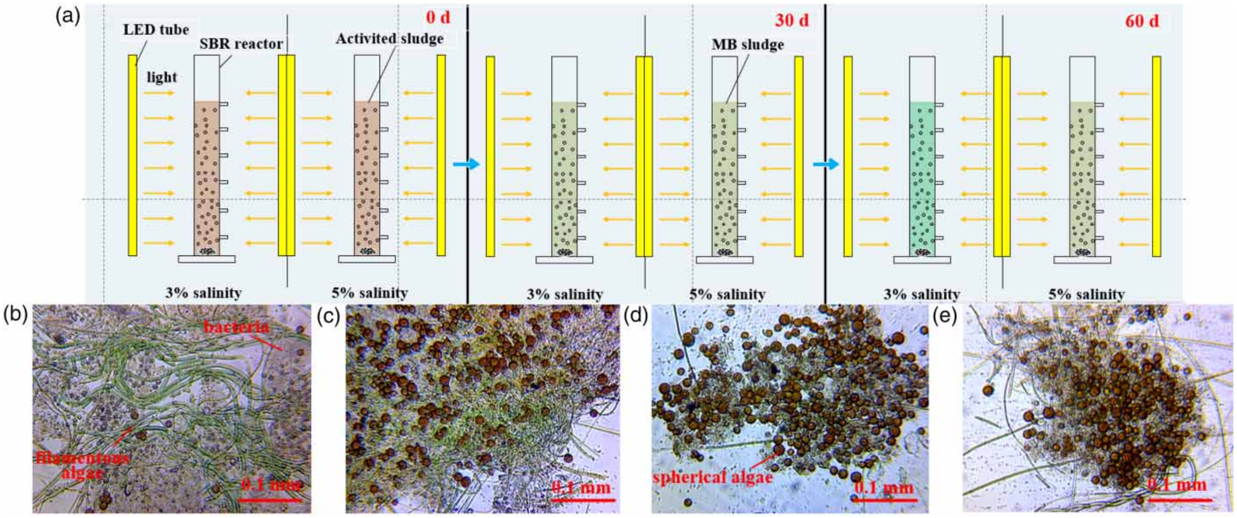

Figure 1 | The change of the MB sludge in two reactors ((a) indicates reactor color change diagram; (b) and (c) indicates microscopic images of sludge in R1 parallelly pictured at 45 days; (d) and (e) indicates microscopic images of sludge in R2 parallelly pictured at 45 days).

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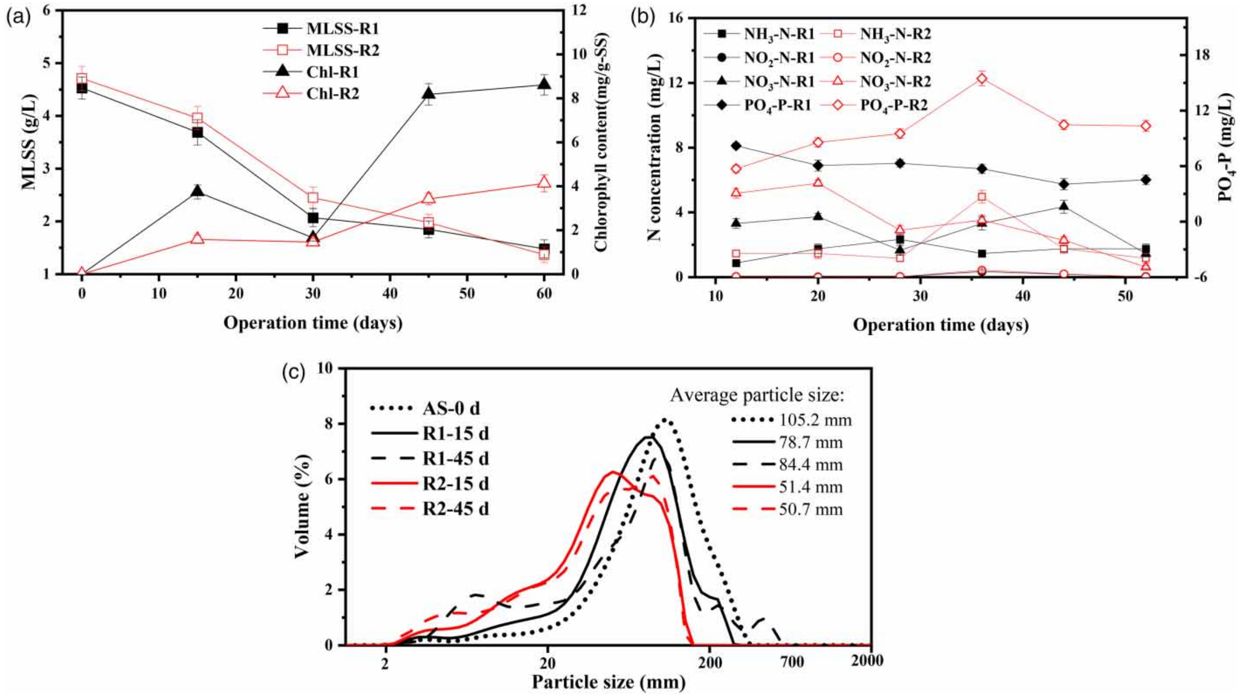

Figure 2 | Changes in MLSS, chlorophyll (a), N and P variations (b), and particle size distribution (c) in the two reactors during the operation.

respectively (Figure 2(a)), indicating algae largely grew with the sludge biomass reduction. And the higher chlorophyll *a* content in R1 showed the algae content was more abundant at 3% salinity condition. It was also worth noting that the chlorophyll *a* content in R1 had a big reduction at 30 days, this might be because the dominant green spherical algae probably have turned to another genus/species (likely the *Porphyridium* from its morphology) (Figure 1(b)–1(e)). The literature reported that spherical algae *Porphyridium sordidum* and *Porphyridium purpureum* had green and red color, respectively (Medina-Cabrera *et al.* 2020). While the big increment of chlorophyll *a* during 45–60 days was probably due to the big proliferation of filamentous algae at this period (Figure 1(b)–1(e)).

The inconsistency of the algae content and the total MLSS might be because the autotrophic microalgae grew using nutrients released from the decomposed sludge biomass under famine and light illumination conditions. Microalgae can grow at famine conditions and can produce organic compounds through photosynthesis. On the other hand, sludge degradation might bring inorganic nutrients (N, P, CO_2) from bacteria and EPS, due to part of the bacteria and extracellular polymers that can be decomposed by other microorganisms, releasing some organic (soluble protein and polysaccharide) and inorganic compounds (N and P) into the water (Liu *et al.* 2017; Lara-Moreno *et al.* 2022; Wang *et al.* 2022a, 2022b). These nutrients are essential for algae growth, which might accelerate the microalgae increment.

To prove this point, EPS content in sludge (Table 1) and the N, P concentration (Figure 2(b)) in water were detected from both reactors. Results showed that the total EPS content of sludge in R1 and R2 was 195.2 mg/g-VSS and 194.2 mg/g-VSS, respectively, lower than the original AS sludge (263.3 mg/g-VSS), indicating a certain extent of decomposition for EPS. Also, the main decomposed substance of EPS was found to be the protein (PN) component, while the polysaccharide (PS) showed

Table 1 | EPS in the MB consortia during the cultivation (unit: mg/g-SS)

Sample	Protein	Polysaccharide	Total EPS
AS-0 day	234.3	29.0	263.3
R1-60 days	124.2	71.1	195.2
R2-60 days	82.8	111.3	194.2

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a large increase compared with the AS sample. The released nitrogen species concentration (mainly NH_4^+-N and NO_3^--N) were measured between 0.9 and 5.8 mg/L, phosphorus (PO_4-P) was between 4.0 and 15.5 mg/L, which was sufficient for many algae growth (Salo & Salovius-Laurén 2022). This explained why the algae largely grew during the 60 days of cultivation, due to algae being reported to flourish when nutrients exceeded a certain threshold value ($N > 1.2$ mg/L, $P > 0.1$ mg/L) (Hu *et al.* 2017).

Another evidence of the sludge decomposition was that most of the sludge volume of R1 and R2 was distributed at smaller particle size area than AS (Figure 2(c)), and the average particle size in R1 and R2 decreased from 105.2 μm (AS, 0 day) to 84.4 and 50.7 μm (60 days), respectively. This implied the sludge flocs were severely decomposed due to famine conditions. On the other point, the relatively larger size of R1 than R2 was most likely due to the more abundant filamentous algae in R1 that had a stronger binding effect on the decomposed flocs (Cao *et al.* 2022).

Therefore, it was approved that the microalgae largely grew in the self-reliant system, which utilized abundant nutrients (including N and P) released from decomposed AS flocs.

3.1.3. Settleability

One of the big obstacles for traditional microalgae cultivation is the biomass harvesting problem (Álvarez *et al.* 2021; Ahmad *et al.* 2022). As it is known, to obtain pure microalgae, solids need a series of downstream processing such as sedimentation, flocculation, flotation, centrifugation, and filtration. These processes are costly to about 20–30% of biofuel production's overall expense (Ahmad *et al.* 2022). It is quite beneficial for reducing these costs when the algae could automatically flocculate to a dense concentration before taking downstream processing methods.

Figure 3 and Supplemental material, Figure S4 show the sludge volume change in a 6-h sedimentation experiment. Obviously, the algae growth in both R1 and R2 negatively affected the settleability of the MB consortia, since the 2-h settling velocity (SV) of R1 and R2 were 53.5 and 59.5%, respectively, much higher than normal AS (34%) (Zhang *et al.* 2018b). This indicated that the presented MB sludge had more difficulties in biomass collection than the original AS (Zhang *et al.* 2018b). However, sludge volume could still significantly reduce to 23.0% (R1) and 33.5% (R2) ($p = 0.46 > 0.05$) after 6 h of sedimentation, indicating the MB consortia were able to self-flocculate into a more condensed mixture liquid.

EPS is known as a biological flocculant, which is important for the settleability in AS systems (Nouha *et al.* 2018). Despite the large decomposition of EPS in R1 and R2, there still remained 195.2 mg/g-VSS and 194.2 mg/g-VSS in the two MB consortia reactors. The undecomposed EPS might contribute a lot to maintain the settleability of the MB systems. On the other hand, filamentous algae helped to scavenge free unicellular algae (Supplemental material, Figure S5), which probably also improved the sedimentation of the spherical algae in MB consortia.

Obviously, the MB consortia in the two reactors could self-flocculate to a smaller sludge volume probably with the help of the filamentous algae and residue EPS. Also, it is quite meaningful in reducing biomass harvest costs.

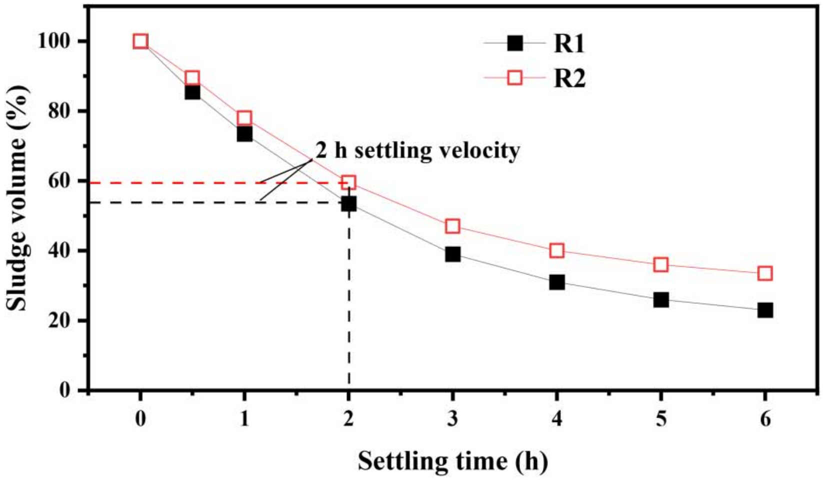

Figure 3 | Changes in sludge volume during a sedimentation experiment.

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3.2. Lipid analysis

3.2.1. Lipid content

Lipid content in MB consortia was shown in Figure 4(a), aiming to illustrate the effect of famine and saline environment on lipid biosynthesis. Original sludge in R1 and R2 had low lipid content of 12.1 mg/g-SS. While this value dramatically increased to 136 mg/g-SS (R1) and 119 mg/g-SS (R2) after 45 days of cultivation ($p = 0.66 > 0.05$), about 11.2 and 9.8 times that in original AS, respectively.

The primary reason for the lipid enrichment could be attributed to the algae growth in the two reactors, thus the lipid in R1 was slightly higher than R2 because the algae was more abundant in 3% salinity (Figure 2(a)). In addition, there are two more aspects of reasons for lipid increment. First, biomass decomposition could largely reduce the low lipid component (EPS and bacteria) in the MB consortia, as the lipid in original AS (1.2% w/w) was much lower than pure algae (20–40% w/w) (Zhang *et al.* 2021). Second, 3–5% salinity has been proved to have a stimulating effect on the algae growth and lipid biosynthesis in MB consortia (Cao *et al.* 2022), which might also have positive influence on the lipid production in the present systems.

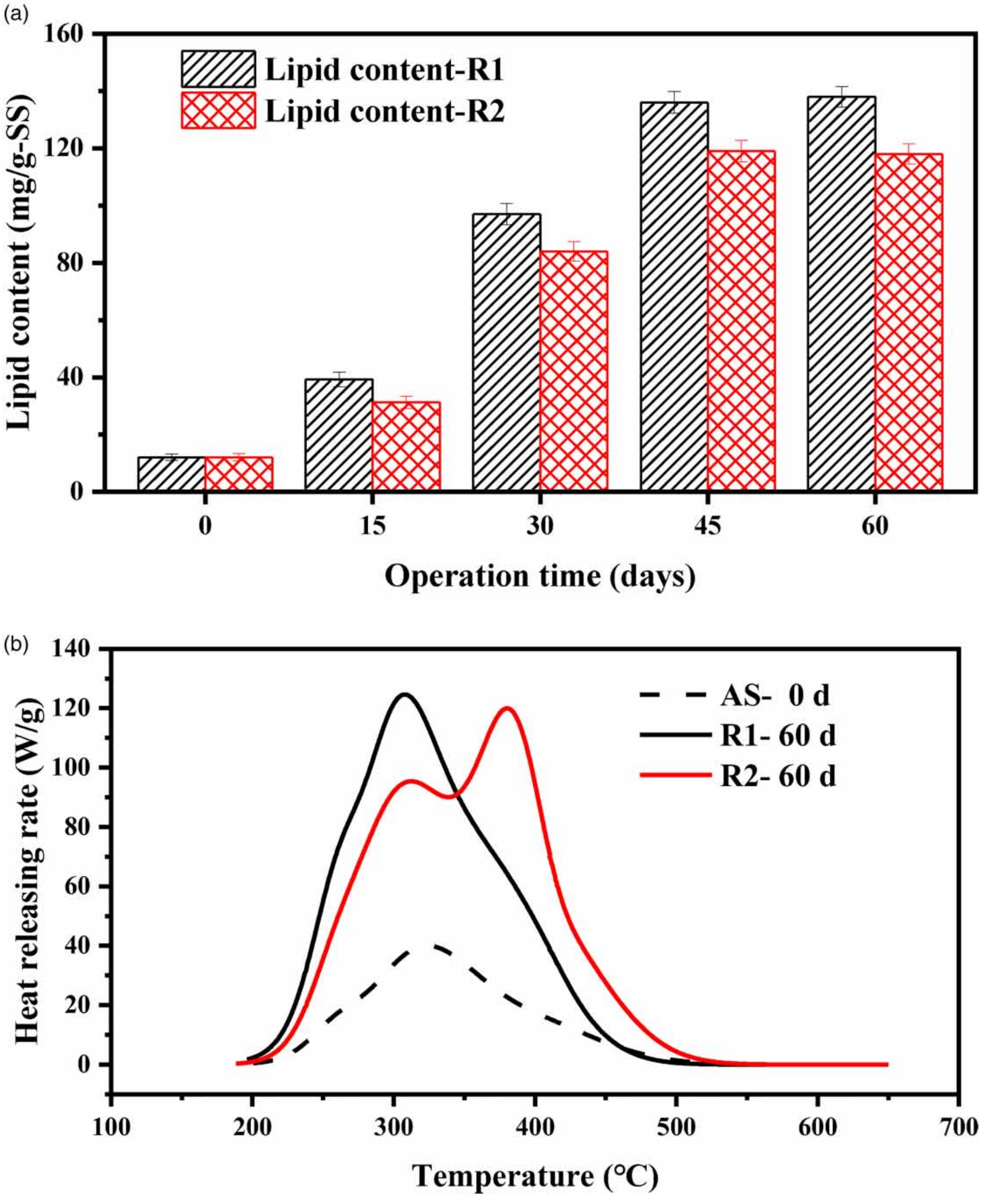

Figure 4 | Lipid content (a) and heat releasing rate (b) in the sludge from the two reactors.

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Clearly, in the self-reliant systems, the algae growth and saline condition can highly enhance the lipid production in MB consortia to 9.8–11.2 times of AS sludge, which will greatly benefit the reuse of sludge biomass to extract biodiesel products.

3.2.2. Microscale combustion calorimetry

In China, sludge incineration is currently used as one of the most important sludge disposal methods for the waste-activated sludge (Zhao *et al.* 2022). In order to test the flammability of MB biomass as burning material, the microscale combustion calorimetry (MCC) was conducted and results are shown in Table 2 and Figure 4(b). It was shown that the heat releasing rate (HRR) of the MB consortia in two reactors (60 days) peaked at 124.6 W/g (R1) and 119.9 W/g (R2), much higher than original AS (40 W/g) (Table 2, Figure 4(b)). Compared with only one big peak (307.6 °C) of HRR in R1, there are two peaks for biomass in R2, indicating the burning component in the sludge of R2 was more complex and mainly could be separately burned at two temperature sites (312.5 °C, 380.0 °C). Meanwhile, THR of R1 and R2 also significantly increased from 5.3 kJ/g (AS, 0 day) to 16.3 and 17.7 kJ/g (60 days), respectively. This illustrated the MB consortia in the two reactors had much enhanced flammability due to the lipid enrichment. One point should be noted that the THR of R2 was slightly higher than R1 despite a relatively lower lipid content. This might be because lipid components of FAMEs were not the same in the two systems, due to which various fatty acids could donate different THR values with long or short molecular chains.

Therefore, the current MB consortia cultivation process can significantly improve the flammability of waste sludge biomass, which will have a big meaning in decreasing the energy consumption in sludge incineration processes.

3.2.3. Lipid composition

Lipid compositions in MB consortia were identified by converting them to FAMEs (Table 3). Main FAMEs in original AS were myristic acid (C14:0, 21.9%), hexadecanoic acid (C16:0, 47.3%), heptadecanoic acid (C17:0, 13.4%), and oleic acid (C18:1, 8.5%). Also, the saturated FAMEs amounted for 89.2%. While after 45 days of cultivation in saline conditions, the saturated FAMEs of C14:0, C16:0, C17:0 in R1 and R2 were largely transformed into mono-unsaturated C18:1 (48.1% R1, 53.3% R2) at 45 days. Meanwhile, another dominant component of FAMEs (45 days) in R1 and R2 was still C16:0 (34.0% R1, 26.7% R2), but was much lower than C18:1. It could be found that the MB consortia (45 days) had more mono-unsaturated FAMEs content than seed AS, which accounted for 49.0 and 57.1% in R1 and R2, respectively.

High-quality biodiesel was known to mainly consist of fatty acids with 14–18 carbon atoms in their molecular chain (Huang *et al.* 2010). Poly-unsaturated FAMEs (PUFA) are unsuitable for long time storage because they are likely to be oxidized, and saturated FAMEs (SFA) are reported to have weak fluidity at low temperature (Vimali *et al.* 2022). There were no or tiny PUFA in lipids of MB consortia, meanwhile the SFA also decreased to 50.7% (R1) and 42.9% (R2) from 89.2% (AS). Therefore, the biodiesel products from the systems might be superior in stability and cold flow properties after cultivation.

On the other hand, it could be found that the MB consortia in R2 had relatively more C18:0 and C18:1, but much lower C16:0 than R1. This explains why the THR in R2 was slightly higher (Table 2), which might be because fatty acids with longer molecular chain contained more heat value rather than short ones. Meanwhile, longer chain fatty acids were more stable in thermal conditions, thus a higher temperature (380.0 °C) of a heat-releasing rate peak was also observed for R2 (Table 2) (Kahwaji *et al.* 2017).

Restated, the lipid contents were much enriched in 3 and 5% salinity self-reliant MB consortia systems, and the biodiesel quality was also improved. Meanwhile, flammability of sludge biomass in two systems were significantly enhanced due to lipid increment.

3.3. Microalgae community

In order to find out the main microalgae genera that grew in two reactors during the cultivation, metagenomic sequencing, and microscopic methods were used for identification. Metagenomic sequencing can identify microbial community composition, including bacteria, eukaryota, archaea, and viruses in sludge, as well as their genera relative content, and algae can be

Table 2 | Microscale combustion data of the MB consortia at 60 days

Sample	pHRR (W/g)	THR (kJ/g)	T_{max} (°C)
AS	40	5.3	323.6
R1	124.6	16.3	307.6
R2	119.9	17.7	380

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Table 3 | FAME compositions of lipid in MB consortia from the two reactors (45 days)

FAME compositions (%)	AS	R1	R2
Saturated FAMEs	89.2	50.7	42.9
Mono-unsaturated FAMEs	10.8	49.0	57.1
Poly- unsaturated FAMEs	0.0	0.3	0.0
C5:0 (Pentanoic acid)	3.5	8.6	6.5
C13:0 (Tridecanoic acid)	0.0	0.4	0.0
C14:0 (Myristic acid)	21.9	1.0	0.9
C16:0 (Hexadecanoic acid)	47.3	34.0	26.7
C16:1 (Hexadecenoic acid)	2.3	0.5	3.8
C17:0 (Heptadecanoic acid)	13.4	0.0	0.3
C18:0 (Stearic acid)	1.5	5.0	7.4
C18:1 (Oleic acid)	8.5	48.1	53.3
C18:2 (Linoleic acid)	0.0	0.3	0.0
C20:1 (Eicosenoic acid)	0.0	0.4	0.0
C22:0 (Docosanoic acid,)	0.0	0.2	0.2
C28:0 (Octacosanoic acid)	0.0	0.7	0.5
Others	1.7	0.8	0.4

selected from all genera items. Meanwhile, the microscopic method can also be used for supporting the main algae existence through morphology observation.

Ten largest microalgae genera in R1 included *Geitlerinema* (68.8%), *Leptolyngbya* (7.1%), *unclassified_p__Cyanobacteria* (6.4%), *Porphyridium* (3.1%), *Nodosilinea* (2.3%), *Jacksonvillea* (1.6%), *Synechocystis* (1.6%), *Phormidium* (1.3%), *Oscillatoriales* (0.6%), *Cyanothece* (0.5%) (Figure 5(a)). While 10 largest genera in R2 included *Nodularia_f__Aphanizomenonaceae* (30.6%), *Geitlerinema* (14.4%), *Leptolyngbya* (8.8%), *unclassified_p__Cyanobacteria* (7.6%), *Porphyridium* (7.5%), *Synechocystis* (6.9%), *Nostoc* (3.8%), *Nodosilinea* (3.2%), *Phormidium* (1.7%), and *unclassified_o__Nostocales* (1.6%) (Figure 5(b)).

It could be noticed that all these above algae genera except for *Porphyridium* (phylum Rhodophyta) belonged to phylum Cyanobacteria. Among these genera, the filamentous *Geitlerinema* was dominant in R1, the filamentous *Nodularia_f__Aphanizomenonaceae* was dominant in R2. Meanwhile, filamentous *Leptolyngbya* was also rich in the two reactors. This was consistent with the large content of filamentous algae presented in microscopic pictures (Figure 1). Filamentous algae that belonged to Cyanobacteria was known to easily proliferate in eutrophication coast or lagoons with a relatively low N and P nutrients (Mazor *et al.* 2022). The high salinity water in the present self-reliant systems (R1 and R2) had low N and P concentrations (Figure 2(b)) similar to the eutrophication sea condition, which could create a suitable environment for the filamentous Cyanobacteria growth.

On the other hand, a type of spherical algae with dark brown color was quantitively observed in microscopic images (Figure 1), which was identified as *Porphyridium* from the phylum Rhodophyta in metagenomic sequencing (Figure 5). The *Porphyridium* was known to be a marine unicellular microalgae which possessed abundant lipid and exopolysaccharides (Huang *et al.* 2021). Lipid content of *Porphyridium* was reported to reach as high as 37.4% w/w in an artificial seawater medium (Seemashree *et al.* 2022). Also, together with the lipid in filamentous *Nodularia* (1.0–2.6% somatic wet weight), *Geitlerinema* (16.5% w/w), and *Leptolyngbya* (32.1% w/w), *Porphyridium* significantly improved the total lipid content in the present MB consortia (11.9–13.6% w/w) (Persson *et al.* 2011; Singh & Kumar 2020, Ruiz-Domínguez *et al.* 2021). The contribution from *Porphyridium* might be prominent, due to its higher lipid content than the filamentous genera. *Porphyridium* in saline condition was also able to synthesize over 50% (w/w) carbohydrates (Ferreira *et al.* 2021). As the *Porphyridium* largely expanded to 3.1 and 7.5% in R1 and R2, respectively, this is probably the reason for the EPS in MB consortia (60 days) to have much enriched PS component than original AS (0 day) (Table 1).

Therefore, filamentous *Geitlerinema*, *Nodularia,* and *Leptolyngbya* as well as the spherical red algae *Porphyridium* were dominant in high salinity self-reliant reactors. These genera of microalgae utilized nutrients from decomposed sludge

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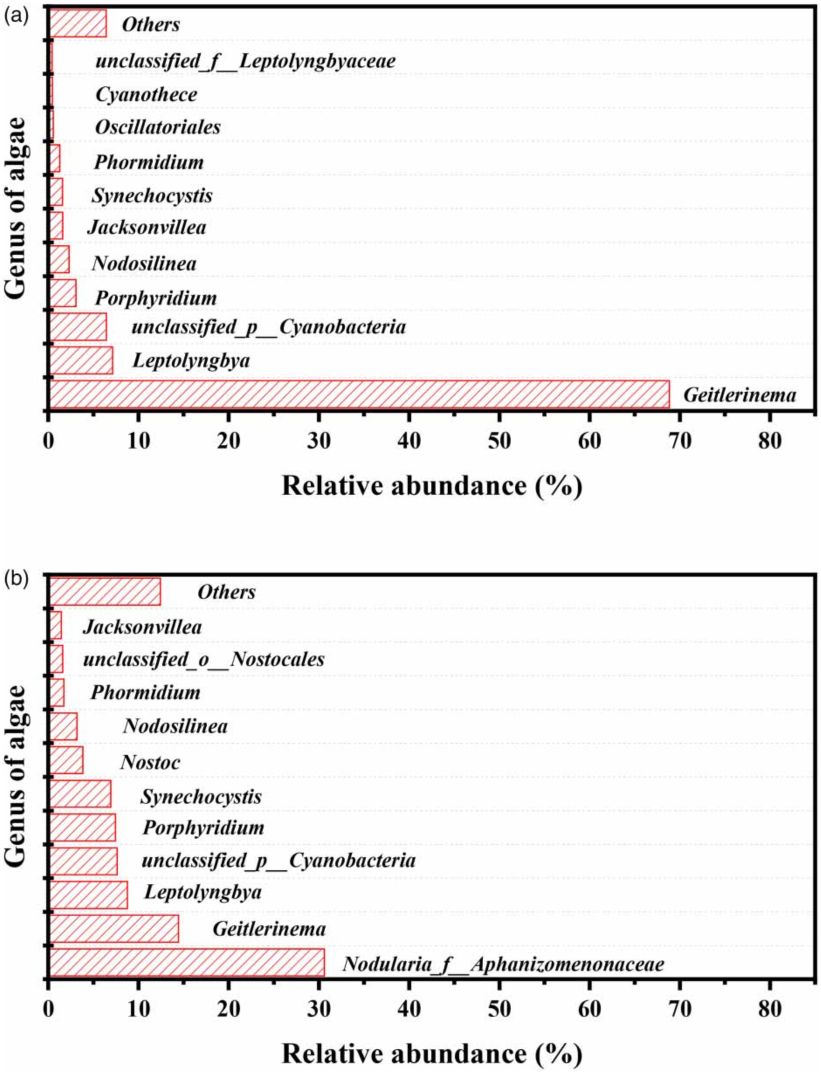

Figure 5 | Microalgae community in R1 (a) and R2 (b) at 45 days.

biomass, and produced high-value-added lipid products, along with the meaningful transforming processes from waste-activated sludge to algae.

4. CONCLUSIONS

In order to improve the lipid content in waste-activated sludge, self-reliant MB consortia systems with 3 and 5% salinity water were constructed for cultivating lipid-rich microalgae from an AS. Sludge biomass was decomposed due to famine conditions, releasing nutrients of N and P into water for the algae assimilation. Meanwhile, lipid contents were multiplied to 9.8–11.2 times that of AS due to the algae growth, which included filamentous *Geitlerinema*, *Nodularia*, *Leptolyngbya*, and spherical *Porphyridium*. Thereby, much enhanced flammability was also achieved for the consortia biomass. This work presents a new strategy for consuming the massive waste-activated sludge, and reusing the N, P resources for producing valuable biodiesel.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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