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# Advanced treatment of food processing effluent by indigenous microalgae-bacteria consortia: Population dynamics and enhanced nitrogen uptake

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ABSTRACT

The potential of indigenous microalgae-bacteria consortia (IMBC) to recover nutrients from food processing effluents (FPE) supports the basis for advanced effluent polishing and value-added biomass generation. In this study, the effluent polishing potential of an FPE-borne IMBC treating FPE and synthetic wastewater (SWW) was investigated regarding nutrient, coliform bacteria, and chemical oxygen demand (COD) removal as well as the IMBC species evolution, and pigment production. Species evolution and diversity of the IMBC in FPE and SWW were influenced by nitrogen levels (3.83 mg/L and 32.61 mg/L $NH_4^+$, respectively). More blue-green microalgae were observed in SWW (0.96 mg/L phycocyanin) whilst diatoms dominated in FPE (0.05 mg/L phycocyanin). Total coliform bacteria removal influenced COD reduction and this had a significant effect on dissolved oxygen production. The study offers new insights into the feasibility of using IMBC biofilm for advanced FPE polishing and nutrient recovery (0.98 mg/L $NH_4^+$, 0.85 mg/L $PO_4^{3-}$, 0.84 mg/L COD, 3.2 g/L protein, and 2.8 g/L carbohydrates), demonstrating that it is possible to use IMBC biofilm for post-treatment of FPE, removing the residual N and P to prevent eutrophication.

## 1. Introduction

Food processing effluents (FPEs) are rich in biodegradable organic carbon compounds such as proteins, fats, and carbohydrates. FPEs are usually treated by anaerobic or aerobic processes with simultaneous bioenergy generation such as methane production. Unfortunately, these treatment processes still leave effluents with high organics, nitrogen (N), and phosphorus (P) levels. The direct discharge of these compounds causes water pollution problems in the receiving environments [1]. Thus, sustainable tertiary treatment technologies for FPEs are required for further effluent polishing and resource recovery.

Microalgae-based wastewater treatment technology has been extensively explored as a more sustainable alternative for advanced or tertiary effluent treatment with the benefits of enhanced nutrient uptake, value-added biomass production, and reduced greenhouse emissions [2,3]. For example, microalgae monocultures (e.g., *Chlorella vulgaris*, *Scenedesmus vacuolatus*) have been shown to have great nutrient removal capacities for the bioremediation of raw effluents and synthetic wastewater [4]. However, these microalgae monoculture wastewater treatment systems still face problems such as low microalgal activities in wastewater, which limits their largescale application. These are important factors to consider while trying to establish a cost-efficient microalgal effluent treatment process.

Previous studies have mostly been centered on the application of commercial microalgae and bacteria in MBC systems. With regard to microalgae, numerous species are just as efficient in removing specific pollutants in wastewater. *C. vulgaris* has been successfully applied in the removal of lead (Pb) and *Tetraselmis suecica* for cadmium (Cd) removal

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[5].

An ideal solution to achieving optimal wastewater treatment is the utilization of indigenous MBC (IMBC) obtained from the targeted wastewater [6]. In doing this, the microalgae and bacteria strains need to be selected and bred from the same environment through adaptive laboratory evolution to ensure proper acclimatization of the indigenous species to particular wastewater conditions [7] as opposed to commercial strains. Diatoms are well known to tolerate low nutrient levels and high conductivity in wastewater [8], thus, employing native diatom species in the MBC could also be a practical application of IMBC in effluent treatment. It should be noted, however, that the feasibility of IMBC in treating FPE remains unclear, although one attempt was reported using monocultures of *C. vulgaris and Scenedesmus obliquus* for FPE bioremediation with enhanced N (54.8 %) and P (79.4 %) removal efficiencies and value-added biomass production. After 93 days of operation and intermittent biomass harvesting, the study proved that FPE could be utilized for the production of food-grade algae biomass [9]. However, pathogenic microbes such as coliform bacteria exist in FPEs, with their impact on IMBC growth/physiology and their nutrient uptake from FPE still obscure. This therefore, necessitates further research.

Thus, the goal of this study was to demonstrate the feasibility of applying FPE-originated IMBC for simultaneous nutrient uptake and biomass production. The FPE used in this study represents secondary effluents that do not meet the discharge standards of the Ghana Standards Authority (Table 1). Specifically, the performance of the wastewater-borne IMBC isolated from FPE at Ghana's industrial hub (Tema Industrial Area) was observed in real and synthetic wastewater. The IMBC performance with regard to population dynamics, nutrient reduction, coliform bacteria, and chemical oxygen demand (COD) removal, pigments production, such as industrially important phycocyanin (PC), chlorophyll (Chl), and extracellular polymeric substances (EPS) was established. The link between the population dynamics and nutrient uptake was also investigated as well as the influence of the intrinsic wastewater characteristics on the species evolution. The environmental significance and resource recovery potential of the study were also highlighted.

## 2. Materials and methods

### 2.1. FPE source and characteristics

Effluents from the food processing plant were collected from the four chambers of the treatment plant at the Tema industrial area, Ghana (DD: 5.657696, 0.003353) (Fig. S1). The initial physicochemical characteristics of the effluents were analyzed within 24 h (Table 1). The remaining effluent samples were refrigerated (4 °C) for subsequent use.

**Table 1**
Effluent characteristics of FPE at time of collection.

| Parameter | Sedimentation chamber | GS 1212:2019 |
|---|---|---|
| pH, (pH units) | 7.50 | 6.00–9.00 |
| Conductivity, (mS $cm^{-1}$) | 5.862 | 1.500 |
| Turbidity (NTU) | 115 | 75.0 |
| Apparent colour (Hz) | 20.0 | 200 |
| Total suspended solids (TSS) (mg/L) | 106 | 50.0 |
| Total dissolved solids (TDS) (mg/L) | 3224 | 1000 |
| Alkalinity (mg/L) | 396 | 150 |
| Chloride (mg/L) | 1966 | 250 |
| Nitrate–nitrogen ($NO_3$-N) (mg/L) | 0.147 | 50.0 |
| Phosphorous – $PO_4^{3-}$ (mg/L) | 1.19 | 2.00 |
| Ammonium – $NH_4^+$ (mg/L) | 3.83 | 1.00 |
| Biological oxygen demand (BOD) (mg/L) | 43.7 | 50.0 |
| Chemical oxygen demand (COD) (mg/L) | 163 | 250 |

GS 1212 – Ghana standards No. 1212 - 2019.

Sterile 500 mL plastic bottles were used to collect effluents for microbiological analysis.

### 2.2. Generation of IMBC and isolation of individual species

Aliquots (10 mL) of the FPE samples were added to a 250 mL Erlenmeyer flask with a 100 mL working volume of BG-11 as previously described [10]. Flasks were kept in a shaking incubator with a 12 h: 12 h light/dark regime to generate native microorganisms. By Day 14, IMBC cells were observed at the bottom of the flasks (Fig. S2 a & b). 100 μL of the IMBC culture was streaked onto agar plates to isolate pure cultures. The plates were placed by a window at room temperature (28 ± 2 °C) and 12 h: 12 h, light/dark regime. Cells on the agar plates were gently scrapped and introduced into fresh nutrient broth in conical flasks and subsequently into microplate wells for uniform biofilm discs and inoculant production.

### 2.3. Experimental setup

The experiment was conducted in two parts. In the first part, effluent-polishing potential of the IMBC was monitored. Thus, only water samples were drawn for analysis to avoid disturbance of the biofilm structure. In the second part of the experiment, the biofilm biomass was applied directly for analysis. To monitor the performance of the isolated microbial consortia, experiments were conducted using two types of wastewater media, FPE and synthetic wastewater (SWW). SWW with higher, COD, N and P levels (Table S1) was used to ascertain the performance of the IMBC in high-strength wastewater without real wastewater stress such as bacteria, high turbidity, alkalinity or dissolved solids which impede light penetration and microalgae growth. The cultures were operated at room temperature (28 ± 2 °C) and a 12 h: 12 h, light/dark photoperiod. To investigate microalgae-bacteria interactions, non-axenic cultures without antibiotic treatment was used in the main experiment. In a preliminary experiment (Table S2), the growth media (FPE and SWW), were sterilized (121 °C, 15 min) prior to analysis to determine the nutrient removal efficiencies of the cultures without bacteria interactions. The nutrient levels were monitored every other day. All experiments were set up in triplicates.

To understand the dynamics of the IMBC structure formation, separate cultures were set up alongside the effluent polishing experiment. The biofilm growth was monitored and divided into 4 stages according to visual and microscopic (Nikon Eclipse 90i) observations. In the acclimatization stage (Phase 1, Day 0–6), cells adjusted to the culture environment. During the attachment stage (Phase 2, Day 7–14), cells attached to the substrate (glass beaker) and spread as the cells matured in the maturation stage (Phase 3, Day 15–21). Subsequently, in the detachment stage (Phase 4, Day 21–28), cells detached from the beakers, and the biofilms floated in the growth media, marking the end of their growth cycle. The species evolution, pigments, proteins, and carbohydrate production of the IMBC were investigated during the second part of the experiment.

### 2.4. Water quality analysis

To monitor the effluent polishing ability of the IMBC, growth media was drawn with a 10 mL syringe and filtered through a 0.45 μm membrane filter (Xinya 50 mm, Shanghai, China) before analysis. Physicochemical parameters, including alkalinity, chloride, COD, ammonium ($NH_4^+$), and phosphates ($PO_4^{3-}$) were analyzed every other day. COD, $NH_4^+$, and $PO_4^{3-}$ were measured using the potassium dichromate, Nessler reagent spectrophotometry, and ammonium molybdate spectrophotometry methods, respectively. All protocols were performed according to standard methods [11]. Conductivity and dissolved oxygen (DO) were measured using a EUTECH COND610 meter and a HACH portable probe (HQ40D) respectively. Nutrient reduction efficiencies (nRE, %) were calculated according to Eq. (1), where $P_i$ = initial parameter value; $P_f$ =

final parameter value.

$$nRE = \left(P_f – P_i / P_i\right) \text{ x } 100\% \quad (1)$$

Total coliform (TC) bacteria was determined through serial dilutions (1 mL aliquots) and the membrane filtration method [11]. The membrane filters were subsequently cultured on chromogenic coliform/*E. coli* agar (Park Scientific Ltd) and the plate-count method was used to count the coliform forming units (CFU) on the membrane filters.

### 2.5. Pigment analysis

The population composition of the cultures was determined through pigment analysis. Phycocyanin was extracted by the freeze-thaw method (after 5 cycles) [12]. Briefly, the IMBC biofilm was scraped from the flasks at the end of the cultivation cycle. The biomass was transferred into 50 mL falcon tubes and frozen (−30 °C). The samples were allowed to thaw and the supernatants were collected with a micropipette into a separate falcon tube. The procedure was repeated five times to obtain the maximum pigment contents. The absorbance of the resultant extracts was read at 615 and 652 nm and phycocyanin content was calculated using Eq. (2). Chlorophyll *a* and *b* were extracted from the cultures with 80 % acetone, following the Lichtenthaler [13] method. The chlorophyll content was calculated with Eq. (3), where $PC$ = phycocyanin; $C_a$ = Chlorophyll *a*.

$$PC = (A_{615} – 0.474 \text{ x } A_{652})/5.43 \quad (2)$$

$$C_a = 12.25(A_{663}) – 2.79(A_{646}) \quad (3)$$

### 2.6. Estimation of EPS and nitrogen recovery efficiency

EPS plays a major role in microbial biofilm aggregation and attachment. Thus, to determine the effect of EPS on IMBC formation, the carbohydrates and protein contents of the cultures were measured at the end of each experimental phase. This facilitated estimation of the biofilm formation, maturation, and disintegration. Carbohydrate was measured by the phenol sulfuric acid method described elsewhere [10] and protein was quantified by the Kjeldahl nitrogen block digestion method [14]. Since a majority of the inorganic nitrogen assimilated by IMBC will be transformed into amino acids and proteins, the nitrogen recovery efficiency (NRE) was represented by the amount of protein obtained after each experimental phase. Eq. (4) was used to calculate the NRE, where gp is the growth phase and Dw refers to the dry weight [15].

$$\text{NRE (Protein (mg)/(L.gp))} = \text{Protein content x Dw of biomass} \quad (4)$$

### 2.7. Statistical analysis

Data were expressed by means ± standard deviations. An unpaired *t-test* was used to determine the statistical differences between the groups.

## 3. Results and discussion

### 3.1. Effluent polishing performance varied in IMBC and Chlorella sp.

To ascertain the effect of microalgae-bacteria interactions in effluent polishing, the nutrient and coliform reduction potential, dissolved oxygen (DO) production, and COD removal of the IMBC were monitored.

#### 3.1.1. Nutrient removal

The preliminary experiment (Table S2) indicated that the rate of nutrient removal in the sterilized effluent group was lower than that of the unsterilized group. By Day 2, for instance, $NH_4^+$ levels had reduced by only 58 % compared to a 95 % reduction in the non-sterilized experiment. In this study, as shown in Fig. 1 a & b, nutrient removal in the FPE IMBC cultures was the fastest during the first phase due to lower nutrient levels. In FPE, $NH_4^+$ reduction was 95.3 % by Day 2. However, in SWW, $NH_4^+$ reduction was 0.4 % on Day 2. Higher nutrient reduction was observed from Day 6 onwards (56.0 %) compared to previous days. This could be due to the acclimatization of cells to high $NH_4^+$ levels [16]. Generally, there was a wider preference for $NH_4^+$ over $PO_4^{3-}$ in all scenarios (Fig. 1 a & b). By Day 2, the IMBC cultures had reduced $PO_4^{3-}$ levels in FPE by 13.4 % and 1.2 % in SWW. By Day 6, $PO_4^{3-}$ reduction was 64.7 % in FPE and 11.0 % in SWW. The significant differences between the removal performance in FPE and SWW were likely due to high nutrient shock which required the IMBC to acclimatize to the nutrient-rich environment, leading to low nutrient removal in Phase 1 of the SWW. At the end of Phase 2 (Day 12), $NH_4^+$ levels in FPE were reduced by 99.0 %. In SWW however, $NH_4^+$ was reduced by only 66.4 %. Thus, comparing both growth media, IMBC biofilm cells in SWW were still actively growing and consuming nutrients steadily after Phase 2. Regarding $PO_4^{3-}$ removal, by Day 12, the FPE treatment group was reduced by 73.1 % and 14.3 % in SWW. The lower reduction rate of $PO_4^{3-}$ in both treatment groups compared to $NH_4^+$ could be due to

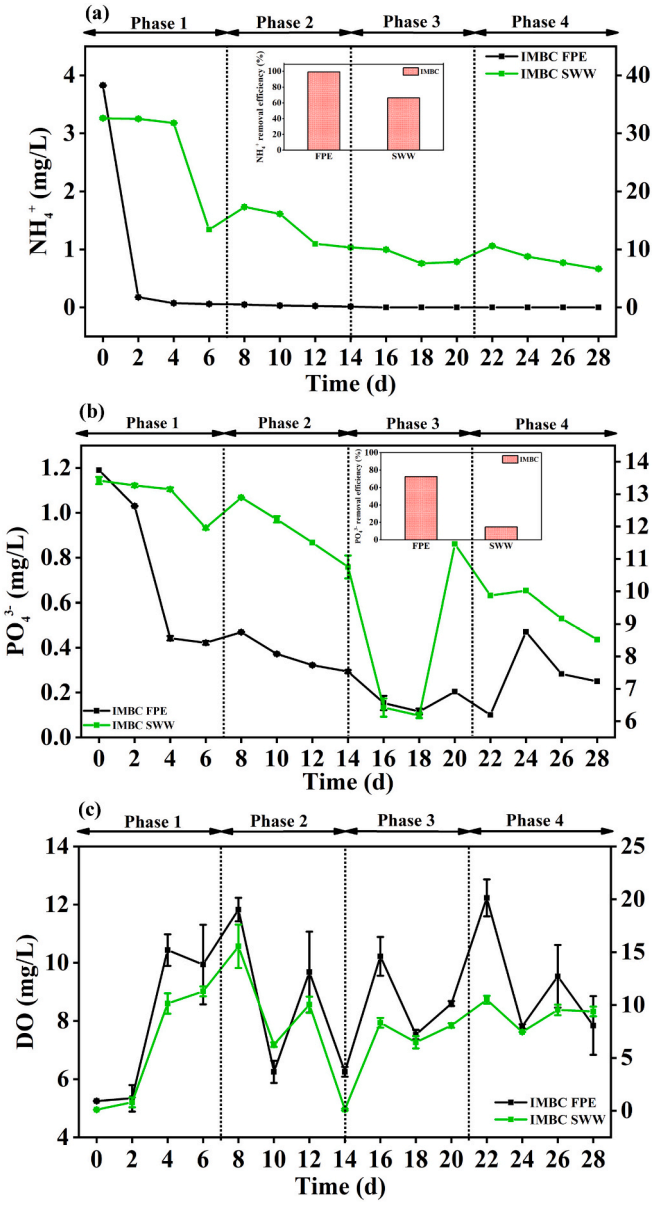


**Fig. 1.** Effluent polishing potential of IMBC: $NH_4^+$ reduction (a), $PO_4^{3-}$ reduction (b); and DO production (c). Insert represents the IMBC nutrient removal efficiencies (%). The right Y-axis represents SWW and the left represents FPE.

wastewater stress conditions such as high conductivity, TDS, and TSS levels hindering $PO_4^{3-}$ uptake. Increasing loads of N and P lead to silicon (Si) shortages which might have influenced the population dynamics of IMBC in various wastewater conditions [17]. Therefore, the high N and P in SWW could have led to a dominance of non-siliceous blue-green microalgae over diatoms while in FPE, high silica levels facilitated the proliferation of diatoms with few blue-green microalgae filaments. Microalgae species are efficient scavengers of $NH_4^+$ with the order of nitrogen utilization being $NH_4^+ > NO_3^- > N_2$ and they do not utilize other nitrogen sources while $NH_4^+$ is available [16]. It is noteworthy that the nitrate levels in the FPE at the start of the experiment were less than 0.5 mg/L (Table 1), suggesting that N removal was by microalgae assimilation rather than the dissimilation route as corroborated by [18]. Table 2 summarized the utilization of microalgae either as a mono-culture or in co-culture for treating FPE with simultaneous bioproduct production.

Generally, FPE is considered a sustainable source of growth nutrients for microalgae to thrive, while producing other metabolites such as lipids, EPS, proteins, and pigments. For instance, the high N levels in brewery effluents led to high protein and lipid recovery [9], while, MBC treatment of soybean fermentation effluent led to fish feed production from nutrient-rich biomass of *Chlorella* sp. and acidogenic bacteria [19]. Similarly, 103.0, 57.0, and 30.0 mg/g dry weight of phycocyanin, allophycocyanin, and phycoerythrin, respectively, were obtained from blue-green microalgae; *Nostoc* sp. *Arthrospira platensis* and *Porphyridium purpureum,* respectively, while treating 98 % COD, 94 % N, and 100 % P of food industry effluents [20]. These scenarios demonstrate the feasibility of simultaneously achieving enhanced effluent polishing and high-value bioproduct recovery from FPE using IMBC.

### 3.1.2. Oxygen evolution

Microalgal photosynthetic oxygenation supports bacteria growth, thus, influencing microalgae-bacteria interactions. The DO levels in FPE increased slightly from 5.2 to 7.84 mg/L in the IMBC cultures, representing a 1.5-fold change at the end of the experiment (Fig. 1c). In SWW however, initial DO levels of 0.1 mg/L increased to 9.39 mg/L in IMBC cultures, indicating a 93.9-fold change at the end of the experiment. Currently, blue-green microalgae are the only known prokaryotes that perform oxygen-evolving photosynthesis [20,21]. Air pockets were observed in IMBC-SWW cultures throughout the cultivation period, possibly due to blue-green microalgae photogranules [22]. The presence of bacteria in the FPE effluents led to increased DO consumption, thus, influencing the final DO levels. Although some researchers consider microalgae a nuisance because of clogging issues, one cannot ignore the fact that the oxygen produced by algae is beneficial to the bacteria in the microalgae-bacteria system for organic matter breakdown as well as complete nitrification [23].

### 3.1.3. Coliform bacteria and COD reduction

TC bacteria naturally present in FPE was analyzed a few hours after field collection. The initial TC bacteria in FPE was $1.1 \times 10^7$ CFU/mL. This was reduced to 300 CFU/mL and 0 CFU/mL on Days 2 and 6 respectively by IMBC (Fig. 2a), suggesting that by the end of Phase 1, all the TC bacteria had been entrapped in the biofilm matrix and were no longer free-living in the growth media. In a side trial experiment with *Chlorella* sp. (Fig. S3), TC levels reduced from $1.1 \times 10^7$ CFU/mL to $3.72 \times 10^4$ CFU/mL on Day 6 and by Day 12 (end of phase 2), TC content of *Chlorella* sp. was 4800 CFU/mL. It is worth noting that, microalgae suspension cultures possess a lower capacity to remove TC in wastewater compared to biofilm cultures [24]. This was corroborated in the present study as it was demonstrated that IMBC biofilm completely removed TC while monoculture suspensions did not. Blue-green microalgae are known to excrete molecules with herbivore-repellent properties that reduce their palatability to predators such as phage bacteria and grazers [25].

COD reduction in wastewater involves the breakdown of organic compounds into carbon dioxide ($CO_2$) and water in aerobic or anaerobic conditions. In this experiment, COD reduction occurred in aerobic conditions, where microalgae added DO to the culture, while bacteria consumed part of the DO. Organic chemical degradation has been observed in blue-green microalgae-microbial biofilms, which are often initiated by associated bacteria and are enhanced through oxic/anoxic diurnal shifts created within the biofilm [26]. COD reduction in FPE was 84.0 % and 54.0 % in SWW at the end of the experiment (Fig. 2b). Thus, generally, there was a higher COD reduction in FPE than in the SWW cultures compared, possibly due to the presence of bacteria in FPE cultures. This was consistent with another study reporting that COD removal efficiency was lower (2.2 %) in the control experiments with

**Table 2**
Simultaneous nutrient reduction and bio-product production by microalgae growing in FPE.

| Food industry | Wastewater characteristics | Microorganisms | N & P removal | Reactor & operations time | Metabolites | Reference |
|---|---|---|---|---|---|---|
| Brewery | CIP effluent | *Chlamydomonas* sp.<br>*Chlorella* sp.<br>*Scenedesmus* sp. | N – 48.3 - 54.8 %<br>P – 65.5 - 79.4 % | Batch / 93 days | Protein – 44.5 % DW<br>Lipid – 15.6 % DW | [9] |
| Indian snack | Raw & AD wastewater | *Chlorella sorokoniana*<br>*Scenedesmus obliquus*<br>*Scenedesmus abundans* | N – 84 %<br>P – 70 % | Batch / 8 days | Lipid - 27.5 mg/L/day | [38] |
| Plant-based food products (Alpro) | UASB effluent | *Nostoc* sp.<br>*Arthrospira platensis*<br>*Porphyridium purpureum* | N – 94 %<br>P – 100 % | Batch photobioreactor / 20 days | Phycocyanin 103 mg/g DW<br>Allophycocyanin 57 mg/g DW<br>Phycoerythrin 30 mg/g DW | [20] |
| Meat processing | DAF effluent | Microalgae/ bacteria (co-immobilized) *Scenedesmus obliquus*<br>*Chlorella vulgaris*<br>*Chlorella sorokoniana* | N – 8 - 33 % | Annular batch reactor / 7 days | N/A | [39] |
| Soybean fermentation | SFE | Microalgae (*Chlorella* sp.) & acidogenic bacteria consortia (*Prevotella* sp., *Acidaminococcus* sp., *Lactobacillus* sp.) | N – 35.35 % P – 57.70 % | Batch / 44 days | Fish feed | [19] |
| FPE | CIP & reverse osmosis effluents | *Leptolyngbya* sp.,<br>*Phormidium* sp.,<br>*Nitzchia palea.* | N – 97.9 %<br>P – 84.9 %<br>COD –84.3 % | Batch / 28 days | Protein – 35.8 - 90.61 %<br>Carbohydrate – 9.39–64.17 % | This study |

N – Nitrogen; P – Phosphorus; CIP – Clean-in-place; DW – Dry weight; AD – Anaerobic digestion; N/A – Not applicable; Upflow anaerobic sludge blanket (UASB); Dissolved air flotation (DAF); Soybean fermentation effluent (SFE); FPE – Food processing effluen.

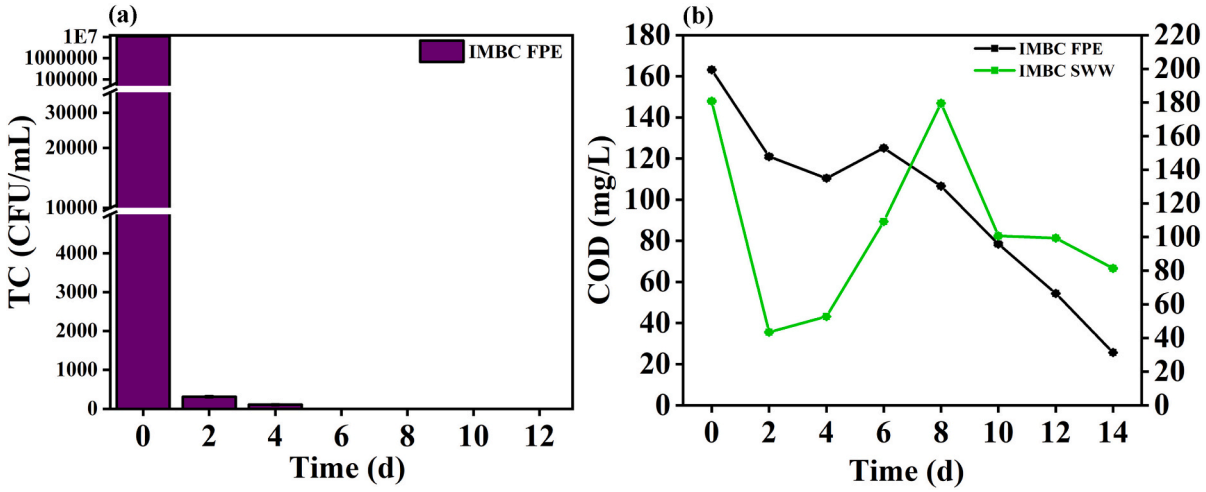


**Fig. 2.** Total coliform bacteria (TC) (a) and COD (b) removal by IMBC cultures in FPE and SWW. The right Y-axis represents SWW and the left represents FPE.

only native bacteria compared to 14.7 % in treatment groups with microalgae-bacteria cultures. The higher COD removal in treatment groups was attributed to the cooperation between algae and bacteria in the elimination of organic carbon [9].

The absence of TC bacteria in SWW explains the reduced/lower TC removal compared to the scenario in FPE. In other words, the absence of sufficient bacteria to help remove the COD led to lower removal efficiency (54.0 %) in SWW. Conversely, in FPE, the presence of more bacteria facilitated higher COD removal (84 %). Similarly, mutual metabolic interactions demonstrated ammonium cross-feeding between *Rhodobacteraceae* and *Phaeodactylum tricornutum* where methylamine degradation showed species-specific patterns, and bacterial glycine betaine degradation led to diatom growth. In this study, low ammonium levels and high conductivity in FPE led to the proliferation of diatoms in contrast to low conductivity and high ammonium concentrations in SWW which have been demonstrated to be toxic to marine diatoms [8].

### 3.1.4. Nutrient limitation induced a reduction in pigment production

Pigment production in microalgae is a result of a photosynthetic activity that relies mostly on light and $CO_2$. In addition to the main photosynthesis pigment chlorophyll, microalgae possess ancillary pigments such as phycobiliproteins that increase light energy use efficiency and carotenoids that protect microalgal cells against solar radiation. In this study, the filamentous blue-green microalgae (see details in Section 3.2) including *Leptolyngbya* sp., and *Phormidium* sp., possess phycocyanin (*PC*). *PC* and chlorophylls *a* (Chl *a*) were extracted from the cultures at the end of the cultivation period to confirm the dynamics of the species population under the two wastewater conditions. Results showed that the dominance of blue-green microalgae in SWW led to higher *PC* content (0.96 mg/L; $p = 0.04$) compared to that of the FPE (0.05 mg/L) which was dominated by diatoms (Fig. 3). This was consistent with the observation that the FPE culture appeared paler than the SWW culture owing to the loss of blue-green pigmentation. Similarly, Hernández et al. [27] observed a yellowish colouration in their photobioreactor after 31 days of cultivation, which was attributed to induced chlorosis under the nutrient limitation condition. The degradation of the pigments in FPE might be linked with nutrient limitation since $NH_4^+$ (3.93 ± 0.97 mg/L) was depleted within 2 days and phosphorus was consumed by Day 16 (Fig. 1). This was consistent with a study demonstrating that in response to the depletion of external nitrogen sources, chlorophyll content decreased drastically by 2.0-fold as an intracellular nitrogen source for cell utilization [28]. In addition, Chl

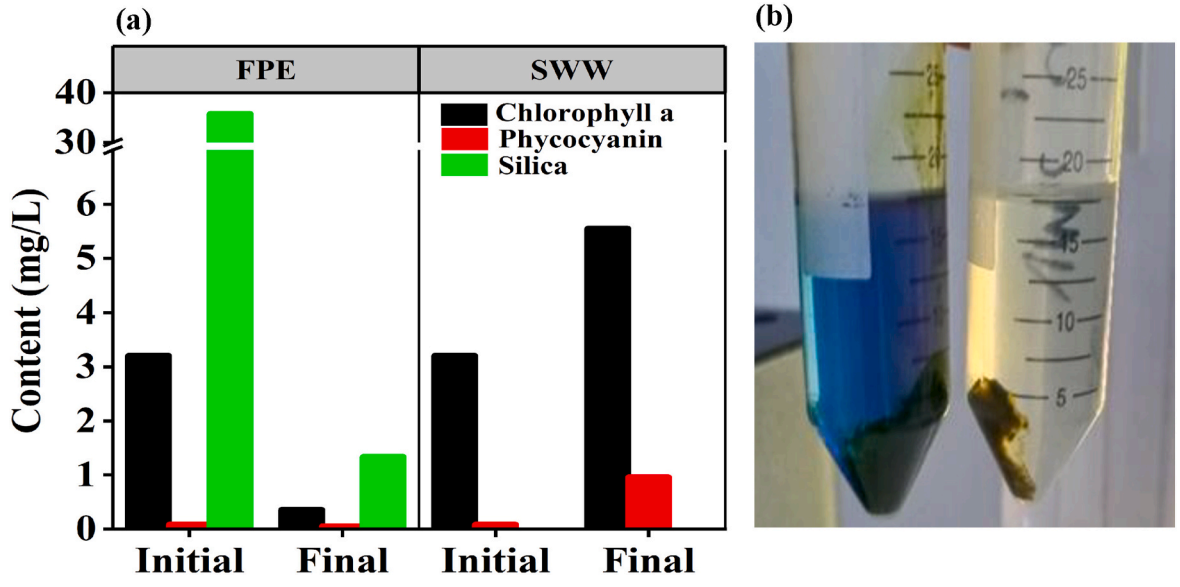


**Fig. 3.** Phycocyanin, silica, and chlorophyll content (mg/L) (a) and actual extracted pigments (b) of IMBC.

*a* extracted from IMBC in FPE was lower (0.36 mg/L) compared to SWW (5.55 mg/L) by Day 28 ($p = 0.0025$). This might be attributed to the differences in the compositions of species as well as the various $NH_4^+$ removal rates between IMBC-treated FPE and SWW groups. Overall, it is likely that nutrient depletion of FPE induced a reduction of pigment production in IMBC.

### 3.2. Wastewater characteristics affected IMBC species evolution and diversity

IMBC consisting of different microalgae and bacteria species grew differently under the two wastewater conditions. The observed formation of a blue-green mat-like biofilm with brown and green patches in the inoculum material confirmed the presence of blue-green filaments, diatoms, and green microalgae, respectively, prior to inoculation (Fig. 4).

Microalgae in the inoculum were identified as green unicellular cells of *Chlorella* sp. (*Chlorophyceae*), non-heterocystous blue-green filaments of *Leptolyngbya* sp. and *Phormidium* sp. (*Cyanophyceae*), and pennate diatoms of *Nitzchia palea* (*Bacillariophyceae*). After cultivation, it was believed that the presence of grazers in FPE, potentially led to the selective feeding of certain microalgal groups, thus, contributing to the differences in population composition. For instance, although the starting material was the same IMBC biofilm, after 10 days of cultivation, the FPE biofilm transformed from a filamentous blue-green microalgae-dominated culture, into a pennate diatom-dominated culture, while the SWW biofilm was still dominated by blue-green filamentous microalgae throughout the cultivation period (Fig. 4). The low light intensity in the laboratory from the natural day/light setting (without additional illumination) possibly led to the high silica content in diatom frustules, making them unpalatable and indigestible to grazers, thus serving as a mechanical defense. It has been demonstrated that diatoms cultivated under low light intensity accumulated more silica compared to higher light intensity cultures [29]. Thus, the high TSS levels (106 mg/L) of the FPE, could have led to shading and low light penetration, enabling the diatoms to accumulate more silica. Thus, the wastewater composition influenced the differences in microbial population composition and their pigment content in FPE and SWW cultures (Fig. 3). The high conductivity (5.9 mS/cm) observed for the FPE might have also accounted for the lower diversity which is supported by [30], reporting that some species are unable to tolerate high conductivity stress. Thus, in this study, species unable to tolerate high conductivity might not have been favored, leading to lower species diversity in FPE. However, Boelee et al. [4] observed a larger diversity of microalgae in their study with real municipal wastewater effluents compared to SWW. This could be due to higher nitrates in their effluents, favoring diverse microalgae growth.

The biofilm growth was monitored by observing its spread along the edges of the beakers up to the water level (Fig. 4). The species composition in SWW was more diverse at the end of the experiment compared to that in the FPE. The population in SWW was comprised in descending order: blue-green microalgae > green microalgae > diatoms. However, in FPE, the reverse was true (diatoms > green microalgae > blue-green microalgae). This could be attributed to a couple of factors explained subsequently. Firstly, the high nutrient levels in SWW favored more microalgae species. Differences in the nutritional composition of the wastewaters such as higher $NH_4^+$ and $PO_4^{3-}$ levels in SWW (32.6 mg/L and 13.4 mg/L, respectively) led to more blue-green microalgae proliferation compared to lower levels in FPE (3.83 mg/L and 1.19 mg/L respectively) [31]. Additionally, the growth of many organisms might be inhibited by the conductivity stress of the FPE. This might be due to the food processing treatment plant's reverse osmosis tank that released saline water into the effluent channel (Fig. S1), thereby, increasing the alkalinity (396 mg/L), chloride content (1966 mg/L), as well as conductivity (5.9 mS/cm) of the FPE (Table 1). The high silica concentration in the FPE was another factor that contributed to the proliferation of diatoms compared to other microalgae species, considering that the silica content in FPE was 7-fold higher ($p = 0.0001$) than that of the SWW. Other wastewater characteristics such as turbidity in FPE (115 NTU) influenced light penetration and also provided a conducive microenvironment for diatoms to thrive in. All these conditions could have hindered the proliferation of several organisms as conductivity and silica have been known to influence phytoplankton communities [32]. These aforementioned intrinsic characteristics of the culture media influenced the IMBC performance in terms of nutrient reduction, pigment production, EPS formation, oxygen evolution, and coliform bacteria removal rates.

### 3.3. Extracellular mucilage production influenced IMBC development

Microalgal colonies and filaments within granular consortia and biofilms are typically embedded in a matrix of exopolymers (EPS) [5]. Depending on the species, diatoms, green, blue-green microalgae, and bacteria secrete various compositions of extracellular substances made of complex carbohydrates and glycoproteins. These components of EPS promote the formation of a cohesive three-dimensional framework between the resident microorganisms. The EPS-polysaccharide (EPS-PS) content of IMBC in both wastewater media increased from the time of growth to detachment (2.70–3.69 mg/L in FPE and 2.40–3.67 mg/L in SWW) while EPS-protein (EPS-PN) content decreased with time in both

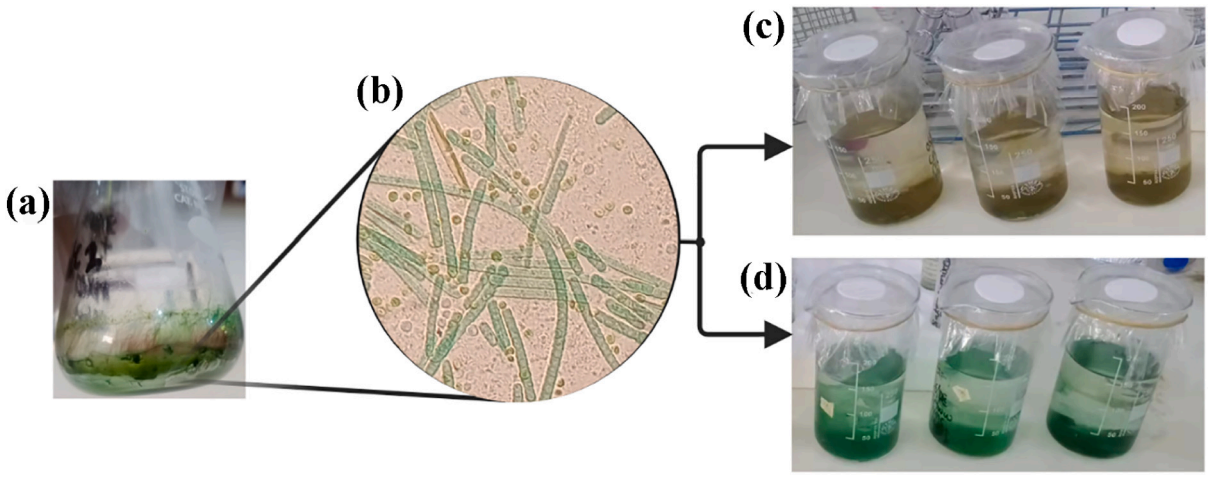


**Fig. 4.** 7-day-old IMBC in a flask (a), microscopic observation of IMBC (x400 magnification) (b), 26-day-old cultures of FPE showing brown biofilm (c) and SWW dominated by blue-green microalgae (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

conditions (26.25–2.06 mg/L in FPE and 14–0.7 mg/L in SWW) (Fig. 5 a & b). This corroborated the observation that EPS-PS are the primary components of biofilm EPS and they increased with the increase in biofilm age [33]. The inverse relationship between EPS-PS and EPS-PN production in both real and synthetic wastewater demonstrates the importance of EPS-PS in triggering biofilm formation. Previous studies also proposed that EPS-PS played an important role in the adhesion and physical strength of biofilms even when in lower concentrations compared with EPS-PN. Overall, EPS-PN and EPS-PS components have both been considered to be significant for the establishment and stabilization of the biofilm, especially through the contribution of filamentous blue-green microalgae [31]. During the attachment phase (Phase 2), the individual biofilm cells grew actively to attach to the substrate. The EPS-PS content in the young biofilm increased steadily over time up to the maturation stage (Phase 4). Between Phases 1 and 4, there was an observed 1.3-fold and 1.5-fold increase in EPS-PS content in FPE and SWW respectively ($p = 0.009$). However, EPS-PN content was reduced by 12.7-fold in FPE and 20-fold in SWW in Phase 4. EPS production has been linked to nutrient limitation in several studies. Therefore, nutrient limitation in FPE could have caused the microalgae-bacteria biofilm to accumulate more EPS-PN and EPS-PS than in SWW.

Moreover, the population dynamics of the two media might account for the differences in EPS as well. Diatoms are known to secrete mucilage for gliding purposes, in addition to bacteria EPS production which facilitated the biofilm development process. The mucilage is primarily composed of proteins and polysaccharides [34]. In response to the cohabitation of algae, bacteria excrete more EPS to keep their structure [5]. During the early growth stage (Phase 1), N removal was faster, compared to P removal. This corroborates with [35] who observed steady carbon and N removal during the adaptation stage (0–5 days). It was observed in their study that P removal was affected by the disintegration and re-aggregation of their IMBC. During the first growth phase, the adaptation of algae and bacteria to their environment, led to rapid consumption of N while P consumption was slow. Thus, the high diatom population in the FPE supports the faster $NH_4^+$ reduction and high EPS-PS content compared to the blue-green microalgae-dominated population of the SWW.

The strength and adhesive properties of the biofilm reduced with age, as seen in the reduction in EPS-PN content from growth Phase 1–4 (Fig. 5 a & b). The blue-green filament in SWW slowly detached from the flask after Phase 3 (Day 15 onwards) and eventually floated freely in the culture media at the end of the experiment. In FPE however, the majority of the blue-green filaments (*Leptolyngbya* sp. and *Phormidium* sp.) died off during Phase 2, leaving the diatoms (*Nitzchia palea*) and a few green unicellular cells (*Chlorella* sp.) settled at the bottom of the flasks within the sediments. These in combination with the associated wastewater bacteria, formed a brown microalgae-bacteria biofilm (Fig. 4). Similarly, the importance of diatom-associated bacteria has been demonstrated in the aggregation of diatoms, thus aiding in the formation of IMBC [36]. Moreover, the bacterial EPS matrix plays a major role in enhancing microalgal settleability. Thus, in the absence of bacteria, microalgal aggregates settled slowly [35]. This could also explain the floating of the blue-green microalgae-dominated biofilm in the SWW compared to the settled microalgae-bacteria biofilm in the FPE aside from the presence of gas vacuoles in blue-green microalgae filaments.

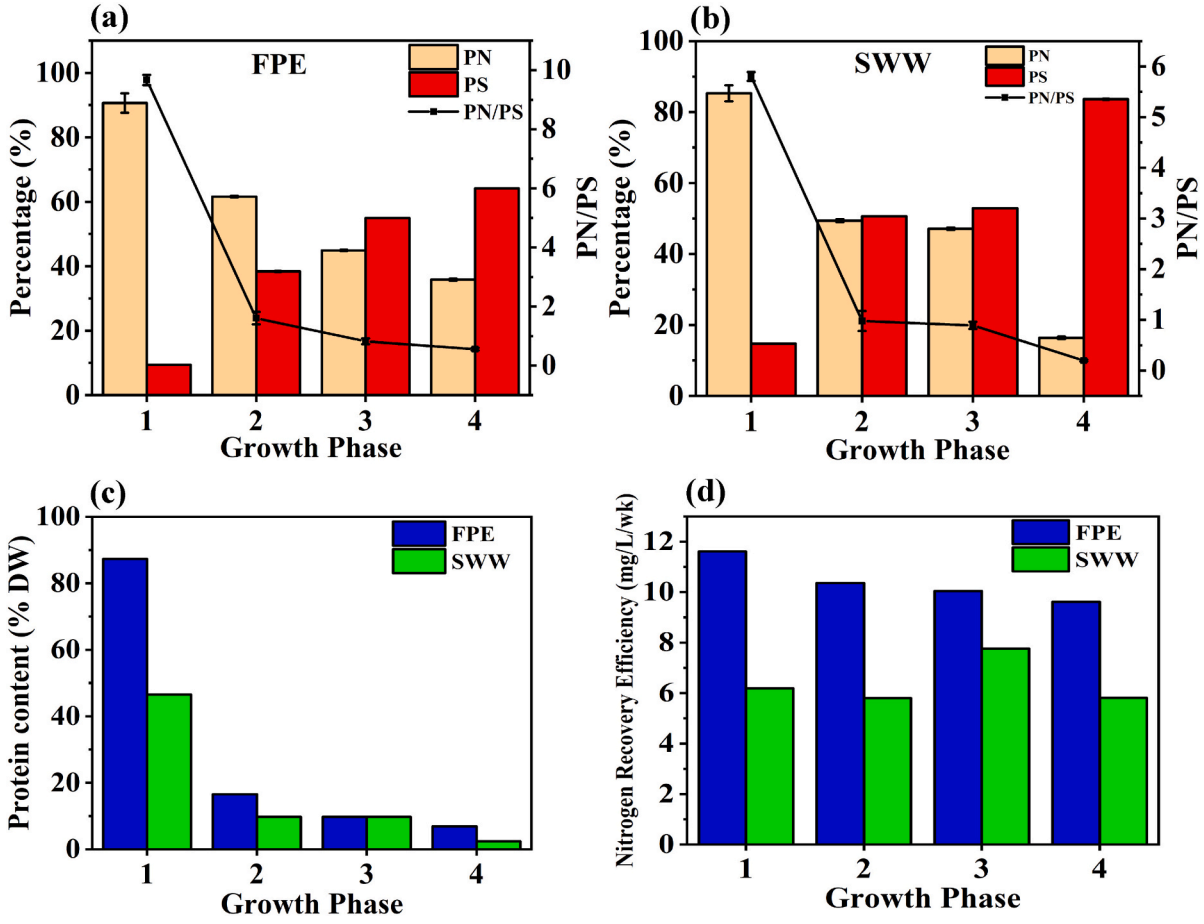


**Fig. 5.** Protein (PN) and Polysaccharide (PS) content of FPE (a) and SWW (b) at different growth cycles and Protein content (% DW) (c) and Nitrogen recovery efficiency (mg/L/wk) (d) of IMBC in FPE and SWW.

### 3.4. Resource recovery potential of IMBC and environmental significance of the study

The capacity of IMBC in nutrient removal from FPE supports the basis for the application of MBC in simultaneous effluent polishing and resource recovery. This is because FPE with low N and P levels but high COD and BOD still supported diatom growth while FPE with high N and P levels supported cyanobacteria proliferation. Thus, both scenarios could serve as a sustainable source of nutrients for IMBC species growth and evolution. The establishment of an IMBC would lead to better effluent polishing due to proper acclimatization of indigenous species which will enhance the performance of wastewater treatment facilities and allow them to meet discharge standards in wastewater treatment plants. Currently, the source of FPE employed in this study was designed to remove TSS, COD, and BOD with little emphasis on nutrient reduction due to the low nutrient levels in the effluents, although the nutrient levels are generally higher than the discharge standards (Table 1). The treatment facility has an annual treatment capacity of approximately 0.10 mg/L $NH_4^+$, −0.09 mg/L $PO_4^{3-}$, and 0.47 mg/L COD per year. Based on the findings in this study, the IMBC treatment capacity was 0.98 mg/L $NH_4^+$, 0.85 mg/L $PO_4^{3-}$, and 0.84 mg/L COD per month which resulted in 8.56 g/L biomass. This demonstrates the practicality and sustainability of employing IMBC in effluent polishing.

The intracellular N content of the IMBC cells was translated into nitrogen recovery potential based on the weight of the harvested biomass. It was found that the FPE cultures had higher N content ($p = 0.0007$) compared to SWW cultures. Generally, the highest N content and NRE were observed in growth Phase 1 in FPE and SWW cultures (87.3 % and 46.6 % N, respectively) (Fig. 5 c & d). The NRE of the IMBC in this study is a promising avenue for the biofertilizer industry which was valued at $1.6 billion in 2020 and is projected to grow to $3.98 billion by 2028 [37]. Additionally, The IMBC in this study can consume 413.16 mg/L silica per year and this has implications on the microalgae-bacteria community structure as well as the biogeochemical cycles of carbon and nutrients in saline environments such as highly saline industrial wastewater like the FPE. Besides, the protein content of the IMBC which is estimated at 3.2 g/L per month, it is also possible to achieve a carbohydrate content of 2.8 g/L per month, making the FPE IMBC a third-generation biomass for protein and carbohydrate production that can be applied as animal feed, especially in the aquaculture industry.

## 4. Conclusion

The study demonstrated the feasibility of cultivating IMBC in FPE and SWW. Interestingly, the nutrient content of the wastewater influenced the species evolution of the IMBC. More blue-green microalgae (*Leptolyngbya* sp. and *Phormidium* sp.) were observed in SWW with higher N and P levels while diatoms (*Nitzchia palea*) dominated in FPE with higher conductivity. Moreover, the IMBC reduced COD, $NH_4^+$, and $PO_4^{3-}$ significantly in both FPE and SWW. Additionally, IMBC had an advanced removal potential of TC bacteria due to the biofilm nature of the consortia. Aside from the effluent polishing effect of the IMBC, industrially important pigments such as phycocyanins can be extracted from the cultures through environmentally friendly methods while recovering nutrient-rich biomass for applications in biofertilizer and other useful avenues with high market value such as animal feed production.

## CRediT authorship contribution statement

**Ayesha Algade Amadu:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **Abdul-Wahab Abbew:** Data curation, Formal analysis, Writing – original draft, Investigation. **Shuang Qiu:** Funding acquisition, Investigation, Project administration, Writing – original draft. **Gloria Naa Dzama Addico:** Methodology, Writing – review & editing. **Isaac Hodgson:** Methodology, Writing – review & editing. **Samuel Duodu:** Writing – review & editing. **Serapis Asiedu Appiah:** Data curation, Writing – review & editing. **Shijian Ge:** Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2022.102913.

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