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SHORT RESEARCH AND DISCUSSION ARTICLE

Microalgal-bacterial treatment of ice-cream wastewater to remove organic waste and harvest oil-rich biomass

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Abstract
The diversity of microalgae and bacteria allows them to form beneficial consortia for efficient wastewater treatment and nutrient recovery. This study aimed to evaluate the feasibility of a new microalgal-bacterial combination in the treatment of ice cream wastewater for biomass harvest. The bacterium *Novosphingobium* sp. ICW1 was natively isolated from ice cream wastewater and the microalga *Vischeria* sp. WL1 was a terrestrial oil-producing strain of Eustigmatophyceae. The ice cream wastewater was diluted 4 folds for co-cultivation, which was relatively less inhibitory for the growth of *Vischeria* sp. WL1. Four initial algal–bacterial combinations (v:v) of 150:0 (single algal cultivation), 150:1, 150:2, and 150:4 were assessed. During 24 days of co-cultivation, algal pigmentation was dynamically changed, particularly at the algal–bacterial combination of 150:4. Algal growth (in terms of cell number) was slightly promoted during the late phase of co-cultivation at the combinations of 150:2 and 150:4, while in the former the cellular oil yield was obviously elevated. Treated by these algal–bacterial combinations, total carbon was reduced by 67.5 ~ 74.5% and chemical oxygen demand was reduced by 55.0 ~ 60.4%. Although single bacterial treatment was still effective for removing organic nutrients, the removal efficiency was obviously enhanced at the algal–bacterial combination of 150:4. In addition, the harvested oils contained 87.1 ~ 88.3% monounsaturated fatty acids. In general, this study enriches the biotechnological solutions for the sustainable treatment of organic matter-rich food wastewater.

Keywords Algal–bacterial consortium · Ice cream wastewater · Nutrient removal · Biomass harvest · Oil production

Introduction

Microalgae are photoautotrophic microorganisms with the high potential to produce industrially valuable metabolites such as proteins, oils, carbohydrates, and pigments, especially oil feedstocks for biodiesel production (Yasir Arafat Siddiki et al. 2022; Mittal and Ranade 2023). Microalgae can be grown in various types of wastewater to recover nutrients and degrade toxins, which serves as a promising alternative to conventional technologies such as those based on activated sludge (Acién Fernández et al. 2018; Mohsenpour et al. 2021; Abdelfattah et al. 2023). Microalgal cultivation with organic-rich wastewater offers a cost-effective approach for both removing organic nutrients and harvesting value-added biomass.

In recent years, microalgae-bacteria consortia have attracted great attention in wastewater treatment to harvest algal biomass (Muñoz and Guieysse 2006; Anand et al. 2023; Li et al. 2023). Theoretically, there exist at least three interaction relationships, including mutualism, commensalism, and parasitism (Ramanan et al. 2016; Lee and Lei 2019). A basal mutualistic relationship between microalgae and bacteria is the exchange of metabolites (González-González and De-Bashan 2021; Saravanan et al. 2021; Li et al. 2023). Bacteria utilize extracellular organic carbon and oxygen released by algal photosynthesis, while algae assimilate carbon dioxide, nutrients, vitamins, or phytohormones produced by bacteria. More complex mutualistic interactions include signal transduction and gene transfer (Kouzuma and Watanabe 2015; Saravanan et al. 2021), which should be

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universal in those tightly symbiotic consortia. Although some advantages have been well-known for the utilization of microalgae-bacteria consortia in wastewater treatment, several factors must be carefully considered. Firstly, the selection of a suitable microalgal strain is most important. Different algal strains may have different adaptability to the specific wastewater and show different capabilities in organic nutrient removal. Secondly, the section of a cooperative or beneficial bacterium for co-cultivation is also very important. Some bacteria can lyse the algal cell and use its intracellular compounds as nutrients (Wang et al. 2020). The aggregation of algal cells facilitated by bacterial extracellular polymeric substances may affect algal light utilization efficiency. Thirdly, the pollution character of wastewater is decisive in the selection of a suitable algal–bacterial combination. The organic matter is the major contaminant to be removed in whatever wastewater treatment process (Acién Fernández et al. 2018). Thus, the coordination of microalga, bacterium, and wastewater is required in the practice of wastewater treatment.

Thus far, dozens of microalgal species/strains, including *Scenedesmus*, *Chlorella*, *Botryococcus*, *Phormidium*, *Limnospira*, and *Chlamydomonas*, have been used in microalgae-bacteria consortia for wastewater treatment (Jiang et al. 2021; Abdelfattah et al. 2023; Li et al. 2023). Within them, *Chlorella vulgaris* is most widely used in the treatment of municipal, agricultural (Piggery), and domestic wastewater. The bacterial partners are more diverse, including *Rhodococcus*, *Bacillus*, *Gordonia,* and *Arthrobacter* (Das et al. 2021; Li et al. 2023). Usually, more than one bacterial strain exists in the wastewater. Selectively isolating the dominant strain for co-cultivation with a suitable microalga for wastewater treatment is necessary.

Ice cream is a popular food and consumed worldwide. It is prepared from drinking water, sugar, milk products, egg products, edible fats, and other additives (Yan et al. 2022). The wastewater from the ice cream industry is an important organic pollution source of municipal wastewater, and its sustainable management is a global challenge. Up to now, the application of microalgae-bacteria consortia in the treatment of ice cream wastewater has been rarely reported. In previous studies, we identified a terrestrial oil-producing microalga *Vischeria* sp. WL1 (Eustigmatophyceae), which exhibits remarkable capability to utilize proteic nitrogen resources and can tolerate mild salt stress (She et al. 2022; Zhu et al. 2023). It can be cultivated by diluted salt lake water (Gao et al. 2024). It might be also a good candidate for treating organic matter-rich ice cream wastewater and harvesting oil-rich biomass. In this study, we first attempted to isolate native bacteria in the wastewater sourced from an ice cream factory and identified two *Novosphingobium* strains. Then we assessed the effects of the coupled application of *Vischeria* sp. WL1 and one *Novosphingobium* strain in the treatment of ice cream wastewater. The changes in algal biomass and oil yield as well as the nutrient removal efficiency from the wastewater were analyzed. The present study will provide a sustainable solution for the treatment of ice cream wastewater with a novel microalgal-bacterial combination.

Materials and methods

Ice-cream wastewater, pretreatment, and nutritional analysis

The ice cream wastewater was sourced from a food factory in Xi'an City, China. The wastewater was first pretreated by filtration with eight-layer gauze to remove insoluble solids. Then the wastewater was suction-filtrated with 10 μm neutral filter paper in a Buchner funnel. Finally, the filtered wastewater was sterilized at 121 ℃ for 20 min and stored at 4 ℃ for use. The nutrient indicators of the ice cream wastewater were determined with the following methods: total carbon (TC), total organic carbon (TOC), and total inorganic carbon (TIC) were measured with a Shimadzu TOC-L CPN analyzer (Shimadzu, Japan); total nitrogen (TN) was measured using the alkaline potassium persulfate digestion-ultraviolet spectrophotometric method (Koistinen et al. 2020); Kjeldahl nitrogen (KN) was measured using the Kjeldahl method (Sáez-Plaza et al. 2013) and ammonia nitrogen (NH_3-N) was measured using the Nessler's reagent spectrophotometry (Demutskaya and Kalinichenko 2010); total phosphorus (TP) was measured by the inductively coupled plasma atomic emission spectrometry (ICP-AES) method (Wang et al. 2020); chemical oxygen demand (COD) was measured using the potassium dichromate method after digestion (Dedkov et al. 2000). The nutritional compositions in the wastewater before and after sterilization are shown in Table 1.

Table 1 The nutritional compositions in the ice cream wastewater before and after sterilization

Indices of analysis	Raw water (mg L^{-1})	After sterilization (121 ℃, 20 min) (mg L^{-1})
TC (total carbon)	762.7	685.0
TOC (total organic carbon)	735.8	642.7
TIC (total inorganic carbon)	26.95	42.37
TN (total nitrogen)	36	19.1
TP (total phosphorus)	3.23	3.25
KN (Kjeldahl nitrogen)	23.6	10.4
NH_3-N (ammonia nitrogen)	15.8	1.4
COD_{Cr} (chemical oxygen demand)	93.6	390

Microorganisms and cultivation conditions

Bacterial strains were isolated from the ice cream wastewater using the gradient dilution method and the streak plate method (Ogodo et al. 2022). The solid medium for bacterial isolation was prepared with the wastewater solidified with 1.2% agarose. Two obtained strains were subjected to molecular identification based on the 16S rRNA gene (Gao et al. 2011). The V_4–V_5 region was amplified using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 926R (5'-CCGTCAATTCMTTTGAGTTT-3′). Their 16S rRNA gene sequences were deposited into the NCBI database (Accession nos. OR342076 and OR342076). The growth of two strains (named *Novosphingobium* sp. ICW1 and ICW2) was evaluated in sterilized ice cream wastewater at 37 ℃ in a shaker (220 rpm). The optical density at 600 nm (OD_{600}) was measured after the vibration dispersion of bacterial cells.

The microalgal strain *Vischeria* sp. WL1 used in this study was previously isolated from a dryland biological soil crust (She et al. 2022). It belongs to the Eustigmatophyceae and shows the remarkable capability to accumulate oils (She et al. 2022; Zhu et al. 2023). The growth of *Vischeria* sp. WL1 in diluted ice cream wastewater was evaluated in a shaker (130 rpm) at 25 ℃ under constant LED illumination of 60 μmol photons m^{-2} s^{-1}. For comparison, the control medium was the nitrogen-free solution $BG11_0$ (Stanier et al. 1971) supplemented with 4.5 mM $NaNO_3$, which is a relatively optimal medium for biomass and oil production of *Vischeria* sp. WL1 (She et al. 2022). The optical density at 750 nm (OD_{750}) was measured. The chlorophyll fluorescence parameter Fv/Fm was measured using an AquaPen AP110 fluorometer (Czech Republic) as previously described (Yuan et al. 2021).

Co-cultivation design, phenotypic observation, and pigment measurement

For the co-cultivation experiment, the ice cream wastewater was diluted with $BG11_0$ solution in a ratio of 1:3 (v:v). $BG11_0$ is a basal medium for microalgal cultivation and can supplement necessary mineral nutrients for wastewater. *Vischeria* sp. WL1 was propagated in the nitrogen-rich solution BG11 (Stanier et al. 1971) and collected by centrifugation at 1776 × *g* for 5 min. Then algal cells (approximately 2 ml) were added into 150 mL of diluted wastewater in each triangular flask with a final OD_{750} of 0.2. *Novosphingobium* sp. ICW1 was propagated in the ice cream wastewater to reach OD_{600} of 0.2. Then different volumes of bacterial culture (0, 1, 2, and 4 mL) were added into the above 150 mL algal cultures for co-cultivation. Correspondingly, the algal–bacterial combinations were presented as 150:0, 150:1, 150:2, and 150:4 (v:v). The co-cultivation was conducted for 24 days in a shaker (130 rpm) at 25 ℃ under constant illumination of 60 μmol photons m^{-2} s^{-1}. The phenotypes of the microalgal-bacterial aggregate were observed by light microscope and scanning electron microscopy (SEM).

For pigment measurement, 1 mL of each culture was collected by centrifugation (6000 × *g*, 5 min) and suspended in 1 mL methanol. Then the suspension was crushed by steel beads (180 rpm, 10 min) in a high-throughput tissue homogenizer (Wonbio-L, Shanghai Wonbio Biotechnology Co., Ltd., China). The supernatant was measured at 665.2, 652.4, and 470 nm using a UV–Vis spectrophotometer. The chlorophyll a and carotenoid concentrations were calculated using the equations (Lichtenthaler and Buschmann 2001): (1) chlorophyll a (μg ml^{-1}) = $16.72 \times A_{665.2} - 9.16 \times A_{652.4}$; (2) carotenoid (μg ml^{-1}) = ($1000 \times A_{470} - 1.63 \times$ chlorophyll a)/221. The ratio of carotenoids to chlorophyll a content was calculated.

Biomass, lipid, and fatty acid composition determination

For biomass determination, 20 mL of each culture was centrifugated at 1776 × *g* for 5 min to remove the floating bacterial biofilms, and then the pellets were collected. The pellets (mainly consisting of algal cells) were freeze-dried and weighed. For oil extraction, the dried samples were crushed in a 2-mL centrifuge tube with the above high-throughput tissue homogenizer, and then extracted using the chloroform–methanol method (Folch et al. 1957). Briefly, the broken cells were transferred to a 10 mL tube, mixed with 3 ~ 4 mL chloroform–methanol (v:v = 2:1) mixture, and vortexed for 2 min. After centrifugation, the lower chloroform layer containing oils was collected and dried using a nitrogen blower. For calculating oil yield per cell, the algal cell number was scored in a manual hemocytometer. The fatty acid composition of oils was determined using a GC2010-Pro Gas Chromatograph (Shimadzu, Japan) as previously described (She et al. 2022).

Collection of treated wastewater for nutritional analysis

After 24 days of co-cultivation, *Vischeria* sp. WL1 and *Novosphingobium* sp. ICW1 in the 4 folds diluted ice cream wastewater were removed by centrifugation (1776 × *g*, 5 min) and filtering (0.45 μm membrane filter). Seven nutritional parameters (TC, TOC, TIC, TN, KN, TP, and COD) were measured as above-mentioned. For comparison, a single bacterial strain was cultivated in the non-diluted ice cream wastewater (initial OD_{600} = 0.046) in a shaker (220 rpm, 37 ℃) for 10 days, and then the treated wastewater was collected for nutritional analysis.

Statistical analysis

The experiments were conducted in three replicates and their results were expressed as the mean ± SD. The statistical analysis was performed using the one-way analysis of variance with Tukey's test at $p < 0.05$ (IBM SPSS Statistics 26).

Results and discussion

Bacterial isolation in the wastewater from an ice cream factory

The ice cream wastewater usually contains a high amount of organic matter such as protein and fat, which contributes to high biological oxygen demand (BOD) and COD levels (Konstantas et al. 2019; Enteshari and Martínez-Monteagudo 2020). The bacteria well adapted to the wastewater should have a high potential to decompose organic matter. We thus attempted to isolate bacterial strains from the wastewater discharged from a local ice cream factory. Based on the bacterial colony morphology and growth rate on solid plates, two kinds of strains were distinguished. According to the 16S rRNA gene sequencing, two types of strains were identified and named *Novosphingobium* sp. ICW1 and ICW2, respectively. Their phylogenetic tree analysis is shown in Supplementary Figure S1. During liquid shake cultivation, both strains exhibited the self-flocculation feature (Fig. 1A) but *Novosphingobium* sp. ICW1 showed relatively slower growth (Fig. 1B). Generally, the bacteria in the genus *Novosphingobium* are capable of degrading a wide range of xenobiotic aromatic compounds and can be applied in bioremediation (Krishnan et al. 2017; Wang et al. 2018). The suspension culture of *Novosphingobium* sp. ICW1 appeared more turbid, implying a relatively weaker self-flocculation ability. Thus, the strain ICW1 was chosen for use in the following co-cultivation experiments.

Evaluation of the growth of *Vischeria* sp. WL1 in ice cream wastewater

Vischeria sp. WL1, an oil-producing unicellular strain, was previously found to grow well with organic nitrogen sources (She et al. 2022). However, its potential in treating organic matter-rich wastewater remains to be assessed. The growth of *Novosphingobium* sp. WL1 in ice cream wastewater was evaluated (Fig. 2). In terms of OD_{750}, 4, 6, and 8 folds diluted wastewaters were relatively favorable for the cultivation of *Vischeria* sp. WL1, although the growth rates in them were less ideal than that in the control medium (Fig. 2A). The chlorophyll fluorescence parameter Fv/Fm is widely used as a sensitive indicator of algal photo-physiological performance (Zhao et al. 2008; Gao et al. 2023). In terms of Fv/Fm, the photo-physiological activity of *Vischeria* sp. WL1 was not or weakly inhibited in 4, 6, or 8 folds of diluted wastewater (Fig. 2B). In contrast, two folds of diluted ice cream wastewater were obviously inhibitory for the growth of *Vischeria* sp. WL1. Based on these results, we chose the 4 folds diluted ice cream wastewater for co-cultivation experiments.

Co-cultivation of *Novosphingobium* sp. ICW1 and *Vischeria* sp. WL1

Effects on algal pigmentation

Co-cultivation of *Vischeria* sp. WL1 and *Novosphingobium* sp. ICW1 was performed in the diluted ice cream wastewater. During the cultivation, microscopic and SEM observations showed that the bacterial colony surrounded algal cells but

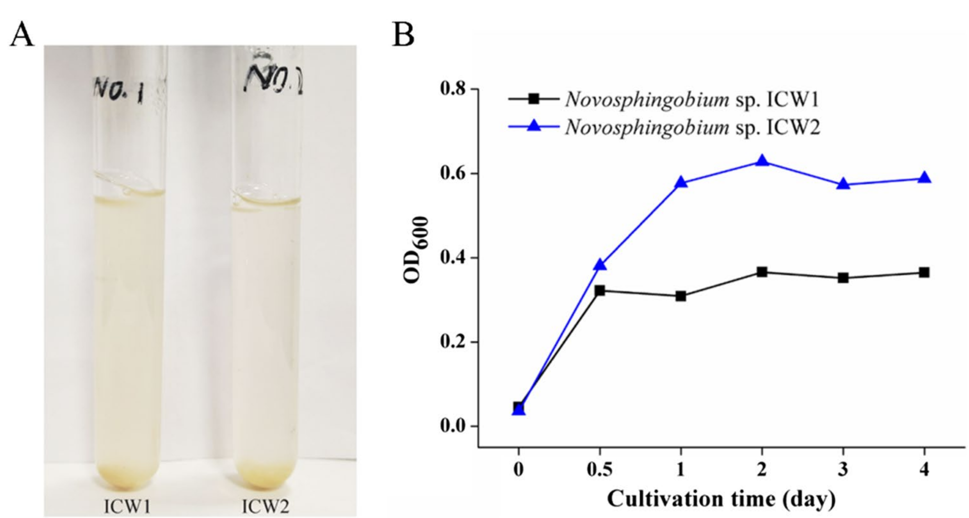

Fig. 1 The appearances in tubes (**A**) and growth rates (**B**) of *Novosphingobium* sp. ICW1 and ICW2. Two strains were shake-cultivated in sterilized ice cream wastewater

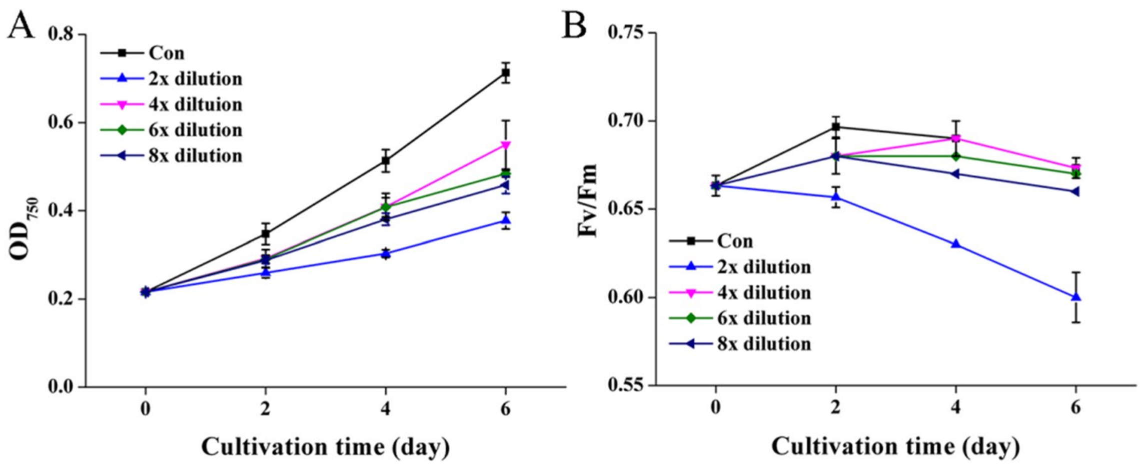

Fig. 2 The growth of *Vischeria* sp. WL1 in diluted ice cream wastewater. **A** Cell growth (in terms of OD_{750}). **B** The photo-physiological performance (in terms of Fv/Fm) of cells

some algal cells still remained dissociative (Supplementary Figure S2). Their physical contact may be attributed to the sticky extracellular polysaccharides (Matsuyama et al. 2003; Prasad and Purohit 2023). The effects of *Novosphingobium* sp. ICW1 on the pigmentation (chlorophyll a and carotenoid contents) of *Vischeria* sp. WL1 cultures were further investigated (Fig. 3). Different volumes of *Novosphingobium* sp. ICW1 (OD_{600} of 0.2) were added into algal cultures of 150 mL with initial algal–bacterial combinations (v:v) of 150:0 (as control), 150:1, 150:2, and 150:4, respectively. It was found that increased addition of the bacterium generally led to reduced chlorophyll contents but increased carotenoid contents (Fig. 3A, B). The carotenoids/chlorophyll a ratio was highest at the algal–bacterial combination of 150:4; and at this combination, the ratio peaked on day 12, earlier than other combinations (Fig. 3C). Carotenoids and chlorophyll a are essential pigments in photosynthesis and abiotic stresses usually induce the accumulation of carotenoids (Kato and Shinomura 2020). For example, higher light and nitrogen deficiency were accompanied by an increase in carotenoid content and a decline in chlorophyll content in *Parietochloris incisa* (Solovchenko et al. 2009). Thus, high concentrations of *Novosphingobium* sp. ICW1 may impose a stressful effect on *Vischeria* sp. WL1.

Effects on algal growth and oil production

Algal growth and oil production were further investigated during the algal–bacterial co-cultivation (Fig. 4). At the algal–bacterial combinations of 150:2 and 150:4, algal growth (in terms of cell number) seemed to be slightly promoted during the later phase of cultivation, as compared to the control group (150:0) (Fig. 4A). The combination of 150:2 achieved relatively higher cellular oil yield than the control after 12 days of cultivation; on day 24, the oil yield was 18.7% higher than the control (Fig. 4B). The biomass concentration on day 24 was slightly reduced (8.1%) at the combination of 150:4 (Fig. 4C), while the oil contents were not significantly different among these combinations (Fig. 4D). Nevertheless, the highest oil production was achieved at the combination of 150:2, being 1.67 g/L, which is higher than that (1.2 g L^{-1}) in our previous study using peptone as nitrogen source (She et al. 2022). This result suggests the existence of algal–bacterial synergy in promoting algal oil production.

Effects on fatty acid profiles of oils

The fatty acid profiles of the harvested oils were measured to assess their suitability for use in biodiesel or food areas (Fig. 5). Generally, palmitoleic acid (C16:1) and oleic acid (C18:1) accounted for the majority of total fatty acids in the oils. Palmitoleic acid shows numerous health benefits and also serves as an ideal biodiesel feedstock (Wu et al. 2012). On day 12, the palmitoleic acid content in the control was 53.5%, while the contents were 61.1%, 61.5%, and 56.6% in the algal–bacterial combinations of 150:1, 150:2, and 150:4, respectively. On day 24, the palmitoleic acid contents were around 68.2% on all four cultural conditions. Oleic acid is the principal monounsaturated fatty acid (MUFA) present in olive oil and nuts and is one of the better fats for humans to consume (Tutunchi et al. 2020). The oleic acid contents were not much different among the four conditions on day 12 (17.5 ~ 19.4%), while on day 24 the contents became more similar, being around 19.4%. Considering that the total MUFA content accounted for 66.2 ~ 77.6% on day 12 and 87.1 ~ 88.3% on day 24 of the total fatty acids (Supplementary Figure S3) as well as other unknown health risks from the bacterium, these oils seem more desirable for biodiesel fuel (Maltsev and Maltseva 2021).

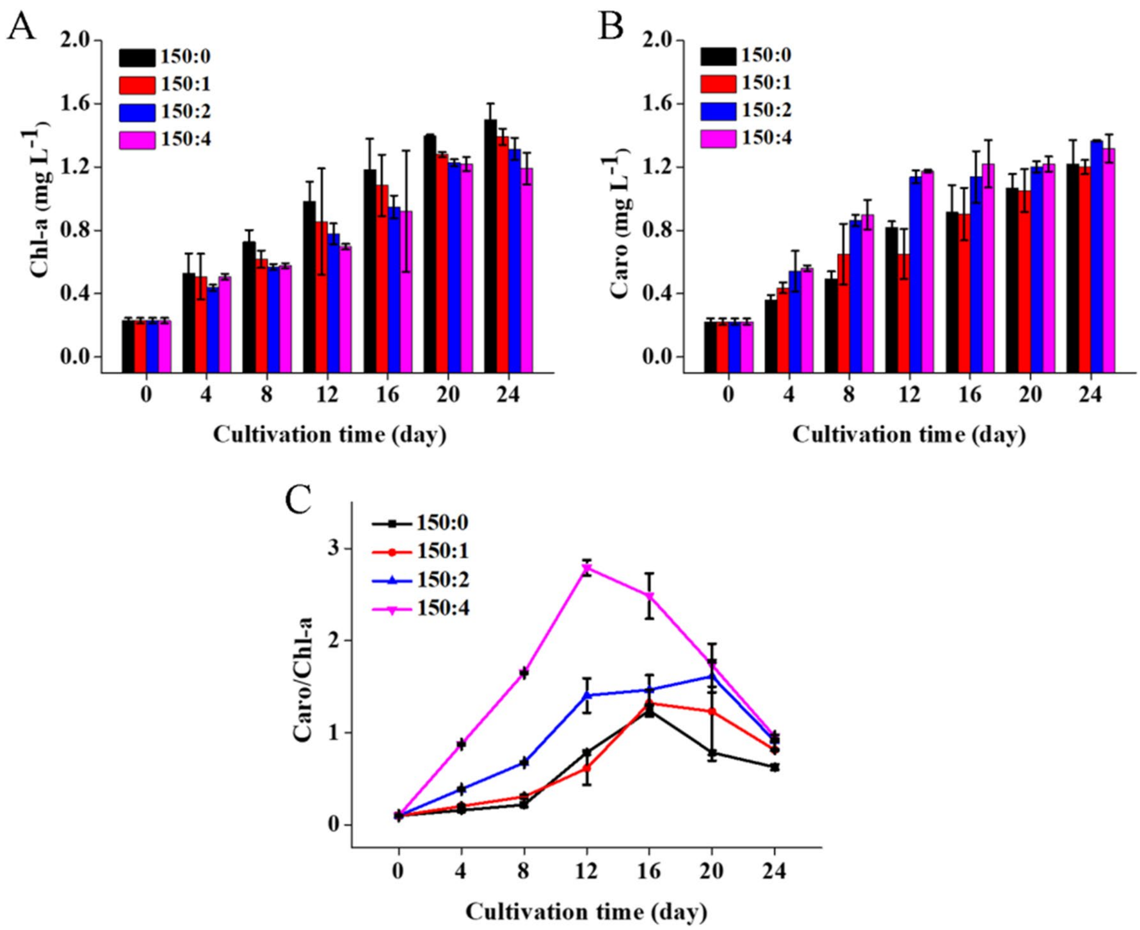

Fig. 3 The effects of *Novosphingobium* sp. ICW1 on chlorophyll a (**A**) and carotenoid (**B**) contents of *Vischeria* sp. WL1 cultures as well as the ratio of carotenoids to chlorophyll a (**C**) during the co-cultivation. Chl-a, chlorophyll a. Caro, carotenoids

Effectiveness of the co-cultivation on wastewater treatment

The nutritional changes of the ice cream wastewater after treatments were evaluated (Fig. 6). As shown in Fig. 1B, *Novosphingobium* sp. ICW1 grew well in raw ice cream wastewater but rapidly entered into the lag phase. Treated alone by *Novosphingobium* sp. ICW1 for 10 days, the nutrients of the wastewater were dramatically decreased (Fig. 6A). TC was reduced by 39.5%, TOC was reduced by 35.3%, TN was reduced by 67.7%, KN reduced by 49.3% and COD value was reduced by 78.4%. Therefore, *Novosphingobium* sp. ICW1 alone was effective in the bioremediation of ice cream wastewater. That should also be why this strain was dominant in the ice cream wastewater. When *Vischeria* sp. WL1 was applied for the treatment of the diluted ice cream wastewater, combined with *Novosphingobium* sp. ICW1 or not, TC was reduced by 67.5 ~ 74.5%, TOC reduced by 75.0 ~ 81.3%, TN reduced by 73.2 ~ 79.9%, KN reduced by 93.4 ~ 95.0% and COD value reduced by 55.0–60.4% (Fig. 6B). However, at the algal–bacterial combination of 150:4, the values of these parameters were relatively more reduced (Fig. 6B). In addition, TIC was reduced by 96.2% in the single bacterial treatment but was increased by 29.7 ~ 52.2% in the algal–bacterial treatments. The increase in TIC content may be related to algal growth-caused pH change (Liu et al. 2016). Together, these results demonstrate the greater potential of the coupled application of *Vischeria* sp. WL1 and *Novosphingobium* sp. ICW1 in organic nutrient removal.

The effective combination of microalgae and bacteria is crucial for treating food wastewater. The ice cream wastewater is rich in organic matter that is required to be removed before its discharge. Bacteria of the genus *Novosphingobium* are widely distributed in nature and have been isolated from diverse environments, e.g., toxic chemical-contaminated soils, plant rhizospheres, seawater, activated sludge, hot springs, and freshwater (Liu et al. 2021). In this study, we reported a new strain, *Novosphingobium* sp. ICW1, which was isolated from the ice cream wastewater and showed remarkable capability to remove organic nutrients. In addition, it was also the first time that we used a terrestrial strain of Eustigmatophyceae for nutrient assimilation from the ice cream wastewater to produce

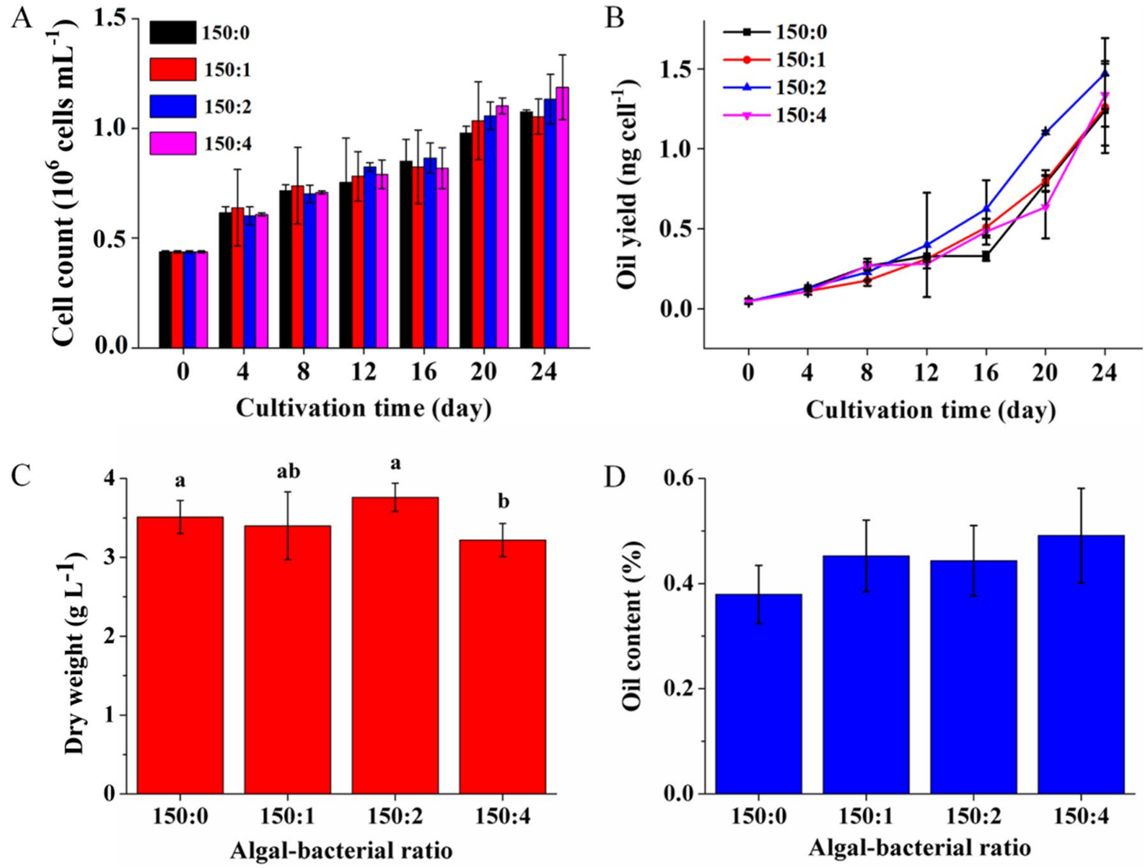

Fig. 4 The growth and oil production of *Vischeria* sp. WL1 during the co-cultivation. **A** Cell count. **B** Oil yield per cell. **C** Dry weight on day 24. **D** Oil content in biomass on day 24

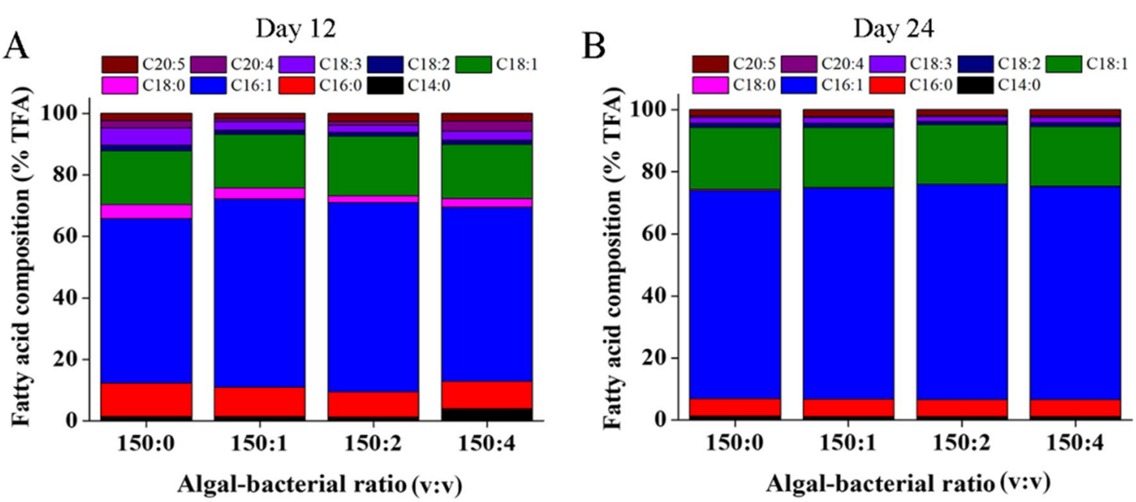

Fig. 5 The fatty acid profiles of the harvested oils after 12 (**A**) and 24 (**B**) days of co-cultivation

oils. Most importantly, the *Vischeria-Novosphingobium* consortia did not lead to reduced oil production in *Vischeria* sp. WL1 cells, although some interactive effects existed. Instead, at a proper concentration combination, algal oil production and nutrient removal efficiency were enhanced. Previous studies also reported the promotion of algal oil

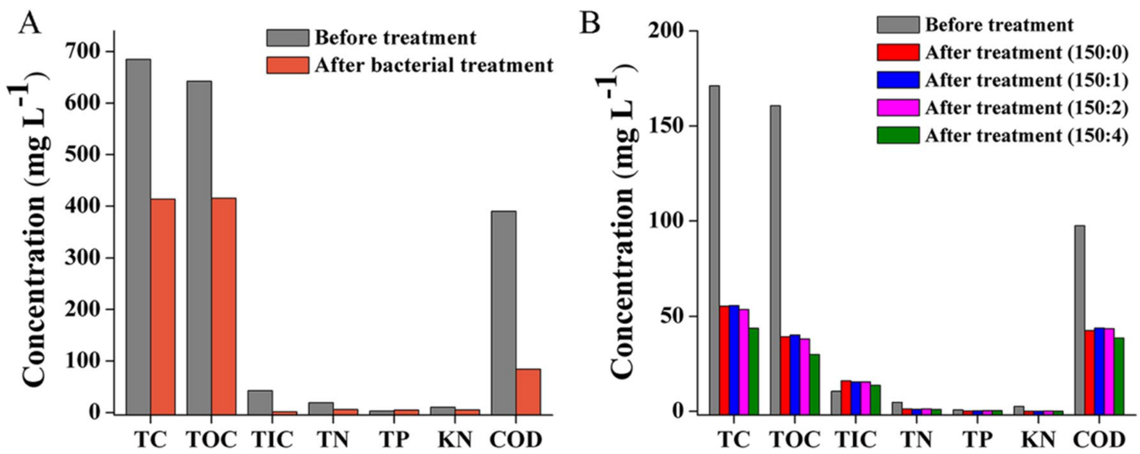

Fig. 6 The nutritional changes in the ice cream wastewater before and after treatments by single bacterial strain (**A**) and the algal–bacterial consortium (**B**)

production by bacteria, such as the siderophore-producing bacterium *Idiomarina loihiensis* RS14 on *Chlorella variabilis* and the nitrogen-fixing aerobic bacterium *Mesorhizobium sangaii* on *Chlorella vulgaris* (Rajapitamahuni et al. 2019). However, their beneficial interactions were limited to certain conditions, such as iron deficiency or nitrogen deficiency conditions. By comparison, our study presented a novel example of an algal–bacterial combination applied in treating ice cream wastewater to harvest oil-rich biomass. The concrete interaction mechanisms of *Vischeria* sp. WL1 and *Novosphingobium* sp. ICW1 remain unclear. Nevertheless, an inhibitory or competitive effect was implied in Fig. 3C and Fig. 4C, and a mutualistic relationship was implied in Fig. 4B and Fig. 6B. The former phenotype may be largely attributed to the formation of algal–bacterial aggregate, which will block the light needed for algal photosynthesis and growth. The latter phenotype may be related to the self-flocculation feature of the bacterium that will slow cell proliferation and thus weaken its potential adverse effect. Of course, it cannot exclude the basal mutualistic interactions that function between them.

Conclusions

Vischeria sp. WL1 and *Novosphingobium* sp. ICW1 were co-cultivated to treat ice cream wastewater. A synergic relationship for promoting algal growth and oil yield was observed for proper algal–bacterial concentration combination at the late growth phase. An algal–bacterial combination of 150:2 seemed most effective for oil production, while an algal–bacterial combination of 150:4 was more effective in removing nutrients in organic form. More than 50% of organic nutrients were removed by the algal–bacterial consortium on the tested conditions. The harvested oils, which contained more than 80% of MUFA, are desirable for use in biodiesel fuel. This study enriches the biotechnological solutions for the sustainable treatment of organic matter-rich food wastewater. It also suggests the feasibility of diverse combinations of microalgae and bacteria in wastewater treatment. However, it is worth noting that in this study *Vischeria* sp. WL1 cannot well adapt to the non-diluted ice cream wastewater and also the co-cultivation experiment was conducted at the laboratory level. In the next step, an enlarged co-cultivation experiment and further domestication of this microalga are very important for practical application.

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Authors' contributions Conceptualization: XG, DZ; methodology: XJ, CL, MG; investigation: XJ, CL; writing—original draft: XG, CL, XJ; writing—review and editing: MG, KL, DZ; supervision and funding acquisition: XG, DZ. All authors read and approved the final manuscript.

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Data availability The authors declare that the data supporting the findings of this study are available within the main article and its supplementary files.

Declarations

Ethics approval Not applicable.

Consent to participate All authors consented freely to participate in the study.

Consent for publication All authors consent for this work to be published.

Conflict of interest The authors declare that they have no conflict of interest.

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