



Algal Lysis by *Sagittula stellata* for the Production of Intracellular Valuables

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Abstract

The purpose of this study was to examine the efficacy of the algicidal bacterium *Sagittula stellata* on the cell lysis of *Nannochloropsis oceanica*, a microalga found in the marine environment, in order to extract intracellular valuables. Algicidal bacteria are capable of lysing algal cell walls while keeping lipids and proteins intact yet separated. We obtained these microbes from locations with consistent algae blooms and found that the bacterium *Sagittula stellata* displayed significant algicidal properties toward *Nannochloropsis oceanica*, achieving an algicidal rate of 80.1%. We detected a decrease of 66.2% in in vivo fluorescence intensity in algae cultures, obtained a recoverable crude lipid content of 23.3% and a polyunsaturated fatty acid (PUFA) ratio of 29.0% of bacteria-treated algae, and observed the lysis of the cell membrane and the structure of the nucleus of algae. We also identified the inhibited transcription of the ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (*rbcS*) gene and proliferating cell nuclear antigen (PCNA)-related genes and the upregulated heat shock protein (*hsp*) gene in algal cells during bacterial exposure. Our results indicate that *Sagittula stellata* effectively lysed microalgae cells, allowing the recovery of intracellular valuables. The algicidal method of *Sagittula stellata* on *Nannochloropsis oceanica* cells was confirmed to be a direct attack (or predation), followed by an indirect attack through the secretion of extracellular algicidal compounds. This study provides an important framework for the broad application of algicidal microorganisms in algal cell disruption and the production of intracellular valuables.

Keywords Microalga · Algicidal bacteria · Cell lysis · Intracellular valuables · *Sagittula stellata* · *Nannochloropsis oceanica*

Introduction

Researchers have recently cataloged several species of bacteria capable of lysing algae from locations known to have consistent algae blooms [1–3]. These algicidal bacteria target particular kinds of phytoplankton and slow the growth of harmful algae in various aquatic and marine ecosystems. These bacteria contribute to the regulation of harmful algae growth [2, 4, 5]. Research interest in algicidal bacteria has increased in recent decades due to their wide distribution and availability [6–8]. Stewart and Brown [9] first identified a unicellular, predatory, and Gram-negative microorganism, *Cytophaga* sp. N-5, that predated a wide array of organisms. *Alcaligenes denitrificans* is a gliding freshwater organism, inducing cell lysis or

death in particular species of cyanobacteria [1]. Zhang et al. [10] identified a bacterial strain CH-22 from Chaohu Lake, China, which displays significant phytoplankton-lytic ability against bloom-forming cyanobacteria, reducing the number of cells and the concentration of chlorophyll a. The bacterial algicide IRI-160AA induced mortality in dinoflagellates but not in other species of algae, suggesting that this class of phytoplankton is vulnerable to algicide [11]. Similarly, the potent algicide *Streptomyces* U3, isolated from Zhangjiangkou Mangrove National Nature Reserve, absorbs nutrients from algal cultures to support its growth and produces algicidal compounds to remove harmful algae [12]. However, there is a lack of knowledge on algicidal microorganisms, how they affect algal blooms, and how they extract nutrients from algae.

Several strains of algae can both alleviate environmental concerns and satisfy energy demand. Algae has the potential to generate significant quantities of renewable biofuels due to their rapid rate of growth and their ability to produce significant amounts of lipids (for biodiesel) and carbohydrates (for bioethanol) [13, 14]. However, the primary challenge of using algae for biofuels remains the high cost of algal biomass production and processing. For example, algae cell walls resist disintegration, presenting challenges for the scaling and cost-effectiveness of lipid extraction and the industrial use of algal cells [15, 16].

Currently, several approaches have been tested to disrupt algal cells (Table 1). These methods are generally categorized as either non-mechanical or mechanical. Some examples of non-mechanical cell disruption are chemical (e.g., using detergents, solvents, or antibiotics), physical (e.g., electric or osmotic shock, pressure, or supercritical CO₂), and enzymatic treatments (e.g., lytic and autolysis). While cell disruption is convenient for additional extraction, the algal lysis method also presents several challenges [17]. For example, chemical additions are potentially toxic toward humans and are environmentally unfriendly. Enzymatic degradation is expensive and less effective. Physical treatments are more affordable, but most of these methods are either ineffective for treating tiny algae cells or are difficult to scale. Mechanical methods include bead/grind milling, high-shear mechanical processing, microwave, high-pressure homogenization, and ultrasonication [18, 19]. Unfortunately, these methods are only effective at small scales or are not energy efficient [20]. The optimal solution is to identify a cell disruption method that not only induces high extraction yields and requires little energy, but also maintains comparable lysis efficiency (even for microalgae with rigid cell walls).

The algicidal method is a viable biological method for microalgal cell disruption (Table 1) for the following reasons: (1) Alga lysis by algicidal microorganisms which secrete compounds responsible for lysing or direct attack in a strain- and cell wall-specific way avoids the difficulty of selecting compounds to add, and increases cell disruption efficiency. (2) Algicidal microbes can feed on alga to maintain their population growth, while the proliferated microbes consume more and more alga. This not only saves the cost of cultivating additional nutrients but could be a sustainable, environmentally friendly, and easy method for managing large-scale operations [25].

In this study, *Sagittula stellata* was chosen to lyse algal cells. *S. stellata* has previously been studied with respect to environmental concerns or biosynthesis. Boden et al. [26] determined that dimethylsulfide could be oxidized to dimethylsulfoxide using *S. stellata*. Nanomolar concentrations (20 $\mu\text{g L}^{-1}$) of hydrophobic exopolymers given off by *S. stellata* could stimulate dissolved organic matter and the formation of microgels in seawater [27]. Additionally, *S. stellata* hydrolyzed cellulose and solubilized lignin in the runoffs from pulp mills [28]. *S. stellata* can use several disaccharides and monosaccharides to generate fatty acids. *S. stellata* was able to disrupt the cell wall of *Nannochloropsis oculata* UTEX 2164 and

Table 1 Pros and cons of different algal lysis methods from recent studies

Methods	Advantages	Limitations	References
Ultrasonication	Disrupts algal species, high yield of extraction	Energy intensive, ineffective on concentrated algae	[21]
High-pressure homogenization	Positive disruption, high yield of extraction	High energy cost, significant cost of scaling	[22]
Bead milling	Significant cell disruption, effective at disrupting cells on a large scale	Bead-dependent disruption rate, significant cost of scaling	[23]
Physical methods	No contamination, easily used	Significant cost prevents scaling	[18]
Chemical methods	Inexpensive materials, reproducible	Materials leave traces, toxic, high cost	[24]
Enzymatic methods	Easily combined with other approaches for fast and easy extraction	High cost	[19]
Algicidal methods	Safe, easily scaled, cheap, sustainable	Bacteria levels difficult to control, slow	[25]

facilitate cell lipid recovery [29]. However, the mechanisms behind how *S. stellata* extracts nutrients from algae are still unknown.

The objective of this study was to identify a biological method capable of effectively lysing the microalga *Nannochloropsis oceanica* to extract its intracellular components. In this study, the algicidal mode of *S. stellata* was identified; the algal crude lipid, protein, and sugar contents, and fatty acid profile were determined; and the bacterial toxicity on algal *rbcS*, *hsp*, and PCNA gene expression was studied. Furthermore, we established the theoretical framework and mechanisms behind *S. stellata* lysing algae for the release of valuable intracellular compounds, and proposed potential methods for addressing the negatives of bacteria-based algal cell disruption for further biorefineries. This study provides information regarding innovative microalgae cell breakdown techniques that show promise as environmentally sustainable tools during algal production.

Materials and Methods

Algae Sample Preparation

Nannochloropsis oceanica samples were obtained from the Culture Collection of Algae at the University of Texas at Austin (Austin, TX). *N. oceanica* was cultivated in a seawater mixture [21]. This strain was selected because it has previously been researched and produces bioproducts such as lipids, proteins, sugars, fatty acids, and amino acids [20]. Cultures were performed in 1-l bubble column photobioreactors with aeration (0.2–0.4 L/min) and CO₂ supplements (5–8 mL/min) at (25 ± 1) °C. Fluorescent lamps provided the light (60–70 μmol photons m⁻² s⁻¹) with 12:12 dark/light cycles.

Bacteria Inoculum Preparation

The bacterium *Sagittula stellata* (ATCC 70007 3) was obtained from the lab of Dr. Mary Moran at the University of Georgia and was subsequently grown in a Marine Broth 2216 medium [28]. The bacterium was grown for 24 h in 250-mL Erlenmeyer flasks at 30 ± 2 °C,

which included a 150-mL growth media on a shaker at 150 rpm in dark conditions. This mixture was then used in subsequent experiments.

Interactions Between *S. stellata* and *N. oceanica*

We used the centrifugation method (2020g for 5 min) to extract algal and bacterial cells in their mid-exponential growth phases. We then washed the extracted pellets twice with a saltwater solution (19.45 g/L NaCl). The bacterial supernatant (BS) was collected and purified using 0.22- μm polycarbonate filters, resulting in a cell-free filtrate. We prepared the modified Marine Broth 2216 medium (MMBM) and removed extracts of yeast or peptone in the control Marine Broth 2216 medium. Other compounds were kept the same. Removing extracts of peptone and yeast deprived the bacteria of nitrogen and carbon sources and forced the bacteria to feed on algae. Bacteria feed directly on algae when no nitrogen and carbon sources are available, which could enhance cell lysis efficiency. The primary difference between medium BS and MMBM is whether the bacteria consume extracellular substances.

The incubation methods of algae and bacteria are summarized in Table 2: (1) algal cell pellets were incubated into MMBM (treatment #1), the first control, to determine if the added nutrients had any effect on the algae; (2) bacterial cell pellets were incubated into MMBM (treatment #2) to determine bacterial growth in a modified medium; (3) algal and bacterial cell pellets were incubated together into MMBM (treatment #3) to determine if algicidal activity is from the bacterial cells; (4) algal cell pellets were incubated into BS (treatment #4) to determine if the algicidal activity was due to extracellular substances; (5) bacterial cell pellets were incubated into BS (treatment #5), the second control, to determine bacterial growth in its own medium; (6) algal and bacterial cell pellets were incubated together into BS (treatment #6) to determine general algicide activity. In all treatments, the incubation ratio (in volume) of algal and bacterial cell pellets was 50:1; the ratio (in volume) of cell pellets with incubation medium was 1:10. These ratios were set up based on the preliminary experiments and our previous study [29]. It is because under these ratios, the bacterial cells displayed the best cell lysis effect on algae. All treatments were arranged in randomized blocks and performed in triplicate, then placed on a shaker at 150 rpm in the dark at 30 ± 2 °C. They were incubated for 6 days. A sample from each treatment was freeze-dried and stored in a sealed container inside a desiccator for further analysis.

Analysis

Algal and Bacterial Cell Number

We counted the number of algal cells each day using bright-field Epifluorescence microscopy (Nikon Eclipse microscope, Model E600) via the direct counting method and a hemocytometer. The rate of algicide was calculated according to the following:

$$\text{Algicidal rate} = \frac{N_c - N_e}{N_c} \times 100\% \quad (1)$$

where N_c is the number of algae cells in the control group (with no additional bacteria) and N_e is the number of algae cells in the variable group (with additional BS or bacteria).

The number of bacterial cells was counted via colony-forming units (CFU) and the working dilution of each treatment was added and spread over an agar plate in triplicate. These were then stored overnight at 30 °C, after which the number of CFUs was counted and calculated.

Table 2 Experimental design

Treatment #	1	2	3	4	5	6
Algal cell pellets	+		+	+		+
Bacterial cell pellets		+	+		+	+
MMBM	+	+	+			
BS				+	+	+

Lipid and Fatty Acid Profile

Hexane was used to extract the lipids in the sample, which were then determined gravimetrically. The dried algae were treated with hexane at a ratio of 1:30 (g/mL), with constant exposure to magnetism at 60 °C and 500 rpm for 10 h. The mix was then centrifuged for 10 min at 4500g. This removed the algal biomass, after which the supernatant was evaporated using N₂ and the remaining lipids were obtained and weighed. The lipids were then stored at −70 °C for subsequent experiments.

Following a direct transesterification, gas chromatography (GC) was used to examine the composition of the fatty acids of the extracted lipids. The procedure was described in a previous study [20]. A 7820A gas chromatographer (Agilent, USA) using a flame ionization detector, an automated injector, and a DB-23 column (60 m × 0.25 mm × 0.25 μm, Agilent J&W, USA) was used to analyze the fatty acid methyl esters (FAMES). The carrier gas was nitrogen.

Fluorescence Intensity

A Synergy Mx monochromator-based multi-mode microplate reader (Synergy Mx, Winooski, VT) was used to measure the chlorophyll a fluorescence intensity of each sample with an emission wavelength of 680 nm and an excitation wavelength of 450 nm. The algal fluorescence intensity of each treatment was calculated by deduction of the corresponding background ($CAFD_{\text{Treatment \#1}} - CAFD_{\text{MMBM}}$, $CAFD_{\text{Treatment \#3}} - CAFD_{\text{Treatment \#2}}$, $CAFD_{\text{Treatment \#4}} - CAFD_{\text{BS}}$, $CAFD_{\text{Treatment \#6}} - CAFD_{\text{Treatment \#5}}$).

Protein and Sugar

We obtained the crude protein yield using Kjeldahl nitrogen with freeze-dried algal biomass according to established methods [20]. The amount of soluble protein was measured using a Bradford assay, and spectrophotometry (specifically, the phenol-sulfuric method) was used to measure total sugars using glucose as a standard. The 3,5-dinitrosalicylic acid (DNS) method was used to find the reducing sugar's yield.

Extraction of RNA, Reverse Transcription, and Analysis in Real-time

Given the cell size difference between *S. stellata* (0.9 μm in width and 1.4 μm in length) and *N. oceanica* (3–8 μm, round-shaped), the algal cells were collected using a 1.5-μm nylon membrane filter following treatment. The RNA of the algal samples was extracted using an RNAiso kit (Tiangen, Shanghai, China) according to the manufacturer's instructions. Reverse transcription of total RNA and real-time PCR was conducted according to the manufacturer's

instructions. We quantified the relative expression of genes in groups treated using the $2^{-\Delta\Delta Ct}$ method [30].

Morphological Observation

The algal cells under different treatments were separated as described in the “Interactions Between *S. stellata* and *N. oceanica*” section and examined via transmission electron microscopy (TEM). The samples were prepared according to the methods described by Furusawa et al. [31]. The samples were then examined under a TEM (JEOL JEM-2011, Tokyo, Japan).

Table 3 summarizes the analytical methods including the extraction, the standard, and the instrument information.

Each experiment was performed three times for each treatment. The listed results are the mean \pm standard deviation (SD). The resulting data were analyzed with ANOVA via SPSS version 12.0 software (SPSS Inc., Chicago, IL, USA). The Waller-Duncan test was used to determine the differences between means, with $p < 0.05$, and other extent p values of 0.01, 0.001, and 0.0001 can provide a more understanding of the data, but there was no statistical difference between some specific treatments under p value of less than 0.01; thus, $p < 0.05$ was applied.

Results and Discussion

Lipids and Lipid Profiling

The lipid yield of *N. oceanica* was significantly higher under each kind of treatment when compared to the control (treatments #2 and #5 contained no algal cells), indicating that *S. stellata* treatment effectively disrupted algal cells and recovered intracellular lipids (Fig. 1A). Treatment #6 achieved the highest lipid yield, reaching 23.3% (about 62.1% of the total neutral lipid content of *N. oceanica*) following treatment by both *S. stellata* and its supernatant. Treatments #3 and 4 were also effective in increasing lipid yield, indicating that

Table 3 Summary of analytical methods used in this study

Analysis parameter	Extraction method	Standard	Instrument (model and producer)
Fatty acid profile	Direct transesterification	Heptadecanoic acid	7820 A gas chromatography (Agilent, USA)
Chlorophyll a fluorescence intensity	N/A	N/A	A Synergy Mx monochromator-based multi-mode microplate reader (Synergy Mx, Winooski, VT)
Crude protein	Kjeldahl nitrogen	N/A	N/A
Soluble protein	Bradford assay	N/A	N/A
Total sugar	Phenol-sulfuric method	Glucose	N/A
Reducing sugar	3,5-Dinitrosalicylic acid (DNS) method	N/A	N/A
RNA extraction	RNAiso kit	N/A	Tianguen, Shanghai, China
Morphological observation	N/A	N/A	TEM (JEOL JEM-2011, Tokyo, Japan)

N/A, not available or not applicable

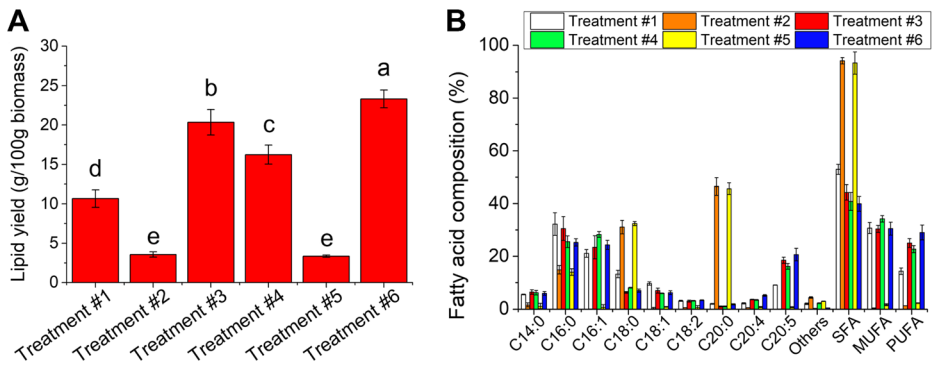


Fig. 1 Recoverable crude lipid yield (A) and compositions of fatty acids (B) from *N. oceanica* in different experimental groups. A statistically significant difference is indicated by different letters above the bar. Treatment #1: algae only; treatment #2: bacteria only; treatment #3: algae+bacteria; treatment #4: algae+BS; treatment #5: bacteria+BS; treatment #6: algae+bacteria+BS

both *S. stellata* cells and its cultivation medium played important roles in lysing algal cells, and that combining the two was most effective. This suggested that the lytic activity of *S. stellata* could involve both direct and indirect interactions with target algal cells. However, the particular makeup of microalgae and its cell wall characteristics vary between species. Volkman et al. [32] determined the biochemical composition of different strains of microalgae and found that carbohydrate contents varied from 5.2% (*N. salina*) to 8.9% (*N. oculata*), protein contents varied from 17.8 to 22.1%, and lipid content varied from 8.2 to 16.9% of the dry weight of the cell. The polyunsaturated fatty acids (PUFAs) displayed significant ranges among species and classes of algae. The greatest levels of eicosapentaenoic acid (EPA) were in *Phaeodactylum tricoratum* (28.4 mg g⁻¹). Docosahexaenoic acid (DHA) was found in high levels in *Pavlova* sp. (13.2 mg g⁻¹) and *Isochrysis galbana* (15.8 mg g⁻¹) [33]. Therefore, the effectiveness of *S. stellata* on *N. oceanica* does not necessarily imply that results would be similar with other algal species. Further study is required to determine viable applications.

Figure 1B displays the compositions of fatty acids after various treatments. Compared to the control (treatment #1), the proportion of algal PUFAs after applying treatments #3, 4, and 6 increased, and saturated fatty acids (SFAs) decreased. This demonstrates that *S. stellata* was able to disrupt the membrane and cell wall of microalgae, because the distribution of PUFAs with 20 carbons (arachidonic acid (ARA), 20:4 ω -6 and EPA, 20:5 ω -3) is much higher in membrane lipids [34]. The increased ratio of PUFAs could indicate more severe cell wall disruption or de-clumping of *N. oceanica* after incubation with *S. stellata*. ARA and EPA serve important roles in the human diet. They are membrane components in the neural system, and their role as hormone precursors is believed to play an important role in the immune system [20]. In this study, we obtained the maximum percentage of PUFAs (29.0%) after treatment #6, indicating that the combination of the filtrate of *S. stellata* and the washed mycelial pellets effectively disrupted *N. oceanica* cell walls and resulted in the isolation of related intracellular lipids. However, the filtrate and cells of *S. stellata* did play minor roles in algal cell lysis, compared with the effects of their combined application. The killing mechanism is likely to be direct cell-to-cell physical contact as well as exuding lysing compounds into the surrounding media without physically coming into contact with the target. Additionally, *S. stellata* could also accumulate some lipids (treatments #2 and 5 in Fig. 1A based on 100-g biomass). In this study, the ratios of monounsaturated fatty acids (MUFAs) and PUFAs

in treatments #2 and 5 (containing *S. stellata* cells, no algal cells) were much lower than those in other treatments (Fig. 1B), indicating that the cell wall or membrane of *S. stellata* in the medium was intact and not degradable. Moreover, the incubation ratio of algal to bacterial cells was low, mitigating the effect of *S. stellata* on algal lipid yield. However, if *S. stellata* could accumulate lipids in cells and grow by feeding on algal cells, it could have useful applications in other fields, such as the fermentation industry.

Algal Chlorophyll a Fluorescence Intensity and Cell Number

When a cell is disrupted into smaller fragments containing chlorophyll a or when its cell wall is ruptured though the cell but remains physically intact, it typically emits stronger fluorescence than a similar intact cell. Furthermore, when a cell containing chlorophyll dies, cell synthesis is rapidly inhibited and the chlorophyll could degrade. For this reason, CAFD is an important metric measuring the viability of the cell or cell lysis [21]. CAFD increased on the first day and then decreased significantly under the bacterial treatments when compared to the control treatment (treatment #1), as shown in Fig. 2a. After 6 days of cultivation, we observed a fluorescence signal that was roughly 70.4% lower than the control (Fig. 2a). Generally, the cell culture is destroyed when the intensity of relative fluorescence is more than 10% below the control [4]. Low levels of chlorophyll a fluorescence intensity demonstrate that the breakdown of DNA or the partial lysis of cells has caused cells to lose more of their nucleic acids [35]. This suggests that *S. stellata* treatments were effective in changing the algal cell structure.

The treatment of algal cells using *S. stellata* is shown in Fig. 2b. The number of cells relating to algal strain under treatments #3 and 4 did not significantly decrease during the initial cultivation compared to the control. The change in the algal cell structure occurred prior to whole-cell disruption. Alternatively, during the first incubation, the lysis effect from bacteria was not strong enough to degrade all of the algal cells, and some algal cells still proliferated. Afterward, the algal cell numbers in the groups with additional bacteria or bacterial supernatant addition quickly decreased compared to the control treatment, particularly after 3 or 4 days. The combination of *S. stellata* cells with its cultivation supernatant was most effective when lysing *N. oceanica* due to the degradation of CAFD and a decrease in the algal cell number.

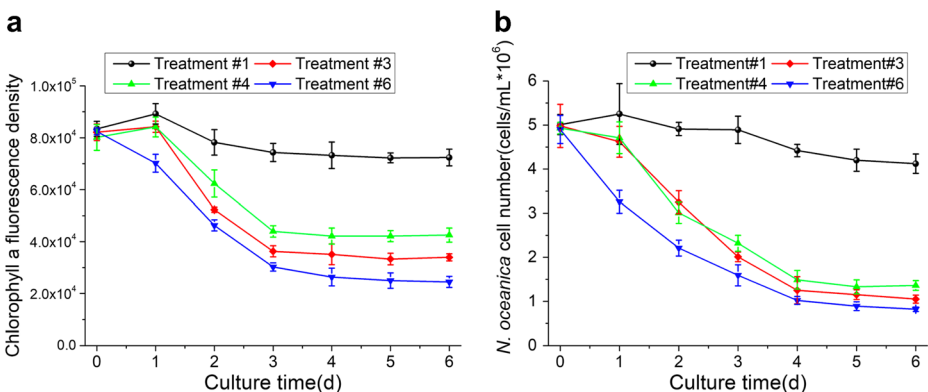


Fig. 2 Dynamics of chlorophyll a fluorescence intensity (a) and cell number (b) of *N. oceanica* in cultures with different treatments. Treatment #1: algae only; treatment #3: algae+bacteria; treatment #4: algae+BS; treatment #6: algae+bacteria+BS

Protein and Sugar

Significant increases in yields of protein and sugar from *N. oceanica* were observed (Fig. 3) under different applications of *S. stellata*, compared to the control. This further confirmed that either the bacterial cells or the suspension could effectively degrade the cell walls, destroy the protein/sugar structure of *N. oceanica*, and increase the cell protein/sugar yield. This indicates that bacteria-based algal cell lysis could be a promising technique for the extraction of intracellular parts. Bacteria treatment affected the solubility of proteins differently from the way it affected the protein content. Only a moderate increase in the solubility of protein was observed (Fig. 3A). This indicates that until sampling, bacterial treatments had not yet denatured algal proteins. Conversely, the yield of reducing sugars increased after each bacteria treatment (Fig. 3B). This can be explained by the fact that converting polysaccharides into reducing sugars, including hemicellulose, pectin, and cellulose into galactose, fructose, or glucose, was promoted by the presence of bacteria and its suspension. Moreover, polysaccharides are the primary components of the rigid cell wall in algal species like *Nitzschia*, *Phaeodactylum*, and *Fistulifera* [36]. As such, the degradation of algal polysaccharides is highly beneficial for the production of cellular valuables.

Algal Gene Expression

Organisms under attack must maintain cell-specific metabolisms via biochemical mechanisms or changes in gene expression. This study examined the effects of bacterial toxicity on the functioning of the nucleus and chloroplast in algae cells, including *rbcS*, *hsp*, and PCNA gene expression. As shown in Fig. 4, PCNA and *rbcS* transcription were severely hindered by bacterial treatment, while *hsp* was promoted by bacterial treatment. Groups of genes related to the *rbcS* nucleus contain the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunits, which are involved in targeting rubisco to the algal pyrenoid and concentrating CO₂ for optimal photosynthesis. This can influence the efficiency of carboxylation catalysis and the specificity of CO₂/O₂ [29, 37]. The downregulation of *rbcS* expression in the algalicidal bacteria treatment suggests that algal photosynthesis was impeded, which explains why the CAFD of *N. oceanica* cells degraded after they were incubated with the filtrate of *S. stellata* and the washed mycelial pellets (Fig. 2, treatments #3, 4, and 6).

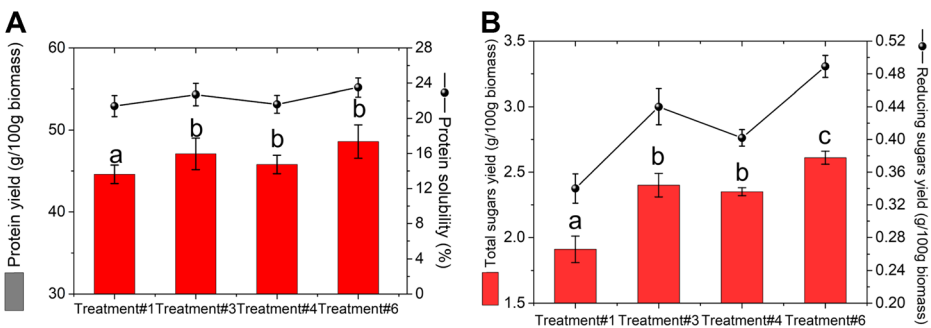


Fig. 3 A, B The sugar and protein in *N. oceanica* under different treatments. A statistically significant difference is indicated by different letters above the bar. Treatment #1: algae only; treatment #3: algae+bacteria; treatment #4: algae+BS; treatment #6: algae+bacteria+BS

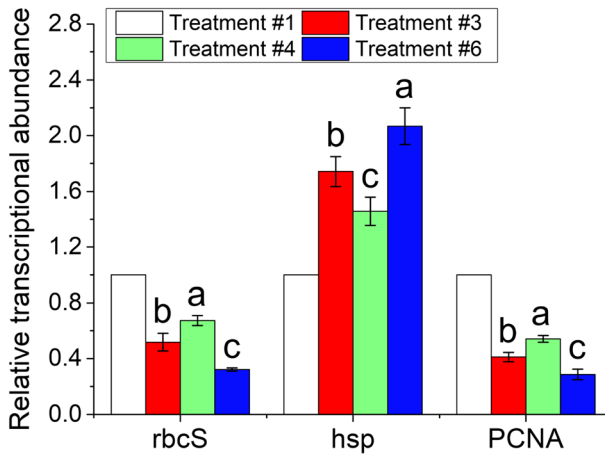


Fig. 4 The transcription of *rbcS*, *hsp*, and PCNA genes in *N. oceanica* following treatment. A statistically significant difference is indicated by different letters above the bar. Treatment #1: algae only; treatment #3: algae+bacteria; treatment #4: algae+BS; treatment #6: algae+bacteria+BS

The *hsp* gene is different from *rbcS* and is usually overexpressed under stress conditions such as low or high temperatures, high salinity, or drought. The HSP family molecular chaperones serve a housekeeping role in cells and are a primary factor affecting whether or not these cells survive in environments stressed by biotic or abiotic factors [38]. Therefore, in the present study, the upregulated *hsp* gene indicated that the stress from bacteria or a bacterial supernatant inhibited algal growth and induced algal cells to self-repair. The PCNA gene plays critical roles in cell DNA synthesis and cell proliferation, and its expression is correlated with cell growth rate [39]. Figure 4 demonstrates that PCNA expression was severely hindered by treatments #3, 4, and 6 after 6 days of treatment, which suggested that the nucleus of the algae cells could not function normally under the stress exerted by *S. stellata*. The greatest impact on *rbcS*, *hsp*, and PCNA gene transcription was observed in treatment #6, followed by treatments #3 and #2. For example, in treatment #6, the transcription of *rbcS* and PCNA was inhibited 0.32- and 0.29-fold, while *hsp* was promoted by 2.07-fold, compared to the control (treatment #1). These results indicate that the combination of bacteria and a bacterial supernatant induced the most severe cell stress, nucleus malfunction, and cell lysis of *N. oceanica*.

Cell Integrity Observation

Following bacteria treatment, there was an observable change in the ultrastructure of *N. oceanica* (Fig. 5). Several cells subjected to treatment #6 demonstrated the effects of cell lysis and the loss of organelle integrity by algicidal bacteria or the bacterial supernatant treatment. Under the control treatment, there were no changes to the wall or membrane of the cell, the cytoplasm remained complete, and mitochondrion and chloroplasts (normal cells) were observed (Fig. 5a). However, clear plasmolysis was observed and morphological and structural differences in the algae cell wall were observed (Fig. 5b). The membrane of the cell was damaged and the components of the inner cell such as the nucleus and chloroplast emerged (Fig. 5c). The cell membrane structures were obscured (Fig. 5d). Damage to the integrity and permeability of the cell membrane could cause the cells to lose components of the inner cell and facilitate the extraction of algal lipids.

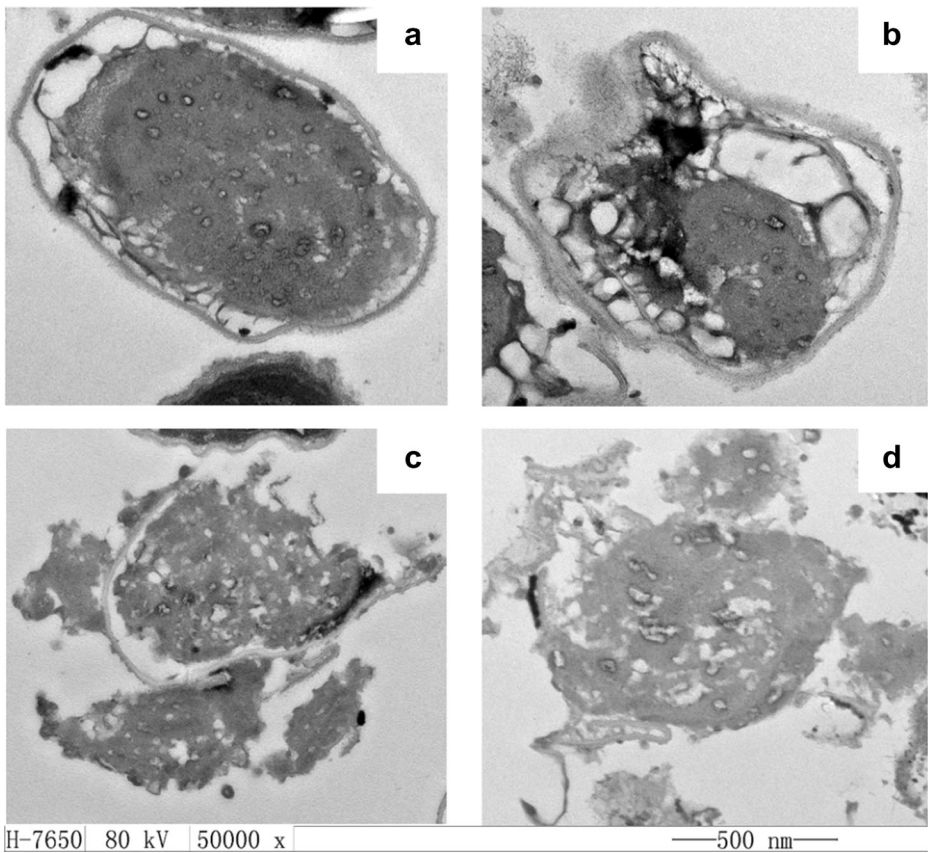


Fig. 5 TEM images displaying lysis in *N. oceanica* (a is the control; b, c, and d are chosen from treatment #6), scale bar 500 nm. Treatment #6: algae+bacteria+BS

Algicidal Mode Analysis

Various applications of bacterial cultures showed differing degrees of algicidal activity toward *N. oceanica*. The rate of algicide in cultures with bacteria (80.1%, $t = 6$ days, treatment #6) was roughly 5.4% higher than in the cells with re-suspended bacteria in MMBM (74.6%, $t = 6$ days, treatment #3) and was 13.1% greater than the cultures with filtrate-free bacteria (67.0%, $t = 6$ days, treatment #6) (Table 4). These results suggest that *S. stellata* exerted algicidal activity on *N. oceanica* cells primarily by a direct attack (predation), followed by an indirect attack via the possible secretion of extracellular algicidal compounds (Fig. 6). Algicidal bacteria perform direct and indirect attacks on algal cells. Direct attacks necessitate a physical interaction between the target algal cell and the bacterium preceding lysis for the cell, which could include algae surface colonization and invasion. The indirect method requires algicidal bacteria to exude the lysing compound into the surrounding media and does not require physical interaction between the algal bacteria and the target [4]. The algicidal bacteria secrete extracellular compounds, such as agarase, lipase, amino-peptidase, alkaline phosphatase, glucosaminidase, proteins, benzoic acid, antibiotics, biosurfactants, bacillamide, and protease [40–43]. Additionally, algicidal compounds induced the lysis of algal cells mainly

Table 4 Growth of algicidal strain *S. stellata* in different experimental groups and related algicidal activity. Treatment #1: algae only; treatment #2: bacteria only; treatment #3: algae+bacteria; treatment #4: algae+BS; treatment #5: bacteria+BS; treatment #6: algae+bacteria+BS

Treatment	#1	#2	#3	#4	#5	#6
<i>N. oceanica</i> cell number (cells/mL × 10 ⁶)	4.12±0.22 a	–	1.05±0.09 c	1.36±0.11b	–	0.82±0.05d
<i>S. stellata</i> cell number (CFU/mL × 10 ³)	–	5.62±0.29c	7.82±0.38 b	–	7.29±0.58 b	9.01±0.82 a
Algicidal rate (%)	–	–	74.6	67.0	–	80.1

by elevating oxidative stress, by the destruction of cellular integrity and alteration of enzymatic activities and gene expression, and by influencing algal photosynthesis/respiration during the algicidal process [11, 44]. Recently, autophagy was found to be a possible cell death pathway induced by microbes, which has rarely been observed [12]. In contrast, some algicidal bacteria hinder algae growth by the direct interaction, penetration, or entrapment of algal cells [25]. In fact, direct interaction can confer ecological benefits for algicidal bacteria because the chemical components involved in algicide could be diluted by freshwater or marine water [5].

The increased *S. stellata* cell number was positively correlated with the algicidal rate in treatments #3 and 6 (Table 4), indicating that bacteria fed on algae and obtained carbon/nitrogen sources from algal cells. This resulted in algal cell lysis and the release of the cell contents. The cell wall plays a primary role in resistance to algae. Bacteria can detect whether or not algae will be resistant and target the cell walls of the less resistant strains [25]. Additional study is required to better understand the relationship between algicidal bacteria and their prey.

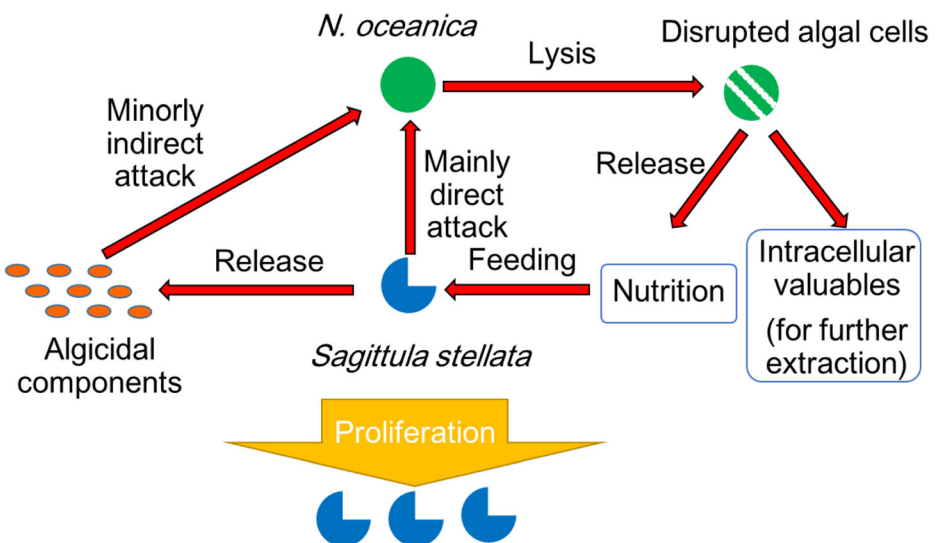


Fig. 6 Schematic representation of the mechanisms of algicidal bacteria *S. stellata* attacking cells of *N. oceanica*

Perspectives and Further Directions

The recent advances of different methods of algal lysis from the perspectives of energy usage and environmental impact are summarized in Table 5. Mechanical disruption requires energy inputs such as shear forces, electrical pulses, waves, or heat [19]. Chemical additives are potentially toxic to humans and are environmentally unfriendly. Additionally, these steps can account for up to 26% of the total biofuel cost [20]. The traditional approach typically focuses on a single type of product such as lipids or ethanol, instead of maximizing product revenue in a refinery [5, 18]. Using microorganisms for microalgal degradation is an energy-efficient, environment-friendly, and sustainable approach for producing biofuels (Table 5). This novel refinery approach could facilitate and improve the recovery of macromolecules (lipid extraction or increasing carbohydrate/protein availability) without affecting the biomass. Furthermore, considering its similarities with “on-site production,” the microbial degradation method can be used for large-scale production in biorefineries [25]. This research will contribute to affordable large-scale algae biofuel manufacturing that can benefit the global economy, energy security, natural resource sustainability, the environment, and society as a whole.

It should be noted that algicidal bacteria are highly species-specific in destroying algal cells in many cases. For example, Fukami et al. [51] previously reported that *Flavobacterium* sp. 5 N-3 acted against *Gymnodinium nagasakiense*, but failed to control *Chattonella antiqua*, *Heterosigma akashiwo*, or *Skeletonema costatum*. *Pseudoalteromonas haloplanktis* AFMB-08041 was recently shown to quickly lyse the harmful dinoflagellate *Prorocentrum minimum*, but it did not negatively affect other algae such as *Alexandrium tamarense* and *Akashiwo sanguine* [52]. However, the molecular mechanism(s) permitting algicidal bacteria to discriminate between target organisms, even at the species level, remains unresolved.

It is extremely difficult to ensure that the target cells are not consumed by bacteria during algal cell lysis when bacteria are used for the production of bioproducts or biofuels. For example, when manufacturing algal biodiesel, the membranes of the cell must be lysed while keeping the proteins and lipids fully functional. This can be achieved by changing the amount of time the bacteria are cultured or choosing species that prey only on the wall or membrane of the cell (but not other components) [29]. However, in order to perfect these methods, strains of algicidal bacteria might need to be genetically engineered. Recently, a genomic analysis of the algicidal bacterium *Bacillus subtilis* strain JA demonstrated that a putative AI-2 type quorum-sensing gene in its genome cluster was responsible for the algicidal activity of this bacterium [50]. Algicidal bacterial lysis takes a long time because of the slow nature of the interactions between cells compared to non-biological methods, meaning the process could be much longer. One possible solution uses both traditional methods of cell disruption and bacterial lysis. For example, conventional methods of cell disruption requiring significant energy can damage the cell walls; however, since lysis of the whole cell is unnecessary, the energy usage can be moderated. Algal cells with existing damage to their cell walls are more susceptible to algicidal bacteria, making cell lysis via algicidal bacteria following the conventional method a more efficient approach to lysis. However, there is currently no published research on this method.

Conclusions

During the lysis of alga *Nannochloropsis oceanica*, the algicidal bacterium *Sagittula stellata* was unable to extract intracellular lipids, proteins, and sugars. After 6 days of treatment, the

Table 5 Summary of different algal lysis methods reported in recent studies from energy and environmental perspectives

Cell lysis method	Species	Scale of use	Cell lysis condition	Input energy (MJ/kg dry biomass)	Chemical use	Reference
Bead milling	<i>Botryococcus, Chlorella, Scenedesmus</i>	Laboratory, industrial	100 mL, 5 kg m ⁻³ , 840 W, 5 min	504	Not applicable	[45]
Ultrasonication	<i>Chlorococcum</i> sp.	Laboratory, industrial	200 mL, 8.5 kg m ⁻³ , 750 W, 5 min	132	Not applicable	[46]
Microwave	<i>Botryococcus, Chlorella, Scenedesmus</i>	Laboratory	100 mL, 5 kg m ⁻³ , 700 W, 5 min	420	Not applicable	[45]
Steam explosion	<i>Nannochloropsis oceanica</i>	Laboratory	Steam at set pressure (1.0 to 2.1 MPa), 0.1-s pressure release for 5 min	8.56	Not applicable	[47]
Acid	<i>Chlorella vulgaris</i>	Laboratory	1% H ₂ SO ₄ (120 , 60 min)	Not applicable	Yes	[48]
Surfactant	<i>Chlorella vulgaris</i>	Laboratory	0.2% sodium dodecyl benzene sulfonate, 2% H ₂ SO ₄ (120 , 1 h)	Not applicable	Yes	[48]
Enzymatic treatment	<i>Nannochloropsis oceanica</i>	Laboratory	Cellulose, lipase, protease	Not applicable	Yes	[49]
Algal lysis	<i>Nannochloropsis oceanica</i>	Laboratory, industrial	Coculture with <i>Sagittaria stellata</i> (continuous)	Not applicable	Not applicable	Current study

algicidal rate reached 80.1%. We achieved a recoverable crude lipid content of up to 23.3% and a polyunsaturated fatty acid ratio of up to 29.0% from bacteria-treated algae. The increases in protein and sugar yields of algal cells further confirmed that using *S. stellata* was an effective method of algal cell disruption and the production of more intracellular valuables. Lysis of the algal cell membrane and the nucleus structure integrity was observed. Cell lysis was further confirmed by the inhibited transcription of ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit and proliferating cell nuclear antigen genes and the upregulated heat shock protein gene. The algicidal method of strain *S. stellata* on *N. oceanica* cells was primarily that of a direct attack, while indirect attacks played a minor role. The results demonstrate that the algicidal bacteria *S. stellata* and its algicidal molecules are a promising and environmentally sustainable tool for biofuel production from *Nannochloropsis oceanica* cells, representing significant promise for the practical biorefinement of microalgae.

Nomenclature *ARA*, Arachidonic acid; *BS*, Bacterial supernatant; *CAFD*, Chlorophyll a fluorescence intensity; *CFU*, Colony-forming units; *DHA*, Docosahexaenoic acid; *EPA*, Eicosapentaenoic acid; *FAMES*, Fatty acid methyl esters; *GC*, Gas chromatography; *hsp*, Heat shock protein; *MMBM*, Modified Marine Broth 2216 medium; *MUFAs*, Monounsaturated fatty acids; *N. oceanica*, *Nannochloropsis oceanica*; *PUFAs*, Polyunsaturated fatty acids; *PCNA*, Proliferating cell nuclear antigen; *rbc*, S: Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit; *S. stellata*, *Sagittula stellata*; *SFAs*, Saturated fatty acids; *TEM*, Transmission electron microscopy

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Data Availability The data sets supporting the results of this article are included within the article.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

Ethical Approval Not applicable

Consent to Participate Not applicable

Consent to Publish Not applicable

References

1. Manage, P. M., Kawabata, Z., & Nakano, S. (2000). Algicidal effect of the bacterium *Alcaligenes denitrificans* on *Microcystis* spp. *Aquatic Microbial Ecology*, 22, 111–117.
2. Natrah, F. M. I., Bossier, P., Sorgeloos, P., Yusoff, F. M., Defoirdt, T. (2014). Significance of microalgal–bacterial interactions for aquaculture. *Reviews in Aquaculture*, 648–661.

3. Zohdi, E., & Abbaspour, M. (2019). Harmful algal blooms (red tide): a review of causes, impacts and approaches to monitoring and prediction. *International Journal of Environmental Sciences*, 16(3), 1789–1806.
4. Roth, P. B., Twiner, M. J., Mikulski, C. M., Bamhorst, A. B., & Doucette, G. J. (2008). Comparative analysis of two algicidal bacteria active against the red tide dinoflagellate *Karenia brevis*. *Harmful Algae*, 7, 682–691.
5. Meyer, N., Bigalke, A., Kaulfuß, A., & Pohnert, G. (2017). Strategies and ecological roles of algicidal bacteria. *FEMS Microbiology Reviews*, 41(6), 880–899.
6. Anderson, D. M., Cembella, A. D., & Hallegraeff, G. M. (2012). Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. *Annual Review of Marine Science*, 4(1), 143–176.
7. Li, Z., Geng, M., Yang, H. (2015). Algicidal activity of *Bacillus* sp. lzh-5 and its algicidal compounds against *Microcystis aeruginosa*. *Applied Microbiology and Biotechnology*, 99(2), 981–990.
8. van Tol, H. M., Amin, S. A., & Armbrust, E. V. (2017). Ubiquitous marine bacterium inhibits diatom cell division. *ISME Journal*, 11, 31–42.
9. Stewart, J. R., & Brown, R. M. (1969). Cytophaga that kills or lyses algae. *Science, New Series*, 164(3887), 1523–1524.
10. Zhang, H., Yu, Z. L., Huang, Q., Xiao, X., Wang, X., Zhang, F., et al. (2011). Isolation, identification and characterization of phytoplankton-lytic bacterium CH-22 against *Microcystis aeruginosa*. *Limnologica*, 41, 70–77.
11. Pokrzywinski, K. L., Tilney, C. L., Modla, S., Caplan, J. L., Ross, J., Warner, M. E., et al. (2017). Effects of the bacterial algicide IRI-160AA on cellular morphology of harmful dinoflagellates. *Harmful Algae*, 62, 127–135.
12. Yu, X., Cai, G., Wang, H., Hu, Z., Zheng, W., Lei, X., et al. (2018). Fast-growing algicidal *Streptomyces* sp. U3 and its potential in harmful algal bloom controls. *Journal of Hazardous Materials*, 341, 138–149.
13. Deshmukh, S., Bala, K., & Kumar, R. (2019). Selection of microalgae species based on their lipid content, fatty acid profile and apparent fuel properties for biodiesel production. *Environmental Science and Pollution Research*, 26(1), 24462–24473.
14. Chen, Y., Xu, X., & Vaidyanathan, S. (2020). Influence of gas management on biochemical conversion of CO₂ by microalgae for biofuel production. *Applied Energy*, 261, 114420.
15. Reis, A., & Gouveia, L. (2016). Low cost microalgal production for biofuels: a review. *Current Biotechnology*, 5(4), 266–276.
16. Menegazzo, M. L., & Fonseca, G. G. (2019). Biomass recovery and lipid extraction processes for microalgae biofuels production: a review. *Renewable and Sustainable Energy Reviews*, 107, 87–107.
17. Kanda, H., Hoshino, R., Murakami, K., Wahyudiono, Z., & Q. Goto, M. (2020). Lipid extraction from microalgae covered with biom mineralized cell walls using liquefied dimethyl ether. *Fuel*, 262, 116590.
18. Show, K. Y., Lee, D. J., Tay, J. H., Lee, T. M., & Chang, J. S. (2015). Microalgal drying and cell disruption—recent advances. *Bioresour. Technol.*, 184, 258–266.
19. Lee, S. Y., Cho, J. M., Chang, Y. K., & Oh, Y. K. (2017). Cell disruption and lipid extraction for microalgal biorefineries: a review. *Bioresour. Technol.*, 244, 1317–1328.
20. Wang, M., Cheng, H., Chen, S., Wen, S., Wu, X., & Zhang, D. (2018). Microalgal cell disruption via extrusion for the production of intracellular valuables. *Energy*, 142, 339–345.
21. Wang, M., Yuan, W., Jiang, X., Yun, J., & Wang, Z. (2014). Disruption of microalgal cells using high-frequency focused ultrasound. *Bioresour. Technol.*, 153(2), 315–321.
22. Spiden, E. M., Yap, B. H., Hill, D. R. A., Kentish, S. E., Scales, P. J., & Martin, G. J. O. (2013). Quantitative evaluation of the ease of rupture of industrially promising microalgae by high pressure homogenization. *Bioresour. Technol.*, 140, 165–171.
23. Prabhakaran, P., & Ravindran, A. D. (2011). A comparative study on effective cell disruption methods for lipid extraction from microalgae. *Letters in applied microbiology*, 53(2), 150–154.
24. Enamala, M. K., Enamala, S., Chavali, M., Donepudi, J., Donepudi, R., Kolapalli, B., et al. (2018). Production of biofuels from microalgae - a review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. *Renewable and Sustainable Energy Reviews*, 94, 49–68.
25. Demuez, M., Gonzalez-Fernandez, C., & Ballesteros, M. (2015). Algicidal microorganisms and secreted algicides: new tools to induce microalgal cell disruption. *Biotechnology Advances*, 33(8), 1615–1625.
26. Boden, R., Murrel, J. C., & Schäfer, H. (2011). Dimethylsulfide is an energy source for the heterotrophic marine bacterium *Sagittula stellata*. *FEMS Microbiology Letters*, 322(2), 188–193.
27. Ding, Y. X., Chin, W. C., Rodriguez, A., Hung, C. C., Santschi, P. H., & Verdugo, P. (2008). Amphiphilic copolymers from *Sagittula stellata* induce DOM self-assembly and formation of marine microgels. *Marine Chemistry*, 112(1), 11–19.

28. González, J. M., Mayer, F., Moran, M. A., Hodson, R. E., Whitman, W. B. (1997). *Sagittula stellata* gen. nov., sp. nov., a lignin-transforming bacterium from a coastal environment. *International Journal of Systematic Bacteriology*, 47(3), 773–780.
29. Wang, M., & Yuan, W. (2014). Bacterial lysis of microalgal cells. *Journal of Sustainable Bioenergy Systems*, 4, 243–248.
30. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*, 25(4), 402–408.
31. Furusawa, G., Yoshikawa, T., Yasuda, A., Sakata, T. (2003). Algicidal activity and gliding motility of *Saprospira* sp. SS98-5. *Canadian Journal of Microbiology*, 49(2), 92–100.
32. Volkman, J. K., Brown, M. R., Dunstan, G. A., & Jeffrey, S. W. (2010). The biochemical composition of marine microalgae from the class *Eustigmatophyceae*. *Journal of Phycology*, 29(1), 69–78.
33. Patil, V., Källqvist, T., Olsen, E., Vogt, G., & Gislerød, H. R. (2007). Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquaculture International*, 15(1), 1–9.
34. Melo, M. M. R., Sapatinha, M., Pinheiro, J., Lemos, M. F. L., Bandarra, N. M., Batista, I., et al. (2020). Supercritical CO₂ extraction of *Aurantiochytrium* sp. biomass for the enhanced recovery of omega-3 fatty acids and phenolic compounds. *Journal of CO₂ Utilization*, 38, 24–31.
35. Hu, X., Yin, P., Zhao, L., & Yu, Q. (2015). Characterization of cell viability in *Phaeocystis globosa* cultures exposed to marine algicidal bacteria. *Biotechnology and Bioprocess Engineering*, 20(1), 58–66.
36. Domozych, D. S., & Domozych, C. E. (2014). Multicellularity in green algae: upsizing in a walled complex. *Frontiers in plant science*, 5, 1–8.
37. Genkov, T., Meyer, M., Griffiths, H., & Spreitzer, R. J. (2010). Functional hybrid rubisco enzymes with plant small subunits and algal large subunits: engineered *rbcS* cDNA for expression in *Chlamydomonas*. *Journal of Biological Chemistry*, 285(26), 19833–19841.
38. Liu, F., Sun, X., Wang, W., Liang, Z., & Wang, F. (2014). De novo transcriptome analysis-gained insights into physiological and metabolic characteristics of *Sargassum thunbergii* (Fucales, Phaeophyceae). *Journal of Applied Phycology*, 26(3), 1519–1526.
39. Boehm, E. M., Gildenberg, M. S., & Washington, M. T. (2016). The many roles of PCNA in eukaryotic DNA replication. *The Enzymes*, 39, 231–254.
40. Kim, Y. S., Son, H. J., & Jeong, S. Y. (2015). Isolation of an algicide from a marine bacterium and its effects against the toxic dinoflagellate *Alexandrium catenella* and other harmful algal bloom species. *Journal of Microbiology*, 53(8), 511–517.
41. Petropoulos, K., Bodini, S. F., Fabiani, L., Micheli, L., Porchetta, A., Piermarini, S., et al. (2019). Remodeling ELISA kits embedded in an automated system suitable for on-line detection of algal toxins in seawater. *Sensors and Actuators B: Chemical*, 283, 865–872.
42. Shi, X., Liu, L., Li, Y., Xiao, Y., Ding, G., Lin, S., et al. (2018). Isolation of an algicidal bacterium and its effects against the harmful-algal-bloom dinoflagellate *prorocentrum donghaiense* (dinophyceae). *Harmful Algae*, 80, 72–79.
43. Wang, Y., Li, S., Liu, G., Li, X., Yang, Q., Xu, Y., et al. (2020). Continuous production of algicidal compounds against *Akashiwo sanguinea* via a *Vibrio* sp. co-culture. *Bioresource Technology*, 295, 122246.
44. Cai, G., Yang, X., Lai, Q., Yu, X., Zhang, H., Li, Y., et al. (2016). Lysing bloom-causing alga *Phaeocystis globosa* with microbial algicide: an efficient process that decreases the toxicity of algal exudates. *Scientific Reports*, 6(1), 20081.
45. Lee, A. K., Lewis, D. M., & Ashman, P. J. (2012). Disruption of microalgal cells for the extraction of lipids for biofuels: processes and specific energy requirements. *Biomass and Bioenergy*, 46, 89–101.
46. Halim, R., Harun, R., Danqua, M. K., & Webley, P. A. (2012). Microalgal cell disruption for biofuel development. *Applied Energy*, 91(1), 116–121.
47. Cheng, J., Huang, R., Li, T., Zhou, J., & Cen, K. (2015). Physicochemical characterization of wet microalgal cells disrupted with instant catapult steam explosion for lipid extraction. *Bioresource Technology*, 191, 66–72.
48. Park, J. Y., Nam, B., Choi, S. A., Oh, Y. K., & Lee, J. S. (2014). Effects of anionic surfactant on extraction of free fatty acid from *Chlorella vulgaris*. *Bioresource Technology*, 166, 620–624.
49. Chen, L., Li, R., Ren, X., & Liu, T. (2016). Improved aqueous extraction of microalgal lipid by combined enzymatic and thermal lysis from wet biomass of *Nannochloropsis oceanica*. *Bioresource Technology*, 214, 138–143.
50. Zhang, S., Du, X., Zhu, J., Meng, C., Zhou, J., & Zou, P. (2020). The complete genome sequence of the algicidal bacterium *Bacillus subtilis* strain JA and the use of quorum sensing to evaluate its anti-algal ability. *Biotechnology Reports*, 25, e00421.
51. Shi, S. Y., Liu, Y. D., Shen, Y., Li, G., & Li, D. (2006). Lysis of *Aphanizomenon flos-aquae* (cyanobacterium) by a bacterium *Bacillus cereus*. *Biological Control*, 39(3), 345–351.

52. Kim, J. D., Kim, J. Y., Park, J. K., & Lee, C. G. (2009). Selective control of the proroentrum minimum harmful algal blooms by a novel algal-lytic bacterium *Pseudoalteromonas haloplanktis* AFMB-008041. *Marine Biotechnology*, 11(4), 463–472.

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