Enhanced growth and lipid productivity by living Chlorella sorokiniana immobilized in Ca-alginate beads

Alfaro-Sayes, Daniel A ; Amoah, Jerome ; Rachmadona, Nova ; Hama, Shinji ; Hasunuma, Tomohisa ; Kondo, Akihiko ; Ogino, Chiaki

(Citation)
Journal of Physics: Energy,5(1):014019

(Issue Date)
2023-01

(Resource Type)
journal article

(Version)
Version of Record

Enhanced growth and lipid productivity by living *Chlorella sorokiniana* immobilized in Ca-alginate beads

Journal of Physics: Energy

PAPER

Enhanced growth and lipid productivity by living *Chlorella sorokiniana* immobilized in Ca-alginate beads

Daniel A Alfaro-Sayes[1], Jerome Amoah[2], Nova Rachmadona[1,4,5], Shinji Hama[3], Tomohisa Hasunuma[2], Akihiko Kondo[1,2] and Chiaki Ogino[1,*]

[1] Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, Kobe, Japan
[2] Graduate School of Science, Technology and Innovation, Kobe University, Kobe, Japan
[3] Research and Development Laboratory, Bio-energy Corporation, Amagasaki, Japan
[4] Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Padjadjaran, Jatinangor, West Java 45363, Indonesia
[5] Research Collaboration Center for Biomass and Biorefinery between BRIN and Universitas Padjadjaran, Jatinangor, West Java 45363, Indonesia
[*] Author to whom any correspondence should be addressed.

E-mail: ochiaki@port.kobe-u.ac.jp

Keywords: *Chlorella sorokiniana*, biomass, lipids, alginate, immobilized cell

OPEN ACCESS

RECEIVED
31 August 2022

REVISED
10 January 2023

ACCEPTED FOR PUBLICATION
16 January 2023

PUBLISHED
30 January 2023

Abstract

The bottleneck for the production of biofuels from microalgae consists on costly harvesting processes and low lipid production, immobilization technology could play a part on making the production of biofuels more feasible. The aim of this study was to evaluate the effect of alginate immobilization on the growth and lipid productivity of the microalgae *Chlorella sorokiniana*, so far, the main focus of immobilization technology has been its use for wastewater treatment and nutrient removal from effluents. The microalgae *Chlorella sorokiniana* was cultured in both free and immobilized forms under optimal autotrophic growth conditions. Microalgae were immobilized in calcium alginate beads generated by mixing algal cells with a sodium alginate solution, followed by extrusion into a $CaCl_2$ solution. The results obtained in this study showed that the growth of the microalgae immobilized in alginate beads, was enhanced and achieved a dry cell weight 1.4-fold higher than that of a free cell culture, a higher light transmittance was also achieved in the alginate immobilized culture, and the lipid productivity was increased from 54.21 ± 2.48 mg l^{-1} d in the free cell culture to 82.22 ± 8.48 mg l^{-1} d in the immobilized culture. These results demonstrate the effectiveness of immobilization technology for promoting growth and lipid productivity in the microalgae *Chlorella sorokiniana*.

1. Introduction

Energy is the primary commodity in the world for the growth of any nation; thus far, this necessity has been fulfilled through the use of fossil fuels. However, this reliance on fossil fuels has resulted in negative effects such as climatic change [1].

Microalgae are fast-growing microorganisms with many advantages over terrestrial plants. These advantages include higher rates of carbon fixation and the ability to grow without the need for arable land to produce high biomass yields [2, 3]. Various species of microalgae can store considerable amounts of energy-rich compounds, specifically lipids and polysaccharides, which can be utilized to produce biofuels such as biodiesel and bioethanol [4, 5]. Microalgae cells can double the biomass of these energy-rich compounds by 1–3 times in 24 h, and under specific conditions, they can store 40%–60% of their dry weight in fatty acids [6].

A bottleneck in the use of microalgae for biofuel production involves the cost, which can be 2–3-fold higher than when using first- and second-generation biomass [7]. Harvesting, drying, and oil extraction account for half of the total production cost. Harvesting alone occupies almost 30% of total production cost, which can amount to as much as US $10 kg [8]. To achieve economic feasibility in biofuel production from

microalgae, it is necessary to focus on the most important areas, such as low lipid accumulation, expensive cultivation steps, and unproductive downstream processes [7].

Alginate is one of the most abundant biopolymers of natural origin available after cellulose, with many useful properties such as low toxicity, biodegradability, biocompatibility, and good hydrogel-forming ability upon the addition of divalent cations such as Ca^{2+} [9].

Immobilization technology has been proposed as an alternative technique to facilitate the extraction of microalgal cells in the aqueous phase, from which immobilized cells can be harvested through simple filtration without the need for large amounts of energy [10]. Immobilization can be either physical (adsorption) or chemical (entrapment, cross-linkage, or covalent bonding) [11], entrapment in a polymeric matrix, such as calcium alginate, is one of the most widely used immobilization techniques for microalgae [12]. Alginate beads are formed when a solution of sodium alginate and microalgae cells is extruded into a Ca^{2+} solution, at that moment an instantaneous interfacial polymerization occurs, producing the beads with the immobilized microalgae inside, the bead hardness is increased with the permeation of calcium ions through the beads [11].

It has been reported that the cost of harvesting suspended cells by centrifugation is approximately US $1.8 per cubic meter of culture volume, however, the cost of alginate for cell immobilization is about US $0.2 per cubic meter of culture volume [13], taking into account that the cost of the alginate used for immobilization is much lower than harvesting by centrifugation, makes the use of alginate-immobilized cells more economically viable and attractive.

The chlorophyll-a content, which is considered to be a cell activity indicator, is higher in immobilized cultures [14], immobilized cells have also shown a higher level of chloroplast formation and higher oxygen production rates than free microalgae cells [15]. Immobilized microalgae cells are able to retain 90% of their chlorophyll even after 3 months, whereas in a free cell culture, the occurrence of pheophytinization occurs just after 7 d [15].

Currently, the most frequent applications of immobilized microalgae include wastewater treatment [16, 17], removal of nutrients from aquatic media [14, 18], removal of organic or metal pollutants [19, 20], toxicity measurement [21, 22], and energy production via hydrogen [23, 24] or electricity [12, 25]. In the previously mentioned applications of immobilized microalgae, their main focus has not been the improvement of lipid accumulation, which has not been developed to the same level, making the information regarding this topic to remain limited and scarce, with most studies achieving a similar growth between free and immobilized cells.

Cheirslip *et al* [13] evaluated the growth of *Nannochloropsis* sp. immobilized on alginate beads in a secondary effluent of palm oil mill effluent, they were able to cultivate the immobilized cells to achieve a comparable growth and lipid production to the free cells by varying the bead volume and inoculum size. Li *et al* [26] found that the optimum immobilization conditions for *Chlorella vulgaris* were of 2.42% sodium alginate and 2.69% $CaCl_2$ to treat fracturing flowback fluids, achieving a higher chemical oxygen demand (COD) removal compared to the free cells, and obtained a growth of 1.97 g l^{-1}. Lee *et al* [14] studied the effect of the alginate bead size for optimal growth and nutrient removal of *Chlorella vulgaris* and *Chlamydomonas reinhardtii*, their results showed that the optimal bead size was of 3.5 mm. The growth of free and immobilized cells of *Chlorella vulgaris* was studied by Sepian *et al* [27], with a combination of matrices of sodium alginate, calcium alginate, and sodium carboxymethyl cellulose, and nearly identical cell densities were achieved between free and immobilized cells.

This study focused on the immobilization of *Chlorella sorokiniana* for lipid accumulation. It is a robust industrial species that tolerates high temperatures and high levels of intense light [28]. This strain possesses levels of lipid accumulation that can reach >30% of its dry cell weight, *Chlorella* strains has been studied for commercial production of biodiesel because of their high growth rates, with some species being able to double their biomass and lipid yield within 24 h [29].

The aim of the present study was to demonstrate the potential of immobilization not only as a tool to simplify the harvesting process of microalgal cells but also as a method to increase the lipid productivity of the cells, addressing two of the disadvantages for achieving biodiesel economic feasibility: lipid accumulation and simplification of downstream processes.

2. Materials and methods

2.1. Strain and culture conditions

Chlorella sorokiniana UTEX 1230 cells, obtained from the University of Texas Culture Collection of Algae, as described by Amoah *et al* [30], were precultured for 96 h at 30 ± 2 °C in 250 ml of modified Bold 6 N medium, supplied with CO_2 (2%) at 0.5 vvm, under continuous light irradiation of approximately 250 μmol photons m^{-2} s^{-1} . The optimal growth conditions for *C. sorokiniana* are approximately 30 °C–35

°C and light irradiation above 100 μmol photons m^{-2} s^{-1} [31], the optimum temperature for high fatty acid production in *C. sorokiniana* is 30 °C [29].

2.2. Optimization of alginate concentration for beads production

To produce stable beads, it was necessary to adjust the concentration of alginate utilized for bead production and the concentration of the gelling solution, $CaCl_2$ was utilized as the gelling solution in this study. Alginate and $CaCl_2$ concentrations were varied between 1% and 4% in order to produce stable and cylindrical beads.

2.3. Alginate immobilization of *C. sorokiniana* cells

An aliquot of 0.1 OD_{680} cells was gathered by centrifugation at 7600 xg for 5 min and washed twice with 50 ml of Milli-Q water [30]. The Microalgae cells were resuspended and mixed with a 2% (w/v) sodium alginate solution (Nacalai Tesque, Japan). The solution was then dripped with a syringe into a 100 ml sterile solution of 2% (w/v) $CaCl_2$ to form spherical beads with a diameter of approximately 4.0 mm, 1 ml of beads contained approximately 40 beads. The beads were cured for 1 h and then washed with distilled water.

2.4. Immobilized culture of *C. sorokiniana* in alginate beads

The immobilized cells of *C.* sorokiniana were cultured in 500 ml cylindrical flasks for a period of 9 d utilizing a volume of 20% beads (100 ml) and 400 ml of Bold 3 N medium, at 30 $\pm$ 2 °C supplied with CO_2 (2%) at 0.5 vvm under continuous light irradiation of approximately 250 μmol photons m^{-2} s^{-1}. A free-cell culture with an 0.1 OD_{680} was also performed as a control under the same culture conditions. An LI-250 A light meter (LI-COR, NE, USA) was used to measure light intensity. The light intensity was measured at both the back and front of the culture flask to calculate transmittance. Three flasks of immobilized beads and three flasks of free *C. sorokiniana* cells were utilized.

2.5. Dry cell weight measurement

To measure the dry cell weight, 10 ml of alginate beads (approximately 400 beads) were taken from the culture, dissolved in a 2% (w/v) Na_2CO_3 solution, and freeze dried to obtain dry cells that could then be weighed. The biomass produced is shown as the mass of dry cell weight per volume of culture.

2.6. Lipid content analysis

Lipid analysis was performed by crushing 3 mg of dried *C. sorokiniana* cells in a multi-bead shocker (Yasui Kikai, Japan) with 0.5 mm glass beads. Using a fatty acid methylation kit (Nacalai Tesque, Japan), the lipids were esterified and extracted, followed by quantification using gas chromatography-mass spectrometry (GCMS-QP2010 Plus, Shimadzu, Japan), as described by Amoah *et al* [30]. Purified fatty acid methyl esters (FAMEs) were identified by their retention times, using margaric acid as the internal standard.

2.7. Evaluation of *C. sorokiniana* cells growth and distribution inside the alginate beads

To observe the growth and condition of the cells inside the alginate beads, they were analyzed using a Keyence BZ-X810 All-in-one Fluorescence Microscope, for this 1 ml of the alginate beads was taken, followed by degradation of the beads using a 2% Na_2CO_3 solution; after extracting the cells from the alginate beads, they were resuspended in 1 ml of Milli-Q water, 100 μl of the suspended cells were washed with 100 μl of phosphate-buffered saline (PBS), followed by a resuspension in 45 μl of PBS and 5 μl of BODIPY (Invitrogen, Thermo Fisher Scientific, Japan) and left staining for 12 h. To observe their lipid accumulation, 1 ml of the free cultured cells was also submitted to staining and observed under the fluorescence microscope as a control.

Analysis of *C. sorokiniana* cell distribution inside the alginate beads was performed using an Olympus Fluoview FV31S-SW confocal laser scanning microscope (CLSM). One bead was taken from the culture, followed by cell staining with BODIPY (Invitrogen, Thermo Fisher Scientific, Japan) as previously described, and a cross-section cut of the bead was observed using CLSM.

2.8. Statistical analysis

For the statistical analysis, the results data was subjected to a one-way ANOVA using JMP Pro® 16 (JMP Statistical Discovery LCC, USA) to evaluate significant differences, where $p \leqslant 0.05$. The data presented in this study are the average of triplicate measurements, and the values were expressed as mean $\pm$ standard deviation. The experiments were conducted three times to further verify the results.

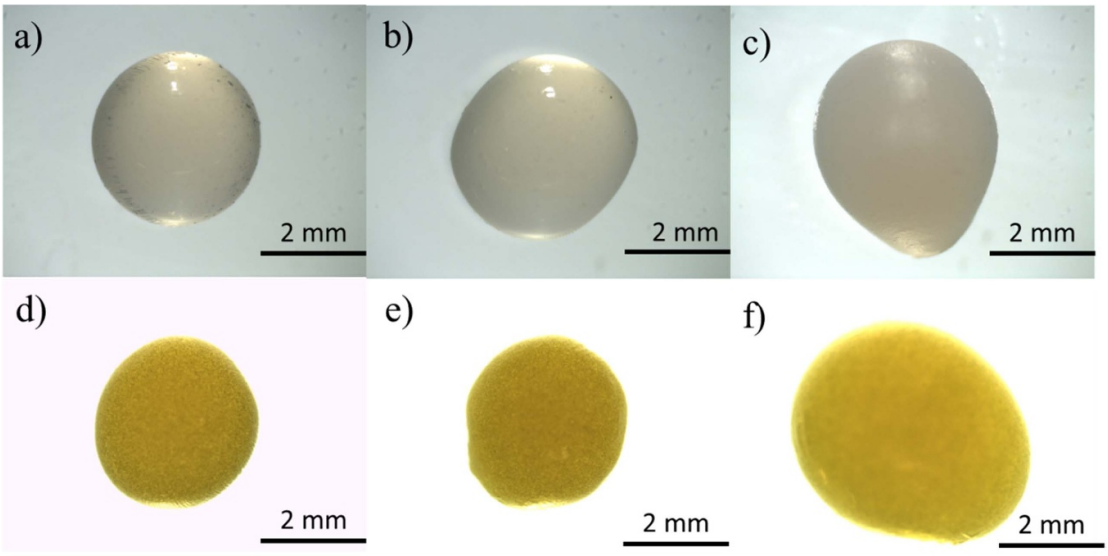

Figure 1. Alginate beads images at different concentrations. (a) Blank alginate bead (2% Alginate, 2% $CaCl_2$), (b) blank alginate bead (2% Alginate, 4% $CaCl_2$), (c) blank alginate bead (4% Alginate, 2% $CaCl_2$), (d) immobilized cells in alginate bead (2% Alginate, 2% $CaCl_2$), (e) immobilized cells in alginate bead (2% Alginate, 4% $CaCl_2$), (f) immobilized cells in alginate bead (4% Alginate, 2% $CaCl_2$).

3. Results

3.1. Alginate concentration optimization for stable alginate beads production

In this study, the beads produced with different concentrations of alginate and $CaCl_2$ were evaluated, the concentrations ranged from 1% to 4%. The alginate beads prepared with alginate and $CaCl_2$ concentrations below 2% did not have a well-defined shape and they broke or dissolved easily due to their low stability.

The beads produced with 2% alginate and 2% $CaCl_2$ had a well-formed spherical shape, as shown in figure 1, a size of 4 mm, good stability, not breaking after submitting them to vortex, and were easily degraded by a 2% Na_2CO_3 solution.

By utilizing alginate concentrations higher than 2%, the alginate solution became very viscous, making it harder to achieve a good mix with the microalgae cells. Moreover, because of its high viscosity at the time of dropping the alginate solution into the $CaCl_2$ to form the beads, the drops could not form completely and the bead shape was not spherical, their size was bigger compared to the beads produced with lower alginate concentrations, and the degradation of the beads by a 2% Na_2CO_3 solution was not possible; a higher concentration of Na_2CO_3 solution was needed, the time it took for the beads to dissolve was three times higher than that of the beads produced by a 2% alginate solution. For these reasons in the following experiments, the concentrations used to produce the beads were of 2% alginate and a 2% $CaCl_2$.

3.2. Immobilized *Chlorella sorokiniana* growth

The time course of the increase in the dry cell weight of the free and immobilized cells is shown in figure 2. Compared with the free-cell culture, the immobilized cells showed a higher dry cell weight that began on day 1, and this tendency continued until day 9, revealing that the immobilized culture had a positive effect on the cell growth compared with the suspended culture. On day 9 the dry cell weight for the free cell culture was of 2.09 ± 0.03 g l^{-1}, compared with 2.87 ± 0.24 g l^{-1} in the immobilized culture, which was about 1.4-fold higher.

3.3. Light transmittance in an immobilized culture

The variation in light transmittance during the growth of both the free and immobilized cell cultures is shown in figure 3. On day 1 the light transmittance was higher in the free cell culture than in the immobilized culture, this was because the number of cells inside the culture medium was still relatively low, for this reason the medium remained transparent, however by day 3 the number of cells had already increased, causing the culture medium to become darker and decreased the light transmittance in the culture medium from 58% on day 1%–6% on day 9.

In the immobilized culture, the cells grew inside the alginate beads but not in the culture medium, and the leaching of the cells into the culture medium was minimal. The alginate beads maintained their stability,

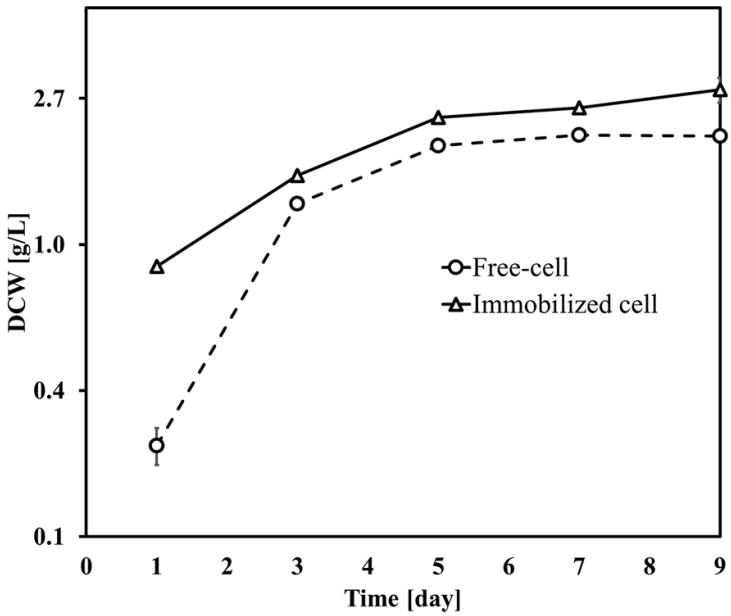

Figure 2. Dry cell weight (DCW) of free-cell and alginate-immobilized culture of *C. sorokiniana*. The error bars show the standard deviation of triplicate values.

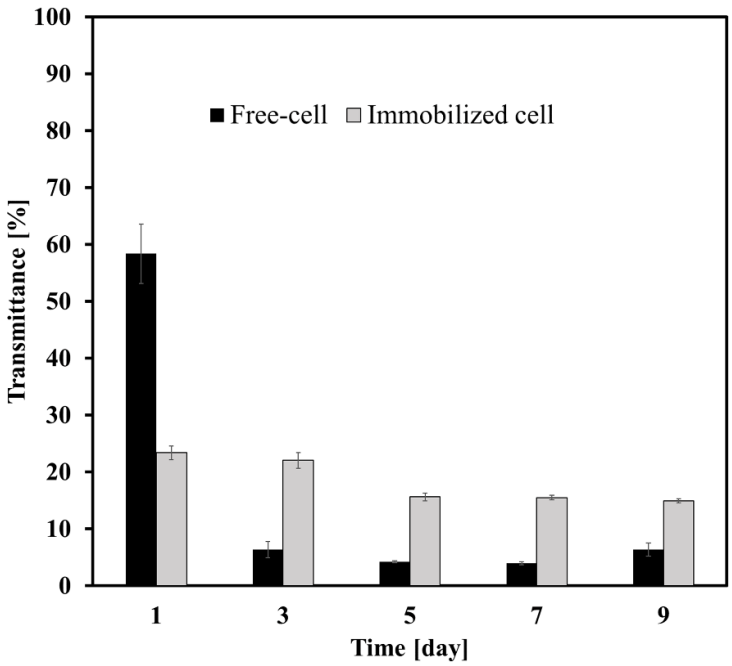

Figure 3. Light transmittance in a free-cell *C. sorokiniana* culture and in an alginate-immobilized culture. The error bars show the standard deviation of triplicate values.

and they did not break or dissolve, allowing the medium to remain transparent during the nine days of culture; only the beads became darker. In the immobilized culture, the light transmittance experienced a lower decrease compared to that of free cell culture, going from 23% on day 1%–15% on day 9.

3.4. Lipid productivity of immobilized *C. sorokiniana*

The time courses for the increase in lipid content and lipid productivity are shown in figure 4(a) for free cells and in figure 4(b) for immobilized cells. The lipid content in the immobilized culture was higher than that in the free-cell culture, reaching a 23.3% in the suspension culture and 25.8% in the immobilized cells. Lipid productivity in the immobilized culture was higher than that in the free-cell culture; this tendency remained during the nine days of culture, and the difference was especially noticeable on days 7 and 9, where it achieved 1.3- and 1.5-fold higher lipid productivity than that in the free-cell culture.

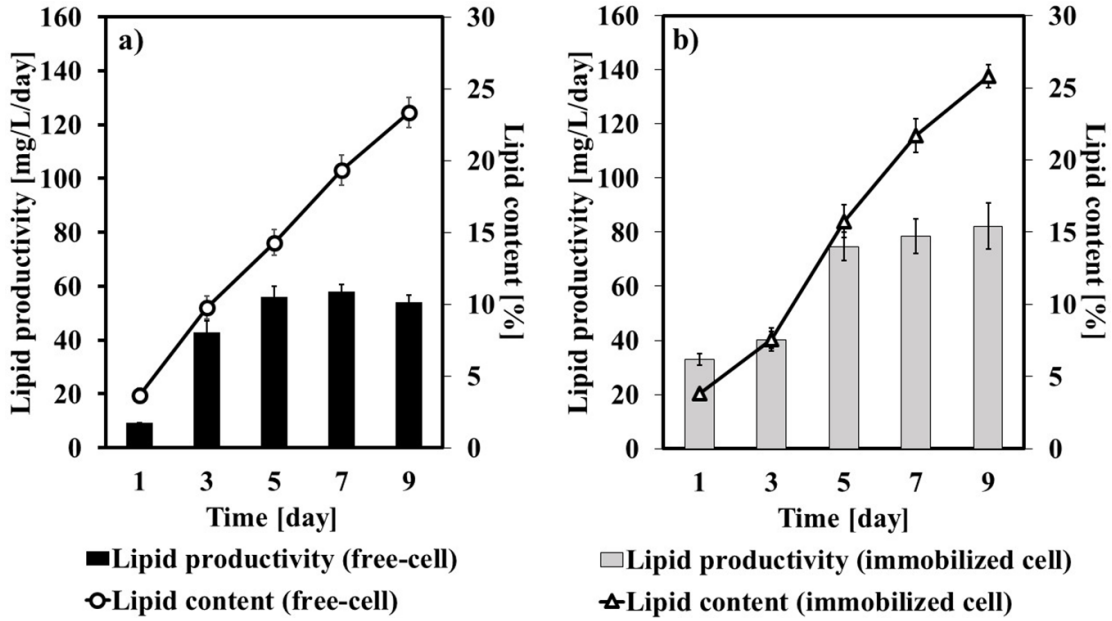

Figure 4. Lipid content and lipid productivity in (a) free-cell culture *C. sorokiniana* and (b) alginate immobilized culture *C. sorokiniana*. The error bars show the standard deviation of triplicate values.

Table 1. Fatty acid profile of lipids from free and immobilized *C. sorokiniana*.

FAME composition	Free cells (%)	Immobilized cells (%)
Palmitic Acid (C16:0)	34.9	34.6
Palmitoleic Acid (C16:1)	1.9	2.3
Stearic Acid (C18:0)	7.7	7.8
Oleic Acid (C18:1)	20.4	21.6
Linoleic Acid (C18:2)	24.9	25.1
Linolenic Acid (C18:3)	9.7	8.3
Eicosanoic Acid (C20:0)	0.6	0.3

The highest lipid productivity was obtained on day 9 at 82.22 ± 8.48 mg l^{-1} d for the immobilized alginate culture and 54.21 ± 2.48 mg l^{-1} d for the free-cell culture, this clearly shows how immobilization enhances the microalgae growth and is beneficial for both lipid content and lipid productivity.

The fatty acid profiles of free and immobilized cultures are shown in table 1. In both configurations, the fatty acids consisted of long-chain fatty acids; the predominant fatty acid was palmitic acid, followed by linoleic acid and oleic acid. The composition of the fatty acids in the immobilized culture included concentrations of oleic acid (21.6%) and linoleic acid (25.1%), which were slightly higher than those found in the free-cell culture (20.4% and 24.9%, respectively). The fatty acid profile of palm oil, which is one of the most utilized feedstocks for biodiesel production, contains mainly oleic and palmitic acids [32], which is similar to the fatty acid profile obtained by immobilized *C. sorokiniana*, indicating that these lipids have the potential to be used as biodiesel feedstock.

3.5. *C. sorokiniana* cells condition and distribution inside of the alginate beads

The alginate immobilized cells were stained and observed in a fluorescence microscope, for both culture forms, the highest lipid productivity occurred between day 5 and 9 of the culture. Fluoroscopic images of the cells grown in suspension culture and cells grown in immobilized culture are shown on figure 5, it can be observed that the cell density of the immobilized cells was higher than that of the free cells.

The CLSM images of the alginate beads are shown in figure 6, where it can be observed that on day 5, the cells were mostly around the edge of the bead, and there was a small number of cells in the center; however, by day 9, the total cell number increased, and they were distributed all over the bead.

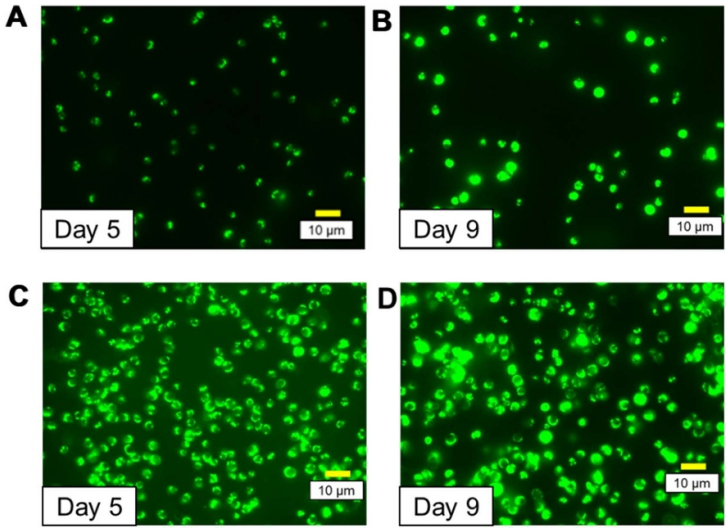

Figure 5. Fluoroscopic images of *C. sorokiniana cells,* (A) suspension culture day 5, (B) suspension culture on day 9, (C) immobilized culture on day 5, (D) immobilized culture on day 9.

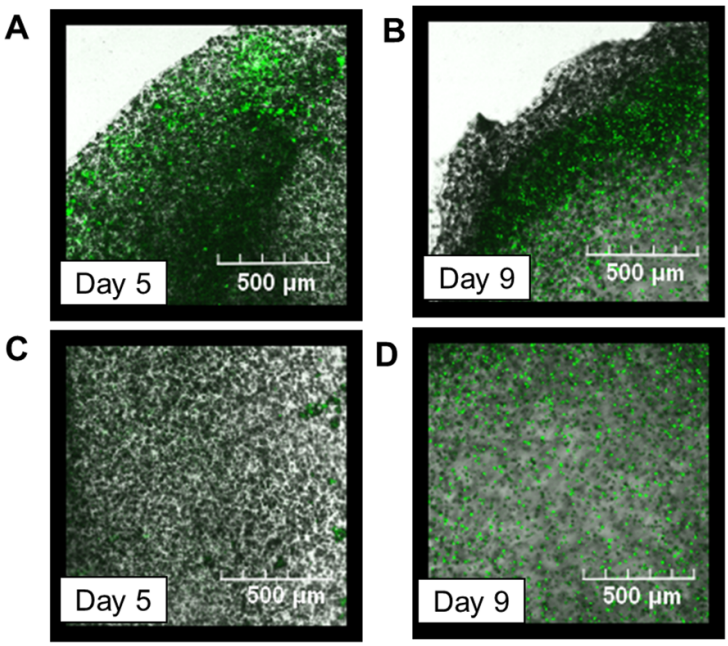

Figure 6. CLSM images of *C. sorokiniana* cells entrapped in an alginate bead, (A) side of alginate bead on day 5, (B) center of alginate bead on day 5, (C) side of alginate bead on day 9, (D) center of alginate bead on day 9.

4. Discussion

The results of the experiments conducted in this study showed an overall positive effect on the growth and lipid productivity by the immobilized microalgae *C. sorokiniana*. Previous studies focused on the utilization of alginate immobilized microalgae for the treatment of wastewater or nutrient removal, in each of them different optimized parameters were found, such as the optimum bead volume of 25% to obtain a comparable growth to that of free cells [11], optimum concentrations for alginate 2.42% and calcium chloride 2.69% [24], and optimum bead size of 3.5 mm [12], the optimized conditions found in each of these studies were applied to this research which allowed the growth and lipid productivity of *C. sorokiniana* to be enhanced.

A comparison between the results obtained in this study and previous studies regarding the use of *C. sorokiniana* for lipid accumulation is shown in table 2, where it can be seen that the dry cell weight and lipid content obtained by the immobilized culture under autotrophic conditions are higher than those

Table 2. Previous studies of *C. sorokiniana* for biodiesel production.

Microalgae	Growth condition	Initial glucose ($g\,l^{-1}$)	CO_2 (%)	Temperature (°C)	DCW ($g\,l^{-1}$)	Lipids (%)	References
C. sorokiniana	Autothropic (Free cell)	0	5	37	1.0	10%	[33]
C. sorokiniana	Autotrophic (Free cell)	0	10	37	1.2	11%	[33]
C. sorokiniana	Autotrophic (Free cell)	0	15	37	1.1	20%	[33]
C. sorokiniana	Heterotrophic (Free cell)	6	1	25	2.8	12%	[34]
C. sorokiniana	Mixotrophic (Free cell)	4	1	25	3.6	7%	[34]
C. sorokiniana	Autotrophic (Free cell)	0	2	30	2.1	23%	Present study
C. sorokiniana	Autotrophic (Alginate immobilized)	0	2	30	2.9	26%	Present study

obtained by a free cell culture in other studies, and it is comparable to the growth and lipid content obtained by utilizing heterotrophic or mixotrophic growth conditions, showing the potential that immobilization has to increase the lipid productivity of this microalgae strain.

One of the reasons for this increase in growth could be that in the immobilized system, a more efficient mixing was achieved compared with that of a free-cell culture, this is because the microalgae cells grew inside the alginate beads in the immobilized culture, rather than in the culture medium as in the free cell culture [12, 13], allowing the beads to remain suspended and in constant movement inside the culture medium by simple stirring or CO_2 bubbling, without the need for expensive stirring methods.

Mixing is a very important parameter in microalgae cultivation, and its function is to homogenize the culture medium and prevent the clumping of cells or settling inside the reactor [35]. Mixing also allows for a more uniform distribution of nutrients in the medium and it is critical to enhance the biofixation of CO_2. Poor mixing can lead to undesirable pH and nutrients gradients [36]. As immobilized cells remain in constant movement inside the system, they have better access to nutrients. However, it is difficult to achieve perfect cell mixing in suspended cultures.

As shown in figure 6, the microalgae cells were able to grow and disperse inside the alginate beads and their movement was not restrained. Lee *et al* [14] reported that the optimum bead size for cell growth was approximately 3.5 mm, if the bead size was bigger, there would be empty spaces inside the bead, caused by the cells inside the alginate beads migrating from the inside of the beads to the edge to prevent limited substrate diffusion and to find the optimal light conditions [14]. However, the bead size utilized in this study seemed to be appropriate because by day 9 of culture, the cells were localized both at the edge and in the center of the bead, allowing them to receive light and nutrients efficiently.

Light is important for microalgal growth; this basic energy source affects cell growth, carbon fixation ability, and productivity [37]. In an immobilized culture, the cells grow inside the beads rather than in the culture medium, which allows for better light penetration than that in the free cell culture. The light transmittance results shown in figure 3 indicate that the immobilized culture had a higher level of light transmittance than the free cell culture, which is beneficial for the cells. This could have been one of the reasons for the improvement in dry cell weight because it prevents the process of self-shading which usually occurs in free cell cultures, where the cells are unable to receive the same amount of light, and cells closest to the surface receive most of the light [38]. In an immobilized system, on the other hand, the cells grow inside a matrix rather than in the culture medium, which remains transparent and allows the cells to receive an equal amount of light.

From the results in figure 2, it can be seen that by day 7, the free cell culture had already reached its stationary phase and was approaching its death phase. However, for the immobilized culture, even after day 7, it continued to grow, resulting in a higher dry cell weight and a higher lipid productivity than that of the free cell. In addition, the immobilization strategy offers a shorter lag phase and acclimatization period than free cell culture [14], allowing lipid productivity to begin sooner.

5. Conclusions

The results of the present study showed the potential for immobilization to improve the growth and lipid productivity of the microalgae *C. sorokoniana* by achieving a growth that was 1.4-fold higher than that of a free cell culture, leading also to an increase in lipid content and productivity. Immobilization also simplifies the harvesting process, and its application could lower the cost associated with the harvesting of suspension cultures, which should promote the economic feasibility of biofuels production from microalgal biomass.

A limitation of this technology is the need to separate the cells from the alginate beads, in order to extract the lipids accumulated inside the cells, however, this could be avoided by utilizing a strain that secretes the desired chemical to the medium, future studies should apply the immobilization technology to these kinds of strains, to take advantage of the increase in growth by the immobilization and the simplification of harvesting, an example of these types of strains would be the cyanobacteria *Synechocystis* sp. pasteur culture collection (PCC) 6803 which secretes succinate and lactate into its fermentation medium [39].

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Acknowledgments

This research was partially supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), the New Energy and Industrial Technology Development Organization (NEDO) Feasibility Study Program, Japan, G-7 Scholarship Foundation, Fuji Foundation for Protein Research, and Yanmar Environmental Sustainability Support Association (YESSA).

Conflict of interest

The authors declare no competing financial interests that could influence the work reported in this paper.

CRediT author statement

Daniel A Alfaro-Sayes: Conceptualization, Investigation, Formal analysis, Data curation, Writing—original draft. Jerome Amoah: Conceptualization, Writing—Review & Editing. Nova Rachmadona: Writing—Review & Editing. Shinji Hama: Supervision, Resources. Tomohisa Hasunuma: Methodology, Resources, Supervision. Akihiko Kondo: Supervision, Resources, Project administration. Chiaki Ogino: Conceptualization, Resources, Writing—Review & Editing, Supervision, Project administration, Funding acquisition.

ORCID iDs

Daniel A Alfaro-Sayes https://orcid.org/0000-0002-4034-3505
Chiaki Ogino https://orcid.org/0000-0002-8906-7724

References

[1] Mathimani T, Uma L and Prabaharan D 2015 Homogeneous acid catalysed transesterification of marine microalga Chlorella sp. BDUG 91771 lipid—an efficient biodiesel yield and its characterization *Renew. Energy* **81** 523–33
[2] John R P, Anisha G S, Nampoothiri K M and Pandey A 2011 Micro and macroalgal biomass: a renewable source for bioethanol *Bioresour. Technol.* **102** 186–93
[3] Chia S R, Nomanbhay S B H M, Chew K W, Munawaroh H S H, Shamsuddin A H and Show P L 2022 Algae as potential feedstock for various bioenergy production *Chemosphere* **287** 131944
[4] Aikawa S, Joseph A, Yamada R, Izumi Y, Yamagishi T, Matsuda F, Kawai H, Chang J S, Hasunuma T and Kondo A 2013 Direct conversion of Spirulina to ethanol without pretreatment or enzymatic hydrolysis processes *Energy Environ. Sci.* **6** 1844–9
[5] Peter A P, Chew K W, Pandey A, Lau S Y, Rajendran S, Ting H Y, Munawaroh H S H, Van Phuong N and Show P L 2023 Artificial intelligence model for monitoring biomass growth in semi-batch Chlorella vulgaris cultivation *Fuel* **333** 126438
[6] Khan S A, Rashmi Hussain M Z, Prasad S and Banerjee U C 2009 Prospects of biodiesel production from microalgae in India *Renew. Sustain. Energy Rev.* **13** 2361–72
[7] Jacob A, Ashok B, Alagumalai A, Chyuan O H and Le P T K 2021 Critical review on third generation micro algae biodiesel production and its feasibility as future bioenergy for IC engine applications *Energy Convers. Manage.* **228** 113655
[8] Tasić M B, Pinto L F R, Klein B C, Veljković V B and Filho R M 2016 Botryococcus braunii for biodiesel production *Renew. Sustain. Energy Rev.* **64** 260–70
[9] Lee K Y and Mooney D J 2012 Alginate: properties and biomedical applications *Prog. Polym. Sci.* **37** 106–26

[10] Lam M K and Lee K T 2012 Immobilization as a feasible method to simplify the separation of microalgae from water for biodiesel production *Chem. Eng. J.* **191** 263–8
[11] Urrea D A M, Gimenez A V F, Rodriguez Y E and Contreras E M 2021 Immobilization of horseradish peroxidase in Ca-alginate beads: evaluation of the enzyme leakage on the overall removal of an azo-dye and mathematical modeling *Process Saf. Environ. Prot.* **156** 134–43
[12] Moreno-Garrido I 2008 Microalgae immobilization: current techniques and uses *Bioresour. Technol.* **99** 3949–64
[13] Cheirsilp B, Thawechai T and Prasertsan P 2017 Immobilized oleaginous microalgae for production of lipid and phytoremediation of secondary effluent from palm oil mill in fluidized bed photobioreactor *Bioresour. Technol.* **241** 787–94
[14] Lee H, Jeong D, Im S J and Jang A 2020 Optimization of alginate bead size immobilized with Chlorella vulgaris and Chlamydomonas reinhardtii for nutrient removal *Bioresour. Technol.* **302** 122891
[15] Mallick N 2002 Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review *Bio. Met.* **15** 377–90
[16] Kube M, Mohseni A, Fan L and Roddick F 2019 Impact of alginate selection for wastewater treatment by immobilised Chlorella vulgaris *Chem. Eng. J.* **358** 1601–9
[17] Kube M, Mohseni A, Fan L and Roddick F 2020 Energy and nutrient recovery by treating wastewater with fluidised-beds of immobilised algae *J. Water Process Eng.* **38** 101585
[18] Kube M, Spedding B, Gao L, Fan L and Roddick F 2020 Nutrient removal by alginate-immobilized Chlorella vulgaris: response to different wastewater matrices *J. Chem. Technol. Biotechnol.* **95** 1790–9
[19] Hashim M A, Tan H N and Chu K H 2000 Immobilized marine algal biomass for multiple cycles of copper adsorption and desorption *Sep. Purif. Technol.* **19** 39–42
[20] Sharmila A, Altaf A, Anagha C and Shreyas M 2020 Cr(VI) removal from industrial wastewater using immobilized algae *Biosci. Biotechnol. Res. Commun.* **13** 108–14
[21] Zhang L J, Ying G G, Chen F, Zhao J L, Wang L and Fang Y X 2012 Development and application of whole-sediment toxicity test using immobilized freshwater microalgae Pseudokirchneriella subcapitata *Environ. Toxicol. Chem.* **31** 377–86
[22] Al-Hasawi Z M, Abdel-Hamid M I, Almutairi A W and Touliabah H E 2020 Response of *Pseudokirchneriella subcapitata* in free and alginate immobilized cells to heavy metals toxicity *Molecules* **25** 2847
[23] Hahn J J, Ghirardi M L and Jacoby W A 2007 Immobilized algal cells used for hydrogen production *Biochem. Eng. J.* **37** 75–79
[24] Maswanna T, Phunpruch S, Lindblad P and Maneeruttanarungroj C 2018 Enhanced hydrogen production by optimization of immobilized cells of the green alga Tetraspora sp. CU2551 grown under anaerobic condition *Biomass Bioenergy* **111** 88–95
[25] Ng F L, Phang S M, Periasamy V, Yunus K and Fisher A C 2017 Enhancement of power output by using alginate immobilized algae in biophotovoltaic devices *Sci. Rep.* **7** 1–8
[26] Li R, Yang J, Pan J, Zhang L and Qin W 2018 Effect of immobilization on growth and organics removal of chlorella in fracturing flowback fluids treatment *J. Environ. Manage.* **226** 163–8
[27] Sepian N R A, Yasin N H M and Ramesh N 2022 Immobilization method to separate microalgae biomass for fatty acid methyl ester production *Chem. Eng. Technol.* **45** 1474–81
[28] Cuaresma M, Janssen M, Vílchez C and Wijffels R H 2009 Productivity of Chlorella sorokiniana in a short light-path (SLP) panel photobioreactor under high irradiance *Biotechnol. Bioeng.* **104** 352–9
[29] Choi Y Y, Hong M E, Chang W S and Sim S J 2019 Autotrophic biodiesel production from the thermotolerant microalga chlorella sorokiniana by enhancing the carbon availability with temperature adjustment *Biotechnol. Bioprocess Eng.* **24** 223–31
[30] Amoah J, Hasunuma T, Ogino C and Kondo A 2019 5-Hydroxymethylfurfural production from salt-induced photoautotrophically cultivated Chlorella sorokiniana *Biochem. Eng. J.* **142** 117–23
[31] Lizzul A M, Lekuona-Amundarain A, Purton S and Campos L C 2018 Characterization of chlorella sorokiniana, UTEX 1230 *Biology* **7** 1–12
[32] Juanssilfero A B, Kahar P, Amza R L, Yopi Sudesh K, Ogino C, Prasetya B and Kondo A 2019 Lipid production by Lipomyces starkeyi using sap squeezed from felled old oil palm trunks *J. Biosci. Bioeng.* **127** 726–31
[33] Varshney P, Beardall J, Bhattacharya S and Wangikar P P 2018 Isolation and biochemical characterisation of two thermophilic green algal species- Asterarcys quadricellulare and Chlorella sorokiniana, which are tolerant to high levels of carbon dioxide and nitric oxide *Algal Res.* **30** 28–37
[34] Li T, Zheng Y, Yu L and Chen S 2014 Mixotrophic cultivation of a Chlorella sorokiniana strain for enhanced biomass and lipid production *Biomass Bioenergy* **66** 204–13
[35] Posten C 2009 Design principles of photo-bioreactors for cultivation of microalgae *Eng. Life Sci.* **9** 165–77
[36] Assunção J and Malcata F X 2020 Enclosed "non-conventional" photobioreactors for microalga production: a review *Algal Res.* **52** 102107
[37] Alami A H, Alasad S, Ali M and Alshamsi M 2021 Investigating algae for CO_2 capture and accumulation and simultaneous production of biomass for biodiesel production *Sci. Total Environ.* **759** 143529
[38] Jayakumar S, Bhuyar P, Pugazhendhi A, Rahim M H A, Maniam G P and Govindan N 2021 Effects of light intensity and nutrients on the lipid content of marine microalga (diatom) Amphiprora sp. for promising biodiesel production *Sci. Total Environ.* **768** 145471
[39] Hasunuma T, Matsuda M and Kondo A 2016 Improved sugar-free succinate production by Synechocystis sp. PCC 6803 following identification of the limiting steps in glycogen catabolism *Metab. Eng. Commun.* **3** 130–41