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A Novel 3D Paper-Based Immobilized Cultivation Platform for Accelerated Microalgae Growth

Jinxin Guo, Chaocan Li, Yufei Wang, Yuanyuan Song, Daohong Zhang*
Tianjin Key Laboratory of Aquatic Science and Technology, School of Environmental and Municipal Engineering, Tianjin Chengjian University, Tianjin, China.
*Corresponding Author.

Abstract:

Compared to suspended cultivation, immobilized cultivation methods of microalgae were reported to enhance biomass productivity and reduce harvesting cost. In this paper, a 3D microalgae immobilized cultivation method was developed on a paper platform, composed by six 5 mm-diameter wells fabricated with a wax printer. The microalgae immobilized in agar gel were loaded on the top of the wells, which was exposed to air. Agar with higher density was loaded on back of the well (immersed in medium) to both prevent leakage of microalgae and transport nutrients to cells. Results showed that the microalgae entrapped in gels stand on the top of medium grew faster than the microalgae gels immersed in medium, owing to high carbon dioxide concentration and faster transmission rate of air. Meanwhile, six wells under same culturing condition equaled to six cultures in Erlenmeyer flasks, which saved space and medium for cultivation. Furthermore, the microalgae could be obtained easily by just collecting the paper platform. No microalgae growth out of the gel also indicated the recyclable of the culture medium. Microalgae were cultivated on this platform with medium containing different salts as a test. Therefore, this platform represents an alternative for microalgae flask culture and a promising microalgae immobilized cultivation method, with decreased culture cost, time and space, which can be utilized for easier and faster study on microalgae wastewater treatment and biofuel production.

Keywords*: Microalgae, Immobilized cultivation, Paper-based, Agar gel.*

I. INTRODUCTION

About 40% of global photosynthesis is performed by microalgae[1]. Microalgae are good candidates for wastewater treatment[2], and their byproducts are of great value for cosmetic, food, pharmaceutical and biofuels industries[3,4]. However, the utilization of microalgae in water treatment should be under inspection to avoid algal blooms, while the byproducts are still far away from commercialization, majorly due to the high cost raised from microalgae harvesting (centrifugation or chemical flocculants)[3,5].

In order to solve those problems, non-suspended cultivation method for microalgae were developed, which could prevent microalgae from diffusion to water and reduce cost for microalgae collection[1,6]. Compared to suspended microalgae cultivation method, such as open, unlined ponds, and closed photobioreactors, the biomass productivities of non-suspended cultivation methods were largely

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improved[7], with lower energy and water requirements[8], and higher efficiency for light utilization[9].

Non-suspended cultivation of microalgae can be classified into two categories, immobilized cultivation (microalgae entrapment) and attached cultivation (microalgae surface attachment)[9]. However, all of the cultivation methods required microalgae immersing in medium, so the transportation of gas in liquid may be one of the bottlenecks preventing microalgae growth and byproducts synthesis. Furthermore, microalgae or fixation chemicals may leak to the medium in the immobilized cultivation systems[9,10]. In order to solve those problems, a 3D microalgae immobilized cultivation method supported by agar hydrogel on fabricated paper platform is proposed. The filter paper is employed as a carrier for the microalgae with printed hydrophobic barrier to separate different cultural zones. Microalgae absorbed in low density agar gel on the top of the paper platform, are exposed to air. High density agar gel casted on the back of the paper platform is immersed in medium, in order to bridge the microalgae with the nutrition and prevent the migration of microalgae to medium. Lab-based microalgae cultivation was tested with this method, which showed rapid growth and was able to perform multi-tests in one platform. Utilization of this method can minimize the operation and time for microalgae selection and cultivation parameter, which is a great platform for easier and faster study on microalgae wastewater treatment and biofuel production.

II. MATERIALS AND METHODS

2.1 Materials

Chlorella vulgaris (C. vulgaris, FACHB-8), Cholrellapyrenoidosa (C. pyrenoidosa, FACHB-9), Chlorella ellipsoidea (C. ellipsoidea, FACHB-40) were purchased from algal-species database of Wuhan Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). All the chemicals and culture plates were bought from Sangon Biotech (Shanghai, China). Whatman #1 cellulose paper was from Whatman (Piscataway, NJ).

2.2Design and Fabrication of Paper Platform

The paper platform was designed to a 2×3 array format (Fig 1), and fabricated according to literature protocols[11,12]. Briefly, the pattern for the paper platform was designed using Adobe Illustrator software (Adobe Systems Incorporated, San Jose, CA). A Xerox ColorQube 8580 solid ink printer (Xerox Corporation, Norwalk, CT) was utilized to spray the wax pattern on Whatman #1 cellulose paper. The resulted paper was heated on a heating plate at 150 °C for 2 min. The wax-fabricated paper platform was stored in ambient condition before usage.

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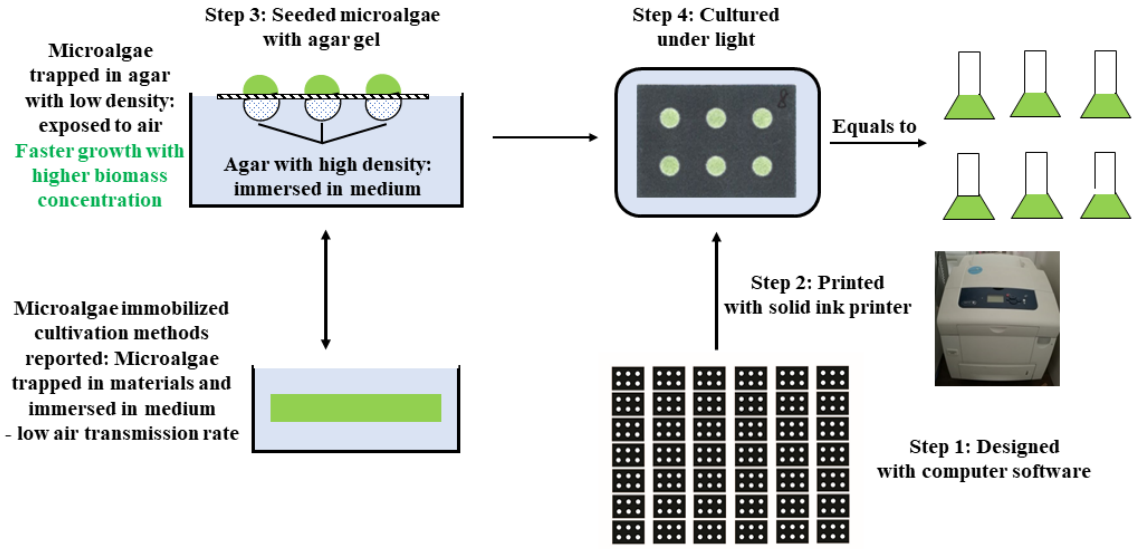

Fig 1: scheme for the development of 3D paper-based microalgae immobilized cultivation platform

2.3Analysis of Microalgae Amount on Paper

A 4 µL aliquot of *C. ellipsoidea* with different OD (range 0-8.0, λ_{abs} =680 nm) was added on paper wells and dried. The result was scanned, and the color intensity was quantified with ImageJ software. Microalgaebiomass concentration was measured by cell counting with a hemocytometer under a microscope (XSZ-138, AOK International Group Ltd., Shanghai, China).The microalgae array was dried under a vacuum drier, and the microalgae with agar were cut out and weighed with a balance. The dry weight of microalgae was calculated by subtracting the weight of agar from the measured dry weight.

2.4Development of 3D Paper-Based Microalgae Cultivation Array

Steps for development of3D paper-based microalgae cultivation array was illustrated in Fig 1.First, a 20 µL aliquot of agar (1%, w/v) was added on the back of each well. Then on the top of the well, a 10 µL mixture of agar (0.3%, w/v) and microalgae (*C. pyrenoidosa*, 2.0 µL, OD 1.0) was added. Finally, the microalgae plate was put in a 90 mm glass plate with the support of one cotton layer and ten filter paper layers. The resulted paper-based microalgae array was utilized in following experiments.

2.5Paper-Based Microalgae Cultivation with BG11 Medium

For microalgae growth in BG11 medium, the back agar gels of the paper platform were soaked in 10 mL medium. The microalgae were cultured under continuous illumination (about 10000 W/m^2) at 25 °C for 0-8 days. The paper platform with gels was collected and dried under ambient condition. The result was scanned and the color intensity of each gel was analyzed with ImageJ software.

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2.6 Eleven-Day Microalgae Serial Cultivation Study

Microalgae arraywere cultivated for eleven days in two steps. First, the microalgae gel was cultured on paper platform under continuous illumination (about 10000 W/m^2) at 25 °C for 1-10 days. Then the resulted microalgae gel was cut and immersed in BG11 medium in a 1.5 mL centrifuge tube under continuous illumination (about 10000 W/m^2) at 25 °C for another 1-10 days. The paper platform with gels was collected and dried under ambient condition. The result was scanned and the color intensity of each gel was analyzed with ImageJ software.

2.7 Paper-Based Microalgae CultivationwithSingle Salt

The effect of single salt upon microalgae growth was evaluated on paper platform. Five salts were tested, including K_2HPO_4 (1.0 g/L), $NaNO_3$ (37.5 g/L), Na_2CO_3 (0.50 g/L), $CaCl_2{\cdot}2H_2O$ (0.90 g/L), and $MgSO_4{\cdot}7H_2O$ (1.875 g/L). A 10 μL aliquot of each salt was added on the top of well. Microalgae paper plate was cultivated (with back gels soaked) in 10 mL water under continuous illumination (about 10000 W/m^2) at 25 °C Then the paper platform with gels was collected and dried under ambient condition. The result was scanned and quantified with ImageJ software.

2.8Paper-Based Microalgae Cultivation with One Salt absent in BG11 Medium

The effect of single salt absent upon microalgae growth on paper platform was evaluated with paper-based microalgae plate. Five salts were tested, including K_2HPO_4 (1.0 g/L), $NaNO_3$ (37.5 g/L), Na_2CO_3 (0.50 g/L), $CaCl_2{\cdot}2H_2O$ (0.90 g/L), and $MgSO_4{\cdot}7H_2O$ (1.875 g/L). Salt mixtures containing four of salts listed above was made (totally five mixtures), and a 10 μL aliquot of one mixture was added on the top of well. Microalgae paper plate was cultivated (with back gels soaked) in 10 mL water under continuous illumination (about 10000 W/m^2) at 25 °C. The paper platform with gels was collected and dried under ambient condition. The result was scanned and quantified with ImageJ software.

III. RESULTS AND DISCUSSION

3.1 3D Microalgae Agar Array on Paper Platform

Microalgae culture is usually performed in Erlenmeyer flasks or culture plates for microalgae selection or culture condition adjustment, which is time-consuming, expensive and labor intensive. Here, we designed a 3D microalgae immobilized cultivation method on a paper platform. Microalgae were trapped in agar gels, standing on a hydrophilic well in wax-fabricated filter paper. Paper array was fabricated with a solid ink printer, generating hydrophobic area with wax and hydrophilic wells for microalgae cultivation. A low density of agar gel was utilized to support microalgae and leave space for microalgae growth and air penetration, which was loaded on the top on the paper platform. A high density of agar gel was sealed on the back of the paper platform, in order to prevent leakage of the microalgae, help the paper platform stand above the medium, and transport nutrition to the microalgae. Agar was chosen as an example, which was a biopolymer extracted from certain red algae[13], and were widely utilized in DNA electrophoresis and

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microalgae immobilization[8].

3.2 Immobilized Cultivation of Microalgae in Air-Exposed Agar Gel

No microalgae was found out of the gel after culturing for 1-8 days in the paper array, indicating the microalgae adsorbed to the agar gel. In an Erlenmeyer flask, the highest final OD of microalgae can hardly reach 4.0 by our experience and other reports[14]. Fig 2 showed that the final density of microalgae gels on paper platform was much higher than the ones growing in flasks. Meanwhile, the cell density and biomass dry weight can hardly be reached by traditional cultivation methods. The higher microalgae cell density and dry weight (Fig 3) may due to well separated cells in the gel, forming small microalgae aggregates without hindering air and nutrient penetrating to cells in the middle.

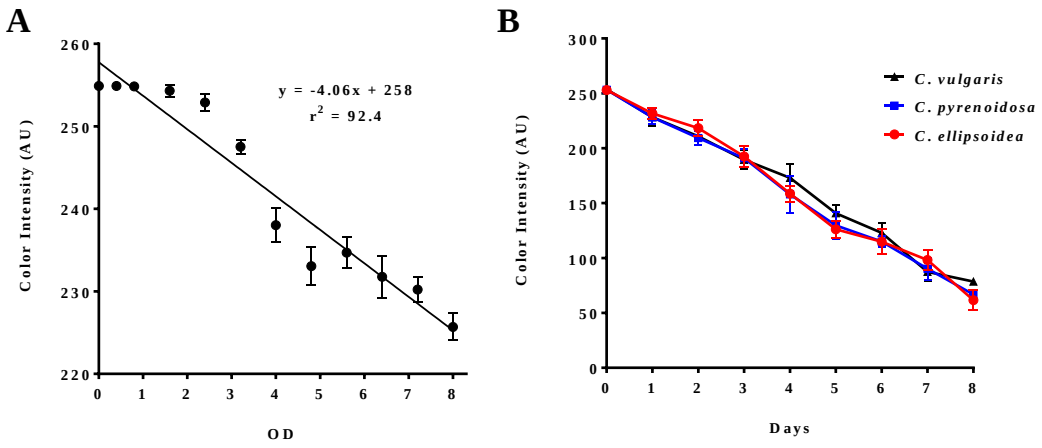

Fig 2: color density vs. OD (A) and color density for eight-day cultivation of three microalgae species (B)

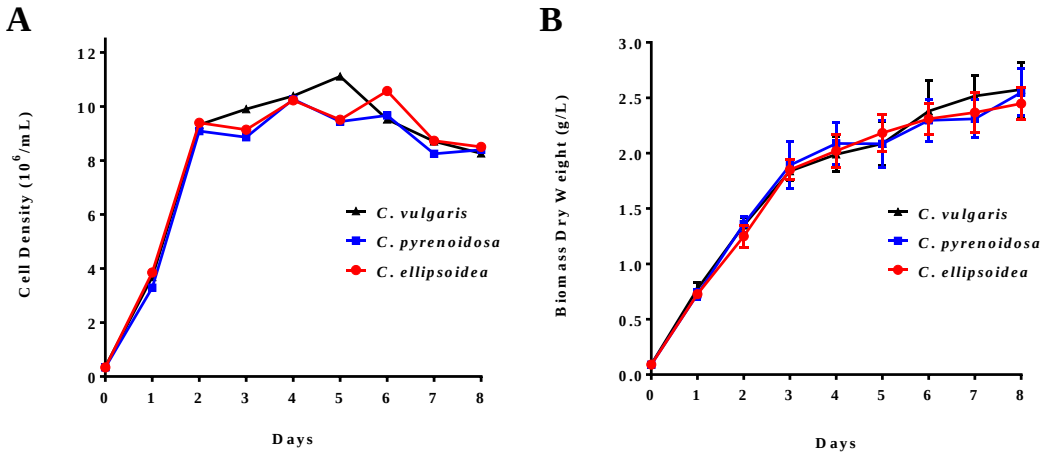

Fig 3: cell density (A) and biomass dry weight (B) increased during eight-day microalgae cultivation

3.3 Comparison of Microalgae Gel Cultivation Standing on Paper Platform and Immersion in BG11

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Medium

An eleven-day microalgae serial cultivation was performed on paper platform first, followed by immersion in BG11 medium. The gels were collected and compared with the microalgae gel culture on paper platform. Results showed that the microalgae on paper platform grew much faster than the ones in medium (**TABLEI**). The microalgae cultured on paper platform for less than 5 days could only grow to a relatively low density (equaled to 4-5 days' microalgae culture on paper platform). While the microalgae cultured on paper platform for more than 5 days hardly grew after immersed in medium. Therefore, compared to the microalgae grew in medium, the ones culture on paper platform grew faster, and could accumulate to a much higher final density than the ones growing in medium.

TABLE I. Eleven-day serial cultivation for microalgae trapped in gels

C. vulgaris				***C. pyrenoidosa***				***C.ellipsoidea***			
Days for cultivating on paper platform	**Days for cultivating in medium**	**Color Intensity (AU)**	**OD equals to how many days cultivation on paper platform**	**Days for cultivating on paper platform**	**Days for cultivating in medium**	**Color Intensity (AU)**	**OD equals to how many days cultivation on paper platform**	**Days for cultivating on paper platform**	**Days for cultivating in medium**	**Color Intensity (AU)**	**OD equals to how many days cultivation on paper platform**
1	10	111.983	4	1	10	112.475	3	1	10	94.122	3
2	9	96.634	4-5	2	9	81.311	4-5	2	9	78.248	3-4
3	8	120.282	4	3	8	83.639	4-5	3	8	96.424	3
4	7	123.966	4	4	7	98.185	4	4	7	101.151	3
5	6	85.672	4-5	5	6	58.189	5	5	6	63.265	4
6	5	61.492	5-6	6	5	28.613	8	6	5	43.462	6
7	4	50.092	5-6	7	4	4.303	>8	7	4	39.790	6-7
8	3	27.584	7-8	8	3	0.672	>8	8	3	10.752	8
9	2	8.714	>8	9	2	0.718	>8	9	2	0.987	>8
10	1	5.807	>8	10	1	0.571	>8	10	1	0.370	>8

High carbon dioxide concentration and transmission rate by exposure in air might contribute to the increased growth rate. In surface water, carbon dioxide is usually less than 10 ppm, while the carbon dioxide concentration in air is around 412 ppm[15]. High concentration of carbon dioxide combining with elevated transmission rate of the air leads to faster gas penetration and cell growth rate. Meanwhile, stirring or shaking, which is usually required in all microalgae cultivation methods, can be avoided, since microalgae were mixed well with agar solution before seeding, forming evenly separated cells in the gel and preventing settling down to the bottom. Therefore, energy cost of this method can be reduced.

3.4 Paper-Based Microalgae Cultivation with Single Salt or One Salt absent in BG11 Medium

Paper-based microalgae array was cultivated in different medium as a preliminary test. First, one of the five main salts in BG11 was used as single nutrition (Fig 4A). The growth rate of three main nutrients (i.e., the P source (K_2HPO), the N source ($NaNO_3$) and the C source (Na_2CO_3)) were similar to that of negative

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control (water only). However, the growth rate of the microalgae added with $CaCl_2$was slower than that of the negative control (water only), possibly because excessive single Ca ions inhibit the growth of the microalgae or promote the oil accumulation. The color intensity of the microalgae added with $MgSO_4$was lower than that of the negative control, possibly because the addition of Mg results in the accumulation of chlorophyll. Paper-based microalgae array was also cultivated in BG11 medium with one salt absent (Fig 4B). Microalgae cultured in most salt mixtures and in BG11 medium (positive control) showed similar rates. However, the growth rate of microalgae cultured with $NaNO_3$ was much slower, indicating the importance of N source for microalgae cell growth. All the experiments were performed in six parallel tests. Therefore, microalgae cultivation on paper platform can be utilized as a replacement for Erlenmeyer flask tests, which can save both cost and space, and offer a good model in the studies on microalgae wastewater treatment and biofuel production.

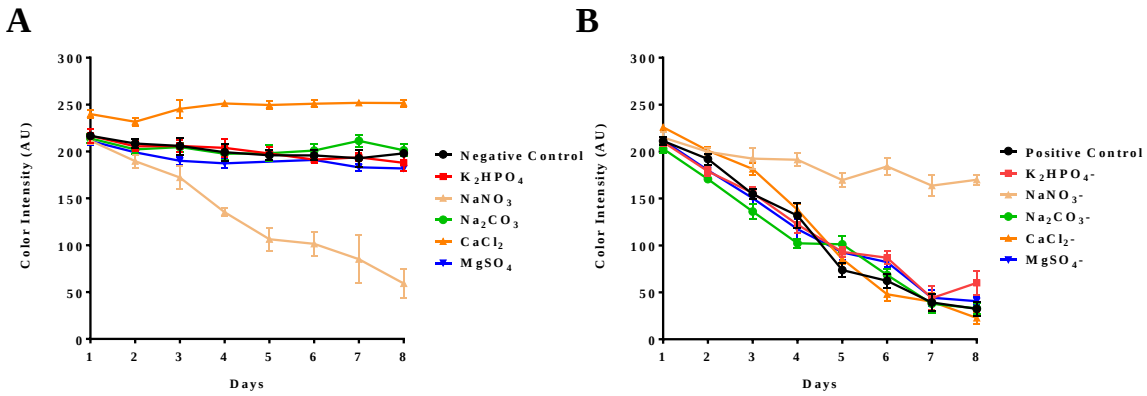

Fig 4: microalgae cultivation with single salt (A) and with one salt absent in BG11 medium (B)

IV. CONCLUSION

A paper-supported 3D microalgae immobilized cultivation method was developed, which showed faster growth than microalgae cultivation in medium. Microalgae were trapped in agar gels standing on the top of5 mm paper wells, which were blocked by denser agar gels on the back of the filter paper. Nutrients were transferred by the bottom gels immersed in the medium, and gas were diffused directly to microalgae. The air-exposed microalgae grew faster, which can be harvested easily by collecting the top gels. This platform can serve as an easier and faster model for microalgae wastewater treatment and biofuel production. Further utilizationof this platform, such as microalgae biofuel production, will be performed in future studies.

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