Microalgae *Chlorella Vulgaris* Harvesting Via Co-Pelletization with Filamentous Fungus

Ghaidaa Alrubaie 1* Rana H. H. Al-Shammari 2

Received 8/10/2017
Accepted 29/1/2018

This work is licensed under a Creative Commons Attribution 4.0 International License.

Abstract:

The objective of this study was to progress another method for coagulation/flocculation of the microalga *Chlorella vulgaris* via pellet-forming of the fungal species *Aspergillus niger* which was isolated from municipal wastewater mud and the facultative heterotrophic microalga *C. vulgaris* was used. The main factors studies were spore inoculums, organic carbon concentration in medium as well as pH variation which had considerably positive effects on microalgae/fungi co-pelletization formation. The process parameters are an inoculum 1×10^4 spores/ML, 15 g/l sucrose as carbon source and pH ranged from 5 - 7.0 were found optimal for efficient microalgae/fungi co-pelletization formation. For autotrophic growth, when pH of culture broth was adjusted to 5.0 - 7.0 with organic carbon addition (15 g/L sucrose), almost complete harvesting efficiency of the microalga was achieved. Furthermore, it was observed that diameter and the concentration of microalgae/fungi pellets were pretentious by the shaker rotation. The new harvesting technology established in this study will decrease the microalga harvesting cost and will be possible to adapt this technique to all microalgal species as an alternative to other old-style harvesting approaches.

Keywords: bio flocculation, autotrophic, heterotrophic microalgae, filamentous fungi.

Introduction:

Microalgae are photosynthetic organisms that can grow rapidly and live in hard conditions because of their simple cellular structure and short life cycle (1, 2). The production of biodiesel from microalgae is not economically feasible because of the very high cost, mainly water pumping, mixing, harvesting the microalga biomass and maintenance require very high-energy inputs (3, 4). Harvesting of algae from water or other liquids culture media is currently a difficult process. Algal cultivation can account for up to 50% of the total cost of biomass production (5,6) due to the physical properties and nature of algae such as a low density, and the small size and tendency to grow as single cells, they are challenging to harvest (7).

Several methods were used to harvest the microalgae and provide the high value of biomass such as flotation, centrifugation sedimentation, filtration, ultrasonic combination and flocculation can also added to these techniques (7, 8). Bioflocculation is a natural flocculation process hastened with biomolecules from microbial cells(9).

In recent years, increased attention was focused on cultivation of microalgae with fungi (Co-cultivation) for several reasons, the most important of which is the high efficiency of bio-flocculation of microalgal cells for added chemicals and low energy inputs (10,11,12,13). Different microalgae and fungal strains can be applied for this transformative technique. This design was applied to in all areas like in the microbial oil production, wastewater treatment to remove nutrients and heavy metals (11). Aims of the study: Isolation of aquatic filamentous fungi from the municipal wastewater mud in Baghdad City, Evaluating the efficiency of filamentous fungi for the formation of bioflocculation under different growth conditions; autotrophic, heterotrophic and mixotrophic studying the main factors that affect co-culture of microalgae / fungal pellets formation.

Materials and Methods:

Experimental project

The experiments were carried out in three steps. In the first step, filamentous fungal strains were isolated from municipal wastewater. The following isolated fungi: *Aspergillus flavous*, *A. niger*, *Penicillium* sp., *Alternaria* sp. The spores of...
applicant fungal strains (1×10^4 spore/ml as standard spore suspension) were cultured on glucose peptone broth (GPB) for 5 days and pellets-forming fungal strains were partitioned and identified based on their morphological analyses (14). In the second stage, the isolated promising fungal strain Aspergillus niger was studied for its pellet-forming capability under several culture conditions including different initial inoculate of A. niger (approximately 1×10^6 spores/ml), pH variation (ranged from 5 to 7.0), and different sucrose concentrations (5, 10, 15, 20, and 25 g/l) in the medium when cultivated heterotrophically. In the third step, bioflocculation of chlorella vulgaris by pelletization of filamentous fungus A. niger under heterotrophic condition 15 g/l glucose was added to the culture broth with different concentrations, and pH of culture broth was adjusted accordingly by addition of 1 M sulfuric acid solution to culture broth.

**Spore inoculum**
Fungal spore suspension was obtained by rinsing the PDA slant with 10 ml PBS buffer and collected in a sterile tube prior to use (19). Fungal spores were counted using a hemocytometer under light microscope.

**Microscopic imagining of microalgae/fungi complex**
The pelletized microalgae/fungi biomass was picked up in order to perform a microscopic study and was photographed under the microscopes (Olympus) with objective lenses of 40 magnifications.

**Determination of microalgal growth**
Daily measurement of the inoculated medium algal culture of Optical density (OD) at 680 nm using a spectrophotometer as an indicator for algal growth density. A linear relationship between OD and dry weight (DW, g/L) was determined for algal strain (20): Dry weight (g/l) = 0.34 OD_{680} - 0.0068, R^2 = 0.997. Calculate the harvesting ratio of microalga according to the following formula:

\[
\text{The microalgae harvest (\%)= } \left( \frac{\text{total algal biomass} - \text{suspended biomass}}{\text{total algal biomass}} \right) \times 100
\]

All microalgae-fungi co-cultures were done in triplicate and mean value and standard error of results was calculated to assess the difference between samples. The concentration of glucose was strong-minded using the dinitrosalicylic acid examine (21).

**Results and Discussion:**
**Screening the capability of isolated fungal strains for formation of pellets**
The isolated fungal strains belong to filamentous fungi (as recorded in Table 1) revealed that some of isolated candidate fungal strains showed fungal mycelium on PDA plates after 5-days of cultivation. The isolated fungal strains were more examined for pellets-forming capability by growing fungal spores of these candidate fungal strains on PGB medium. It was found that only one fungal
strain (A. niger) established pellet-forming ability (1–5 mm diameter) while other fungal strains formed loose ones, suggesting that pellet-formation for filamentous fungi is strain-specific. This was in agreement with other investigations (22). Filamentous fungi characterize suitable bioflocculating agents because of their self-pelletization and extraordinary microalgal trapping efficiencies. Fungal self-pelletization has been observed for numerous filamentous strains (23, 24). Aggregates/pellets were representatives of Aspergillus spp., Basidiomycete spp, Phanerochaete spp. (25).

Table 1. Pellets-forming of isolated filamentous fungal strains derived from municipal wastewater mud

<table>
<thead>
<tr>
<th>Fungi Isolates</th>
<th>Pellet formation capability</th>
<th>Early pH</th>
<th>Last pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>+</td>
<td>6.7</td>
<td>4.8</td>
</tr>
<tr>
<td>A. flavus</td>
<td>-</td>
<td>7</td>
<td>5.7</td>
</tr>
<tr>
<td>Penicillum sp.</td>
<td>-</td>
<td>6.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Alternaria sp.</td>
<td>-</td>
<td>6.5</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Note: + = pellets-forming and - = non-pellets-forming

Fungal assisted bio-flocculation of heterotrophic microalgal cells

The filamentous fungus A. niger could assist bioflocculation of heterotrophic microalgal cells by formation of pellets under certain conditions (Fig. 1). The algae lost culture most of its green color, and turned transparent; microalgal cells were pelleted (15).

Spores inocula of different concentrations were added to culture media ranged from 1×10² to 1×10⁵ spores/mL could help the formation of fungi–algae pellets under heterotrophic growth conditions (Table 2). It was found that when initial spores inoculum was 1 × 10⁴ spores/mL, fungi–algae pellets was not observed even after 7 days of cultivation., which is in agreement with reports by Foster (26), who explained that fungal cell pelletization ,number and size of pellets depending on the inoculum concentration of fungal spores(27, 28). Meanwhile the early spore concentration of 1×10⁴ spores/ ml was suitable for well-organized pellets formation in short growth period. This inoculum size was used in the following experiments.

Table 2. Effect of inoculum size on the fungi–algae pellet formation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Inoculum sizes (spores/mL)</th>
<th>1×10⁻³</th>
<th>1×10⁻⁴</th>
<th>1×10⁻⁵</th>
<th>1×10⁻⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>High pH</td>
<td>7.01</td>
<td>7.04</td>
<td>7.04</td>
<td>6.94</td>
<td>7.11</td>
</tr>
<tr>
<td>Low pH</td>
<td>5.02</td>
<td>4.9</td>
<td>4.5</td>
<td>4.02</td>
<td>4.05</td>
</tr>
<tr>
<td>Culture time (day)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Pellet formation</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
</tr>
</tbody>
</table>

Note: + stands for pellets-forming; - stands for non-pellets-forming.

Results show that pH was the major factor that effected on the formation of the algae-fungal pellets. It was suggested that the algae have negative surface charge. The reason for this is the occurrence of proton-active carboxylic, phosphoric, hydroxyl and amine functional groups (29). Fungal hyphae and mycelia are positively charged because they contain polysaccharides. This can possibly neutralize the negative charges on the algal surface, allowing the attachment to the fungal cell wall. Moreover, fungi secret hydrolytic enzymes in the presence of the microalgal cells, the surface charge and the cell size and density were the main factors for the stability of microalgae suspension in the culture (30). Filamentous fungi A. niger cells with microalgae cells C. vulgaris cells (co-cultured) formed cell pellets. These pellets were sphere-shaped, relatively homogeneous in size and dense the pellets in the co-culture showed green color instead of grey one (Fig. 2). The observed green color of pellets was formed due to microalgal cells entrapped in or attached to the hyphae of the filamentous fungus A. niger, and the size of the pellets was about 3–6 mm (Fig. 3) This technique can provide an easy way to harvest microalgal cells through filtration with a sieve. (31,32).

Figure 1. Flocculation of the microalgal Chlorella vulgaris by Aspergillus niger. (A) A. niger culture (B) C.vulgaris autotrophically grown culture (C) Flocculation of C.vulgaris autotrophically grown co-cultured with A. niger (D) C.vulgaris Mixotrophically grown (E) Flocculation of C.vulgaris heterotrophic culture mixed with A. niger pellets, time 24 h later.
Figure 2. Comparison of fungi pellets/fungi–algae pellets grown on shaker under different rotation speed: (A: heterotrophic algae and B: autotrophic algae) fungi and fungi-pellets formation with rotation speed at 150 rpm/min. after 72h of incubation.

Figure 3. Microscopic photo of fungus–algae complex biomass visualized under Olympus-BH2 with 40 magnifications.

Figure 4. harvesting ratio by filamentous fungus isolate Aspergillus niger under numerous cultivation conditions. AC: autotrophic algal culture; HC: heterotrophic algal culture.

Microalgae harvesting ratio by filamentous fungus under heterotrophic conditions was shown to be better than autotrophic condition for the formation of pellets (Fig. 4). In heterotrophic condition, the competition occurred between cells of A. niger and cells of C. vulgaris for the glucose in the co-culture because both cells need an organic carbon source to maintain their life (33). In mixotrophic conditions, competing for nutrients with heterotrophic algal cells, while a symbiotic relationship formation between fungi and autotrophic microalgal cells, also it seemed that the concentration of fungal biomass in the co-culture was moderately higher at glucose than sucrose, maltose source of carbon. Glucose consumption rate by the fungus is much faster than that by the microalgal when the amount of glucose was abundant. This may be due to the high efficient metabolic ability of fungi (34). Under autotrophic cultures, phototrophic cultivations after 3 days, no pellets were formed in the co-culture. the growth of microalga was very slow and the fungal spores were not germinated. It was apparent that co-pelletization relies on the existence of an organic carbon (35). Experiments with different carbon source of glucose, sucrose, maltose showed that the higher harvest efficiency of microalga was more in glucose source than sucrose and maltose. The size of the pellets formed in the co-culture is larger, and the total number of the pellets increases on glucose which provided a sufficient carbon source to sustain the cell growth, especially the fungal cells, which therefore resulted in better pelletization and harvest efficiently (31).

References:
لاحصاد الطحلب الدقيق كلوريلا فولجارس عند طريق التلبد المشترك مع الفطريات الخيطية

زياء حسين الربيعي 1
هادي الشمري 2

1 قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية، بغداد، العراق.
2 قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية، بغداد، العراق.

الخلاصة:
الهدف من هذه الدراسة هو تطوير بديلة لتربيب/تلبد الطحالب الدقيقة كلوريلا فولجارس تتغذية التغذية عن طريق زرعها مع الفطر المكون للتلبد الأسيرجليس نبحر المعزول من أطيان مياه الصرف الصحي. أن العوامل الرئيسية المدروسة هي تركيز اللاقحة الجرثومية وتركيز الكاربون العضوي في الوسط والتغيير في الأس الهيدروجيني والتي لها تأثير إيجابي في تكوين تلبد في الطحالب/فطر. أن استخدام 1 × 10⁷ جرثومة/مل، 15 غرام/لتر كوكوز و10 غرام/لتر الريسول هي الأفضل لتكوين التلبد، تم الحصول على كفاءة تربيب كاملة عند تنمية الطحلب تحت ظروف التنمية الذاتية أما قطر وتوزع الحبل فلن تتأثر بسرعة تدوير الهواء. هذه التقنية الجديدة لحصاد طحلب الكوليلا يمكن أن تكون مفيدة لباقي أنواع الطحالب الدقيقة ويمكن اعتبارها بدلة لطرق حصاد التقليدية.

الكلمات المفتاحية: التربيب البيولوجي، طحالب متغايرة التغذية، طحالب ذاتية التغذية، فطريات خيطية.