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Research Article

Harvesting of *Chlorella sp.* by Co-cultivation with Some Filamentous Fungi

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Abstract

Algae are play a major role as straight producers of biofuels, so expansion of a new. harvesting-technology is important to achieve economic feasibility of biofuel production from algae.. Fungal pelletization-assisted.. Microalgal harvesting has Emerged as new research area for decreasing the harvesting cost and energy inputs in the algae-to-biofuel method. The present study tried to optimize process circumstances as (substrate inputs, process time and pH). Through choice of a robust fungal strain. Four fungal strains (*Aspergillus terreus*, *Trichoderma* sp., *Mucor* sp. and *Rhizopus* sp.**)** were screened for their pelletizing efficiency in fresh/supplemented chu-10 with selected media nutrient (glucose, nitrogen and phosphorous). Results showed that *Aspergillus terreus* was the most efficient strain for pelletizing in the nutrient supplemented chu-10 with its neutral pH (7) and acidic pH (5). Stimulatingly, *A. terreus* was capable to harvest nearly 100 % of the *Clorella* sp. cells (1×10^6 spore/ml at optical density (OD) approximately 2.5 initial working algal concentration) within only 24 h. at supplementation of (10 g/l glucose, 2.5 mg/l aNH_4NO_3 and 0.5 mg/l mK_2HPO_4) also performed well at lower glucose level (5 g/l) can also results in similar harvesting but its need relatively higher incubation time. The procedure kinetics in term of harvesting index (H. I) as well as the variation of residual glucose and pH with time was also studied. The mechanism of harvesting process was studied through microscopic, examination. *A. terreus* strain investigated in this study could emerge as an efficient, sustainable and economically viable tool in microalgae harvesting for biofuel production and time conservation.

Keywords: Co-cultivation, *Chlorella sp.*, Harvesting, Filamentous fungi.

الخلاصة

تلعب الطحالب دورا رئيسيا كمنتج مباشر للوقود الحيوي، لذلك يجب تطوير تكنولوجيا حصاد جديدة ضرورية لتحقيق الجدوى الاقتصادية لإنتاج الوقود الحيوي من الطحالب لذا برز دور الفطريات لحصاد الطحالب الدقيقة كساحة أبحاث جديدة لتقليل تكاليف الحصاد في انتاج الطحالب للوقود حيوي. انجزت الدراسة الحالية لتحسين ظروف التفاعل (المغذيات الاضافية، وزمن العملية ودرجة الحموضة). اختبر كفاءة اربع عزلات فطرية لحصاد الطحلب وهي *Trichoderma* sp., *Aspergillus terreus*, *Mucor* sp. و*Rhizopus* sp. اذ زرعت في وسط chu-10 الغير محور والمحور باضافة (كلوكوز10 غرام\لتر, نتروجين2.5 ملغم \لتر, فوسفات 0.5 ملغم\لتر) تم اختيار العزلة الاقوى في هذه العملية وهي *A. terreus* اذ اظهرت كفاءة حصاد مقاربة لنسبة 100% بعد 24 ساعة من الحضن في الوسط المحور المتعادل (درجة حموضة 7) وفي الوسط الحامضي (درجة حموضة 5) اذ زرع العالق الفطري بتركيز 1×10^6 سبور\مل مع عالق الطحلب ذو الكثافة الضوئية≈ 2.5. تم دراسة معامل الحصاد مع التراكيز المتبقية من الكلوكوز ودرجة الحموضة خلال فترة الحضن اذ اظهر الفطر *A. terreus* كفاءة حصاد جيدة بتركيز كلوكوز واطيء (5 غرام\لتر) لكنه يحتاج فترة حضانة اطول. تم دراسة ميكانيكية هذه العملية تحت المجهر الضوئي المركب. اظهر الفطر *A. terreus* كفاءة عالية في استخدامه كأداة مجدية في حصاد الطحالب لانتاج الوقود الحيوي باقل كلفة وعدم هدر الوقت.

Introduction

The limitation of non-renewable energy supplies and the serious threat to the environment, alternative energy sources based on biomass appear increasingly attractive [1]. Several candidates appeared to replace the current energy supplies, with no more pollution [2]. Recently microalgae gained great attention as one of the most promising renewable energy sources [3].

Microalgae produce much larger amounts of "biofuels" in a shorter time than other sources depend on plant wastes and seeds. However, there are several obstacles impeding algae-based biofuel production, from these complica-

tions: microalgae cultivation, harvesting process which contributes to substantial cost and energy demand [4].

The current harvesting approaches as sedimentation, centrifugation, filtration and flocculation had low efficiency due to small sizes and unicellular microalgae [5], thus we need novel harvesting processes.

In nature, associations between microbes particularly in aquatic habitats. Free living algae and fungi coexist in the same habitat without direct structural contact between them, however the physiology and ecology of algal-fungal associations found in freshwater in which algae live as epiphytic on fungi which could be considered a kind of artificial lichen [6], so pelletization characteristic of filamentous fungi could be utilized to harvest microalgae [7].

Recent studies indicate that the pelletization characteristic of filamentous fungi as Aspergillus oryzae had a wanted harvesting effect on Synechocystis sp. ,though Rhizopus oryzae had no effect [8]. other studies reveled that co-pelletization of Chlorella vulgaris. with filamentous fungi could improve biomass production [9].

This study aimed to achieve the following objectives: (1) Screening for the efficient fungal isolate for pelletization in chu-10; (2) exploring the usability of chu-10 for fungal pelletization; (3) selecting a strain with pelletization ability under wide range of pH; and (4) investigating the harvesting kinetics and mechanism (5) reducing the time and glucose inputs required for achieving complete harvesting.

Materials and Methods

Microorganisms isolates and Growth Media

Four filamentous fungal strains, Aspergillus terreus, Trichoderma sp., Mucor sp. and Rhizopus sp. were collected from domestic wastewater. (Al-Jaesh canal-Baghdad), and streaked straight on potato dextrose agar (PDA) plates by applying the method designated by [10].

The isolated fungal strains were tested for pellets formation ability by cultivating spores of contender strains in 100 ml of PG broth which consist of (4 peptone and 6 glucos g /l for each) and pH 6.0, those which could form pellets were further identified based on mycelia and spores morphological analysis under the microscope and compound- secretion ability grown on the PDA agar plates, depending on the taxonomic key [11].

Chlorella sp. isolated from Al-Mustansiriyah University garden by Patterson Method [12]. Algal strain identified by using an optical microscope according to [13], this strain had been stably cultivated in our lab under both autotrophic (cultured in chu-10 media) and mixotrophic with (modified chu-10 by adding 10 g /l glucose) modes.

Screening of Pellet Forming Fungal Strains in chu-10 media (PFF)

The method for screening of PFF described by previous study [14], experiments were carried out in 250 ml conical flasks with 100 ml working volume. Two weeks-old Chlorlla sp. culture with optical density of ≈2.5 at 680 nm) was used as inoculum (10 % v/v). Flasks were inoculated aseptically with fungal spore suspension containing $\approx 1.0\times10^6$ spores/ml at inoculum size of (2% v/v), then incubated under shaking (150 rpm) in a bench top orbital shaker at 30°C for 72 h to achieve pelletization. The growth and pelletization were recorded with the visual observations. The chu-10 medium was supplemented with (10g/l glucose) in order to provide carbon source to fungi.

In second set of experiments, glucose and nitrogen (as NH_4NO_3, 2.5 mg/l) was added to the chu-10 media. The third set of experiment was accomplished with supplementation of phosphate (K_2HPO_4, 44 0.5 mg/l) in order to adding the glucose and nitrogen with adjusted pH (≈6.8) was tested for fungal growth and pelletization. All above flasks were then inoculated and incubated under shaking (150 rpm) at 30 °C for 72 h.

Fungal-algal Pelletization

After the optimization of fungal pelletization process in supplemented chu-10 media, the next study investigated the harvesting of Chlorlla sp. by fungal pelletization. Chlorella sp. was cultivated in a 250-ml flask using 100 ml sterile chu-10 medium at 25°C. Chlorella sp. culture (two weeks-old; $OD_{680}\approx2.5$) was used as inoculum at (10% v/v). Chlorella sp. cultivation was under light intensity of 4.5 Klux with dark/light cycle of 12 h. After two weeks of growth, flaks (containing microalgal broth) were substituted with optimized media inputs (glucose, NH_4NO_3 and K_2HPO_4) and inoculated with selected fungal spores' suspension (1×10^6 spore/ml). The kinetics of fungal pelletization assisted Chlorella harvest-

ing was studied by withdrawing samples after every 3 h for determination of residual suspended algal cells by measuring optical density at 56808 nm (OD_{680}). The harvesting efficiency of the selected fungus was calculated using the harvesting index (HI) calculated as:

$$\mathbf{HI}= (1 - \frac{\lambda t}{\lambda 0}) \quad (1)$$

Where λ0 is the OD_{680} of the initial microalgal culture and λt is the OD_{680} of the residual algal cells at time t. harvesting efficiency (%) was estimated using the following equation:

$$\textbf{Harvesting efficiency (\%) =HI×100} \quad (2)$$

Residual glucose concentration was also determined in order to measure the glucose utilization rate of the fungal strains during the algal harvesting process. The residual glucose in the broth was estimated using the" phenol- sulphuric acid method [15] briefly, the powdered biomass (100 mg) was hydrolyzed with 5 ml of 2.5 N hydrochloric acid in boiling water bath for 3 h, cooled at room temperature and neutralized with solid sodium carbonate. After neutralization, an aliquot of 0.1 mL was pipette out in a clean test tube and diluted to 1 ml. After dilution, 1 mL phenol solution and 5 ml of 36N sulfuric acid were added, well mixed and cooled to 25 ^{0}C in a water bath.
The optical density of the samples was measured at 490 nm and the total carbohydrates were than calculated using standard calibration curve. Changes in pH were also monitored using pH meter.

Effect of Variation in Glucose Concentration on Fungal–algal Pelletization
According to the hypothesis that fungus can use microalgal biomass as carbon source, attempts were made to decrease the glucose inputs during fungal assisted microalgal harvesting. Depending on the method that achieved by [16], glucose concentration was lowered to 5 and 2.5 g/l, while NH_4NO_3 and K_2HPO_4 concentrations were the same as used previously. High glucose level (15 g/l) was also tested to check if it can further speed up the algal harvesting process. Whenever needed sterile distilled water was used for diluting to maintain the initial OD_{680} at≈2.5.
The HI and efficiency of algal harvesting through fungal pelletization process was then estimated depending on the (Equation 2).

Mechanism of Fungal Pelletization-Assisted Microalgae Harvesting
The changes in the color of the culture, and the aggregations of suspended Chlorella sp. cells in the culture, were monitored. So, to understand the interaction of algae with fungal spores and mycelium, samples from experimental flasks were collected every three hours and visualized under optical microscope (Olympus compound) with objective lenses of 40× magnification.

Statistical Analysis
All the tests were achieved in triplicates and results presented here are either mean of triplicate reported as mean ± standard deviation or with error bars.

Results and Discussion
Screening of Efficient (PFF) in the initial run with fresh chu-10 media without glucose supplementation, it was showed that chu-10 media alone is unable to support growth of any fungal strain. Hence, the glucose supplemented chu-10 media was used for further studies. The pelletization ability of the tested fungal strains in glucose supplemented chu-10 media at different time intervals is shown in (Table 1).
With glucose supplementation, *A. terreus*, *Trichoderma* sp. showed good pelletization within 12h, *Mucor* sp. and *Rhizopus* sp. started to grow within 24h. However, despite the good growth of *Mucor* sp. and *Rhizopus* sp. was not able to pelletize even after 48h (Table 1).
According to microscopic examination, it was observed that the pellets formed were uniform (within fungal strains) with pellet size varying from 0.5 to 1.2 mm, so *A. terreus* was the best.

Table 1:Occurrence of fungal growth and pelletization in fresh /supplemented chu-10 media with glucose (10 g/l), NH_4NO_3 (2.5 mg/l) and K_2HPO_4 (0.5 mg/l).

Time (h)	fresh chu-10 media				Supplemented chu-10 media											
					Glucose (10 g/ l) only				Glucose+ NH_4NO_3				Glucose+ NH_4NO_3+K_2HPO_4			
	At	Tri.	Muc.	Rhi.	At	Tri.	Muc.	Rhi.	At	Tri.	Muc.	Rhi.	At	Tri.	Muc.	Rhi.
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	+	+	G	G
36	-	-	-	-	-	-	-	-	-	-	-	-	+	+	G	G
48	-	-	-	-	-	-	-	-	+	-	-	-	+	+	G	G
60	-	-	-	-	-	-	-	-	+	-	-	-	+	+	G	G
72	-	-	-	-	-	-	-	-	+	+	-	-	+	+	G	G

"+" growth with pelletization, "-"no growth, "G" growth without pelletization.
At=*Aspergillus terreus*, Tri. =*Trichoderma* sp., Muc. =*Mucor* sp., Rhi. =*Rhizopus* sp.

Moreover, glucose level of 10 g /l was found to be suitable for proper pelletization of selected fungal strains grown in chu-10. Based on the screening studies, the selected PFF was then tested for growth and pelletization on chu-10 media. In the first set of experiments, chu-10 media (with 10 g/l) was used as nutrient source for fungal pelletization. Interestingly, no palletization or growth was observed in any flask with fresh chu-10 media (Table1), even up to extended incubation period of 72 h (table 1). The causes for this could be either (1) the neutral pH of the chu-10 media (≈7) or (2) absence of the basic nutrients (N and P) other than glucose, as prolonged cultivation of microalgae in chu-10 media results in utilization of nutrients as well as shift in pH towards alkaline range. At 2.5 and 0.5 mg/l as $NO^{3-}$and $PO4^{3-}$, respectively, further confirmed the hypothesis of neutral limitation in chu-10 media. Supplementation with NH_4NO_3 and glucose in chu-10 media also did not stimulate the fungal growth and pelletization (table 1) these results agreed with [17] informed that *Rhizopus oryzae* had fast and well peletization ability when carbon was supplemented to the broth media during co-cultivation with algae, suggesting that organic carbon have profound impact on fungi growth and cell palletization of filamentous fungi species. Only *A. terreus* showed growth as well as palletization but after 48 h. Moreover, pelletization in the case of *Trichoderma*sp was observed in 72 h. The biomass and pellets obtained in both the cases were relatively small as compared with that obtained in fresh chu-10 media supplemented with same glucose level. However, addition of K_2HPO_4 with NH_4NO_3 and glucose resulted in dramatically enhanced growth and pelletization of the *A. terreus* as fugal pellet were observed within 12 h, *Trichoderma* sp. observed after 24 h (table 1). However, with rest fungal strain, there were growth and no pelletization was observed, even at 72 h. These results established the significance of particular combination of nutrients for fungal growth and pelletization. It is not worthy that in previous attempts on fungal-assisted microalgae harvesting, addition of any inorganic nutrients (N and P) was not reported [18, 19]. At the end of the exponential phase of microalgal growth, no primary nutrients might be available in the chu-10 medium. Thus, it is very unlikely for the fungal strains to grow and pelletize without the addition of primary nutrients along with glucose, as confirmed with current observations.

Such nutrient limitation was possibly not encountered in the study conducted by [20] as fungus was cocultivated with microalgae in nutrient-rich fresh medium. Similarly to [21] utilized precultured fungus (in nutrient medium) to harvest microalgae and hence did not required further addition of any primary inorganic nutrients. Nevertheless, in order to develop a realistic and economically competent harvesting protocol, the availability of data on minimum nutritional demand for fungal pelletization is must. In the present study, only *A. terreus* was able to efficiently pelletize in glucose and nutrient supplemented chu-10, followed by *Trichoderma* sp. with rela-

tively delayed and poor pelletization. No or very little pelletization (and growth) of other tested PFF could be attributed to their inability to survive and pelletize under unfavourable (≈ neutral pH) growth conditions further strengthen the hypothesis that the pH has vital role in fungal growth and pelletization. Similarly [19] also observed the crucial role of pH in fungal pelletization-assisted algal harvesting. However, there is no studies reported earlier on fungal-assisted microalgal harvesting could establish fungal pelletization and harvesting at alkaline pH. However, in the present study, *A. terreus* showed remarkable behavior of ample growth and fast pelletization at the actual pH (≈7) of Chu-10. Hence, the growth and pelletization of *A. terreus* was unaffected with variation in pH. The versatile nature of this fungal strain with respect to growth and contaminant removal at various pH has been previously reported by [22, 23]. The acid- and alkali-tolerant characteristics have rendered this organism equally capable of treating acidic electroplating effluent [24] and alkaline textile effluent [22] without the need of any pH adjustment. In this study, the ability of *A. terreus* to pelletize under neutral pH for algal harvesting has been established. Therefore, it can be concluded that *A. terreus* has high potential for microalgal harvesting at any pH, which further strengthens the applicability of this strain at commercial scales. Therefore, *A. terreus* was selected as the most efficient PFF for further studies.

Harvesting Microalgae Using Selected PFF Nutrient supplementation and spore inoculation resulted in an immediate pH drop of chu-10 from initial value of 7 to 6.5 this decline in pH due to the fungal growth, filamentous fungi grow fast and show compound performance when suitable organic carbon is supplied in the medium [17], pH of the chu-10 medium after fungal assisted micro algal harvesting decreases during incubation period and the final pH values were increased as in Table 1, these results confirm the harvesting efficiency was not pH-dependent, unlike previous studies [25, 26] reported that low pH supports micro algal harvesting by fungi. The culture pH is possibly an important factor for the pelletization of fungal spore, but not for the adhesion of *Chlorella* sp. cells to the surface of the fungal pellet [25]. Significant harvesting phenomenon was observed during the 24 h observation period.

The variation in efficiency of fungal assisted microalgae harvesting process (in terms of HI), residual glucose and pH with time is shown in (Table 2). The fungal assisted micro algal harvesting started within the first 3 h with (≈20 % glucose utilization). The harvesting continued at very fast rate up to 12 h (≈75 % of glucose utilization) with harvesting efficiency of more than 80 % during this period. After 9 h, harvesting process as well as the glucose utilization continued but at relatively slower rate and resulted in more than 66 % harvesting within 18 h table 2. Eventually, up to 100 % harvesting of microalgal cells was recorded within 24 h with utilization of 86 % of the added glucose (table 2), these results as compared with previous studies on co-cultivation of fungi with microalgae [20] reported about 60 % harvesting of *Chloreela vulgaris* cells in 72 h of co-cultivation system using pre-cultured *Aspergillus niger*. similarly [25] stated the best harvesting of *C. vulgaris* in 418 h at high glucose level (260 g/1) with *Aspergillus* sp.

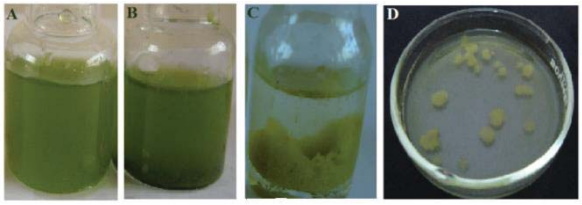

Figure1: pictorial illustration of various stages of microalgal–fungal pellet formation at different time intervals. A: *Chlorella* sp. culture at 0 time, B: co-culture after 6 h, C: co-culture after 18 h D: pellet on completion of harvesting after 24 h.

Table 2: Variation in pH, Residual glucose and harvesting ratio (%) and with time (h). data for pH and Residual glucose as (Mean± SD).

Incubation time(h)	pH	Residual glucose concentration (g/l)	Harvest ratio (%)
0	6.8±0.8	10±0.7	0
3	5.7±0.1	8.1±0.18	20
6	4.8±0.2	5.2±0.3	41
9	5±0.8	4.3±0.3	73
12	5±0.7	4.1±0.2	78
15	5.3±0.2	3.9±0.1	80
18	5.8±0.9	3.8±0.2	90

21	5.6±0.2	3.2±0.1	93
24	6.9±0.1	3.1±0.5	100

As shown (Figure 1) the initially microalgal cells were suspend in medium (0 h). Sooner the fungal spores were geminated, microalgal cells started aggregating (along with fungal biomass) and eventually turned into tightly packed pellet on completion of harvesting process (24 h). These pictures taken at different time intervals, showing the aggregation stages of harvesting process further validated the profile of observed harvesting kinetics After 24 h, the microalgal fungal pellets settled down at the bottom of the flask with separation of the clear supernatant. The settled pellets were harvested easily by simply decantation of supernatant or filtering though filter paper. Hence, the present study makes important contribution by depicting the correlations between fungal growth, glucose consumption and accompanied pH change in the medium to best algal harvesting.

The latter will effect of Glucose level variation on *Chlorella* sp. through PFF. It is clear that *A. terreus* was the most efficient in harvesting *Chlorella* sp. from the liquid culture at glucose concentration of (10 g /l). Further attempts were then made to reduce the glucose input in order to make the fungal assisted microalgal harvesting economically feasible at large scale. The variation of HI at different glucose levels is shown in (Figure 2). It was interesting to notice that the reduced glucose level (5 g/l) was enough to support the efficient fungal growth and subsequently the harvesting of microalgal cells at (5 g/l), the recorded harvesting was more than 75 % within 24h. Moreover, 30 % harvesting of microalgal cells was observed approximately in 24h at (2.5 g/l) glucose concentration. Though, the harvesting efficacy was relatively poor at (110 g/l glucose) with own harvesting 93%. Farthermore, as realized from Fig.2, the raised glucose level (1053 g/l) does not have any significant differences from the concentration (10 g/l) on the fungal pelletization assisted algal harvesting progression. As confirmed by [9] the heigh concentrations of glucose up to (10 g/l) increase productivity of the fungus about (800 mg/l. day^{-1}) which was about seven times higher than the fungal productivity with low glucose concentrations. [26] confirm higher concentrations of glucose increase fungal biomass in the co-culture, whereas the microalgal biomass in the co-culture was much lower compared with the biomass produced in the pure culture of only microalgae So it strong to be determined that although 10 g/l glucose concentration was optimal for 100 % harvesting of microalgal cells within 24 h, the reduced glucose level (5 g/l) can also results in similar harvesting but maybe with relatively higher incubation time. Hence, the efficient microalgal harvesting can be done either at (10 or 5 g/l) glucose concentration, depending up on the time of incubation, similarly to [27] improved the ability of *A. lentulus* to harvest was nearly 100 % of the *Chroococcus* sp. within only 24 h at supplementation of(10 g/l) of glucose also performed well at lower glucose level (5.0 g/l) resulting in 92 % harvesting within 24 h and up to 98 % harvesting within 52 h.

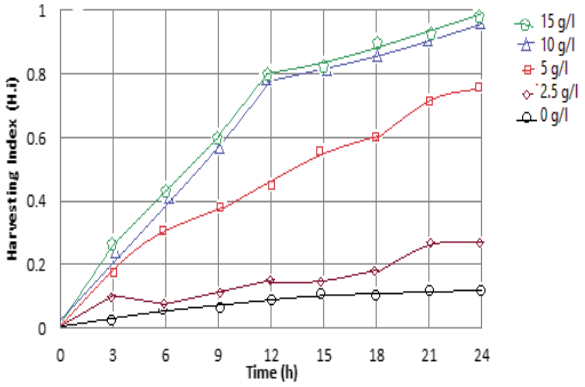

Figure 2: Comparison of H. I % at different glucose concentration (supplemented with 2.5 mg/l NH_4NO_3 and 0.5 mg/l K_2HPO_4) ranging from (0 -15 g/l).

The Possible Mechanism of Microalgal–Fungal Pelletization

The microscopic analysis revealed that in this particular study, the fungal pelletization assisted microalgal. Harvesting mainly talented through coagulative type pelletization (Figure 3). From microscopic observations it is clear that the microalgal and fungal spores coagulated in form of algal–fungal (spore) cluster as soon as the spore germination started. This cluster then turned into "microalgal cell containing fungal hyphal network", which subsequently evolved as algal–fungal pellet after further hyphal growth and binding of more algal cells. Previous studies [26, 27] state that fungal growth in submerged culture undergoes mainly by three phases including micromorphological growth (germ tube elongation), macromorphological growth (pelletization) and fungal cell autolysis. Hence, the fungal as-

sisted microalgal harvesting could result from the interaction of microalgal cells with fungal biomass at any of the above stated stages of fungal pelletization process as described by [14], in the coagulative-type pelletization, germinating spores possibly may cluster together along with the microalgal cell due to germinating spore to spore interaction and spore to microalgal cell interaction. Moreover, in the hyphal–elemental agglomeration, the microalgal cells might bind to the exposed hyphal tips of the geminating spores as soon as the germination starts followed by fungal hyphal growth resulting in its further interaction with other fungal hyphae and microalgal cells and subsequently the formation of algal–fungal pellet.

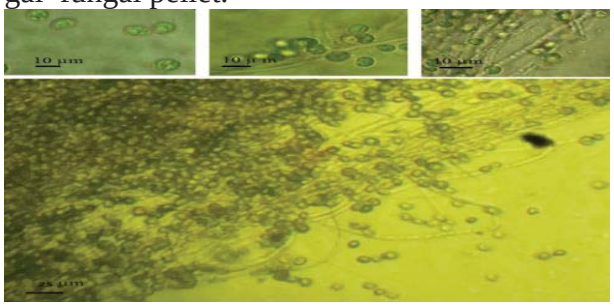

Figure 3: optical microscopic pictures Illustrate various stages of fungal pelletization assisted algal harvesting process

It is widely reported that fungal growth (in submerged culture) results in secretion of various metabolites including organic acids, polysaccharides, proteins and enzymes [28, 29]. Hence, the interaction and attachment of microalgal cells to fungal spore and hyphal surface may be facilitated by growth associated metabolite secretion by the fungi.

Conclusions

The present study was focused on the identification of the limiting factors and subsequent optimization of the process of fungal assisted microalgal harvesting. From the present observations, it was found that the fresh chu-10 does not support growth of selected PFF and hence needs to be supplemented with carbon and nutrient sources. Supplementation of the chu-10 media with glucose (10 g/l), NH_4NO_3 (2.5 mg/l) and K_2HPO_4 (0.5 mg/l) was optimal for efficient fungal pelletization of selected strains. Among the tested PFF, only *A. terreus* was efficient to grow and pelletize in the nutrient supplemented chu-10, irrespective of its pH. Moreover, *A. terreus* resulted in nearly 100 % harvesting of *Chlorella* sp. from the suspended culture within 24 h. However, the mechanism behind the fungal pelletization-assisted microalgae harvesting was observed to be coagulative type. Based on the above observations, the process of microalgal–fungal pelletization can be summarized in the following possible steps: (1) germination of fungal spore, (2) interaction followed by binding of microalgal cell to active sites (on germinating spore or fungal hyphae) resulting in the nucleation of microalgal–fungal pelletization process, (3) further growth of fungal pellet with more active sites on exposed hyphae and finally (4) binding of microalgal cells on other exposed active sites and completion of pelletization process. This technique is yet to be tested at pilot level in order to replace energy intensive harvesting methods currently being employed at industrial scale. If the investigated process is scaled up and optimized properly, it could be the most efficient and the economically feasible process at industrial scale in the world of microalgal biofuel.

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