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Utilizing novel *Aspergillus* species for bio-flocculation: A cost-effective approach to harvest *Scenedesmus* microalgae for biofuel production

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ABSTRACT

The present study aimed to isolate a bioflocculating fungal strain from wastewater collected from a local bike garage. The isolate showed maximum similarity to *Aspergillus* species. The fungus was identified as *Aspergillus flavus* species *F_GTAF1 IU* (accession no OP703382). The isolated fungus was evaluated in terms of biomass recovery efficiency in *Scenedesmus* Sp. GTAF01. The extent of algal fungal co-pelletization was evaluated as a function of the algae-to-fungi ratio, volume of fungal culture in broth, agitation rate, and pH. results showed that at fungal culture volume of 60%v/v, fungal culture volume of 1:3%w/w, 100 rpm, and pH 3, 93.6% biomass was obtained during the initial 5 h. At wavenumbers 1384 and 1024 cm^{-1} a significant alteration in the transmission percentage was observed in co-pellet compared to algae and fungal cells. This shows the significant role of C-H–H and C-N stretches in co-pellet formation. This study provides deep insight into effective microalgal harvesting along with the simultaneous extraction of lipids that can be used for the sustainable production of biodiesel.

1. Introduction

The increase in population has urged an unremitting global need for feed, food, and energy. Energy is considered the most important factor in the industrialization and urbanization of the country. Fluctuations in the energy market have resulted in increased investment in the development of alternatives for energy production (Banerjee et al., 2020). Alternatively, microalgal biomass has emerged as one of the most assuring raw materials to combat rising global demand (Samoraj et al., 2024; Vandamme et al. 2013). Microalgae are known for their high growth rates and lipid, protein, and vitamin concentrations. They are also known for their ability to grow under various biotic and abiotic stresses (Shamim et al. 2022). Among the known microalgal strains, the genus *Scenedesmus* is known for its rich lipid content, which makes it a plausible source for biodiesel production, offering a sustainable and environmentally friendly fuel option. It has also gained significant attention due to its ability to accumulate lipids rapidly while thriving in various aquatic environments, including freshwater and aquaculture sources (Dao et al., 2024). Researchers have also explored the dual nature of *Scenedesmus* where it is not only aid in biodiesel production but also contributes to the treatment of wastewater from sources like fish ponds and chicken farms (Alavianghavanini et al., 2023). The biodiesel derived from Scenedesmus meets both Indian and international standards, highlighting its viability as a fuel source. Its lipid composition, particularly rich in palmitate and oleate, makes it suitable for biodiesel production. This underscores its potential as a renewable energy resource with minimal environmental impact, demonstrating the genus role in sustainable energy solutions.

However, despite these advantages, overpriced dewatering remains a major challenge. This is because of the small cell size, high dilution rate, and negatively charged cell surface. The algal biomass concentration depends on the surface charge, pH of the medium, ionic strength of the culture, and flocculant concentration. Considering this dependency, most algal biomass harvesting or dewatering methods are limited in

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terms of energy and economic intensiveness (Li et al. 2020). Utilizing microalgae for biofuel production, the harvesting step accounts for approximately 30 % cost of the biofuel production (Shokravi et al. 2022). Thus, economical harvesting of microalgal cells from media is still one of the biggest challenges for the production of biofuel. Conventionally, centrifugation, gravity sedimentation, electrophoresis, flotation, and flocculation are used as harvesting methods (Bharathiraja et al. 2022). Among these, centrifugation is the most widely used technique for extracting high-value products. However, it accounts for cost- and energy-intensive processes for the production of high-volume and low-value products such as biofuels. Among all the conventional methods, filtration is considered a low-cost harvesting method, but it is appropriate for algae with large cell sizes. Filtration is not sufficient for cells with smaller sizes, as it blocks the pores by forming clogs in the filter membrane, and thus requires regular replacement. The flotation or sedimentation method directly depends on microalgal cell density and growth medium. The drawback associated with sedimentation is the long time required for settling the microalgal cells. Floatation is found to be effective at the pilot scale but is least effective at the industrial scale, as it makes harvesting expensive in comparison to the centrifugation process (Farooqui et al. 2021).

Flocculation is a method in which microalgae aggregate, resulting in larger flocs. The larger flocs were easily harvested by filtration. Flocculation can be induced either by adjusting the culture pH or using external chemical flocculants (Zhang et al., 2024; Musa et al. 2020). Chemical flocculants contaminate microalgal biomass. This also adds an additional step to downstream processing, making it economically unsustainable (Mubarak et al. 2019). To overcome these challenges, scientists have started utilizing microorganisms as bioflocculants. In this process, the microalgae are mixed with bacterial or fungal cells or extracellular polymeric substances from these microorganisms to form flocs (Khoo et al., 2021). This results in an increase in the size that can be easily filtered or sedimented (Pahariya et al., 2024).

The present study aimed to isolate and screen a novel fungal strain from wastewater that could effectively co-pelletize microalgal cells (Nishshanka et al., 2023). The isolated fungal species *Aspergillus flavus* F_GTAF01 IU was cultivated in a yeast extract-peptone-dextrose medium. As soon as the fungal cells reached the mid-exponential phase, they were harvested and used for bioflocculation. The extent of algal fungal co-pelletization was evaluated as a function of algae-to-fungi ratio, volume of fungal culture in broth, agitation rate, and pH. The results were confirmed through various physicochemical techniques.

2. Materials and methods

2.1. Isolation of biofloculating fungus

Wastewater was collected in June 2021, at 39 °C. The collected wastewater was stored in sterile screw-cap bottles (amber coated) in the dark at 4 °C until further use. As per the American Public Health Association (APHA) guidelines, the collected water samples were analyzed for suspended solids, temperature, pH, biological oxygen demand, ammonia, nitrate, and phosphate.

The pour plate method was used to isolate bio-flocculating fungi from the wastewater. Serial dilutions were performed from $10^{\text{to } 1}$ to 10^{-10} using 1 ml of wastewater in normal saline. The desired volume from the 10^{-3} dilution was poured onto Potato Dextrose Agar (PDA) (Himedia make, India) plates. The BG11 agar plates were incubated for 5 days at 25±5 °C. After incubation for 5 days, four morphologically distinct colonies were selected. The selected colonies were inoculated into the pre-cultured microalgal broth of *Scenedesmus* sp. GTAF01 IU. Out of all colonies, a fungal colony with the highest bioflocculation efficiency was selected for further study.

2.2. rRNA sequencing and molecular identification

The 18S rRNA gene sequencing analysis was performed by Biokart, Bangalore, India Pvt. Ltd. The forward primer 5′-TCCGTAGGTGAACCTGCGG-3′ and reverse primer 5′-TCCTCCGCTTATTGATATGC-3′ were used for the sequencing. The amplified PCR products were analyzed using agarose gel electrophoresis. The amplified 18S rRNA gene product was purified from the gel and sequenced using the same primers used for the PCR reaction. The nucleotide sequence was analyzed using the Basic Local Alignment Search Tool (BLAST), National Center for Biotechnology Information (NCBI), USA. The BLAST sequence search was based on the maximum sequence similarity with other microalgal species. A phylogenetic tree was constructed for the fungal isolate using the reference sequence available in GenBank (NCBI, USA). The ClustalW alignment tool was used for the nucleotide sequence alignment. The phylogenetic tree was constructed in MEGA X software using the Maximum Likelihood method and the Tamura-Nei model. The 18S rRNA gene sequence of the fungal isolate was submitted to GenBank and NCBI, and an accession number was obtained (Amir et al. 2022).

2.3. Cultivation of microalgae and fungus

The microalgae, *Scenedesmus* sp. GTAF01 IU was isolated and examined. Isolated strains were procured from the Department of Bioengineering, Integral University, Lucknow, India. The pure culture (test organism) was maintained in a culture room at a temperature of 27 ± 2 °C. BG11 medium (pH 7.0) was used for optimum growth of the culture under 75 μmol $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD) with a 14 h photoperiod. The manual shaking of cultures was done for two–four times per day. Biomass harvesting was performed during the stationary phase of microalgal growth.

The isolated fungal species *Aspergillus flavus* F_GTAF01 IU was cultivated in a yeast extract-peptone-dextrose medium. The media contained 20 gL^{-1} of peptone, 10 gL^{-1} of dextrose and 10 gL^{-1} yeast extract (Himedia, make, India). The pH of the media was maintained at 5.5 ± 0.2 and incubated at 25 ± 5 °C. 48 h (Stationary phase) culture was used for the harvesting experiments.

As soon as the fungal cells reached the mid-exponential phase, they were harvested at 5000 rpm for 5 min. The microalgal cells were inoculated at an initial concentration of 0.4 gL^{-1} in 200 mL PDA medium in 500 mL glass flasks in three replicates. All the experiments were conducted at 25 ± 5 °C.

2.4. Bio-flocculation experiment

The flocculation study was carried out in a 500 mL conical flask at room temperature. 48 h old fungal culture was used as the flocculating agent. At 750 nm, the absorbance of the supernatant was recorded by dual-beam spectrophotometer (Shimadzu, model UV- 1601 PC make, Japan), and UV–visible spectroscopic measurements were performed in a quartz cuvette with a resolution of 1 nm. The recovery efficiency was calculated using Eq. (1) (Kumar and Das 2012).

$$\textit{Biomass recovery efficiency}\ (\%) = \frac{(\mathrm{ODi} - \mathrm{ODf})}{\mathrm{ODi}} \times\ 100 \qquad (1)$$

where, OD_i = Initial optical density and OD_f = final optical density

2.5. Bio-flocculation parameters

Different essential parameters such as algae-to-fungi ratio, volume of fungal culture in broth, agitation rate, sucrose concentration, and pH were studied to assess biomass recovery through co-pelletization.

2.5.1. Algae to Fungi ratio

The bioflocculation efficiency was evaluated at different algal:

fungal ratios (1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, and 1:4. The algal and fungal cultures were cultivated separately in a 500 mL flask under optimum conditions. The cultures were dried and the dry cell weight was recorded. The dried cell weight in the required amount was used as the weight: weight ratio for the experiment.

2.5.2. Volume of fungal culture in broth

The effects of different volumes of fungal culture in the range of 10 %, 20 %, 30 %, 40 %, 50 %, and 60 %v/v) were studied. For this, at optimum condition the algal and fungal cultures were cultivated separately in a 500 mL flask. Thereafter, the required range of fungal broth culture was added to% volume/volume for the experiment.

2.5.3. Agitation rate

The co-pelletization of algal-fungi was studied between 50 and 200 rpm. Zero-rpm was used as the control.

2.5.4. pH

The flocculation efficiency was evaluated in range of pH 2 to 8.

2.6. Biomass and lipid concentration

Algal-fungal microalgae powder co-pellets (10 mg) were suspended in deionized water and sonicated. The Concentrated sulfuric acid (150μL) and 30μL of 5 % phenol were added. The resulting solution was used to calculate the carbohydrate concentration equivalent to glucose units (at 490 nm). A dried algae suspension and 10 ml of 0.5 M NaOH were prepared to extract protein from microalgae (El Agawany et al. 2021). The suspension was vortexed, sonicated, and incubated at 100 °C for 120 min. Subsequently, the sample was centrifuged, and 1 mL of the supernatant was mixed with 4 mL of Bradford reagent and analyzed at 595 nm (Rosero-Chasoy et al. 2022). Dried algae (10 mg) were suspended in 5 ml methanol and 2.5 ml chloroform. The mixture was vortexed, sonicated, and centrifuged to obtain the supernatant. The same procedure was repeated for half the amount of solvent. The supernatants were collected and 4 ml of 1 % NaCl and with 4 mL of chloroform were added to the test tube. The resulting dark-green layer formed after centrifugation, including lipids, was carefully collected in a glass vial. Lipid content was calculated gravimetrically after evaporation of the chloroform phase (Yilancioglu et al., 2014).

2.7. Fourier transform infrared (FTIR) spectroscopy

To analyze the functional groups present on the surface that are associated with the algae and fungi interaction, the FTIR Spectra of the fungi, algae, and algal-fungal co-pellet were obtained. The spectra were recorded between 4000 and 450 cm^{-1}. Pellets were prepared by mixing photometric KBr (Himedia make, India, AR grade) and the harvested dried biomass at a ratio of 1:3.

2.8. Field emission scanning electron microscopy (FESEM)

In the present study, FE-SEM was used to analyze the attachment of algal-fungi in co-pellet. A small amount of dried sample was visualized (JEOL India Pvt. Ltd., India).

3. Results and discussion

3.1. Wastewater analysis

Physicochemical parameters of wastewater characterization are enlisted in Table 1.

The pH of the collected water was marginally acidic and harmful to several organisms in marine ecosystems. The concentration of suspended particles was also greater than the permissible limit. This hinders water flow and reduces the breeding of aquatic animals. Phosphate, ammonia, and biological Oxygen Demand (BOD) values in the wastewater were also beyond the permissible limit of discharge. The concentration of dissolved oxygen (DO) is also very low, indicating a significant pollution level in the wastewater (Maiti et al. 2019).

Table 1
Physico-chemical parameters of collected wastewater.

Physicochemical parameters	Wastewater	Central Pollution Control Board (CPCB), India
Temperature (°C)	37.40 ± 0.70	20.00–30.00
pH	6.3 ± 0.40	5.50–9.00
Phosphate (mg L^{-1})	42.3 ± 1.8	5.00
Suspended solids (mg L^{-1})	823 ± 2.4	100.00
Nitrate (mg L^{-1})	29.08 ± 0.04	10.00–50.00
Ammonia (mg L^{-1})	61.7 ± 2.3	5.00
DO (mg L^{-1})	1.54± 0.23	3.00–5.00
BOD (mg L^{-1})	35.42± 2	30.00–150.00

3.2. rRNA gene sequencing

Fig. 1 shows the amplified 18 s rRNA gene sequencing of isolated microalgae.

The gel image confirmed the amplified 18 s rRNA gene. A 500 bp DNA marker is shown in lane L (ladder). In lane 1, the 18S gene product was amplified using the 18S gene. In lane 1, the band size was 2500–3000 bp. This confirmed substantial 18 s rRNA amplification (Seto et al. 2020). The evolutionary relationship between the isolated fungus and the reference sequence present in the NCBI database is shown in (Fig. 2).

The fungal isolate showed maximum similarity to *Aspergillus flavus*. The gene sequence of *Aspergillus flavus* strain F_GTAF01 IU was deposited in the NCBI GenBank database (accession number: OP703382). Evolutionary analysis of the fungal isolate *Aspergillus flavus* strain F_GTAF01 IU showed the most common evolutionary inter-relationship with *Aspergillus flavus* strain F10. *Aspergillus flavus* strain F_GTAF01 IU exhibited high similarity with other strains of *Aspergillus Flavus* (Fig. 2). Similarly, Patel and Lakshmi (2021) isolated *Aspergillus fumigatus* A2DS from a motor garage sample.

3.3. Bio-flocculation efficiency

3.3.1. Initial inoculum concentration

Fig. 3 shows the algal bio-flocculation efficiency of the fungus obtained at 10, 20, 30, 40, 50, 60, and 70 % v/v fungal culture. The Maximum biomass efficiencies of 66.4 % and 65.2 % were obtained with a fungal culture volume of 60 and 50 %v/v. Fig. 4 shows the algal flocculation efficiency of fungi obtained at 1:0.5, 1:1, 1:1.5, 1:2, 1.2.5, 1:3, and 1:3.5 w/w ratios of algae to fungus.

Fig. 4 shows the algal flocculation efficiency of fungi obtained at 1:0.5, 1, 1, 1, 1.5, 1:2, 1.2.5, 1:3, and 1:3.5 w/w ratios of algae to fungus.

The Maximum biomass efficiencies of 86.6 % and 83.2 % were obtained at a fungal culture volume of 1:3 and 1:2.5 w/w (Fig. 4). Further

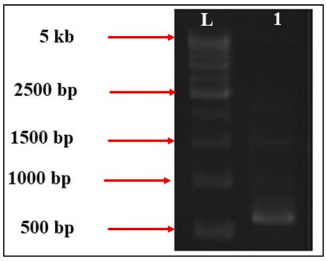

Fig. 1. Amplified 18 s rRNA gene of isolated microalgae.

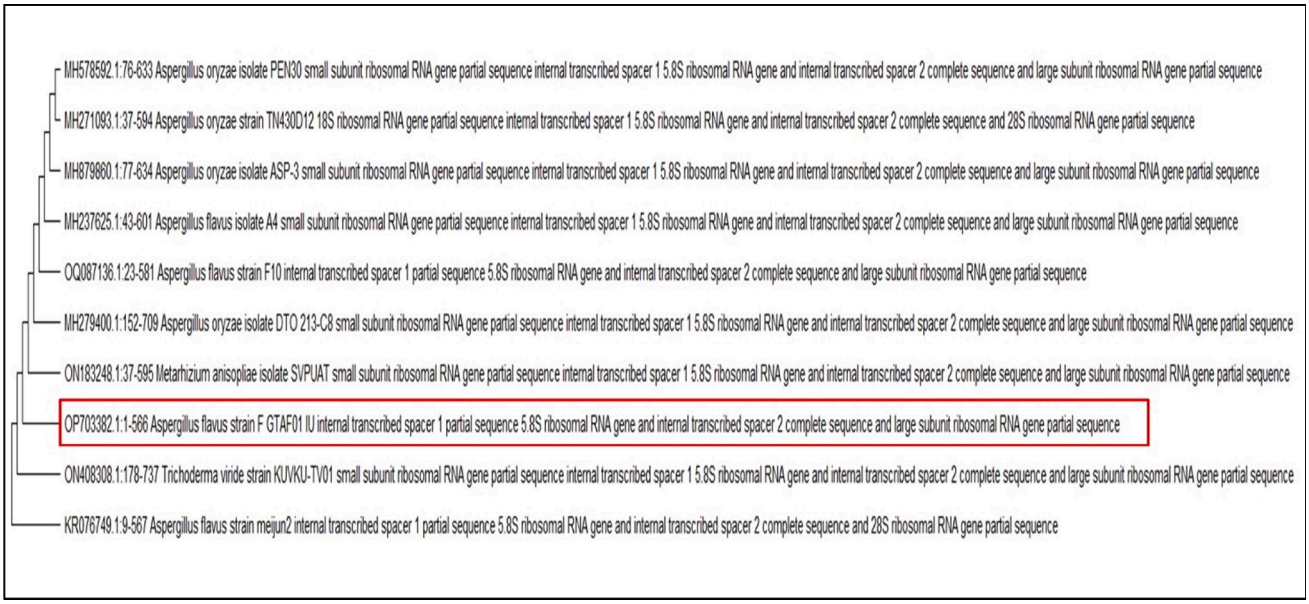

Fig. 2. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Ne 1993). The tree with the highest log likelihood (−822.00) is shown.

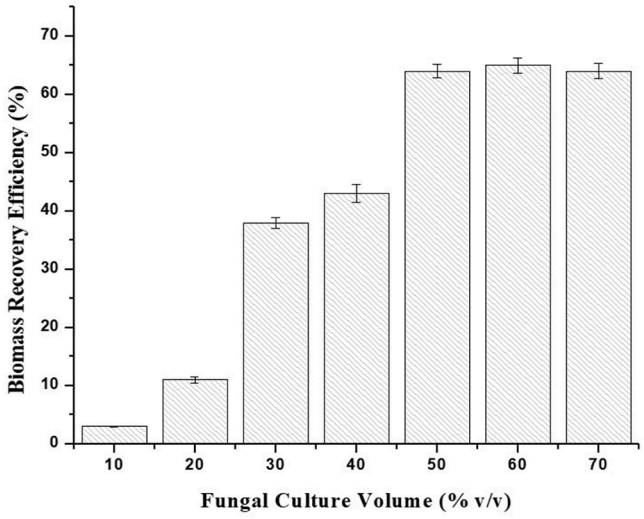

Fig. 3. Biomass recovery efficiency of *Scenedesmus* sp. GTAF01 IU.

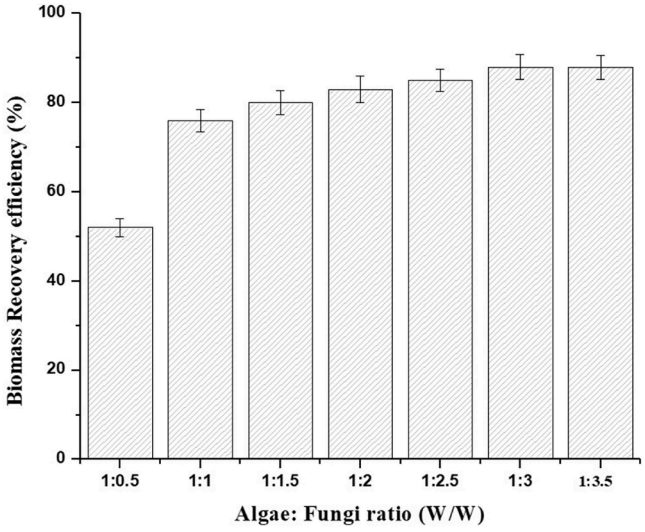

Fig. 4. Biomass recovery efficiency of *Scenedesmus* Sp. GTAF01 IU at different algae: Fungi ratio.

increasing the algae-to-fungus ratio did not affect recovery efficiency. This incorporation of whole fungal cell pellets (by weight) resulted in an increased harvesting efficiency compared to fungal culture. This is because the algal cells become entrapped in the filamentous fungal hyphae. Increasing the ratio to 1:3 resulted in the maximum biomass recovery. This was due to a 3-fold increase in fungal hyphae for entrapping microalgal cells (Fig. 4). This entrapment of algal and fungal cells results in the formation of pellet-like structures. These pellets settle rapidly and can thus be easily separated by filtration techniques or even by gravity sedimentation. Thus, an algae/fungi ratio of 1:3 w/w was used for further experiments. Similarly, Lal et al. (2021) obtained results in which > 80 % recovery was achieved when *Chlorella* sp. MJ 11/11, and *Aspergillus* sp. was added at a 1:3 ratio. Nazari et al. (2021) obtained > 80 % recovery when *Aspergillus niger and Spirulina platensis* were added at 1:2.

3.3.2. Agitation rate

Fig. 5 shows the biomass recovery efficiencies obtained at 50, 100, 150, 200, and 250 rp

Agitation in culture medium plays a major role in cell growth. An algal-fungal complex was obtained when fungal cell pellets were added to the suspension of algal cells under agitation. maximum algal-fungal complex, reflecting maximum biomass recovery efficiency of 92.1 %, was obtained at 100 rpm followed by 84.6 % at 150 rpm. Thereafter, increasing agitation above 150 rpm did not influence the recovery. The high recovery efficiency at a shaking speed of 150 rpm resulted in a better oxygen diffusion rate inside the pellet, which supported fungal hyphal growth (Fazenda et al. 2008). In addition, algal cells have sufficient time for contact with fungal pellets to create adhesive forces. A high agitation rate increases the shear stress on algal culture, causing its breakage without attaching to itself or the fungal hyphae (Gultom and Hu 2013). It was also observed that no algal-fungal co-pellets were formed during non-shaking mode. Similarly, Ndikubwimana et al. (2016) obtained the maximum biomass recovery with increasing agitation rate.

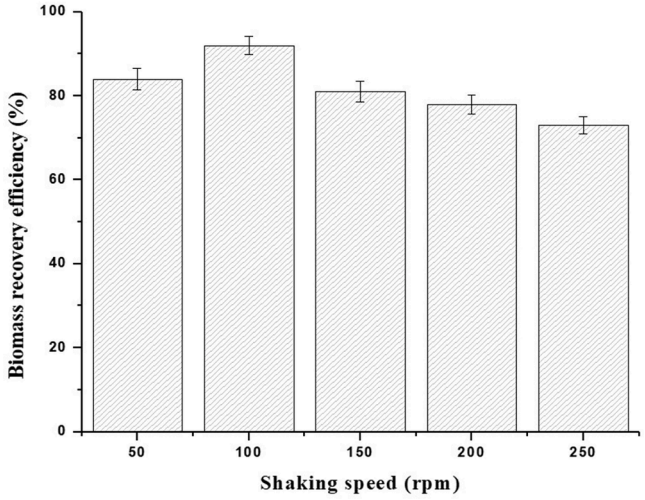

Fig. 5. Biomass recovery efficiency of *Scenedesmus* sp. GTAF01 IU at different agitation rate.

3.3.3. *pH concentrations*

The algal flocculation efficiency of fungi obtained at pH values of 2, 3, 4, 5, 6, 7, and 8 is shown in Fig. 6.

The Maximum biomass recovery (93.6 % and 92.5 %) was obtained at a pH 3 and 4. A further increase in pH did not significantly affect the recovery. The high biomass recovery was due to preferred fungal growth at low pH (Deng et al. 2005). The least recovery was obtained at pH above 7 because of the high number of OH^- charges. A similar result was observed when the pH was increased above 7 and low biomass recovery was obtained (Lal et al. 2021). It has also been reported that an acidic pH favors spore formation in fungi. This is due to the lesser extent of electrostatic repulsion (Kaushik and Malik 2009). In the present study, high biomass recovery was recorded at acidic pH due to lower electrostatic repulsion, which resulted in the development of a greater number of algal-fungal co-pelletizations. Lal et al. (2021) observed 90 % biomass recovery of *Chlorella* sp. MJ 11/ 11 with *Aspergillus* sp. at a pH of 3. It decreased to 21 % by increasing the pH to 8.

In the present study, it was noted that pH level emerged as a critical factor in the formation of algal-fungal co-pellets. Specifically, the co-pellets exhibited small spherical structures at pH 3, as shown in Fig. 7. This confirmed the attachment of the spherically shaped *Scenedesmus* sp. GTAF01_IU on the *Aspergillus fumigatus* F GTAF01_IU hyphae. Studies

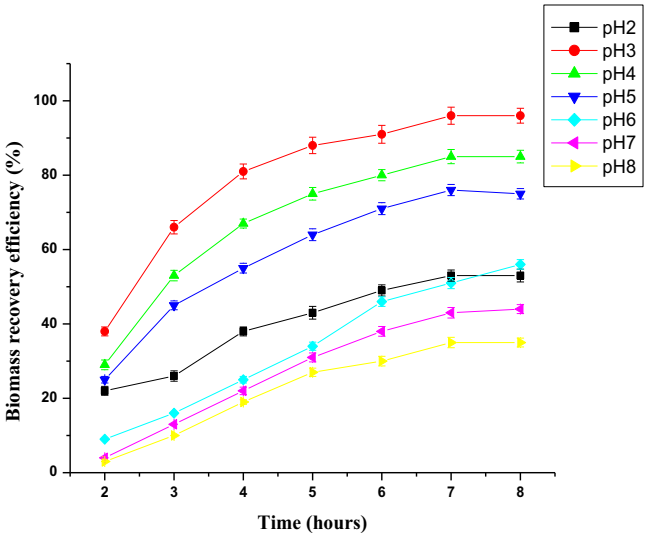

Fig. 6. Biomass recovery efficiency of *Scenedesmus* sp. GTAF01 IU.

reveal that alteration in the pH of culture solution significantly affects the degree of ionization of the bioflocculant, and oxidation–reduction. It also alters the functional groups present on the surface of the fungi, especially NH_2, COOH, and OH, thereby impacting the flocculation process (Pu et al., 2020; Patil et al., 2011). It was also observed in the present results that decreasing the pH below 3 has decreased the flocculation efficiency. This could be possible because an extremely low pH fungal secretes extracellular substances and its mycelia structure must be destroyed.

3.4. *Fourier-transform infrared (FTIR) spectroscopy*

FTIR analysis of algal, fungal, and algal-fungal co pellet is shown in (Figs. 8 and 9).

The presence of alkenes (2924 cm^{-1}; 1084 cm^{-1}), carboxylic (1644 cm^{-1}), and phosphate (1051 cm^{-1}) was observed on the surface of the algal cell were observed (Bhattacharya et al. 2017). Similarly, FTIR spectra of the fungal strain suggested the presence of alkenes (2926 cm^{-1}), amines (1618 cm^{-1}), and phosphate (1049 cm^{-1}).

At wavenumbers 1384 and 1024 cm^{-1} a significant increase in the transmission percentage was observed in the co-pellet compared to the algae and fungal cells. At these wavenumbers, functional groups with C-N stretches are present, reflecting their role in co-pellet formation.

The study conducted by Zhang et al. (2020) revealed that the peak intensity of fungal-kaolin particles at 1654 cm^{-1} was notably lower than that of the fungi. This suggests that the carboxyl functional group (C—O) positively influences the flocculation process by altering surface charge under strongly polar conditions. Peaks measured at approximately 1368 cm^{-1} were less intense because to bending vibrations and metabolite deformation in C–H.

Qamar et al. (2022) showed that the cell wall of *Aspergillus* sp. is mainly composed of galactosaminogalactan and N-acetylglucosamine. These sugars mainly contribute to the C-N functional groups and increase their intensity. A significant decrease in the number 1384 cm^{-1} has also observed. At this wavelength, the C-H groups of the alkenes were present. This decline in wavelength indicated that the C-H groups were also exposed to algal-fungal pellet formation. In algae, a high intensity at 1644 cm^{-1} suggested that there were a significant number of C-H groups. These results suggest that at the time of bio-flocculation, a noteworthy change occurred in the groups present on the surface of the algal-fungal co-pellet than in the monoculture of fungi and algae. The present results of the FTIR study were parallel with earlier research in that they indicated that the primary causes of successful flocculation were a range of metabolites (amino acids, polysaccharides, alcohols, and esters) released by fungus in culture and the presence of functional groups on the surface (Nie et al. 2021).

3.5. *Biochemical composition*

The total carbohydrate, protein, and lipid contents of *Scenedesmus* sp. GTAF01_IU was 20.4 %, 54.1 %, and 34 % in *Aspergillus fumigatus* F GTAF01_IU were 17.3 %, 8.1 %, and 11.6 % and in co-pellet were 19.3 %, 53.7 %, and 29.3 %, respectively. A slight decrease in carbohydrate, protein, and lipid content was observed in co-pellet compared to the monoculture of *Scenedesmus* Sp. GTAF01_IU and *Aspergillus fumigatus* F GTAF01_IU. A non-significant change in carbohydrate and protein content in the co-pellet was observed in the monoculture. This was due to a significant increase in C-N stretches, reflecting their role in co-pellet formation. These stretches were attributed to carbohydrate and protein formation. *Aspergillus* sp. are mainly composed of galactosaminogalactan and N-acetylglucosamine, which are sugars (Qamar et al. 2022).

A significant decrease in the lipid content was observed in the co-pellet. Analogous results were obtained for *Chlorella* sp. MJ11/11, and *Aspergillus* spp. (Lal et al. 2021). Muradov et al. (2015) reported that the lipid concentrations of *Aspergillus fumigatus-Tetraselmis suecica* and

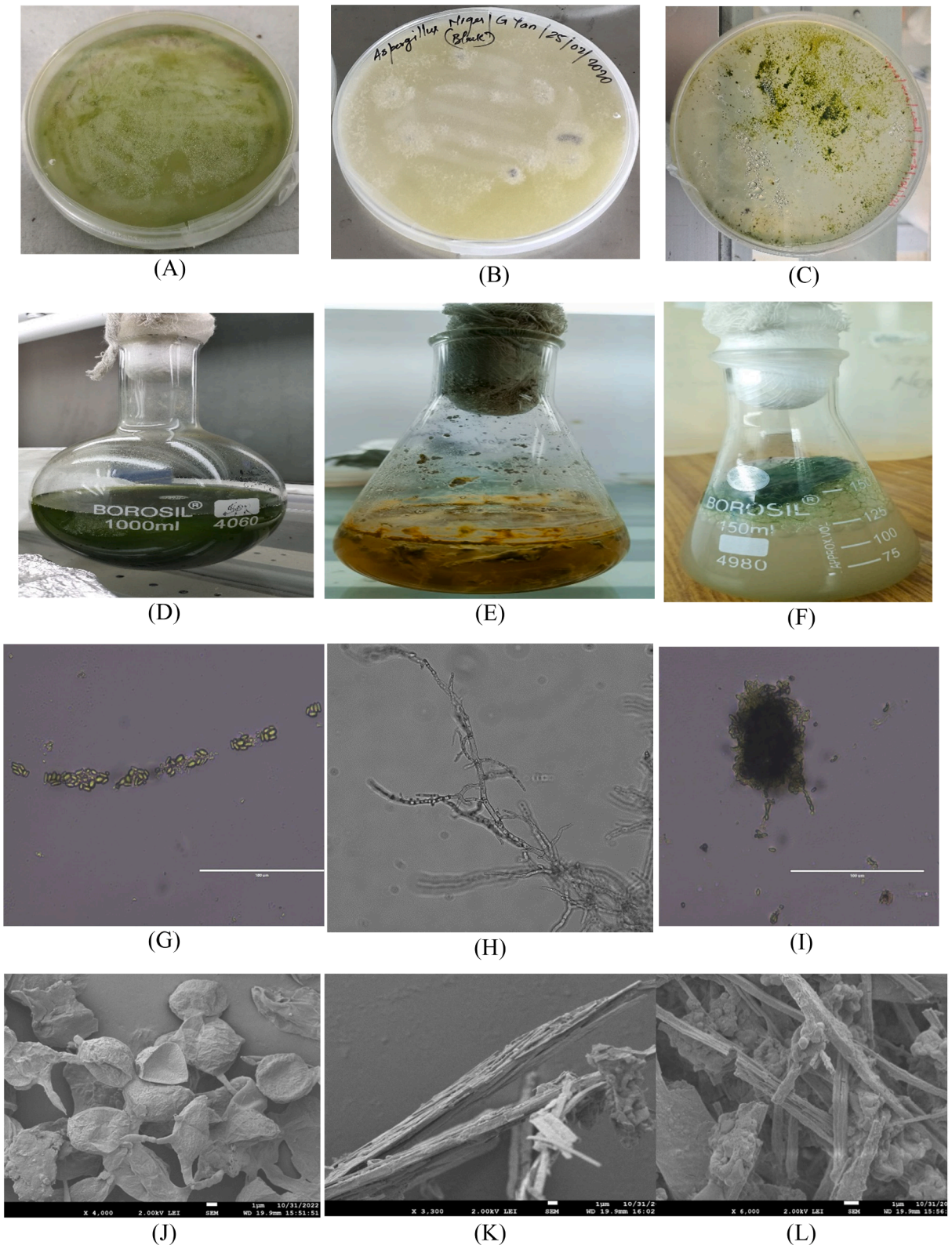

Fig. 7. Co-pelletization of *Scenedesmus Sp. GTAF01_IU* with *Aspergillus fumigatus* F GTAF01_IU in a petridish of volume 20 Ml. (A) *Scenedesmus* sp. GTAF01_IU (B) *Aspergillus fumigatus* F GTAF01_IU and (C) *Scenedesmus* sp. GTAF01_IU -*Aspergillus fumigatus* F GTAF01_IU co-pellet. Bio-flocculation of fungi and algae at pH 3 in 1:3 ratio in a flask after 5 h of incubation time (D) *Scenedesmus* sp. GTAF01_IU, (E) *Aspergillus fumigatus* F GTAF01_IU and (F) *Scenedesmus* sp. GTAF01_IU -*Aspergillus fumigatus* F GTAF01_IU co-pellet. Microscopic analysis of (G) *Scenedesmus* sp. GTAF01_IU, (H) *Aspergillus fumigatus* F GTAF01_IU and (I) *Scenedesmus* sp. GTAF01_IU -*Aspergillus fumigatus* F GTAF01_IU co-pellet. FESEM analysis of (J) *Scenedesmus* sp. GTAF01_IU, (K) *Aspergillus fumigatus* F GTAF01_IU hyphae and (L) the attachment of *Scenedesmus* sp. GTAF01_IU on the *Aspergillus fumigatus* F GTAF01_IU hyphae.

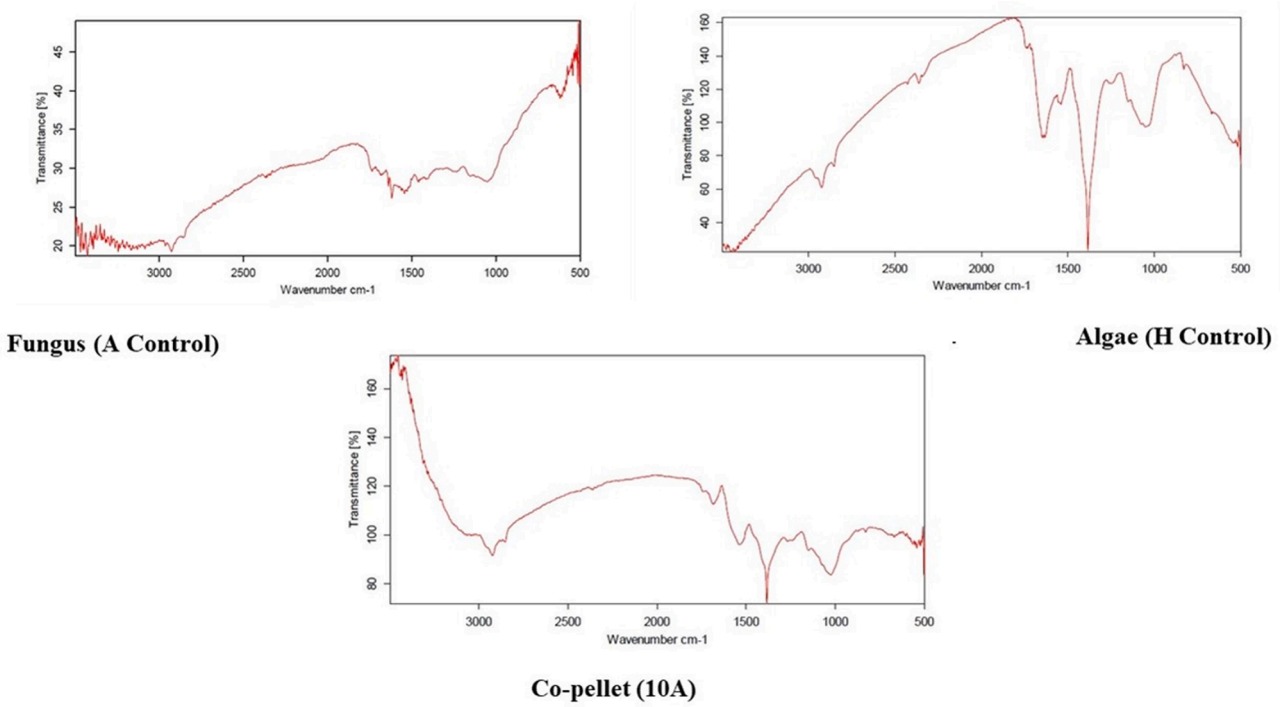

Fig. 8. FTIR spectrum of (a) *Scenedesmus Sp.* GTAF01_IU (b) *Aspergillus fumigatus* F GTAF01_IU and (c) algae and fungi co-pellet.

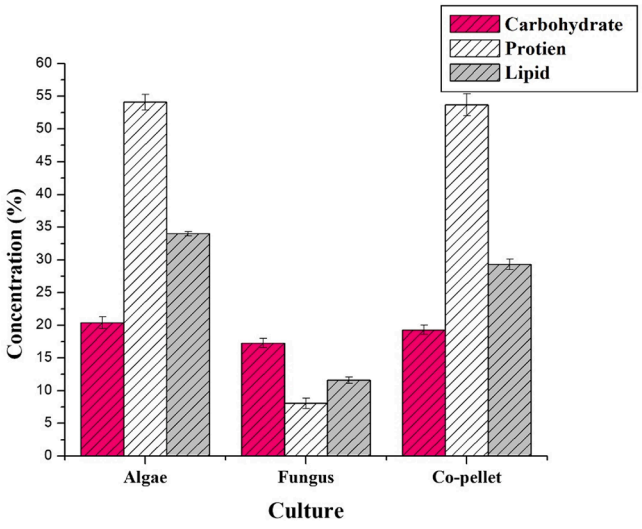

Fig. 9. Biochemical composition.

Aspergillus fumigatus-Chlorella protothecoides pellets were slightly reduced by 15 % and 18 % after 48 h of co culturing. A significant reduction in the lipid content was observed in co-pellet formed by the *Chlorella protothecoides Aspergillus fumigatus* (21.35 %) as compared to lipid content in monoculture of algae (28.20 %) (Muradov et al. 2015). The results indicate that the bio-flocculation significantly alters the metabolic profile of the algae, fungus, and co-pellet. The most notable changes were observed in the levels of biofuel precursor molecules such as carbohydrates and lipids. Supporting the present results, a study conducted by Bhandari et al., 2024 suggested that the nutrient starvation condition and high abiotic stress have altered the lipid and carbohydrate assimilation. They also suggested that the inorganic carbon enhances the Ribulose-1,5-bisphosphate carboxylase activity which is important for the conversion of phosphoglycerate into lipids and carbohydrates. Thus, it can be concluded that the co-pelletization of algae-fungus can be significantly used for harnessing its biofuel potential.

4. Conclusion

In the present study, fungal-assisted bio-flocculation of algae emerged as an effective algal harvesting technique. Optimization of process parameters like, algae: fungi ratio, pH, and agitation, resulted in an enhanced algal biomass recovery up to 90 %. Diminishing the pH up to 3 enhanced the recovery (in the form of spherical co-pellets) up to 93.6 % in 7 h. The primary surface charges were affected by co-pelletization. Algal-fungal co-pelletization represents a sustainable harvesting method that mitigates the risk of biomass contamination commonly associated with traditional approaches employing chemical flocculants. Additionally, the substantial size of the co-pellet streamlined the separation process, presenting an effective strategy for biomass utilization in biofuel production.

CRediT authorship contribution statement

Gyanendra Tripathi: Conceptualization, Writing – original draft, Writing – review & editing. **Vinay Kumar Pandey:** Writing – original draft, Writing – review & editing. **Suhail Ahmad:** Writing – original draft, Writing – review & editing. **Irum:** Writing – original draft, Writing – review & editing. **Nortoji A. Khujamshukurov:** Conceptualization, Writing – original draft, Writing – review & editing. **Alvina Farooqui:** Conceptualization, Writing – original draft, Writing – review & editing. **Vishal Mishra:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data relevant to this study are presented in the manuscript. No additional datasets were generated or analyzed during the current study.

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