




Application of Microbial Consortium to the Pilot-Scale Treatment of Primary Treated Sewage Wastewater

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Abstract Huge discharge of different organic and inorganic waste compounds into water sources is the prime reason for water pollution. To protect the environment, appropriate biological treatment methods of wastewater with high removal efficacy are needed. To meet this end, indigenously available microbial consortiums were explored for their possible bioremediation efficiency. Cyanobacteria purified from microbial consortium was identified as *Desertifilum* sp. based on 16 s rRNA gene sequencing, and its biochemical characteristics were determined. High-rate algal pond (HRAP) of 6 m³ volume with dimensions of 3 m×2 m×1 m was inoculated @ 0.25% and operated in an open environment at a light intensity of 38,000 to 62,000 lx with a hydraulic retention time (HRT) of 12 days. Results obtained after 12 days showed removal efficiencies of 78.26, 76, 79.55, 4.77, and 58.74% for soluble chemical oxygen demand (sCOD), total chemical oxygen demand (tCOD),

biochemical oxygen demand (BOD), nitrates, and total phosphorus, respectively. The results from the study inferred that *Desertifilum* sp. is a suitable candidate for secondary-stage wastewater treatment without any additional amendment. Moreover, the biochemical composition of the biomass obtained unraveled its potential application in the field of nutraceuticals.

Keywords *Desertifilum* sp. · High-rate algal ponds (HRAT) · Hydraulic retention time (HRT) · Percent reduction · Wastewater

1 Introduction

Urbanization and industrialization have generated tons of solid wastes and wastewater which affect the ecosystem's stability and cause soil, water, and air pollution. Eutrophication-like problems are caused by nutrient enrichment of water bodies due to the dumping of untreated or partially treated wastewater in nearby rivers or streams (Mahapatra et al., 2013). To remove nutrients from wastewater, several chemical treatments and conventional physical methods are used. However, high cost, excessive generation of greenhouse gases (GHGs), and more sludge production are the major disadvantages limiting their use (Zhang et al., 2019). Moreover, through physical and chemical processes, contaminants and pollutants in wastewater are not fully removed from wastewater.

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Microalgae are unicellular or simple multicellular, microscopic (2–200 μm), photosynthetic microorganisms that can thrive in harsh conditions and can grow autotrophically as well as mixotrophically (Daneshvar et al., 2019). Microalgae as well as cyanobacteria metabolize nitrogen, phosphorus, and carbon present in wastewater and can help in the potential treatment of wastewater. The microalgae-bacterial process works on self-synchronized microalgal photosynthesis and bacterial respiration. In this process, microalgae can effectively assimilate carbon dioxide and nutrients to generate oxygen, which is in turn utilized by bacteria for organic oxidation to produce carbon dioxide. They also sequester carbon dioxide from the environment and, hence, contribute to a reduction in the greenhouse effect (Ramanan et al., 2016). Microalgae-bacterial consortium-mediated bioremediation of domestic wastewater is supported by complex nutritional and metabolic interaction between microalgae and associated bacteria (Nagarajan et al., 2022). Cyanobacteria can be used either as monoculture or in symbiosis with other microbial populations for the treatment of wastewater. Microbial consortia are one of the most important types of symbiosis used for wastewater treatment. Each microbial population in consortia maintains cooperative interactions with each other by metabolite exchange leading to enhanced nutrient reduction potencies (Gonçalves et al., 2017).

Punjab is a state of agriculture with a total geographical area covered of 50,362 km^2 and is located between 29°33′–32°32′N latitudes and 73°55′–76°51′E longitudes (Baweja et al., 2017). Most of the crops cultivated in Punjab are irrigated using groundwater. Excessive use of these groundwater sources has caused a serious threat to sustainable agriculture development (Sidhu et al., 2010). Many ponds and lakes existing in Punjab's villages are severely polluted due to improper sanitation practices by nearby dwellers. Hence, to address the distressing rate of water decline in Punjab, it becomes inevitable to recycle and reuse wastewater generated from sewage treatment plants (STP) and village ponds. Most developing countries employ only secondary levels of wastewater treatment which does not address nutrient removal from wastewater efficiently as effluent-treated water still contains a vast amount of nutrients such as phosphorus and nitrogen (Cai et al., 2013). Hence, alternative methods that are economical and

eco-friendly are needed for efficient wastewater management practices and nutrient removal from wastewater within permissible limits before its discharge.

The use of wastewater for the cultivation of cyanobacteria has several potential benefits such as minimizing freshwater use, reducing the extra cost of nutrient addition, removing nitrogen and phosphorus from wastewater, producing microalgal biomass as bioresources for biofuel or high-value by-products, and reducing investment and operating costs as compared to conventional chemical and mechanical wastewater treatments. Moreover, harvested microalgal biomass from wastewater treatment can be used to produce high-value by-products viz., bioethanol, biodiesel, biogas, etc.

Unlike our research, many studies are conducted with purified microalgal strains. Strains like *Chlorella vulgaris*, *Chlorella variabilis*, *Botryococcus braunii*, *Nitzschia* sp., and *Nannochloropsis* sp. were explored for their bioremediation potential and additional value-added products (Alibas et al., 2023). But the potential of indigenous consortia is least explored especially in the ambient environmental conditions. Many researchers supported the integration of the microalgal process as a successful secondary wastewater treatment process. Most of the research carried out in the field of microalgae-bacterial-mediated wastewater treatment is performed in photobioreactors in controlled environmental conditions.

To meet this end, the present investigation was carried out to evaluate the potential of microbial consortium for the treatment of wastewater at a pilot scale with possible potential target application of biomass.

2 Material and Methods

The wastewater was collected from the sewage treatment plant of the Thapar Institute of Engineering and Technology, Patiala, Punjab after the primary treatment. The physicochemical behavior of wastewater has been characterized.

2.1 Collection and Cultivation of Consortium

The selective microbial consortium under study was collected from a wastewater pond in the village of Haripur district of Jalandhar, Punjab, India. The geographical location of the village wastewater

pond is 30°22'42.23" N and 76°20'40.01" E with an altitude of 223 m. The climatic conditions of Patiala are semi-arid, and the average annual rainfall is 698.0 ± 297.3 mm. The consortium is cultivated in BG-11 media at 10% (v/v) with a 14:10 photoperiod cycle at 24 ± 2 °C for 8 days. The aeration was provided continuously at 1.5 LPM (liter per minute) at a light intensity of 12,000 lx.

2.2 Experimental Setup for Pilot-Scale Wastewater Treatment

The study was carried out in a high-rate algal pond of 6000 L (6 m³) with dimensions of 3 m × 2 m × 1 m. The working volume was kept at 4800 L. The experiment was conducted in an ambient environment under variable natural sunlight intensity ranges between 38,000 and 62,000 lx with a hydraulic retention time of 12 days. After 12 days of treatment, biomass was harvested, filtered using fine sieve mesh, dried, and finally weighed.

2.3 Purification of Dominant Microalgae Strain with Effective Wastewater Treatment Efficacy from Indigenous Microbial Consortium

The dominant microalgae strain was isolated from a pre-assessed indigenous consortium at the Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India. Isolation was performed on BG-11 media with the light intensity of 8000 lx and selective light and dark cycle of 14:10 at 28 ± 2 °C.

2.4 Biochemical Characterization and Molecular Identification of Potential Isolate with Wastewater Treatment Efficacy

2.4.1 Biochemical Characterization

Chlorophyll content was determined by the method of El-Baky et al. (2008) with little modifications. The method of DuBois et al. (1956) was used to determine carbohydrates. The lipids were estimated by sulpho-phospho-vanillin (SPV) assay given by Mishra et al. (2014). Proteins were estimated according to Lowry et al. (1951). The total phenolic content was quantified using Folin Ciocalteu's method as described by Taga et al. (1984). Total antioxidant activity was

measured by the method of Prieto et al. (1999). Screening of phytochemicals viz. quinones, saponins, and terpenoids was also performed (Tyagi, 2017).

2.4.2 Molecular Identification

Molecular identification of the isolate was done at the National Chemical Laboratory, NCIM, Pune. The biomass was harvested at the logarithmic growth phase. Extraction of Genomic DNA was done by employing the HiPurA Fungal DNA purification spin column kit (MB543-250PR, HiMedia, India). PCR amplification was carried out by using cyanobacterial specific 16SrRNA gene (1000 bp) primers, i.e., *cya106F* (5'CGG ACG GGT GAG TAA CGC GTG A 3') and *cya1281R* (5' GCA ATT ACT AGC GAT TCC TCC 3') (Nübel et al., 1997). The PCR reaction was performed in 25- μ L volume containing 10 μ L EmeraldAmp GT PCR Master Mix, 2x (Takara Bio USA), 1 μ L DNA template (50–100 ng), 1.25 μ L (10 μ M) of each primer (forward and reverse), and 11.5 μ L of free-nuclease water using Applied Biosystems Veriti Thermal Cycler. The specifications of the PCR reaction were as follows: Initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min and a final extension at 72 °C for 10 min. Detection of PCR products was done by staining with GelRed Nucleic Acid Gel Stain on 1% agarose electrophoresis gel in (1X) TBE buffer. Amplified products were visualized under a UV transilluminator (Protein Simple Red Imager SA-1000). Final purification of the PCR product was done by using Exonuclease I and Shrimp Alkaline Phosphatase Purification Kit (New England Biolabs, Inc) and cycle sequenced using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA) under following conditions: Denaturation at 96 °C (1 min) followed by 25 cycles of 96 °C (10 s), 50 °C (5 s), and 60 °C (4 min). Cycle-sequenced amplicons were further purified using sodium acetate ethanol method (Thermo Fisher Scientific), and sequencing reactions were run on a 3500xL Genetic Analyzer (Applied Biosystems, USA).

2.5 Phylogenetic Analysis

Sequencing files (.ab1) were edited using CHROMASLITE (version 1.5). Basic Local Alignment Search Tool (BLAST), search was carried out to find the

closest culture sequence retrieved from the National Centre for Biotechnology Information (NCBI) database that finds regions of local similarity between sequences (Altschul et al., 1990). The phylogenetic tree was constructed by Mega11 software using the neighborhood joining method with 1000 bootstrap replicates. The sequence was submitted to the NCBI GenBank database, and an accession number was allotted.

2.6 Analytical Methods

Physico-chemical parameters of wastewater such as BOD, COD, nitrates, and phosphates were analyzed by following the standard protocols of APHA (2012). pH was measured using a Lab 860 pH meter (Schott, Germany).

2.7 Statistical Analysis

Experiments were performed in triplicates. Error bars in figures display mean \pm standard deviation. Two-way analysis of variance (ANOVA) was performed for statistical analysis using IBM SPSS Statistics 22. Data were considered statistically significant at $P < 0.001$. The differences between the means were calculated using Tukey's test at the 0.05 level of significance test employing IBM SPSS Statistics 22.

3 Result and Discussions

In the present study, the potential of the selective microbial consortium was evaluated for the reduction in various physicochemical parameters of domestic wastewater to explore its potential for reusing in agricultural irrigation. The efficiency of wastewater treatment is the function of several parameters such as the growth rate of cyanobacteria which further depends on the metabolism of micro-organisms present in the consortium and available nutrients, system operating conditions, and harvested biomass for nutrient removal (Olguín, 2003). Under nutrient stress conditions, microalgal growth tends to slow down and use the nutrients available for the production of lipids or carbohydrates (Rodolfi et al., 2009). Cyanobacteria can grow under autotrophic, heterotrophic, or mixotrophic conditions (Mata et al., 2010) which makes it most suitable for wastewater treatment.

3.1 Wastewater Treatment at Pilot Scale

The selective microbial consortium under study was cultivated in BG-11 medium at 10% (v/v) inoculum rate with a 14:10 photoperiod cycle at 24 ± 2 °C for 8 days. The grown microbial consortium was inoculated in the algal pond at 0.25% inoculum size. Light intensity and photoperiod regime along with temperature are the important operational factors for the microalgae culture system because they can affect the wastewater treatment system by altering the proportions of specific microalgal species in the consortium (Moondra et al., 2020). The initial pH of the wastewater was 7.18 ± 0.01 . The concentration of total solids in STP water was 772 ± 0.11 mg/L. The total solid content of wastewater is constituted by the presence of compounds like carbonates, bicarbonates, chlorides, sulfate, phosphate, nitrate, Ca, Mg, Na, K, Mn, and organic matter (Sahana and Shirnalli 2018). BOD and COD of wastewater were observed around 122 ± 2.30 and 230 ± 7.35 mg/L, respectively. The COD content of wastewater in this study is higher than that of BOD. The COD value may be attributed to the presence of organic matter and inorganic matter in wastewater as COD is a measure of non-biodegradable organic matter in biologically treated industrial effluents (Malaviya & Rathore, 2001). Nitrates present in wastewater were 32.6 ± 0.80 mg/L, and total phosphorus was 14.2 ± 0.22 mg/L. Domestic wastewater contains pathogens, suspended solids, organic matter, and organic and inorganic nutrients (Al-Jalil, 2009). Therefore, to minimize the health hazards caused by these pollutants present in wastewater, it is necessary to bring down them to the permissible limits. Nitrate contamination in water bodies includes anthropogenic activities, chemical fertilizers, sewage, and landfill by domestic waste (Shrimali & Singh, 2001). Several industrial effluents like plastic, metal treatment, textile, plywood, household cleaning, and pharmaceuticals further increase the nitrate pollution in groundwater (Singh et al., 2006). Phosphate pollution in groundwater occurs through non-point sources such as the natural decomposition of rocks and minerals, agricultural runoff, erosion, sedimentation, animals, and by-point sources such as sewage effluents and industrial discharges (Singh, 2013).

3.2 Analysis of Physio-Chemical Parameters of Wastewater after Treatment

For sustaining microalgal growth, organic carbon, nitrates, and phosphates are required. Organic carbon is mineralized by cyanobacteria by consuming the dissolved oxygen in wastewater to fulfill their carbon and energy demands. This organic carbon is taken in the form of BOD and COD. BOD is defined as the capability of microorganisms to oxidize organic material into CO₂ and water. Hence, BOD uses dissolved oxygen in water causing the death of water-bodies. The primary goal of wastewater treatment is BOD removal (Sarfraz et al., 2021).

Higher COD may be attributed to the higher organic matter in wastewater (Malaviya & Rathore, 2001). The reduction of COD content might be due to the inherent potential of microbes to utilize organic compounds as an energy source besides carbon dioxide (Hu et al., 2012).

In the present study, the maximum percent reduction in soluble COD is 78.26% (Fig. 2) and 76% total COD (76%) (Fig. 1) occurred after 12 days of treatment as compared to initial values of soluble COD and total COD of 153.33 and 233.33 mg/L,

respectively. On the other hand, natural reduction was also observed in control without inocula addition, i.e., 48.70% in soluble COD and 30.57% in total COD after 12 days of treatment (Fig. 2). Our results are in agreement with Maza-Márquez et al. (2017) who recorded an $80.7 \pm 9.7\%$ COD removal efficiency of the microalgae-bacteria consortium consisting of microalgae *Scenedesmus obliquus* and *Chlorella vulgaris* and bacteria *Raoultella terrigena* and *P. agglomerans* in Olive-washing water at Pilot-scale Photobioreactor.

The rate of COD reduction during the initial phase was low due to the acclimatization phase of organisms in a new environment, and secondly, the carbon might be colloidal, gradually biodegradable material. Generally, carbon is the restricting variable when algae are used in sewage. However, the carbon requirement for algal photosynthesis is fulfilled by atmospheric CO₂ if found insufficient (Su et al., 2011).

A similar rate of reduction in BOD was observed after 12 days of treatment. After 72 h of treatment, a 30.11% BOD reduction was achieved. A BOD reduction of 61.36% was observed after 7 days of treatment. However, a decrease in BOD reduction was found with a further increase in treatment days.

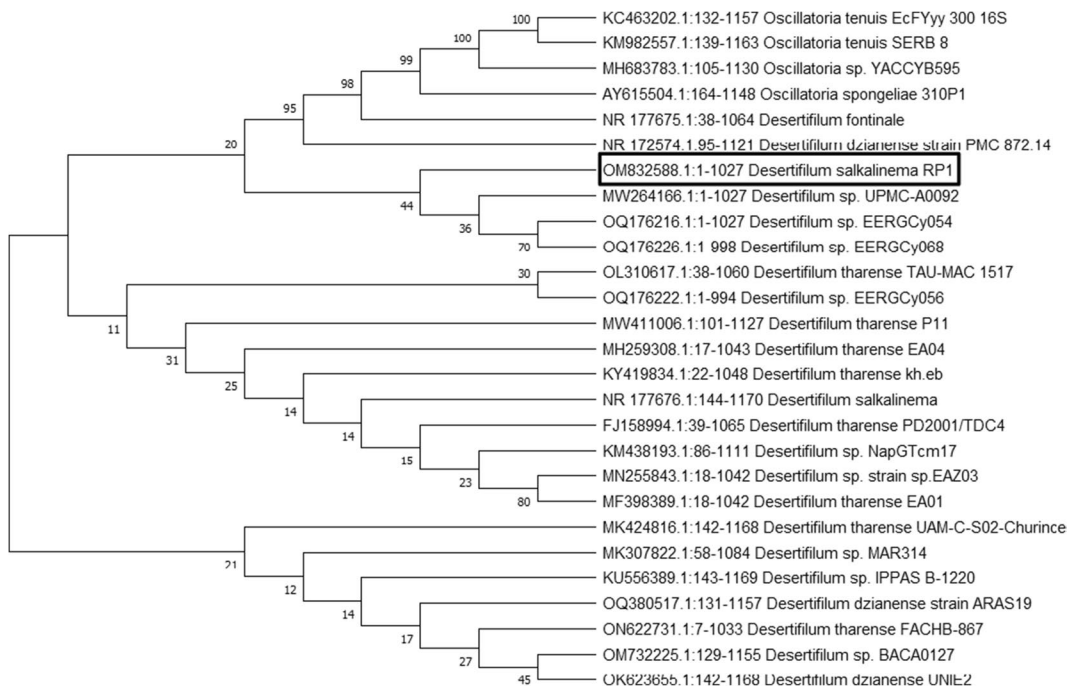
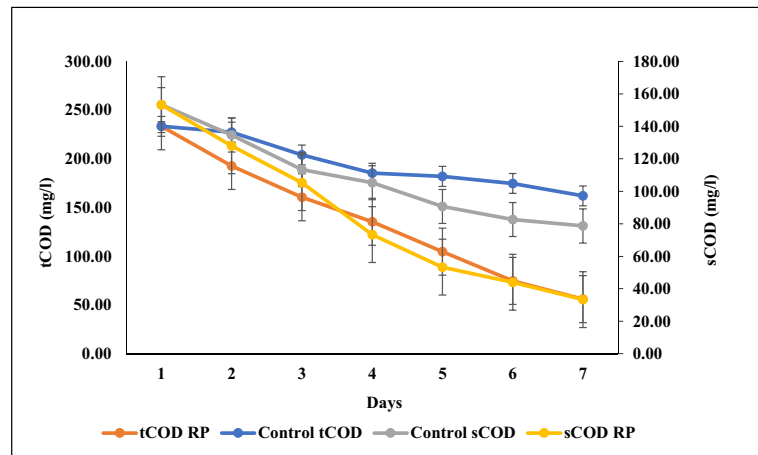


Fig. 1 Phylogenetic tree of *Desertifilum* sp

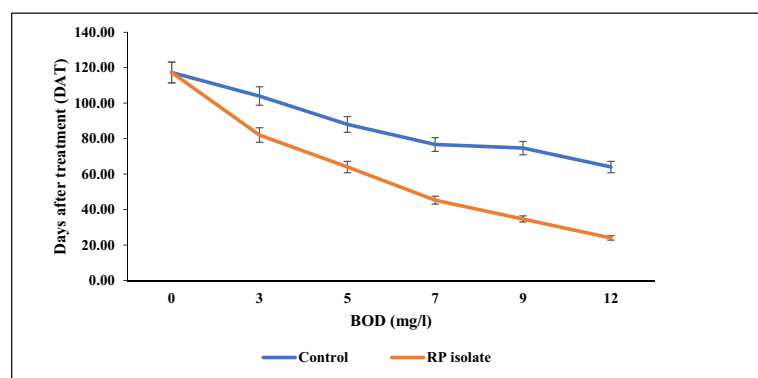
Fig. 2 Variation in tCOD and sCOD

Consequently, the present study reported a 79.55% maximum percentage reduction in BOD after 12 days of reduction. Control treatment resulted in a lower percentage reduction, i.e., 11.36% as compared to microbial consortium treatment after 12 days of treatment (Fig. 3). Natural wastewater treatment without inoculation was observed initially. However, with an increase in treatment days, the rate of BOD and COD reduction has slowed down as compared to wastewater inoculated with the consortium. Comparative results showed a significant difference between control and consortium treatment. Khemka and Saraf (2017a, 2017b) reported a 70% reduction in the BOD content of dairy wastewater after 15 days of treatment with *Desertifilum tharense* MSAK01.

The removal of dissolved organic compounds and their derivatives might be the reason behind the reduction of BOD levels (Kotteswari et al., 2012). Biological oxygen demand (BOD) is the measure of oxygen present in wastewater for utilization by

microorganisms for breaking down decomposable organic matter into simpler substances. It is also an indicator of the amount of organic matter present in wastewater (Divya & Santhanam, 2018). Photosynthetic microbes during the wastewater treatment process build their bodily substances for growth and development by converting BOD and COD into carbon sources (Hadiyanto & Soestrisnanto, 2013).

The two utmost important macronutrients for microalgal growth are Nitrogen and Phosphorus. They are required for growth, nutrient content, enzyme functioning, biochemical processes, and cell integrity of plants. Ammonium (NH_4^+) and nitrate (NO_3^-) are two preferred forms of nitrogen used by plants. Deficiency of nitrogen harms crop productivity and growth, while excess nitrogen has detrimental effects on humans and causes groundwater contamination (Akao et al., 2021). A deficiency of phosphorus adversely affects chlorophyll synthesis, growth, and metabolism of cells because it is necessary for

Fig. 3 Variation in BOD

the functioning Calvin cycle and many phosphorylation syntheses (Liang et al., 2013), whereas a surplus amount of phosphorus can retard growth, cause leaf necrosis, and reduce the availability of zinc elements resulting in loss of nutritional elements and organic metabolites. Thus, nitrogen and phosphorus should be within permissible limits in water as well as in soil, because both excess and deficiency of these nutrients adversely affect living organisms.

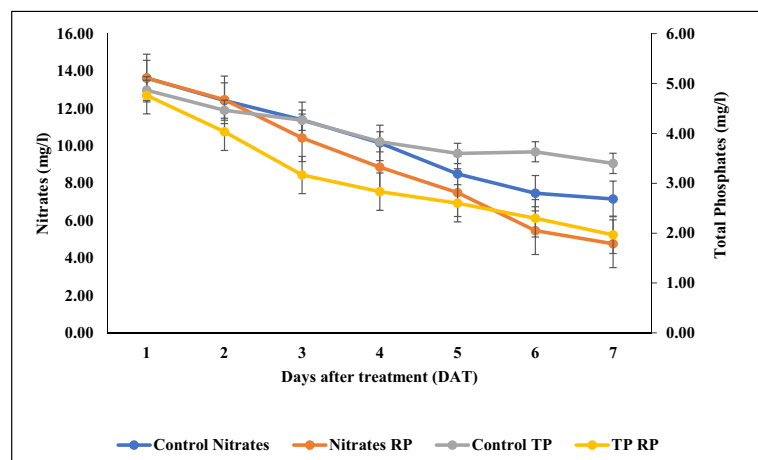
After 12 days of treatment, phosphate content was reduced to 1.97 mg/L as compared to the initial concentration of phosphate, i.e., 4.77 mg/L in STP water, while in control, phosphate concentration was reduced to only 3.40 mg/L after 12 days of treatment as compared to the initial concentration of 4.87 mg/L on day 1. Reduction in the control was very low as compared to the wastewater treated with the microbial consortium (Fig. 4). According to the Food and Agriculture Organization, permissible limits of phosphorus in irrigation water are 0–2 mg L⁻¹ (Ayers and Westcot, 1985). After microbial consortium treatment, the permissible limit of phosphorus has been achieved making treated water suitable for irrigation. Fito and Alemu (2019) reported a reduction of 59% in total phosphorus content of municipal wastewater after treatment with a consortium consisting of microalgae *Chlorella* sp., *Chlamydomonas* sp., *Scenedesmus* sp., and bacteria carried out in 1 L of bioreactors at batch scale. However, in the present study under natural conditions, a 58.74% reduction in total phosphorus was achieved.

The major problem for most wastewater sources is the higher concentration of nutrients, particularly

the TN and TP concentrations, which require costly chemical-based treatments to remove them during wastewater treatment. The TN and TP concentrations range from 10 to 60 mg/L in municipal wastewater, >1000 mg/L in agricultural effluent, and 500–600 mg/L in farm wastewater (Chiu et al., 2015). Photosynthetic microbes have an astounding ability to grow in wastewater and to efficiently consume nutrients and accumulate metals from wastewater which makes them an extremely attractive means for sustainable and low-cost wastewater treatment (Devi et al., 2012).

Total phosphorus is reduced owing to its consumption by microbes, because phosphorus is required by algae for sustaining its growth and development such as for the synthesis of algal biomass and other intracellular polyphosphate compounds, phospholipids, adenosine triphosphates (ATP), and nucleic acids which are generally assimilated as inorganic orthophosphate, primarily as H₂PO₄⁻ or HPO₄²⁻ (Ding et al., 2015). The phosphorus removal mechanism is more complex as compared to nitrates as it is commonly assimilated in the form of orthophosphate. It is also utilized for growth, nucleic acid synthesis, and value-added compounds like astaxanthin and polyunsaturated fatty acids. Algae do not always uptake phosphorus stoichiometrically. It is affected by factors like the physiology of algae, concentration of phosphorus, light intensity, pH, and temperature affect. Cell phosphorus concentration is inversely related to phosphorus uptake (Choi, 2014). Hence, the cellular concentration of phosphorus controls the kinetics of phosphorus uptake (Choi, 2016).

Fig. 4 Variation in nitrates and total phosphorus



Similarly, nitrate reduction after treatment has followed a similar trend. After 12 days of treatment nitrates were reduced to 4.77 mg/L as compared to 13.63 mg/L. On the other hand, in the control treatment, nitrate reduction was very low after 12 days of treatment, and it displayed a decrease to only 7.17 mg/L as compared to the initial value (Fig. 4). According to the Food and Agriculture Organization, permissible limits of nitrates in irrigation water are $<5 \text{ mg L}^{-1}$ (Ayers and Westcot, 1985). After microbial consortium treatment, the permissible limit of nitrates has been achieved making treated water suitable for irrigation. Huo et al. (2020) reported that wastewater produced after vinegar generation was treated with a consortium consisting of *Chlorella* sp. along with *Bacillus firmus* and *Beijerinckia fluminensis* and observed with 20% reduction in nitrate content after treatment.

Nitrogen reduction in wastewater is the result of several processes occurring simultaneously including nitrification, biomass uptake of nitrogen, ammonia stripping, and loss in the atmosphere (Lv et al., 2017). Protein synthesis in cyanobacteria requires nitrogen; hence, it is an essential element contributing to microalgal growth. Nitrate is a preferential form for utilization by algae, and algal cells contain 5–10% of it. Under nitrogen-deficient conditions in a culture medium, lipids and carbohydrates are synthesized (Voltolina et al., 2005).

Results of statistical analysis after 12 days of treatment showed that the pH of wastewater treated with microbial consortium increased significantly displaying a high increase in pH (8.74) as compared to day 1 where the pH was 7.03. Control treatment showed a similar trend with a pH increase from day 1 to day 12 ranging from 7.05 to 8.02 (Fig. 1). However, the pH increase in wastewater treated with the microbial consortium was higher than in the control. The increase in pH from day 1 to day 2 was attributed to microalgal growth and hence photosynthesis. Cyanobacteria take up dissolved CO_2 in the water during the process of photosynthesis, then by the action of the enzyme carbonic anhydrase, bicarbonate is converted into CO_2 , releasing hydroxyl ions, leading to an increase in pH (Morales et al., 2018).

Elevated pH levels not only increase the microalga's ability to assimilate nutrients by changing the available form of nutrients but also affect the cellular physiology of the microalgal-bacterial consortium

(Kube et al., 2018). The increased pH content also helped in the significant reduction of ammonical nitrogen and phosphates through ammonia volatilization and phosphate precipitation, respectively. This further increases the overall efficiency of the wastewater treatment system by the removal of the suspended algae from the wastewater leading to optimal productivity of the microalgal-bacterial consortium (Moondra et al., 2020).

After 12 days, about 258.3 g g^{-1} dw of microalgal biomass was obtained. The obtained potential amount of biomass can have applications in the field of biofuel and nutraceutical production as determined by its biochemical composition.

The interaction between microalgae and bacteria in the context of wastewater treatment was observed and applied extensively in stabilization ponds with microalgal growth. Photosynthetic oxygenation provided by microalgal metabolism proved to be effective for organic matter removal by bacteria. The stabilizing ponds are termed 'high-rate algal ponds' which are low-cost and energy-efficient treatment systems. Typically, any wastewater treatment system that treats non-sterile or unsanitized wastewater with microalgae develops a microalgal-bacteria consortium. The microbial community in the consortium varies throughout the treatment period, depending on the nutrient availability. Microalgae and bacteria in consortium interact in a mutualistic manner. Bacteria act as natural mineralizers in wastewater treatment participating in the degradation of organic carbon in a dissimilatory manner releasing CO_2 . Algal photosynthesis assimilates nitrogen, phosphorus, and the CO_2 released by bacteria as beneficial biomass. The major macronutrients in wastewater that can be removed by microalgal systems are carbon (dissolved organic carbon, and gaseous CO_2 from industrial waste), nitrogen, and phosphorus. Organic carbon is assimilated into algal biomass by heterotrophic or mixotrophic growth. Degradation of organic carbon by symbiotic bacteria could provide CO_2 for photosynthesis with algae (Ramanan et al., 2016).

3.3 Biochemical Characterization

Several types of antioxidants, bioactive compounds, and compounds with biological functions were synthesized by cyanobacteria as a result of either primary or secondary metabolism. Keeping this in

mind, a study of the biochemical composition of the methanolic extract of isolate RP was done.

Microalgae extracts are complex mixtures of the compounds present in microalgal biomass such as polysaccharides, phenols, polyunsaturated fatty acids, proteins, pigments, vitamins, terpenoids sterols. The constitution of extracts varies with season, age, species, and other environmental factors. Microalgal bioactive compounds belong to various chemical groups such as tannins, alkaloids, glycosides, lignans, terpenoids, which show different solubilities in different solvents. The most commonly used solvents for extract preparation are benzene, chloroform, diethyl ether, ethyl acetate, ethanol, hexane, methanol, and distilled water (Patil & Kaliwal, 2019). In the present study, methanolic extract of dried biomass of microalgal isolate RP was prepared and used for the detection of biochemical components. Due to its aptness to extricate both polar as well as non-polar components from plant material, methanol was employed as a polar solvent for the extraction of bioactive molecules from microalgae (Ukwubile et al., 2019).

A wide variety of pigments are synthesized by microalgae sp. Pigments like β -carotene (β -Car), lutein, canthaxanthin, astaxanthin (Asx), and fucoxanthin (Fcx) are known to be produced by the microalgae such as *Botryococcus braunii*, *Chlorella* sp., *Chlorococcum* sp., *Coelastrrella striolata*, *Haematococcus pluvialis*, *Dunaliella salina*, *Nanochloropsis* sp., *Scenedesmus* sp., *Spirulina platensis* (Vignaud et al., 2023). Results illustrated that the chlorophyll content of RP was 54.30 mg L⁻¹. Pigment content varies in microalgae sp. In a previous study conducted by Grubišić et al. (2022) the total chlorophyll content of microalgae *Tetraselmis* sp. C6 and *Picochlorum* sp. D3 was 54.03 mg/100 g DW. Chlorophyll is the green pigment found in green algae and plants and has a role in several biological functions like induction of apoptosis cancer cells, and anti-mutagenic, and antioxidation properties (Hashem et al., 2021). Pigment chlorophyll has several biotechnological applications. For example, it may be used as a natural food colorant, deodorant, or as a component of dentifrice and skin care cosmetics. It also has antitumor and anti-inflammatory functions. Chlorophyll has been reported to reduce the severity of facial acne vulgaris lesions with minimal side effects, after photodynamic therapy (Song et al., 2014).

Different amounts of carbohydrates, proteins, and lipids are present in microalgal biomass. Lipids are

the most energy-rich compound (37.6 kJg⁻¹), followed by proteins and carbohydrates with an energy content of 16.7 kJg⁻¹ and 15.7 kJg⁻¹, respectively (Wilhelm & Jakob, 2011).

Proteins in isolate RP were 366.80 mg g⁻¹ dw. Previous studies on cyanobacterial species such as *Desertifilum tharensense* (Perendeci et al., 2019), *Cyanothece* sp. (Hotos et al., 2023), *Nostoc* sp. (PCC 7936) (Alvarez et al., 2021), and *Arthrospira platensis* (López-Rodríguez et al., 2021) showed the protein content of 281 mg/g (28.1%), 343 mg/g (34.3%), 500 mg/g (50%), 369 mg/g (36.90%), respectively. Proteins found in algae contain essential amino acids, and they are alike other protein sources, e.g., soybean and egg and have several benefits over traditional protein sources in terms of nutritional quality (Bleakley and Hayes., 2017).

RP showed a carbohydrate content of 80.22 mg g⁻¹ dw. Our results are in agreement with Reitan et al. (2021) who showed that the total carbohydrate content in the microalgae varied from 54 mg g⁻¹ DW in *Tisochrysis lutea* to 235 mg g⁻¹ DW in *Chaetoceros muelleri*. Carbohydrates present are easy to digest and good for diabetic patients because they are cellulose and sugar-free (da Silva Braga et al., 2018). Carbohydrates present in microalgal biomass have the lowest energy content as compared to other organic groups, i.e., lipids and proteins, but they are preferred substrates for biofuel production such as bioethanol, biobutanol, biohydrogen. Depending on the type of species and cultivation conditions, the carbohydrate content of microalgal biomass varies. For instance, the carbohydrate content of *Porphyridium cruentum* and *Spirogyra* sp. ranges from 40 to 57% and 33 to 64%, respectively (Harun et al., 2010).

The lipid content found in the RP isolate was 79.83 mg g⁻¹ dw. Previous studies reported the lipid content of 64 mg/g (6.4%), 190 mg/g (19%), 65.5 mg/g (6.55%), 60–110 mg/g (6–11%) in *Chrysothila pseudoroscoffensis* (Moreira et al., 2022), *Desertifilum* sp. (IPPASB-1220) (Bolatkhan et al., 2020), *Nostoc calcicole* (Nagappan et al., 2020), *Syneccoccus* sp. (Singh and Kumar, 2022), respectively. Microalgal lipids are polar membrane lipids, i.e., phospholipids and glycolipids and non-polar lipids, i.e., triacylglycerols, glycerides, carotenoids (Ryckebosch et al., 2014). Lipid production is a function of many factors such as light conditions, temperature and nitrogen concentration, pH, and salt concentration (Zhu et al., 2016).

The total phenols present in biomass were calculated using the most precise Folin Ciocalteu method. Total phenols in isolate RP were 5.78 mg GAE g⁻¹ dw. Jerez-Martel et al. (2017) reported that *Euglena cantabrica* contained 5.87 and 2.97 mg g⁻¹ dry biomass of gallic and protocatechuic acids, respectively. Phenols are aromatic compounds that have a benzene ring with the -OH group attached as a functional group. In biological systems, phenolics have been associated with antioxidant activity and act as scavengers of singlet oxygen and free radicals. In plants, phenolic acids, flavonoids, and tannins are prime compounds that were contemplated as responsible for the antioxidant capacity. A variety of biological functions are performed by these antioxidants, e.g., anti-atherosclerotic, anti-inflammatory, and anti-carcinogenic activities (Patil & Kaliwal, 2019).

Total antioxidants in RP were 9.47 mg AAE g⁻¹. High levels of antioxidant activity detected in isolate RP can be due to the presence of functional groups such as hydroxyl groups of phenols. Phenolic compounds have high redox potential which plays a pivotal role in the adsorption of free radicals and, thus, quench the O⁻¹ and O⁻³ radicals. Phlorotannins are polyphenols that act as major antioxidants and help in resisting oxidative stress. Other functions are also performed by polyphenols. Polyphenol-enriched methanolic extracts of *Ecklonia stolonifera* significantly decreased the plasma glucose concentration and thus showed high anti-diabetic activity in Diabetes Mellitus-induced rats. Further, total polyphenols also play a significant role in the determination of the antiproliferative activity of algae (Thomas & Kim, 2011).

Phytochemical analysis of methanolic extracts of isolate RP indicated the presence of terpenoids and quinones while saponins were absent. Patil and Kaliwal (2019) observed the presence of alkaloids, saponins, glycosides, flavonoids, phenols, anthraquinones, and oils and fats in chloroform, acetone, ethanol, methanol, and aqueous extracts of microalgae *Scenedesmus bajacalifornicus* BBKLP-07.

3.4 Molecular Identification and Phylogenetic Analysis

Dominating photosynthetic isolate in the indigenous microbial consortium was purified on BG-11 Medium by the repeated sub-culturing method. The

purified culture, i.e., RP was observed under a light microscope. Cells were non-branching filamentous with green or bright blue-green in color. The filaments have apical ends and were present alone or organized in separate bundles. Then, the purified culture, i.e., RP was sent to the National Chemical Laboratory, National Collection of Industrial Microorganisms (NCIM), Pune, India for molecular identification. The strain showed a close resemblance with *Desertifilum* sp. as shown by the phylogenetic tree (Fig. 1). The sequences were submitted to the NCIM GenBank database, and the following Accession number, i.e., OM832588 was obtained. *Desertifilum* sp. is an oscillatorean cyanobacterium with several potential applications. *Desertifilum* IPPAS B-1220 was used to fabricate silver nanoparticles that have anticancer and antibacterial activity against different cancer cell lines and pathogenic bacterial strains (Hamida et al., 2020). In another study conducted by Khazi et al. (2021), *Desertifilum* sp. has been characterized for c-phycocyanin production. Khemka and Saraf (2017a, 2017b) reported *D. tharense* as a potential strain for the treatment of dairy wastewater along with the production of lipids, protein, and carbohydrates.

4 Conclusion

It can be concluded that cyanobacteria RP isolated from a village wastewater pond of the Haripur district of Jalandhar (Punjab) was identified as a member of *Desertifilum* sp. The cyanobacteria were able to use contaminants present in wastewater to meet its growth requirement. Wastewater treatment by cyanobacteria can be employed either as a secondary or tertiary treatment and hence has the potential to treat wastewater at a pilot scale. Further, treated water has the potential to be used for irrigation purposes in agriculture.

Author Contribution BS: experimentation. AD: conceptualization and validation. NS: manuscript writing and data analysis. SGS, GSK, HM: review and editing.

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Data Availability The datasets used and analyzed during the current study are available within the article and its supplementary information files or from the corresponding author upon reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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