Sustainable approach for biodiesel production and wastewater treatment by cultivating *Pleurastrum insigne* in wastewater

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**ABSTRACT**

The globalized modern world has been confronted with some of the most challenging problems, most of which arise from human activities. Overexploitation of fossil fuels which leads to energy and environmental crisis, and loss of aquatic ecosystem due to improper disposal of household and industrial waste into water bodies constitute some of the biggest emerging global problems. In this study, an unconventional and sustainable approach to produce biodiesel was analyzed by growing *Pleurastrum insigne* in different wastewater. The growth of *P. insigne* in wastewater in turn resulted in up to 93.61% reduction in biological oxygen demand, 58.62% reduction in total phosphorus content, and up to 76.61% total nitrogen removal in the wastewater. The total lipid content of the organism was highest in wastewater sample 6 (30.47%). The fatty acid profile also showed a high percentage of C16 and C18 fatty acids which are desirable fatty acids for a high-grade fuel. Production of biodiesel conforming to international standards was predicted from *P. insigne* cultivated in wastewater confirming the effectiveness of combining wastewater treatment and biodiesel production.

**NOVELTY STATEMENT**

*Pleurastrum insigne* has never been studied before for phytoremediation of wastewater and biodiesel production. This novel research highlighted the application of *P. insigne* in wastewater treatment and the viable scope in biodiesel production. This work aimed to provide a significant contribution in reducing the cost of production of biodiesel from microalgae while shedding new light on an eco-friendly approach to wastewater treatment.

**Introduction**

The current trend of urbanization, industrialization, and alarming increase in population has led to an intensifying demand for clean energy and water supply (Ansari et al. 2017). The main source of energy for production industries and transportation, fossil fuels, has become a limited resource in addition to the production of harmful greenhouse gases (David 2018; Padmanabhan and Stanley 2012). The UN Report has mandated the reduction of fossil fuel production by ~6% between 2020 and 2030 to mitigate global warming (UNEP 2020). Third-generation biofuels, derived from microalgae, have been proposed to be a potential substitute for conventional fossil fuels (Paddock et al. 2020). Microalgae are effective CO₂ sequesters with a high growth rate and lipid productivity (Tariyal et al. 2013). The lipid produced by microalgae consisted of triglycerides that can be converted to fatty acids methyl esters (biodiesel) by transesterification (Hossain et al. 2008; Yadav et al. 2019). Studies have shown that microalgal biodiesel is non-toxic (contains no sulfur), biodegradable, and releases significantly lesser greenhouse gases than other competing biofuels reducing the release of carbon monoxide, hydrocarbons, and particulate matter into the atmosphere (Murthy and Kumar 2019; Zabed et al. 2019; Ananthi et al. 2021). However, there has been concern over the cost of biofuel production as it involves the high cost of growing and harvesting microalgae. The utilization of wastewater as a growth medium offers a cost-effective approach in reducing the cost of production of microalgal-derived biodiesel, sustainable wastewater treatment, and sequestration of CO₂ (Osundeko et al. 2019; Paddock et al. 2020).

It was reported that more than 70% of the fresh water in India is polluted making it unsuitable for human consumption (Dwivedi 2017). Effluents from industries, domestic waste, and agricultural lands discharged into water bodies contain high concentrations of heavy metals (zinc, cadmium, lead), nutrients like phosphate and nitrate, acids, and alkalis posing a serious threat to the ecosystem and the economy (Chekroun et al. 2014; Egede et al. 2016). Standard wastewater treatment is an expensive process requiring a large amount of space and funding (Solovchenko et al. 2020). Nitrogen and phosphorus, the main nutrients for microalgae are a constituent of wastewater making it a potential medium for microalgal cultivation (Sharma et al. 2020). Nitrogen exists as ammonia, nitrate, and nitrite in wastewater...
assimilating into amino acids to form proteins (Cai et al. 2013). Phosphorus exists as orthophosphate in wastewater which is used to produce phospholipids, adenosine triphosphates (ATP), and nucleic acids by the microalgae. Various studies have shown that microalgae have been successfully grown in wastewaters from animal manure, municipality, brewery, and meat processing units (Mulbury et al. 2005; Farooq et al. 2013; Lu et al. 2015; Otundo et al. 2018; Pham and Bui 2020). Microalgae can adapt to varying environmental conditions including nutrients, salinity, temperature, and UV radiation (Abdullah et al. 2019). They can be grown in different wastewater successfully lowered the chemical oxygen demand (COD), biological oxygen demand (BOD), nutrients like phosphorus and nitrogen which can be detrimental to aquatic life in high concentration (Abdel-Raouf et al. 2012). Coupling wastewater treatment and biofuel production could lower microalgal biofuels. The estimated cost of producing microalgal oil from wastewater was $79.03 barrel⁻¹ ($1.88 gal⁻¹) and the global price of oil vary between $26–107 barrel⁻¹ indicating that cost of microalgal oil is at par and often lower (Pandey et al. 2020). Some microalgae, such as Chlorella vulgaris, C. sorokiniana, Scenedesmus obtusus, Botryococcus braunii, and Phormidium bohneri are extensively used for wastewater treatment (Whitton et al. 2015; Nagi et al. 2020).

The objective of this study is to couple algal bioremediation of wastewater treatment along with biodiesel production reducing the cost involved. In the present study, a novel microalgal strain, Pleurostraum insigne isolated and grown in wastewater from different sources (agriculture, domestic, and tannery). The efficiency of this organism to remediate various parameters (odor, color, BOD, phosphorus, and nitrogen levels) of wastewater was analyzed. The growth and lipid productivity of the organism in different wastewaters was compared with the synthetic growth (Fogg’s) medium. The lipid was extracted and profiled using GCMS. The fatty acids observed from lipid analysis were used to predict the biodiesel properties and were checked if they meet the specifications of Indian Standards (IS), American Society for Testing and Materials (ASTM), and European Norms (EN).

Materials and methods

Microalgal culture

Pleurostraum insigne, with accession number MG940908, isolated from sewage in Mumbai, Maharashtra, India (used previously in the author’s laboratory) was chosen for the study. The organism was maintained in Fogg’s medium at pH 7.0, 25–26°C under natural sunlight (Fogg 1965; Ansari et al. 2017).

Wastewater collection and characterization

Wastewater samples from sewage, agricultural fields, and tanneries from various locations were collected and their source, location, geographical coordinates, color, odor, and pH were noted (Alam et al. 2007). Safety measures for sample collection were followed. The collected samples were carefully transported to the laboratory, stored at 4°C (Pham and Bui 2020), characterized after 24h, and filtered (Whatman Grade 42 filter paper) to remove solid particles and unwanted debris (Chawla et al. 2020). BOD, total phosphorus (TP), and total nitrogen (TN) of the samples were analyzed (APHA 2017). The concentration of orthophosphate (PO₄³⁻) given the TP and the TN was calculated by the addition of known concentrations of ammonia-nitrogen (NH₃-N), nitrate-nitrogen (NO₃⁻-N), and nitrite-nitrogen (NO₂⁻-N) in the samples (Zabed et al. 2019).

Cultivation of microalgae in wastewater and characterization

Two hundred milliliters of each wastewater were autoclaved at 121°C, 15 psi for 15 min to kill all the organisms present (Zhou et al. 2012). Thirty milliliters of a broth culture of P. insigne (~0.06 g mL⁻¹) was inoculated into each sample. Inoculated Fogg’s medium was considered as control. The growth of P. insigne in the test and control samples was analyzed after every two days for 24 days using CL 157 Colorimeter at λ₆70 nm (Otundo et al. 2018). Wastewater was again characterized after culturing P. insigne to test its potential to remediate wastewater (Pham and Bui 2020).

Estimation of dry cell weight and total lipid content

Pleurostraum insigne grown in wastewater and Fogg’s medium was harvested, dried, and powdered after 14 days of incubation (end of log phase) and the total lipid was extracted (Folch et al. 1957). The dry cell weight (dcw) and the total lipid content (%) were calculated (Pandey et al. 2019)

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\text{Total lipid content (\%)} = \frac{\text{Weight of extracted lipid}}{\text{dcw}} \times 100
\]

Lipid analysis

The extracted lipid was analyzed using a Gas Chromatograph (Thermo GC—Trace Ultra Ver: 5.0) and Mass Spectrometer (Thermo MS DSQ II). The column used for the study was a DB 35—MS capillary standard non-polar column with a dimension of 30 m, 0.25 mm ID, and 0.25 µm film thickness. The temperature of the oven was initiated at 70°C for 2 min, which was gradually increased to 260°C at a ramp rate of 6°C min⁻¹. Helium was used as a carrier gas at a 1.0 ml min⁻¹ constant flow rate with a split ratio of 30:1 (Singh et al. 2016).

Analysis of FAME for biodiesel properties

Biodiesel properties can be calculated directly from the fatty acid profile generated from GCMS (Hoekman et al. 2012). In this study, an analytical software BiodieselAnalyzer
Version 2.2 was employed to study the different properties of biodiesel (iodine value, kinematic viscosity at 25°C, density, saponification number, oxidation stability, and cetane number) based on the fatty acid profile as per Talebi et al. 2014. These observed values were compared with standards given by the Indian Standards (IS), American Society for Testing and Materials (ASTM), and European Norms (EN).

**Statistical analyses**

All the estimations were carried out in triplicates and statistical analyses were carried out using IBM SPSS Statistics 20 for Windows. One-way analysis of variance (ANOVA) was used and Duncan’s Multiple Range Test (DMRT) was used to test the significant difference in the characteristics of wastewater, biomass production, and total lipid content, a p-value of 0.05 or less was taken as a significant value.

**Results and discussion**

**Water samples**

Microalgae extensively thrive in wastewater suggesting that the nutrients and their concentration were suitable for growth (Whitton et al. 2015). In this study, wastewater was collected from different sources. The samples were obtained from the agricultural field (sample 1), sewage (sample 2), and tanneries. Tanneries produced different types of effluents based on the production and treatments which were raw soaked (sample 3), treated raw soaked (sample 4), lime bath (sample 5), dye bath (sample 6), treated lime and dye bath (sample 7), chrome (sample 8), and treated chrome (sample 9). The pH of all the water samples ranged between 6.8 and 9.8 except for chrome effluents (3.0) which were highly acidic. Table 1 shows the sources, locations, geographical coordinates, colors, odors, and pH of the different wastewater. The ability of each wastewater sample to promote microalgal growth was further studied.

**Growth analysis**

Growth analysis (Figure 1) showed that *P. insigne* exhibited a full growth cycle (lag phase, exponential phase, and stationary phase) in samples 1, 2, 3, 6, and 7 similar to the control, where the exponential phase ends on the 14th day. But in samples 4, 5, 8, and 9, growth was observed only till the lag phase. The difference in the growth curve may be attributed to the number of nutrients, pH, and the presence of toxic compounds (Pittman et al. 2011; Jeong and Jang 2020). TN: TP ratio higher than 16:1 is optimal for microagal cultivation whereas pH < 5 and pH higher than 8.0 is toxic (Azov and Shelef 1987; Monfet and Unc 2017). The samples where *P. insigne* exhibited a full growth cycle have TN:TP higher than 16:1. and a pH between 6.6 and 7.4. *P. insigne* cannot exhibit a full growth cycle in samples 8 (pH 3.0) and samples 4 (pH 9.0) and 5 (pH 9.8). Sample 9 has a neutral pH of 7.2 but cannot exhibit a full growth cycle as TN:TP is <16:1. Zhang et al. 2021 mentioned the need to supply additional nutrients in wastewater which did not meet the required nutritional value. Even with the supply of additional nutrients and trace elements cultivating microalgae in wastewater still cuts down the cost and the need for water resources. It is important to note that even if *P. insigne* cannot exhibit a full growth cycle in all the samples, the different parameters of the wastewater studied were significantly reduced in all the samples after it was treated with *P. insigne*.

**Characteristics of wastewater**

The BOD, TP, and TN of each wastewater sample were analyzed before and after inoculation of *P. insigne*.

A high level of BOD in the water reduced the amount of dissolved oxygen posing a threat to the aquatic life and therefore reduction of BOD is crucial for the remediation of wastewater (Abdel-Raouf et al. 2012). The BOD removal efficiency of *P. insigne* in different wastewater ranges from 54.84 to 93.61% as seen in Table 2. Our results were found to correlate with previous studies. A study has reported that the BOD removal efficiency of *Chlorella vulgaris* is 82.71% in sewage water from the Gangneung sewage plant, Korea (Choi and Lee 2012). Another study showed that *Chlorella* sp. removed 59.1% of the BOD from sewage wastewater at pH 7.0 (Choi and Lee 2012). A recent study has shown that *Chlorococcum* sp. showed BOD removal efficiency of 83% in a river contaminated with pharmaceutical effluents (Singh et al. 2020).

A study on the TP removal efficiency of *P. insigne* in different wastewater samples indicated that the highest TP removal was observed in sample 3 (58.62%) and sample 1 (54.54%) as seen in Table 3. *C. vulgaris* successfully sequestered 28% of the TP from industrial effluent (Valderrama et al. 2002) and another study observed that *C. vulgaris* removed 36.12% of the TP in sewage water from...
Gangneung sewage plant, Korea (Choi and Lee 2012). It was estimated that Scenedesmus sp. and Chlorella sp. removed 70 and 80.5%, respectively of the TP in untreated wastewater from the primary septic tank of the Environmental Sciences and Engineering Department, Pakistan (Ansari et al. 2017). The TP removal efficiency of Tetraselmis indica in pharmaceutical wastewater was estimated at 70.03% (Amit et al. 2020). When Chlorella vulgaris was grown by Fazal et al. (2021) in textile wastewater it removed 96.3% of the total phosphorus which ultimately led to the high rate of cell propagation. Higher nutrient assimilation resulted in higher cell propagation if there are no harmful substances for the organism in the wastewater.

The two highest TN reduction takes place in sample 1 (76.61%) and sample 6 (73.06%) as seen in Table 4. A previous study reported that Nitzschia sp. reduced the TN by 78% in municipal wastewater (Boelee et al. 2011) and an algal-bacterial coculture removed 70% of the TN in treated domestic wastewater (Posadas et al. 2013). The TN removal efficiency of C. vulgaris in the dairy effluent was found to be 85.47% (Choi 2016). Salgueiro et al. (2016) estimated that C. vulgaris removed 60–86% TN in synthetic wastewater (Salgueiro et al. 2016) whereas Amit et al. (2020) reported 67.17% TN removal by Tetraselmis Indica in pharmaceutical wastewater. Chandra et al. 2021 compared the TN removal of monoculture and a consortium of Chlorella minutissima,

Table 2. Biological oxygen demand (BOD) of wastewater samples before and after cultivation of P. insigne with BOD removal efficiency of P. insigne.

| Samples | Before (mg L⁻¹) | After (mg L⁻¹) | Removal efficiency (%) |
|---------|----------------|----------------|------------------------|
| 1       | 36 ± 0.50      | 2.30 ± 0.04    | 93.61                  |
| 2       | 21 ± 0.30      | 3.60 ± 0.09    | 82.86                  |
| 3       | 113 ± 0.10     | 36.00 ± 0.10   | 68.14                  |
| 4       | 10.4 ± 0.40    | 4.12 ± 0.04    | 60.38                  |
| 5       | 290 ± 0.10     | 116.90 ± 0.20  | 59.68                  |
| 6       | 310 ± 0.20     | 103.00 ± 0.10  | 66.77                  |
| 7       | 18.4 ± 0.20    | 1.20 ± 0.02    | 93.47                  |
| 8       | 220 ± 0.10     | 80.00 ± 0.10   | 63.63                  |
| 9       | 27.2 ± 0.10    | 12.84 ± 0.20   | 54.84                  |

Values are represented as mean ± SE (n = 3). Different letters indicate the significant difference between BOD removal efficiency with p ≤ 0.05. One-way ANOVA followed by DMRT was performed for total lipid content and dry cell weight using IBM SPSS Statistics 20.

*Bold values indicate that there is no significant difference between BOD removal efficiency with p ≤ 0.05.

Table 3. Total phosphorus content of wastewater samples before and after cultivation of P. insigne with a total phosphorus removal efficiency of P. insigne.

| Samples | Total phosphorus (TP) Before (mg L⁻¹) | After (mg L⁻¹) | Removal efficiency (%) |
|---------|---------------------------------------|----------------|------------------------|
| 1       | 0.22 ± 0.01                           | 0.10 ± 0.02    | 54.54                  |
| 2       | 0.13 ± 0.02                           | 0.07 ± 0.03    | 46.15                  |
| 3       | 0.29 ± 0.06                           | 0.12 ± 0.03    | 58.62                  |
| 4       | 0.23 ± 0.02                           | 0.17 ± 0.01    | 26.08                  |
| 5       | 0.38 ± 0.04                           | 0.30 ± 0.02    | 21.05                  |
| 6       | 0.49 ± 0.03                           | 0.25 ± 0.01    | 48.97                  |
| 7       | 0.27 ± 0.02                           | 0.15 ± 0.01    | 44.44                  |
| 8       | 1.74 ± 0.04                           | 1.60 ± 0.06    | 12.28                  |
| 9       | 1.08 ± 0.07                           | 1.00 ± 0.01    | 7.40                   |

Values are represented as mean ± SE (n = 3). Different letters indicate the significant difference between TP removal efficiency with p ≤ 0.05. One-way ANOVA followed by DMRT was performed for total lipid content and dry cell weight using IBM SPSS Statistics 20.

*Bold value indicates that there is no significant difference between TP removal efficiency with p ≤ 0.05.

Figure 1. Growth curve of P. insigne cultivated in various wastewater samples.
Scenedesmus abundans, Nostoc muscorum, and Spirulina sp. and found that nitrogen assimilation differs from species to species in dairy wastewater and that consortium culture generated higher lipid content for biodiesel production which may be due to the presence of both N. muscorum and Spirulina sp. as cyanobacteria are exceptionally capable in nitrogen fixation.

**Biomass and lipid production**

*P. insigne* grown in Fogg’s medium (control) and in samples that promote a full growth cycle (samples 1, 2, 3, 6, and 7) were harvested, dried, and powdered. The dry cell weight and total lipid content of *P. insigne* differ in different samples (Figure 2). The dry cell weight of the organism was highest in control (1.67 g L⁻¹) followed by samples 2 and 1 (1.22 and 1.11 g L⁻¹, respectively). Mahapatra et al. (2014) reported the biomass obtained from a microalgal consortium culture in municipal wastewater was reported to be 1.64 g L⁻¹. A study has shown that pure cultures of *C. saccharophila*, *Scenedesmus* sp., and a consortium culture can be successfully grown in treated and raw dairy wastewater with a higher dry cell weight of 0.276 g L⁻¹ obtained from a consortium culture in treated dairy wastewater (Hena et al. 2015). Nguyen et al. (2019) cultivated *C. vulgaris* in seafood processing wastewater effluent and harvested 0.46 g L⁻¹.

The total lipid content of *P. insigne* was higher in all the samples compared to the control. *P. insigne* was grown in samples 3 and 6 which produced the highest lipid, 30.1 and 30.47%, respectively. The total lipid content in this study was found to be on par with the lipid yielded by various microalgae in different media used for biodiesel production. *C. pyrenoidosa* grew in palm oil mill effluent (POME) and produced 42% lipid (Ponraj and Din 2013). Lipid yield of 28% dry cell weight was obtained from *Chlorococcum* sp. cultivated in pharmaceutical effluents (Singh et al. 2020).

Closer analysis has shown that biomass production is inversely proportional to lipid production. The composition of the growth medium greatly influenced biomass and lipid production (Lam and Lee 2012). Biotic and abiotic stress may lead to a decrease in growth but produce higher lipid (Karpagam et al. 2015). A standard Fogg’s medium has the most suitable concentration of various nutrients and pH for the growth of microalgae. The highest TN:TP ratio was found in sample 2 followed by sample 1. Hence *P. insigne* grew in Fogg’s medium and samples 1 and 2 produced high

### Table 4. Total concentration of ammonia-nitrogen, nitrate-nitrogen, nitrite-nitrogen, and total nitrogen content of wastewater samples before and after cultivation of *P. insigne* with a total nitrogen removal efficiency of *P. insigne*.

| Samples | Concentration of ammonia-nitrogen (mg L⁻¹) | Concentration of nitrate-nitrogen (mg L⁻¹) | Concentration of nitrite-nitrogen (mg L⁻¹) | Total nitrogen (TN) |
|---------|------------------------------------------|-------------------------------------------|------------------------------------------|-------------------|
|         | Before | After | Before | After | Before | After | Before | After | Before | After | Before | After | Before | After |
| 1       | 4.85 ± 0.13 | 0.87 ± 0.02 | 0.11 ± 0.03 | 0.08 ± 0.017 | 1.38 ± 0.12 | 0.52 ± 0.007 | 6.351 ± 0.28 | 1.485 ± 0.044 | 76.61± |
| 2       | 4.29 ± 0.14 | 1.08 ± 0.029 | 0.09 ± 0.025 | 0.05 ± 0.019 | 0.94 ± 0.08 | 0.59 ± 0.1 | 5.331 ± 0.245 | 1.733 ± 0.148 | 67.49± |
| 3       | 5.99 ± 0.2 | 2.11 ± 0.001 | 0.10 ± 0.003 | 0.03 ± 0.009 | 1.34 ± 0.02 | 0.28 ± 0.076 | 7.446 ± 0.223 | 2.434 ± 0.08 | 67.31± |
| 4       | 5.65 ± 0.18 | 2.79 ± 0.07 | 0.23 ± 0.03 | 0.16 ± 0.019 | 0.62 ± 0.027 | 0.45 ± 0.029 | 6.511 ± 0.237 | 3.419 ± 0.129 | 47.46± |
| 5       | 5.49 ± 0.08 | 3.84 ± 0.08 | 0.06 ± 0.003 | 0.05 ± 0.016 | 0.53 ± 0.012 | 0.11 ± 0.057 | 6.097 ± 0.095 | 4.017 ± 0.153 | 34.11± |
| 6       | 6.54 ± 0.01 | 1.42 ± 0.01 | 0.17 ± 0.005 | 0.05 ± 0.003 | 1.51 ± 0.001 | 0.74 ± 0.089 | 8.231 ± 0.016 | 2.217 ± 0.112 | 73.06± |
| 7       | 5.66 ± 0.02 | 3.45 ± 0.07 | 0.05 ± 0.003 | 0.03 ± 0.004 | 2.09 ± 0.003 | 0.42 ± 0.024 | 7.808 ± 0.026 | 3.925 ± 0.098 | 49.73± |
| 8       | 3.84 ± 0.03 | 3.16 ± 0.05 | 0.44 ± 0.023 | 0.43 ± 0.059 | 2.44 ± 0.042 | 0.57 ± 0.018 | 6.735 ± 0.095 | 4.168 ± 0.127 | 38.11± |
| 9       | 4.46 ± 0.01 | 3.21 ± 0.05 | 0.11 ± 0.056 | 0.065 ± 0.012 | 0.367 ± 0.003 | 0.336 ± 0.017 | 4.964 ± 0.083 | 3.612 ± 0.079 | 27.20± |

Values are represented as mean ± SE (n = 3). Different letters indicate the significant difference between TN removal efficiency with p ≤ 0.05. One-way ANOVA followed by DMRT was performed for total lipid content and dry cell weight using IBM SPSS Statistics 20.

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Figure 2. Total lipid content and dry cell weight of *P. insigne* grown in wastewater samples and control.
biodiesel but lesser lipid. The TN:TP ratio is low in samples 3 and 6 compared to other samples 1, 2 and 7, therefore P. insigne produced less biomass but higher lipid in these samples. The same correlation between nutrient content and biomass production and lipid assimilation was observed by Nagi et al. (2020) who explained that nutrient supplements, as well as stress conditions, led to changes in lipids fractions. When nutrient supply is sufficient the cell propagation increases but lowered lipid accumulation. The lipids produced can be further transesterified for the production of biodiesel.

**Fatty acid profile and characterization of biodiesel**

Lipid was extracted from P. insigne grown in sample 6 since lipid yield was the highest. GCMS has revealed the different types of fatty acids (Table 5) present in the lipid as similar to an earlier study (Miranda et al. 2016). Analysis of the fatty acid profile has shown a high percentage of C16 and C18 fatty acids which is required for a good fuel property (Selvarajan et al. 2015). The presence of palmitic acid is also an important indicator for assessing the quality of biodiesel derived from microalgae (Demirbas 2008). The observed values for the different fuel properties tested were given in Table 6. A low iodine value is preferred because a high iodine value promotes the polymerization of glycerides which can hinder the performance of an engine. The minimum cetane number for fuel is 47 because a high cetane number indicates greater ease of ignition and lesser emission of nitrous oxide (N₂O) (Selvarajan et al. 2015). High viscous fuel may result in poor fuel combustion and therefore the kinematic viscosity should be within an acceptable range (Phankosol and Krisnangkura 2015). The density should be within a specific limit for ideal air-to-fuel ratios for complete combustion (Ismail and Ali 2015). Saponification numbers signify the purity of the fuel (Mohan et al. 2019) and oxidative stability refers to the resistance of the fuel to oxidation, which also depends on time (age of the biodiesel) and storage condition (Hoekman et al. 2012). All the observed values of the fuel properties were within the specifications set by IS (1448), ASTM (D6751-08), and Europe (EN 14214) which confirm that biodiesel produced from P. insigne cultivated in wastewater is of high quality.

**Conclusion**

The deteriorating effect of improper disposal of waste into waste bodies and overexploitation of fossil fuels leads to loss of aquatic species and climate change. The development of an alternative form of energy source which is sustainable, economically feasible, and eco-friendly is the need of the hour. When P. insigne was grown in different wastewater samples it decreased the concentration of the organic pollutants while the wastewater served as a culture media for the organism. The lipid content and fatty acid analysis of P. insigne grown in wastewater were shown to be on par with other microalgae studied for biodiesel production. While opening a scope of further research focusing on determining chlorophyll and protein content of P. insigne and the concentration of Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC) of the wastewater before and after cultivating microalgae, this study successfully demonstrated the scope of using microalgae as a feedstock for biodiesel production while mitigating the concentration of BOD, TP and TN content in the wastewater bodies.

**Acknowledgments**

The authors would like to acknowledge the Department of Biotechnology, School of Sciences, JAIN (deemed-to-be University), Bangalore, Karnataka, India for providing infrastructural support to conduct the research.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

| Sl. No. | Fatty acids                  | Retention time (RT) | Molecular weight | Percentage (%) |
|---------|------------------------------|---------------------|------------------|----------------|
| 1       | Myristic acid (C14:0)         | 10.45               | 228.37           | 3.63           |
| 2       | Pentadecanoic acid (C15:0)    | 12.23               | 242.40           | 30.90          |
| 3       | Palmitic acid (C16:0)         | 12.65               | 256.42           | 34.54          |
| 4       | Oleic acid (C18:1)           | 15.25               | 564.90           | 24.72          |
| 5       | Stearic acid (C18:0)         | 15.57               | 284.50           | 2.54           |
| 6       | Linoleic acid (C18:2)        | 18.92               | 280.40           | 3.63           |

Table 5. GCMS showing different types of fatty acids extracted from P. insigne grown in sample 6.

| Properties                              | Observed value | IS (1448) | ASTM (D6751-08) | Europe (EN 14214) |
|-----------------------------------------|----------------|-----------|------------------|-------------------|
| Iodine value (gI₂/100g⁻fat)             | 8.85           | <120      | NA               | <120              |
| Cetane number                           | 69.49          | ≥47       | ≥47              | ≥47               |
| Kinematic viscosity at 25°C (mm²/s)     | 3.84           | 3.5–5.0   | 1.9–6.0          | 3.5–5.0           |
| Density (kg/m³)                         | 866            | 860–900   | NA               | 860–900           |
| Saponification number (mg KOH/g⁻)      | 216            | NA        | NA               | NA                |
| Oxidation stability (h)                 | 35.08          | ≥8.0      | ≥3.0             | ≥6.0              |

Table 6. The observed values of different properties of biodiesel were obtained from P. insigne with international standards.
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