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Research Article

Comparative Analysis of Aerobic Bacteria, Green Microalgae, and Diatoms in Domestic Wastewater Treatment: Pollutant Removal, Lipid Profile, and Biodiesel Properties

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Abstract
Microalgal systems are receiving significant attention for wastewater treatment because of their effective treatment performance while simultaneously generating valuable biomass for energy recovery. This study compares the performance of three biological systems - green microalgae, diatoms, and aerobic bacteria for the treatment of real domestic wastewater. Each system was operated in semi-continuous mode at different solid retention times (SRTs) of 2, 4, 6, 8, 10, and 12 days. Reactor performance was evaluated based on the removal of organic carbon, nutrients, and micropollutants. The maximum dissolved organic carbon removal efficiencies were 89% for aerobic bacteria, 84% for green microalgae, and 87% for diatoms. On the other hand, the microalgal systems outperformed the aerobic bacterial system in terms of nutrient and micropollutant removal. The microalgal systems achieved higher nutrient removal efficiencies, with maximum total nitrogen (TN) removal of 71% for green microalgae and 67% for diatoms, compared with 40% for aerobic bacteria. Diatoms exhibited a higher maximum lipid content of 21.2% at an SRT of 8 days, compared with 15.6% for green microalgae at an SRT of 6 days. Fatty acid analysis revealed the presence of palmitic, palmitoleic, stearic, and elaidic acid esters, which are suitable for biodiesel production. Overall, the results demonstrate that aerobic bacteria were effective for organic carbon removal, whereas green microalgae and diatoms exhibited enhanced nutrient and micropollutant removal while simultaneously generating lipid containing biomass with potential for biodiesel production.

Keywords: Biodiesel, Diatoms, FAME, Green microalgae, Nutrient removal, Wastewater treatment

1 Introduction

The depletion of water resources due to increasing population and rapid urbanization, along with rising global water scarcity, is promoting the reuse of treated wastewater for various purposes [1]. The majority of wastewater generated by human activities comes from domestic sources [2]. Up to 75-90% of the freshwater supplied is discharged as wastewater [3]. Around 75% of the domestic wastewater is generated from households or residential communities, and the remaining is from public facilities, office buildings, and commercial areas [4]. The untreated or partially treated wastewater disposal from urban areas is contributing to significant nutrient loading on the natural water bodies in developing countries [5]. This excess nutrient loading is leading to eutrophication and decreased water quality, resulting in natural ecosystem disruption [6].

Most of the existing conventional domestic wastewater treatment systems employ activated sludge process (ASP) based treatment techniques. However, ASP processes have limitations as they are energy-intensive. Nitrogen removal in ASP occurs through the nitrification-denitrification process, which requires frequent shifting between anoxic-aerobic

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conditions and necessitates significant aeration and an additional carbon source [7]. Furthermore, in the ASP process, there is a significant emission of greenhouse gases, including CO_2 and NO_x, into the atmosphere [8]. Also, the excess sludge generated in ASP imposes an extra economic burden on the operators for its handling and management [9]. Sludge disposal also has negative environmental effects [10]. As the world is transitioning into the low-carbon era, there is a high demand for energy-saving, low-CO_2-emission, and nutrient-recycling wastewater treatment systems. Efficient and sustainable wastewater treatment is reported to contribute to achieving 11 out of 17 sustainable development goals (SDGs) set by the United Nations [11]. It helps to promote the circular economy by enabling the recycling of nutrients, water, and energy.

The diversity and complexity of pollutants in wastewater necessitate the development of more efficient technologies to meet water quality standards. Microalgal wastewater treatment has recently gained significant attention as a sustainable alternative to conventional bacteria-based biological treatment due to its high nutrient removal capability and its ability to simultaneously produce biomass as a valuable resource [8], [12]. The produced biomass can be converted into commercially valuable products like biofuels, biofertilizers, and animal feed, among others [13], [14]. The residual microalgal biomass after biodiesel extraction can be used for biogas and biohydrogen generation and as an adsorbent for heavy metal remediation [15]. Wastewater contains macronutrients, such as carbon, nitrogen, and phosphorus, along with trace elements required for microalgal growth [16]. Microalgae can utilize organic carbon, as well as nitrogen and phosphorus present in wastewater, for their growth, thereby reducing their concentration in the water.

In recent years, emerging contaminants (micropollutants) have been found in various parts of the environment at trace levels ranging from ng/L to µg/L [17]. Micropollutants include several pharmaceuticals, personal care products, pesticides, insecticides, and industrial chemicals. Most of the micropollutants are recalcitrant in nature and are not effectively removed in conventional treatment techniques, raising concerns about their potential release into the environment through effluent disposal [18]. Residual organic micropollutants present in municipal effluents pose a risk to the quality of receiving waters and affect ecosystems because of their biological activity and toxicity [19]. Microalgae are more effective than aerobic bacteria in removing micropollutants due to their involvement in multiple mechanisms. Also, the use of microalgae for micropollutant remediation alleviates the risk of antimicrobial resistance gene development in bacterial systems. Micropollutant removal using microalgae involves processes such as bioaccumulation, biosorption, biodegradation, volatilization, and photodegradation [20]. During photosynthesis, the microalgae produce reactive oxygen species (ROS) such as peroxyl radical, single oxygen, and hydroxyl radical. These ROS take part in the degradation of the recalcitrant pollutants.

Green microalgae and diatoms are the two dominant classes of microalgal species. Microalgal cells grow well in wastewater under photoautotrophic, mixotrophic, and heterotrophic metabolic conditions. Green microalgae are predominantly found in freshwater environments, with few species surviving in marine and terrestrial ecosystems. Diatoms are a diverse group of microalgae having frustule-like cell walls composed of silica. Diatoms are the primary producers in oceans, significantly contributing to global carbon cycling and oxygen production. Although microalgal systems have been increasingly investigated for wastewater treatment, direct comparative assessments with conventional aerobic bacterial systems remain limited, particularly with respect to the performance of distinct microalgal groups under comparable treatment conditions. Existing studies have primarily evaluated microalgae as a broad functional group, with relatively limited attention to the differential treatment characteristics of green microalgae and diatoms and their implications for biomass recovery and valorization. Differences in cellular structure, nutrient assimilation, growth characteristics, and biochemical composition among microalgal groups can influence organic matter and nutrient removal, as well as the composition and quality of the resulting biomass. Furthermore, wastewater treatment and biomass valorization are frequently investigated as independent aspects, limiting the understanding of the relationship between treatment performance and the biochemical characteristics of biomass generated during treatment. In this context, the present study provides a direct comparative assessment of microalgal systems and conventional aerobic bacterial treatment, while linking treatment performance with the characteristics and potential valorization of the resulting biomass. Accordingly, this study aims to evaluate the use of locally available green microalgae and diatom cultures

alongside conventional activated sludge aerobic bacteria for domestic wastewater treatment. The scope of work includes (a) comparing the performance of aerobic bacteria, green microalgae, and diatoms in organic carbon, nutrient, and micropollutant removal from domestic wastewater, and (b) evaluating the lipid content, fatty acid methyl ester (FAME) profile, and biodiesel properties of the microalgal biomass produced in the system.

2 Materials and Methods

2.1 *Wastewater source*

The domestic wastewater used in this study was obtained from the collection tank of a 650 kLD capacity sewage treatment plant (STP) on the IIT Hyderabad campus serving approximately 6000 population. The wastewater sample was collected and passed through an approximate 1 mm double mesh net strainer to remove debris and suspended particulate matter. The collected wastewater sample was stored at -20°C in a refrigerator until further use.

The composition of the wastewater is shown in Table 1. The wastewater in this study falls under the low-strength wastewater category. The pH of the wastewater was observed to be 7.2 ± 0.1. The wastewater has a chemical oxygen demand (COD) of 298 ± 8 mg/L, total nitrogen (TN) of 36.2 ± 1.34 mg/L, dissolved organic carbon (DOC) of 66.0 ± 0.7 mg/L, and phosphorus (P) concentration of 8.7 ± 0.11 mg/L. The carbon-to-nitrogen and nitrogen-to-phosphorus ratios of wastewater were observed to be 1.82 and 4.12, respectively.

Table 1: Domestic wastewater composition.

Parameter	Present study	[21]
pH	7.2 ± 0.1	-
Chemical oxygen demand (mg/L)	298 ± 8	250 – 800
Dissolved organic carbon (mg/L)	66.0 ± 0.7	80 – 260
Total Nitrogen (mg/L)	36.2 ± 1.34	20 – 70
Phosphorus (mg/L)	8.7 ± 0.11	4 – 12

2.2 *Seed culture collection and cultivation*

The heterotrophic aerobic bacterial inoculum culture was collected from the aeration tank of the STP. The collected bacterial biomass was allowed to settle for 2 hours. The biomass was subsequently washed with deionized water to remove impurities. Prior to adding it into the test reactors, the mixed liquor concentration of the biomass was estimated, and accordingly the required quantity was added to achieve a mixed liquor concentration of 1000 mg/L.

Locally available cultures of green microalgae and diatoms were used as the microalgal inoculum. The green microalgal mixed culture was obtained from the Mehboob Sagar Lake situated in Sangareddy Municipality, Telangana state, India. Diatom mixed culture was collected by scraping the bedrock of the Manjeera Dam downstream, located in Sangareddy. The collected culture samples were passed through a double-net strainer to remove the debris and washed with distilled water. The green microalgae were cultured using standard BG 11 medium, and the diatoms were grown on f/2+Si medium. During cultivation, the cultures adapted to the selective environment were used for subsequent experiments. Initially, the cultures were grown in a 1-liter conical flask. During the exponential growth phase, the cultures were transferred to 15-liter master culture reactors. Aeration was provided using stone diffusers at a 3 L/min airflow rate. The cultures were mixed manually three times a day to prevent biomass settlement and to keep it in suspension. The cultures were harvested by centrifuging at 3000 rpm for 10 minutes. The supernatant was discarded, and the biomass was resuspended and washed with deionized water to remove excess nutrients. The harvested biomass was inoculated into the test reactors.

2.3 *Experimental methodology*

Three types of systems with cultures, viz. aerobic bacteria, green microalgal mixed culture, and diatom mixed culture, were set up for the treatment of domestic wastewater. Wide-mouth transparent glass bottles (500 mL, 5-6 cm diameter) were used as test reactors and kept open for adequate air infusion. All the reactors were kept on a stirrer base, and mixing was provided at 200 rpm. Reactor walls were regularly cleaned to remove any attached biomass, which was subsequently returned to the suspension. Natural sunlight (near window) was utilized as the light source at 12:12 h light: dark cycle. The aerobic bacterial reactors were wrapped with aluminium foil to prevent algal growth from light exposure. The temperature was maintained at ambient room conditions. During the experimental period, the temperature varied

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between 25 and 28°C. For each system type, six different reactors were set up and operated simultaneously in semi-continuous draw and fill mode at solid retention times (SRT) of 2, 4, 6, 8, 10, and 12 days. The semi-continuous mode was adopted as it offers better control of substrate input and biomass wastage. Further, the draw and fill system enables the cultures to adjust to high initial substrate doses as in batch tests [22]. In this study, biomass was not recycled. Hence, the SRT was equivalent to the hydraulic retention time (HRT). Based on the SRT of the reactor, daily at the same time, a particular volume of mixed liquor (water and biomass) was withdrawn, and an equal quantity of the influent feed was added. The volume of feed was estimated by dividing the reactor volume by the SRT. For instance, for an SRT of 10 days, the daily feed volume was 50 mL (=500/10). The reactors were maintained in operation for three times the SRT to attain steady state. Tests were conducted after attainment of the steady state. The initial biomass concentration of 1000 mg/L was maintained in all the reactors during the startup.

The Monod/Michaelis-Menten kinetic approach was used to describe the relationship between the applied solids retention time and the observed substrate removal performance of the biological treatment systems. These models were selected as empirical representations of substrate-limited biological processes, allowing comparison of the apparent kinetic behaviour of the bacterial, green microalgal, and diatom systems under the investigated operating conditions. The kinetic relationships were linearized for parameter estimation using the experimental steady-state data obtained at different retention times. The linearized Michaelis-Menten and Monod equations Eq. (1) and (2) were fitted with steady-state experimental data to obtain the kinetic parameters (k, K_s, Y, and k_d).

$$\frac{\theta X}{S_o - S} = \frac{K_s}{k} \times \frac{1}{S} + \frac{1}{k} \quad (1)$$

$$\frac{1}{\theta} = -\frac{Y\,(S_o - S)}{\theta X} - k_d \quad (2)$$

Where X is the active cell concentration (mg volatile suspended solids (VSS)/L); θ denotes the solid retention time (days); S represents the liquid substrate concentration (mg/L); S_o is the concentration of initial substrate (mg/L); k is the maximum specific rate of substrate utilization (mg/mg-day); Y is the yield coefficient; K_s is the half-velocity coefficient (mg/L), and k_d represents the decay coefficient (1/day).

2.4 *Analytical methods, lipid profile, biodiesel properties*

The details of the analytical methods, micropollutant analysis, lipid extraction procedure, fatty acid profiling, and equations used to predict biodiesel properties are provided in the Supplementary Data (Sections S1–S3).

3 Results and Discussion

3.1 *Performance of the reactors*

3.1.1 Carbon removal

Figure 1 shows the DOC removal efficiency of the three systems, aerobic bacteria, green microalgae, and diatoms at different SRTs. A maximum DOC removal efficiency of 89% was observed in aerobic bacteria at a 12-day SRT. In green microalgae and diatom systems, the maximum DOC removal efficiencies of 84% and 87% were observed at an 8-day SRT, respectively. It can be observed that DOC removal efficiency increased with an increase in SRT in aerobic bacteria. However, in both green microalgae and diatoms, the DOC removal efficiency decreased beyond an 8-day SRT. The rise in organic carbon in the effluent might be due to the microalgal cells exudating the extracellular organic matter (EOM) into the surrounding environment at longer retention times. It has been reported that microalgal cells exudate 2-50% of the assimilated carbon as dissolved organic matter [23].

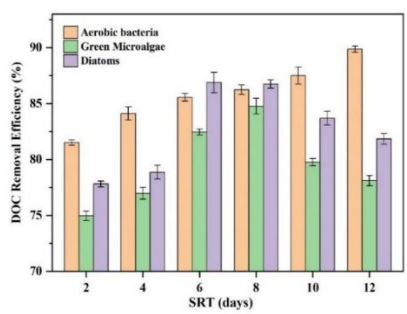


Figure 1: DOC removal at different solid retention times (SRTs) in aerobic bacteria, green microalgae, and diatom systems.

The microalgal EOM is composed of proteins, amino acids, nucleic acids, polysaccharides, peptides, and others [24]. The amount of EOM released by microalgal cells depends on the surrounding environmental conditions such as pH, temperature, nutrient limitations, light availability, and solids concentration [25]. At longer retention times, the algal cells may enter the decay phase, releasing the cellular components into their surroundings and resulting in an increase in the organic content [26]. In this study, the longer retention times and nutrient-limiting conditions might have contributed to the increase in algal EOM, reducing DOC removal beyond 8-day SRT. In the present study, it was observed that aerobic bacteria showed better DOC removal than green microalgae at all the SRTs studied. Aerobic bacteria also showed higher DOC removal than diatoms at 2, 4, 10, and 12-day SRT, while similar DOC removal was observed at 6 and 8-day SRT.

3.1.2 Biokinetic coefficients

The steady-state experimental data were fitted to equations (1) & (2) to determine the biokinetic parameters. Figure S1 shows the linear kinetic plots of the green microalgae, diatom, and aerobic bacteria cultures. The coefficient of determination (R^2) values greater than 0.8 and *p*-values of less than 0.05 indicate the significance of the linear regression (Figure S1). The organic removal kinetic parameters obtained from the plots (Figure S1) are summarized in Table S1. Biokinetic factors play a crucial role in the design of biological treatment plants. The maximal specific substrate utilization rate (k) values for all three systems were observed to be on the lower side of the typical range for domestic wastewater reported by Metcalf & Eddy [21]. The k values for green microalgae and diatom cultures were lower than those of aerobic bacteria in the present study. The observed k values indicate that microalgal cultures exhibit a lower responsiveness towards organic matter removal from domestic wastewater compared to aerobic bacterial cultures. The half-velocity constant (K_s) values for all three systems were observed to be higher than the typical range reported by Metcalf & Eddy [21]. The higher values of K_s indicate that a high substrate concentration is required to achieve the maximum specific substrate utilization rate. The K_s values of microalgal cultures were higher than those of aerobic bacteria, indicating that microalgal cultures have a lower affinity for dissolved organic carbon compared to aerobic bacteria. The yield coefficient (Y) values were within the typical range for the removal of organic matter from domestic wastewater. The decay coefficient (k_d) values for all three systems were observed to be lower than the reported values for domestic wastewater [21]. The apparent kinetic coefficients may reflect variations in substrate utilization and biomass-associated metabolic activity among the respective treatment systems under tested conditions.

3.1.3 Nutrient removal

The effectiveness of the microalgal wastewater treatment is evaluated based on its nutrient removal capacity. The total nitrogen (TN) removal efficiency of all three systems, aerobic bacteria, green microalgae, and diatoms, is shown in Figure 2. It can be observed that, in all the SRTs studied, the TN removal efficiency is significantly higher in both green microalgae and diatoms compared to aerobic bacteria. Green microalgae culture showed a slightly better TN removal than diatoms. The TN removal efficiency was found to increase with an increase in SRT in all three systems, indicating that SRT had a significant role in TN removal. The TN removal efficiency of 71% was observed in green microalgae at SRT ≥ 6 days. Diatoms showed the highest TN removal efficiency of 67% at 8-day SRT. On the other hand, a maximum TN removal efficiency of only 40% was observed with aerobic bacteria at 12-day SRT. It has been reported that aerobic bacteria are comparatively less effective in nutrient removal [27].

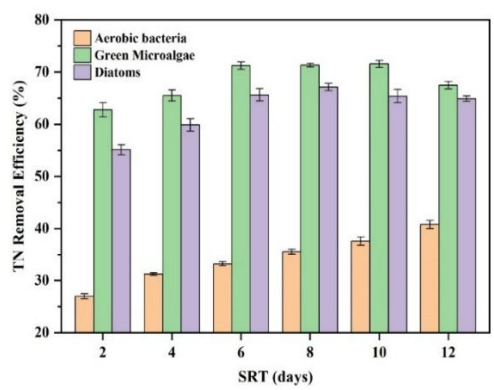


Figure 2: Total nitrogen removal at different solid retention times (SRTs) in aerobic bacteria, green microalgae, and diatom systems.

In aerobic bacterial systems, nitrogen is removed by being utilized for heterotrophic biomass growth and as nitrogen gas through nitrification-denitrification or ammonia stripping at pH levels above 8. In the present study, apart from mixing, no external aeration was provided for aerobic bacteria, which might be limiting nitrogen removal. Microalgae remove nitrogen mainly through assimilation for their growth, and ammonia stripping occurs at elevated pH conditions. During the study period, in all the reactors, the pH was observed to vary between 7.4 and 7.7, which indicates that ammonium stripping was negligible. Microalgae uptake the inorganic nitrogen species ammonia, nitrate, nitrite, and organic nitrogen present in the wastewater for its growth and utilize them for the cellular components, including protein, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) synthesis [28].

Phosphorus (P) is a major component of the cells, essential for synthesizing nucleotides, phospholipids, and adenosine triphosphate (ATP). P removal in microalgal systems occurs through two pathways, viz., by cellular uptake and possible precipitation at elevated pH levels (>8.5). Figure 3 shows the P removal efficiency in green microalgae and diatoms at different SRTs. It was observed that as SRT increased, P removal also increased in both microalgal systems, demonstrating that SRT plays a major role in nutrient removal.

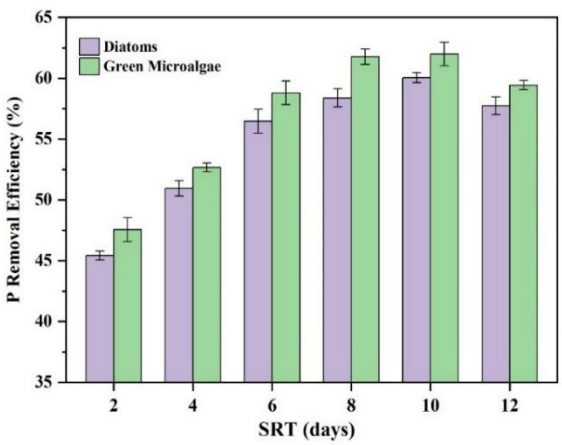


Figure 3: Phosphorus removal at different solid retention times (SRTs) in green microalgae and diatom systems.

During the study, no significant P removal was observed in aerobic bacterial systems. The maximum P removal efficiency of 62% was observed in green microalgae at 8 and 10-day SRT, while 60% removal efficiency was observed in diatoms at 10-day SRT. The pH of the reactors ranged between 7.4 and 7.7, indicating that P removal via precipitation was negligible. This indicates that P removal was mainly through biomass assimilation. Overall, this study indicates that mixed microalgal cultures have a better nutrient removal capability in comparison to conventional aerobic bacterial systems. Table S2 shows the comparison of the organic carbon and nutrient removal obtained in the present study with studies reported in the literature for microalgae treating different wastewaters.

3.1.4 Micropollutant removal

Table S3 shows the abundance of micropollutants present in the influent wastewater and effluent samples from aerobic bacteria, green microalgae, and diatom cultures and their corresponding removal efficiencies at 8-day SRT. The efficacy of compound removal was assessed by estimating the decrease in the abundance values between the influent and effluent samples for each compound. The influent domestic wastewater sample was observed to contain several pharmaceuticals, personal care products, hormones, insecticides, herbicides, and other compounds such as flavoring agents. From Table S3, it can be observed that in most cases, both green microalgae and diatoms have shown better removal of micropollutants compared to aerobic bacteria. The presence of various kinds of pharmaceuticals, pesticides, fungicides, and insecticides in domestic wastewater has been reported by several researchers [29], [30]. The possible sources of herbicides and insecticides could be pest control measures in the gardens and lawns and floor cleaning agents. The microalgal systems removed micropollutants from the wastewater, with an average removal efficiency of over 90% for most compounds. For instance, acetaminophen (paracetamol), a widely used over-the-counter medication, was observed to be removed only 29% by aerobic bacteria; on the other hand, it was below the detection limit in both microalgal systems. Similarly, fluvoxamine, one of the most widely prescribed antidepressants, was found to be removed by 41% by aerobic bacteria, 96% by green microalgae, and was below the detection limit in the diatom system. The green microalgal system showed a better removal of insecticides and herbicides than the other two systems. The insecticide dichrotophos was not significantly removed, with only 26% removal in

aerobic bacteria, 49% in green microalgae, and 21% in diatoms.

In a recent study, Delanka-Pedige *et al.,* [19] analyzed organic micropollutant removal in an algal system (A-WWT) and conventional activated sludge (CAS) using non-targeted analysis and observed that A-WWT outperformed CAS, reducing 306 compounds to below detection limits compared to 206 compounds in CAS. Similarly, in a comparative assessment, Villar-Navarro *et. al.*, [31] also observed better removal of therapeutic antibiotics, diuretics, macrolides, and histamine blockers in a microalgal high-rate algal pond than in an up-flow anaerobic sludge blanket reactor treating real wastewater. The enhanced micropollutant removal in microalgal systems is attributed to diverse pollutant removal mechanisms, including (a) biodegradation, (b) bioaccumulation, (c) adsorption, and (d) photodegradation [32]. During microalgal respiration and photosynthesis, microalgal cells are reported to produce reactive oxygen species, such as peroxide, singlet oxygen, and superoxide radicals, contributing to the oxidative degradation of organic micropollutants [33]. Also, microalgae excrete organic substances, including biopolymers like polysaccharides and proteins, which can function as photosensitizers, facilitating the photodegradation of micropollutants [34].

3.2 *Lipid productivity*

Lipid productivity is one of the important parameters for biodiesel synthesis from microalgae. The lipid content of the green microalgae and diatoms on the dry biomass basis is shown in Figure 4. The lipid content was found to increase with an increase in SRT in both systems. It can be observed from Figure 4 that at all the SRTs studied, diatoms had a higher lipid content than green microalgae. A maximum lipid content of 21.2% was observed at 8-day SRT in diatoms. Whereas a maximum lipid content of 15.6% was observed at 6-day SRT in green microalgae. There is almost double the increase in lipid content at 8-day SRT compared to 2-day SRT in both green microalgae and diatoms. The lipid content of the microalgal biomass depends upon the wastewater characteristics and environmental conditions such as temperature, pH, organics, nutrient availability, and illumination [35].

It has been reported that diatoms have the capacity to accumulate higher lipid content than other classes of microalgal species [36]. Compared to green microalgae, diatoms have larger vacuoles, facilitating increased lipid accumulation [36], [37]. In addition to high lipid content, diatoms also have higher settling rates than other groups of microalgae due to their high-density cell walls [38]. The high settling capacity of diatoms is very useful in biomass harvesting for biodiesel production. The higher lipid accumulation observed in diatoms may not necessarily correspond to higher net DOC removal. In the present study, DOC removal in the diatom system increased up to an SRT of 8 days and subsequently decreased, whereas lipid accumulation remained relatively high at longer SRTs. This may be associated with prolonged biomass retention, which can increase endogenous respiration, cell decay, and the release of extracellular organic matter and soluble cellular components into the liquid phase, contributing to the observed decline in DOC removal. Increasing SRT also enhanced TN and P removal in both microalgal systems, while lipid content generally increased with SRT. However, conditions favoring increased nutrient uptake and lipid accumulation may not necessarily maximize net DOC removal. Under the conditions investigated, an SRT of approximately 8 days provided a favorable balance between carbon and nutrient removal and lipid accumulation. Overall, the results demonstrate that SRT is an important operational parameter for simultaneously achieving wastewater treatment and biomass valorization.

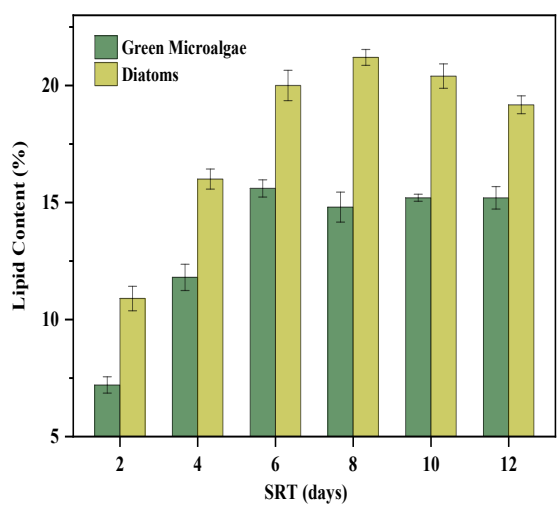


Figure 4: Lipid content of microalgal biomass on a dry weight basis at different SRTs.

3.3 *Correlation analysis*

Figure S2 (a) and (b) show the Pearson's correlation matrix of the parameters, SRT, DOC, TN, P removal, and lipid content for the two systems, green microalgae and diatoms, respectively. It was observed that nearly all parameters exhibited a positive correlation with one another, indicating their role in the performance of the reactors. In both systems, SRT is strongly associated with nutrient removal and lipid productivity. Also, TN and P removal showed a strong relationship with lipid productivity in both systems. The increase in lipid content alongside higher nutrient removal suggests that nutrient assimilation supported microalgal biomass formation and lipid accumulation. Organic carbon removal is weakly associated with SRT in green microalgae compared to diatoms. The green microalgae are reported to exudate cellular organics into the surrounding environment under nutrient-limited conditions.

3.4 *FAME profile and biodiesel properties*

The harvested green microalgae and diatom biomass were used for fatty acid synthesis and analyzed for FAME profile using gas chromatography. The corresponding FAMEs and their relative percentages for both systems at all the studied SRTs are summarized in Table S4. The dominant fatty acids present in the green microalgae and diatoms at different SRTs, and their distribution, are shown in Figure 5(a) and (b), respectively. The dominant fatty acid esters were observed to be palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), elaidic acid (C18:1n9t), and arachidic acid (C20:0). The amount and distribution of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) influence the quality of the biodiesel. Figure 6 shows the SFA, MUFA, and PUFA contents at different SRTs in green microalgae and diatoms. The presence of a high amount of long-chain SFA and MUFA enhances oxidative stability and reduces NO_x emissions. On the other hand, the high content of PUFA has a negative effect on the biodiesel oxidative stability, as they contain reactive sites that are susceptible to free radical attack [39].

In both systems, at all the studied SRTs, the SFA content was found to be higher than 55%, indicating suitability for quality biodiesel production. The SFA content was observed to range from 55 to 66% across different SRTs. It is reported that the fatty acid profile is affected by environmental conditions, stress factors, nutrient availability, growth phase, and light availability [39], [40]. The PUFA content varied between 4.6 and 6.8% in green microalgae, while in diatoms, the PUFA was in the range of 4.6 – 13.9%. A slightly higher proportion of PUFAs was observed in diatom cultures than in green microalgae. Certain species of diatoms are reported to accumulate high PUFA [41]. Although higher PUFA content is not favorable for superior biodiesel quality, the high lipid percentage makes diatoms suitable for biodiesel production. As per the European biodiesel standards (EN 14214), the linolenic acid methyl ester (C18:3) and polyunsaturated fatty acid (with ≥ 4 double bonds) should be ≤ 12% and 1%, respectively. In this study, the linolenic acid ester percentage was observed to be less than 5% in all microalgal reactors. Polyunsaturated fatty acids with ≥ 4 double bonds were not detected in diatoms. In green microalgae, these fatty acids were observed to be less than 1%, except for 1.14% at 4-day SRT.

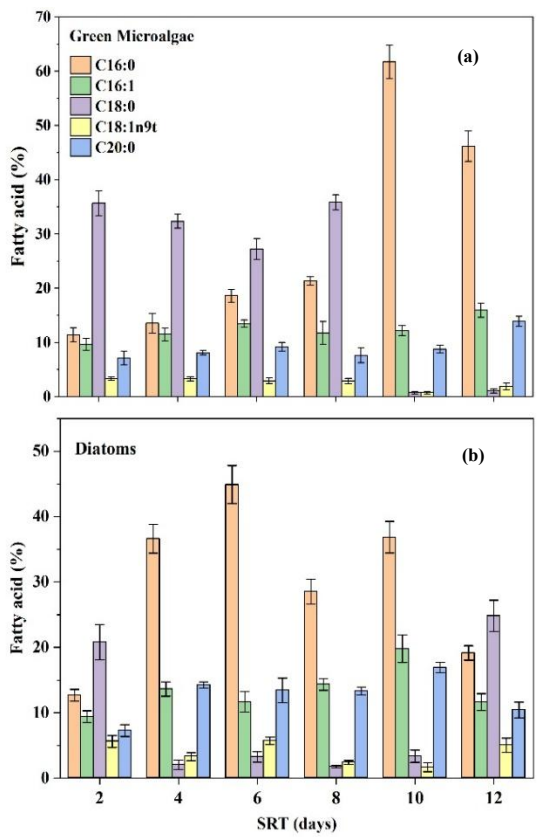


Figure 5: Dominant fatty acids in systems at different SRTs: (a) green microalgae, (b) diatoms.

Table 2: Predicted biodiesel properties.

Parameter	SRT (days)											
	Green Microalgae						Diatoms					
	2	4	6	8	10	12	2	4	6	8	10	12
Cetane Number (CN)	63.03	63.32	63.66	64.54	66.29	63.89	62.34	61.39	63.44	62.76	62.56	61.86
Kinematic Viscosity (ν, mm^2/s)	4.67	4.56	3.78	4.65	4.34	4.31	3.42	4.42	4.41	3.00	4.33	4.43
Density (ρ, g/cm^3)	0.87	0.87	0.87	0.86	0.86	0.86	0.87	0.87	0.86	0.87	0.87	0.87
Higher heating value (MJ/kg)	39.92	39.83	38.97	39.86	39.64	39.67	38.56	39.81	39.75	38.06	39.72	39.78

The physicochemical properties of biodiesel are influenced by the structural characteristics of the fatty acids, including branching, carbon chain length, and degree of unsaturation. Table 2 shows the different calculated biodiesel properties, such as kinematic viscosity (ν), density (ρ), cetane number (CN), and high heating value (HHV) of both green microalgae and diatoms at all studied SRTs. The CN value ranged from 63.03 to 66.29 in green microalgae and from 61.39 to 63.44 in diatom cultures. Even the lowest observed CN value, 61.39, is significantly higher than the minimum CN value of 47 as per ASTM D6751 and 51 by EN 14214 biodiesel standard specifications [42].

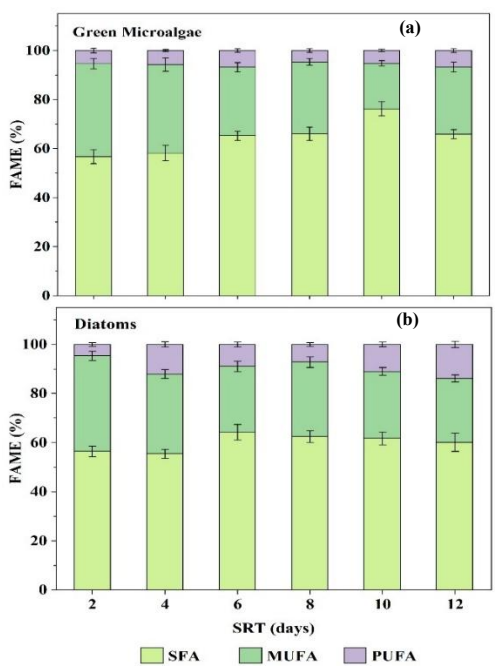


Figure 6: Fatty acids (SFA, MUFA, and PUFA) percentage distribution in systems at different SRTs: (a) green microalgae, (b) diatoms.

These high CN values can be attributed to the high quantities of SFA and MUFA. A high CN number is reported to improve combustion efficiency, smoother engine operation, and easier cold starts [43]. It also reduces emissions of NO_x, unburned hydrocarbons, and particulate matter [43], [44]. The predicted HHV value ranged from 38.06 MJ/kg to 39.97 MJ/kg across all the microalgal reactors. The density of the biodiesel was observed in the range of 0.86 to 0.87 g/cm^3, which is in the EN 14214 specification range of 0.86-0.90 g/cm^3 [42]. The kinematic viscosity values were observed to vary in the 3.78 to 4.67 mm^2/s range in green microalgae and 3.0 to 4.43 mm^2/s in diatoms. In all the microalgal reactors, the observed kinematic viscosity values were within the range of EN 14214 (3.5-5.0 mm^2/s) and ASTM D6751 (1.9-6.0 mm^2/s) standards [42].

Apart from biodiesel production, microalgal fatty acids offer several economic benefits due to their numerous industrial applications, particularly in fields such as nutraceuticals and cosmeceuticals [45]. Myristoleic acid is utilized for its anti-inflammatory, analgesic (pain-relieving), and immune system-regulating properties [46]. Palmitic acid and palmitoleic acid are commonly used in cosmetics due to their emollient, surfactant, and moisturizing properties. Stearic acid and elaidic acid are used in the preparation of food additives and dietary supplements. Oleic acid serves as an excipient in medicines and acts as a solubilizer and emulsifier in aerosols and combustion processes. Meanwhile, linoleic acid is used industrially in the making of skin care products, soaps, anti-inflammatory agents, and emulsifiers [46]. Arachidic acid is employed in industrial lubricants and greases for its high melting point and stability. The nutrient removal, fatty acid profile, and predicted biodiesel properties indicate that mixed microalgal systems can be used as an alternative to conventional aerobic bacteria for effective wastewater treatment, with the produced biomass serving as an effective

feedstock for biodiesel production and other value-added product recovery.

4 Conclusion

A comparative analysis was conducted to assess the efficacy of aerobic bacteria, green microalgae, and diatom cultures in the removal of organic carbon, nutrients, and micropollutants from domestic wastewater. The mixed microalgal cultures have shown lower organic carbon removal than aerobic bacterial cultures. However, a significantly enhanced nutrient removal was observed in both green microalgae and diatoms compared to aerobic bacteria. Solid retention time (SRT) played a significant role in pollutant removal. An SRT of 8 days provided a favorable balance between carbon and nutrient removal and lipid accumulation under the investigated conditions. Diatoms showed a significantly higher lipid-accumulating capacity than green microalgae. The fatty acid profiles of the microalgal biomass indicate a saturated fatty acid (SFA) content of more than 55%, demonstrating suitability for quality biodiesel production. Overall, the results suggest that microalgal systems can provide advantages in nutrient and micropollutant removal, although aerobic bacterial systems were more effective in organic carbon removal under the tested conditions. The integration of microalgal treatment with conventional aerobic processes could therefore be explored as a potential strategy for enhanced wastewater treatment and biomass valorization.

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Author Contributions

R.K.O.: conceptualization, methodology, data curation, investigation, formal analysis, visualization, writing – original draft; D.B.: conceptualization, methodology, funding acquisition, supervision, project administration, resources, writing – reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

This study presents and analyzes experimentally obtained data. The authors declare that no generative AI tools were used in the preparation of the manuscript. The authors utilized the Grammarly tool to enhance the language and readability of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online here.

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