Research Article

Performance of Green Microalgae-Activated Sludge and Diatom-Activated Sludge Co-Cultures in Kitchen Wastewater Treatment: Nutrient Removal Efficiency and Cellular Fatty Acid Profiling

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Abstract
Kitchen wastewater, characterized by elevated levels of organic matter and nutrients, requires efficient treatment solutions to mitigate its environmental impact. Conventional treatment methods are often energy-intensive and inefficient for decentralized or small-scale applications. This study investigates the sustainable treatment of kitchen wastewater by assessing nutrient removal efficiency and fatty acid profile in two different co-culture systems: System A (green microalga and activated sludge) and System B (diatom and activated sludge). The reactors were operated in semi-continuous mode at five distinct solid retention times (SRTs) (2, 4, 6, 8, and 10 days), with monitoring of key parameters including dissolved organic carbon, total nitrogen, and phosphates. The biomass obtained from both systems was analyzed for fatty acid composition after treatment. The removal of carbon and nitrogen was found to be comparable in the two setups. Chlorophyll concentration increased with increasing SRT in these co-cultures. At 10 days of SRT, the average chlorophyll concentration in System A was 6.5 mg/L, while in System B it was 4.9 mg/L. System A generated significantly greater proportions of polyunsaturated fatty acids across several SRTs in comparison to System B. The differences in fatty acid composition make System A more suitable for colder climates, where biodiesel must maintain adequate fluidity, while System B produces biodiesel with superior oxidative stability. This work establishes the feasibility of employing tailored algae-activated sludge co-cultures for integrated wastewater treatment and biodiesel production, demonstrating a sustainable methodology for simultaneous resource recovery and development of application-specific biofuels according to their fatty acid profiles.

Keywords: Activated sludge, Chlorophyll, Fatty acid methyl esters, FTIR, Retention time, Saturated fatty acids

1 Introduction

Kitchen wastewater (KWW) is a primary component of household greywater, originating mainly from food preparation, dishwashing, and cleaning activities. In Indian houses, KWW accounts for 37% of total greywater, generating 9 to 40 liters per capita per day (LPCD), with an average of 23 LPCD [1]. Furthermore, the KWW is generated by the food and restaurant sectors, both of which are substantially growing in India, hence increasing the volume of wastewater. The discharge of nutrient-rich wastewater, primarily from kitchen sources containing elevated levels of organic matter, nitrogen, and phosphorus, considerably contributes to environmental contamination such as eutrophication, deterioration of groundwater and soil quality, and foul odours [2]. When subjected to appropriate treatment methods, KWW becomes suitable for reuse in non-potable uses (such as toilet flushing, gardening, and car washing) and serves as a nutrient-rich resource for agricultural irrigation, hence reducing dependence on synthetic fertilizers [3]. A wide array of treatment technologies has been studied for kitchen wastewater applications. These approaches include biological, physicochemical, and hybrid systems. Biological technologies such as activated sludge processes, phytoremediation, membrane bioreactors, and microbial fuel cells (MFC) use microorganisms to degrade organic contaminants, and in some cases, generate electricity [4]. Physicochemical approaches such as electrocoagulation, flocculation, and advanced filtration remove suspended solids, fats, oils, and grease typically as a pre-treatment to biological processes or in conjunction with the biological steps [5], [6]. Hybrid/integrated systems that combine biological and physicochemical technologies like anaerobic membrane reactors [7] to meet and enhance efficiency and perform effective treatment, considering the complexity of kitchen wastewater. The traditional wastewater treatment processes are mainly energy-dependent and cannot properly remove nutrients, thereby causing eutrophication in water bodies.

In recent years, much interest has been taken in integrating biological treatment processes with microalgae, which demonstrates a very eco-friendly and cost-effective approach to nutrient removal and resource recovery [8], [9]. Microalgae can rapidly utilize nitrogen and phosphorus as nutrients, and research has demonstrated that algae can survive through efficient assimilation processes in wastewater environments, converting hazardous pollutants into biomass [10]. Some species of microalgae can even accumulate lipids in stress when nutrients become scarce, thereby offering a potentially promising feedstock for biofuel production [11]. Co-culturing microalgae with activated sludge was reported to be an effective and sustainable approach toward efficient wastewater treatment that enhances both pollutant removal efficiency and biomass yield due to the synergy between microalgae and microbial communities [12]. Microalgal photosynthesis will supply oxygen to the activated sludge system, facilitating the bacterial degradation of organic matter, and thereby achieving more balanced and sustainable treatment [13].

Numerous studies have been carried out on various microalgal species for the treatment of wastewater as well as biofuel applications. Green microalgae such as *Chlorella* and *Scenedesmus* are of particular interest due to their robust growth and capabilities to assimilate nutrients from diverse environments [14], [15]. Diatoms such as *Nitzschia palea* and *Cyclotella meneghiniana* possess excellent characteristics in wastewater treatment applications [16]. The silicate cell walls in diatoms provide a structural toughness that forms the basis for the robustness of mixed microbial populations, thereby allowing nutrient removal in a stable environment along with the treatment processes using activated sludge. Diatoms also assimilate nitrogen and phosphorus, hence contributing to nutrient removal [17]. More importantly, the rich-lipid cells can be regarded as a renewable source of biofuel, enabling energy recovery from wastewater [18]. Diatoms can also form biofilms, which enhance the settleability of sludge [19]. Despite these advantages, research on the application of diatoms in wastewater treatment, particularly for KWW, which presents a largely unique challenge, has been limited. Secondly, it is proven that solid retention time (SRT) has been acknowledged as one of the important parameters affecting the quantity of biomass and the efficiency of the treatment in activated sludge systems [20]. In this context, an optimal SRT creates a balance between nutrient removal and microbial growth, thereby reducing excess sludge. The effect of variable SRT on the removal efficiencies of carbon, nitrogen, and phosphorus, as well as biodiesel production using diatoms, remains relatively less explored, especially in relation to KWW treatment. This study evaluates the performance of green microalgae-activated sludge and diatom-activated sludge co-cultures for kitchen

wastewater treatment, focusing on nutrient removal efficiency and FAME profile analysis for potential biofuel applications. By comparing these two co-culture systems, we seek to identify which provides superior integration of effective wastewater treatment with biofuel production potential, advancing sustainable wastewater management strategies that combine nutrient recovery and energy generation.

2 Materials and Methods

2.1 *Kitchen wastewater*

Kitchen wastewater samples were collected daily in the morning from the mess wastewater outlet at the Indian Institute of Technology Hyderabad (IITH). The effluent was filtered through a fine mesh tea strainer to eliminate any visible floating particles. The wastewater pH was adjusted to 7–8 using 1 N sulfuric acid (H_2SO_4) and 1 N sodium hydroxide (NaOH).

2.2 *Culture and cultivation*

Activated sludge was collected from a decentralized wastewater treatment plant providing services to approximately 2000 households in Hyderabad, India. The mixed green microalgal seed cultures were collected from the master culture reactor (a controlled bioreactor) maintained at the Environmental Engineering Laboratory of IITH. The green algal culture was acclimated in a synthetic BG-11 medium for a year, kept at room temperature, and exposed to approximately a 12-hour light/dark cycle under direct sunlight. The BG-11 medium, commonly used for cyanobacterial and algal cultivation, contains per liter: 1.5 g sodium nitrate ($NaNO_3$), 0.0367 g calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), 0.006 g ferric ammonium citrate, 0.001 g disodium magnesium EDTA, 0.0314 g dipotassium hydrogen phosphate (K_2HPO_4), 0.036 g magnesium sulfate ($MgSO_4$), 0.02 g sodium carbonate (Na_2CO_3), and 0.0056 g of citric acid.

Diatoms were gathered from the bedrocks of a nearby lake in the Sangareddy district of the state of Telangana in India before the start of the monsoon season. After washing, the diatom culture was centrifuged at a speed of 2000 rpm for fifteen minutes. This was done to remove any pollutants that might have been present in the original seed culture. A diatom culture was grown under conditions similar to those of green microalgae, and an f/2-Si medium was used as a supplement to the culture. The Standard F/2 (Guillard's) medium contains a variety of nutrients, trace metals, and vitamins essential for microalgal growth. The composition per liter of each stock solution includes 84.15 g of sodium nitrate ($NaNO_3$), 6 g sodium molybdate dihydrate ($Na_2MoO_4{\cdot}2H_2O$), 2.9 g ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), and 10 g disodium EDTA dihydrate ($Na_2EDTA{\cdot}2H_2O$). For the silicate source, 33 g of sodium metasilicate nonahydrate ($Na_2SiO_3{\cdot}9H_2O$) are used per liter. Trace metals are supplied by adding 1.96 g copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), 4.4 g of zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$), 36 g of manganese chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$), and 2 g of cobalt chloride hexahydrate ($CoCl_2{\cdot}6H_2O$), all per liter. For vitamins, the solution includes 0.4 g vitamin B_1 (thiamine), 0.002 mg vitamin B_{12} (cyanocobalamin), and 0.1 mg biotin per liter. The use of f/2-Si medium offers advantages for diatom cultivation as it is formulated to match their elemental requirements, following the diatom-specific Redfield ratio of C:N:Si:P ≈ 106:16:15:1 (molar). This balanced nutrient supply ensures that nitrogen and phosphorus are provided in the classical 16:1 ratio while also including sodium metasilicate to meet the high silicon demand necessary for frustule formation. Unlike general algal media lacking silicate, f/2-Si prevents Si limitation, supports robust growth, and promotes healthy frustule development, making it more suitable for sustained diatom culture. The cultures were dominated by the green microalgae genera *Scenedesmus sp*. and *Chlorella sp*. Likewise, the diatom community was dominated by the genera *Meridion, Navicula, Nitzschia,* and *Stauroneis*.

2.3 *Experimental design*

The studies were performed in 500 mL clear glass bottles with large openings (schematics shown in Figure 1).

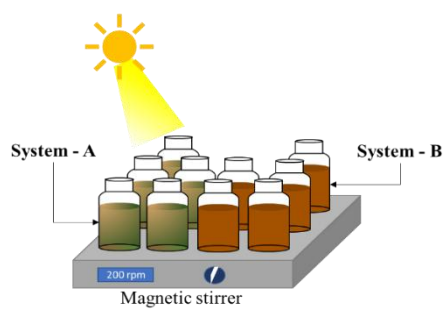

Figure 1: Schematic depiction of experimental configuration: System A and System B.

The test reactors were operated in parallel with different SRTs of 2, 4, 6, 8, and 10 days. These reactors were operated in semi-continuous mode following a daily draw-and-fill procedure. The reactors were maintained at ambient temperatures of 25-30 °C and exposed to sunlight with light intensity between 200 to 500 µmol photons m^{-2} s^{-1}. The reactors were operated for three times their SRTs to stabilize the biomass. At the beginning, identical quantities of algae and activated sludge were introduced to each reactor according to the volatile suspended solids (VSS) concentration. The concentration of VSS in the test reactors was maintained at approximately 1500 mg/L prior to the start of the experiment. The reactors were set on a magnetic stirrer, and they were continually agitated at a speed of 200 rpm.

2.4 *Analytical methods*

2.4.1 Characteristics of biomass and KWW

The water characteristics, such as dissolved organic carbon (DOC), total nitrogen (TN), and total phosphate (TP), were analyzed three times weekly for both the inflow and outflow water. Total phosphates (TP) were measured using 4500 P-C, in accordance with Standard Methods for Water and Wastewater [21]. The DOC and TN in the filtered water samples were analyzed using a TOC-L instrument manufactured by Shimadzu. The biomass (VSS) was determined in accordance with Method 2540 of the Standard Methods for the Examination of Water and Wastewater [21]. The chlorophyll pigments (a, b, and c) were measured following Method 10200 H of the same reference.

2.4.2 FAME analysis

Algae lipids were transformed into Fatty Acid Methyl Esters (FAME) by a direct in-situ transesterification technique as described by van Wychen and Laurens [22]. The dried algal biomass (~10 mg) was combined with 300 µL of 0.6 M HCl in methanol and 200 µL of a chloroform–methanol (2:1 v/v) solvent mixture. The reaction mixture was incubated at 85 °C for 1 hour, promoting simultaneous extraction and acid-catalyzed transesterification. After cooling, FAMEs were extracted into 1 mL of hexane, and an aliquot of the hexane phase was analyzed via gas chromatography. The gas chromatography equipment (Bruker GC-MS 400), in conjunction with a flame ionization detector, was used to conduct the analysis of FAME. The separation of fatty acids was accomplished by employing a BR-5MS column, with helium serving as the carrier gas, and a flow rate of 1 mL per minute. The temperature of the gas chromatography oven was set to rise at a rate of 4 °C per minute, beginning at 80 °C (with a hold of two minutes) and reaching 250 °C. The split ratio was 100:1, the injector was kept at 230 °C, and the detector was set to 300 °C. A combination of 37 FAME compounds (C4–C24) from Supelco was used to develop the standard calibration curve.

2.4.3 FTIR analysis

Organic components in the biomass were identified by Fourier-transform infrared (FTIR) analysis. The FTIR spectrometer (manufacturer: Bruker, model: Alpha II) was utilized to evaluate the presence of lipids, proteins, and carbohydrates in dried powdered biomass samples of System A and System B at the end of the experiment. The FTIR spectra of biomass were recorded in the range of 4000–500 cm^{-1} with a scanning resolution of 4 cm^{-1}.

2.5 *Statistical analysis*

A one-way Analysis of Variance (ANOVA) was used to compare differences in performance of System A and System B in terms of chlorophyll concentration, DOC removal, and nutrient removal. A significance level of p-value < 0.05 was applied, and all analyses were carried out using Excel.

3 Results and Discussion

3.1 *Initial Characteristics of KWW*

The characteristics of KWW are influenced by various factors such as cooking practices, type of meals prepared, and amount of water used in the cleaning [23]. In this study, the concentration of DOC in KWW varied between 113 to 740 mg/L, indicating substantial organic loading. The TN concentration ranged between 9 to 23 mg/L, although occasional spikes to higher concentrations were observed. Comparable concentrations and even elevated nitrogen values have been documented in the literature (Table 1). These elevated nitrogen levels can be attributed to proteins and other nitrogenous compounds commonly found in kitchen/food industry wastewater. The other important nutrient responsible

for eutrophication is phosphorus. The total phosphate concentrations ranged from 2 to 10 mg/L in the wastewater, which is consistent with typical levels found in kitchen effluents (Table 1). Detergents containing phosphorus, food residue, and other household waste serve as sources of phosphorus in kitchen wastewater. The N/P weight ratio of KWW ranged approximately 0.9 to 11.5. The widely recommended N/P weight ratio for optimal algal growth is around 7:1, which ensures balanced nutrient availability and supports high biomass productivity. Since the observed range in KWW overlaps with this recommended value, it can be considered generally suitable for algal cultivation. However, because the ratio may at times fall below or exceed the ideal level, nutrient availability could shift toward nitrogen or phosphorus limitation.

Table 1: Characteristics of KWW.

Type of WW	TN (mg/L)	Phosphate (mg/L)	Ref.
Institutional	9–23	2–10	This study
Institutional	20–30	16–18	[24]
Canteen wastewater	BDL	9.375–13.8	[25]
KWW	381.7 ± 32.4	167.2 ± 22.6	[26]
Employee Mess	52.962 ± 0.018*	2.037 ± 0.008	[27]
kitchen-sink wastewater	21.9–43.5	2.9–14.5**	[28]
Institutional & household	5.35*	5.8	[29]
University restaurant	26–79	3.8–12.5	[23]
Restaurant wastewater	104–136	20–29	[30]

BDL – below detection limit; * nitrates values; **total phosphorus values

3.2 *Algal growth*

The variations of chlorophyll concentrations of two types of co-culture systems - System A of green microalgal-activated sludge and System B of diatom-activated sludge, operated at different SRTs ranging from 2 to 10 days - are given in Figure 2. In this study, chlorophyll analysis was chosen as the primary method to assess algal growth within the mixed co-cultures. Chlorophyll, particularly chlorophyll a, is a key pigment exclusively produced by algae and is directly correlated with their photosynthetic activity and biomass. Measuring chlorophyll concentration provides a rapid, non-destructive, and reliable proxy for estimating algal abundance, even in complex cultures where direct cell counting may be difficult due to the presence of bacteria. In System A, the lowest chlorophyll levels, ranging between 0.25 and 1.9 mg/L, were observed at 2 days of SRT. The maximum value of chlorophyll (6.5 mg/L) was observed at 10-d SRT. Such variability can be due to initial conditions and fluctuations in nutrient availability, which may pose a constraint on the ability of algae to grow. For algae, an increasing trend in chlorophyll concentration with SRT, as observed in System A, is consistent with literature; higher extended SRTs result in a more robust and resilient population of algae, hence higher biomass production [31].

In System B, the chlorophyll concentrations were generally low compared to those in System A throughout the SRTs. The maximum chlorophyll values, ranging from 3.12 to 4.89 mg/L, were observed at 10 days SRT. This difference in total chlorophyll is due to the differences in pigment composition between algal types. The one-way ANOVA test resulted in $p < 0.05$ for all the chlorophyll measurements, confirming significant differences between systems A and B. Diatoms have chlorophyll a and c, as well as significant amounts of the accessory pigment fucoxanthin, which together form the fucoxanthin-chlorophyll protein (FCP) complexes for light harvesting [32]. In contrast, green algae have chlorophyll a and b, with a often present in larger amounts. Since diatoms lack chlorophyll b, total chlorophyll measurements (which usually means the sum of a, b, and c) will tend to be less for diatom cultures than for green algae cultures, even when there is equal biomass or photosynthetic capacity. Therefore, the low chlorophyll values in System B cannot indicate lower productivity or cellular activity of diatoms.

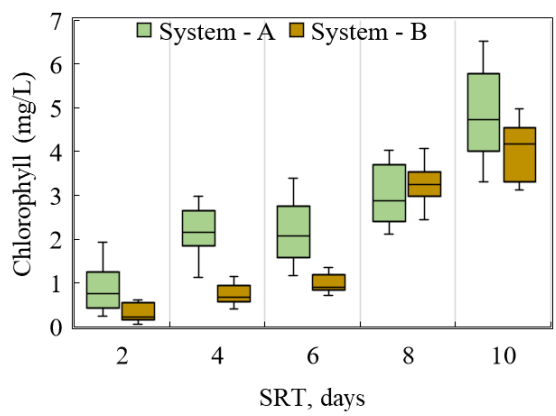

Figure 2: Chlorophyll concentrations at different SRTs in Systems A and B.

3.3 *Removal of carbon and nutrients*

The DOC, TN, and TP removal in two systems - System A and System B at different SRTs - is shown in Figure 3. The diatoms and activated sludge co-culture exhibited reduced effluent values relative to the algal-activated sludge co-culture reactor across all the parameters. The removal efficiency improved with an increase in SRT. The DOC (Figure 3(a)) effluent values were 80 mg/L at SRT 2 days, thereafter decreasing to roughly 13 mg/L at SRT 10 days. For System B, DOC levels ranged from 6 to 9 mg/L for SRTs of 8 and 10 days, with removal effectiveness above 95% for SRTs greater than 6 days (Table 2).

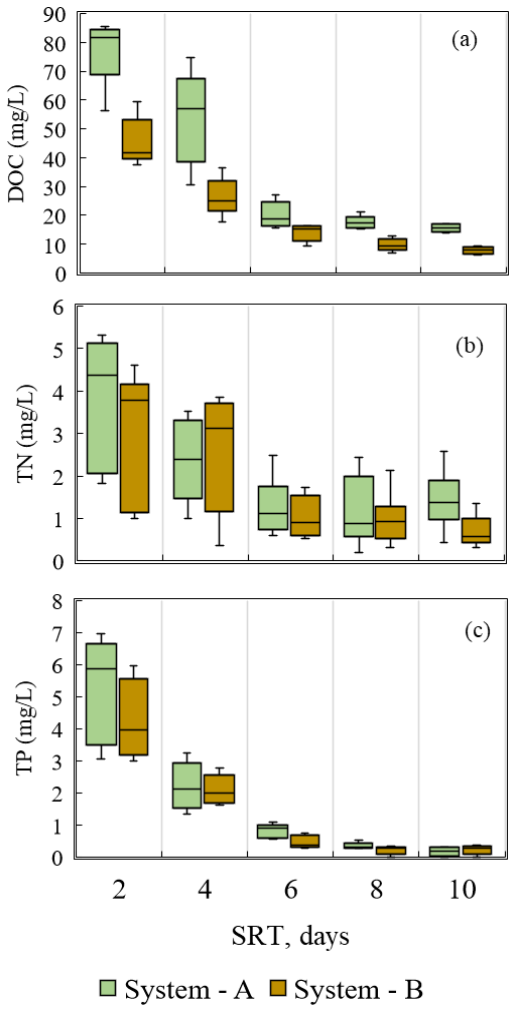

Figure 3: Carbon and nutrient (nitrogen and phosphate) concentrations at different SRTs in Systems A and B.

The enhanced organic carbon removal in System B indicates that diatoms, in association with activated sludge, facilitate organic carbon decomposition more efficiently than algae-based systems. This improvement can be attributed to synergistic interactions between diatoms and the heterotrophic microorganisms in activated sludge, enhancing organic matter degradation and carbon assimilation. Likewise, the effluent TN removal effectiveness of System B consistently exhibited a somewhat superior removal rate compared to System A (Figure 3(b)). The effluent TN levels for both systems are below the acceptable discharge limit of 10 mg/L set by the Central Pollution Control Board and the Ministry of Environment, Forest and Climate Change (MoEFCC) of India. At 2-d SRT, System A and System B attained TN removal efficiencies of 73.8% and 77.5%, respectively. After a retention time of 10 days, TN removal achieved 89.8% in System A and 95.2% in System B. Both System A and System B attained near-zero levels at SRT 10, with more than 95% phosphate removal. The one-way ANOVA results indicated that for TN and TP removal, p-value > 0.05, suggesting no statistically significant differences between the systems. In contrast, DOC removal showed p-value < 0.05, indicating a significant difference.

Table 2: Carbon and nutrient removal efficiencies (in percentage) of different technologies in KWW treatment (COD, nitrates, and phosphates).

Treatment Technology	Species	COD	N	P	Ref.
Algae - Activated sludge	Mixed	94[a]	91[b]	94	This study
Diatom - Activated sludge		96[a]	94[b]	96	
Microalgae	*Chlorella sp., Scenedesmus sp., Coelastrum sp.,* and *Pediastrum sp.*	55	83	96	[29]
Microalgae	Mixed green algae	88[a]	85[b]	80	[33]
Biofilter - MFC	Bacteria	88	87	94	[34]
Silver nanoparticles	-	65	72	93	[29]
Constructed wetland	-	36	76	77	[35]

[a] TOC removal; [b]TN removal

Interactions and removal mechanisms within the co-cultures of bacteria with diatoms or green algae are dynamic, complex processes that are often species-dependent, environment-dependent, and attribute-specific (e.g., lag vs. exponential growth). Co-cultures exhibit mutualistic, facilitative, and antagonistic interactions. For example, two or more mutualisms would occur if the algae or diatoms were to provide organic carbon and oxygen for the bacteria while the bacteria supplied the algae or diatoms with nutrients like vitamins (e.g., vitamin B_{12}), remineralization, or possibly through detoxifying metabolites or preventing toxic algal blooms [36]. However, some bacteria exhibit an antagonistic role by producing algicidal compounds or compete for nutrients and therefore prevent algal or diatom growth [37]. The removal mechanisms of carbon, nitrogen, and phosphorus in these co-culture systems are closely associated with these interactions. In general, algae and diatoms assimilate inorganic nutrients (NH_4^+, NO_3^-, PO_4^{3-}) into biomass while bacteria mineralize organic materials, producing CO_2 and inorganic nutrients that can be re-assimilated by the photosynthetic partner. In terms of nitrate nitrogen removals, bacteria provide nitrification and denitrification, processes that are supported with oxygen produced by algae or diatoms during photosynthesis. Algae can sequester phosphate into biomass during phosphorus cycling. Bacteria can also sequester nutrients into extracellular polymeric substances (EPS) [38]. Diatoms have unique silica frustules that offer structural protection, allowing survival against environmental stresses and grazing; their frustules also exhibit some phosphorus adsorption, an attractive factor for phosphorus-dominant wastewaters in many ways [39].

Diatoms are capable of surviving in nitrogen-deficient waters by utilizing intracellular nitrate stores and demonstrating alternative metabolic pathways. Marine diatoms can store very high amounts of intracellular nitrate (11–274 mM) that can be respired in the dark under anoxic conditions by the metabolic process of dissimilatory nitrate reduction to ammonium (DNRA), allowing diatoms to continue their metabolism and viability for weeks to months when external nitrate is limited or absent (in sediment layers or oxygen minimum zones) [40]. In other words, DNRA allows for a resting stage for diatom cells, and ultimately, this allows for long-term survival and subsequent recovery into active metabolic states upon reestablishing favorable environmental conditions. Additionally, diatoms have a highly integrated nitrogen metabolism within their chloroplasts and mitochondria that provides them with the ability to effectively assimilate and redistribute nitrogen in relation to the changing availability of nitrogen. The integrated nitrogen metabolism in diatoms is more inclusive than the integrated nitrogen metabolism in green algae; thus, it allows diatoms to respond more flexibly and rapidly to episodic nitrogen availability in their environments [41]. This ecological sensibility to nitrogen resource variability is an evolutionary advantage for diatom survival in variable nitrogen environments. Overall, diatoms have adaptability to nutrient concentration, which represents operational flexibility important for nutrient removal from wastewater streams with biomass valorization. In comparison, green algae are predominantly utilized because they are fast-growing, photosynthetically efficient organisms with relatively good carbon capture and oxygen generation, which facilitates the bacterial decomposition of organic waste. Green algae tend to require more consistent environmental nutrient supply, particularly nitrogen and phosphorus, and are more sensitive to changes in wastewater composition and environmental stress compared to diatoms. Green algae also lack the physical protection of a silica shell and, therefore, may be more susceptible to grazing and environmental perturbations.

3.4 *Biochemical composition*

FTIR spectroscopy was used to study the biochemical composition of biomass from Systems A and B at various SRTs (Figure 4). The functional groups and molecular structures of lipids, carbohydrates, proteins, and valuable extracellular compounds were determined to assess their potential for biodiesel production and other valuable bioproducts. The FTIR spectra of 2-d SRT biomass of System A (2A - Figure 4(a)) and System B (2D - Figure 4(b)) showed prominent lipid peaks near 1740 cm^{-1} (indicative of ester C=O bonds), as well as stretching vibrations of C-H in aliphatic chains (-CH_2 and -CH_3) in the ~2850–2950 cm^{-1} region, marking high initial lipid content. However, a significant decline in these peaks was observed with an increase in SRT, particularly in System A (10A - Figure 4(a)), indicating progressive lipid degradation, likely due to bacterial metabolism and cellular aging.

FTIR spectra demonstrated obvious distinctions with protein concentration, as indicated by the

occurrence of amide bond peaks at 1650 cm^{-1} (Amide I, reflecting C=O stretching vibration) and ~1540 cm^{-1} (Amide II, reflecting N-H bending vibration) [42]. Peaks within the 2-d retention period biomass were far more intense, reflecting high protein concentration. This means that lower SRT is beneficial for protein accumulation, which is desirable for different applications such as the production of single-cell proteins (SCP), biofertilizers, and animal feed [43]–[45]. The biomass at higher retention time, however, showed extensive loss of these amide peaks. A comparable pattern was found to be present in diatom co-culture (System B), 2-d SRT biomass having a high protein content, and 10-d SRT diatom biomass showed a wide range decrease in peaks associated with protein.

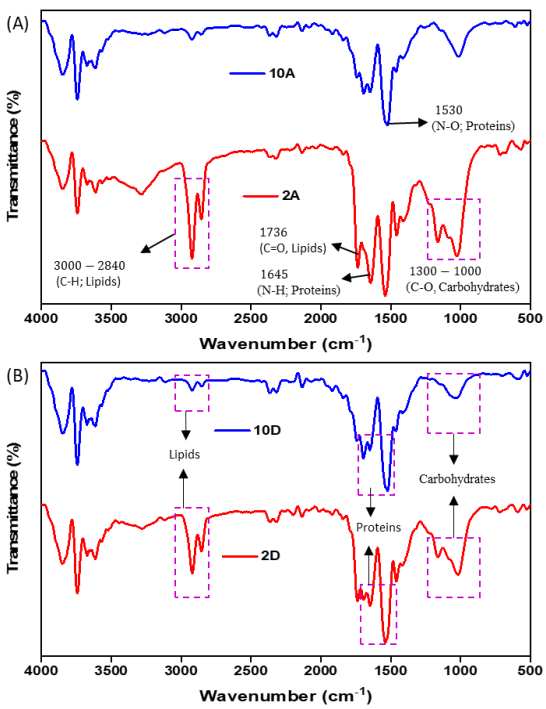

Figure 4: FTIR spectra of dried co-culture biomass at 2-day and 10-day SRT in (a) System A (2A denoting 2-day SRT and 10A denoting 10-day SRT) and (b) System B (2D denoting 2-day SRT and 10D denoting 10-day SRT).

Carbohydrate content was also observed in FTIR, the C-O-C and C-O stretching vibration at ~1030 cm^{-1} that corresponds to polysaccharides and carbohydrate molecules. In both System A and B, biomass showed a decreasing trend in carbohydrate accumulation with an increase in retention times. Conversely, the carbohydrate region (~1030 cm^{-1}) remained more stable in System B, especially at higher SRTs (6 – 10 d), indicating the superior preservation of polysaccharides in diatoms. This resilience may be attributed to the siliceous frustules of diatoms, which offer structural protection against enzymatic degradation [46], [47]. These carbohydrates can be utilized in bioethanol production or high-value polysaccharides for the pharmaceutical and food industry [48]. The ability to engineer SRTs enables the targeted production of biodiesel, protein-rich bioproducts, or chemicals of interest based on specific industry needs. Metabolic shifts at different retention times of 2 and 10 days also highlight the importance of optimizing growth conditions to maximize the commercial value of algal and diatom biomass.

3.5 *Fatty acid profile*

In general, green microalgae have higher lipid and tolerance levels, and diatoms produce unique fatty acid profiles of PUFAs, which may influence the quality and properties of biodiesel. The fatty acid composition impacts biodiesel characteristics such as oxidative stability, cold-flow performance, and combustion efficiency [49]. Saturated fatty acid (SFA) consistently dominates the fatty acid composition in both systems (Figure 5), while polyunsaturated fatty acid (PUFA) values are lower in both systems. PUFA values remained relatively higher (between 6–10%) in System A compared with those of System B, where PUFA content is significantly reduced to nearly zero at later stages. In comparison to previous studies, our PUFA results align well with reported ranges in similar algal systems. For instance, PUFA content in diatom cultures treating KWW has been reported between 0.9% and 3.8% [50], while mixed green algal cultures show higher PUFA levels, ranging from 8% to 29% [33]. Additionally, mixed blue-green algae cultures typically contain approximately 3% to 5% PUFA when treating KWW [24]. These findings are consistent with reported PUFA profiles in Chlorella-based systems, which often exhibit comparable or higher PUFA percentages. Monounsaturated fatty acid (MUFA) levels varied between 20–28% and remained almost similar at different SRTs in both systems.

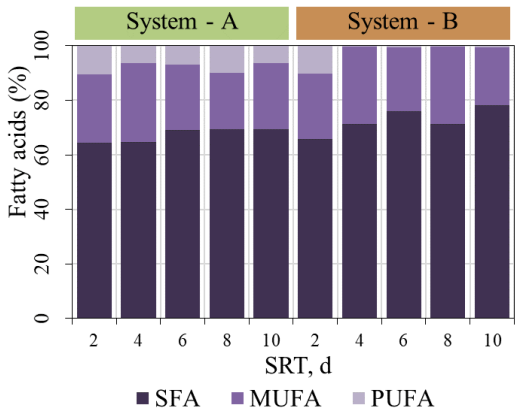

Figure 5: Fatty acid profile at different SRTs in Systems A and B.

Comparing it to the literature, it is well documented that diatoms tend to produce higher PUFA levels, especially eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) under optimal growth conditions [51]. However, activated sludge-associated interactions may lead to increased SFA proportions, potentially due to bacterial metabolism influencing lipid saturation levels [52]. An increase in SFA at later time points indicates either possible oxidative stress or lipid metabolism change in favor of stability rather than fluidity of membranes. The presence of a high saturation percentage in a fatty acid is indicative of superior fuel qualities, including a reduced risk of combustion and ignition delay [53]. Changes in MUFA levels might represent species-specific synthesis or interactions with bacteria.

In both the microalgae–activated sludge and diatom–activated sludge co-cultures, we detected palmitic acid (C16:0), oleic acid (C18:1), myristoleic acid (C14:1), and pentadecenoic acid (C15:1). The dominant saturated fatty acid, palmitic acid, is a well-known feedstock for biodiesel production due to its favorable combustion properties and stability [54]. Myristoleic, pentadecenoic, and oleic, monounsaturated fatty acids have potential antimicrobial, anti-inflammatory, and nutraceutical applications, and may serve as specialty chemical feedstocks [54]. Notably, in the diatom co-culture, we also detected linoleic acid methyl ester (C18:2n6c), erucic acid methyl ester (C22:1n9), and arachidonic acid methyl ester (C20:4n6), all of which have been reported in various diatom species. Linoleic acid is an essential omega-6 PUFA with key roles in human and animal nutrition. Erucic acid is valued in the industrial sector as a precursor for high-performance lubricants, plasticizers, and specialty polymers [54]. Arachidonic acid is a bioactive omega-6 PUFA with pharmaceutical and nutraceutical importance due to its involvement in cell signaling and inflammatory processes.

The quality and yield of fatty acid methyl esters (FAMEs) in grams produced per gram of dried biomass are important factors to assess the viability of algae and diatoms as biodiesel feedstocks. Various studies have shown that diatoms typically have higher lipid content than many green algae, often above 30% of their dried weight, meaning there is a higher yield of FAMEs [55]. The quality of FAME (mg) per g dried biomass of both Systems A and B in comparison with the literature is given in Table 3.

Table 3: FAME yield of algal cultures cultivated in different types of wastewater.

Type of Wastewater	Culture	FAME (mg/g)	Ref.
KWW	Algae-activated sludge	54–95	This study
	Diatom-activated sludge	204–315	
Domestic WW	Algae-activated sludge	34–135	[56]
Mixed (industry + municipal)	*Chlorella sorokiniana*	62.4	[57]
Textile industrial wastewater	*Chlorella vulgaris*	61.65	[58]
	Chlorella vulgaris + *Biochar*	76.8 ± 2	[59]
BG 11	*Chlorella sp.* 227	160.4	[60]

The findings indicate that the productivity of FAME was different between systems, and it was influenced by the type of wastewater and the algal species or consortium. Of the systems tested, diatom-activated sludge cultivated in KWW produced the highest quantity of FAME (204–315 mg/g dry weight (DW)) than algae-activated sludge (54–95 mg/g DW). Similar moderate yields were reported for domestic wastewater, producing 34–135 mg/g DW FAME in algae-activated sludge [56]. *Chlorella sorokiniana* (via mixed industrial & municipal wastewater) and *Chlorella vulgaris* (via textile wastewater) produced moderate yields (62.4 mg/g DW and 61.65 mg/g DW, respectively) [57], [58]. When biochar was added to the textile wastewater system, FAME yield improved significantly in *Chlorella vulgaris* [59]. BG-11 control medium showed high lipid yields for *Chlorella sp. 227,* which produced 160.4 mg/g DW lipid due to the synthetic nature and increased nutrient environment of the growth medium. *Chlamydomonas sp.* grown in fertilizer wastewater produced 15.99% FAME

(~160 mg/g DW) [61], and *Scenedesmus sp.* in cattle manure effluent produced a lipid productivity of 81.9 mg/L/d, indicating a high capacity for biodiesel conversion [62]. Overall, the diatom co-culture system gave better FAME yield than System A. Further studies are needed to determine how the composition of the activated sludge community is directly related to fatty acid accumulation. Although real kitchen effluent may fluctuate greatly in organic load and nutrient content, mixed co-culture reactors showed resilience in adjusting to these changes. Additionally, the reactors can be scaled up by modular design, allowing for staged treatment and buffering against influent variability. However, we acknowledge that pre-treatment or blending may be necessary to moderate extreme variations for optimal performance at larger scales.

4 Conclusions

This study demonstrates that the diatom-activated sludge system (System B) performs similarly to that of the algae-activated sludge system (System A) in treating kitchen wastewater, particularly in the removal of carbon and nutrients. The SRT had significantly affected the performance of the system. The performance of algal co-cultures in nutrient removal can be attributed to their higher photosynthetic efficiency, faster growth rates, enhanced nutrient uptake mechanisms, and stronger mutualistic interactions with activated sludge. Green microalgae-activated sludge systems effectively assimilate nitrogen and phosphorus into intracellular polyphosphate granules and generate oxygen to support activated sludge degradation of organic matter. In contrast, diatoms exhibited stable carbohydrate buildup and marginally higher efficiency in phosphate removal due to their silica cell wall. The results indicate that an algae-activated sludge system is more suitable for high-rate wastewater treatment applications, which mainly focus on treatment, and biodiesel and diatoms-activated sludge appear better suited for extended cultivation when carbohydrate retention is prioritized, relevant for bioethanol or fermentative processes.

Author Contributions

K.K.: conceptualization, research design, investigation, data curation, writing an original draft and editing; S.D.: FTIR data analysis, writing, and reviewing; A.B.T.: experimentation and data curation; V.V.: experimentation and data curation; A.T.: reviewing and editing; D.B.: funding acquisition, 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.

References

[1] I. N. Shaikh and M. M. Ahammed, "Quantity and quality characteristics of greywater from an Indian household," *Environmental Monitoring and Assessment*, vol. 194, no. 3, Mar. 2022, doi: 10.1007/s10661-022-09820-0.

[2] P. R. Rout et al., "Nutrient removal from domestic wastewater: A comprehensive review on conventional and advanced technologies," *Journal of Environmental Management*, vol. 296, p. 113246, Oct. 2021, doi: 10.1016/j.jenvman.2021.113246.

[3] S. J. Mane, P. Kumbhare, and A. Pandav, "Experimental study of treatment of kitchen waste water using rice husk ash," *Materials Today: Proceedings*, Feb. 2024, doi: 10.1016/j.matpr.2024.01.056.

[4] P. Moñino et al., "A new strategy to maximize organic matter valorization in municipalities: Combination of urban wastewater with kitchen food waste and its treatment with AnMBR technology," *Waste Management*, vol. 62, pp. 274–289, Apr. 2017, doi: 10.1016/j.wasman.2017.02.006.

[5] V. S. Neeraj, A. Arunangiri, and K. Muthukumar, "Treatment of Kitchen Wastewater in a Batch Electrochemical Reactor," *Journal of The Institution of Engineers (India): Series E*, vol. 104, no. 1, pp. 185–194, Jun. 2023, doi: 10.1007/s40034-022-00262-4.

[6] J. Zhang et al., "Enhanced purification and disinfection of restaurant wastewater by electro-coagulation coupled with an electro-oxidation process: From lab scale to field scale," *ACS ES and T Engineering*, vol. 3, no. 7, pp. 932–943, Jul. 2023, doi: 10.1021/acsestengg.2c00381.

[7] T. S. Le et al., "Two-stage anaerobic membrane bioreactor for co-treatment of food waste and kitchen wastewater for biogas production and nutrients recovery," *Chemosphere*, vol. 309,

p. 136537, Dec. 2022, doi: 10.1016/j.chemosphere.2022.136537.

[8] K. Katam, Y. Tiwari, T. Shimizu, S. Soda, and D. Bhattacharyya, "Start-up of a trickling photobioreactor for the treatment of domestic wastewater," *Water Environment Research*, vol. 93, no. 9, pp. 1690–1699, Sep. 2021, doi: 10.1002/wer.1554.

[9] S. F. Mohsenpour, S. Hennige, N. Willoughby, A. Adeloye, and T. Gutierrez, "Integrating micro-algae into wastewater treatment: A review," *Science of The Total Environment*, vol. 752, p. 142168, Jan. 2021, doi: 10.1016/J.SCITOTENV.2020.142168.

[10] V. Matamoros, E. Uggetti, J. García, and J. M. Bayona, "Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: A laboratory scale study," *Journal of Hazardous Materials*, vol. 301, pp. 197–205, Jan. 2016, doi: 10.1016/j.jhazmat.2015.08.050.

[11] S. Pandey, I. Narayanan, R. Selvaraj, T. Varadavenkatesan, and R. Vinayagam, "Biodiesel production from microalgae: A comprehensive review on influential factors, transesterification processes, and challenges," *Fuel*, vol. 367. p. 131547, Jul. 01, 2024. doi: 10.1016/j.fuel.2024.131547.

[12] A. Saravanan et al., "A review on algal-bacterial symbiotic system for effective treatment of wastewater," *Chemosphere*, vol. 271, p. 129540, May 2021, doi: 10.1016/j.chemosphere.2021.129540.

[13] C. S. Lee, S.-A. Lee, S.-R. Ko, H.-M. Oh, and C.-Y. Ahn, "Effects of photoperiod on nutrient removal, biomass production, and algal-bacterial population dynamics in lab-scale photobioreactors treating municipal wastewater," *Water Research*, vol. 68, pp. 680–691, Jan. 2015, doi: 10.1016/J.WATRES.2014.10.029.

[14] M. A. M. Condori, M. M. Condori, M. E. V. Gutierrez, F. J. Choix, and F. García-Camacho, "Bioremediation potential of the *Chlorella* and *Scenedesmus* microalgae in explosives production effluents," *Science of the Total Environment*, vol. 920, p. 171004, Apr. 2024, doi: 10.1016/j.scitotenv.2024.171004.

[15] J. J. Ortiz-Betancur et al., "Application of *Chlorella* sp. and *Scenedesmus* sp. in the bioconversion of urban leachates into industrially relevant metabolites," *Applied Sciences (Switzerland)*, vol. 12, no. 5, p. 2462, Feb. 2022, doi: 10.3390/app12052462.

[16] T. K. Marella, N. R. Parine, and A. Tiwari, "Potential of diatom consortium developed by nutrient enrichment for biodiesel production and simultaneous nutrient removal from waste water," *Saudi Journal of Biological Sciences*, vol. 25, no. 4, pp. 704–709, May 2018, doi: 10.1016/j.sjbs.2017.05.011.

[17] M. K. Kumara, R. K. Oruganti, R. Tejavath, M. Sriariyanun, A. Tawai, and D. Bhattacharyya, "The potential application of diatom-aided constructed floating wetlands for domestic wastewater treatment," *Applied Science and Engineering Progress*, vol. 18, no. 2, 2025, Art. 7662, doi: 10.14416/j.asep.2024.12.004.

[18] I. Rawat, R. R. Kumar, T. Mutanda, and F. Bux, "Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production," *Applied Energy*, vol. 88, no. 10, pp. 3411–3424, Oct. 2011, doi: 10.1016/J.APENERGY.2010.11.025.

[19] C. Koedooder et al., "Diatom-bacteria interactions modulate the composition and productivity of benthic diatom biofilms," *Frontiers in Microbiology*, vol. 10, p. 454984, Jun. 2019, doi: 10.3389/fmicb.2019.01255.

[20] L. K. Akula, R. K. Oruganti, D. Bhattacharyya, and K. K. Kurilla, "Treatment of marigold flower processing wastewater using a sequential biological-electrochemical process," *Applied Science and Engineering Progress*, vol. 14, no. 3, pp. 525–542, 2021, doi: 10.14416/j.asep.2021.04.001.

[21] APHA/AWWA/WEF, *Standard Methods for the Examination of Water and Wastewater*, 22nd ed. Washington, DC, USA: American Public Health Association, 2012.

[22] S. V. Wychen and L. M. L. Laurens, "Determination of total lipids as fatty acid methyl esters (FAME) by in situ transesterification: Laboratory analytical procedure (LAP)," National Renewable Energy Laboratory (NREL), Golden, CO, USA, Tech. Rep. NREL/TP-5100-60958, May 2013.

[23] C. Gurd, B. Jefferson, and R. Villa, "Characterisation of food service establishment wastewater and its implication for treatment," *Journal of Environmental Management*, vol. 252, p. 109657, Dec. 2019, doi: 10.1016/j.jenvman.2019.109657.

[24] A. Babu, K. Katam, M. P. Gundupalli, and D. Bhattacharyya, "Nutrient removal from wastewater using microalgae: A kinetic evaluation and lipid analysis," *Water Environment Research*, vol. 90, no. 6, pp. 520–529, Jun. 2018, doi: 10.2175/106143017X15054988926299.

[25] A. Gupta and J. R. Nath, *Kitchen Greywater Treatment in a Constructed Wetland Microcosm Using Aquatic Macrophytes*. Singapore: Springer, 2018, pp. 141–149, doi: 10.1007/978-981-10-5795-3_13.

[26] X. Zheng et al., "Increasing municipal wastewater BNR by using the preferred carbon source derived from kitchen wastewater to enhance phosphorus uptake and short-cut nitrification-denitrification," *Chemical Engineering Journal*, vol. 344, pp. 556–564, Jul. 2018, doi: 10.1016/j.cej.2018.03.124.

[27] P. K. Kumar, S. V. Krishna, S. S. Naidu, K. Verma, D. Bhagawan, and V. Himabindu, "Biomass production from microalgae Chlorella grown in sewage, kitchen wastewater using industrial CO2 emissions: Comparative study," *Carbon Resources Conversion*, vol. 2, no. 2, pp. 126–133, Aug. 2019, doi: 10.1016/j.crcon.2019.06.002.

[28] A. Huelgas, M. Nakajima, H. Nagata, and N. Funamizu, "Comparison between treatment of kitchen-sink wastewater and a mixture of kitchen-sink and washing-machine wastewaters," *Environmental technology*, vol. 30, no. 1, pp. 111–117, 2009, doi: 10.1080/09593330802445549.

[29] A. Bindhuraj, S. V. Paulose, S. Asharaf, and S. Joseph, "A comparative study on the treatment of kitchen grey water using microalgae consortia and microalgae-synthesized silver nanoparticles," *Environmental Science and Pollution Research*, vol. 31, no. 60, pp. 67521–67533, Dec. 2024, doi: 10.1007/S11356-024-33655-6/METRICS.

[30] N. Hendrasarie, M. Nugraha, and K. Fadilah, "Restaurant wastewater treatment with a two-chamber septic tank and a sequencing batch reactor," *E3S Web of Conferences*, vol. 328, p. 01011, 2021, doi: 10.1051/e3sconf/202132801011.

[31] K. Katam and D. Bhattacharyya, "Simultaneous treatment of domestic wastewater and bio-lipid synthesis using immobilized and suspended cultures of microalgae and activated sludge," *Journal of Industrial and Engineering Chemistry*, vol. 69, pp. 295–303, Jan. 2019, doi: 10.1016/j.jiec.2018.09.031.

[32] P. Kuczynska, M. Jemiola-Rzeminska, and K. Strzalka, "Photosynthetic pigments in diatoms," *Marine Drugs*, vol. 13, no. 9. MDPI AG, pp. 5847–5881, Sep. 01, 2015. doi: 10.3390/md13095847.

[33] K. Katam and D. Bhattacharyya, "Comparative study on treatment of kitchen wastewater using a mixed microalgal culture and an aerobic bacterial culture: kinetic evaluation and FAME analysis," *Environmental Science and Pollution Research*, vol. 25, no. 21, pp. 20732–20742, 2018, doi: 10.1007/s11356-018-2209-6.

[34] A. Y. Radeef, A. A. Najim, H. A. Karaghool, and Z. H. Jabbar, "Sustainable kitchen wastewater treatment with electricity generation using upflow biofilter-microbial fuel cell system," *Biodegradation*, vol. 35, no. 6, pp. 893–906, Oct. 2024, doi: 10.1007/s10532-024-10087-0.

[35] G. Baskar, V. T. Deeptha, and A. A. Rahman, "Treatment of wastewater from kitchen in an institution hostel mess using constructed wetland," *International Journal of Recent Trends in Engineering*, vol. 1, no. 6, pp. 54–58, 2009.

[36] E. Kazamia et al., "Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation," *Environmental Microbiology*, vol. 14, no. 6, pp. 1466–1476, 2012, doi: 10.1111/j.1462-2920.2012.02733.x.

[37] J. J. Cole, "Interactions Between Bacteria and Algae in Aquatic Ecosystems," *Annual Review of Ecology and Systematics*, vol. 13, no. 1, pp. 291–314, Nov. 1982, doi: 10.1146/annurev.es.13.110182.001451.

[38] A. W. Decho and T. Gutierrez, "Microbial extracellular polymeric substances (EPSs) in ocean systems," *Frontiers in Microbiology*, vol. 8, p. 922, May 2017, doi: 10.3389/FMICB.2017.00922.

[39] H. Xu, F. Chen, X. Zhang, Z. Zhang, K. Pan, and H. Liu, "Grazer-induced changes on mechanical properties of diatoms frustule: A new proof for a watery arms race," *Limnology and Oceanography Letters*, vol. 9, no. 6, pp. 754–763, Dec. 2024, doi: 10.1002/LOL2.10419.

[40] A. Kamp, D. D. Beer, J. L. Nitsch, G. Lavik, and P. Stief, "Diatoms respire nitrate to survive dark and anoxic conditions," *Proceedings of the National Academy of Sciences of the United*

States of America, vol. 108, no. 14, pp. 5649–5654, Apr. 2011, doi: 10.1073/pnas.1015744108.

[41] C. Wilhelm et al., "The regulation of carbon and nutrient assimilation in diatoms is significantly different from green algae," *Protist*, vol. 157, no. 2, pp. 91–124, Jun. 2006, doi: 10.1016/j.protis.2006.02.003.

[42] M. Sweiss et al., "Qualitative and quantitative evaluation of microalgal biomass using portable attenuated total reflectance-Fourier transform infrared spectroscopy and machine learning analytics," *Journal of Chemical Technology and Biotechnology*, vol. 99, no. 1, pp. 92–108, Jan. 2024, doi: 10.1002/jctb.7512.

[43] M. Janssen, R. H. Wijffels, and M. J. Barbosa, "Microalgae based production of single-cell protein," *Current Opinion in Biotechnology*, vol. 75, p. 102705, Jun. 2022, doi: 10.1016/J.COPBIO.2022.102705.

[44] E. E. Ammar et al., "Algae as Bio-fertilizers: Between current situation and future prospective," *Saudi Journal of Biological Sciences*, vol. 29, no. 5, pp. 3083–3096, May 2022, doi: 10.1016/J.SJBS.2022.03.020.

[45] P. Parmar, R. Kumar, Y. Neha, and V. Srivatsan, "Microalgae as next generation plant growth additives: Functions, applications, challenges and circular bioeconomy based solutions," *Frontiers in Plant Science*, vol. 14, p. 1073546, Mar. 2023, doi: 10.3389/FPLS.2023.1073546/XML/NLM.

[46] M. Cabrera-Brufau, L. Arin, M. M. Sala, P. Cermeño, and C. Marrasé, "Diatom Dominance Enhances Resistance of Phytoplanktonic POM to Mesopelagic Microbial Decomposition," *Frontiers in Marine Science*, vol. 8, p. 683354, Jun. 2021, doi: 10.3389/FMARS.2021.683354/XML/NLM.

[47] B. Moriceau et al. , "Si–C interactions during degradation of the diatom *Skeletonema marinoi*," *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 56, no. 18, pp. 1381–1395, Aug. 2009, doi: 10.1016/J.DSR2.2008.11.026.

[48] P. Sarkar, T. K. Bandyopadhyay, K. Gopikrishna, O. Nath Tiwari, B. Bhunia, and M. Muthuraj, "Algal carbohydrates: Sources, biosynthetic pathway, production, and applications," *Bioresource Technology*, vol. 413, p. 131489, Dec. 2024, doi: 10.1016/J.BIORTECH.2024.131489.

[49] G. Knothe, "Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters," *Fuel Processing Technology*, vol. 86, no. 10, pp. 1059–1070, Jun. 2005, doi: 10.1016/J.FUPROC.2004.11.002.

[50] K. Katam, M. P. Gundupalli, and D. Bhattacharyya, "Production of biofuel from kitchen wastewater by using a mixed culture of diatoms: Treatment, kinetic evaluation, and lipid analysis," in ASCE India Conference 2017, Dec. 2018, pp. 278–287, doi: 10.1061/9780784482025.029.

[51] G. A. Dunstan, J. K. Volkman, S. M. Barrett, J. M. Leroi, and S. W. Jeffrey, "Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae)," *Phytochemistry*, vol. 35, no. 1, pp. 155–161, Dec. 1993, doi: 10.1016/S0031-9422(00)90525-9.

[52] J. B. Parsons and C. O. Rock, "Bacterial lipids: Metabolism and membrane homeostasis," *Progress in Lipid Research*, vol. 52, no. 3. pp. 249–276, Jul. 2013. doi: 10.1016/j.plipres.2013.02.002.

[53] W. N. A. W. Osman, M. H. Rosli, W. N. A. Mazli, and S. Samsuri, "Comparative review of biodiesel production and purification," *Carbon Capture Science and Technology*, vol. 13, p. 100264, Dec. 2024, doi: 10.1016/j.ccst.2024.100264.

[54] M. P. Devi, G. V. Subhash, and S. V. Mohan, "Heterotrophic cultivation of mixed microalgae for lipid accumulation and wastewater treatment during sequential growth and starvation phases: Effect of nutrient supplementation," *Renewable Energy*, vol. 43, pp. 276–283, 2012, doi: 10.1016/j.renene.2011.11.021.

[55] A. Saxena and A. Tiwari, "Biodiesel Production and Advancement from Diatom Algae," in *Bioenergy Research: Evaluating Strategies for Commercialization and Sustainability*, New Jersey: John Wiley & Sons, Ltd., 2021, pp. 261–277. doi: 10.1002/9781119772125.ch12.

[56] K. Katam and D. Bhattacharyya, "Effect of solids retention time on the performance of alga-activated sludge association in municipal wastewater treatment and biofuel production," *Journal of Applied Phycology*, vol. 32, no. 3, pp. 1803–1812, Mar. 2020, doi: 10.1007/s10811-020-02076-6.

[57] D. De Francisci, Y. Su, A. Iital, and I. Angelidaki, "Evaluation of microalgae production coupled with wastewater treatment," *Environmental Technology (United Kingdom)*,

vol. 39, no. 5, pp. 581–592, Mar. 2018, doi: 10.1080/09593330.2017.1308441;SUBPAGE:STRING:ACCESS.

[58] F. Javed, F. Rehman, A. U. Khan, T. Fazal, A. Hafeez, and N. Rashid, "Real textile industrial wastewater treatment and biodiesel production using microalgae," *Biomass and Bioenergy*, vol. 165, p. 106559, Oct. 2022, doi: 10.1016/j.biombioe.2022.106559.

[59] R. Rehman et al., "Algal-biochar and Chlorella vulgaris microalgae: A sustainable approach for textile wastewater treatment and biodiesel production," *Biochar*, vol. 6, no. 1, pp. 1–16, Dec. 2024, doi: 10.1007/s42773-024-00358-7.

[60] S. Cho et al., "Microalgae cultivation for bioenergy production using wastewaters from a municipal WWTP as nutritional sources," *Bioresource Technology*, vol. 131, pp. 515–520, Mar. 2013, doi: 10.1016/j.biortech.2012.12.176.

[61] I. Ambat, S. Bec, E. Peltomaa, V. Srivastava, A. Ojala, and M. Sillanpää, "A synergic approach for nutrient recovery and biodiesel production by the cultivation of microalga species in the fertilizer plant wastewater," *Scientific Reports*, vol. 9, no. 1, p. 19073, Dec. 2019, doi: 10.1038/s41598-019-55748-w.

[62] L. Luo et al., "Simultaneous nutrition removal and high-efficiency biomass and lipid accumulation by microalgae using anaerobic digested effluent from cattle manure combined with municipal wastewater," *Biotechnology for Biofuels*, vol. 12, no. 1, p. 218, Sep. 2019, doi: 10.1186/S13068-019-1553-1.