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Potential for Corroborative Microalgal Energy Storage from Brewery Wastewater

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Industrial wastewater contains some organic and inorganic pollutants that are detrimental to the ecosystem but good for the aquatic environment's energy economy. This was observed in the carbon footprint of this study's microalgal growth paradigm change. The brewing industrial wastewater from Durban, South Africa, was characterized using contemporary techniques for analyte quantification. The brewery wastewater was treated using the microalga *Scenedesmus sp.*, and the physicochemical parameters were monitored and determined using the reference beam Hatch DR 3900 Vis spectrophotometer 7TFT WVGA version 1.01 and the multipurpose metre Aqua Lytic AL 15, while BOD_5 was measured using the manometric BOD bottle method. TS, TDS, and TSS were measured using gravimetric techniques. On a dry basis, the average biomass productivity of the microalga *Scenedesmus sp.* culture broth was 0.457±0.167 $gL^{-1}d^{-1}$, and the optical density increased from 0.014 to 0.680. This growth response resulted in a 96.82 % decrease in COD, an 81.75 % decrease in BOD_5, and a 97.71 % decrease in TDS. Similar to this, TKN (76.22 %), NO_3-N (73.95 %), NO_2-N (54.05 %), NH_3-N (84.21 %), PO_4^{3-} (75.03 %), and Cl^- (91.67 %) all saw nutritional loss during the twenty-nine-day operation. The skewed growth response, which led to high biomass output, validated the synergistic nutrient scavenging and *Scenedesmus sp.* cell proliferation in corroborative energy storage.

1. Introduction

The manufacturing and process industries produce so much wastewater, introducing pollution factors in the receiving water bodies. Industrial wastewater from food and agro-based processing, electroplating, metal finishing, munition production, mineral oil refineries, boiler blowdown, paint shops, cooling towers, photo processing, laundries, hospitals, and others varies by industry and location. The composition of wastewater is 99.9 % water and 0.1 % dissolved and particulate matter (Pang et al*.,* 2019). This 0.1 % contains microorganisms, organic matter and inorganic compounds. The 0.1 % typically has 75 % suspended particles and 25 % soluble materials from which 40 % are dissolved organic components. Rezagama et al. (2017) averred that dissolved organic components comprise proteins (65 %), carbohydrates (25 %), lipids (9 %), and detergents (1 %). According to recent study trends (Razzak et al., 2017), industrial wastewater has been discovered as a possible tropic growth medium that can alter the carbon economy of the aquatic ecosystem and increase energy sustainability. *Scenedesmus sp.* was used in earlier experiments by Kukwa and Chetty (2022) to collect lipids while also demonstrating the microalga's ability to remove minerals from brewing industrial wastewater from Durban, South Africa. This work emphasizes *Scenedesmus sp.*'s potential feasibility to combine biomass synthesis and nutrient scavenging from industrial wastewater and produce energy storage.

2. Materials and methods

In this study, the laboratory sessions addressed sample preparation, brewery wastewater characterization using physical, chemical, and biological parameters, algal biomass generation, and an outline of the metrics for biomass output.

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2.1 Sample collection and preparation

Durban, South African brewery effluent was collected in UV-sterilized 25-litre plastic jars and sent to the lab within an hour of collection. At both the collection site and the laboratory, the Aqua Lytic AL 15 Germany multipurpose metre assessed temperature, pH, EC, and DO. In the lab, 2 litres were removed for biochemical oxygen demand (BOD) study, and the remaining volume was filtered through fibreglass, autoclaved at 121 ºC and 103.421 kPa of pressure for 30 min.

2.2 Brewery wastewater characterization

The study used a reference beam Vis spectrophotometer (DR 3900 7TFT WVGA version 1.01, Germany) to monitor nutrient depletion in culture broth. The TNTplus 880 s-TKN method measured total nitrogen, total Kjeldhal nitrogen, nitrate-nitrogen, and nitrite-nitrogen. The salicylate TNTplus 830 method measured ammonia-nitrogen. PhosVer 3 methods measured reactive phosphorus, and manometric BOD bottles measured BOD and COD. The Vis spectrophotometer measured wastewater and microalgal culture broth colours in platinum-cobalt (Pt-Co).

2.3 *Scenedesmus sp.* biomass production

The microalga *Scenedesmus sp.* was cultivated in the brewing effluent using a 3-L bubble-column reactor with a working volume of 2.5 L. The broth was sparged with 2 L/min of carbon dioxide gas as the OD increased, and microalgal biomass was monitored at 680 nm.

2.3.1 Biomass production metrics

The production of microalgal biomass in this study was determined by measuring the dry mass in a batch mode operation. The specific growth rate, denoted as μ, was calculated using Eq(1) as follows:

$$\mu = \frac{ln\left[{}^{m_f}/{m_i}\right]}{\Delta t} \tag{1}$$

The variable Δt denotes the temporal interval between the initial and final states during the exponential phase of cell reproduction, measured in units of days. The dry mass concentrations at the final and initial stages of this phase, denoted as m_f and m_i respectively, are measured in gL^{-1}. Eq(2) was employed to evaluate the productivity of algal biomass based on its dry mass. The variable t, denoted in terms of a day (d), represents the duration of the batch operation period.

$$\mathrm{P} = \frac{[m_{f-}\,m_i]}{t}\ (\mathrm{gL^{-1}d^{-1}}) \tag{2}$$

Algal biomass productivity P is related directly to the rate of CO_2, $R_{(CO_2)}$ fixation per unit volume of algal culture broth (Eq 3). Where the proportionality constant, α, in Eq(3), specifies the mass of CO_2 gas, which is trapped,

$$\mathrm{R}_{CO_2} = \alpha\mathrm{P}\ (\mathrm{gCO_2L^{-1}d^{-1}}) \tag{3}$$

and fixed on the backbone of every unit mass of microalgal biomass, taking into account that the dry biomass has a carbon content of 50 %.

$$\alpha = 0.5\left(\frac{44}{12}\right) = 1.833 \tag{4}$$

In Eq(4), 12 is the atomic mass of carbon and 44 is the molar mass of CO_2. Eq(5) was used to calculate the efficiency, η, of CO_2 gas sequestration. where q is measured in grams of CO_2 per litre of microalgal cultivation broth per day and indicates the rate of CO_2 gas bubbling through each litre of microalgal cultivation broth.

$$\eta = \frac{\alpha P}{q} * 100\ \% = 1.833\ \frac{P}{q} * 100\ \% \tag{5}$$

3. Results and discussion

Tables 1, 2, and 3 display the findings of the analyses in this study. Some of the analyses are shown in Figure 1. The physical, chemical, and biological properties of *Scenedesmus sp.,* as well as its ability to sequester carbon dioxide and store energy, are discussed in this research. Particular growth rates, levels of productivity, and CO_2 sequestration effectiveness were highlighted.

The physical characteristics of brewery wastewater are displayed in Table 1 as investigated in this study.

Table 1: Physical characteristics of brewery wastewater

Parameter	Brewery wastewater		Discharge standard	
	Before Treatment	After Treatment	*EPA, 2014	**DEA, 2014
Temperature (°C)	22.50±0.50	25.60±1.20	20 - 35	25 - 35
EC at 25 °C (µS/cm)	85.25±5.83	1.31±0.42	250 mS/m	250 mS/m
TDS (mg/L)	7056.71±12.61	161.55±0.47	≤1200	
TSS (mg/L)	585.84±6.41	56.75±0.25	≤30	≤90
TS (mg/L)	7642.50±4.20	29.31±0.51		
Turbidity (NTU)	319.62±54.14	73.28±3.82	300	
Colour (PtCo)	167.00±5.20	61.00±2.60		

*EPA – the United States Environmental Protection Agency; **DEA – South Africa Department of Environmental Affairs.

3.1.1 Temperature of brewery wastewater

Since temperature has an impact on the water's chemistry, it is a significant factor. At higher temperatures, chemical and biological processes proceed more swiftly. Temperature influences a water body's residents as well as biological activity and growth. Chemical equilibrium and industrial wastewater treatment system's effectiveness are both impacted by temperature. This hinders biological processes to the point where only cryophilic species can survive during cryogenic preservation at freezing temperatures. While certain microbes are thermophilic and grow best above 45 °C, the majority of them are mesophilic and thrive between 15 and 30 °C. Higher groundwater temperatures dissolve more minerals from rocks, improving electrical conductivity. *Scenedesmus sp.* flourished in the mesophilic range of 22.5-25.6 °C (Table 1) because of fluctuating weather conditions observed in this investigation. According to Mohan et al. (2015), microalgae experience changes in membrane lipid content as temperatures rise over 25 °C.

3.1.2 Colour of brewery wastewater

Minerals in water produce a colour shift. Brewery wastewater, however, turns dark due to putrefaction. Brown is commonly associated with the presence of iron, but black can be attributed to the presence of either manganese or biodegraded organic matter. Industrial effluent gets its "true colour" from metal ions such as chromium, platinum, iron, manganese, conjugated compounds, and dyes. However, wastewater has an "apparent colour" due to suspended particles. The evaluation of colour in wastewater is mostly based on the visual observation of its hue. Microalgae's scavenging actions remove colour from wastewater. Table 1 demonstrates a significant decrease in colour intensity, namely from 167 to 61 PtCo, corresponding to a colour loss of approximately 63.5 %, seen in the brewery effluent during the microalgal growth process.

3.1.3 Solids in brewery wastewater

Two types of total solids (TS) are identified in wastewater: suspended (non-filterable) solids (SS) and total dissolved solids (TDS). The investigated brewery wastewater had SS weighing 585.84±6.41 mg/L before treatment, and 56.75±0.25 mg/L after treatment (Table 1). Two potential mechanisms could explain this decrease, namely disintegration (or aggregation) followed by sedimentation. High SS may decrease water's natural dissolved oxygen levels and increase water temperature, which may induce the migration of aquatic life, such as fish. Before treatment with microalgae, TDS in the brewery wastewater was 7056.71±12.61 mg/L and 161.55±0.47 mg/L after treatment. This showed the efficiency of the microalga *Scenedesmus sp.* as a biological TDS removal remedy for brewery effluent, leading to a 97.71 % drop in TDS. *Scenedesmus sp*. has the potential to remove as much as 99.62 % of TS from brewery wastewater resulting in an average biomass production of 1.275±0.505 gL^{-1}. The initial turbidity of 319.62±54.14 NTU decreased by 77.07 % to 73.28±3.82 NTU.

3.1.4 Electrical Conductivity (EC) of brewery wastewater

Solution electrical flow capacity is measured by EC. Electricity flowing through a solution reveals ions, the only electrical current carriers. EC monitors wastewater ion concentrations to measure NO_3^- and PO_4^{3-} ion mobility as microalgae cells grow. EC values consider corrosion and wastewater mineralization. Water samples with dissolved solids had higher ECs. High temperature increases ion mobility and decreases viscosity, raising EC. The study found that brewery wastewater's EC was 85.251±5.826 µS/cm before treatment and 1.314±0.421 after treatment; removing 98.56 % of the EC from the brewery wastewater. This shows that *Scenedesmus sp.* could potentially remove ions from solution to register biomass productivity of 0.079 $gL^{-1}d^{-1}$.

3.2 Chemical characteristics of brewery wastewater

The chemical characteristics that are monitored frequently in water and wastewater samples include COD, pH, Cl^-, TKN, NO_3^- + NO_2^-, NH_3, PO_4^{3-}, S^{2-}, SO_4^{2-} and DO. Table 2 displays those chemical characteristics monitored in this study.

Table 2: Chemical characteristics of brewery wastewater

Parameter	Brewery wastewater		Discharge standard	
	Before treatment	After treatment	EPA, 2014	DEA, 2014
pH	5.02±0.06	6.14±0.03	6.0 - 9.0	5.5 - 7.5
Alkalinity (mg/L)	105.10±0.20	23.76±2.34		
*COD (mg/L)	5855.0±15.2	186.00±1.2	100	75
**TKN (mg/L)	8.62±1.03	2.05±0.08	10	
Nitrate Nitrogen, NO_3^- - N (mg/L)	4.30±0.23	1.12±0.09	50.0	1.5
Nitrite Nitrogen, NO_2^- - N (mg/L)	0.37±0.02	0.17±0.01	2.0	
NH_3 – N (mg/L)	0.382±0.021	0.064±0.401		1.0
Ortho-phosphate, PO_4^{3-} (mg/L)	23.71±1.07	5.92±1.31	5.0	1.0
Dissolved oxygen (mg/L)	76.30±2.30	8.7±0.1	75 % sat.	75 % sat.
Sulphate, SO_4^{2-} (mg/L)	19.23±2.18	6.80±0.24	500	

*Chemical oxygen demand; **Total Kjeldhal Nitrogen

3.2.1 The pH of brewery wastewater

For a neutral solution at 25 °C, the pH is expressed as in Eq(6).

$$\mathrm{pH} = -\log_{10} C_{H_3O^+} = 6.998 \approx 7 \tag{6}$$

H_3O^+ and OH^- ions increase as water molecules dissociate at higher temperatures. K_w rises and pH falls. At 37 °C, empirical data (Kula et al., 2017) showed that K_w = 2.7 x 10^{-14} mol^2L^{-2}, which gives a neutral pH of 6.784. At 40°C, K_w = 2.916 x 10^{-14} mol^2L^{-2} with a neutral pH of 6.767. The temperature has an impact on the pH scale's neutral point, although this does not necessarily mean the solution is acidic or alkaline. As temperature rises, the neutral solution pH decreases to 6.145 at 100 °C. Lower temperatures reduce K_w because fewer water molecules dissociate. Temperatures below 25 °C require a pH greater than 7. At 20 °C pH = 7.083, neutral point, and K_w = 0.681*10^{-14} $mmol^2L^{-2}$. Water contamination causes a hydroxonium-hydroxide imbalance. The microbial breakdown of organic compounds increases or decreases wastewater acidity. In this study, biomass formation increased the optical density and raised the pH value, which was regulated by CO_2 gas sparging.

3.2.2 Alkalinity of brewery wastewater

Anions such as CO_3^{2-}, HCO_3^-, OH^-, PO_4^{3-}, SiO_4^{4-}, and BO_3^{3-} contribute to freshwater alkalinity. Thus, ionic species determine the alkalinity type in water. Raw materials and industrial operations determine species dominance, leading to carbonate-phosphate, phosphate-hydroxide, or carbonate-hydroxide alkalinity. Laboratory protocols measure either carbonate or total alkalinity. Thermal pollution from industrial wastewater discharge reduces pH, boosts the carbonate-to-bicarbonate ratio, and increases H^+ ion concentration. In this study, 77.4 % alkalinity was removed, revealing the potential of *Scenedesmus sp.* in this area.

3.2.3 Chemical oxygen demand (COD) of brewery wastewater

COD is the oxygen required for the reactive oxidation of organic compounds with strong chemical oxidants, and it is used to quantify pollution in residential as well as industrial wastewater. Since COD doesn't consider biodegradability, it only measures organic matter in freshwater and wastewater sources indirectly. In this investigation, *Scenedesmus sp*. treatment of brewery effluent reduced COD by more than 96 % signifying the efficacy of the process to produce biomass.

3.2.4 Nitrogen content of brewery wastewater

Wastewater chemistry concerns ammonia-nitrogen ($\mathrm{NH_3}$-N), nitrate-nitrogen ($\mathrm{NO_3}$-N), nitrite-nitrogen ($\mathrm{NO_2}$-N), and total Kjeldahl nitrogen (TKN). TKN includes organic and NH_3 nitrogen. $\mathrm{NH_3}$-N concentrations as low as 0.5 mg/L can kill freshwater fish, while concentrations as high as 1,600 mg/L can harm microalgae (Pang *et al*., 2019). Little $\mathrm{NO_2}$-nitrogen in treated wastewater streams, and $\mathrm{NH_3}$ nitrification bio-agents swiftly convert unstable $\mathrm{NO_2^-}$ to $\mathrm{NO_3^-}$. Wastewater treatment bio-organisms need $\mathrm{NH_3}$, $\mathrm{NO_2^-}$, $\mathrm{NO_3^-}$, and organic-N, which are regulated and require treatment. Anaerobic environments eliminate obligatory aerobes like nitrifying bacteria. Nitrification is most efficient at around 3 mg DO/L and requires 4.6 kg of oxygen to convert $\mathrm{NH_4^+}$ ions to $\mathrm{NO_3^-}$.

DO should be 1.5 mg/L for nitrification. From Table 2, *Scenedesmus sp.* sequestered $\mathrm{NH_3}$-N (83 %), $\mathrm{NO_3}$-N (74 %), $\mathrm{NO_2}$-N (54 %) and TKN (76 %) to produce a significant amount of biomass.

3.2.5 Phosphorus as Orthophosphate ions (PO_4^{3-} -P) in brewery wastewater

Phosphorus found in industrial waste streams is from the automobile industry as corrosion inhibitors, the detergent industry as builders, the process industry as process chemical reagents, and sanitary wastes as reactive orthophosphate. Depending on upstream industrial activity, waste streams may contain polyphosphates, pyrophosphates, and metaphosphates. $PO_4{}^{3-}$-P is a vital ingredient in biochemical activities that make phospholipids and other bioproducts. Typically, biological wastewater treatment systems require a 100:1 BOD:P ratio (Razzak et al., 2017). This study showed an initial BOD:P ratio of 123:1, which dropped 27 % to 90:1 at microalgal biomass harvest. *Scenedesmus sp.* in brewery wastewater sequestered 75 % of reactive phosphorus.

3.3 Biological characteristics of brewery wastewater

Bacteria in wastewater, some of which are pathogenic, reduce maintenance costs and enhance wastewater treatment. Table 3 shows this study's biological properties.

Table 3: Biological characteristics of brewery wastewater

Parameter	Brewery wastewater		Discharge standard	
	Before treatment	After treatment	EPA, 2014	DEA, 2014
BOD_5 (mg/L)	2953.3±55.42	539.07±32.05	30	

3.3.1 Biochemical oxygen demand (BOD) of brewery wastewater

BOD measures dissolved oxygen aerobic microbes need to metabolise organic materials. Biological oxidation slowly converts organic pollution substrates into carbon dioxide and water using dissolved oxygen. Dissolved oxygen is being lost due to carbonaceous BOD, as shown in Eq(7). Nitrogenous BOD considers both organic and inorganic nitrogen sources for oxidation and reduction. Microalgae can use both cBOD and nBOD to form biomass. Aerobic biological organisms (ABO) degrade water sample organic load at a certain temperature and time. ABO rapidly deoxygenate high-BOD water. High temperatures speed up biological oxidation, while soluble cBOD inhibits nitrification. They can infiltrate nitrifying bacteria and deactivate enzyme systems.

$$\text{Organic matter} + \mathrm{O_2} \xrightarrow{\textit{Aerobic bacteria}} \mathrm{CO_2} + \mathrm{H_2O} + \mathrm{NH_3} + \text{new cells} \tag{7}$$

Organotrophs must degrade this form of cBOD for nitrifying bacteria to oxidise ammonium and nitrite ions.

3.4 Energy storage capacity of *Scenedesmus sp.*

By absorbing visible light from the electromagnetic spectrum, a microalgal biomass suspension may measure its optical density (OD), which represents its growth. *Scenedesmus sp.* responded to brewery wastewater nutrient sequestration and spent the first 10 days in brewery effluent in the lag phase, acclimating to the new habitat. This protracted induction period indicates that the seed culture was virgin. The exponential growth and nutrient availability supported the second phase when cell density rose logarithmically as in Eqs(8) and (9).

$$C = C_0 * e^{\mu t} \tag{8}$$

$$lnC = lnC_0 + \mu t \tag{9}$$

Where C and C_0 represent cell concentrations at periods t and 0, and μ is the specific growth rate. Microalgal strain, temperature, and light intensity determine μ. After development slowed further in the third phase, medium depleted and cellular processes like replication, nutrient sequestration, and COD and BOD removal reduced. Thus, microalgal development phase two removes COD and BOD better. Microalgae die when the culture broth's optical density drops below light penetration. As microalgal cells in suspension multiplied, effluent colour changed from dark brown to light brown (Table 1). As a result, we may conclude that *Scenedesmus sp.* was effective in removing the taint from the brewery's wastewater. Figure 1 illustrates *Scenedesmus sp.*'s average biomass production, productivity and specific growth rate throughout cultivation. The dry *Scenedesmus sp.* biomass production was, on average, 1.275±0.505 gL^{-1} with productivity of 0.079 $gL^{-1}d^{-1}$ and the specific growth rate, μ, of 0.116 d^{-1}.

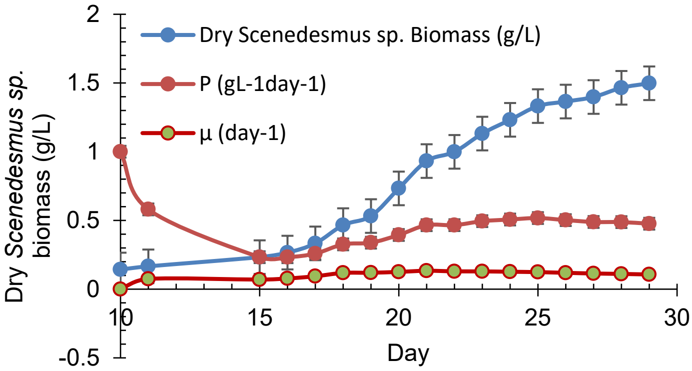

Figure 1: Exponential growth of Scenedesmus sp. showing dry biomass, biomass productivity (P) and specific growth rate (µ)

During this twenty-nine-day investigation (Figure 1), there was a notable attenuation in the growth of microalgae, accompanied by a decrease in productivity; however, the reduction eventually reached a state of equilibrium. The performance of the system was contingent upon the prevailing conditions, resulting in an effective rate of CO_2 gas sequestration of 0.144 $gL^{-1}d^{-1}$, which corresponds to a removal efficiency of 7.2 %. Biomass is a biological renewable energy resource that can be used as a long-term substitute for fossil fuels due to its high energy density. Direct combustion produces heat, thermochemical conversion yields solid, gaseous, and liquid fuel, and chemical conversion yields liquid fuel from the stored energy.

4. Conclusions

Scenedesmus sp. showed a high capacity to efficiently remove several pollutants, including nutrients, from brewery effluent, with removal rates ranging from 54 % to 99.62 %. The mean biomass output, measured in terms of dry mass, was found to be 1.275±0.505 gL^{-1}. The productivity of the biomass was determined to be 0.079 $gL^{-1}d^{-1}$, while the specific growth rate was calculated to be 0.116 d^{-1}. The growth indices observed indicate the potential of utilizing wastewater from the brewing sector as a viable source for efficient energy storage in *Scenedesmus sp.*

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