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Biomass Production and Simultaneous Minerals Sequestration from Brewery Wastewater with Concomitant Lipid Accumulation using Algae

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Some industrial wastewaters contain high levels of non-biodegradable organic and inorganic matter, and in-house physical and chemical treatment produces secondary pollutants that are released into the receiving environment and can harm the ecosystem. Over the years, conventional biological treatment of these industrial effluents has proven ineffective. Advanced biological aerobic and anaerobic methods, in which biomass is nurtured for growth and biochemical manipulations are used to present a variety of value-added products with potential applications, have been adopted. As CO_2 gas is sparged through the broth, the microalga Scenedesmus sp. accumulates lipid as it mops up minerals and nutrients from brewery wastewater to produce biomass. Standard methods were used to determine the mineral content of brewery wastewater from Durban, KwaZulu-Natal. Scenedesmus sp. was then grown in wastewater using bubble-column photobioreactors, with the broth being CO_2-sparged at regular intervals. The optical density reading on the DR 3900 spectrophotometer was used to track biomass production, and lipid accumulation was measured using the chloroform-methanol solvent system. The findings revealed that the minerals in the effluent brewery wastewater contained high levels of cadmium, exceeding the WHO limit of 0.03 mg/L. Zinc (1.578 mg/L) and nickel (0.053 mg/L) concentrations were both within WHO limits of 5.0 mg/L and 1.0 mg/L, respectively. All of the minerals in the effluent were significantly reduced after treatment with Scenedesmus sp. As a result, during the exponential growth period, biomass production increased in tandem with proportional lipid accumulation.

1. Introduction

Under phototrophic conditions, microalgae can use both soluble organic compounds and carbon dioxide as carbon sources for photosynthetic engagements. Microalgae can remove dissolved organic matter (DOM) and other nutrients such as nitrogen (N) and phosphorus (P) from municipal and industrial wastewaters when grown in a mixotrophic environment. Additionally, Nielsen (2015) asserted that the mixotrophic growth of microalgae in industrial wastewater mixtures effectively removed more than 99 per cent of ammonium-nitrogen (NH_4^+-N), more than 99 per cent of nitrate-nitrogen (NO_3—N), more than 84 per cent of sulphates, and up to 98 per cent of total phosphorus (TP); and the growth of microalgae in industrial wastewater mixtures, with dissolved organic carbon (DOC) concentration. CO_2, an inorganic carbon source, is highly soluble in water, accounting for over 99 per cent as dissolved gas and less than 1% as carbonic acid H_2CO_3, which partially dissociates to form H^+, HCO_3^-, and CO_3^{2-} ions (Yew et al. 2019).
Process wastewater (PWW), sanitary wastewater (SWW) from toilets and kitchens, and rainwater runoff make up the brewery's wastewater. The amount of yeast present and the raw materials used in the process determine the levels of nitrogen and phosphorus in brewery wastewater (Armah et al., 2020). Cleaning solutions such as nitric acid may contribute to the total nitrogen content, which is derived from malt and adjuncts. The amount of yeast discharged, the cleaning agents used, and the amount of water flowing down the drain will all affect concentration. Cleaning agents can also be used to obtain phosphorus. Several lipid-producing microalgae were isolated and identified after evaluating their ability to grow and accumulate lipids (Zhang and Hong 2014). They make a wide range of lipid compounds that are both highly valuable and commercially viable.

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Copper, iron, manganese, nickel, and zinc are essential micronutrients involved in a variety of biological processes as precursors of vitamins, catalytic cofactors for numerous metalloenzymes, and membrane structural proteins. Microalgae have been demonstrated to be efficient and effective at mineral removal and to be able to tolerate high concentrations of these minerals via a variety of mechanisms, including metal-protein coupling. Toxic metal accumulation can result in the production of reactive oxygen species (ROS), inhibition of chlorophyll synthesis, and negative disruption of cell proliferation, affecting lipid accumulation within the microalgae cell. Numerous studies have demonstrated that heavy metal stress increases the lipid content of certain microalgae. Patel et al. (2019) examined the effects of Fe(III), Mg(II), and Ca (II) ions on lipid accumulation in Scenedesmus sp. cells and discovered that when biodegradable organic matter was added during cultivation, total lipid content and lipid accumulation increased by 28.2 and 29.7 per cent, respectively. Ammary (2004) examined the effect of Mn (II) and Co (II) ions on the lipid content of C. vulgaris in an earlier study and discovered a significant increase in lipid content. This research examines the removal of mineral pollutants from brewery wastewater by microalgae and the lipid accumulation in the cellular matrix.

2. Materials and methods

The laboratory protocol in this study covered four stages, including (i) wastewater characterization and mineral analysis before and after algal cultivation (ii) microalgae cultivation and harvesting (iii) microalgal biomass characterization, and (iv) microalgal lipid extraction and quantification.

2.1 Brewery wastewater characterization and mineral analysis

Wastewater samples were collected from the brewery industry in Durban, South Africa and processed as described by Khan *et al*. (2017) to remove the suspended solids. The pretreated brewery wastewater was analysed for temperature, pH, electrical conductivity (EC), dissolved oxygen (DO) and total dissolved solids (TDS) using standard methods adopted from the American Public Health Association (APHA, 1999). Chemical oxygen demand (COD), total nitrogen (TN), total Kjeldahl nitrogen (TKN), nitrate-nitrogen (NO_3^--N), nitrite-nitrogen (NO_2^--N), ammonia nitrogen (NH_3-N), and phosphate (PO_4^{3-}) were analysed using reagent vials, which allowed determination using the water lab (DR/3900 HACH, USA).

2.2 Microalgae cultivation and harvesting

The microalga *Scenedesmus sp*. was cultivated in brewery wastewater and the broth was bubbled through with CO_2 gas at the rate of 2 L/min in a 3-L bubble column reactor with a working volume of 2.5 L. Biomass production was monitored as the optical density (OD) of the broth at 680 nm changed with time. About 50 mL portion of the microalgal broth was collected at the end of the exponential growth period to determine the biomass concentration and nutrient consumption as shown in Eq(1) – (5). The algal cells were harvested by centrifugation at 1000g (HITACHICR22G, Japan) for 10 min.

$$\text{Dry mass }(\text{DM}, \tfrac{\text{g}}{L}) = 0.3834 \text{ x } \text{OD}_{680} - 0.0122 \quad (1)$$

$$\text{Biomass concentration }\left(\frac{\text{mg}}{L}\right) = \text{DM x } 20 \quad (2)$$

$$\text{Nitrate consumed }\left(\frac{\text{mg}}{\text{L}}\right) = \text{N}_i - \text{N}_f \quad (3)$$

$$\text{Phosphate consumed }\left(\frac{\text{mg}}{\text{L}}\right) = \text{P}_i - \text{P}_f \quad (4)$$

$$\text{DOC consumed }\left(\frac{\text{mg}}{L}\right) = (\text{DOC})_i - (\text{DOC})_f \quad (5)$$

where DM is the dry biomass weight from the 50 mL of broth taken on the day of harvest, N_i and N_f represent the nitrate concentration in the broth at the beginning and last day of the microalgal growth period, P_i and P_f represent the phosphate concentration in the broth at the beginning and last day of the growth period; and $(\text{DOC})_i$ and $(\text{DOC})_f$ represent the dissolved organic carbon concentration in the broth sampled on initial and final days, respectively. The harvested microalgae samples were dried in a vacuum drying oven at 40 °C for 1.0 h and ground to a powder for analysis.

2.3 Microalgal biomass characterization

Using a scanning electron microscope and energy dispersive crystallography (SEM-EDX), a basic analysis of C and N in dry biomass was carried out in duplicate. The nitrogen content was used to calculate the crude protein content. The protein content of Scenedesmus sp. was calculated using a nitrogen-to-protein (NTP) conversion factor of 6.25. (Wong & Cheung, 2000). The carbohydrate content of the lyophilised and ground algal biomass was determined using a modified two-step acid hydrolysis procedure developed at the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2004). (2012).

2.4 Lipid content of *Scenedesmus sp.*

Gwak et al. described a slightly modified method for lipid extraction, which we used (2014). After centrifugation at 3000g for 3 minutes, the biomass pellet was collected from suspension and lyophilized using the Scanvac Coolsafe freeze dryer (Gene company Ltd., Hong Kong). The dried biomass pellet was ground and vigorously agitated in a solvent system of chloroform:methanol:water (2:2:1, v/v/v). To separate the extract into two phases, centrifugation at 3000g for 5 minutes was used. A new vial was filled with the lipid-rich lower organic phase. To obtain and quantify gravimetrically the total algal lipid content, chloroform in the organic phase was removed using a nitrogen evaporator (DC-12, ANPEL, China).

3. Results and discussion

Membrane filtration at the microfiltration (MF) level was used to pre-treat brewery wastewater. This was done on purpose to get rid of the majority of COD, BOD, and TSS, which can stifle algal growth. However, fouling and energy consumption remain a challenge for this technique; however, new types of anti-fouling membranes are gaining attention in the research world, and if successful, the membrane process will become a viable and preferred treatment option (Tobias, 2016). Figures 1 and 2 as well as Tables 1, 2 and 3 show the experimental data from this study. Figure 1 depicts the depletion of nutrients during the mixotrophic cultivation of Scenedesmus sp. The most commonly used nutrient was ammonium-nitrogen, followed by nitrate-nitrogen, and only a small amount of phosphate was used. The mixotrophic productivity of Scenedesmus sp. is shown in Figure 2. The plot clearly shows that different initial nutrient substrate concentration provides different growth stimulus for the alga Scenedesmus sp. to thrive. It can then be seen that algal growth would be sustained if nutrients were always available.

3.1 pH

The biochemical reaction characteristics of microalgae are normally influenced by the pH of the culture medium. The most favourable pH range for microalgae growth is between pH 7 and 9.5, and the optimal pH is between 8.2 and 8.7, which varies depending on the strain. The ideal pH range for heterotrophic cultures is between 6 and 7. Bicarbonates, which are used by microalgae via CO_2 concentrating mechanisms (CCMs), are commonly used to control pH. The pH range for this operation was between 6.65 and 8.15 as Table 1 depicts.

Table 1: Physicochemical characterization of brewery wastewater

Property	Influent	Effluent	WHO
Temperature (^oC)	25.5	23.5	
pH	6.54	8.15	8.5
COD (mg/L)	3448	2017	80
EC (µ*S*/cm)	4.864	2.261	1
TN (mg/L)	38.701	17.362	
NH_3-N (mg/L)	0.612	0.257	
NO_3^- + NO_2^- (mg/L)	2.693	2.071	
TKN (mg/L)	19.001	15.903	
PO_4^{3-} (mg/L)	44.804	23.041	
DOC (mg/L)	910	204	

Table 2: Scenedesmus sp biomass profiling

Property	Profile before treatment	Profile after treatment
Carbohydrate (%)	24	33
Protein (%)	52	35
Lipid (%)	18	28
Ash (%)	6	4

3.2 Temperature

The rate of algal growth and the solubility of most chemicals both increase as the temperature rises. Elevated temperatures, on the other hand, can reduce oxygen solubility, which harms fish and other oxygen-dependent organisms. Temperature changes can have a big impact on microalgal metabolic processes and enzyme activity (Cheng et al., 2019). Many microalgae species thrive best at temperatures between 25 and 30 degrees Celsius. Some microalgae species experience negative growth rates at temperatures above 35 °C. Duygu (2019) investigated the effect of temperature on the productivity of S. obliquus in a mineral medium and found that the highest biomass growth of 1.84 mg/L h was obtained at 30 °C, while the lowest biomass production of 1.1 mg/L h was observed at 35 °C. the average temperature of this operation was 24.5 °C which falls within the favourable growth region.

Table 3: Mineral composition of brewery wastewater

Property	Influent	Effluent	WHO
Cr (mg/L)	23.5	3.42	0.05
Cd (mg/L)	0.142	0.051	0.03
Zn (mg/L)	5.671	1.578	5.00
Ni (mg/L)	1.234	0.053	1.00
Pb (mg/L)	0.254	0.052	0.03
As (mg/L)	0.018	0.002	

3.3 COD and dissolved Oxygen

COD is a metric for determining the total amount of organic and inorganic chemically oxidisable matter in a sample, and thus the feedstock's energy content. COD is generated by residual food and beverage waste from cans and bottles, as well as antifreeze and emulsified oils from industrial food processing and agricultural activities. Since more organic compounds can be oxidized chemically than biologically, the majority of CODs are water-soluble, and their values are typically greater than biochemical oxygen demand (BOD). Dissolved oxygen (DO) concentrations in surface water are proportional to temperature and atmospheric pressure. At one atmosphere of pressure, DO concentrations range from 14.6 mg/L at 0 degrees Celsius to 7 mg/L at 35 degrees Celsius. Because these concentrations decrease as atmospheric pressure increases, a source at a higher elevation with the same temperature will contain less oxygen than a source at a lower elevation (Goldman and Horne, 1994). Depletion of DO stresses aquatic organisms, resulting in an unfit environment for life.

3.4 Nitrogen

Nitrogen is an essential nutrient for the growth of all living things. 2014 (Nandeshwar) Organic nitrogen can be found in peptides, proteins, enzymes, chlorophylls, energy transfer molecules (ADP, ATP), and genetic materials (RNA, DNA), among other biological substances. Nitrate (NO_3^-), nitrite (NO_2^-), nitric acid (HNO_3), ammonium (NH_4^+), ammonia (NH_3), and nitrogen gas are all inorganic sources of organic nitrogen (N_2). Microalgae play a crucial role in the assimilation process, which converts inorganic nitrogen to its organic form. The production of biological components such as protein and chlorophyll requires nitrogen. Ammonia (NH_3 and nitrate (NO_3^-)), ammonium (NH_4^+), and nitrite (NO_2^-) are examples of inorganic nitrogen in surface water systems. Ammonia and ammonium are reduced forms of nitrogen that are commonly found in anaerobic conditions; at very low concentrations, the ammonia form is toxic to fish and other aquatic organisms. The oxidized forms of nitrogen are nitrite and nitrate. Figure 1 shows the uptake of nutrients and subsequent increase in biomass production.

3.5 Phosphorus

Phosphorus compounds can be found in soil, plant and animal tissues, and animal waste in nature. Phosphorus is found in water and tends to adsorb to soil particles. Phosphates (PO_4^{3-}) are non-toxic compounds that pose no threat to human health. They do, however, cause significant water quality issues because they are a limiting nutrient for many aquatic plants. Their presence usually leads to an overabundance of plants and algae, as well as an increased risk of eutrophication. At very low concentrations, phosphates can also interfere with coagulation processes in water treatment.

3.6 Mineral sequestration from wastewater

Heavy metals are known to be sequestered by microalgae (Rai *et al.,* 1981). The increased discharge of toxic pollutants into wastewater collection systems has resulted from the advancement of industrialization. Heavy metals and toxic organic compounds are present in high concentrations in municipal wastewater. As a result, the ability of wastewater treatment systems to tolerate and eliminate toxicity is critical. Heavy metals can be absorbed very effectively by microalgae. Metal bioaccumulation by algae could be a viable method for metal-contaminated wastewater remediation (Abdel-Raouf *et al*., 2012). Algae, on the other hand, has the advantage of being able to grow in ponds without a lot of nutritional input or upkeep. While heavy metal concentrations in some drainage systems are lower than in industrial effluents, particularly those from metal processing plants, the problems caused by their presence, especially in densely populated areas, are of public concern. Many marine and freshwater microalgae have been shown to selectively absorb and accumulate heavy metals from aqueous media (Afkar *et al.,* 2010). As shown in Table 3, the mineral composition of brewery wastewater decreased significantly, while the lipid content of Scenedesmus sp. biomass increased.

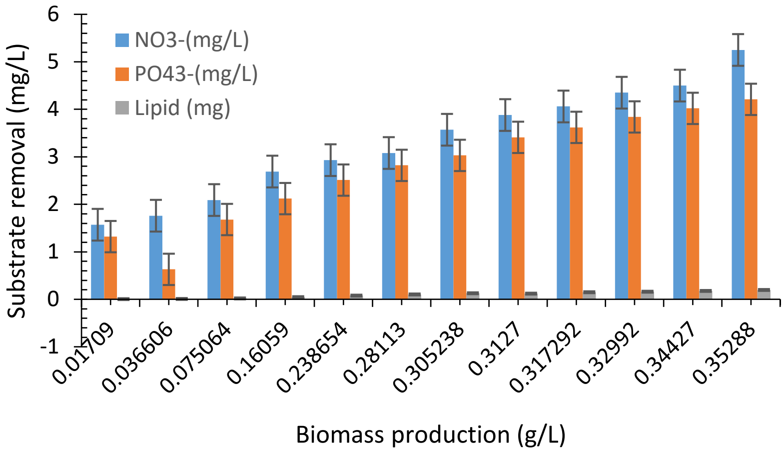

Figure 1: Nutrient removal trend during mixotrophic Scenedesmus sp., cultivation in brewery wastewater

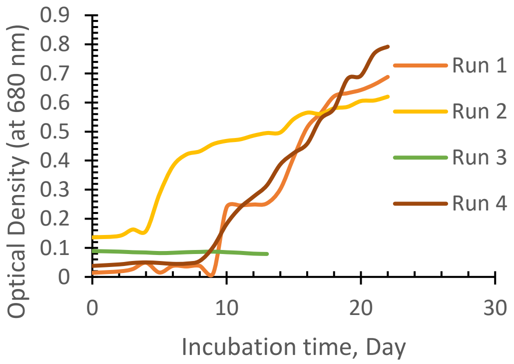

Figure 2: Mixotrophic productivity of Scenedesmus sp

3.7 Lipids in microalga *Scenedesmus sp.*

Microalgae produce two types of lipids: polar lipids, such as glycerophospholipids, which play a critical role in cell structure, and nonpolar lipids, such as triacylglycerols, which are primarily responsible for energy storage. These structural lipids typically contain long chains of fatty acids that can be converted to polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) (Sivaramakrishnan *et al*., 2020). Non-polar or storage lipids such as sterol esters, glycerides, hydrocarbons, and carotenoids are linked to other lipids and hydrophobic regions of proteins in microalgae via relatively weak non-covalent forces (Van der Waals or hydrophobic associations) via their hydrocarbon chains. Fats and oils, waxes, phospholipids, and steroids are the major types. Fats, also known as triacylglycerols or triglycerides, are a form of stored energy.Carbon availability in the growth medium is critical for microalgal lipid synthesis. Glucose is the most frequently used carbon source for heterotrophic microalgal cultures, as it results in the highest rates of growth and respiration of any substrate (Mohan *et al*. 2015). The biomass profile in Table 2 illustrates the accumulation of lipids following *Scenedesmus sp.* treatment of brewery wastewater.

4. Conclusions

The findings revealed that the minerals in the effluent brewery wastewater contained high levels of cadmium, exceeding the WHO limit of 0.03 mg/L. Zinc (1.578 mg/L) and nickel (0.053 mg/L) concentrations were both within WHO limits of 5.0 mg/L and 1.0 mg/L, respectively. All of the minerals in the effluent were significantly reduced after treatment with Scenedesmus sp. As a result, during the exponential growth period, biomass production increased in tandem with proportional lipid accumulation.

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