Wastewater Treatment by Microalgae through Biosorption

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Bacteria naturally present in wastewater contribute to nutrient removal. The analysis here are promising for the potential environmentally friendly application of *C. vulgaris* in the development of an integrated biorefinery in sugar beet processing plants for improved and cost-effective wastewater treatment. It could also be considered particularly important for a multifaceted approach to managing the environmental sustainability of wastewater bioremediation.

Keywords: wastewater treatment ; microalgae cultivation ; biosorption ; nutrient removal ; biorefinery concept

1. Introduction

The sugar industry, with global production of sugar exceeding 18 million tons annually, is one of the most important agro-based industries, in which sugar beet accounts for more than 20% of global sugar production [1].

As the European Union set the goal to reach carbon neutrality by 2050, the sugar industry has decreased its CO_2 emissions by 51% compared to 1990, but achieving climate neutrality still presents a real challenge for this sector. The generation of enormous amounts of pulp, the consumption of large quantities of lime (which are transformed into sludges), the production of vinasse, and high consumption of energy and water are the main sources of sustainability challenges and environmental management problems in traditional beet sugar processing [2].

Generally, the sugar beet industry is one of the top water users and wastewater producers, although water consumption depends on technological processes within the plant [3]. Even though modern wastewater treatment technologies provide more efficient water use, such as water reuse, regeneration, and recycling, in older factories, the consumption of 25–45 kg water per 100 kg beet and discharges of an even larger quantity of wastewater (including water contained in the beet processed) are still considered normal [4]. Processing wastewaters, if not properly managed, are a serious risk to human beings, the environment, and the recipient's aquatic life, as they contain a high concentration of organic compounds, especially soluble and insoluble polysaccharides, which presents an ideal environment for the proliferation of microbes [4].

Various physical, chemical, and biological methods for the treatment of sugar factory wastewater have been proposed aiming at the reduction of chemical oxygen demand (COD). Some advanced technologies involving anaerobic digestion are considered to be the preferred methods of wastewater treatment; however, these processes are unable to remove biological nitrogen and phosphorus, require frequent adjustments for alkalinity, and are yet to be feasible due to large land requirements, byproduct formation, and high operational costs [3].

Recently, concern has grown over the sustainability of conventional wastewater treatment systems in terms of economic feasibility and environmental impact given the fact that standards to improve water quality have become more stringent. This implies higher energy consumption and greenhouse gas emissions, aspects that have become key factors concerning the overall performance of wastewater treatment. It is estimated that annual CO_2 emissions from electricity consumed for wastewater treatment in Germany are 2.2 million tons, around 2.1 million tons in the United Kingdom, and approximately 11.5 million tons in the United States [5].

In recent years, the idea of integrating mixotrophic microalgae into wastewater treatment has received much attention given the fact that the use of microalgae in wastewater treatment is a cost-effective and feasible method for biofixation of CO_2 [6]. Apart from their ability to utilize organic and inorganic C, N, and P for their growth, the principal advantage of incorporating microalgae into wastewater treatment is the generation of O_2 through photosynthesis, necessary for heterotrophic bacteria to biodegrade carbonaceous materials [7]. It is already known that multiple factors such as light, pH, nitrogen-to-phosphorus ratio, temperature, and carbon source and bacteria concentration influence algal productivity, so it is difficult to compare the effect of algal culture in processing wastewater treatment [8][9][10][11]. In addition to removing pollutants, the cultivation of microalgae in conjunction with wastewater treatment can provide lipids that can be converted into biodiesel [8][12].

However, several practical and economic challenges still hinder the implementation of microalgae to treat wastewater on a large scale. In a number of papers, wastewater pretreatment, such as dilution, nutrient addition, and anaerobic digestion, was necessary before microalgal inoculation [8][9][10][11]. Among others, the challenge the researchers tried to address in this research is harvesting, which constitutes one of the main technoeconomic limitations of this technology [5].

2. Current Insights

Microalgae can effectively utilize nutrients from sugar plant wastewater and assist in bioremediation. Mixotrophic growth was chosen for microalgal cultivation, since it was previously stated as the most reliable and efficient in wastewater treatment, due to the fact that the mixotrophic type of cultivation overcomes the limitation of light requirement present during wastewater treatment, in contrast to the photoautotrophic nutrition mode [13]. During mixotrophic cultivation, microalgae could simultaneously use inorganic (for instance, CO_2) and organic compounds as carbon sources [14]. As a result, microalgae grown in a mixotrophic system synthesize compounds that are typical for both autotrophic (photosynthetic) and heterotrophic metabolisms at large rates. In addition, mixotrophic cultivation has been related to lower energy costs as compared to photoautotrophic cultivation, owing to its lower light intensity requirements [15]. Both control experiments did not show comparable removal rates: aerobic control showed a significant decrease in COD, BOD, and nitrates, while anaerobic treatment showed a noticeable decline in orthophosphates and suspended solids. These reactions are contingent on oxidation–reduction processes carried out by a variety of bacteria and fungi that are abundant in wastewaters [16].

It can be assumed that changes in pH values during the main experiment were the result of inorganic carbon assimilation by microalgae, as well as nitrogen consumption. As previously reported, increased ammonia volatilization and nitrate ions absorption could lead to higher pH values. However, if ammonia becomes the main nitrogen source for microalgae, it may lead to a significant pH decrease and inhibition of microalgae growth due to an excessively acidic environment [17][18][19]. Hawrot-Paw et al. [20] reported a pH range from 7.98 to 8.54 during aquaculture wastewater treatment with *Chlorella minutissima* over a span of 10 days. Eze et al. [21] also described a significant rise in pH values from 8.0–8.5 to 10.5 after 28 days during the experimental purification of wastewater sampled from a wastewater treatment plant with *Desmodesmus* sp. Moreover, it was proven that the CO_3^{2-} forms of inorganic carbon predominates in solutions with a pH above 10, and microalgae cannot fully utilize this form, which leads to a decrease in their biomass production and overall nutrient removal rates [22]. As the process was mixotrophic (i.e., both light as inorganic and nutrients as an organic source of carbon were used by microalgae for growth), microalgae were produced and released oxygen by the process of photosynthesis in the presence of daylight, which led to a rise in dissolved oxygen levels [19]. The corresponding conclusion is also in agreement with previous studies, where the correlation between the rise in dissolved oxygen levels and the increase in microalgae biomass was reported during microalgae cultivation in wastewater [23][24][25]. In contrast, control treatments without microalgae showed no (anaerobic) or very little (aerobic) dissolved oxygen level increase compared to the main treatment. Such results indicate that microalgae are essential for wastewater saturation with oxygen, hence stimulating the nitrification process by oxygenic photosynthesis [24]. Both pH and dissolved oxygen levels are important markers for monitoring and characterizing microalgae growth patterns.

The sharp increase in concentration of nitrates from Week 3 to 4 could be explained by the process of oxidation of ammonium nitrogen provoked by bacterial nitrification, and a similar effect was previously reported by Eze et al. [21]. Moreover, they reported 62% of total nitrogen removal efficiency after 28 days of wastewater cultivation using *Desmodesmus* sp.; however, the actual nitrogen removal efficiency by microalgae accounted only 48% out of 62%, as material N/P balance predicted that around a 14% loss of the initial ammonium nitrogen occurred due to NH_3 volatilization. Additionally, Aslan and Kapdan [26] reported that during algal treatment of synthetic wastewater, the NH_4-N removal rate was around 50% in water with an ammonia concentration of 41.8–92.8 mg-N/L, and it dropped to around 24% in wastewater samples where the NH_4-N concentration was above 129 mg-N/L. McGaughy et al. [27] reported a drop of nitrate levels in wastewater produced from hydrothermally treated septage during microalgae treatment with *Chlorella* sp., from 9.3 ± 1.3 to 5.2 ± 0.2 and then 1.7 ± 0.2 mg-N/L at Days 0, 5, and 10, respectively. The authors concluded that microalgae initially consume ammonia and other TKNs, while nitrates and nitrites are consumed secondarily. Therefore, nitrate- and nitrite-containing wastewaters require longer periods of time to be efficiently treated with microalgae. It is known that microalgae consume NO_3 and NO_2 at slower rates compared to NH_4^+, as ammonia in contrast to nitrates and nitrites can be included directly into the composition of amino acids, which is necessary for microalgae metabolic functioning and growth, whereas specific enzymes nitrate reductase and nitrite reductase should firstly reduce NO_3 and

NO_2 to ammonium in order for them to later be utilized by microalgae. Moreover, being an energy-dependent process, NO_3 transportation to the cell membrane requires direct consumption of ATP [28].

Phosphorus removal from municipal wastewater is possible through chemical precipitation at a pH higher than 8.6 or through microalgae assimilation of phosphorus from wastewater. Due to the fact that pH increased up to 9.4 in the main experiment, both possibilities are relevant in the conducted experiment. Other studies achieved a 55% phosphorus removal rate from agroindustrial wastewater using *C. vulgaris* and *Scenedesmus dimorphus* [19]. In the study conducted by Choi [29], 90.84% of phosphorus was removed from municipal wastewater in the microalgae membrane bioreactor. Removal rates obtained in the current research are average; however, phosphorus removal rates highly depend on the initial nutrient concentration, light, pH, and microalgal species or strain.

As can be seen from the data, the concentration of Na, K, and Zn increased by the end of the wastewater treatment process. A steep increase in Na concentration from 3.24 to 26.20 is explained by the addition of NaOH at the beginning of the main experiment in order to adjust the pH value to the microalgae growth optimum. The K and Zn elevation might be a result of water evaporation, as microalgae did not absorb K and Zn after the total volume reduction elevation took place. Since during the experiment aeration was used, it could cause mechanical disturbance and breakup of the particles, leading to the release of metal ions [30]. Additionally, some bacteria are able to accumulate metals while consuming organic matter [31]. During wastewater treatment, some of these bacteria die, which also causes the release of ions. The same trend of the increasing concentration of Zn, Cu, Ni, and As during the microalgae wastewater treatment process was reported by Krustok et al. [32]. Microalgae are known for their abilities of metal removal through various mechanisms, such as physical adsorption, complexation, precipitation, chelation, and ion exchange with the help of metallothioneins, crystallization on the cell surface, and chemisorption [30][33][34]. Additionally, removal of metals strictly depends on biotic factors such as microalgal species, tolerance capacity, biomass concentration, and bacterial abundance, as well as abiotic factors such as pH, temperature, metal ionic strength, salinity, hardness, effect of combined metals and metal speciation, and initial concentration [30]. In the study of Mubashar et al. [35], the removal of Cr, Cd, Cu, and Pb from textile wastewater by *C. vulgaris* was studied. The concentration of all metals was above permissible limits of 1 mg/L Cr, 1 mg/L Cu, 0.5 mg/L Pb, and 0.1 mg/L Cd, so 5%, 10%, and 20% dilutions were made to improve removal of metals. The final removal efficiency for all metals was between 25 and 93% in all dilutions. In the present study, no dilutions were made to the initial wastewater, which can provide a great benefit to potential industrial implementation.

In the present study, Ca, besides having the highest initial concentration in the wastewater, had also the second biggest removal rate of 82.7%. This value is in agreement with a previous study conducted by Wang et al. [36], where 80.2% of Ca was removed by *Chlorella* sp. from reverse osmosis concentrate during 16 days of batch cultivation. It was reported earlier that a pH increase may promote the uptake of heavy metals from the water by microalgae, as with pH elevation, the surface of the microalgae cell becomes negatively charged [37][38]. However, Wang et al. [36] concluded that chemical precipitation has the most significant impact on Ca and Mg removal rates during the cultivation of microalgae, which occurs due to pH increase as a result of biomass growth. Thus, an increased pH is likely to facilitate metal removal in both ways: because of microalgal sorption and chemical precipitation. Moreover, the presence of certain bacteria can enhance metal removal, as it was studied by Mubashar et al. [35] that the addition of *Enterobacter* sp. MN17 to textile wastewater during microalgae cultivation with *C. vulgaris* showed better removal rates of Pb, Cu, Cd, and Cr by decreasing wastewater toxicity and intensifying microalgal growth. A potential explanation for the higher removal efficiency of some metals than others may be that microalgae better utilize these metals to maintain their functions and growth [39].

In previous studies of wastewater treatment with microalgae, COD, BOD, and TOC removal rates varied significantly, depending on the initial quality and type of wastewater, treatment duration, and microalgae species used. Hongyang et al. [8] reported a 77.8% removal of COD after cultivation of *Chlorella pyrenoidosa* in soybean processing wastewater. Travieso et al. [9] reported an 88% removal rate of COD after 190 h of treatment in piggery wastewater using *Chlorella* spp.; however, initial wastewater COD was significantly lower compared to the current research and was composed of 250 mg/L. Usha et al. [10] reported an 82% and 75% removal rate of BOD and COD, respectively, and a 75% reduction in TOC in pulp and paper mill effluent after 28 days of cultivation, with a mixed microalgae culture of two *Scenedesmus* species. One more study obtained 85% and 89% TOC removal in two open photobioreactors treating domestic wastewater with mixed microalgal–bacterial consortium [11].

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