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Cultivation of Microalgae (*Scenedesmus* sp.) Using Coal Mining Wastewater and Separation via Coagulation/Flocculation and Dissolved Air Flotation (DAF)

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Abstract: Algae growth can be carried out in treated mine waters, providing biomass and helping to achieve the standards for water discharge. However, efficient separation of algae from the aqueous medium is crucial. The present work investigated the stability of *Scenedesmus* sp. in treated acid drainage from coal mining and assessed the harvesting of microalgae via coagulation/flocculation and dissolved air flotation (DAF). Successful algae growth was achieved, with cells remaining suspended in the water at a wide range of pH values, requiring the use of reagents for destabilization/aggregation. Algae coagulation/flocculation was attained with the use of tannin or ferric chloride associated with an anionic polymer flocculant at a pH of 8.0 ± 0.1. When combined with the flocculant, both tannin and the inorganic coagulant proved effective in enhancing floc stability and hydrophobicity for the DAF process. In summary, this operational approach facilitated algae biomass recovery and significantly reduced turbidity in the treated water. Finally, a schematic diagram illustrating the algae cultivation and harvesting process is presented, offering a practical alternative to acid mine drainage (AMD) treatment refinement associated with algae biomass production.

Keywords: mining; water treatment; tannin; microalgae harvesting; biofuel; energy

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1. Introduction

The active treatment of acid mine drainage (AMD) typically involves the addition of alkaline agents to elevate the pH, leading to the precipitation of metals in the form of hydroxides [1–3]. Recently, following a more sustainable perspective of mining activities, there has been a growing interest in studying the utilisation and recycling of mine water [4,5]. A noteworthy initiative involves the use of pre-treated mining water for promoting microalgae growth. This innovative approach offers benefits, including the potential production of materials for biofuel generation and the removal of residual pollutants that may persist after conventional acid mine drainage treatment processes [6–13]. In particular, *Chlorella* and *Scenedesmus* are microalgae of choice for metal removal, but it is necessary to develop strategic multidisciplinary approaches to develop commercially viable technologies [14].

Active treatment of acidic mine waters involves the addition of alkaline reagents such as NaOH or $Ca(OH)_2$ to increase the pH and precipitate the dissolved metals. The treated water is clear and some ionic species are suitable as a source of essential nutrients for algae growth, such as Ca^{2+}, Zn^{2+}, Mn^{2+}, and $SO_4{}^{-2}$ [7,10], although the addition of other nutrients may be necessary. In a previous study [12], the algae *Scenedesmus* sp. was grown in treated acid mine drainage from coal mining. It was found that mining water was suitable for the growth of this microalgae, with biomass generation productivity values of

around 65 mg L^{-1} day^{-1} and additional gains in removing residual metals and reducing the toxicological response. However, this approach requires the successful integration of the operations involved in microalgae cultivation, cell aggregation, and biomass harvesting.

The algae genus *Scenedesmus* sp. has been cultivated to produce biodiesel and biogas [15,16]. Current forms of microalgae harvesting/separation include microfiltration, sedimentation, flotation, and mixed filtration and centrifugation systems [17]; however, the economic feasibility of these processes remains an issue [18]. Gravimetric sedimentation, despite its low cost, has low efficiency due to the specific weight of the cells being very close to the water; this requires long sedimentation times, leading to a large equipment area. Thus, dissolved air flotation (DAF), which involves injecting microbubbles into the system, has the potential to promote effective algae removal [19]. The use of proper reagents to promote algae flocs with characteristics associated with effective solid–liquid separation via DAF [20] is required. Considering the future applications of biomass, biodegradable reagents, such as acacia tannin, may present advantages over inorganic reagents [21–26].

In this context, employing maturation ponds with pre-treated water from coal mining to cultivate microalgae for subsequent utilization in energy generation emerges as a potential alternative for enhancing water quality for discharge, while simultaneously extending the energy production chain. Therefore, this study aims to investigate the growth of *Scenedesmus* sp. suspension in mining water and assess the performance of tannin (nature-based coagulant) and ferric chloride (conventional, but with some environmental advantages compared to aluminium-based coagulants) in promoting cell aggregation, with both coagulants associated with polymeric flocculants. Subsequently, the dissolved air flotation process was employed as a solid–liquid separation step for harvesting algae to determine whether the DAF process would respond well to the use of those two combinations of reagents. The results are discussed in terms of an estimate of algae production and the new advances in mine water management.

2. Experimental

2.1. Mine Water

The sample of AMD originated from water percolation in a coal waste deposit obtained from the municipality of Forquilhinha, Santa Catarina, Brazil (28°47′36″ S; 49°26′02″ W). In this mining site, coal waste is strongly associated with iron sulphides, with a content of pyrite ranging up to 12% [27]. Reject-dump-bearing waters contained dissolved metals (Fe^{3+}, Fe^{2+}, Al^{3+}, Mn^{2+}, and Zn^{2+}) and sulphates ($SO_4{}^{2-}$), resulting from the low pH and the activity of acidophilic bacteria [28].

The treatment of AMD was conducted based on the conventional procedure carried out by Brazilian coal mining companies [4,29]. First, the pH was raised to be circumneutral or slightly alkaline by adding the alkaline reagent (calcium hydroxide—$Ca(OH)_2$) under stirring, with the pH monitored using a pH meter. This was followed by the precipitation of the metals as hydroxides. The supernatant was filtered and then separated for additional treatment with algae, due to their metal sorption capacity [30]. The analysis of raw and treated effluent is presented in Table 1.

2.2. Reagents

The composition of the Guillard Modified culture medium [32], a recognized cultivation broth for microalgae growth, is shown in Table 2. The pH adjustment for zeta potential measurements was made using solutions of 0.5 M HCl and 0.2 M NaOH. Neutralization of AMD was carried out using a solution of 200 g L^{-1} of $Ca(OH)_2$. The coagulant $FeCl_3{\cdot}6H_2O$ was prepared in a stock solution of 50 g L^{-1}. These reagents were all of analytical grade (supplied by Dinâmica Química, São Paulo, Brazil). The company TANAC® (Montenegro, Brazil) provided the coagulant Tanfloc SG, a tannin-based coagulant manufactured from acacia forestry in the state of Rio Grande Sul. This product is sold in liquid form, with 30 to 35% of active component. The polymeric flocculant applied was AN 956 SH (SNF Floerger®, Andrézieux-Bouthéon, France), an anionic polyacrylamide of high molecular

weight—(as powder), which was prepared in a stock solution of 500 mg L^{-1} and used within a period of 24 to 48 h after preparation. Deionized water, obtained using Mac Clean reverse osmosis equipment (model 90APGE BUBE), was used in the dilutions and media for algae growth.

Table 1. Characteristics of raw acid mine drainage (AMD) and after active treatment via neutralisation/precipitation with $Ca(OH)_2$.

Parameter	AMD	AMD after Neutralisation with $Ca(OH)_2$	Emission Standards CONAMA 430/2011 [31]
pH	2.9	7.0	5–9
Conductivity (µS cm^{-1})	784	568	-
Na (mg L^{-1})	2.1	0.4	-
K (mg L^{-1})	0.4	1.3	-
Ca (mg L^{-1})	1.5	116.7	-
Mg (mg L^{-1})	0.4	1.2	-
Zn (mg L^{-1})	0.64	<0.01	5
Fe (mg L^{-1})	323.3	0.53	15
Mn (mg L^{-1})	0.05	<0.01	1
Al (mg L^{-1})	67.2	<0.01	-
Sulphates (mg L^{-1})	490.6	302.5	-

Table 2. Composition of the Guillard Modified culture medium, employed for algae inoculum growth.

Salt	Concentration (g L^{-1})
$CaCl_2 \cdot 2H_2O$	36.76
$MgSO_4 \cdot 7H_2O$	36.97
$NaHCO_3$	12.6
K_2HPO_4	8.71
$NaNO_3$	85.01
$Na_2SiO_3 \cdot 7H_2O$	28.42

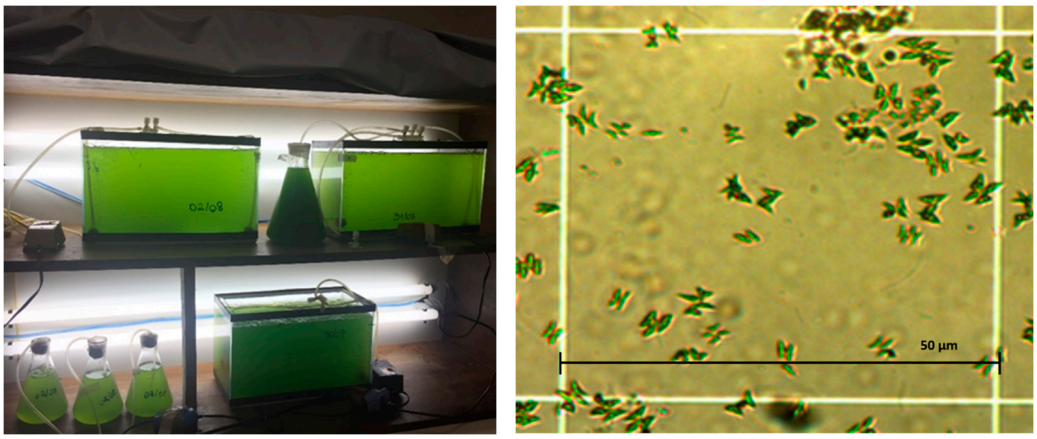
2.3. Algae Growth

The algae species selected was *Scenedesmus* sp., used due to its known metal uptake capacity and its use as a biofuel resource [15,16,27]. In preparation for the experiments, water, equipment, and supplies were sterilized in an autoclave. The handling of biological and chemical material was carried out in a chamber with UV radiation.

The inoculation of microalgae cultures was conducted in 1000 mL Erlenmeyer flasks with the Guillard Modified culture medium. The flasks were incubated in an orbital shaker (Cientec® model CT-712RN, Piracicaba, São Paulo, Brazil) at 125 rpm and 27.5 °C, with a photoperiod of 8 h day^{-1} under illumination with a light intensity of 5500 lux (measured via a digital lux meter—model MLM 1011). Dry weight and optical density (OD) were measured daily to determine microalgae growth and the relationship between them. OD was measured at a wavelength of 570 nm (UV1100 spectrophotometer Pro-tools®, Porto Alegre, Brazil) and the dry matter was measured by filtering the samples through 0.7 µm pre-weighted membranes, which were dried at 60 °C for 24 h. The inoculums were used to introduce the biological material when the growth reached the exponential phase, which was achieved on the tenth day of growth.

The production of algae for flotation studies was conducted in vessels of 10,000 mL capacity, as depicted in Figure 1a. These vessels contained 8868 mL of treated AMD, 1000 mL of a solution composed of an inoculum containing a strain of *Scenedesmus* sp., and 132 mL of nitrogen and phosphorus solution to provide essential macronutrients for microalgae growth (prepared with 85.01 g of $NaNO_3$ and 8.71 g of K_2HPO_4 diluted in 1 L of water). The culture was developed for 10 days with an 8 h day^{-1} photoperiod, under the same conditions of light and temperature employed on the inoculums, but agitation

was promoted in this case via air bubbling. Algae growth was periodically monitored via microscopy on samples (Figure 1b) using a transmitted illumination microscope (Bel Photonics Bio3 Series, BEL Engineering, St Peters, UK), at a magnification of up to 100×, coupled to a digital camera of 1.3 megapixels.



(**a**) (**b**)

Figure 1. (**a**) Inocula and containers for the growth of the algae *Scenedesmus* sp. (**b**) Photomicrograph of *Scenedesmus* sp. grown in an aquarium during the study, as visualised under optical microscopy at 100× magnification.

2.4. Microalgae Destabilization

The dispersion of algae was evaluated as a function of the pH of the medium. The tests were carried out following the NBR 10561 (Water—Determination of Settable Solids—Imhoff Cone Method). The pH was adjusted using solutions of 0.5 M NaOH and 0.2 M HCl.

Ferric chloride and the tannin-based coagulant were employed at various concentrations while the pH was continuously monitored. Coagulation was performed in 0.5 L beakers, stirring at 300 RPM for 1 min (rapid mixing) and 50 RPM for 5 min (slow mixing). The flocculation was performed using SNF® Floerger AN 956 SH (1 to 7 mg L^{-1}) added 1 min after the addition of the coagulant under rapid mixing, and then extending it for 1 min. The flocs settling was performed for 10 min and clarified water samples were collected at the top for the determination of algae concentration (via spectrophotometry at 570 nm) and turbidity.

2.5. Algae Separation by Dissolved Air Flotation (DAF)

Flotation (depicted in Figure 2) was conducted in a bench-scale glass column (1.5 L), featuring inlets for air-saturated liquid entry and outlets for sample collection, both located at the bottom of the column. The mixing conditions and reagent additions for algae aggregation followed the same procedure previously described. The main operational variables were the saturation pressure (2, 3, 4, and 5 atm) and recycle ratios (10, 20, and 30%), which provided different air/solids ratios that were calculated from Equation (1), assuming a saturation pressure efficiency of 90% from Henry´s Law.

$$\frac{A}{S} = \frac{1.3 \cdot S_{air} \cdot (f \cdot P - 1) \cdot q}{TSS} \quad (1)$$

where:

1.3—air density (mg mL^{-1});
A/S—air/solids ratio (mg mg^{-1});
S_{air}—air solubility in the water (20 mL L^{-1} at 25 °C and 1 atm of pressure);
f—efficiency of gas dissolved at a given pressure (90%);
P—pressure (atm);

TSS—total suspended solids (mg L^{-1});
q—recirculation ratio (recirculation flowrate for air saturation per effluent flowrate).

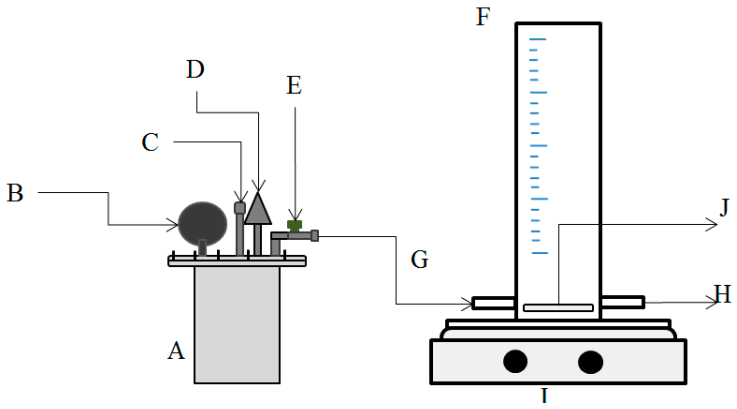


Figure 2. Bench-scale flotation system: (A) saturator vessel; (B) manometer; (C) pressurized air inlet; (D) pressure relief valve; (E) needle valve; (F) flotation column; (G) DAF microbubbles inlet for air-saturated liquid; (H) outlet for sample collection for analysis; (I) magnetic stirrer; (J) magnetic stir bar.

The bubbles were generated via depressurization and hydrodynamic cavitation through a needle valve with a saturation manometric pressure range of 2 to 5 atm, generating a cloud of microbubbles with a diameter ranging between 1 and 60 µm [33]. Samples were collected from the bottom of the column 2 min after the bubbles were injected. The separation of algae via DAF required a comparative assessment of the reagents ferric chloride and tannin (Tanfloc SG), in combination with the flocculant, at their previously determined optimal concentrations.

2.6. Analytical Methods

Turbidity was measured using a nephelometric turbidimeter (DIGIMED DM-TU, Digimed, São Paulo, Brazil). *Scenedesmus* sp. dry mass was determined through visible-range spectrophotometry at a wavelength of 570 nm, employing a benchtop spectrophotometer (UV-1100 Spectrophotometer Pro Tools, Environmental XPRT, Madrid, Spain).

The pH and conductivity of the suspension were measured using a benchtop pH meter (AKSO AZ Water Quality Meter—model 86505, São Leopoldo, Brazil). The zeta potential of the algae was also measured as a function of the pH. The measurements were carried out in the culture medium itself using the microelectrophoresis technique (Zetasizer Nano-ZS ZEN 3600, Malvern®, Malvern, United Kingdom). Algae suspension aliquots were collected from the cultivation vessel after 10 days and had their pH adjusted using HCl and NaOH. The same reagents were applied to Imhoff cones sedimentation tests to evaluate the volume of cells in 1 L of the suspension that settles in a period of 1 h. Measurements at each pH were carried out in triplicate.

The biochemical composition of the algae biomass was characterized in terms of lipids, proteins, and ash according to the Association of Official Agricultural Chemists [34]. The concentrated microalgae biomass was dried in a De Leo Bacteriological oven, with 440 watts of power at a temperature of 65 °C, and was transformed into powder using a ceramic pestle to a particle size less than 0.263 mm. Lipids were determined using the classical Soxhlet method. When determining the amount of protein from the Kjeldahl nitrogen, a multiplication value of 6.25 was used for the nitrogen–protein ratio. The carbohydrate content was calculated from the difference between the total weight of solids and the weight of lipids, protein, and ash.

Samples for metal analyses were preserved using HNO_3, and an optical spectrophotometer with inductively coupled plasma (ICP-OES) was employed. The sulphate content was measured using turbidimetry after precipitation with barium chloride. All analyses

followed the methods described in 'Standard Methods for the Examination of Water and Wastewater' [35].

3. Results and Discussion

3.1. Microalgae Biomass Production

Under the conditions applied in this work, the concentration of algae (in terms of dry mass) reached 570 mg L^{-1} in 10 days (Figure 3) in the treated AMD with nutrients added. This is slightly below that obtained using the modified Guillard medium, where values reached 650 mg L^{-1} in the same period. Therefore, the productivity was 0.057 g L^{-1} day^{-1}, which is very similar to that obtained in another study in southern Brazil using a tubular compact photobioreactor (0.060 g L^{-1} day^{-1}) [36]. Biochemical analysis of the material indicated a composition comprised of 8.9% total lipids, 20% proteins, 30% carbohydrates, and 41.1% ash. The lipid content for *Scenedesmus* sp. is low, even though values in this range have been reported by other researchers [37,38]. However, the high ash content is notable, which is at the maximum threshold of that found in algae [39]. This is possibly due to the assimilation of inorganic components from the mine water [12,40]. If the values are normalized to only organic components, as demonstrated in many studies, the composition becomes 15.1% total lipids, 34% proteins, and 50.9% carbohydrates. This composition is consistent with results found in the literature for strains of the genus *Scenedesmus* sp. without optimizing the conditions of genetics, light, culture medium composition, and temperature [41]. Reported strategies for increasing lipid content in *Scenedesmus* sp. species are algae growth under high light conditions, nutrient deficiencies, and other environmental conditions of stress [42–44].

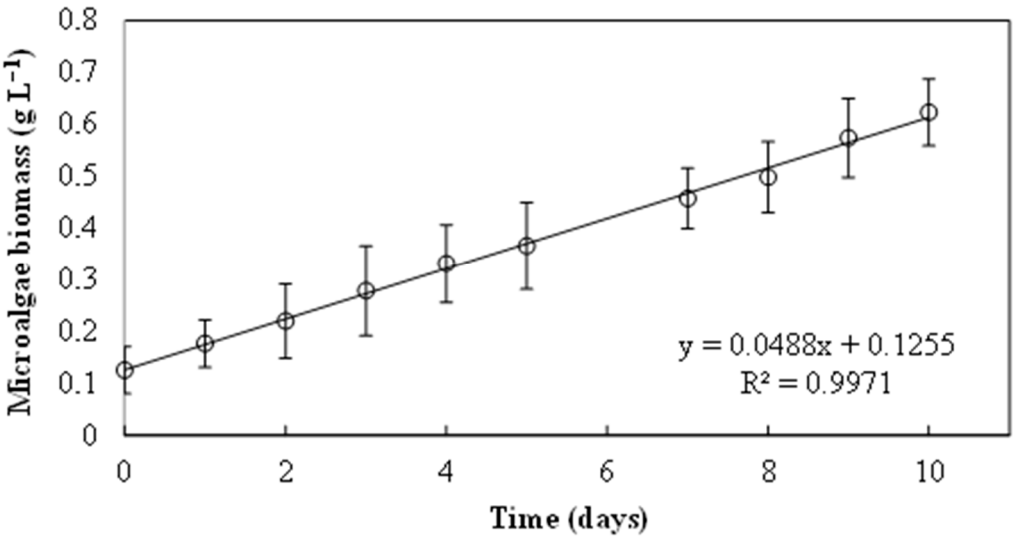


Figure 3. Evolution of biomass concentration during the cultivation of microalgae in acid mine drainage supplemented with nutrients.

3.2. Microalgae Removal

3.2.1. Microalgae Removal via Sedimentation

Initially, the natural sedimentation tendency of microalgae was evaluated without the addition of reagents. Figure 4 expresses the volume of settled microalgae as a function of the pH. The higher volumes were obtained at a pH of 3.0 (28 mL L^{-1}) and 8.0 (47 mL L^{-1}), while at other pH values the volume was less than 10 mL L^{-1}. However, even in the best conditions, the clarification was not complete, with many algae still dispersed in the aqueous environment.

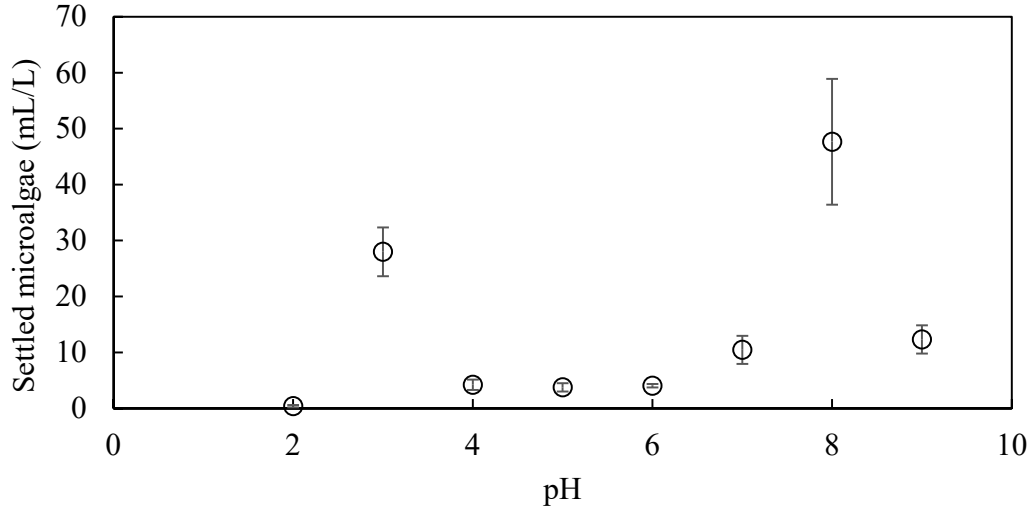


Figure 4. The volume of *Scenesdesmus* sp. cells settled in the Imhoff cone as a function of pH.

The destabilization at a pH of 3.0 can be explained in terms of electrokinetic behaviour (Figure 5). The isoelectric point of microalgae was measured at a pH close to 3.0 and, in the absence of electrostatic repulsion, cell aggregation is induced. At a pH of 8.0, aggregation and sedimentation must have occurred via another mechanism, since at this pH the algae are electronegative (zeta potential in the order of −11 mV). This is explained by the occurrence of calcium phosphate precipitation at a pH of 8, arising from the components of the cultivation medium. The Guillard Modified culture medium, added as an inoculum in a proportion of 10%, is composed of doses of calcium ($CaCl_2 \cdot 2H_2O$—36.7 g L^{-1}) and phosphorus (K_2HPO_4—8.71 g L^{-1}), which can react to form calcium phosphate—$Ca_3(PO_4)_2$ [45].

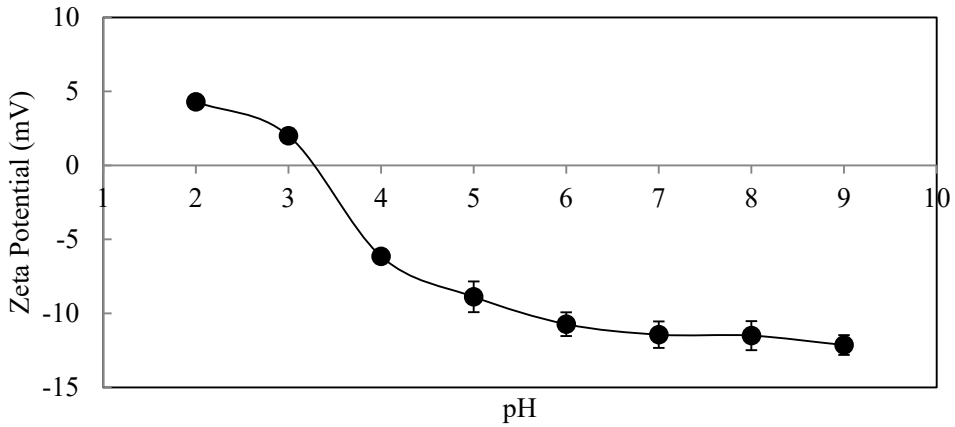


Figure 5. Zeta potential of the *Scenesdesmus* sp. cells as a function of pH.

However, even at pH values where natural aggregation and sedimentation were more prominent, clarification was not complete, with a substantial number of algae cells still dispersed in the medium. Therefore, the use of coagulants to destabilize the suspension is necessary. Tannin-based coagulants are effective over a wide range of pH, according to the manufacturer. Other research works obtained great algae removal in a neutral medium (pH below 8) [23,24,26]. In addition, Brazilian legislation (CONAMA 430) [30] restricts the release of effluents into water bodies at a pH between 5.0 and 9.0. Therefore, separation studies at a pH of 8 ± 0.1 were conducted, coinciding with the final pH of the medium after microalgae cultivation.

Table 3 lists the best conditions found to aggregate the algae using $FeCl_3 \cdot 6H_2O$ and tannin in association with the anionic polyacrylamide. In the first case, the proper dosages were 50 to 200 mg L^{-1} of $FeCl_3 \cdot 6H_2O$ (equivalent to 10 to 40 mg L^{-1} of Fe^{3+}) and 1 to

3 mg L^{-1} of the flocculant, as no efficiency improvement was obtained using higher reagent concentrations. The pH had to be adjusted back to 8 after the addition of the iron salt, due to iron hydrolysis. The alternative is the addition of 0.2 to 0.3 mL L^{-1} of the Tanfloc SG (equivalent to 70 to 100 mg L^{-1} of the reagent active component) followed by 1 to 3 mg L^{-1} of the polymer. In both situations, this results in large microalgae-containing flocs resistant to the stirring speed applied. Following the cessation of agitation, flocs slowly settled in a flocculent settling regime—as defined by Fitch and Stevenson (1977) [46]. After a few mins of settling time, the sedimentation process was complete. However, the presence of small residual flocs suspended in the effluent was always observed (Figure 6), which can be explained by the low density of algae aggregates. It could also be caused by the adhesion of algae aggregated to small air bubbles resulting from the agitation process, or even the adhesion of micro- and nanobubbles of O_2 released by algae during photosynthesis. The polymer provides hydrophobicity to the algae aggregates, enabling the adhesion of air bubbles [20,47]. These observations prompted us to investigate DAF as a more effective process for algae harvesting.

Table 3. Best condition attained for coagulation and flocculation of *Scenedesmus* sp. biomass grown in a treated AMD.

Condition	pH Range	Algae Removal (%)	Residual Turbidity Range (NTU)	Description
50–200 mg L^{-1} of $FeCl_3 \cdot 6H_2O$ 1–3 mg L^{-1} of an anionic polyacrylamide flocculant	5–10	≈98	8–13	Excellent flocculation. Good sedimentation. However, there is the presence of small residual floccules suspended in the effluent.
0.2–0.3 mL L^{-1} of Tanfloc SG 1–3 mg L^{-1} of an anionic polyacrylamide flocculant	5–9	≈99	6–13	

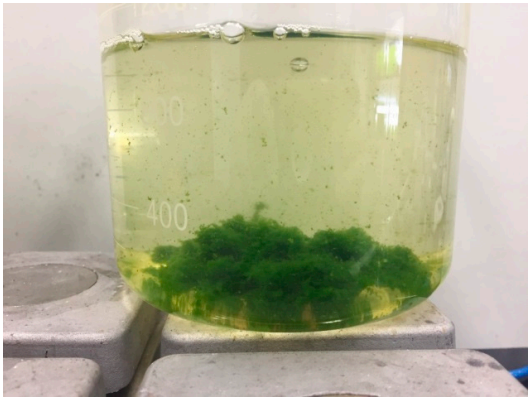


Figure 6. Settled flocs of microalgae after coagulation and flocculation. Conditions applied: a pH of 8.0+/−0.1; 0.3 mL L^{-1} of Tanfloc SG; and 2 mg L^{-1} of flocculant (Floerger AN 956 SH).

3.2.2. Microalgae Removal by Dissolved Air Flotation

DAF studies were conducted using both combinations of reagents, operating at a bench scale with recycle ratios of 10, 20, and 30% and saturation pressures ranging from 2 to 5 atm (relative pressures). When applying more than 2 atm of pressure and at a 20% recycle ratio, the results showed biomass removal consistently close to 100% and turbidity values lower than 10 NTU.

In comparison to settling, which typically takes longer for efficient flocs separation (in the order of min in this work), algae separation via DAF proved significantly faster, requiring only a few seconds (10 to 20 s) for complete separation. This notable difference in separation time should be carefully considered when selecting a technique, especially when aiming for efficient separation and utilising compact equipment.

With the appropriate physical arrangements, DAF studies were conducted using the best chemical conditions previously attained in terms of algae aggregation. Algae harvesting efficiency via DAF was similar—and very high—with both studied coagulants (ferric chloride and Tanfloc SG) in combination with the flocculant (anionic polyacrylamide) reaching values of 100% of biomass removal (Table 4).

Table 4. Separation results of *Scenedesmus* sp. cells aggregated in flocs via dissolved air flotation with a saturation pressure of 5 atm and a recycle ratio of 20%.

Condition	pH	Algae Removal (%)	Residual Turbidity Range (NTU)	Description
150 mg L^{-1} of $FeCl_3 \cdot 6H_2O$ 2 mg L^{-1} anionic polyacrylamide flocculant Air/solids ratio: 0.036 mg mg^{-1}	8.0 ± 0.1	100	8–13	Excellent flocculation and flocs separation. Complete removal of algae flocs.
0.3 mL L^{-1} of Tanfloc 2 mg L^{-1} anionic polyacrylamide flocculant Air/solids ratio: 0.034 mg mg^{-1}	8.0 ± 0.1	100	6–13	

In this study, the optimal operational conditions have already been achieved with a 20% recycle ratio and a manometric saturation pressure equal to or greater than 3 atm, which demonstrates the potential of DAF for easily separating microalgae. Low saturation pressures and recycling ratios in DAF operation provide benefits in terms of energy consumption. Recycling ratios between 10 and 50% and saturation pressures between 2 and 6 atm are commonly reported in the literature for DAF applications in clarifying public water supplies and industrial effluents [48,49]. Therefore, DAF may represent an effective process in terms of separation efficiency and due to its recognized advantage in terms of its hydraulic loading (expressed in $m^3\ m^{-2}\ h^{-1}$ or $m\ h^{-1}$) compared to sedimentation [50,51].

Under optimized coagulation and flocculation conditions of 150 mg L^{-1} of $FeCl_3 \cdot 6H_2O$ or 0.3 mL L^{-1} of Tanfloc SG and 2 mg L^{-1} of flocculant polymer, open and branched flocs were formed, providing space for microbubble adhesion and/or entrapment, resulting in aerated floc formation [47]. In this condition, the buoyancy force of the bubble–cell aggregates is increased, resulting in the formation of a stable floated layer of biomass on the top of the bench flotation column in just a few seconds (Figure 7).

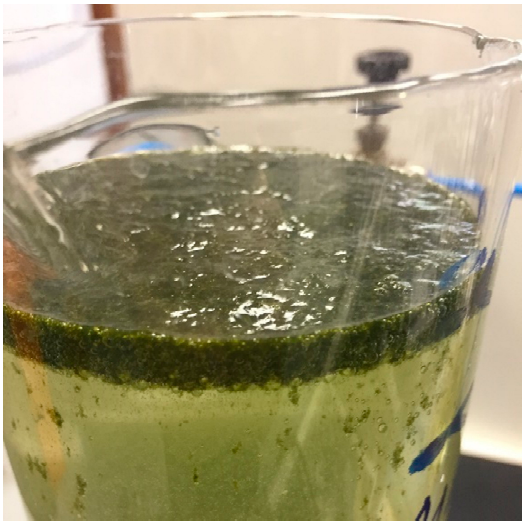


Figure 7. Floated biomass in the coagulation-DAF experiments for microalgae removal. Conditions: 0.3 mL L^{-1} Tanfloc SG; 2 mg L^{-1} anionic polyacrylamide; pH = 8 ± 0.1; a saturation pressure of 5 atm; and a recycle ratio of 20%.

Regarding the thickening of algae flocs, the results were comparable to those achieved after 1 h of sedimentation (33 mL L^{-1}) but notably faster, taking only about 10 s. These rapid separation velocities indicate the potential for operating DAF at its maximum capacity, with hydraulic loadings reaching up to 30–40 m h^{-1} (as reported in [48,52,53]).

Additionally, experiments were conducted using lower reagent dosages in coagulation-DAF separation, resulting in satisfactory biomass removal (>90%). However, the separation time for microalgae flocs through flotation with microbubble adhesion was slightly longer (approximately 1 min). Figure 8 illustrates microphotographs of the aerated flocs post-microbubble adhesion under optimized conditions of coagulation and flocculation.

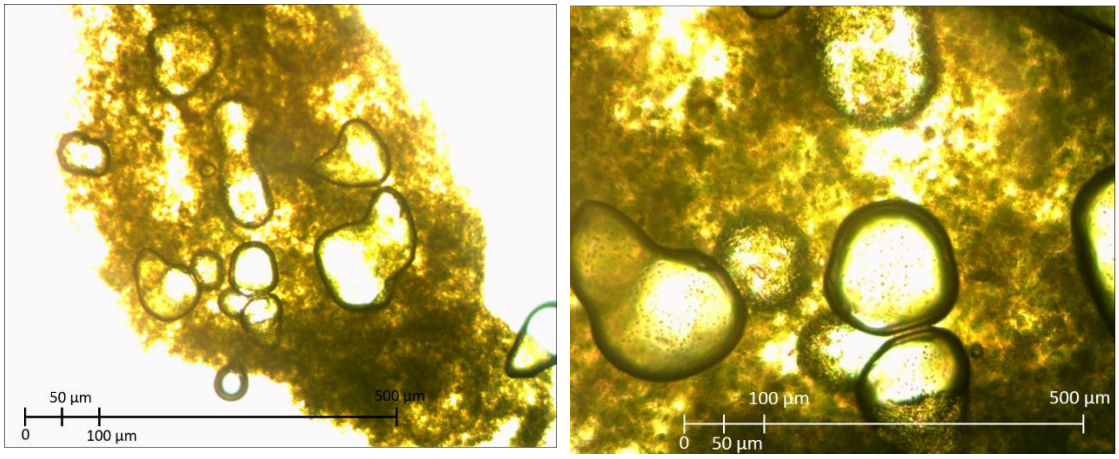


Figure 8. Aerated floc of microalgae with multiple bubbles, captured during ascension in the DAF column. Conditions: 0.3 mL L^{-1} Tanfloc SG; 2 mg L^{-1} anionic polyacrylamide; pH = 8 ± 0.1; a saturation pressure of 5 atm; and a recycling ratio of 20%. Visualised under optical microscopy at 100× magnification.

At the scale of Brazilian coal mining, considering a medium-sized mine with a production of 550 t $year^{-1}$ of thermal coal CE-4500 (with the calorific value of 18.828 kJ kg^{-1}) [54] and a mine water bearing flowrate of 150 m^3 h^{-1}, the dry mass of algae production could reach 750 t $year^{-1}$. Considering the lipid content is 8% and lipids are extracted via saponification, with a conversion rate of 90% [36], the amount of oil required to be obtained is 54 t $year^{-1}$ with approximately 40 MJ kg^{-1}. Converted into energy, it is equivalent to 2,160,000 MJ per year. Assuming that the energy provided by the coal extracted from the mine is 10,335,400 MJ, a *Scenedesmus* sp. growth farm may increase energy production by up to 20% (not counting the energy costs of operating the effluent treatment plant). Considering raceway ponds 0.5 m deep [55], the area required for this purpose would be 7.2 ha, which could be divided into six 1.2 ha ponds (Figure 9). For algae harvesting, DAF is already a technology known to the sector and the unit may have an area of ≈15 m^2, considering the conservative approach in this project, with a hydraulic loading of 10 m h^{-1} [53].

It should be noted that this approach was made for biodiesel; however, microalgae have multiple uses that could be contextualized regionally within the concept of a circular economy [56]. Nevertheless, it is important to note that the same global challenges, regarding the industrial implementation of microalgae production, are encountered here. These include the development of strains, the control of external biological agents, developments of technologies for algae biomass drying after harvesting, and, of course, the adaptation of mining companies to new directions.

Figure 9. Layout of a proposed active treatment system for AMD, integrating high-rate ponds for algae growth (raceways) and a unit for algae harvesting via DAF: (1) coal tailing deposit; (2) acid mine drainage; (3) effluent treatment via neutralization and metals precipitation; (4) treated effluent (first stage); (5) dewatering of the inorganic sludge using a filter press; (6) microalgae "raceway" pond; (7) dissolved air flotation (FAD) unit; (8) treated effluent (second stage); (9) microalgae biomass harvesting.

4. Conclusions

This study investigated the utilization of acid mine drainage (AMD) after neutralization–precipitation treatment as a growth medium for *Scenedesmus* sp. microalgae. Augmenting AMD with nitrogen and phosphorous from inorganic sources created an optimal environment for algal proliferation, yielding a productivity rate of 0.057 g L^{-1} day^{-1}. Destabilization of the suspended cells was necessary. Excellent flocculation was attained at a pH of 8.0 ± 0.1 with the addition of 50–200 mg L^{-1} of $FeCl_3 \cdot 6H_2O$ and 1–3 mg L^{-1} of anionic polyacrylamide, or 0.2–0.3 mL L^{-1} of commercial tannin reagent followed by the same dosage of the anionic flocculant. Complete removal of algae was achieved via flocculation followed by dissolved air flotation (DAF), using 150 mg L^{-1} of ferric chloride or 0.3 mL L^{-1} of tannin flocculant, both associated with 2 mg L^{-1} of polyacrylamide. DAF emerged as a robust solid–liquid separation technique for microalgae harvesting, boasting high separation efficiency and a high application rate. Based on the results obtained in this work, a process flow diagram was proposed. This incorporated pre-sized units, the integration of a conventional AMD treatment unit with raceway ponds for microalgae growth, and a solid–liquid unit for separation via DAF.

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