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A N I N T E R N A T I O N A L J O U R N A L

Harvesting Microalgal Biomass grown in Anaerobic Sewage Treatment Effluent by the Coagulation-Flocculation Method: Effect of pH

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ABSTRACT

Harvesting is a critical step in microalgal biomass production process for many reasons. Among the existing techniques available for harvesting and dewatering microalgal biomass, recovery from aqueous medium by coagulation-flocculation has been the most economically viable process, althoughit is highly dependent on pH. This study aims to assess alternative coagulants compared to the standard coagulant aluminum sulfate for microalgal biomass recovery from anaerobic effluent of domestic sewage treatment. The effluent quality was also analyzed after biomass recovery. Coagulants represented by modified tannin, cationic starch and aluminum sulfate recovered more than 90% of algae biomass, at concentrations greater than 80 mg/L, in the pH range 7-10. Cationic starch promoted higher microalgal biomass recovery with a wider pH range. Powdered seeds of Moringa oleifera *and* Hibiscus esculentus(okra) *gum promoted biomass removal of 50%, only in the acidic range of pH. After sedimentation of the microalgal biomass, the effluents showed a removal of >80% for phosphorus and nitrogen values and >50% for BOD and COD when using aluminum sulfate, cationic starch and modified tannin as coagulants. Natural organic coagulants in a wide pH range can replace aluminum sulfate, a reference coagulant in microalgal biomass recovery, without decreasing microalgal biomass harvesting efficiency and the quality of the final effluent.*

Key words: microalgae, anaerobic effluent, coagulation-flocculation, pH

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INTRODUCTION

Microalgae is a general, rather than taxonomic, designation generally referring to photosynthetic unicellular microorganisms, with visible growth in water bodies as a major component of phytoplankton. The wide variety of microalgal species include prokaryotic algae (cyanobacteria) and eukaryotic algae, such as diatoms (*Bacillariophyta*), green algae (*Chlorophyta*) and red algae (*Rhodophyta*)[1].

For microalgalbiomass (MBM) production, microalgae rely on sources of inorganic carbon and other nutrients, which mostly include nitrogen (N) and phosphorus (P). When provided these nutritional requirements, microalgae can be grown in natural water matrices (fresh and marine), synthetic growth media and effluent from domestic sewage or industrial treatment systems[2,3].

The use of wastewater for microalgal growth provides two significant benefits: the tertiary treatment of effluent at the end of the biomass growth and recovery process, which incorporates the nutrients required by microalgae and generates bulky biomass for many purposes[4,5].Microalgal biofuels could be an important alternative to conventional biofuels, as microalgae can be produced at high rates without the need of arable land or potable water and with out having competition with food production[2]. In addition to the benefits of microalgal biomass production in the effluent medium, microalgae are able to assimilate CO_2 as a carbon source for growth. Approximately 1.6 to 2.0 g of CO_2 can be captured per gram of MBM produced, thus contributing to a reduction in greenhouse gases[6].

The energy-intensive harvesting processes are limiting the commercial production of microalgal biofuels and other products that could be commercially viable. Harvesting microalgal biomass from the aqueous media is a critical step for microalgal biomass production because it represents 20 to 50% of the total cost[7,8]. The main microalgal harvesting processes include filtration, centrifugation, magnetic or electromagnetic separation, coagulation-flocculation, flotation and sedimentation or a combination of these processes. However, despite the abundance of microalgae recovery studies for microalgal biomass recovery, there is no consensus on the most appropriate methodology for this process[9,10]. The coagulation-flocculation process, followed by sedimentation, has been the most widely adopted method because it has been shown to be economically viable for large production volumes of biomass[11,12].

The coagulation-flocculation process can promote microalgal aggregation by the addition of coagulants (electrolytes), which can be metal salts, natural or synthetic polymers, or through pH adjustment, providing floc formation which facilitates microalgal biomass sedimentation[13]. According to Duan and Gregory[14], this process is critically dependent on pH values. Inorganic coagulants are very effective for MBM harvesting, but require a high dosage of coagulant addition, and thus, contaminates the MBM with aluminum and iron. Unlike inorganic metal salts, biodegradable organic coagulants do not contaminate the recovered biomass, and generally require low application doses, considering a wide pH range. Therefore, coagulants obtained from natural polymers are better alternatives for MBM harvesting than inorganic coagulants[15,16,17].

In this work, aluminum sulfate wasused as the reference coagulant, and natural organic coagulants were obtained from *Moringa oleifera* seeds, Acacia tannin, *Hibiscus esculentus* gum and cationic starch and were evaluated for MBM recovery by thecoagulation-flocculation-sedimentation method. The optimal dose and pH values were evaluated, which provide the best efficiency rates for recovery of MBM for each polymer. Furthermore, the quality of the effluent after sedimentation of the three best MBM recovered coagulants was assessed.

MATERIALS AND METHODS

Microalgal Growth Conditions

Microalgal growth was performed in effluent media from the anaerobic domestic experimental sewage treatment plant, located at Federal University of Espírito Santo (UFES), Goiabeiras campus, Vitoria, ES, Brazil. The pH, alkalinity, COD, BOD, total phosphorus (TP), and NTK nitrogen (TN) of the anaerobic effluent were analyzed, according to methodologies proposed by the Standard Methods for the Examination of Water and Wastewater[18], as shown in Table 1.

Table 1 – Averaged values ($\bar{x}$) andcoefficients of variation (CV) of the main parametersevaluated for anaerobic effluentquality(n=3).

Parameter	$\bar{x}$	**CV (%)**
pH	7.8	0.5
Alkalinity (mg/L $CaCO_3$)	207	2
COD (mg/L O_2)	110	9
BOD (mg/L O_2)	66	8
Total Phosphorous (mg/L)	9.8	1.4
Total Nitrogen (mg/L)	37.2	6.0

The anaerobic effluentwas previously filtered through a sand-gravel filter to remove large particles. The effluent was inoculated with a *Chlorella sp*. strain previously isolated and grown in sewage anaerobic effluent, at a final concentration of $2.0x10^7$ cells/mL. The production of microalgal biomass was conducted in an outdoor batch pilot – scale photobioreactor (FBR), which consisted of a 1.0 m³ plastic tank with an aeration system programmed to operate for 15 minutes, every three hours, and maintained at a temperature of approximately 30°C during day time hours.

Optical microscopy images of the main species of microalgae grown in the anaerobic effluent were obtained through optical microscopy (CARL ZEISS Axioplan), using a digital camera (MC80DX) coupled to the equipment, on a 40 µm scale.

Coagulation-Flocculation Assays

The coagulation-flocculation process was performed in jar tests under hydraulic parameters according to Di Bernardo & Di Bernardo[19] and Richter[20]. Four alternative coagulants were evaluated (modified tannin Tanfloc® Pop, *Moringa oleifera* seed powder, *Hibiscus esculentus* gum and SUPERION® 300 cationic starch). The coagulant aluminum sulfate ($Al_2(SO_4)_3$) was used as a reference for the microalgal coagulation process.

The SUPERION® 300 cationic starch solution was obtained from the mixture of the powder with distilled water at a concentration of 10 g/L and solubilized at 60°C, until the formation of a gelatinous product. Tanfloc® Pop (TANAC©) and aluminum sulfate (Dinamica, $Al_2(SO_4)_3.(14\text{-}18)H_2O$) solutions were established in accordance with the guidelines of the product manufacturer. The dry seeds of *M. oleifera*we reprocessed in accordance with the recommendations of Teixeira *et al.*[13].Okra (*Hibiscus esculentus*) gum prepared from raw okra involved the following macro-steps: (1) Raw pods of *H. esculentus*, obtained from a local market, were washed with distilled water, and then, the seeds were removed from the pods, according to Mishra *et al.*[21]. (2) Forgum extraction, the pods were triturated for one minute, with distilled water at 50°C, in a ratio 1:7 w/v. After grinding, the contents were filtered as recommended by Pranne & Tanatcha[22].(3) The liquid fraction was mixed with ethanol 3:1 v/v and stirred at 150 rpm for an hour. The obtained gum was washed in acetone 1:1 v/v, stirred at 150 rpm for 20 minutes, and then filtered through a muslin

fabric vacuum system to remove excess of ethanol and acetone. (4) The solubilized material was placed in distilled water at 65°C, until the formation of a gelatinous product; this product was used as the okra coagulant.

The coagulation diagrams were created according to the methodology proposed by Paixão[23] and Di Bernardo & Di Bernardo[19], and plotted in the software Surfer® 11. The microalgae biomass nutrient removal efficiency was determined by Equation 1, where E is the removal efficiency in percent, C_0 is the value determined for the parameter evaluated, and C is the concentration of the parameter evaluated in the final effluent after MBM sedimentation.

$$E(\%) = \frac{C - C_0}{C} \times 100 \text{ (Eq. 1)}$$

Microalgal Biomass and Final Effluent Characterization

MBM characterization was determined by the ratio of volatile solids to total solids VSS/TS, according to Andreoli *et al*.[24]. The physical-chemical characterization of the final effluent after the separation process was determined by measuring pH, alkalinity, COD, BOD, total phosphorus and NTK nitrogen. All assays were performed in accordance tothe Standard Methods for the Examination of Water and Wastewater[18].

Statistical Analysis

Statistical analysis was performed by using an analysis of variance (ANOVA), followed by a Tukey's post hoc test, to determine the significance in differences among the physical-chemical parameter values, with a significance level of α = 5.0%.

RESULTS AND DISCUSSION

The dominant genera of microalgae naturally associated with the anaerobic effluent were *Scenedesmus sp*. (Fig. 1A), *Chlorella sp.*(Fig. 1B) and *Synechocystis sp.* (Fig. 1C).Additionally, other microorganisms, such as fungi and protozoa, were associated with the anaerobic effluent due to natural growth in the non-sterile medium.

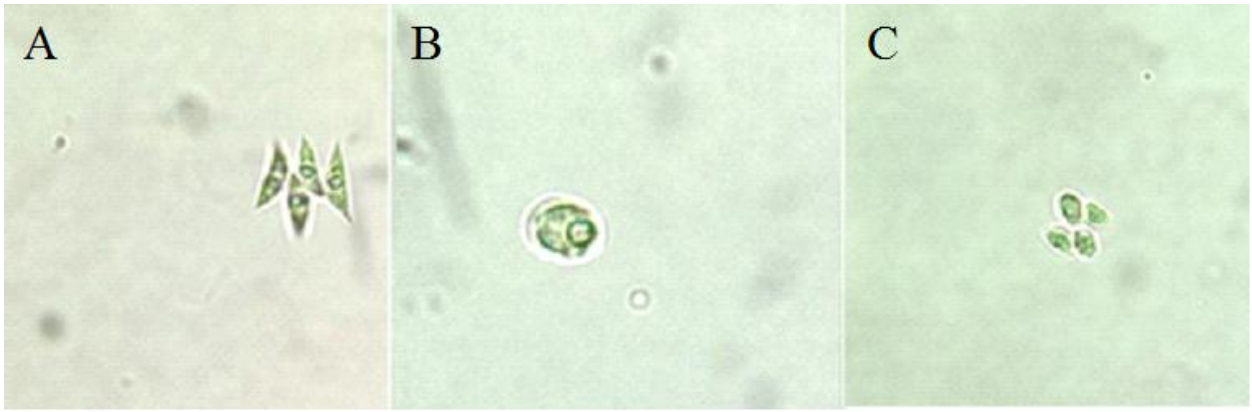

Figure 1 -Microalgae species found in the anaerobic effluent after 6 days of MBM growth: (A) *Scenedesmus sp.*, (B) *Chlorella sp.*,and (C) *Synechocystis sp.*

In study reported by Torres[25], six microalgae, identified as *Desmodesmus sp*., *Chlorococcum sp*., *Coccomyxa sp*., *Chlorella sp*., *Scenedesmus sp*. and *Tetradesmus sp*., were isolated from a Domestic Sewage Anaerobic Experimental Treatment Plant. Unlike the results of this study, Torres[25]observed abundant growth of *Chlorella sp*. in the anaerobic effluent, which showed a survival greater than 90%

compared to other species.The evaluation of the microalgal growth in a photobioreactor, using previously primary- and secondary-treated effluent from a meat processing industry as the growth medium, was performed in the study by Tango[26]. Similar to the present study, *Scenedesmus sp*. was the dominant microalgae in both evaluated effluents.

The growth of microalgae was dependent on pH and temperature of the medium, characterized as a daily variation cycle, as shown in Figure 2.

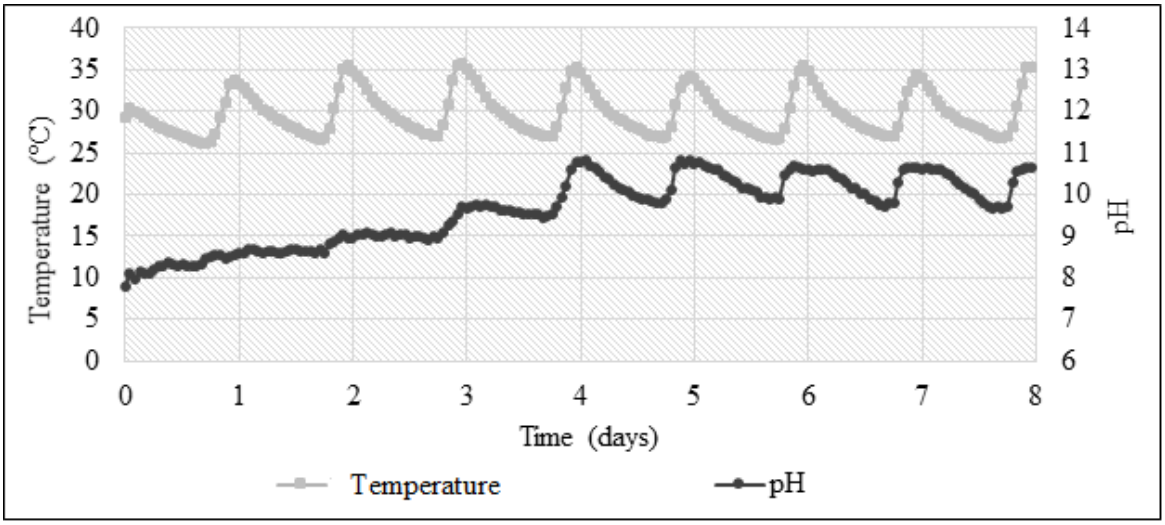

Figure 2 – Daily cycle variation in pH and temperature of the MBM batch growth over 8 days.

The microalgal biomass growing in culture showed a large variation in pH values varying from approximately 8 to apeak of 11 over the course of the daily diurnal cycle. Initial pH values were less variable and after stable growth was achieved by day 4, the diurnal cycle was fully operating. This could be associated with the CO_2/ CO_3^{2-} balance, as seen in Equation 2.

$$CO_{2(aq)} \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \qquad \text{(Eq.2)}$$

The inorganic carbon species normally used by microalgae are CO_2 and HCO_3^-. The assimilation of HCO_3^-, assisted by the enzyme carbonic anhydrase, likely represents a true H^+ removal from water medium and consequently results in anincrease in pH values. At higher pH values, e.g., pH >9, most of the inorganic carbon is in form of carbonate (CO_3^{2-}) which is also used by microalgae in high alkalinity values, resulting in H^+ removal from water medium and consequently results in an increase in pH. Night respiration and other metabolic processes replenish the CO_2 in the medium, lowering the pH values and restoring the pH cycle[27].

Addition or removal of CO_2 have no direct effect on the alkalinity, but these processes do affect the pH and are responsible for the significant diurnal changes in pH. Because of the pH values observed in the daily cycle of microalgal growth, the choice in coagulant type became a critical factor. Thus, we propose optimized microalgal biomass recover ythrough a coagulation-flocculation mechanism based on the pH range of the medium. The results of coagulation assays performed with jar tests and plotted by diagrams are shown in Figure 3.

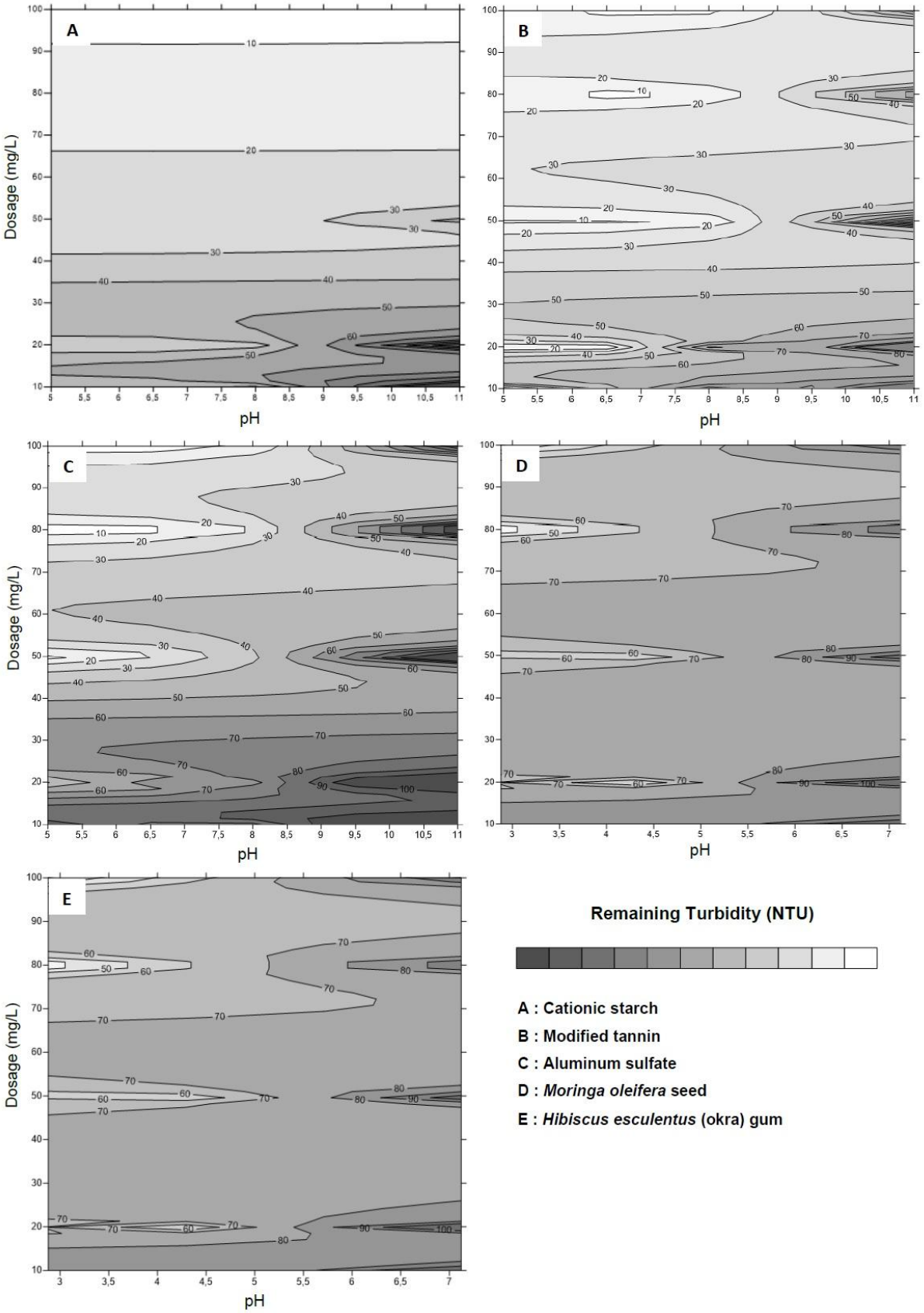

Figure3 -Coagulation diagrams based on Jar tests datafor different coagulants. The lightest are as represent lower turbidity values, while the darker areas have the greatest remaining turbidity values.

Based on the results obtained from the diagrams, it was possible to determine the microalgal biomass (MBM) removal efficiency values, as a function of dose and pH, as recorded in Table 2.

Table 2. Dose, optimum pH values and MBM removal efficiencies for each coagulant when evaluated by means of the coagulation-flocculation process followed by sedimentation.

Coagulant	pH	Dose (mg/L)	Removal efficiency(%)
Aluminum sulfate	7.0	50.0	95.9
SUPERION® 300 cationic starch	10.0	80.0	92.5
TANFLOC® POP	9.0	100.0	95.6
Moringa oleifera seed	5.0	150.0	85.8
Hibiscus esculentus (okra) gum	5.0	100.0	80.3

From the analysis of the results presented in this study and in the related literature, it could be inferred that aluminum sulfate is an effective coagulant in the recovery of MBM for neutral and acidic pH ranges, in doses less than 80 mg/L. At basic pH values, aluminum sulfate reached a biomass recovery of 60 to 70% with doses higher than 80 mg/L. Efficiencies greater than 90% were obtained by applying doses greater than 50 mg/L, at neutral pH; however, according to this study, neutral pH is not within the operating pH range for microalgal growth. In a study by Teixeira *et al*.[13],*C. vulgaris* biomass recovery was 93.8%, applying 1000 mg/L of aluminum sulfate at pH 9.24. Gerde *et al*.[16]applied aluminum sulfate to recover *Scenedesmus sp*. grown in synthetic medium at an adjusted pH between 8.5 and 9.5. Doses of 250 mg/L of aluminum sulfate were required to achieve 90% of *Scenedesmus sp*. removal by means of coagulation-flocculation. The data presented in the literature corroborate the findings of this study because a high MBM efficiency removal requires higher doses of aluminum sulfate by coagulation-flocculation at high pH.

For doses greater than 80 mg/L of modified tannin, recovery efficiencies were greater than 80%, with pH ranging from 7 to 10. Similar results were obtained to remove *Microcystis aeruginosa* cultured in BG-11, using a modified tannin coagulant (Q-TN)[28]. Recovery efficiencies obtained were greater than 90% in a pH range from 6 to 9[28]. In a recent study, *Chlorella vulgaris* biomass cultivated in swine effluent treated by phytoremediation had a 97% removal efficiency rate using 11 mg/L of commercial modified tannin at neutral pH[29]. In a study to recover *C. vulgaris* and *Nannochloropsis oculata*, grown in synthetic medium with pH of 8, the removal efficiencies for *C. vulgaris* were 99, 100 and 100% for concentrations of 5, 15 and 45 mg/L, respectively. In contrast, *N. oculata* removal efficiencies were 92, 96 and 99% for concentrations of 5, 15 and 45 mg/L, respectively[30].In this study, the effect of pH at the same low concentrations of modified tannin (5, 15 and 45 mg/L), the removal efficiencies were lower, between 30 and 60%. For pH values of approximately 8, the MBM recovery from a raceway pond, which treats wastewater, were greater than 90% by using 50 mg/L of Tanfloc® SG[30]as the coagulant. In contrast, the same study conditions of Gutierrez *et al*.[31] were established in this study efficiency of 96.7% using Tanfloc® Pop modified tannin as coagulant.

When cationic starch is used as a coagulant in MBM recovery, with doses between 80 and 150 mg/L, the removal efficiencies were above 80% for a wide range between pH 7and 11. Two types of cationic starch (GREENFLOC® 120 and CARGILL C*BOND RH 35 849) were tested for *Parachlorella sp*. and *Scenedesmus sp*. recovery in astudy by Vandamme*et al*.[32,33]. With a pH range between 7 and 8, than 80% at cationic GREENFLOC® 120 was more efficient in biomass recovery than Cargill C*BOND RH 35 849 for both genera of microalgae. The removal efficiency was greater than 80% for starch doses ranging from 20 to 40 mg/L (*Parachlorella sp*.) and 5 mg/L (*Scenedesmus sp*.); these results corroborate

those observed in the current study for MBM removal efficiency using the same dosage and pH ranges. Gerde *et al*.[16] used two types of cationic starch (DS02 and DS05) to harvest *Scenedesmus sp*. and *Chlamydomonas reinhardtii* in synthetic medium at pH adjusted between 8.5 and 9.5. The specie *Scenedesmus sp*. required 20 mg/L of DS5 to remove 90% of the biomass. The specie *C. reinhardtii* required approximately 40 mg/L of DS2 and 20 mg/L of DS5 to obtain 85 and 90% of removal, respectively. Hansel *et al*.[34] used modified cationic potato starches for flocculation of *Scenedesmus dimorphus* cultures. The results showed that all cationic starches were highly effective and the best removal rate used 10 mg/L of the tested starches, resulting in recovery efficiency above 95%.

According to Vandamme *et al*.[32,33], the cationic starch positive charge is due to quaternary ammonium salts introduced in the manufacturing process, which are able to maintain their positive charge even at high pH values and can contribute for MBM autoflocculation in higher pH; this may have promoted an increase in the recovery efficiency of BMA at high pH. Furthermore, according to the authors, the efficiency of flocculation by cationic starches and modified tannins can also be related to the degree of substitution of the quaternary groups, the site of substitution and the molecular weight of the polymers.

The use of *M. oleifera* seed coagulant was not the best option for MBM recovery in this study based on alkaline pH range. Amaximum removal efficiency of approximately 80% was achieved by adjusting the pH to the acidic range (4 to 5) and applying doses of 100 to 150 mg/L of the *M. oleifera* seed coagulant. Teixeira *et al*.[13] achieved an efficiency of 88.6% in *C. vulgaris* biomass removal, using WC medium and adjusting the pH to 9.2 and applying 1000 mg/L of *M. oleifera* powdered seeds, although the long 2 hours settling time. It should be noted that the selected concentration and settling time in the study by Teixeira *et al*.[13]were significantly higher than those observed in the present work. Hamid *et al*.[35] obtained efficiencies greater than 95% for *Chlorella sp.* removal using a *M. oleifera* coagulant, but using Bold basal medium, at an approximate pH value of 6, and the coagulation process with 30 mg/L *M. oleifer a*powdered seeds witha settling time of 2 hours.

Using polymers extracted from okra (*H. esculentus*) as a coagulant was not adequate for biomass growth in FBR alkaline pH range as observed in the MBM coagulation diagram charts. Low removal rates were observed in acidic pH ranges, approximately 4 and 5, reaching efficiencies of up to 80%. There is no sufficient literature data for MBM harvesting studies with microalgae grown in anaerobic sewage treatment effluent, but several studies investigate turbidity removal pH range domestic sewage effluent treated with polymers extracted from okra. Anastasakis *et al*.[36] presented data ofturbidity removals of 70 to 74% from biologically treated effluent, applying 2.5 mg/L of polymers extracted from okra seeds in pH 6.09. In contrast, Agarwal *et al*.[37] achieveda suspended solids removal efficiencyof approximately 86% for raw wastewater treatment, with only 0.12 mg/L of extracted okra gum at neutral pH.

Modified tannin, aluminum sulfate and cationic starch as coagulants were the most efficient for MBM recovery after the sedimentation process. The consolidated results for three best coagulants for MBM recovery for pH range of microalgal growth are presented in Table 3.

Table 3 - Means ($\bar{x}$) and coefficients of variation (CV%) of the main parameters for the evaluation of anaerobic effluent used for MBM growth (EUASB) and of the effluents after MBM removal by aluminum sulfate (ESAL), modified tannin (ETFP) and cationic starch (EACS). The different letters on each line indicate significant differences ($p < 0.05$) by Turkey's test.

Parameter	EUASB		ESAL		ETFP		EACS	
	$\bar{x}$	CV(%)	$\bar{x}$	CV(%)	$\bar{x}$	CV(%)	$\bar{x}$	CV(%)
pH	7.8[a]	0.5	3.4[b]	4.0	4.1[b]	14.8	7.1[a]	1.2
Alkalinity (mg/L $CaCO_3$)	207[a]	2	15[b]	12	15[b]	25	21[b]	9
BOD (mg/L O_2)	66[a]	10	43[b]	2	45[b]	7	21[c]	5
COD (mg/L O_2)	110[a]	8	21[b]	29	22[b]	8	11[b]	13
TP (mg/L)	9.8[a]	1.4	1.3[b]	22.5	1.4[b]	7.5	0.9[b]	5.1
TN (mg/L)	37.2[a]	6.0	3.6[bc]	20.8	5.4[b]	15.8	1.8[c]	15.8

From the analysis of the main physical-chemical parameters, the results suggest that the effluent pH values varied significantly after biomass removal and were generally more acidic, except for the effluent after biomass removal with the cationic starch, which provided an effluent with a nearly neutral pH.

The results presented in Table 3 illustrate one of the major advantages of MBM growth in anaerobic sewage treatment effluents: the growth of MBM represents a tertiary sewage treatment, because of the removal of phosphorus and nitrogen from the aqueous phase. The total P and total N decrease were more evident after using cationic starch as a coagulant, show in gremoval efficiencies of 90.64 % and 95.14%, respectively. Nutrient removal could be related to the MBM assimilation and the other associated biomass, such as fungi and bacteriaas microalgal growth was not performed in sterile medium.

Interestingly, a decrease in chemical and biochemical oxygen demands (BOD and COD) of 44% and 84%, respectively, were observed (Table 3). The removal of BOD and COD may be related to the removal of the suspended and dissolved organic material with negative charges present in anaerobic sewage treatment effluent (EUASB), microalgal cells and other microorganisms associated with EUASB; therefore, this process decreases the oxygen demand, particularly the chemical oxygen demand (Table 3).

Alkalinity is another parameter that showed the same trend with decreasing values as compared to the anaerobic sewage treatment effluent, taking on average by 91%. As mentioned previously, growth medium pH and alkalinity increasingly coincide with the exponential MBM growth phase; this is a phenomenon that can be explained by the consumption of CO_2 for photosynthesis, increasing the concentration of HCO_3^- and CO_3^{2-} and consequent rising in pH and alkalinity from carbonates and bicarbonates. As the culture moves to a lower stage of growth, during which stage the samples were collected for biomass removal, the medium alkalinity slightly reduced due to a reduction in dissolved CO_2 consumption.

CONCLUSIONS

It was possible to very fypolymer dosage, the optimum pH values and MBM removal efficiencies through the process of coagulation-flocculation followed by sedimentation. It is worth noting that, for high pH values from 8 to 10, only the cationic starch and modified tannin coagulants provided optimal MBM removal efficiencies with doses of 80 and 100 mg/L of the polymers, respectively.

From the analysis of the coagulation diagrams and the biomass removal efficiency data, cationic starch had the lowest dose required with highest recovery efficiency in

the target pH range observed for the MBM growth; however, the pH range of application and recovery efficiencies were similar to those reached using the modified tannin. Aluminum sulfate showed a smaller pH range for application than the range of modified tannin and cationic starch polymers, and required larger doses to achieve the same MBM recovery efficiency.

M. oleifera seeds and *H. esculentus* (okra) gum promoted MBM removals of only approximately 50%, in the acidic pH range. With aluminum sulfate, modified tannin and cationic starch as coagulants, the effluents generated after MBM floc settling revealed a total P removal of greater than 80%, and total N, BOD and COD removals were all greater than 50%.

Although aluminum sulfate is commonly used as a reference coagulant in microalgal biomass recovery, this work verified that it can be replaced by natural coagulants (e.g.,modified tannin and cationic starch) under the conditions presented here, without decreasing the biomass removal efficiency and causing negative impacts to the final effluent and, consequently, to the recipient water bodies.

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