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Assessment of a tannin-based organic polymer to harvest *Chlorella vulgaris* biomass from swine wastewater digestate phycoremediation

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ABSTRACT

This study investigated the efficiency of an organic tannin polymer alone or amended with polyacrylamide to harvest *Chlorella vulgaris* biomass grown in a laboratory-scale photobioreactor treating swine wastewater digestate. The effect of biomass concentration, tannin (TAN) dosages and changes in pH were evaluated in jar test experiments. Among the TAN concentrations tested (11, 22, 44, 89, 178 mg L^{-1}), 11 mg L^{-1} showed the highest biomass recovery (97%). The highest coagulation/flocculation efficiencies were obtained at pH 5 to 7. Flocculation efficiency improved from 50 to 97% concomitant with the increasing biomass concentrations from 45 to 165 mg L^{-1}, respectively. Recovery efficiencies above 95% were achieved with the same TAN dosage (11 mg L^{-1}) irrespective of the concentration of organic carbon present (75 to 300 mg TOC L^{-1}). Overall, the results suggest that TAN could become an interesting alternative choice of non-toxic organic polymer for harvesting *Chlorella* sp. from organic-rich wastewater.

Key words | chemical harvesting, *Chlorella vulgaris*, phycoremediation, swine wastewater, tannin, water reuse

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INTRODUCTION

Microalgae have been extensively studied because of their nutrition value and potential as feedstock for the commercial production of biomass (Mata *et al.* 2010). Despite microalgae's many advantages over agricultural crops, the economic feasibility of biomass production at large scales can be impaired to some extent by harvesting and dewatering processes (Mata *et al.* 2010). Commercially available technologies used to harvest microalgae biomass from media culture are based on mechanical, electrical, biological and/or chemical processes (Pahl *et al.* 2013; Vandamme *et al.* 2013). Among these, coagulation/flocculation seems to offer competitive advantages due to operational simplicity and lower costs (Mata *et al.* 2010; Vandamme *et al.* 2013).

Chemical precipitation with polyvalent metal salts has long been used in water and wastewater treatment to facilitate the removal of dissolved and suspended particles by sedimentation (Metcalf & Eddy Inc. 1991). The most common salts used in wastewater treatment plants are aluminum sulfate, ferric chloride, ferric sulfate, ferrous sulfate and lime. These chemicals have also been tested to flocculate microalgae efficiently (De Godos *et al.* 2011; Vandamme *et al.* 2011). However, the inherent toxicity characteristics of these chemicals could pose a threat to water quality or even interfere with downstream biofuel extraction processes (Uduman *et al.* 2010; Anthony *et al.* 2013). Therefore, the use of organic polymers, comprising either polysaccharides or proteins, has also been considered for harvesting microalgae since minimal (if any) environmental implications are anticipated at the very low dosages required (Yin 2010; Pahl *et al.* 2013). Microalgae biomass recoveries between 70 to 100% were demonstrated with the use of chitosan or plant-based coagulants (De Godos *et al.* 2011; Vandamme *et al.* 2012; Rashid *et al.* 2013; Banerjee *et al.* 2014).

Tannins (TAN) are secondary metabolites from higher plants that can be extracted from the bark and wood of trees, for example, *Acacia*, *Castanea* and *Schinopsis* (Özacar & Şengıl 2000; Yin 2010). These natural polyelectrolytes have been studied thoroughly in an attempt to improve its flocculant capacity for the removal of suspended solids and metals from water and wastewater (Özacar & Şengıl

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2000; Sánchez-Martín *et al.* 2010). Turbidity removal efficiencies of 63 to 97% have been documented with tannin dosages from 0.03 to 6 mg L^{-1} (Özacar & Şengıl 2000; Sánchez-Martín *et al.* 2009). Steinmetz (2007) studied the efficiency of solid–liquid separation of swine manure and obtained a turbidity removal efficiency of 94.8% using TAN combined with polyacrylamide (PAM) at 1.5 g L^{-1} and 0.5 mg L^{-1}, respectively. Wang *et al.* (2013) studied the flocculation of cyanobacterium *Microcystis aeruginosa* from water using larch tannin. Their results showed that more than 90% removal efficiency was obtained from tannin dosages ranging from 0.5 to 20 mg L^{-1}. Camacho *et al.* (2012) used a new tannin-based coagulant-flocculant agent (commercially available as Tanfloc SG, TANAC S.A., Brazil) at a concentration of 175 mg L^{-1} to achieve *Cylindrospermopsis raciborskii* removal efficiencies above 90%. The use of modified larch tannin to harvest pure culture of cyanobacteria from a culturing medium was recently published (Wang *et al.* 2013). Nonetheless, to our knowledge, the use of TAN to harvest microalgae *Chlorella* sp from either pure cultures or organic-rich piggery wastewater has not yet been documented.

Therefore, the aim of this study was to evaluate the use of TAN extracted from the leguminous *Acacia mearnsii* bark for the coagulation/flocculation and harvesting of *Chlorella vulgaris* biomass grown within a photobioreactor treating swine wastewater digestate. Emphasis was placed on determining the effects of microalgae biomass concentration, TAN dosages (with or without PAM as a coagulant aid), pH, and organic carbon content on coagulation/flocculation recovery efficiencies.

METHODS

Chlorella vulgaris cultivation

Microalgae inoculum was obtained directly from facultative open ponds treating effluent from an anaerobic biodigestor (covered lagoon) at the EMBRAPA swine wastewater treatment facility (Concórdia, SC, Brazil). *Chlorella vulgaris* was the dominant species in the enrichment. Approximately 110 to 120 mg/L of cell dry weight (DW) was used as inoculum. Cells were cultivated in a laboratory-scale 9 L batch photobioreactor fed diluted swine wastewater digestate from an upflow anaerobic sludge blanket (UASB) as previously described (Mezzari *et al.* 2013). The use of raw piggery wastewater was not tested in this study because *Chlorella vulgaris* growth was severely impaired by the intrinsic characteristics of raw piggery effluents such as the high carbon content (1,500 mg TOC L^{-1}, Mezzari *et al.* 2013), high concentrations of toxic ammonia (900 mg NH_3-N L^{-1}) and very high turbidity that limits light penetration and photosynthesis, ultimately affecting microalgae proliferation (data not shown).

Biomass concentration

Microalgae used for the coagulation/flocculation tests were obtained directly from the photobioreactor during their exponential growth phase. Gravimetric assays were used to measure biomass DW. Cell suspensions were filtered on a 0.45 μm cellulose acetate filter and allowed to dry at 105 °C for 24 h. Algal concentration within the photobioreactor was monitored by optical density (OD) at 570 nm (A_{570}) (DR2000, Hach). Significant correlation between OD and DW biomass were obtained. For simplicity, OD measurements were used to estimate DW biomass (see Supplemental information, available online at http://www.iwaponline.com/wst/070/307.pdf).

Polymers used

The organic tannin (TAN) used in this study was extracted from Acacia tree (*A. mearnsii*) bark. It is a cationic polyphenolic organic polymer produced through ammonium chloride and formaldehyde reaction. It is available commercially in liquid form with 30% w/v of tannic acid solution (flavan-3,4-diol) with weight distribution of 830-1940Da (CAS # 85029-52-3; Veta Organic™, Brazilian Wattle Extracts, Canoas, Brazil). TAN was chemically modified through the Mannich reaction to improve cationic strength properties by the addition of an ammonium quaternary functional group. A commercial PAM (CAS # 9003-05-8; Activator QTM; Brazilian Wattle Extracts, Canoas, Brazil) was also tested in combination with TAN to investigate whether its presence could aid coagulation/flocculation recovery efficiency.

Coagulation/flocculation assays

A series of assays was performed in jar tests in an attempt to determine the effects of tannin (with or without PAM) on coagulation/flocculation biomass recovery efficiencies. The jar test operational conditions are shown in Table 1. Changes in microalgal biomass concentration, PAM and tannic acid (TAN) dosages and pH on the coagulation/flocculation process were evaluated. Coagulation/flocculation experiments were conducted in 1 L jar tests (JT102 Milan®, Brazil) containing 700 mL of cell suspension. Different initial

Table 1 | Jar test experimental assays

	Values	
Parameters	**TAN**	**TAN + PAM**
Stir velocity (rpm)	50	50 (5 min) and 10 (10 min)
Stir time (min)	5	15
PAM (μg L^{-1})	0	10 or 15
Average biomass (mg L^{-1})	45, 86, 122 or 165	62, 154, 244 or 338
Tannin (mg L^{-1})	11, 23, 45, 90 or 180	
Sedimentation time (min)	15	
pH	5, 6, 7, 8, or 9	

microalgae concentrations were prepared by dilution, and pH was adjusted with either HCl 0.5 mol L^{-1} or NaOH 1 mol L^{-1}.

Coagulation/flocculation tests were performed at a fixed velocity of 50 rpm for 5 min. PAM was tested in combination with TAN at concentrations of 10 and 15 μg L^{-1} to investigate its potential effect on enhancing interparticle bridging and flock size formation and sedimentation. PAM dosage was fixed at 10 μg L^{-1} for 62, 154 and 244 mg biomass L^{-1} and increased to 0.15 mg L^{-1} for 338 mg biomass L^{-1}. Jar test operational conditions for TAN + PAM were changed to an initial velocity of 50 rpm for 5 min with TAN followed by PAM addition at a stirring velocity of 10 rpm for 10 min. After the coagulation/flocculation process, the flocks were allowed to settle for 15 min. An aliquot of the supernatant was collected from half the height of the clarified layer for turbidity analysis (Hach, 2100P, Colorado, USA).

Estimation of biomass recovery efficiency

Recovery efficiency (ηa) was estimated by differences in nephelometric turbidity as follows:

$$\eta a(\%) = \left(\frac{NTU_0 - NTU}{NTU_0}\right) \times 100$$

where: NTU_0 = initial turbidity; NTU = turbidity after sedimentation.

Water recycle

The resulting supernatant water from the coagulation/flocculation process (with >95% biomass recovered) was recycled into the photobioreactor to investigate its effects on cell viability and growth. For this experiment, 10 L glass cylinder batch-type photobioreactors (18.6 cm diameter, 60 cm height) were filled with either 60% v/v fresh tap water or recycled water, 10% v/v swine wastewater digestate and 30% v/v cell inoculum (230 mg L^{-1} biomass DW). The culturing media were maintained under continuous agitation using submersible vortex type aquarium pumps (S300, Sarlo Better®, Brazil). Cell growth was estimated by OD analyses as previously described in the section on biomass concentration.

RESULTS AND DISCUSSION

Microalgae harvesting with TAN and PAM

Microalgae were harvested by chemical precipitation using TAN and PAM as coagulants (Table 2). The addition of PAM (0.5 to 1.25 mg L^{-1}) resulted in larger flocks and faster sedimentation rates (data not reported). Buelna *et al.* (1990) reported that 5 mg L^{-1} of the PAM Zetag 63 was required to reach 100% recovery of *Chlorella* sp. grown on diluted pig-waste. De Godos *et al.* (2011) showed that dosages of 25–50 mg L^{-1} of cationic PAM-based flocculants were needed for maximum biomass recovery (84–99%) of *Chlorella* sp., *Chlorococcum* sp., *Scenedesmus obliquus* and *Chlorella sorokiniana* fed with piggery wastewater. It is worth noting that the use of PAM for separation processes must be done with caution, since it contains trace amounts of toxic acrylamide, which can render the harvested microalgae biomass unsuitable as a source of animal food (Buelna *et al.* 1990). In the present study, a small dosage of 0.1 to 0.15 mg L^{-1} PAM was sufficient to attain more than 93% recovery efficiency. Whereas the use of TAN amended with PAM has been shown to increase the coagulation efficiencies of suspended solids from swine wastewater (Kunz *et al.* 2009), its use for microalgae recovery was negligible, indicating that TAN alone is sufficient to reach the desirable coagulation goals.

According to Granados *et al.* (2012), a minimum flocculant dosage required to reach at least 90% recovery efficiency should be considered. In this work, more than 93% removal efficiency was obtained for all test conditions. The minimum dosage of TAN required for recovery efficiencies above 99% was 75, 180, 285 or 360 mg L^{-1} for 62, 154, 244 or 338 mg L^{-1} of *Chlorella vulgaris* DW concentration, respectively (Table 2).

A slight decrease in pH by only 0.4 unit was observed after the coagulation/flocculation reaction (Table 2). This was not

Table 2 | Biomass recovery efficiencies (%) obtained in jar tests from different TAN and PAM dosages

Dry biomass (mg L^{-1})	Polymer dosage (mg L^{-1}) TAN	PAM	Initial pH	Final pH	Initial turbidity (NTU)	Final turbidity (NTU)	Biomass recovery (%)
62	60	0.01	7.43	7.36	47	3.1	93.5
	69		7.43	7.30		2.4	95.0
	75		7.43	7.28		0.5	98.9
	90		7.36	7.23		0.7	98.4
	105		7.36	7.22		0.7	98.5
154	120	0.01	7.24	7.02	134	9.2	93.1
	150		7.24	7.27		2.9	97.9
	165		7.24	7.08		2.6	98.0
	180		7.27	7.14		0.7	99.5
	195		7.27	7.09		0.9	99.2
244	210	0.01	7.29	7.12	248	9.8	96.1
	240		7.29	7.07		5.0	98.0
	270		7.29	6.99		1.1	99.5
	285		7.29	6.96		1.1	99.6
	300		7.29	6.94		1.5	99.4
338	300	0.015	7.36	6.99	394	5.1	98.7
	330		7.36	6.93		4.0	99.0
	360		7.36	6.95		0.9	99.8
	375		7.36	6.95		2.0	99.5
	390		7.36	6.90		2.8	99.3

surprising since natural plant-based polymers likely TAN are unlikely to significantly change the pH of treated water (Yin 2010). Therefore, pH adjustments downstream from the coagulation/flocculation process may not be required, which minimizes operational costs at a large scale.

Effect of TAN dosages on recovery efficiencies

The ionic strength of the cultivation media and the surface charge of microalgal cells have a significant effect on the optimal coagulant dose, with higher ionic strength systems requiring larger coagulant doses (Pahl *et al.* 2013). The efficacy of microalgae removal (mg cells per mg of TAN) was pronounced at greater cell concentrations (Figure 1). Maximal flock size and settling velocities were observed with increasing TAN concentrations (data not shown). The highest harvesting efficiencies were obtained with the lowest TAN dosage tested (11 mg L^{-1}; Figure 2). Other conventional natural organic based polymers, such as chitosan and cationic starch may require much higher dosages than TAN in order to achieve similar recovery efficiencies (Table 3).

For biomass concentrations of 86, 122 and 165 mg L^{-1} DW, biomass recovery efficiencies reached 90, 95 and 97%, respectively. Biomass recovery of only 50% was attained with the use of 45 mg L^{-1} biomass. The lowest TAN concentrations had the highest coagulation efficiencies. The mechanism of chemically induced microalgae flocculation based on concentration-dependent coagulation efficiency phenomena has already been previously demonstrated and discussed (Tenney *et al.* 1969). The degree of flocculation is directly associated with polymer coverage extent; that is, polyelectrolyte concentrations lower than or above optimum will result in either insufficient bridging or

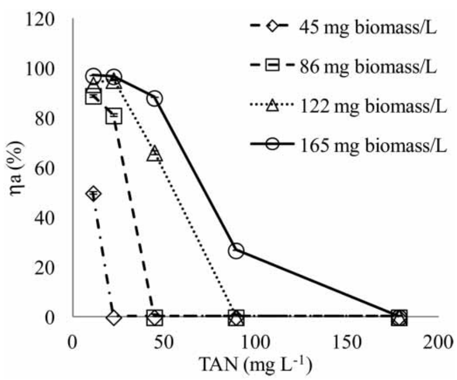

Figure 1 | Effect of TAN dosages on the coagulation/flocculation recovery efficiency (ηa) as a function of *Chlorella vulgaris* biomass concentrations (45, 86, 122 and 165 mg L^{-1} DW). Experiments conducted at pH 7. Bars represent standard errors from the mean.

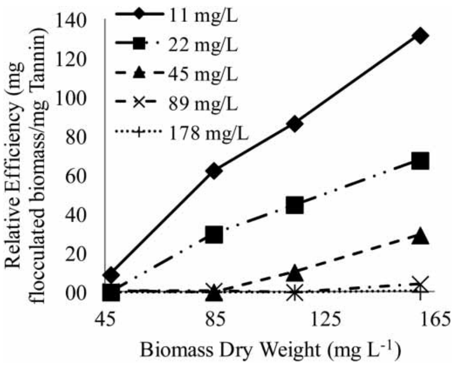

Figure 2 | Effect of different TAN dosages on coagulation/flocculation recovery efficacy (mg flocculated *Chlorella vulgaris* biomass per mg TAN).

hindered bridging. Therefore, the higher TAN concentrations utilized in this work most likely interfered with coagulation reaction by promoting a charge inversion, for example, by surface saturation and polymer excess.

It has been demonstrated that the amount of polymer demand is governed not only by biomass concentration but also by organic matter content in the medium (Vandamme *et al.* 2012). It is known that the presence of organic matter in the media can inhibit *Chlorella vulgaris* flocculation, thus requiring higher coagulant dosages (Vandamme *et al.* 2012). For instance, to achieve a minimum of 85% *Chlorella* biomass recovery from media containing 5 mg TOC L^{-1} the concentration of chitosan and the cationic starch were increased from 8 to 75 mg L^{-1} and from 0.02 to 90 g L^{-1}, respectively. Therefore, much higher dosages of chitosan and cationic starch would likely be needed to recover microalgae biomass efficiently from organic-rich swine wastewaters (digestate contains total organic carbon (TOC) of 1,500 mg L^{-1}; Mezzari *et al.* 2013). Interestingly, the increased organic matter content in the photobioreactor (75 to 300 mg TOC L^{-1} from diluted digestate) did not require an incremental TAN dosage to achieve satisfactory recovery efficiencies (Figures 1 and 2). Recovery efficiencies above 95% were achieved with only 11 mg L^{-1} TAN, even in the presence of 300 mg TOC L^{-1}. Therefore, compared to other organic polymers, the use of TAN could become an interesting alternative to harvest microalgae from organic-rich wastewaters with competitive lower operational costs (Table 3).

Effect of pH on recovery efficiencies

The effect of pH on coagulation/flocculation of microalgae using TAN at 22 mg L^{-1} was evaluated (Figure 3). Flocculation reactions with cationic tannin polymers occur via charge bonding between polymers and the surface of the algae at one or more sites, bridging cells together in a three-dimensional algal-polymer matrice (Uduman *et al.* 2010). The pH of the medium plays an important role on particles' surface charge density, thus altering flocculation efficiency (Metcalf & Eddy Inc. 1991). For biomass concentration above 86 mg/L, slightly acidic conditions (pH 5–6) resulted in higher removal efficiencies (Figure 3). Although TAN is known to be effective at pH ranging from 4.5 to 8, flocculation response is also dependent on tannin chemical modifications (Sánchez-Martín *et al.* 2009). Zeta potential and flock morphology analyses demonstrated that the removal of cyanobacteria from media with cationic TAN occurred through charge neutralization and the formation of bridges to absorb and wrap the algae cells (Wang *et al.* 2013). Therefore, low pH can contribute by negatively charging the surface of the cells, thus augmenting attraction with cationic TAN polymer. Interestingly, however, very diluted microalgal biomass (45 mg L^{-1} DW), showed the best recovery efficiencies at a slightly alkaline pH of 8 (Figure 3) probably because of variations in cell surface charge density as result of a decrease in the ionic strength of the diluted media. In this case, considering the inherent alkaline pH (near 8) of the medium in the photobioreactors treating swine wastewater digestate (Mezzari *et al.* 2013), pH adjustment prior to microalgae coagulation may not be needed.

Investigating the effects of modified tannin structures on *C. vulgaris* recovery efficiencies was beyond the scope of

Table 3 | Comparison between TAN and other conventionally used organic polymers for coagulation/flocculation of *Chlorella* sp.

Natural polymers	Best recovery efficiency (%)	pH	Dosages (mg L^{-1})	Average costs (USD m^{-3})[a]	References
Chitosan	90–99	5–7	5–200	0.1–4	Divakaran & Sivasankara Pillai (2002); Rashid *et al.* (2013); Xu *et al.* (2013)
Cationic starch	85	Not adjusted	90	0.27	Vandamme *et al.* (2012)
Tannin	99	7	11	0.09	This study

[a]Considering an exchange rate of 2.34 R$ (Brazilian Reais)/USD.

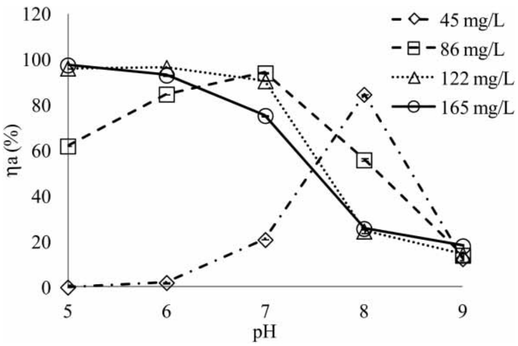

Figure 3 | Effect of pH on coagulation/flocculation recovery efficiencies (*ηa*) for different concentrations of *Chlorella vulgaris* biomass. Coagulant TAN tested at 22 mg L^{-1}. Bars represent standard error from the mean.

this work. However, it should be mentioned that *Mannich* reactions can alter TAN pH specificity and dosage requirements (Michael *et al.* 1998; Wang *et al.* 2013).

Water reuse

Turbidity values obtained at the end of coagulation/flocculation experiments (Table 2) were far below the permissible wastewater discharge of 100 NTU (see Supplemental information, available online at http://www.iwaponline.com/wst/070/307.pdf). The supernatant clear water was recycled into the photobioreactor to investigate the potential effects of residual TAN on microalgae growth. Compared to fresh culturing media, the use of recycled water had negligible effects ($p < 0.005$) on microalgae cell growth over time (Figure 4). Similar results were demonstrated for cyanobacteria (Wang *et al.* 2013). Therefore, effluent clean water could be recycled into the microalgae culturing system, thus minimizing the toxicity effects that raw digestate exerts on microalgae proliferation. Water use and costs related to microalgae biomass production are very recent (Pate *et al.* 2011; Yang *et al.* 2011). Borowitzka (1992) has performed an economic modeling of the microalgae production process for aquaculture feed purposes and has stated that water reuse after harvesting can significantly reduce water costs by 42%.

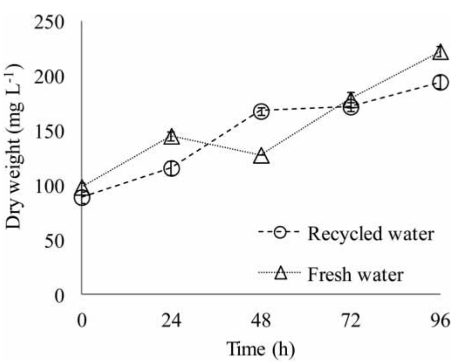

Figure 4 | Microalgae growth over time in two independent photobioreactors fed with swine wastewater digestate effluent diluted with either fresh tap water or recycled supernatant water from coagulation/flocculation process. Bars represent standard error from the mean.

CONCLUSIONS

TAN-based organic polymer served to recover *Chlorella vulgaris* efficiently from a laboratory-scale photobioreactor treating swine wastewater digestate. TAN concentrations as low as 11 mg L^{-1} were effective to recover above 95% of microalgae. A slightly acidic pH of 5 to 7 enhanced flocculation/coagulation efficiencies. The increase in organic carbon content from 75 to 300 mg L^{-1} did not require an incremental dosage of TAN to achieve recovery efficiencies above 95%. Thus, TAN could become an interesting alternative choice of organic polymer for harvesting *Chlorella* sp. from organic-rich wastewater. The very low turbidity water obtained at the end of the coagulation/flocculation process did not affect microalgae growth and thus could be recycled into the system for the dilution of toxic raw digestate.

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