

***Aloe* sp. leaf gel and water glass for municipal wastewater sludge treatment and odour removal**

Thameur Jaouadi, Mounir Hajji, Mariam Kasmi, Amjad Kallel, Abdelwaheb Chatti, Hichem Hamzaoui, Adel Mnif, Chedly Tizaoui and Ismail Trabelsi

ABSTRACT

Aloe gel (Alg), which is a natural extract from the *Aloe* sp. plant, was evaluated in this study for its potential use as a bioflocculant to treat urban wastewater sewage sludge. The gel was used alone and combined with water glass (WG) under controlled conditions in laboratory experiments. Alg was found effective to settle the flocculated sludge rapidly and remove distinctive unpleasant odours of the sludge as highlighted by gas chromatography–mass spectrometry (GC/MS) analysis.

Furthermore, Alg was pH tolerant and had no effect in changing the pH of the wastewater. The optimum dose of Alg was 3% at which a sludge volume index (SVI) of 45.4 mL/g was obtained within 30 min settling time. To enhance the treatment performances of Alg, WG was also evaluated as an alkali agent to further reduce the chemical oxygen demand (COD) and ammonia (NH₄-N) in the wastewater. At equal doses of 3% of WG and Alg each, the combined treatment outcomes showed high turbidity and NH₄-N removals of 83 and 89%, respectively, but the overall COD removal was at best 25%. The settling rate of treated sludge with combined Alg/WG was very rapid giving an SVI of 25.4 mL/g within only 5 min.

Key words | *Aloe* sp. gel, COD removal, odour removal, sewage sludge, turbidity, water glass

Thameur Jaouadi
Office National de l'Assainissement (ONAS),
Tunis, Tunisia

Thameur Jaouadi
Mariam Kasmi (corresponding author)
Abdelwaheb Chatti
Ismail Trabelsi
Laboratoire de Traitement et Valorisation des
Rejets Hydriques (LTVRH),
CERTÉ,
Technopark of Borj-Cedria, 8020, Soliman,
Tunisia
and
University of Carthage,
Avenue de la République, P.O. Box 77, 1054
Amilcar, Tunis, Tunisia
E-mail: mariam.kasmi@certe.mrt.tn

Mounir Hajji
Hichem Hamzaoui
Adel Mnif
Laboratoire de Valorisation des matériaux utiles,
Centre national des sciences et recherche de
matériaux University of Carthage,
Tunis, Tunisia

Amjad Kallel
Laboratoire Eau-Energie-Environnement (3E), Sfax
National School of Engineering,
University of Sfax,
P.O. Box 1173, 3038, Sfax, Tunisia

Chedly Tizaoui
College of Engineering, Bay Campus,
Swansea University,
Swansea SA1 8EN, UK

INTRODUCTION

In municipal wastewater treatment, sludge treatment accounts for about a third of the total wastewater treatment plant costs (Nowak 2006), making it one of the important treatment sections of the process. New cost-effective and environmentally friendly sludge treatment technologies are hence needed by wastewater undertakers. In Tunisia, where this study was carried out, the activated sludge process (AS) is the most used biological process for

wastewater treatment and the solid–liquid separation of the sludge from the treated water remains a challenge due to overload resulting in poor quality of treated wastewater (Jemli *et al.* 2015). To enhance the performance of the secondary clarifiers, addition of organic and/or inorganic flocculants is widely practiced. The coagulants/flocculants used in wastewater to aid the separation of sludge can be either inorganic such as aluminium sulphate, or chemically

synthetic organic flocculants such as polyacrylamide derivatives (Zahrim *et al.* 2010). Although these coagulants/flocculants have been successfully used for many decades in the water and wastewater sectors, there have been concerns about their environmental impacts, cost and the sustainability of over-extraction of raw materials, which are not renewable resources. The leaching of monomers from sludges treated with synthetic organic polymeric flocculants has also been highlighted as a significant barrier for the use of wastewater sludge in other areas such as agricultural applications (Abdelaal 2004). Therefore, demand for new coagulants/flocculants to improve the characteristics of the produced sludge to be safely disposed of or utilized in other sectors (e.g. land spraying) is increasing. In recent years, bioflocculants have emerged as potential substitutes to traditional inorganic and synthetic polymeric coagulants/flocculants because they are effective in treating water, generally nontoxic, harmless, less sensitive to pH changes, and are biodegradable (Giri *et al.* 2015; Liu *et al.* 2010, 2015). Bioflocculants such as *Moringa* seeds (Menkiti & Onukwuli 2010), *Lablab purpureus* peels (Shilpa *et al.* 2012), Moroccan cactus extract (Abid *et al.* 2009), dates seeds (Al-Sameraiy 2012) and *Opuntia dillenii* (Nougbo *et al.* 2013) have been investigated for turbidity removal providing removal percentages in the range 78–94%. *Moringa oleifera* seed, maize and chitosan were also used in direct filtration of lake water and were successfully evaluated for turbidity and microorganism removal as reported by Mandloi *et al.* (2004). A widely reported Indian grown natural coagulant (Nirmali seeds), Okra seeds pod tips, sap, plant stalk, and roots have also been studied (Al-Samawi & Shokralla 1996). Al-Samawi & Shokralla (1996) have used okra extract to treat clay suspensions and have concluded that the okra extract was a powerful polyelectrolyte coagulant whether it was used as a primary and/or as a coagulant aid with alum. They also confirmed that the natural okra extract performed much better than alum at high turbidity waters. *Aloe vera* has also been used in water and wastewater treatment to remove suspended solids, turbidity, chemical oxygen demand, heavy metals and textile dyes (Lee *et al.* 2015; Adugna & Gebresilasie 2018). *Aloe* leaves are well known for their mucilaginous jelly, which is referred to as *Aloe* gel (Femenia *et al.* 1999; Hamman 2008). *Aloe* gel contains mainly monosaccharides such as glucose and mannose, vitamins, minerals, polysaccharides and phenolic compounds together with some organic acids (Hamman 2008; Mazzulla *et al.* 2012). In their study on textile wastewater, Adugna and Gebresilasie (Adugna & Gebresilasie 2018) reported that *Aloe steudneri* performed

as polyacrylamides for treating the wastewater and suggested that this natural flocculant can substitute the synthetic flocculant. Nougbo *et al.* (2016) have also confirmed that leaf gels from several *Aloe* species could be used as natural flocculants for water treatment. Adsorption was suggested as the mechanism by which *Aloe* gel provides water treatment due to its high fibre content (Adugna & Gebresilasie 2018). In addition, other constituents of the gel such as glyco-aloe-modinanthrone and tannins are postulated to be responsible for the gel's coagulation property. Despite being effective to treat water, *Aloe* gel, similarly to other natural flocculants, could increase the residual organic matter in the treated water. According to literature, the chemical composition of *Aloe* plants largely depends on the species analysed but overall the organic matter can reach up to 81% of the mass of *Aloe* plants (Eugene *et al.* 2011; Radha & Laxmipriya 2015). In this study, *Aloe* gel was evaluated for the first time as a bioflocculant to enhance the gravity settling of municipal wastewater sludge. The study also highlights the increased performance of using *Aloe* gel in combination with sodium silicates to obtain faster settling velocities of the treated wastewater sludge. Several jar tests were carried out to select the optimal doses of *Aloe* gel and sodium silicates and the effects on pH changes and the volume of sludge produced.

MATERIALS AND METHODS

Sludge collection

The sludge samples were collected in two 20 L plastic jerry cans from the wasted line of a secondary settling reactor of an activated sludge process of the municipal wastewater treatment plant (Chotrana II, Tunis, Tunisia). The Chotrana treatment plant serves a population equivalent to 400,000 with a total wastewater flow of 110,847 m³ per year and serves several industries (textile, slaughterhouse and food wastewater). The average total solids concentration in the sludge is TS = 33 g/L. The sludge samples were immediately stored after collection in a fridge at 5 °C. In certain experiments, the pH of the sludge was adjusted using 1 M NaOH and 1 M HCl solutions.

Preparation of *Aloe* sp. gel

The *Aloe* sp. leaves were harvested in March 2018 from a 2-year-old *Aloe vera* plant grown in the garden of the Biotechnology Center of Borj Cedria, Soliman, Tunisia. The

gel was prepared as described by [Kasmi *et al.* \(2018\)](#). Briefly, the whole leaves were washed with a tap water and the spikes which were placed along their margins were removed before slicing the leaf to separate the skin from the filet. The resulting filets were mixed and homogenized using a hand electric blender (Moulinex, model genuine). The gel was used freshly in each experiment immediately after its preparation.

***Aloe* sp. gel analysis**

Aloe gel composition in terms of fats, sugars, proteins and minerals content was determined. Fats assessment was performed according to the methodology reported by [Hamman \(2008\)](#). Sugars were assessed using Dubois protocol ([Dubois *et al.* 1956](#)). Proteins content was determined using Bradford methodology ([Bradford 1976](#)). As for minerals, phosphorus and magnesium concentrations were determined by means of colorimetric method using vanadomolybdic complex and atomic absorption. Calcium concentration was determined using EDTA method; zinc, copper, and iron assessments were performed using atomic adsorption method ([Rodier *et al.* 2009](#)). Sodium and potassium contents were determined by the flame photometer method. Lyophilized *Aloe* sp. leaf gel was incubated in (1N) H₂SO₄ at 80°C for 30 min to extract cations. Concentrations of Cd, Zn and Fe in the extracts were determined by atomic absorption spectrophotometry (SpectrAA 220, Varian, Australia). Protocol adopted from [Zorrig *et al.* \(2010\)](#).

Preparation of water glass

Water glass (i.e. sodium metasilicate (Na₂SiO₃)) was prepared by reacting Tunisian silica sand (SiO₂ > 99%) with sodium carbonate, Na₂CO₃ (>99%, Honeywell) in a 1:1 M ratio at temperatures between 1,200 and 1,300°C as indicated by [Bouaoun *et al.* \(2017\)](#).

Coagulation and flocculation process

The coagulation and flocculation tests were carried out in a jar apparatus equipped with four steel paddles. The doses of the *Aloe* gel varied according to the values 1, 3, 5 and 7% (v/v) of sludge. The coagulation experiments were performed using a modified protocol as described by [Patil & Hugar \(2015\)](#). The coagulation step was done at high speed of 200 rpm for 2 min followed by the flocculation

step at 50 rpm for 30 min. The settling time was set to 60 min after flocculation.

Settling experiments were made using a glass column (46 cm high and 7.8 cm in diameter) in which the height of the liquid/sludge interface was recorded at regular time intervals ([Zodi *et al.* 2009](#)). The effect of pH on sludge settleability was also studied using initial pH values of 7, 11, 12 and 13 adjusted by 1 M NaOH solution.

Flocculating rate measurement

To calculate the flocculating rate of *Aloe* gel, Kaolin clay was used to make suspensions at 5 g/L in which Alg was added and stirred for 2 min. After settling for 1 min, the absorbance at a wavelength of 550 nm of the supernatant sample was measured by a spectrophotometer UV/VIS Lambda 25 (PerkinElmer). The flocculating rate was calculated according to Equation (1) ([Liu *et al.* 2010](#)), where A₁ is the absorbance of the supernatant sample at 550 nm and A₀ is the absorbance of the control at 550 nm.

$$\text{Flocculating rate} = (A_0 - A_1)/A_0 \times 100\% \quad (1)$$

Analytical methods

The total solids content (TS), suspended solids (SS) and volatile suspended solids (VSS) were measured according to the standard methods described by [Rodier *et al.* \(2009\)](#). The total organic carbon (TOC) was measured using a total carbon analyzer Shimadzu TOC 5050. The total nitrogen and phosphorus contents were determined following the standard methods proposed by [Rodier *et al.* \(2009\)](#). The pH, conductivity (mS/cm) and total dissolved solids (TDS) (g/L) of each sample were determined using a multi-parameter instrument (C860, Consort bvba, Belgium). Chemical oxygen demand (COD) values were measured by the potassium dichromate colorimetric method using an open reflux system ([Rodier *et al.* 2009](#)). Ammoniacal nitrogen was determined according to the NF T90-15 method (AFNOR). Sludge settling ability is expressed by means of the sludge volume index (SVI) which is often recommended for the characterization of the sludge formation ([Tchobanoglous *et al.* 2003](#); [Zodi *et al.* 2009](#); [Kallel *et al.* 2017](#)). The SVI is defined by Equation (2).

$$\text{SVI} = \frac{H_{30}}{H_0\text{SS}} 1000 \text{ (mL/g)} \quad (2)$$

where H_{30} is the sludge height after 30 min settling (cm), H_0 the initial height of the sludge in the settling column (cm) and SS is the initial sludge concentration after treatment (g/L).

Odour's measurement

The odour from raw and treated sludge were qualitatively assessed by the mean of GC-MS. Four samples identified as raw sludge (control), sludge treated with Alg, sludge treated with water glass and sludge treated with *Aloe* gel (3%) plus water glass (3%), were collected and sealed in 250 mL glass. 100 mL of raw and treated sludge were used as reactor for 24 h incubation at room temperature. All samples were subjected to VOCs analysis.

Volatile organic compounds (VOCs) were analyzed using a headspace (TELEDYNE TEKEMAR HT3TM) coupled with an Agilent GC-MS system (GC with 7890A, mass detector 5975C with Triple-Axis, insert XL MSD). A 30 mL headspace vial was used for incubation of the sample performed during 30 min in headspace oven at 50 °C, then transferred in a heated line at 85 °C to avoid condensation of VOCs and injected in the GC inlet for 2 min. An HP-5 ms (5% phenylmethylsiloxane) column (Agilent 19091S-433: 2169.66548) was used (30 m × 250 μm × 0.25 μm) at 1.6 mL/min flow with helium N60 (99.9999%) as carrier gas and the run was performed over 25 min. The temperature programme of the oven was: 70 °C for 2 min, then, 230 °C for 20 min and 270 °C for 25 min. The inlet had the following characteristics: temperature of 250 °C, helium gas carrier, split flow of 20 mL/min, splitless split/splitless inlet: splitless injection mode, 60.688 kPa as inlet pressure, 50 mL/min and 2 min for purge flow and time, respectively; gas saver on and 20 mL/min for gas saver flow and 2 min for gas saver time. In this analysis, the used MS had the following specifications: ms quadrupole at 150 °C, from 50 to 550 m/z full scan, 70 eV ion ionized energy, source and transfer line temperature at 250 °C. The volatile compounds were identified by reference to mass spectra and the retention time of Wiley09 NIST2011 library.

Particle size distribution was determined with a Master-sizer 2000 laser light scattering instrument (Malvern Instruments Ltd, UK) as reported by Rahsepar et al. (2017). The samples of treated and untreated sludge were observed qualitatively with a scanning electron microscope (SEM) (FEI Quanta FEG 650).

RESULTS AND DISCUSSION

Sludge characterization

The main characteristics of the collected sludge are summarized in Table 1. The results reveal that the mean value of 7.15 for pH, 11,200 mg O₂/L for COD and 16 g/L for SS. VSS content was 2.6 g/L, the TOC was about 473 mg/kg and the ammoniacal nitrogen and phosphorus contents were 400 mg/L and 6.53 mg/kg, respectively. Compared to a similar sludge reported in Ramirez et al. (2018), we observed a low carbon-nitrogen content of Tunisian sludge and quite high level of COD which might be attributed to the industrial effluent co-treated at the Chotrana wastewater treatment plant.

Aloe sp. gel characterization

In order to provide meaningful discussion on the effect of the bio-flocculants on the sludge treatment, the *Aloe* sp. leaf gel characteristics were determined (Table 2). The analysis revealed an important amount of soluble sugars of about 22.8 g/100 g of lyophilized gel and high content of proteins (7.8 g/100 g). Fats minerals and metals were also detected. Table 2 illustrates other plants composition such as *Moringa oleifera* seeds and cactus (*Opuntia ficus indica*) plants, that various studies pointed out the importance of their use as flocculant, coagulant or coagulant/flocculants aid for the removal of turbidity, COD and heavy metal (Lopez-cervantes et al. 2011; Leone et al. 2016; Ben Rebah & Siddeeg 2017). As it can be easily noticed, *Aloe* sp. fat, protein and total sugars contents are always in the range of the other plants' values.

Table 1 | Characteristics of sludge generated by Chotrana municipal wastewater treatment plant

Measured parameters	Unit	Recorded values
pH	–	7.15 (at 25 °C)
Moisture content	%	84 ± 1
Total solids content (TS)	g·L ⁻¹	33 ± 1.2
Suspended solids (SS)	%	16 ± 0.5
VSS	g·Kg ⁻¹	2.6 ± 0.5
TOC	mg·Kg ⁻¹	473 ± 5
Ammoniacal-nitrogen	mg·L ⁻¹	400 ± 0.5
Total phosphorus	mg·Kg ⁻¹	6.53 ± 0.05
COD	mg O ₂ L ⁻¹	11,200 ± 10

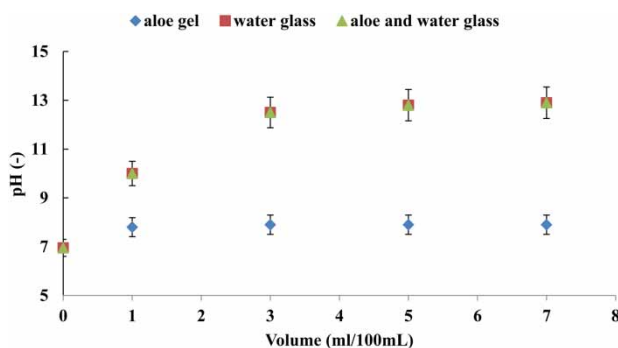
Table 2 | *Aloe* leaf gel biochemical characterization compared to *Moringa oleifera* seeds and cactus (*Opuntia ficus indica*) flours

Content (g/100 g)	Lyophilized <i>Aloe</i> sp. leaf gel	<i>Moringa oleifera</i> seeds (Leone et al. 2016)	Cactus flours (Lopez-Cervantes et al. 2011)
Fats	4.8	36.7	2.38
Soluble sugars	22.78	18.4	67.6
Proteins	7.82	31.4	7.24
Ca	3.02	–	–
Mg	0.98	–	–
Na	3.03	–	–
K	3.89	–	–
P	0.01	–	–
Fe	0.87	–	–
Cu	0.04	–	–
Zn	0.01	–	–

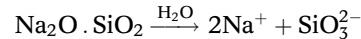
Treatment of sludge with *Aloe* gel and water glass

Effect of Alg and water glass addition on sludge's pH

Figure 1 illustrates the effect of the *Aloe* sp. gel (Alg) addition on the sludge's pH. The figure also shows the effect of adding water glass (WG) alone or combined with Alg (Alg-WG) on pH values. Following the addition of 3 mL/100 mL Alg, the pH showed only a modest increase from 6.95 to 7.9 to remain constant at 7.9 regardless of the increased amount of Alg added. However, the addition of 3 mL/100 mL water glass exhibited a remarkable increase in pH from 6.95 to 12.5. A further increase in WG has increased pH modestly to reach a value of pH 13 after adding 7 mL/L of water glass. In fact, the alkaline character of sodium silicate is the main cause of pH increase as shown

**Figure 1** | pH values variation following the *Aloe* sp. leaf gel (Alg), water glass and *Aloe* + water glass addition to sludge at different volume.

by the reaction equation:

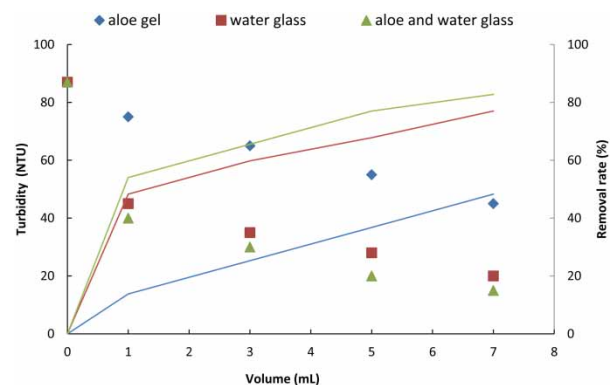


Effect on turbidity removal

Figure 2 shows the turbidity changes versus the added volumes of Alg and WG separately and in combination. The results show that as the added volume increases, the turbidity decreases. According to Figure 3, Alg was less effective than water glass to remove turbidity (Alg: 45%; WG: 89%). This could be attributed to the formation of calcium silicate as main precipitate following the reaction of water glass (sodium silicate) and calcium content in the sludge (Table 1). The obtained precipitate had a potential to adsorb fine particles, making the supernatant free of particles.

Effect of Alg and WG addition on SS and VSS

Figure 3 illustrates the effect of the different doses of *Aloe* gel and water glass added separately or in combination on both SS and VSS removal. The obtained results show that the SS content increased instead of being reduced. However, no significant effect was noticed for VSS removal with both treating agents used separately or combined. The progressive addition of WG in the sludge at contents higher than 1% induced a reestablishment of the SS content to its original value (~20.3 g/L) after a drop to 14.5 g/L at 1% WG (Figure 3(b)). In order to investigate the combined effect of *Aloe* gel and WG addition on the sludge treatability, further experiments were carried out at fixed Alg content of 3% while WG dosages were

**Figure 2** | Turbidity variation with different volume of *Aloe* sp. leaf gel (Alg), water glass and *Aloe* gel + water glass, removal rate.

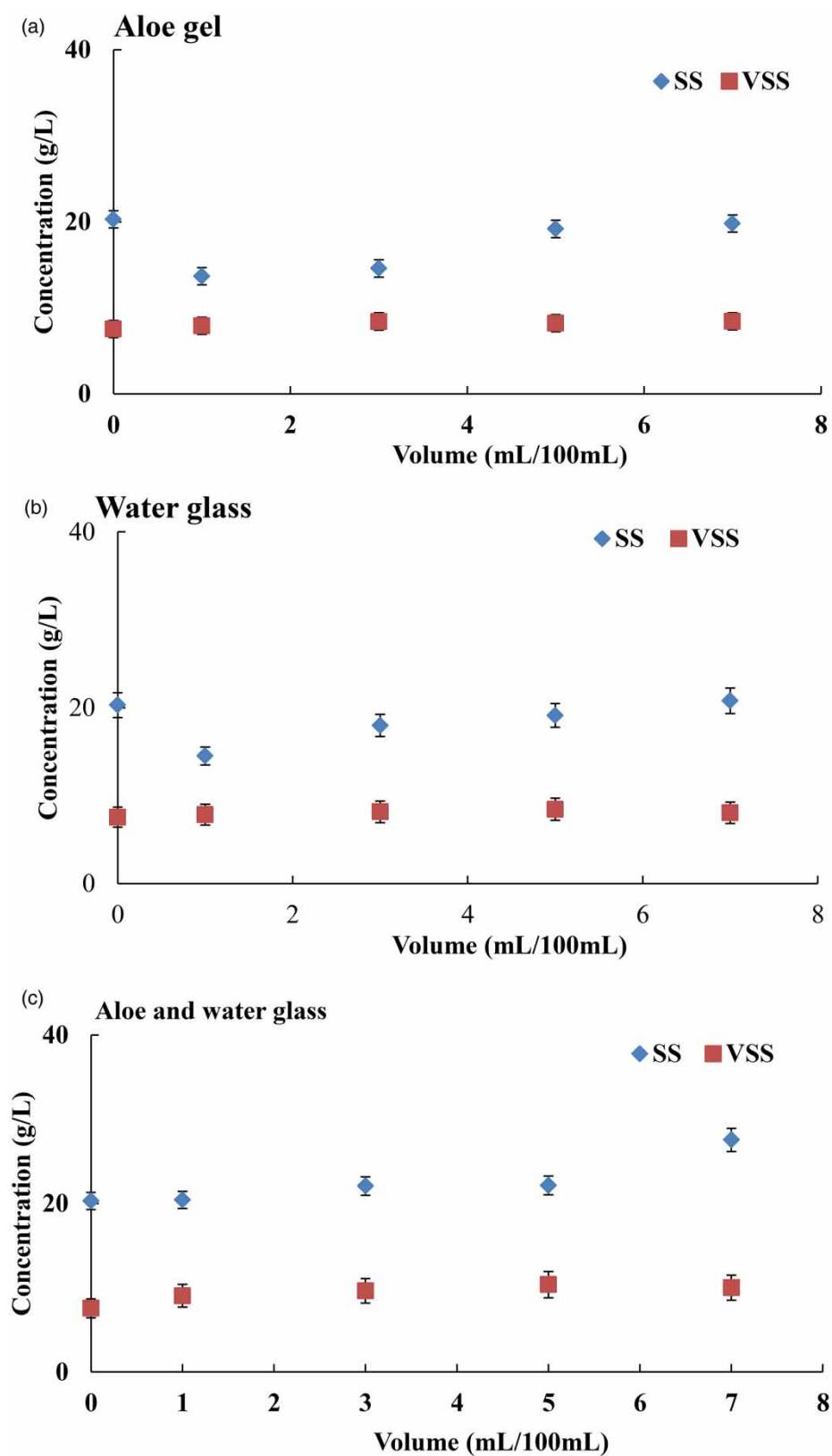


Figure 3 | Variation of suspended solid (SS) and volatile suspended solid (VSS) of sludge following the application of (a) *Aloe* sp. leaf gel (Alg), (b) water glass and (c) *Aloe* + water glass.

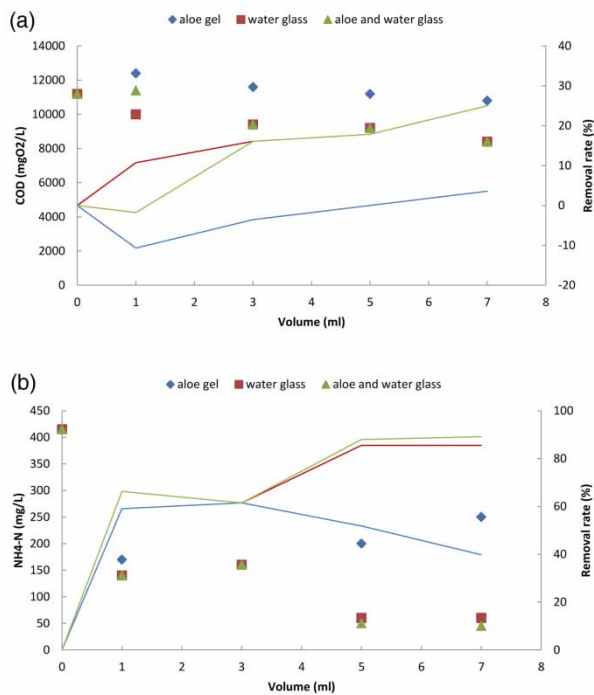


Figure 4 | Variation of COD (a) and ammoniacal nitrogen (b) with volume of *Aloe* sp. leaf gel (Alg), water glass and *Aloe* + water glass removal rates.

varied from 1 to 7%. The more WG was added, the higher the SS content was recorded (ranging from 20.4 g/L at 1% WG dosage to reach 27.5 g/L at 7% WG). The VSS values recorded a moderate increase from 9 g/L at 1% to 10 g/L at 7% (Figure 3(c)).

The mechanistic of flocculation/coagulation as provoked by *Aloe* gel was reported by many studies. In fact, gels from *Aloe* species are composed of low protein and lipid contents, and polysaccharide is considered as the main ingredient. Interestingly, *Aloe* species are known by the polysaccharide mucilage production. Furthermore, the presence of minerals, such as Ca²⁺ and K⁺, is necessary for the gelatinous properties of mucilage (gel) (Hamman 2008). The *Aloe* sp. coagulation/flocculation mechanism could be interpreted as hypothesis by analogy to the cactus, where the high flocculation/coagulation capacity of cactus was mainly attributed to its polysaccharide structure that is composed of various carbohydrates, such as L-arabinose, D-galactose, L-rhamnose, D-xylose and galacturonic acid (Vijayaraghavan et al. 2011). In this context, it was reported that galacturonic acid is significantly implicated as the main active coagulant agent, based on its polymeric structure. This polymeric structure provides a bridge for particles to adsorb. Moreover, the functional

groups of cactus polysaccharides included carboxyl (-COOH), hydroxyl (-OH) and amino or amine (-NH₂) groups, as well as hydrogen bonds. These functional groups are considered as preferred groups for the flocculation process (Sepúlveda et al. 2007).

Effect of Alg and WG addition on COD and NH₄-N

Figure 4 illustrates the changes of COD and ammonia nitrogen concentrations with the added volume of *Aloe* gel and water glass separately and combined. At low doses, the recorded data reveal a slight increase of COD value when *Aloe* gel was added to the sludge (Figure 4(a)). Similar observations were also reported by Ramavandi & Farjadfard (2014). This increase in COD following the addition of Alg was expected due to the organic matter content of *Aloe* gel which is rich in organic substances (e.g. carbohydrates and proteins). Yet, above 1 mL/100 mL of added Alg, the COD values of the solution showed a decreasing trend but the overall COD of the solution remains higher than the initial COD of the sludge until the applied dose of Alg exceeded 5 mL/100 mL Alg. A further increase in Alg doses above 5 mL/100 mL results in COD values slightly lower than the original COD value of the sludge. In contrast, the addition of WG alone or combined with Alg resulted in a much more effective COD removal than Alg reaching a removal percentage of about 25% at a dose of 7 mL/100 mL water glass.

Figure 4(b) shows the removal of NH₄⁺-N at different doses of Alg and WG. According to Figure 5(b), the addition of Alg and WG alone or combined at low doses resulted in a significant reduction in ammonia. At a dose of 1 mL/100 mL, the removal of NH₄⁺-N was 66% for all agents to reach 89% at 7 mL/100 mL WG. A further increase in Alg dose above 1 mL/100 mL did not increase NH₄⁺-N removal but it made it worse (only 40% removal at 7 mL/L), possibly due to a competition with COD removal as illustrated in Figure 4(a) where COD removal with *Aloe* gel becomes relevant only at high doses. In addition, Alg active constituents (e.g. proteins) may aggregate and fold at high doses (Gupta et al. 1998), which reduces the number of active functional groups available for NH₄⁺ accumulation and adhesion. The action of water glass was much more pronounced since its alkali character changes ammonium to ammonia gas (pKa = 9.25 at 25 °C) that can be easily removed from solution via the increase of pH to 12.9 (Figure 1), which justifies the high ammonia removal of 89% obtained following the addition of WG.

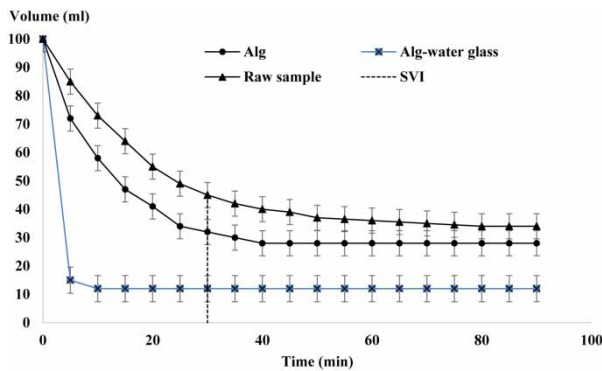


Figure 5 | Effect of Aloe gel (●) and combined Aloe gel-water glass (■) addition on the treated sludge compared to the raw sample (▲) during 90 min.

Performances of Aloe gel and water glass on sludge settling

Figure 5 depicts the sludge settling performance for Alg and for combined Alg-WG treatments as well as the raw sludge without any coagulant being added. The SVI values are also indicated in Figure 5. The combined Alg-WG treated sample exhibited a net drop from 100 mL to reach 15 mL within only 5 min. The settled volume stabilized at 10 mL after 10 min until the end of the experiment (90 min). The Alg treated sample settled volume was limited to 28 mL after 40 min or higher settling times. However, it is noteworthy that both Alg and combined treatments recorded better results compared to the settling of raw samples without addition of any of the coagulants; where, at best, a final settling sludge volume of 34 mL was recorded after 80 min settling. The effect of the optimal dosages of the combined treatment of sludge after settling using 3% of Alg and 3% of WG dosages showed a net difference of settling rates (Supplementary Material).

Figure 6 gives the particle size distribution of the untreated sludge, sludge with Aloe gel and sludge with both Aloe gel and water glass. Particle size shifts are clearly observed for the Alg-WG treated sludge. The sludge particles shifted from fine sizes ($\sim 100 \mu\text{m}$) to larger particles and the agglomerates were found to have a bimodal distribution

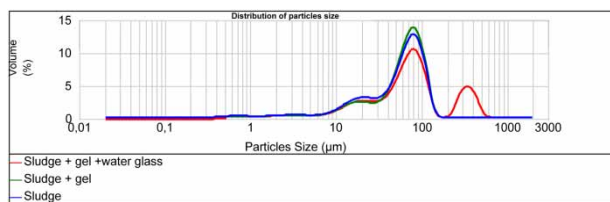


Figure 6 | Raw and treated sludge particle master size distribution.

with a mean size value of $300 \mu\text{m}$. This might explain the accelerated settling velocity observed in the presence of WG. The increased particle size as a result of WG addition could be explained by the formation of large aggregates resulting from the interaction between silicate polymers

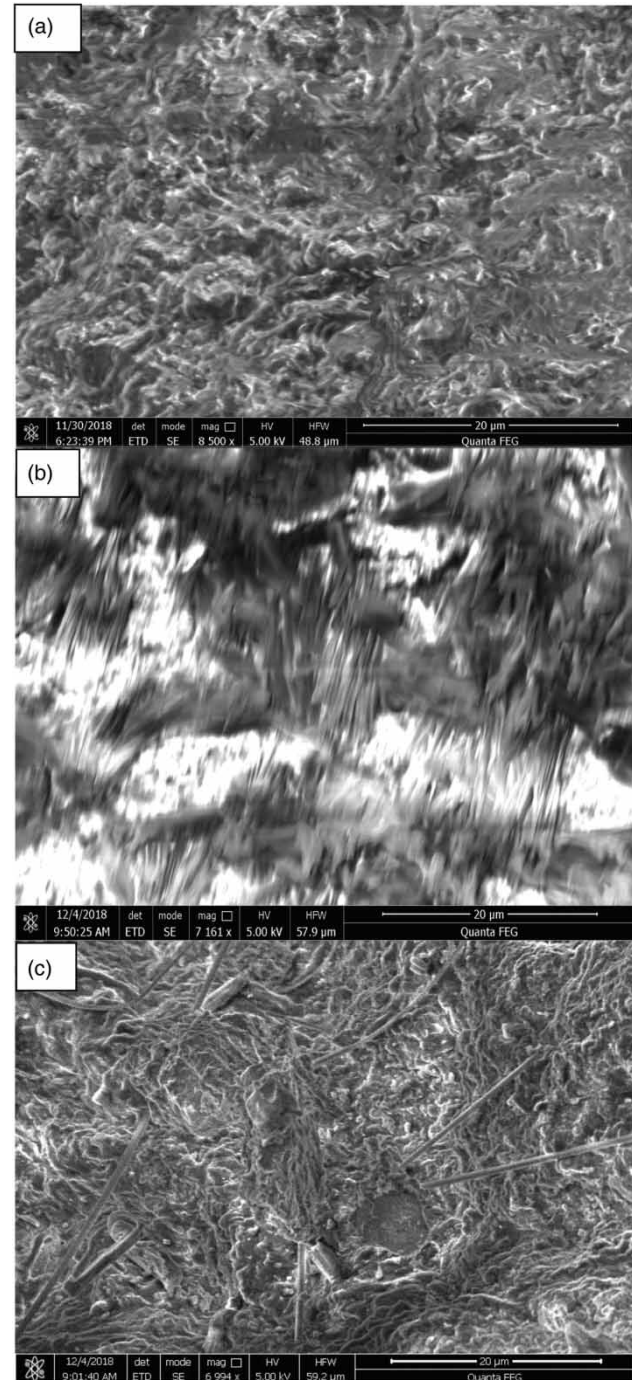


Figure 7 | SEM image of raw sludge (a), and Aloe gel (b) combined Aloe gel and water glass (c) treated sludge.

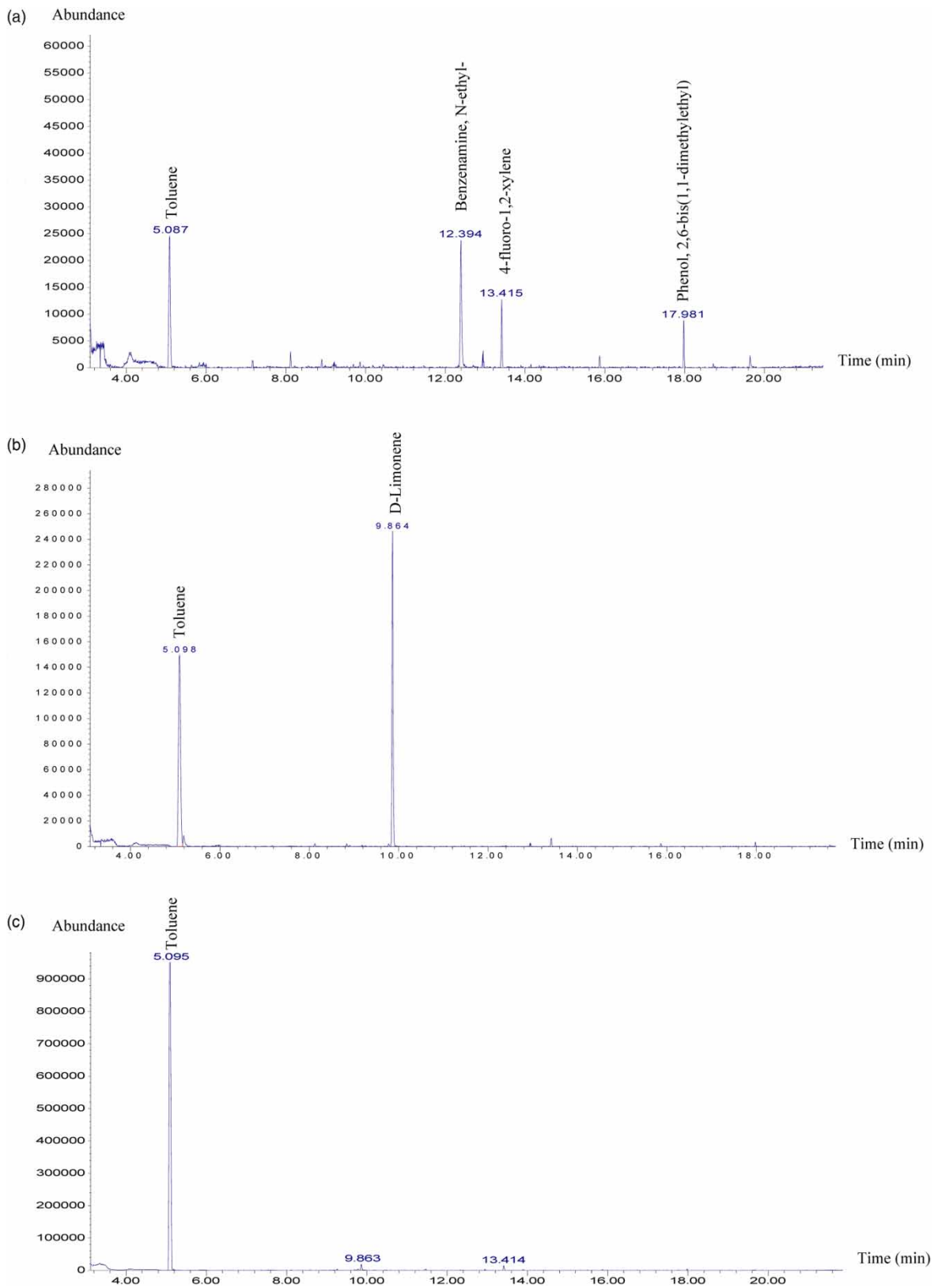


Figure 8 | Mass spectra of VOCs from the untreated sludge (a) and the treated sludge using *Aloe* sp. leaf gel (b) and water glass (c).

(i.e. WG) and sludge with possible ion binding with inorganics in the wastewater (e.g. Ca^{2+}) (Okano *et al.* 2015; Zuo *et al.* 2015).

The SEM images (Figure 7) show that the structures of the raw sludge and the treated sludge with Alg-WG have an abundance of micro pores. The SEM images suggest that significant changes in the floc's morphology occurred following the addition of Alg-WG as evidenced by the formation of large aggregates showing pillars of 10–20 μm of length between aggregates in the presence of Alg-WG. The observed building structure might be attributed to calcium silicate due to the reaction of silicates in the water glass with calcium in the sludge (Okano *et al.* 2015).

The outcome of this novel combined treatment of wastewater sludge by addition of *Aloe* gel and water glass highlighted the clear macroscopic aspect of coarse agglomerates of sludge with raw sludge having suspended fine particles.

The activated sludge flocs are known to have a complex and heterogeneous composition. Furthermore, the flocs characteristics (size, structure, etc.) vary widely according to the surrounding environment variation which could affect their dewaterability mainly the size distribution and the presence of small particles. Next to bacteria, the flocs contain extracellular polymeric substances (EPS) and various inorganic and organic molecules. Water represents the main component of the microbial aggregates, followed by the EPS and biomass. The EPS embody a highly hydrated matrix reaching 98% of water content (Keiding *et al.* 2001). Consequently, considering the reported *Aloe* gel compositions and the involvement of its ingredients in the flocculation/coagulation process, the preeminent role of *Aloe* gel in the flocs formation mechanism could be considered. Moreover, it has been reported that *Aloe* gel can influence sludge floc characteristics, including: morphological aspects; physical properties; and chemical constituents of polymeric substances and metallic ions.

Performances of *Aloe* gel on sludge odour removal

The mass spectrum of VOCs emitted by the untreated sludge is illustrated in Figure 8(a). The analysis allowed the detection of four compounds identified as toluene (peak 1), benzenamine, N-ethyl- (peak 2), 4-fluoro-1,2-xylene (peak 3) and phenol, 2,6-bis(1,1-dimethylethyl) (peak 4) with retention times (min) of 5.087, 12.394, 13.415 and 17.981 and relative areas (%) of 41.03, 38.34, 12.76 and 7.86, respectively. Figure 8(b) and 8(c) illustrate

the mass spectra of VOCs emitted by the treated sludge using Alg and WG, respectively. The spectra show that the addition of either Alg or WG results in the disappearance of peaks 2, 3 and 4 initially detected among the untreated sludge VOCs. Nevertheless, a new compound identified as D-limonene with retention time (min) of 9.867 and relative area of 48.64% was detected after Alg addition. Limonene is one of the most widespread terpenes in the flavour and fragrance industry (Zodi *et al.* 2009). A human olfactory sensing experiment was carried out and showed that the twenty people did not detect any unpleasant smell from the treated sludge, which provides a further evidence of the removal of odour following the application of Alg.

CONCLUSION

This study presented a new insight of the potential use of *Aloe* gel for the treatment of municipal wastewater sludge as a novel bioflocculant. *Aloe* sp. leaf gel, at different doses (1, 3, 5 and 7%), was tested as bioflocculant via sequential treatments that used coagulation, flocculation and sedimentation processes under defined operating conditions. The *Aloe* gel showed good performance for sludge solid-liquid separation. To enhance the *Aloe* gel action, a further step was necessary by the addition of water glass. The combined treatment showed that the use of *Aloe* gel and water glass at 3% yielded a removal of turbidity up to 83%, and 89% of ammonia nitrogen. Moreover, the resulting flocs from the treatment using *Aloe* gel and water glass generate much coarser particles and readily settling sludge, typically with only 5 min of settling time. SEM investigation enabled the observation of multi pillars of 10 to 20 μm length located within the sludge intra particles. Furthermore, the *Aloe* leaf gel odour removal efficiency was revealed through VOCs analysis of the treated sludge which indicated the disappearance of odour-causing substances following application of *Aloe* gel or water glass. The proposed process would be an attractive approach not only to reduce the treatment cost but also to minimize environmental concerns and generate eco-friendly sludge.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at <https://dx.doi.org/10.2166/wst.2020.123>.

REFERENCES

- Abdelaal, A. M. 2004 Using a natural coagulant for treating wastewater. In: *Paper presented at the Eighth International Water Technology Conference Alexandria, Egypt*.
- Abid, A., Zouhri, A. & Ider, A. 2009 Utilisation d'un nouveau bio-floculant extrait de cactus marocain dans le traitement des rejets chargés de chrome(VI) par le procédé de coagulation floculation. *Afriq Sciences* **5**, 25–35.
- Aduana, A. T. & Gebresilasie, N. M. 2018 *Aloe steudneri* gel as natural flocculant for textile wastewater treatment. *Water Practice and Technology* **13**, 495–504.
- Al-Samawi, A. A. & Shokralla, E. M. 1996 An investigation into an indigenous natural coagulant. *Journal of Environmental Science and Health Part A: Environmental Science and Engineering and Toxicology* **31**, 1881–1897.
- Al-Sameraiy, M. 2012 A novel water pretreatment approach for turbidity removal using date seeds and pollen sheath. *Journal of Water Resource and Protection* **4**, 79–92.
- Ben Rebah, F. & Siddeeg, S. M. 2017 Cactus an eco-friendly material for wastewater treatment: a review. *Journal of Materials and Environmental Sciences* **8**, 1770–1782.
- Bouaoun, I., Hammi, H., Aït-Mokhtar, A., El Amine Hamami, A. & M'nif, A. 2017 Effect of calcination temperature of magnesium silicate on the properties of magnesium phosphate cement. *Journal of the Australian Ceramic Society* **53**, 351–359.
- Bradford, M. 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–256.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. 1956 Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350–356.
- Eugene, S., Nidiry, J., Giriya, G. & Loksha, A. 2011 Antifungal activity of some extractives and constituents of *Aloe vera*. *Research Journal of Medicinal Plants* **5**, 196–200.
- Femenia, A., Sánchez, E. S., Simal, S. & Rosselló, C. 1999 Compositional features of polysaccharides from *Aloe vera* (*Aloe barbadensis* Miller) plant tissues. *Carbohydrate Polymers* **39**, 109–117.
- Giri, S. S., Harshiny, M., Sen, S. S., Sukumaran, V. & Park, S. C. 2015 Production and characterization of a thermostable bioflocculant from *Bacillus subtilis* F9, isolated from wastewater sludge. *Ecotoxicology and Environmental Safety* **121**, 45–50.
- Gupta, P., Hall, C. K. & Voegler, A. C. 1998 Effect of denaturant and protein concentrations upon protein refolding and aggregation: a simple lattice model. *Protein Science: A Publication of the Protein Society* **7**, 2642–2652.
- Hamman, J. H. 2008 Composition and applications of *Aloe vera* leaf gel. *Molecules* **13**, 1599–1616.
- Jemli, M., Sabbahi, S. & Ben Ayed, L. 2015 Performance of urban wastewater treatment of four activate sludge treatment plants in Tunisia. *International Journal of Water and Wastewater Treatment* **1**, 1–4.
- Kallel, A., Attour, A. & Trabelsi, I. 2017 Electro-coagulation treatment of raw and autoclaved landfill leachate with aluminum electrodes: case study of Djebel Chakir (Tunisia). *Arabian Journal of Geosciences* **10**, 85–92.
- Kasmi, M., Elleuch, L., Abidi, H., Cherni, Y., Hosni, C., Nouioui, S., Fattouch, S. & Trabelsi, I. 2018 Use of dairy reject and fermented *Aloe sp.* leaf gel mixture in the biological pre-treatment of landfill leachate. *Water Practice and Technology* **13**, 219–228.
- Keiding, K., Wybrandt, L. & Nielsen, P. H. 2001 Remember the water: a comment on EPS colligative properties. *Water Science and Technology* **43**, 17–23.
- Lee, K. E., Hanafiah, M. M., Halim, A. A. & Mahmud, M. H. 2015 Primary treatment of dye wastewater using *Aloe vera*-aided aluminium and magnesium hybrid coagulants. *Procedia Environmental Sciences* **30**, 56–61.
- Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J. & Bertoli, S. 2016 *Moringa oleifera* seeds and oil: characteristics and uses for human health. *Molecular Sciences* **17**, 2141.
- Liu, W., Wang, K., Li, B., Yuan, H. & Yang, J. 2010 Production and characterization of an intracellular bioflocculant by *Chryseobacterium daeguense* W6 cultured in low nutrition medium. *Bioresource Technology* **101**, 1044–1048.
- Liu, C., Wang, K., Jiang, J.-H., Liu, W.-J. & Wang, J.-Y. 2015 A novel bioflocculant produced by a salt-tolerant, alkaliphilic and biofilm-forming strain *Bacillus agaradhaerens* C9 and its application in harvesting *Chlorella minutissima* UTEX2341. *Biochemical Engineering Journal* **93**, 166–172.
- Lopez-Cervantes, J., Sanchez-Machado, D. I., Campas-Baypoli, O. N. & Bueno-solano, C. 2011 Functional properties and proximate composition of cactus pear cladodes flours. *Ciencia E Tecnologia de Alimentos* **31**, 654–659.
- Mandloi, M., Chaudhari, S. & Folkard, G. K. 2004 Evaluation of natural coagulants for direct filtration. *Environmental Technology* **25**, 481–489.
- Mazzulla, S., Sesti, S., Schella, A., Perrotta, I., Anile, A. & Drogo, S. 2012 Protective effect of *Aloe vera* (*Aloe barbadensis* Miller) on erythrocytes anion transporter and oxidative change. *Food and Nutrition Sciences* **3**, 1697–1702.
- Menkiti, M. C. & Onukwuli, O. D. 2010 Coag-flocculation of moringa oleifera coagulant (MOC) in brewery effluent: nephelometric approach. *Journal of American Science* **6**, 788–806.
- Nougbo, Y. A. E. I., Agbangan, C. P., Koudoro, A. Y., Dèdjiho, C. A., Aïna, M. P., Mama, D. & Sohounhlou, D. C. K. 2013 Evaluation of the *Opuntia dillenii* as natural coagulant in water clarification: case of treatment of highly turbid surface water. *Journal of Water Resource and Protection* **5**, 1242–1246.
- Nougbo, Y. A. E. I., Sessou, P., Alassane, A., Youssao, A. K. A., Agbangna, C. P., Mama, D. & Sohounhlou, K. C. D. 2016 Evaluation of *Aloe vera* leaf gel as a natural flocculant: phytochemical screening and turbidity removal trials of water by coagulation flocculation. *Research Journal of Recent Sciences* **5**, 9–15.
- Nowak, O. 2006 Optimizing the use of sludge treatment facilities at municipal WWTPs. *Journal of Environmental Science and Health (Part A)* **41**, 1807–1817.

- Okano, K., Miyamaru, S., Kitao, A., Takano, H., Aketo, T., Toda, M., Honda, H. & Ohtake, H. 2015 [Amorphous calcium silicate hydrates and their possible mechanism for recovering phosphate from wastewater](#). *Separation and Purification Technology* **144**, 63–69.
- Patil, C. & Hugar, M. 2015 Treatment of dairy wastewater by natural coagulants. *International Research Journal of Engineering and Technology* **2**, 1120–1125.
- Radha, M. H. & Laxmipriya, N. P. 2015 [Evaluation of biological properties and clinical effectiveness of Aloe vera: a systematic review](#). *Journal of Traditional and Complementary Medicine* **5**, 21–26.
- Rahsepar, S., Langenhoff, A. A. M., Smit, M. P. J., van Eenennaam, J. S., Murk, A. J. & Rijnaarts, H. H. M. 2017 [Oil biodegradation: interactions of artificial marine snow, clay particles, oil and Corexit](#). *Marine Pollution Bulletin* **125**, 186–191.
- Ramavandi, B. & Farjadfard, S. 2014 [Removal of chemical oxygen demand from textile wastewater using a natural coagulant](#). *Korean Journal of Chemical Engineering* **31**, 81–87.
- Ramirez, K., Possan, E., Bittencourt, P., Carneiro, C. & Colombo, M. 2018 [Physico-chemical characterization of centrifuged sludge from the Tamanduá water treatment plant](#). *Matéria (Rio J.)* **23**. doi:10.1590/s1517-707620180003.0521.
- Rodier, J., Legube, B., Merlet, N. & Brunet, R. 2009 *Eaux Résiduaires*. In: *L'analyse de l'eau: Eaux naturelles, eaux résiduaires, eau de mer* (J. Rodier ed.). Dunod, Paris, France, pp. 987–991.
- Sepúlveda, E., Sáenz, C., Aliaga, E. & Aceituno, C. 2007 [Extraction and characterization of mucilage in Opuntia spp.](#) *Journal of Arid Environments* **68**, 534–545.
- Shilpa, B. S., Akanksha, K. & Girish, P. 2012 [Evaluation of cactus and hyacinth bean peels as natural coagulants](#). *International Journal of Chemical and Environmental Engineering* **3**, 187–191.
- Tchobanoglous, G., Burton, F. L. & Stensel, H. D. 2003 *Wastewater Engineering: Treatment and Reuse*, 4th edn. Metcalf & Eddy Inc., New York, USA.
- Vijayaraghavan, G., Sivakumar, T. & Kumar, A. V. 2011 [Application of plant based coagulants for waste water treatment](#). *International Journal of Advanced Engineering Research & Studies* **1**, 88–92.
- Zahrim, A. Y., Tizaoui, C. & Hilal, N. 2010 [Evaluation of several commercial synthetic polymers as flocculant aids for removal of highly concentrated C.I. Acid Black 210 dye](#). *Journal of Hazardous Materials* **182**, 624–630.
- Zodi, S., Potier, O., Lapicque, F. & Leclerc, J.-P. 2009 [Treatment of the textile wastewaters by electrocoagulation: effect of operating parameters on the sludge settling characteristics](#). *Separation and Purification Technology* **69**, 29–36.
- Zorrig, W., Zaigham Shahzada, A., Abdelly, C., Davidian, J. C. & Berthomieu, P. 2010 [Identification of three relationships linking cadmium accumulation to cadmium tolerance and zinc and citrate accumulation in lettuce](#). *Journal of Plant Physiology* **167**, 1239–1247.
- Zuo, M. Y., Renman, G., Gustafsson, J. P. & Renman, A. 2015 [Phosphorus removal performance and speciation in virgin and modified argon oxygen decarburisation slag designed for wastewater treatment](#). *Water Research* **87**, 271–281.

First received 17 October 2019; accepted in revised form 8 March 2020. Available online 19 March 2020