Application of *Aloe vera* mucilage as bioflocculant for the treatment of textile wastewater: process optimization

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ABSTRACT

Aloe vera is an important commodity plant which has been traditionally used for the treatment of various diseases. This study investigated the use of extracted bioflocculant from *Aloe vera* for the treatment of textile wastewater. The bioflocculant was extracted, purified and characterized using GC-MS, FTIR, SEM, AFM, EDX and XRD analysis. It was mainly composed of carbohydrate (19.5%) and protein (6.0%). Box-Behnken design (BBD), using 3 level-3 variables, was employed to enhance the decolorization process by optimizing the effect of various factors. A significant enhancement from 62.50 ± 0.1 to 82.01 ± 0.8% in decolorization of wastewater was observed under optimized conditions viz. bioflocculant dosage (60 mg/L), pH (5.0) and contact time (180 min). A quadratic polynomial model was adequate beside the actual statistics at an R^2 value of 0.99 for the response decolorization % and was in good agreement with the predicted value (82.01 ± 0.1%) obtained by the RSM model. The results of the present investigation demonstrated that *Aloe vera* mucilage can serve as a promising bioflocculant with high removal efficiency for solids, colour and dye from wastewater. To the best of our information, this is the first report on the use of *Aloe vera* mucilage as a natural bioflocculant for the treatment of dye-bearing wastewater.

Key words | *Aloe vera*, bioflocculant, Box Behnken Design (BBD), decolorization, flocculation efficiency, textile wastewater

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HIGHLIGHTS

- Aloe vera mucilage as a cost effective and eco-friendly natural bioflocculant.
- It can serve as potential bioflocculant for treatment of textile wastewater.
- Box Behnken Design was implemented to optimize the conditions for textile wastewater treatment.
- Significant removal of solids, colour and dye from textile wastewater revealed the novelty of *Aloe vera* mucilage as bioflocculant for the first time.

INTRODUCTION

Water treatment is costly for developing countries, involving the application of synthetic products that have an influence on human health. It has become a significant economic issue for developing countries. The consumption of natural resources in the development of water treatment signifies a favorable way to reduce high costs and environmental impacts. Several treatment techniques are presently available for the treatment of industrial wastewaters. The adoption of the practices depends on the wastewater qualities and treatment objectives. The treatment of water mostly involves flocculation, filtration, and disinfection methods. The treating of a coagulant in wastewater causes the destabilization of negatively charged particles, resulting in better floc formation by a flocculation method termed coagulation. Most of the industries treat their wastewaters with the conventional coagulation-flocculation process (Sellami *et al.* 2014), and this technology is being used extensively for wastewater treatment because of its low cost,

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suitability, energy competence, smooth running, and eco-friendly nature (Dlamini *et al.* 2019).

Dyes are generally used in a variety of industries, viz. food, paper, plastic, textile and so on for coloring their products which create considerable environmental pollution. Toxic dye components significantly threaten the survival of aquatic organisms. Moreover, dye pollution causes skin irritation, heartbeat abnormalities, carcinogenic effects, eye burns etc. Therefore, it is necessary to treat the dye-containing wastewater before releasing it to the environment (Allafchian *et al.* 2019).

Plant-based bio-flocculants are being developed as an alternative to synthetic flocculants and their uses in treatment of wastewater has become important because of their non-toxic and biodegradable nature. They are extensively accessible from renewable resources and have no adverse effect on the environment. Various types of wastewater treatment using plant-derived bio-polymers have been reported.

Based on ten years of research reports, a mini review was provided by Amran *et al.* (2018) on the coagulation/flocculation ability of plant-based materials. A contemporary review was conducted by Saleem & Bachmann (2019) on the efficacy of diverse plants as sources of cationic, anionic and nonionic coagulants, which indicated their scope for wide application and commercialization. More recently, Mohd-Salleh *et al.* (2019) have reviewed more than 16 plants and outlined the future prospects of natural materials as aids and their potential as sustainable composite coagulants/flocculants.

There are reports on bio-flocculants obtained from various plant species such as *Lepidium sativum* (Allafchian *et al.* 2019), *Moringa Oleifera* (Agarwal *et al.* 2019; Zaid *et al.* 2019); *Salvia hispanica* (Tawakkoly *et al.* 2019); *Cyamopsis tetragonoloba* (Dwari & Mishra 2019); *Abelmoschus esculentus* (Lee *et al.* 2018); *Opuntia ficus indica* (Bouaouine *et al.* 2019); *Hibiscus esculentus* and *Trigonella foenum graceum* (Jones & Bridgeman 2019); Albizia gum (Afolabi & Adekanmi 2017); *Aloe vera* (Irma *et al.* 2015); *Plantago ovata* (Mishra *et al.* 2004; Al-Hamadani *et al.* 2011); Date palm (Khiari *et al.* 2010); *Tamarindus indica* (Mishra & Bajpai 2006; Mishra *et al.* 2006).

Textile wastewater has been treated using polyacrylamide-grafted *Plantago psyllium* mucilage as a flocculant (Mishra *et al.* 2004). In the case of the bioflocculant extracted from *Tamarindus indica* (Tamarind), it could not be used as an efficient flocculant for the removal of dyes, viz. direct (direct fast scarlet) and vat (golden yellow) from textile wastewater as it did not show significant removal of dye after a long period of contact time (Mishra *et al.* 2006). In most of the reported studies, the appropriate pH range was neutral for the highest flocculating efficiency of plant-based bio-flocculants. Some were stated to be feasible in acidic or alkali condition depending on the type and features of treated wastewater. Recently, Agarwal *et al.* (2019) reported the use of *Moringa oleifera* seeds as a primary coagulant for the treatment of textile wastewater. Application of cress seed mucilage magnetic nanocomposite has been reported for the elimination of methylene blue dye from water (Allafchian *et al.* 2019). Plant-derived tannin-based naturally derived coagulants has been evaluated for color removal from dye bearing wastewaters (Lopes *et al.* 2019).

Aloe vera is a succulent plant belonging to the family of *Liliaceae*, and grows all over the world. It consists of 99% water, and the remaining 1% represents other ingredients. This plant has beneficial health properties: anti-carcinogenic, anti-inflammatory, anti-diabetic, anti-microbial, digestive, nutritional, dermatological and cosmetic. *Aloe vera* leaf gel was evaluated as a natural flocculant by Irma *et al.* (2015). The presence of significant phytochemical components and some chemical parameters were reported (Saljooghianpour & Javaran 2013) to show the potential of this plant to serve as a flocculant for the reduction of surface water turbidity (Irma *et al.* 2015).

So far, no report is available on the application *Aloe vera* bioflocculant for the treatment of dye bearing wastewater. Therefore, the objective of the present study was focused on the use of *Aloe vera* bioflocculant for the decolorization of textile wastewater. The extracted bioflocculant was purified and characterized using Gas Chromatography-Mass Spectroscopy (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX) and Atomic Force Microscopy (AFM) analysis. The optimization of the dye removal process was done using response surface methodology (RSM) to enhance the decolorization rate of textile wastewater.

Outcomes gained from the present research revealed the first report on process optimization using Box Behnken Design implemented to optimize various parameters for the treatment of textile wastewater using *Aloe vera* mucilage. Remarkable increase towards decolorization percentage and decrease in COD, TSS and TDS after treatment confirmed the novelty of *Aloe vera* mucilage to serve as potential natural and eco-friendly bioflocculant for the treatment of dye bearing wastewater.

MATERIALS AND METHODS

Sample collection

Aloe vera, belonging to family *Liliaceae*, was collected from the Bramhapuram area located at 12.9657° N, 79.1676° E in Tamil Nadu, India. The collection of textile wastewater was done from Manisha textile dying works, Kanchipuram, Tamil Nadu, India. The dye bearing textile wastewater was analyzed adopting the parameters viz. color, smell, turbidity, electrical conductivity, pH, Chemical oxygen demand (COD), Biological oxygen demand (BOD), Total dissolved solids (TDS), Total suspended solids (TSS) following the standard methods (Clesceri *et al.* 1989).

Extraction of bioflocculant

Bioflocculant was extracted following the standard protocol of drying and grinding method with minor modifications (Anastasakis *et al.* 2009). The leaves of *Aloe vera* were washed and sliced into small parts and subsequently dehydrated in an oven at 40 °C. Further, the dehydrated pieces of *Aloe vera* leaves were ground using a mortar and subsequently sieved to obtain a fine powder. The powdered bioflocculant was stored at 40 °C until used.

Purification of bioflocculant

The dried powder of bioflocculant was dispensed into two volumes of chilled solvents such as methanol, ethanol and acetone (1:2 v/v) to precipitate the bioflocculant overnight in the refrigerator at 4 °C. The precipitate was separated by centrifugation (10,000 rpm) for 15 min, washed with Milli-Q water and monitored by reprecipitation. The process was repeated twice and the precipitated bioflocculant was desiccated for 2 hours. The extract was exposed to dialysis against distilled water and kept overnight at 40 °C (Natarajan 2015).

Characterization of bioflocculant

The characterization of the purified bioflocculant was done. The total carbohydrate present in extracted bioflocculant was verified through the phenol-sulphuric acid technique taking glucose as a standard solution (Nielsen 2010). The total protein present in the extracted bioflocculant was determined by Bradford technique using Bovine serum albumin (BSA) as a standard (Bradford 1976). Instrumental analysis viz. Gas Chromatography and Mass Spectroscopy (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX) and Atomic Force Microscopy (AFM) analysis were also performed for characterization.

GCMS analysis

The GC-MS analysis of the bioflocculant was performed using a JEOL GC MATEII chromatograph provided with a flame ionization detector and injector MS transfer line (280 °C), respectively. A fused silica capillary column Hp-5 ms was used. The temperature was automated from 110 °C with a speed of 10 °C/min, to 200 °C and ended at 280 °C (9 min isothermal). Helium (1 ml/min) was used as a carrier gas. Mass spectra (70 eV), scanning rate (1 scan/s), and the run time (90 min) were used for the study. Compound detection was achieved by relating the GC relative retention times and mass spectra to those of reliable substances examined under the identical circumstances by their retention indices (RI) and by evaluation with reference components (Saljooghianpour & Javaran 2013).

FTIR and XRD analysis

The purified bioflocculant was investigated by FTIR (Perkin Elmer) analysis. The dehydrated sample was mixed with KBr powder and compressed into pellets for FTIR spectra measurement spectrum in the frequency range of 500–4000 cm^{-1} (Natarajan 2015).

The XRD analysis of bioflocculant was conducted with X' Pert Pro P analytical diffractometer using monochromatic Cu Kα X-ray radiation (λ 0.154 nm) running at 40 kV and 30 mA (Rasulov *et al.* 2016).

SEM, EDX and AFM analysis

Scanning electron microscopic analysis was done to study the external morphology of the bioflocculant using a HITACHI model high-resolution electron microscope to verify morphology and homogeneity of particles. Bioflocculant was centrifuged (10,000 rpm) for 15 min and the pellets were resuspended in deionized double distilled water, and a drop of suspension was added on a glass coverslip and air-dried. Then the sample was chemically fixed for 24 hours at 40 °C using glutaraldehyde (2.5%). The sample was washed with distilled water to eliminate the traces of glutaraldehyde, then dehydrated in a sequence of graded series of ethanol ranging from (30–100%) and dried under vacuum (Natarajan 2015).

The EDX measurements were performed with an X-ray detector to examine the elemental constitution of the bioflocculant obtained from *Aloe vera*.

The surface topography of the bioflocculant was evaluated using AFM analysis. The sample (5–10 mL) was dispersed on a Pelco mica disc (10 mm) using a rotating spin plate and immersed with absolute ethanol to get fixed on the mica disc. Later, the mica cover was dried to eliminate the residual ethanol, and the AFM micrographs were taken by Nano Surf Easy scan 2 (Saravanan *et al.* 2017).

Flocculation efficiency

Flocculation experiments were carried out using textile wastewater. The bioflocculant (20 mg) was added into 300 mL of textile wastewater in a 500 mL beaker and the pH value was adjusted to 7. The mixture was vigorously stirred (180 rpm) for 60 min and then gradually stirred (80 rpm) for 120 min, and allowed to stand for 10 min. The optical density (OD) of the decolorized solution was calculated using a UV-Vis Spectrophotometer (UV-2450, Shimadzu, Japan) at 650 nm. A control test using waste water was carried out in the same mode without adding bioflocculant (Guo *et al.* 2015). The flocculating activity was calculated.

Effect of parameters on decolorization of wastewater

The effects of different parameters on the decolorization of wastewater (Mishra *et al.* 2006) was tested using different pH values (3–11), bioflocculant dosages (20–100 mg/L), contact times (30–180 min) and temperatures (10–350 °C) keeping the other parameters at optimal conditions.

Statistical optimization

Response surface methodology (RSM) by Box-Behnken design (BBD) was applied to optimize the various parameters, viz. pH, bioflocculant dosage and contact time for the highest decolorization %. The quadratic model was employed to scrutinize the data. All the three factors in the design were studied at three different levels (−1, 0, +1). The lowest and highest ranges of pH, bioflocculant dosage and contact time variables were fixed with different ranges (Table 1). A design of 17 trials was framed, and tests were conducted in 250 ml Erlenmeyer flasks containing textile wastewater (100 ml) with different pH, bioflocculant dosage and contact time maintaining the

Table 1 | Independent factors and its level used in response surface design for decolorization percentage (%)

Factors	Name	Low level (−1)	Level (0^{th})	High level (+1)
A	pH	3	5	7
B	Bioflocculant dosage (mg/L)	20	60	100
C	Contact time (min)	60	180	300

temperature at 25 °C, and the decolorization percentage as Response 1 was evaluated. The 3D and contour plots were made to test the optimized parameters, which affect the response. A second-order polynomial equation analyzed the response, and the statistical data were fitted to the equation by multiple regression measures. Later, a test was performed in triplicates employing the optimum values for variables obtained from response surface optimization to confirm the predicted value and the experimental value of the response. The results of the trial design were examined and elucidated by a statistical software, Design-Expert version 12.0 (Minneapolis, MN, USA).

RESULTS AND DISCUSSION

Analysis of textile wastewater

The wastewater sample collected from Manisha textile dyeing works, Kanchipuram, Tamil Nadu, India, was deep red, foul-smelling, highly alkaline (pH 11.0) and having a temperature of 28 °C. Electrical conductivity was quite low (4.21 μs/cm). Values of COD (1,215.00 mg/L), BOD (593.33 mg/L), TSS (3,768 mg/L), and TDS (3,754 mg/L) from the wastewater sample were calculated. The wastewater sample was subjected to flocculation experiment for decolorization.

Characterization of bioflocculant

The purified bioflocculant extracted from *Aloe vera* was mainly composed of carbohydrates (19.5%) and protein (6.0%). A similar result was confirmed by Ni *et al.* (2004), where the total carbohydrate content from *Aloe* gel was found to be 0.22% (w/v). A study on compositional features of *Aloe vera* was reported, showing total protein content of 6.86 ±0.06%, which was close to our present findings (Ahmed & Hussain 2013).

GC-MS analysis

The GC-MS analysis of *Aloe vera* bioflocculant was done to test the bioactive compounds present in the sample. The chromatogram showed major peaks at retention time 4.119 min, corresponding to D-mannoheptulose, N-hydroxymethylacetamide and D-galactose oxime, as shown in Figure 1. The peaks at 5.6 min, 8.606 min and 5.244 min showed the presence of 2-butanone, ethyl acetate and ethanol respectively. A similar result was demonstrated by Saccù *et al.* (2001); where the peak at retention time 11.72 min, 12.68 min and 5.76 min, respectively, revealed 2-butanone, ethyl acetate and ethanol, which supports the present study. The peak at 7.78 min corresponded to 5-ketofructose, D-arabinitol and pentanoic acid. The sharp peak at 21.58 min corresponded to hexadecanoic acid (Bawankar

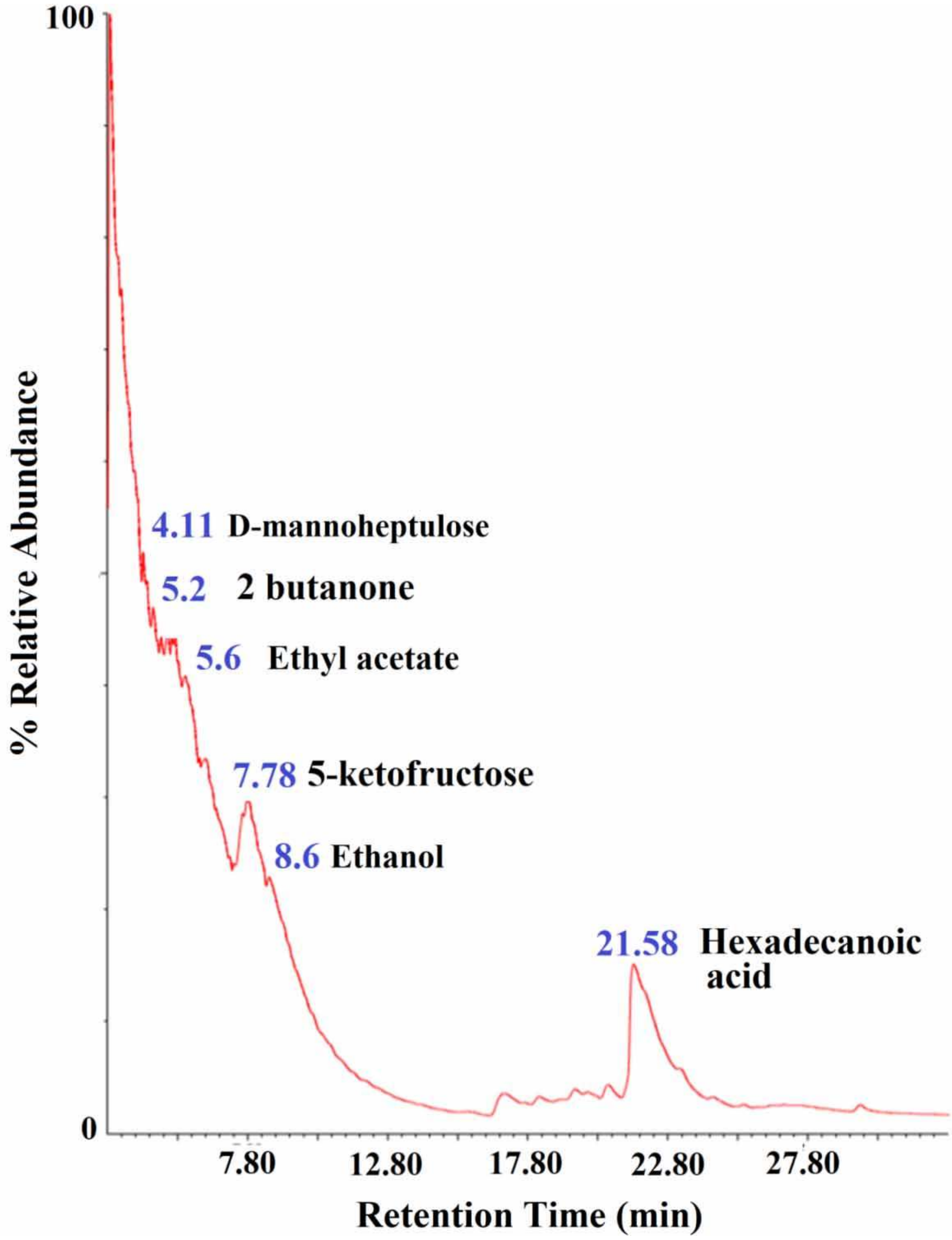

Figure 1 | GC-MS chromatogram of the solvent extracted, purified and dried *Aloe vera* bioflocculant.

et al. 2013). Therefore, the above findings detected from the GC-MS study confirmed the presence of polysaccharide in *Aloe vera* bioflocculant.

FTIR and XRD analysis

The FTIR spectrum of bioflocculant extracted from *Aloe vera* is shown in Figure 2(a), which exhibits different peaks at different positions. The band at 3,269 cm^{-1} resembled the presence of the hydrogen-bonded -OH stretching vibration mode of hydroxyl groups, which was similar to the result as illustrated by Choudhary *et al.* (2019). The sharp peaks at 2,922 cm^{-1} and 2,852 cm^{-1} corresponded to the asymmetric and symmetric stretching of the group -CH-CH_2 that exists in fatty acids; a similar result was described by (Swelam 2019). A significant band in the spectrum was observed between 1,716 cm^{-1} and 1,319 cm^{-1} resembling the presence of the carboxylic group. The peak at 1,716 cm^{-1} is attributed to the C = O stretching mode of either the methylated or protonated (non-ionized) form of a carboxyl group. The band at 1,585.49 cm^{-1} indicated the presence of NH_2 primary alkyl amine groups in the bioflocculant. The peak at 1,392 cm^{-1} was due to ionization of carboxyl groups addressing symmetric stretching vibrations of -COO- group. The band at 1,255 cm^{-1} reflected the presence of o-acetyl groups in the bioflocculant. The peaks between 1,200 cm^{-1} and 800 cm^{-1} indicated the characteristic peaks of polysaccharides, revealing the existence of distinct functional groups such as -OH bending, deforming CH_3 and CO-C stretching vibrations. Similar results were demonstrated by Choudhary *et al.* 2019. The broad band at 1,072 cm^{-1} indicated the existence of C-H stretching in polysaccharides. Thus, the FTIR analysis indicated that the major constituents of the *Aloe vera* bioflocculant are polysaccharides and proteins.

The XRD pattern for *Aloe vera* bioflocculant is shown in Figure 2(b). The diffraction pattern from $2\theta = 10–90$ C illustrated the crystalline nature of bioflocculant. A prominent sharp peak exhibited at 28.05 °C in the XRD spectrum corresponded to its crystalline structure. A similar XRD pattern was reported by Mishra *et al.* (2004).

SEM, EDAX and AFM analysis

The SEM images of the *Aloe vera* bioflocculant, as shown in Figure 3(a), were studied to examine its morphology and microstructure. The surface micrograph of *Aloe vera* revealed a villus structure bearing porous matrix. It can be assumed that the villus plays an essential role in the decolorization, as the spaces between porous matrices may facilitate the adsorption of dye. A similar trend was described by Swelam (2019).

The EDX spectrum of *Aloe vera* bioflocculant is shown in Figure 3(b), indicating the presence of carbonaceous material with a carbon weight percentage of 54.49% and oxygen weight percentage of 42.39%. The presence of C revealed that the *Aloe vera* bioflocculant contained proteins, which contributed to the efficiency of the material in the process of dye adsorption (Swelam 2019). Inorganic element K was also detected in trace amounts.

AFM micrographs showed the surface topology of the *Aloe vera* bioflocculant, as shown in Figure 3(c). Rough surface structures and fissures were observed occasionally on the outward of the villus.

Flocculation efficiency

The flocculation efficiency was checked for the purified bioflocculant using textile wastewater, and was found to be 50%. The effects of different parameters on decolorization were studied.

Effect of parameters on decolorization

The influence of initial pH ranges from 3 to 11 on decolorization was investigated and is shown in Figure 4(a). Maximum decolorization (55.4%) was obtained at acidic pH 5 using *Aloe vera* bioflocculant. At a lower pH, the competition between excess hydroxyl ions, H+ and the cationic groups on the dye for adsorption sites cause the bioflocculant surface to be positively charged. However, at higher pH of the solution, the bioflocculant surface may get negatively charged, which will enhance the attraction between the positively charged dye cations by electrostatic forces of attraction (Anisuzzaman *et al.* 2015). Results also revealed that decolorization efficiency was slightly decreased beyond pH 9 because of electrostatic repulsion. A similar pattern concerning the effects of pH was observed for the adsorption of dye using microbial bioflocculant (Deng *et al.* 2005). Therefore, it was observed that the effect of initial pH of wastewater played an essential role in the decolorization process (Allafchian *et al.* 2019).

The effect of bioflocculant dosage on decolorization percentage is shown in Figure 4(b). The most effective bioflocculant dosage was noted as 60 mg/L with a decolorization efficiency of 62.5%. It was evident that with a rise in bioflocculant dosage, the decolorization percentage was

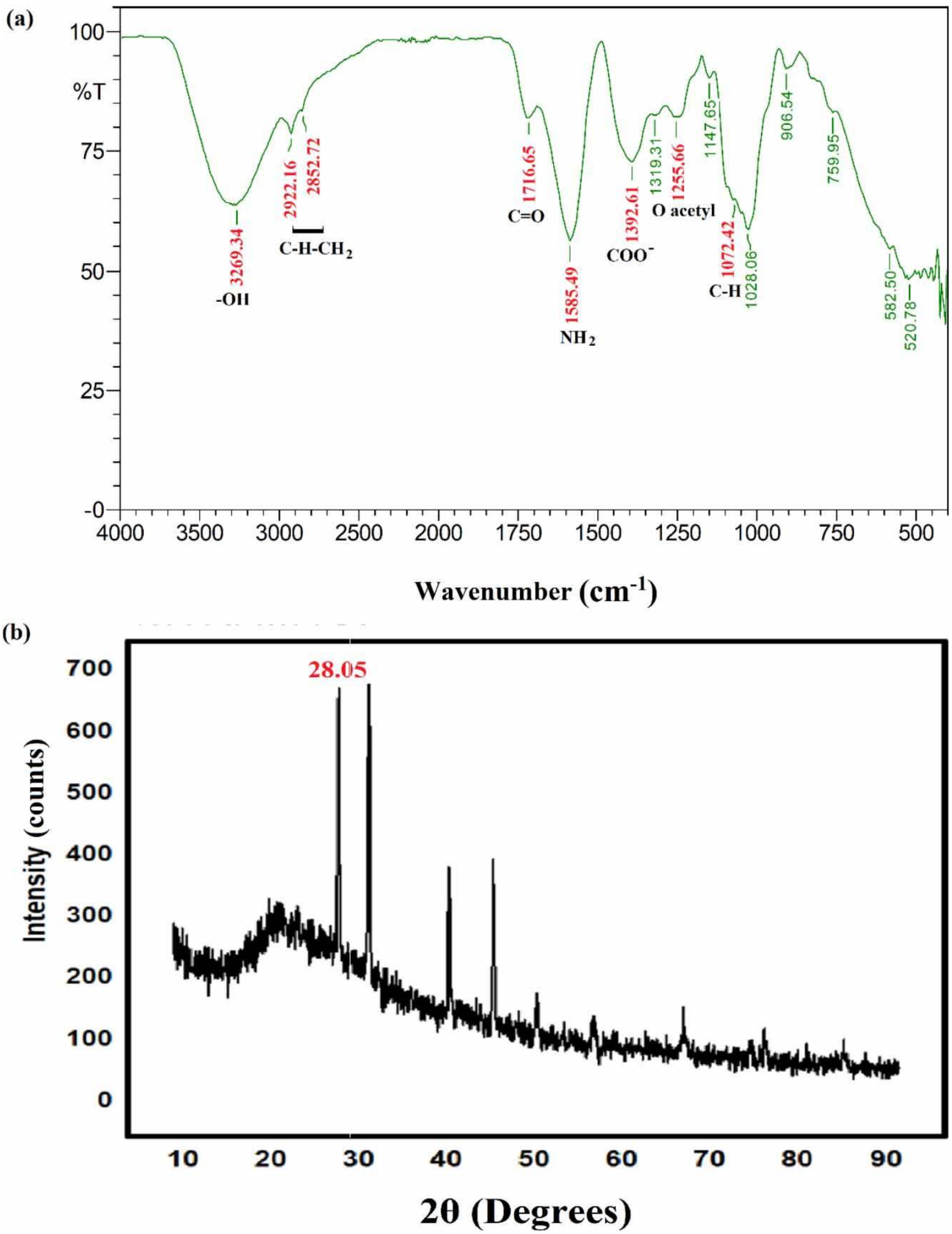

Figure 2 | Characterization of the solvent extracted, purified and dried *Aloe vera* bioflocculant using (a) Fourier-transform infrared spectroscopy (FTIR), (b) X-ray diffraction (XRD) analysis.

increased, but after the optimum dosage of bioflocculant (60 mg/L), a reduction in the decolorization percentage was observed. This finding revealed that the optimal dose of bioflocculant in dye solution caused more significant volume of dye particles to accumulate and settle. However, excess quantity of bioflocculant in textile wastewater would

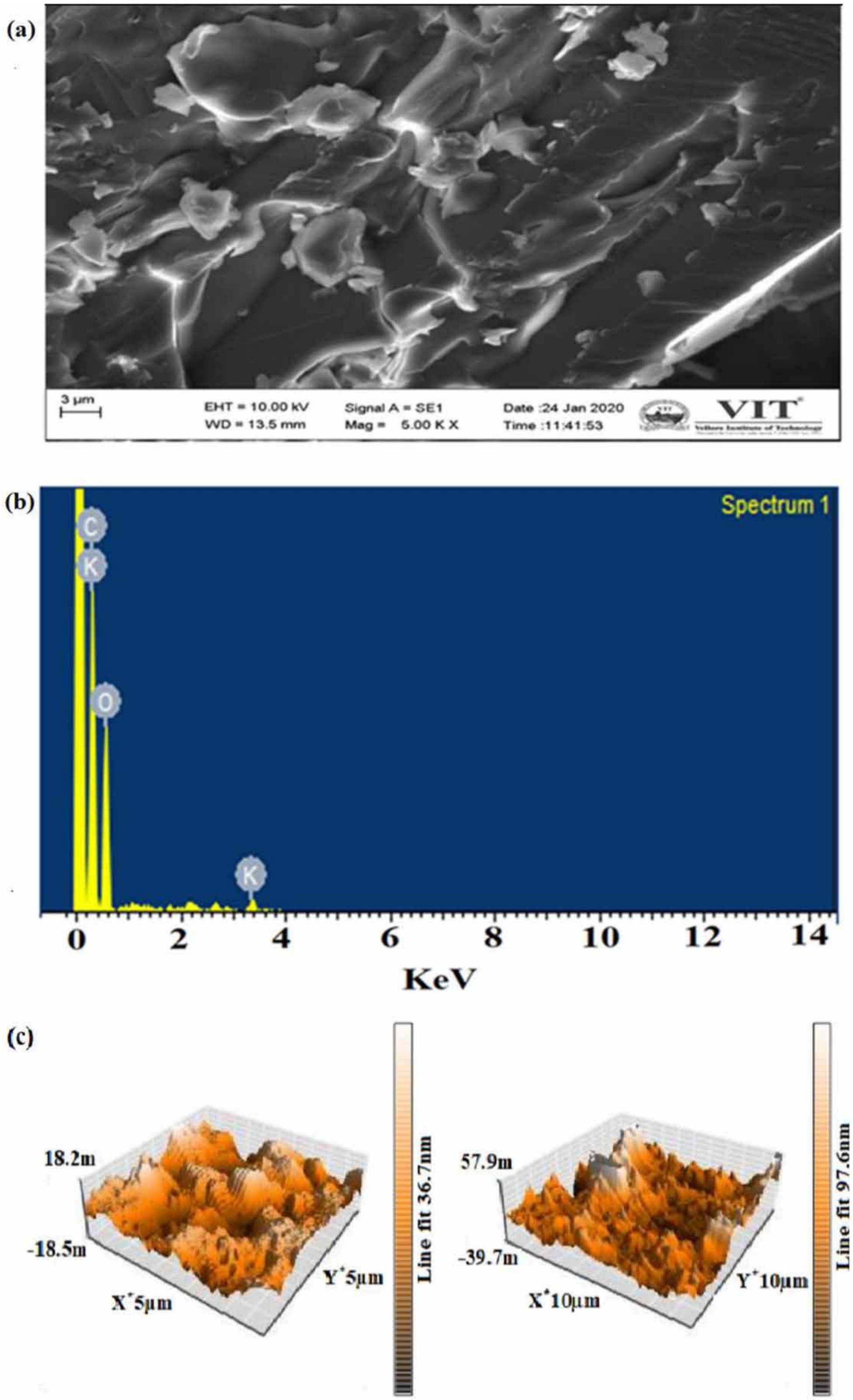

Figure 3 | Surface morphological and elemental mapping of the solvent extracted, purified and dried *Aloe vera* bioflocculant using (a) scanning electron microscopy (SEM), (b) energy-dispersive X-ray spectroscopy (EDAX) and (c) atomic force microscopy (AFM) analysis.

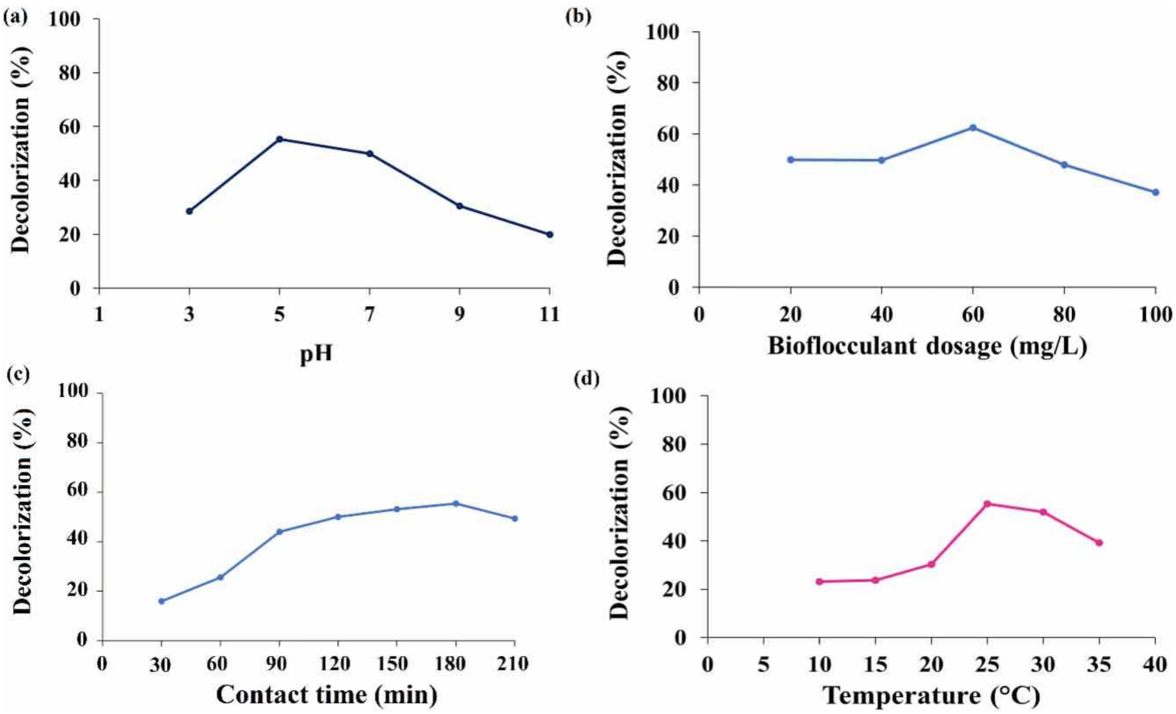

Figure 4 | Effect of parameters on decolorization of textile wastewater using purified and dried *Aloe vera* bioflocculant. (a) effect of initial pH (3–11), (b) effect of bioflocculant dosage (20–100 mg/L), (c) effect of contact time (30–180 min) and (d) effect of temperature (10–350 °C).

produce the accumulated particles to re-disperse in the solution and cause disturbance in particle settling (Mishra *et al.* 2004).

The influence of contact time on decolorization percentage was determined and is presented in Figure 4(c), which shows that the decolorization rate in each case was gradually increased with increase in contact time. The maximum decolorization with *Aloe vera* bioflocculant was obtained after 180 min at pH 5 and after that, no significant changes were observed. A slope curve indicated the decolorization efficiency of the bioflocculant in three different stages. The first stage showed the contact between the dye molecule and bioflocculant, where they started to flocculate. The second stage showed a minor increase in decolorization percentage, which might be due to the formation of larger flocs, and the third phase indicated the stabilization of the flocs (Mishra *et al.* 2006).

The effect of temperature on decolorization is represented in Figure 4(d), which shows that the coagulation-flocculation process was affected by fluctuation of the temperature. When the temperature was in the range of 5–20 °C, the flocculation activity was found to be 30.22%, and the highest flocculating activity of 62.5% was achieved at 25 °C. The flocculating activity started decreasing above 25 °C. The reduction in the flocculation activity at higher temperature could be because of the denaturation of proteins present in the bioflocculant (Manivasagan *et al.* 2015).

A maximum flocculation efficiency of 62.5% was observed at pH 5 using 60 mg/L of bioflocculant dosage after 180 min of contact time at 25 °C, as shown in Figure 4.

Statistical analysis

The statistical model of the BBD was implemented to enhance the decolorization percentage through changing the factors, viz. pH (A), bioflocculant dosage (B) and contact time (C) at various concentration ranges. The ANOVA results for the quadratic model of response 1 (decolorization percentage) is presented in Table 2 and the second-order polynomial equation is shown as follows:

$$Y = +82.01 + 6.93 * A - 3.10 * B + 5.43 * C - 4.20 * AB - 1.66 * AC - 5.01 * BC - 14.93 * A^2 - 10.58 * B^2 - 11.63 * C^2 \quad (1)$$

where Y was the response 1 signifying the decolorization percentage (%) and A, B, and C were coded terms for the three test variables; that is, pH, bioflocculant dosage and contact time respectively. The 3-dimensional (3D) and 2-dimensional (2D) contour plots for the optimal levels of

Table 2 | ANOVA for response surface quadratic model (response: decolorization percentage (%) of textile wastewater (100 ml) with different pH (3–7), bioflocculant dosage (20–100 mg/L) and contact time (60–300 min) at constant temperature of 25 °C)

Source	Sum of squares	df	Mean square	F-value	*p*-value	
Model	3,082.85	9	342.54	413.29	<0.0001	***
A-pH	383.92	1	383.92	463.22	<0.0001	***
B-Bioflocculant dosage	76.76	1	76.76	92.61	<0.0001	***
C-Contact time	235.66	1	235.66	284.34	<0.0001	***
AB	70.73	1	70.73	85.34	<0.0001	***
AC	10.96	1	10.96	13.22	0.0083	**
BC	100.40	1	100.40	121.14	<0.0001	***
A^2	938.36	1	938.36	1,132.18	<0.0001	***
B^2	471.18	1	471.18	568.50	<0.0001	***
C^2	569.36	1	569.36	686.96	<0.0001	***
Residual	5.80	7	0.8288			
Lack of Fit	3.10	3	1.03	1.53	0.3363	NS
Pure error	2.70	4	0.6751			
Cor total	3,088.65	16				
Std. dev.	0.9104					
Mean	64.54					
C.V. %	1.41					
R^2	0.9981					
Adjusted R^2	0.9957					
Predicted R^2	0.9826					
Adeq precision	58.0994					

***Highly significant (*p*-value < 0.0001); **Significant (*p*-value < 0.005); NS, Non-significant *p*-value > 0.005).

each variable for highest decolorization percentage are presented in Figure 5(a)–5(c). The 3D plots indicated significant impact on decolorization percentage (Figure 5) both independently and in contact with each other. The variables, viz. A, B, C, A^2, B^2 and C^2, were significant model terms. When in case of squared terms, all the three variables, viz., pH (A), bioflocculant dosage (B) and contact time (C) showed the positive significance on decolorization percentage (Table 3). The 'Lack of Fit F-value' of 1.53 suggests the Lack of Fit is not significant compared to the pure error. Non-significant lack of fit is excellent and measured that the design is fit. The determination coefficient R-squared value was attained to be 0.9981, representing an accurate fit of the design to the actual data (Table 2). This also indicated that 99% difference of response can be interpreted efficiently and confirms that 1% of the differences arise while carrying out the experiments. The adjusted determination coefficient value was found to be 0.9957, which also confirmed that the design was highly substantial with the coefficient of the difference of 1.41%. The 'Pred R-squared' value of 0.9826 was in logical agreement with the 'Adj R-squared' value of 0.9957. The ratio of 58.0994 indicated a suitable signal. Therefore, this design can be implemented to navigate the model space.

The normal plot for residuals for decolorization % (response), is presented in Figure 5(d). The predicted and the experimental responses of decolorization percentage were quite comparable. The expected values of decolorization percentage were computed by regression analysis and correlated to the actual data, which illustrated that the experimental response values were in good agreement with the expected (predicted) response values (Table 3). The predicted versus actual plots of the decolorization % are presented in Figure 5(e).

A statistical design was confirmed by implementing the point prediction tool of RSM from an optimal value of all the three factors A, B and C that were used for the experiment. The optimized factors of the decolorization percentage attained were A: pH (5), B: bioflocculant dosage (60 mg/L) and C: contact time (180 min.). The

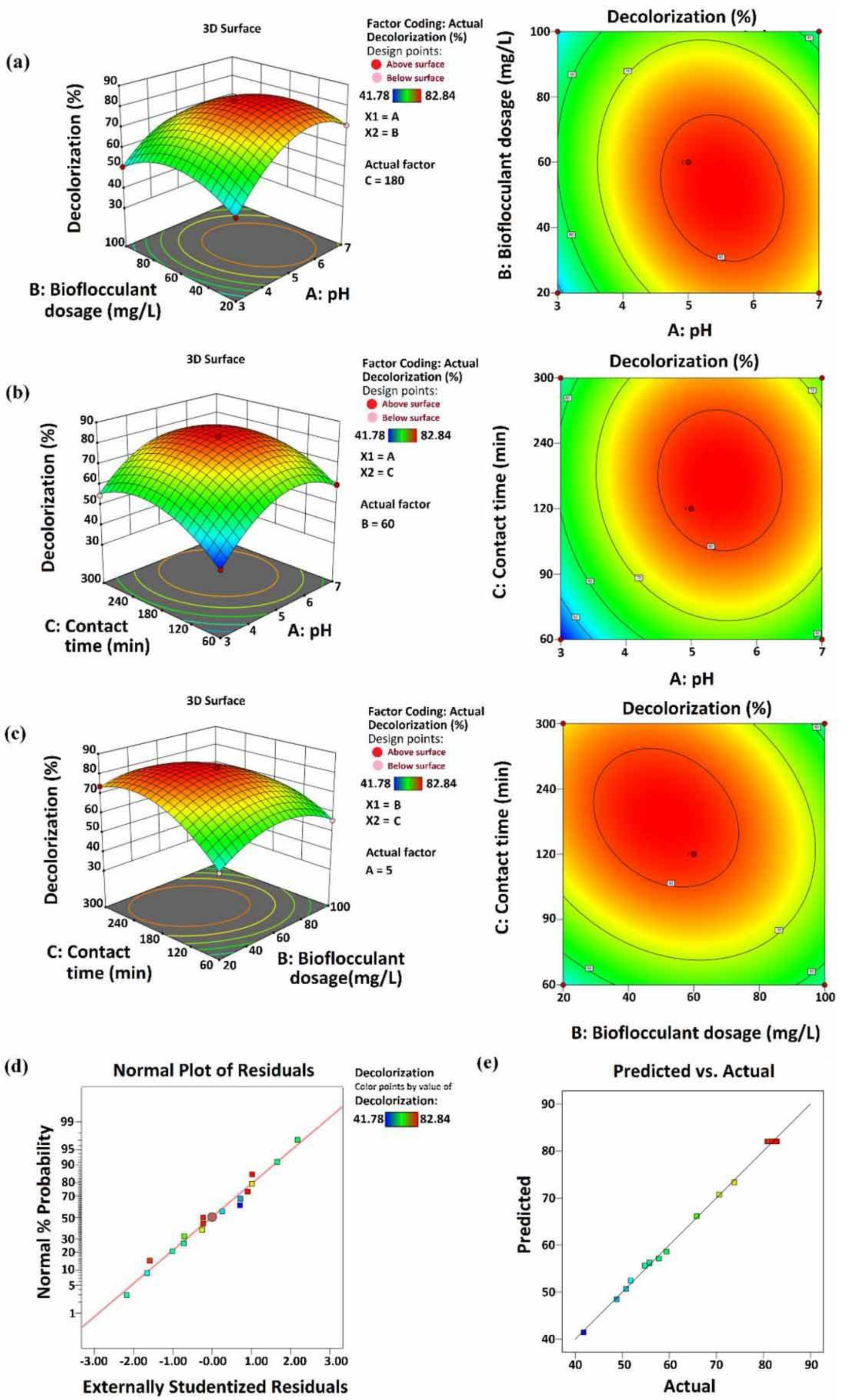

Figure 5 | Process optimization of parameters through Box Behnken Design (BBD) to enhance decolorization using purified and dried *Aloe vera* bioflocculant. Response surface 3D and contour plots showing significant (p-value < 0.005) interaction between (a) A: pH (3–7) vs. B: bioflocculant dosage (20–100 mg/L), (b) A: pH (3–7) vs. C: contact time (60–300 min) and (c) B: bioflocculant dosage (20–100) vs. C: contact time (60–300 min). (d) Normal plot of residuals. (e) The actual vs. predicted plot.

Table 3 | Actual versus predicted value for response: decolorization percentage (%) with different pH (3–7), bioflocculant dosage (20–100 mg/L) and contact time (60–300 min) at constant temperature of 25 °C).

				Response 1 Decolorization (%)	
Run Order	**pH**	**Bioflocculant dosage (mg/L)**	**Contact time (min)**	**Actual value**	**Predicted value**
1	3	20	180	48.81	48.47
2	5	20	60	51.79	52.46
3	3	60	60	41.78	41.45
4	3	100	180	50.81	50.69
5	7	20	180	70.61	70.73
6	7	60	60	59.41	58.61
7	5	100	300	57.8	57.12
8	7	60	300	65.82	66.15
9	5	60	180	81.81	82.01
10	5	100	60	55.83	56.29
11	5	60	180	82.84	82.01
12	3	60	300	54.81	55.61
13	7	100	180	55.79	56.13
14	5	20	300	73.8	73.34
15	5	60	180	80.84	82.01
16	5	60	180	82.76	82.01
17	5	60	180	81.81	82.01

experimental decolorization percentage (82.01 ± 0.8%) was in good agreement with the predicted value (82.01 ± 0.1%), demonstrating the rationality of the design. Thus, a significant increase in the decolorization percentage from 62.5 to 82.0% was attained using *Aloe vera* bioflocculant under optimized condition.

In addition, the physio-chemical properties of the textile waste water were tested after addition of *Aloe vera* bioflocculants under the optimized condition. There was significant reduction in the intensity of the waste water from deep red to almost colorless (82%). No foul smell was found and the alkalinity (pH 11.0) of the waste water was reduced to neutral (pH 7.0). Besides, a remarkable reduction in the concentration of COD (115 mg/L i.e. 90.53%), TSS (68 mg/L i.e. 98.19%) and TDS (45 mg/L i.e. 98.80%) were observed in the *Aloe vera* bioflocculant-treated textile waste water. Similar report was demonstrated by Tawakkoly *et al.* (2019), where mucilaginous seed of *Salvia Hispanica* was used as a natural coagulant for the treatment of landfill leachate and showed reduction in the turbidity (39.76%) and COD (62.4%) of the wastewater under optimum conditions.

Al-Hamadani *et al.* (2011) reported that psyllium husk was found to be more effective as a coagulant aid with poly aluminum chloride (PACl) in the removal of COD (60%), color (90%) and TSS (96%) compared to synthetic flocculant (aluminum sulfate, alum). However, psyllium husk was not effective when used as a primary coagulant. In another report, the grafted copolymer, acrylamide grafted *Tamarindus indica* mucilage (Tam-g-PAM), showed better flocculation efficiency for dye removal than the pure mucilage (Mishra *et al.* 2006). Similar report was demonstrated by Lopes *et al.* (2019), where tannin-based coagulant was used for the decolorization of synthetic effluents. According to the literature survey, there are many reports available on grafted bioflocculants for wastewater treatment. However, no report is available on pure plant- based *Aloe vera* bioflocculant (without grafting) for the treatment of textile wastewater.

Our present study investigated and highlighted the potentiality of *Aloe vera* mucilage as an effective bioflocculant in comparison to other grafted plant-based flocculants reported so far. The application of pure *Aloe vera* mucilage as a cost-effective green bioflocculant and optimization of its treating conditions to enhance the decolorization efficiency of the textile waste water have been documented for the first time in our study.

CONCLUSIONS

Synthetic flocculants used in the coagulation-flocculation method are costly, causing harmful consequences on human health and the environment. In the present study, a sustainable alternative technology has been adopted for textile wastewater treatment using *Aloe vera* bioflocculant, which was found to be quite effective towards decolorization of textile wastewater along with the reduction of total soluble matter present in wastewater. Statistical optimization was carried out using BBD, which resulted in the enhancement of bioflocculant efficiency. Flocculation activity was increased up to 82% after optimization of process parameters such as pH, bioflocculant dosage and contact time. Characterization of purified bioflocculant revealed that it is composed of carbohydrates and protein having hydroxyl and amino groups, which were needed for flocculation. This is the first report that has revealed the probability of using *Aloe vera* as a source of natural, cost-effective bioflocculant that could be effectively used for the treatment of dye bearing wastewater. Future investigation will be carried out on comparative study between pure *Aloe vera* bioflocculant and synthetic flocculants.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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