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Plant-Based *Tacca leontopetaloides* Biopolymer Flocculant (TBPF) Produced High Removal of Turbidity, TSS, and Color for Leachate Treatment

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Abstract: Wastewater treatment is crucial to ensure a sustainable supply of clean water, especially for human use. Natural flocculants can overcome the disadvantages of chemical flocculants in wastewater treatment. This study proposes a new natural-based flocculant from the *Tacca leontopetaloides* plant for leachate treatment. The plant tuber was processed through gelatinization to produce *Tacca leontopetaloides* biopolymer flocculant (TBPF). The characterization of TBPF for flocculant properties was investigated, and the performance of TBPF on leachate treatment using a standard jar test procedure was examined at different pH values of leachate and TBPF dosages. The characteristics of TBPF in terms of amylose/amylopectin fraction, viscosity, and zeta potential were 26:74, 0.037–0.04 Pa·s, and −13.14 mV, respectively. The presence of –COOH and –OH structure in TBPF indicates the flocculant properties. TBPF reduced the turbidity, total suspended solids (TSS), and color from 218 NTU, 214 mg/L, 14201 PtCo to 45.8–54.5 NTU, 19.3–19.9 mg/L, and 852–994 PtCo, respectively, using 240 mg/L of TBPF at pH 3. These results show a high potential of the new plant-based TBPF for leachate treatment and water industry applications.

Keywords: *Tacca leontopetaloides* sp.; *Tacca leontopetaloides* biopolymer flocculant (TBPF); flocculant properties; leachate

1. Introduction

Wastewater treatment requires large land areas, long process lines, as well as large ponds for treatment plants. The dosing of reagents (coagulants and flocculants) to promote aggregation can be advantageous for wastewater treatment. This method reduces settling times in a cost-effective manner and, thereby, can save space [1]. This method is applied to the primary wastewater treatment process to settle heavy metal and suspended solids. The removal of particulate solids in wastewater liquid effluent is crucial. However, when solids are micron and submicron in size, they remain as a suspension in liquid wastewater and cannot be removed by gravity settling [2]. The particles need to agglomerate by coagulation–flocculation, followed by filtration, centrifugation, and sedimentation.

Coagulation or flocculation can occur via four mechanisms: adsorption and charge neutralization, compression of the double layer, adsorption, and interparticle bridging, and enmeshment in the precipitate [3–5]. Normally, in adsorption and charge neutralization mechanisms, the fine particles in wastewater have a negative surface charge. During the coagulation process, the negatives surface charge colloids need to be neutralized to overcome the forces which keep them separated by adding coagulant with positive surface patches that allow some aggregation. This assists any subsequently added flocculant in bridging the destabilized particles to form larger flocs, which will settle at an adequate rate under gravity [1].

In the coagulation process, alum and ferric salts can be used as coagulants to clarify water without flocculant aid. The flocs produced are small and low strength, thus be able to break when external forces are exerted into it. It also led to highly toxic sludge containing alum and ferric metal [6]. Therefore, flocculant addition is needed as it can bind and bridge the coagulated particles. Some of the organic flocculants are synthetic, including poly-acrylamides, poly-acrylic acids, polystyrene sulfonic acids, and their derivatives. These flocculants are mostly linear water-soluble polymers with repeating units of various monomers, high molecular weight, and can be anionic, cationic (both termed as polyelectrolytes) or non-ionic [6]. However, there are concerns with synthetic flocculants, which can have harmful effects on human health when the residual monomer is present in water. Therefore, natural flocculants are still being studied and searched for to overcome the disadvantages of synthetic flocculants.

Natural coagulants and flocculants have been widely used in water clarification treatment, such as guar gum, starch, glue, and sodium alginate. These natural coagulants and flocculants are used not only due to their low cost [7] but also because they are safe for humans, being nontoxic and ecofriendly. Moreover, natural flocculants such as polysaccharides have a high molecular weight that can trap the suspended solids in the large polymer linkage via an interparticle bridging mechanism, thus improving agglomeration and floc formation. Furthermore, the destabilization and aggregation of a suspension can be enhanced using high molecular weight synthetic polymers [8]. Among these natural coagulants/flocculants, polysaccharides possess the highest industrial capacity that can be useful for water treatment due to their prevalence. The most abundant polysaccharide is starch, and it is present in plants as energy storage material. It is made up of mixtures of two polyglucans, amylopectin and amylose, and contain only a single type of carbohydrate, glucose [5]. Starch polysaccharides possess unique chemical and biological properties such as non-toxicity, biocompatibility, biodegradability, poly-functionality, high chemical reactivity, chirality, chelation and adsorption capacity, and high carbon content, which makes it a natural coagulant and flocculant [5]. The excellent adsorption behavior of polysaccharides is due to certain properties such as high hydrophilicity from hydroxyl groups of glucose units, the presence of various functional groups (acetamido, primary amino, and/or hydroxyl groups), the high chemical reactivity of these groups, and the flexible structure of the polymer chain by enhancing the surface area for the interaction between the polymer surface and particulates [5,9]. The high hydrophilicity due to hydroxyl groups of glucose units and the presence of various functional groups (acetamido, primary amino, and/or hydroxyl groups) will promote and broaden the interactions between the polymer adsorbent and the solute in the aqueous matrix. For cross-linked starch materials, physical adsorption occurred in the polymer structure, and, meanwhile, the chemisorption of the pollutant occurred via hydrogen bonding, acid–base interactions, complexation, and ion exchange; both are involved in the adsorption process [5]. There have been limited studies on the use of *Tacca leontopetaloides* starch as a natural flocculant. Therefore, this paper will evaluate the characteristics of *Tacca leontopetaloides* starch as a new *Tacca leontopetaloides* biopolymer flocculant (TBPF) and its effectiveness in reducing turbidity, total suspended solids (TSS), color, and chemical oxygen demand (COD) in leachate treatment.

2. Materials and Methods

2.1. Raw Leachate Sampling and Characterization

Raw leachate was collected from Tanjung Dua Belas sanitary landfill, Kuala Langat Selangor, Malaysia. The samples were collected at several points in the equalization pond and poured into 25 L sealed-cap high-density polyethylene plastic containers and stored in a refrigerator at 4 °C before use for characterization and experimental purposes to minimize chemical and biological reactions. The samples were taken monthly from June until December 2018. The initial characteristics of leachate were determined, such as pH and turbidity. COD was determined using HACH Digital Reactor Block 200 digestion method (HACH Method 8000) in accordance with the United States Environmental Protection Agency (USEPA). A spectrometer (HACH DR 2800) was used to determine TSS (HACH method 8006), dissolved organic (DO), total dissolved solids (TDS), nitrate, color, sulfate, total organic carbon (TOC) and ammoniacal nitrogen. The Al, Cd, Zn, Pb, and Ni in leachate was determined by using the aqua regia digestion method, and zeta potential was determined by using zeta-PALS DelsaMax Pro (Beckman Coulter). The measurements were conducted thrice according to the quality measurements section in the Standard Method of Water and Wastewater [10].

2.2. Preparation of *Tacca leontopetaloides* Powder

Tacca leontopetaloides tuber was collected in December during the monsoon season from Mersing Johor, Malaysia. The tuber with 0.5 to 1 kg of weight was washed, and the skin tuber was peeled off manually. The tuber was blended using an electrical blender and filtered using a muslin cloth to remove the husk. The supernatant was then collected in a biker and left for 24 h for the sedimentation process. After 24 h, the solid phase had settled down, and the water was decanted. The sedimentation and decanting processes were repeated thrice to purify the starch. The solid phase was withdrawn and dried in an oven at 60 °C for 24 h until the moisture content was less than 5%. The dried solid was ground and sieved through a 150 µm sieve. The powder size of less than 150 µm was used in this experiment. The starch extracted procedure was slightly modified from Ogbonna et al. [11] on the equipment used.

2.3. Preparation of *Tacca leontopetaloides* Biopolymer Flocculant (TBPF)

TBPF was prepared via the extraction of starch from the *Tacca leontopetaloides* plant through a gelatinization process. One liter of distilled water was heated to 80 °C, and 30 g (3%) of *Tacca leontopetaloides* powder was subsequently added. During this process, the loss of birefringence and crystalline order occurred due to the breaking of the double helix in the crystalline region and the leaching of amylose in the presence of solvent with the assistant of heat. The mixture was vigorously stirred using a magnetic stirrer at 200 rpm, and the speed was varied when the suspension became more viscous. After 1 h of heating, the solution was left to cool. During the cooling period, a three-dimensional network, commonly called gel, formed, thus increasing the paste viscosity [12]. The TBPF solution was prepared fresh to avoid degradation.

2.4. Jar Test Analysis

Coagulation-flocculation jar test analysis was performed using a six-paddle rotor (24.5 × 63.5 mm) with a 500 mL beaker, and all tests were conducted at room temperature. A total of 500 mL of leachate was transferred into six beakers with the pH values varied from 3 to 9, adjusted using 1 M of HCl or NaOH. The desired amount of coagulant was added to the suspension and mixed rapidly (200 rpm) for 4 min. The speed of the stirrer was reduced to 40 rpm for 30 min to keep the floc particles uniformly suspended and allow flocculation to occur. The mixture was left for 30 min, and then the supernatant was collected 5 cm from the surface of suspension for COD, TSS, turbidity, and color analyses using the standard methods. The effect of pH was studied in the range of 3 to 9, and the effect of dosage was studied in the range of 60–360 mg/L; all analyses were done in triplicate and assigned as Run 1.

The same experiment with a different batch was repeated and assigned as Run 2. The jar test was performed according to the list of coagulation factors described by Yusoff et al. [13].

2.5. Analytical Analysis

Prior to and after the treatment, samples were withdrawn at 3 cm below the surface liquid level using 35 mL volume of syringe for analysis. Leachate, TBPF, and floc solution were distilled in a spherical flask attached to a rotary vacuum pump immersed in a water bath at 95 °C. The sticky and viscous solution was then freeze-dried at temperature −51 °C, and vacuum pressure 10.1 Pa until the samples were fully dried. The dried samples were then analyzed. The sample preparation was according to Shouliang et al. [14] and Cataldo [15].

2.5.1. Element and Mineral of TBPF

TBPF was analyzed and characterized to ensure its suitability as a flocculant. The mineral constituents were determined using an elemental analyzer and inductively coupled plasma (ICP) spectrometer (Thermo Fisher Scientific, London, UK). Carbon, sulfur, and oxygen elements in *Tacca leontopetaloides* were determined by using the CHNS/O Analyzer LECO CHNS932 (LECO Co., St. Joseph, MI, USA) [11]. The viscosity of TBPF was measured by using a Brookfield Viscometer (model DV2T with DV2TLV-01 spindle, (Brookfield Engineering Lab., Middleboro, MA, USA) based on the method applied by Koocheki et al. [16].

2.5.2. Amylose/Amylopectin Fraction

The percentage of amylose in *Tacca leontopetaloides* powder was determined based on the method of Sandhu and Singh [17]. A 20 mg sample of starch was mixed with 0.5 M potassium hydroxide, KOH in 100 mL of volumetric flask. Then, a 10 mL aliquot starch solution was transferred into a 50 mL volumetric flask, and 5 mL of 0.1 M hydrochloric acid (HCl) was added, followed by 0.5 mL of iodine reagent (0.2% I_2 in 20% KI). The absorbance process of the diluted sample was measured at a wavelength of 620 nm by using a UV portable spectrometer DR2800 (HACH, Loveland, Calorado, USA).

2.5.3. Total Phenolic Compound

The phenolic compound analysis of TBPF was based on the method of Bouterfas et al. [18]. A 1 mL sample of a diluted extract of *Tacca leontopetaloides*, diluted by a factor of 50, was mixed with 1 mL of diluted Folin–Ciocalteu reagent (10 times diluted with deionized water). After 4 min incubation, 0.8 mL of 7.5% (*w*/*v*) sodium carbonate solution was added. The mixture was then mixed for 10 s and incubated in the dark at room temperature for 2 h. The absorbance of the mixture was measured against a blank at 765 nm using a UV light spectrometer. The result was expressed in milligrams of gallic acid per grams dry weight of *Tacca leontopetaloides* (mg GAE/gDWT).

2.5.4. Swelling Index

A 0.2 g sample of *Tacca leontopetaloides* powder was suspended in 10 mL of distilled water in a shaken water bath at a temperature of 95 °C. The test tube with *Tacca leontopetalodies* powder and distilled water weight is W1. The mixture was stirred for over 30 min in order to keep the starch granules suspended. The test tube was then dried and cooled rapidly to 20 °C. The test tube with the paste solution was centrifuged at 3000× *g* rpm for 15 min in order to separate the supernatant solution and paste. After 15 min, the supernatant was removed slowly from the paste. The weight of the paste (W2) was determined and used to calculate the swelling index as the weight of paste, W2 minus W1, and divided by the original weight of dry starch [16].

2.5.5. Particle Size

Flocs formed after being treated by TBPF were withdrawn from the surface liquid level and transferred into a 10 mL liquid sample. The size of the floc was measured using zeta-PALS DelsaMax Pro (Beckman Coulter, Indianapolis, IN, United States). By using a 5 mL syringe, the sample was pulled into the sample cell.

2.5.6. Particle Charge

The freeze-dried sample (5 mg) of leachate, TBPF, and flocs were immersed in deionized water and shaken in a 10 mL sample bottle. The particle charge of the solution was analyzed using zeta-PALS DelsaMax Pro (Beckman Coulter) [14].

2.5.7. Functional Group Analysis

The freeze-dried leachate, TBPF, and flocs samples were analyzed using Fourier transform infra-red (FT–IR) for functional group analysis. The samples were scanned over the wavenumber range 400 to 4000 cm^{-1}, and the revealed absorbance was recorded by FT–IR model Spectrum One (Perkin Elmer).

2.5.8. Thermal Analysis

Thermogravimetric analysis (TGA) was performed using a thermogravimetric analyzer (Mettler Toledo), and the samples were scanned between 25 and 600 °C in an air atmosphere at 10 °C/min of airflow rate.

2.5.9. Morphological

The granule starch TBPF and freeze-dried TBPF, leachate, and floc were evaluated using benchtop scanning electron microscopy (JOEL-6000 Plus Versatile Benchtop SEM). The samples were coated with carbon (VC-100 carbon coater) to avoid image charging during the scanning process. The 500-times magnification surface scanning for granule starch, TBPF, and leachate was captured, and 2000-times magnification of floc was captured in order to have a clear image.

3. Results and Discussion

3.1. Raw Leachate Characteristics

Table 1 shows the characteristics of the raw leachate of Tanjung Dua Belas landfill that was collected and analyzed during dry and monsoon seasons from June to December 2018. The leachate was dark brown with high color characteristics (14201 PtCo), and the pH values were between 7.2 and 8.1. This pH range was mainly a consequence of the conversion of intermediate organic acids into methane (CH_4) and carbon dioxide (CO_2) [13]. The pH found in this study is in agreement with Yusoff et al. [13]. On the other hand, a higher range of ammoniacal nitrogen (24–270 mg/L) and nitrate (100–227 mg/L) concentrations were recorded and exceeded the standard discharged limit (Standard A). Besides, the TSS and COD in Tanjung Dua Belas landfill were also recorded with high concentrations ranging between 184–243 and 3425–6800 mg/L, respectively. These values were also in agreement with Yusoff et al. [13] for Matang landfill leachate. The heavy metals such as aluminum, zinc, lead, nickel, and cadmium were higher than the standard discharge stipulated by the Department of Environment, Malaysia (DOE) [19]. The leachate was identified as anionic, where the value of zeta potential was −31.08 mV.

Table 1. The characteristics of raw leachate of Tanjung Dua Belas landfill.

Parameter	**This Study**			**DOE Discharge Standard A** [19]
	Max	**Min**	**Average**	
pH	8.1	7.2	7.6	6.0–9.0
Turbidity, NTU	275	82.3	218	-
COD, mg/L	6800	3425	5150	50
TSS, mg/L	243	184	214	50
DO, mg/L	0.44	0.11	0.30	-
TDS, g/L	0.16	0.15	0.15	-
Nitrate, mg/L	227	100	269	-
Color, Pt/Co	36500	5250	14201	100
Sulfate, mg/L	650	200	341	-
TOC, mg/L	650	50	442	-
Ammoniacal nitrogen, mg/L	270	24	79.16	5
Aluminium, mg/L	21.9	0.20	16.35	-
Cadmium, mg/L	17.01	12	15.33	0.01
Zinc, mg/L	14.27	2.88	10.46	2
Lead, mg/L	18.82	0.63	12.70	0.1
Nickel, mg/L	16.74	8.92	13.62	0.2
Zeta potential, mV	−31.62	−30.55	−31.08	-

Note: Average of six samples collected from June to December 2018.

3.2. Analysis of TBPF

Table 2 shows the physicochemical properties of TBPF and its comparison with other studies. The fraction of amylose and amylopectin in the *Tacca leontopetaloides* starch from Mersing, Johor, was 24:76. The amylose/amylopectin of *Tacca leontopetaloides* starch was higher than rice starch but lower than native sago trunk (Table 2). The higher the amylose/amylopectin ratio, the higher the probability that a bridging mechanism can occur between flocculant and colloid particles in leachate [20]. Based on Nwokocha et al. [21], the molecular weight of *Tacca leontopetaloides* was found at 1.85×10^7 g/mol, which showed that TBPF can still act as a polymer bridge between particulates in the wastewater with the presence of high amylose/amylopectin fractions. As revealed by Tetteh and Rathilal [8], the efficiency of flocculants or coagulants depends on their molecular weight and charge density, but the presence of a high linear chain of amylose can also enhance the flocculating activity. The viscosity of TBPF suspension ranged between 0.037 and 0.04 Pa·s, which is an important flocculant property. The swelling index of 12.5–12.6 g/g indicates a good polymer swelling in water [16]. It also had a high carbon content (34.51–35.33%), where it helps destabilize colloid particles [22]. No phosphorus was found in TBPF, which helped the removal of suspended solid in leachate. According to Teh et al. [23], the presence of phosphate in the amylopectin skeletal structure made this starch less efficient in removing TSS. Overall, it can be concluded that TBPF is a type of anionic flocculant which has a negative zeta potential (−13.14 mV) comparable to flocculant products derived from rice and native sago trunk (NST). Table 3 shows the mineral content in TBPF, which consists of 34 mg/L of calcium, 1.0–1.3 mg/L of magnesium, 26.2–26.25 mg/L of sodium, 1.41–1.42 mg/L of zinc, and 29.28–29.30 mg/L of potassium. Furthermore, the total phenolic content in TBPF, which ranged between 0.384 and 0.386 mg/L, helps flocculation activity. The phenolic group is known to have an anionic nature since it is a good hydrogen donor. In an aqueous solution, it readily deprotonates to the phenoxide anion, which is stabilized via resonance. This will lead to delocalization of electrons within the aromatic ring, which increases the electron density of the oxygen atom, thus it indicates that the more phenolic groups in starch structure, the more it will help in flocculation activity [24].

Table 2. The physicochemical properties of Tacca leontopetaloides biopolymer flocculant (TBPF) and its comparison with other studies.

Properties/Starch	**This Study**	**Rice [23]**	**Native Sago Trunk (NST) [25]**
pH	6	7.55	-
Swelling index, g/g@4 h	12.5–12.6	-	-
Bulk density, g/mL	0.63	-	-
Gelatinization temperature, °C	60–80	-	-
Thermal resistant temperature, °C	280–340	-	-
Fraction amylose/amylopectin	26:74	20.5:79.5	31:69
Viscosity, Pa·s	0.037–0.04	-	-
Zeta potential, mV	−13.14	−2.09	−22.2
Elemental composition, %			
C	34.51–35.33	-	38.94
H	7.7	-	9.77
S	Nil	-	-
N	Nil	-	0.88

Note: The molecular weight of *Tacca leontopetaloides* is 1.85 × 10^7 g/mol [21].

Table 3. The mineral content of TBPF.

Mineral Content (mg/L)	**TBPF**
Calcium	34
Cadmium	2.1–2.19
Iron	0.2–0.21
Magnesium	1.0–1.51
Sodium	26.20–26.25
Zinc	1.41–1.42
Potassium	29.28–29.30
Total phenolic compound	0.38–0.39

The functional groups of *Tacca leontopetaloides* starch were determined using FTIR analysis, and the spectra were compared with those of the original amylose and amylopectin, as shown in Figure 1. *Tacca leontopetaloides* starch spectrum shows a vibration at 764.96 cm^{-1} attributed to the glucose pyranoses ring. The second region between 800 and 1500 cm^{-1} is highly overlapping and complex; thus, it is difficult to assign the exact bands. This region mainly shows the vibration of the monomer glucose unit in polysaccharides (amylose, amylopectin, cellulose, and starch). Based on Kizil et al. [26], the vibration of C–O–C in α-1,4 glycosidic linkages were revealed at 930 cm^{-1}; however, the *Tacca leontopetaloides* spectrum indicates the vibration of C–O–C in α-1,4 glycosidic linkages at 996 cm^{-1} which was similarly observed in the spectra of pure amylose and pure amylopectin. The C–H stretching mode was identified in the region between 2800 and 3000 cm^{-1}. The peak observed at the last region (3000–3600 cm^{-1}) originated from the vibration of the hydroxyl group, OH bending to the water molecule. A broad peak at 3285 cm^{-1} was observed, attributed to the absorption of the hydroxyl group (OH) bending to the water molecule. The presence of –COOH, –OH, and –NH_2 functional groups in the polysaccharides-based coagulant made it preferred for aggregation processes [27]. Therefore, all the functional groups observed in *Tacca leontopetaloides* starch support the capability of starch as the substituted chemical flocculant.

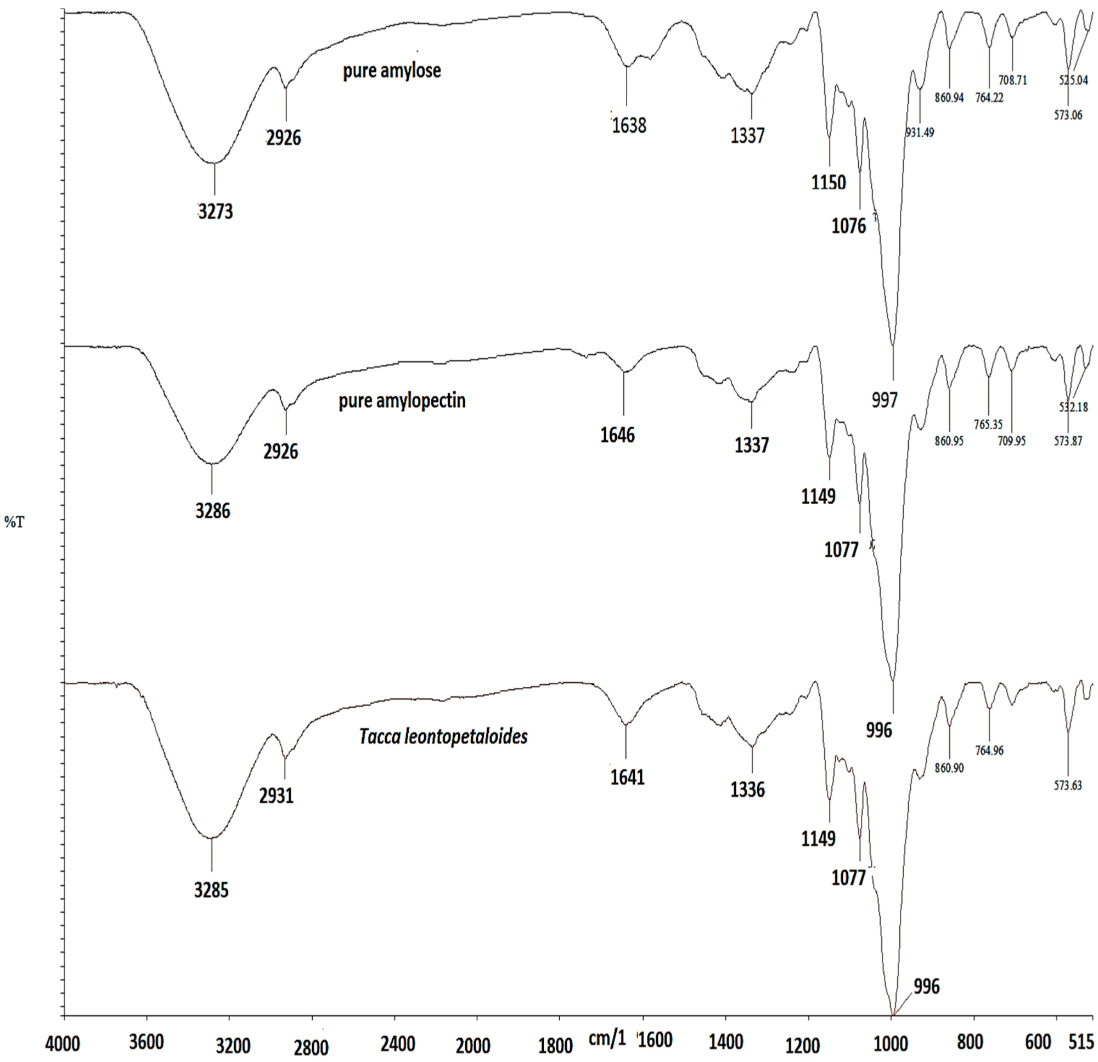

Figure 1. FTIR spectra of pure amylose, pure amylopectin, and *Tacca leontopetaloides* starch.

3.3. Effect of TBPF at Different pH Values of Leachate

Figure 2 shows the effect of TBPF at different leachate pH values on the removal efficiencies of turbidity, TSS, color, and COD at a fixed dosage of 1200 mg/L. At an initial pH of 3, high removal efficiencies of turbidity, TSS, color, and COD over the ranges 25–30%, 84–89%, 91–94%, and 30–55%, respectively, were observed. The effectiveness was due to the protonation of some functional groups such as carboxyl and hydroxyl, forming a high density of positive charge that exerts strong electrostatic forces over negatively charged colloidal particles in the acidic condition [28]. In the presence of TBPF (anionic) and leachate at pH 3 (cationic), the particles were destabilized via charge neutralization and bridging flocculation. This adsorption and interparticle bridging occur when segments of the polymer chain (amylose/amylopectin) are absorbed onto the colloidal particles, thus bridging them together and form loops (segments extending into the solution) and trains (segments absorbed on the surface) [29]. The removal of turbidity, TSS, color, and COD was lower at the initial pH of 7.2–8.1. It was due to the anionic nature of leachate (−31.08 mV of zeta potential), which is similar in TBPF (−13.14 mV of zeta potential), resulting in repulsion between both particles. Meanwhile, the addition of sodium hydroxide to increase the pH to the alkaline phase caused the increased repulsion between leachate particles, and TBPF is strong enough to adsorb and bridge the particles over quite large distances with the extending chain tails. Hence, the polymer chains linked those particles to form bridges that improved the removal of the treated parameters. This bridging process continued to occur until large, rapidly settling flocs were formed [29].

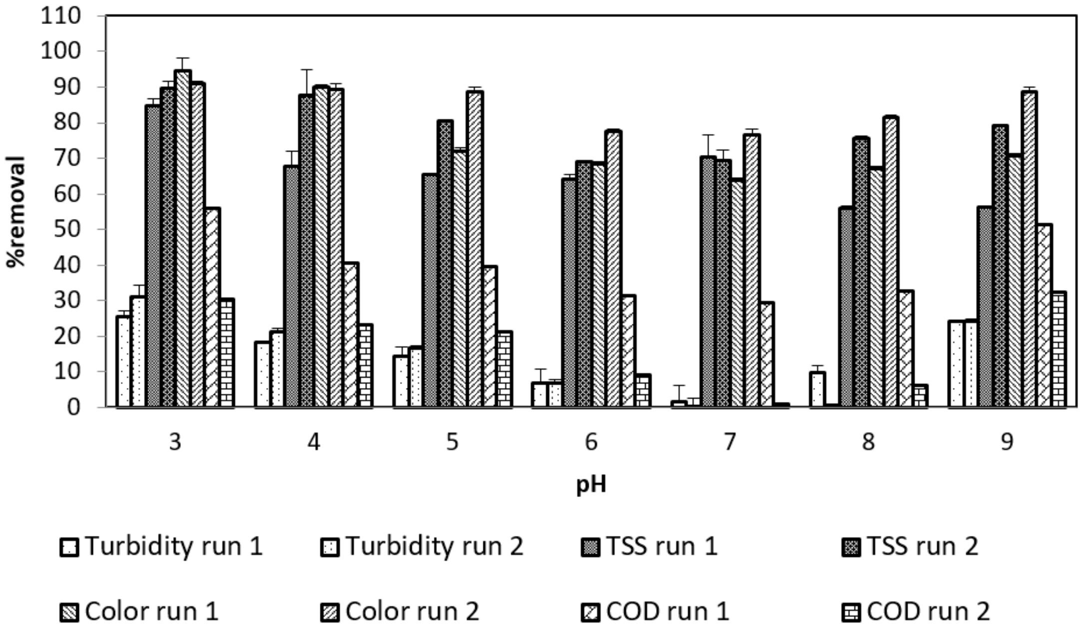

Figure 2. Effect of TBPF at different pH values of leachate on the removal efficiency.

3.4. Effect of Different Dosage of TBPF

The effect of different TBPF dosages on leachate was determined after the best pH was selected. The dosage was varied from 60–360 mg/L at pH 3. The highest removals of turbidity, TSS, and color were observed at 75.1–79%, 90.75–91.9%, and 93.6–94.31%, respectively, using 240 mg/L of TBPF (Figure 3). Nevertheless, less removal of COD (14.2–25%) was observed. The increased dosage of TBPF from 60 to 240 mg/L provided more polymers to be contacted with leachate colloidal particles to form particle–polymer–particle aggregates, leading to the higher formation of flocs. However, a higher dosage (300–360 mg/L) would restabilize the particles due to the surface saturation by the excess amount of absorbed polymer, resulting in insignificant improvement of TSS removal [23]. Smaller flocculant dosage is marginally more effective than larger ones, which may be attributed to the high charge density of the flocculant, whereby lesser dosage is sufficient for the destabilization of suspended particles and larger ones will cause interferences [23]. Moreover, high polymer dosage promotes the restabilization of colloids by repulsion forces between polymer and colloid particles [30]. Therefore, 360 mg/L of TBPF recorded the least removal of turbidity, TSS, color, and COD.

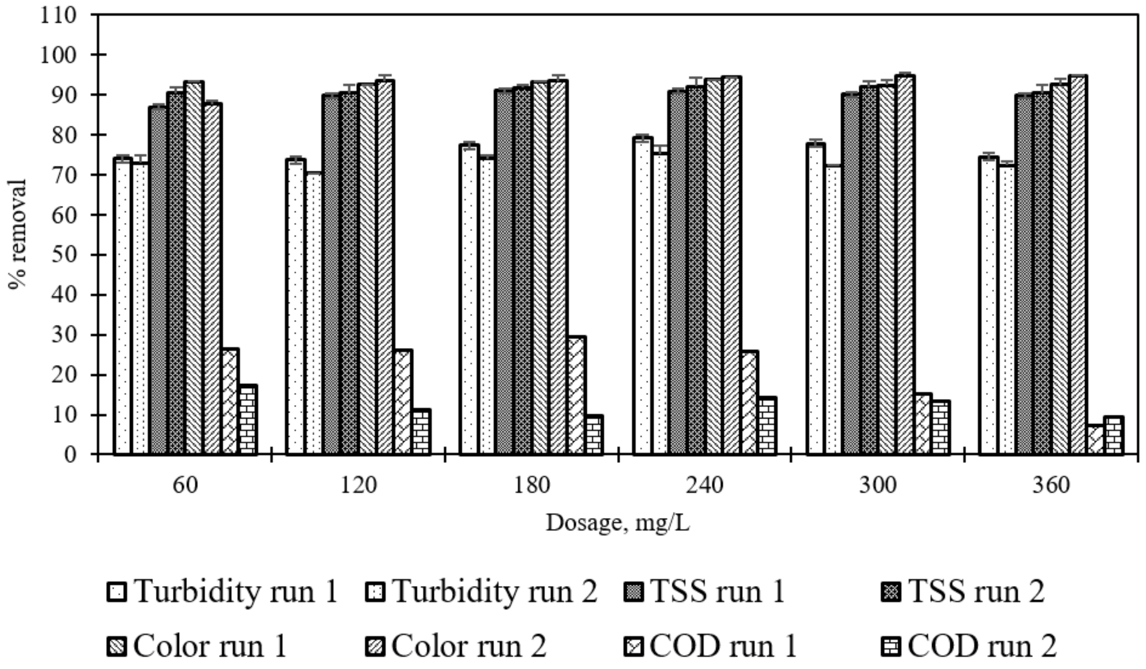

Figure 3. Effect of TBPF dosage on the removal efficiency at pH 3.

3.5. Characteristic of Flocs

3.5.1. Zeta Potential and Size Distribution

Table 4 shows the effect on zeta potential and particle size distribution of TBPF, leachate, and flocs (TBPF + leachate). At first, leachate was observed to have a high negative value (−31.6 ± 1.8 mV) with a size distribution of 94 ± 1.3 nm, which showed the alkaline nature of leachate containing high organic compound and toxicity [31]. TBPF showed as anionic flocculant at zeta value of −13.14 ± 1.8 mV, with a size distribution of 107 ± 6.3 nm, could perform inter-bridging linkage with leachate particles via van der Waal forces, wherein with the flocs at pH 3, the zeta value was recorded as 2.17 ± 0.91 mV with an increase in the size distribution of 337 ± 42 nm. The increasing particle size distribution resulted in the formation of flocs agglomeration and destabilization of particle size. This is in agreement with Yusoff et al. [13], although the flocs size was small. In addition, it can be concluded the coagulation–flocculation process occurred between molecule TBPF and suspended solid in leachate not via charge neutralization but through an interparticle bridging mechanism.

Table 4. Effect of coagulation on zeta potential and size distribution at optimum condition.

Samples	Zeta Potential (mV) ± SD	Diameter (nm) ± SD
Leachate	−31.62 ± 1.8	94 ± 1.3
TBPF	−13.14 ± 1.8	107 ± 6.3
TBPF + leachate	2.17 ± 0.91	337 ± 42

3.5.2. Structural Characteristic of Flocs by FTIR

FTIR spectroscopy was conducted in order to gain further insights on the flocs produced by coagulation–flocculation treatment of leachate using TBPF as flocculant. The viscous flocs sample after rotary vacuum evaporation was freeze-dried in order to preserve the organic and hydroxyl band in the sample, which would be affected if oven-dried [14]. During the bridging mechanism, a high molecular tail and loops provided by the macromolecule of TBPF formed links between particles within the leachate. There was hydrogen interaction that involved –OH attached to the carboxylic acid functional group and TBPF as ionic surface charge and a positive charge of leachate at low pH bridged via hydrogen bonding where it involved a carboxyl group –COO. The dispersion of TBPF in the leachate solution provides an adsorption site along the extending molecules. Figure 4 shows the FTIR spectra of floc (TBPF + leachate), which had a similar trend of leachate and TBPF spectra, indicating the effectiveness of the removal mechanism by TBPF to treat leachate. For all samples, the peaks observed between 2919 and 2935 cm^{-1} were attributed to the aliphatic carbon chain, –C–H, assigned to fatty acids and lipids [29]. The broad peaks centered around 3378 and 3440 cm^{-1} were due to –OH stretching. Meanwhile, peaks observed in the fingerprint region 1750–900 cm^{-1} show peaks commonly exhibited by biowaste materials that contain cellulose, lignin, aldehydes, ketones, esters, and carboxylic acids [29]. A peak of 1639 cm^{-1} shows the aromatic ring of carboxylic acid salts or unsaturated ketones which represent organic substances in leachate. The same peak was revealed in studies by Cataldo [22] and Smidt and Meissl [31]. Meanwhile, an absorption peak at 1560 cm^{-1} is attributed to amide II. Smidt and Meissl [31] stated the peaks that range between 1570 and 1540 cm^{-1} in leachate can be assigned to the N–H plane of amide II or a secondary amide. The presence of amide or nitrate groups supports the existence of ammonical and nitrate substances in leachate properties. When TBPF was used, the aromatic region of leachate and the glucosidic pyronosis ring (982 cm^{-1}) was still observed but with a new shifted band due to the new formation of molecular structures [29]. The aromatic band in leachate was shifted from 1639 to 1641, 1560 to 1564, and 1405 to 1407 cm^{-1}, which indicated it is non-oxidizable [15] but could be the aromatic ring formation in structural flocs. In addition, the band 1460 cm^{-1} in TBPF, which is associated to unsaturated alkene C=C was eliminated (peak in spectra floc) and could be substituted by the S–O (1047 cm^{-1}) from leachate. This indicated that the formation of a new structural compound between TBPF and leachate occurred.

3.5.3. Thermogravimetric Profile by TGA

Thermogravimetric analysis was conducted to understand the thermal profiles of the flocs produced from the coagulation–flocculation treatment of leachate. Each of the degradation ranges shown in Figure 5 is indicative of certain characteristics. The similar trend between the degradation profile of flocs produced with the single thermal profile of TBPF and leachate shows successful TBPF–leachate floc formation. Figure 5 exhibits the initial weight loss (1.85 to 3.5%) due to moisture evaporation from ambient temperature to 150 °C. Above 150 °C, two distinct degradation zones for leachate and flocs and single degradation zones for TBPF were observed from TG-DTG plots. Table 5 summarizes the degradation ranges and the maximum derivative weight loss of the sample with the corresponding degradation temperature. The first degradation zone, approximately in the region of 150 to 350 °C, corresponds to the degradation of light aliphatic compounds such as fatty acids and carbohydrates [32]. This region accounted for 10 to 63% of the total weight loss for all samples. When TBPF was used, the TGA profile showed as overlapping the TG profile between TG leachate and TBPF. The highest temperature for the first region in TG leachate was 237 °C, and it increased to 240 °C when TBPF was used in leachate treatment. TBPF shows a higher maximum derivative loss at 1.1 mg/min compared with the low maximum derivative of leachate, 0.026 mg/min. However, the maximum derivative loss increased to 0.188 mg/min when TBPF was introduced in the treatment. It could be attributed to the decomposition of starch components. The TG-DTG plots for TBPF show a dramatic weight loss at around 250 to 350 °C. Based on Teh et al. [23], in this region, the depolymerization and degradation of starch release CO_2, CO, water, and acetaldeyhyde, furan, and 2-methyl furan.

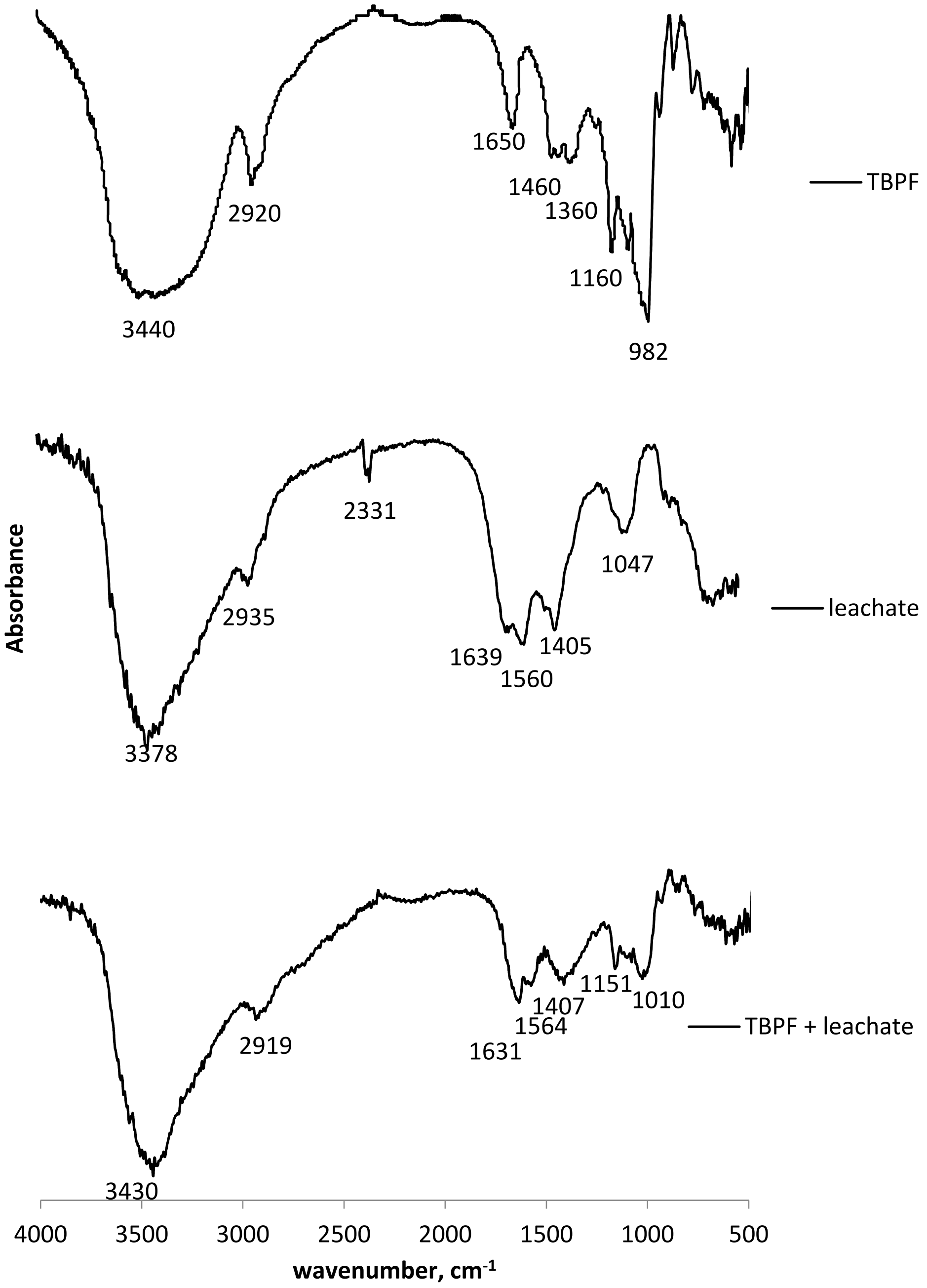

Figure 4. FTIR spectra of leachate, TBPF, and floc produced by coagulation–flocculation treatment using TBPF at optimum pH and dosage.

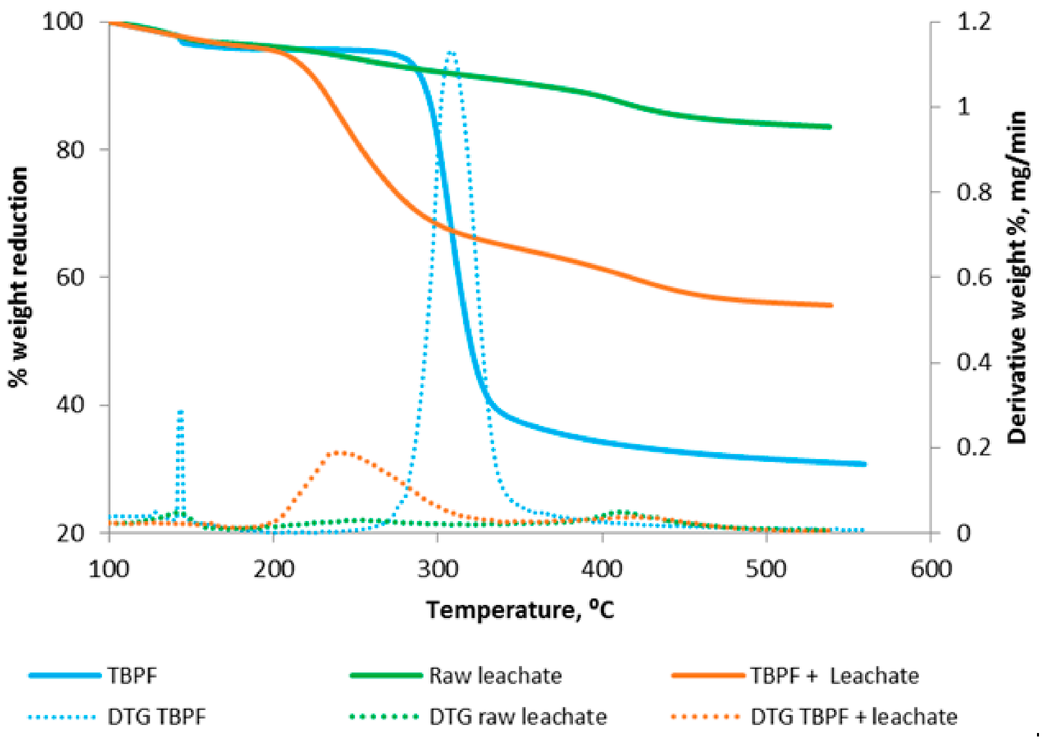

Figure 5. TG and DTG plot of dried leachate, TBPF, and floc produced by treatment using TBPF at optimum condition.

Table 5. Thermal degradation characteristic of leachate, TBPF, and floc produced by coagulation–flocculation process using TBPF.

Parameter	Leachate	TBPF	Floc (TBPF + leachate)
Initial drying range (°C)	25–150	25–150	25–150
Initial moisture loss (%)	1.85	3.5	1.97
Degradation range (°C)	150–350 and 350–530	200–370	150–350 and 350–530
Onset degradation temperature (°C)	236 and 400	272	191 and 388
Temperature at which maximum derivative weight loss occurred (°C)	237 and 403	304	240 and 429
Maximum derivative weight loss (mg/min)	0.026 and 0.044	1.1	0.188 and 0.036
Amount residue left at 500 °C (%)	83	31	55

The second degradation zone was found to be in the region of 350 and 550 °C, corresponding to the degradation of lignin and other more complex aromatic structures [23]. Contrary to the first region, TBPF reduced the maximum derivative loss from 0.044 to 0.036 mg/min. Based on this result, the introduced TBPF in the leachate treatment reduced the thermal decomposition of leachate by decreasing the onset degradation temperature of flocs (TBPF + leachate) from 236 into 191 °C. Overall, the amount of residue leachate left after treatment with TBPF was found to be 55% compared to the initial leachate of 88%.

3.5.4. Morphological Properties by SEM

SEM analysis reveals the morphological properties of the flocs produced using TBPF. Figure 6a depicts the granular swelling of *Tacca leontopetaloides* starch. The starch granules were found to be oval and polyhedral in shape, similar to the images observed by Nwokocha et al. [21]. In this study, starch granules were heated in hot water well above the temperature at which the granules lost their birefringence, also known as gelatinization temperature (80 °C). Continuous heating caused the granules to swell and crystal to melt, leading to complete separation of amylose and amylopectin [12] from the starch (therefore, a swollen and rubbery image was captured in Figure 6b), which would be used for bridging flocculation. After coagulation–flocculation, it was found that a uniform, smoother, and compact surface structure of flocs was formed, indicating the formation of larger, denser, and easier settling flocs as compared with the surface structure of leachate (Figure 6c). The statement is supported and similar surface structure was captured by Teh et al. [23] on suspended solids of palm oil mill effluent (POME).

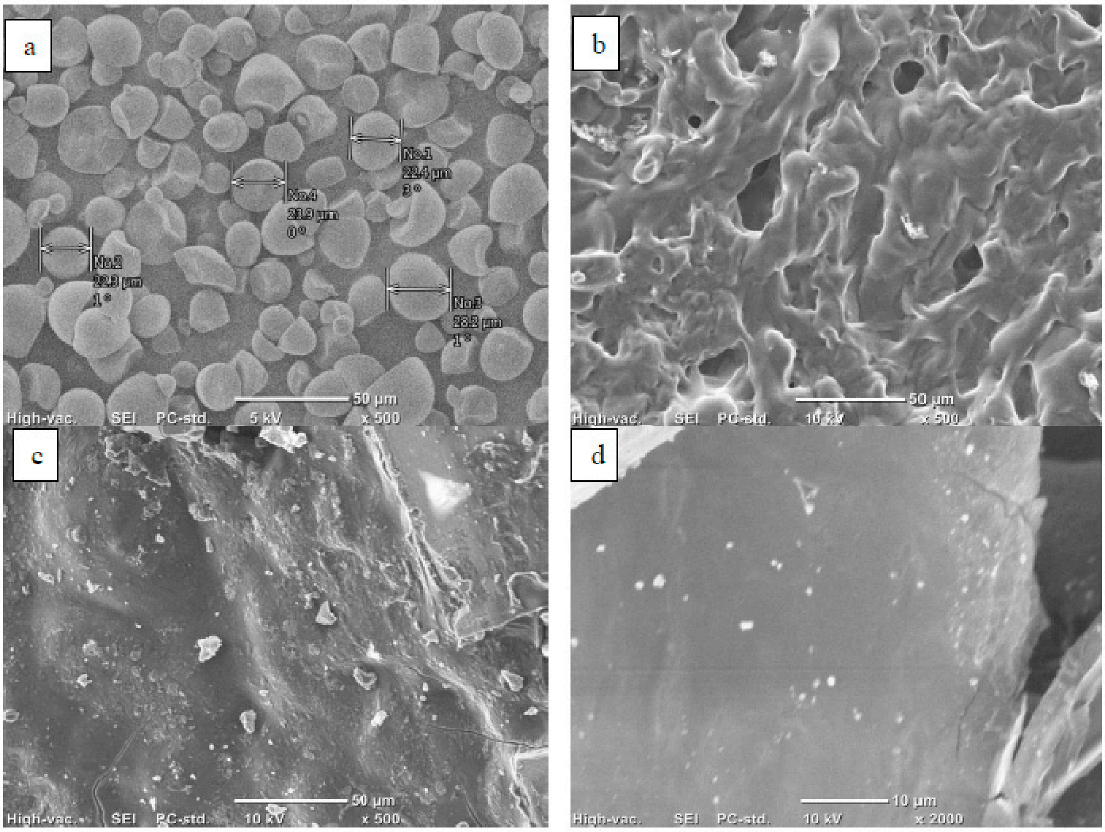

Figure 6. Surface morphology of (**a**) *Tacca leontopetaloides* starch granule, (**b**) TBPF, (**c**) leachate with magnification factor: 500 and (**d**) flocs (TBPF + leachate) at optimum condition (magnification:2000).

3.5.5. Final Treatment and Comparison with Other Studies

A comparison study using this new flocculant with other flocculants/coagulants is important to evaluate the effectiveness of removal. Table 6 shows the treated leachate performance in the removal of turbidity, TSS, color, and COD using TBPF with the final discharge limit (Standard A) stipulated by the Department of Environment, Malaysia (DOE). At the best pH (pH 3) and dosage (240 mg/L), the removal of turbidity, TSS, color, and COD were 45.8–54.5 NTU, 19.3–19.9 mg/L, 852–994 PtCO, and 3820–4429 mg/L, respectively. The treated values were reduced greatly compared to the initial values of the raw leachate at 218 NTU, 214 mg/L, 14201 PtCo, and 5150 mg/L of turbidity, TSS, color, and COD, respectively. Based on the standard discharge limit (Standard A), only TSS comply at lower than 50 mg/L; meanwhile, the levels of COD and color are still high. It can be suggested that TBPF can be used as a primary treatment. The huge reduction of color and turbidity indicates that TBPF can be proposed to be used together with commercial flocculants. The less removal of COD proved that TBPF is moderately effective in the de-stabilization of colloid particles in leachate. Miller et al. [32] reported that the use of natural coagulants might lead to an organic load where there is a possibility for undesired and increased microbial activity. The performance of TBPF was compared with those of other natural flocculants, as shown in Table 7. Compared with *Durio zibethinus* (CDSS), TBPF showed the highest removal of TSS and color. TBPF also showed the highest removal of turbidity, TSS, COD, and color compared with tapioca starch (TS), native sago trunk starch (NSTS), and *Dimocarpus* *longan* seed (LSP). The properties of each plant caused different removal efficiencies. According to Tetteh and Rathilal [8], molecular weight (MW) and charge density (CD) affect the performance of a flocculant. High MW flocculant can improve the agglomeration between particle leachate and TBPF and thus increase floc formation. Therefore, TBPF showed excellent removal of TSS by agglomeration between the TBPF polymer chain and suspended solids in leachate even though its CD was not in similar charges. The CDs of both TBPF and suspended solids were negative, which might obstruct the adsorption onto the undesirable surface. However, it might promote the polymer chain to have links with the leachate particles via mutual charges repulsion between the polymer molecules [8]. The COD removal using all flocculants was low. As stated earlier, it was affected by the properties of starch itself. In addition, it might dissolve the impurities in the leachate that are not contactable and agglomerate with the polymer chain of starch, thus left to consume oxygen and leading to the high COD value. Apart from natural flocculants, the dosage used in this study, as shown in Table 7, was lower compared to other studies that produced high TSS and color.

Table 6. The characteristics of treated leachate using TBPF.

Parameter	**Treatment**		**DOE Discharge Standard A [19]**
	Before	**After**	
pH	7.6	6.7	6.0–9.0
Turbidity, NTU	218	45.8–54.5	-
COD, mg/L	5150	3820–4429	50
TSS, mg/L	214	19.3–19.9	50
Color, Pt/Co	14201	852–994	100

Note: Average of six samples collected from June to December 2018.

Table 7. Comparison of TBPF capacity with other natural flocculants.

Natural Flocculant	**Polymer Element**	**Dosage, mg/L**	**pH**	**Removal, %**				**References**
				Turbidity	**TSS**	**COD**	**Color**	
TBPF	Amylose and amylopectin	240	3	75–79	90.7–91	14–25	93–94	This study
Durio zibethinus (CDSS)	Amylose, amylopectin and epichlorohydrin	250	5	90	87	65	91	[13]
Tapioca starch (TS)	Amylose and amylopectin	2500	4	-	12	-	54.7	[33]
Native sago trunk starch (NSTS)	Amylose and amylopectin	7000	4	0	27.9	1.7	13.1	[25]
Dimocarpus longan seed (LSP)	Protein	2000	4	-	22.2	39.4	28.3	[34]

4. Conclusions

The characteristics and effectiveness of the *Tacca leontopetaloides* plant as a new plant-based TBPF flocculant product for leachate treatment were successfully determined. The physicochemical characteristics of TBPF in terms of amylose/amylopectin fraction, viscosity, and zeta potential were 26:74, 0.037–0.04 Pa·s, and −13.14 mV, respectively. The presence of –COOH and –OH structures in TBPF indicates the flocculant properties. The jar test analysis at pH 3 indicates that 240 mg/L of TBPF produced high removal of turbidity, TSSs, and color from 218 NTU, 214 mg/L, and 14201 PtCo to 45.8–54.5 NTU, 19.3–19.9 mg/L, and 852–994 PtCo, respectively. Through TGA and zeta with size distribution analysis, it was found that flocs produced from the coagulation–flocculation of leachate exhibited certain characteristics similar to the flocculant used. Furthermore, SEM imaging showed that the bridging mechanism by TBPF on leachate produced flocs with more compact, denser, and smoother surfaces. Although the COD and color of treated leachate did not fully comply with the standard discharge limit, nevertheless, it showed a significant reduction and this can be used in the primary stage of leachate treatment.

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