Bio-refractory Dissolved Organic Matter and Colorants in Cassava
Distillery Wastewater: Characterization, Coagulation Treatment and
Mechanisms

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Abstract

An important portion of organic matter and colorants still remain in the biologically treated distillery wastewater, leaving the dark brown and odorous downstream with the heavy loading of chemical oxygen demand and the potential of forming disinfection byproducts. However, those bio-recalcitrant colorants have not been clearly recognized. The current study investigated the features of the bio-refractory organic matter and colorants in a typical distillery effluent, cassava distillery wastewater; special attention was paid to their change and behaviors in the coagulation treatment following the bio-processes. The wastewater analyses denoted that the fraction of high molecular weight (1-50 kDa and > 50 kDa) became predominant after the anaerobic-aerobic processes. Importantly, the lignin breakdown products, melanoidins and lignin phenols were confirmed to be the leading colored components, according to the parallel factor analysis of fluorescence excitation-emission matrixes results. Compared with lignin phenols, the former two types of colorants exhibited stronger bio-refractory activity and resulted in smaller color reduction after the aerobic treatment. Neither advanced oxidation nor adsorption could perform efficiently as post-treatment for decolorization in this study. Nevertheless, high removal of color and dissolved organic matter (~94.0% and ~78.3%, respectively) could be achieved by the FeCl_3 -involved coagulation under the optimal conditions. The ferric coagulant was found to preferably interact with the aromatic compounds (such as lignin derivatives) and melanoidins via either surface complexation or electric charge neutralization, or both. The findings presented herein might provide an insight into the evaluation of bio-refractory organic colorants and the Fe(III)-involved decolorization mechanisms of ethanol production wastewaters.

51 **Keywords:** Bio-refractory organic matter; Colorant characterization; Cassava-based
52 distillery wastewater; Coagulation mechanism
53

1. Introduction

Commercial ethanol has been extensively produced by the distillation of fermented grain or agricultural products; however, a great amount of wastewater with deep color and heavy organic strength is generated during the process. Given to the abundance and availability of cassava over the world, the cassava-based ethanol is attracting an increasing attention in recent years (Campos Benvenga et al., 2016). Take China as an example, approximately 35% of bio-ethanol plants use cassava as feedstock to date, giving rise to a total cassava-based ethanol production of ~400,000 metric tons annually (Baeyens et al., 2015). By 2020, that amount may increase by at least 1,000,000 metric tons (Yishui et al., 2011). The surging cassava ethanol industry in turn results in 8-12 metric tons of distillery wastewater per metric ton of ethanol produced. Its total solid, total chemical oxygen demand (COD) and total carbohydrates is 57.9 g/L, 56.5 g/L and 27.0 g/L, respectively, on average (Intanoo et al., 2014; Luo et al., 2010a, 2010b). The combination of anaerobic and aerobic processes has been applied for the COD reduction in the ethanol wastewater treatment (Zhang et al., 2016). But the downstream still contained as high as 550-1010 mg/L of residual COD with an unpleasantly deep brown color and a strong odor. Recalcitrant color-imparting matters could be barely removed, and thus the biologically treated effluent needed to be further polished before being allowed to be reused in the upstream processes or released to the environment.

That dark color of most distillery wastewater is generally attributed to the existence of naturally polymeric colorants. Previous studies concerning pigments in the molasses- or sugarcane-based distillery wastewater demonstrated that melanoidins, plant polyphenols, alkaline degradation products of hexoses and caramels have the greatest

79 potential for occurrence of color (Pant and Adholeya, 2007). They can form a large
80 part of dissolved organic carbon (DOC). Different feedstocks, fermentation processes
81 and biological treatments may bring about inconsistent structures of individual
82 colorant (Arimi et al., 2014). Different polysaccharide, amino acids and other organic
83 compounds react at various proportions; the polymerization probably occurs in
84 complex ways and extends to different levels. Accordingly, it is not easy to
85 characterize those pigments. It should also be highlighted that multiple colorants
86 instead of one type of pigment alone may result in the undesirable color, which will
87 then increase the difficulty of decolorization and organic elimination. It is reported
88 that caramels and alkaline biodegradation products of hexoses can be reduced by 70%
89 in the aerobic stage. However, melanoidins and plant polyphenols exhibit strong
90 antimicrobial and antioxidant properties (Arimi et al., 2014). Conventionally
91 biological processes are capable of accomplishing the degradation of the melanoidins
92 up to merely 6-7% (Mohana et al., 2009); however, the small size pigments probably
93 re-polymerize during biological processes. In addition to be colored components,
94 melanoidins and polyphenols may also cause the formation of aromatic halogenated
95 disinfection byproducts (DBPs) during chlorine disinfection of wastewater effluents.
96 Recent studies have shown that aromatic halogenated DBPs generally presented
97 significantly higher developmental toxicity and growth inhibition than aliphatic
98 halogenated DBPs (Jiang et al., 2017; Liu and Zhang, 2014; Meng et al., 2016). Given
99 that the chlorine disinfection has been implemented as the last barrier in many
100 wastewater treatments, the removal of those organic colorants could somewhat reduce
101 the potentially adverse effects to the receiving aquatic ecosystems by decreasing the
102 formation of aromatic halogenated DBPs.

103

So far, the molasses distillery wastewater has been mostly studied in the related research, where melanoidins were considered as the leading or even unique type of pigments (Arimi et al., 2015a, 2015b; Hatano et al., 2008; Liang et al., 2009; Liakos and Lazaridis, 2014). For the distillery wastewaters with other types of feedstock, such as the cassava-based ethanol wastewater, the composition and features of refractory colorants have not been well understood, and more efforts are required to address this. Thus, advanced techniques are strongly required to eliminate the organic matter and colorants from the biologically treated distillery wastewater, which has been increasingly recognized as a tough challenge (Arimi et al., 2014; Prajapati and Chaudhari, 2015; Tsioptsias et al., 2015). Coagulation, adsorption, and oxidation processes are possible post-treatment technologies following the biological processes (Arimi et al., 2015a, 2015b; Liang et al., 2009; Prajapati and Chaudhari, 2015). Among those methods, the coagulation technique is attracting an extensive interest due to its sound efficiency and low capital investment at the industrial level. In particular, ferric inorganic salts performed distinctively in the decolorization of distillery wastewaters (Arimi et al., 2015b; Liakos and Lazaridis, 2014). The Fe(III)-coagulant could remove up to 96% of color and 86% of COD from the molasses wastewater (Liang et al., 2009). Nevertheless, the investigations concerning the change of bio-refractory organic colorants during the tertiary process and the involved mechanisms are far from sufficient to the authors' knowledge.

Therefore, this study aimed at an emerging fermentation wastewater, cassava-based distillery wastewater. Firstly, the characterization and assessment of colored dissolved organic matter (DOM) were conducted on distillery wastewaters using different analytical techniques. The feasibility of ferric chloride-involved coagulation in the

tertiary decolorization and organic elimination was then studied. In particular, apparent molecular weight distribution, functional groups and fluorescence features of DOM before and after coagulation were comparatively investigated. Hence, the possible interaction between coagulant and colored components was clarified, providing a significant insight into related coagulation mechanisms.

2. Materials and methods

2.1. Chemicals

Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) was purchased from Sinopharm Chemical Reagent Company in China. The pH of solutions was adjusted by adding aqueous solutions of sodium hydroxide (NaOH) and hydrochloric acid (HCl) (Sinopharm Chemical Reagent Co. Ltd., China). All reagents were of analytic pure grade and used as received without further purification. Milli-Q water ($18.2 \text{ M}\Omega \cdot \text{cm}$) was used to prepare all the solutions in this study.

2.2. Cassava distillery wastewater samples

Samples of cassava distillery wastewater were obtained from a cassava ethanol plant in Jiangsu Province of China. Raw stillage was firstly treated by anaerobic digestion and aerobic treatment. The biologically treated wastewater then flowed through advanced treatment and the final effluent was discharged or reused. In this work, for the evaluation of colored organic components, water samples were collected from the raw distillery wastewater and each biologically treated effluent. Sampling was conducted every 7 days. All the wastewater was stored at 4°C prior to use. All samples were passed through $0.45 \mu\text{m}$ membrane filters (Micron-PSE, Polysulfone) to remove non-dissolved solids before analysis.

2.3. Coagulation treatment procedure

Jar tests were performed on a programmable jar test apparatus (ZR4-6, China), and FeCl_3 was chosen as coagulant herein. The removal of color and DOM was investigated on 400 mL of aerobically treated wastewater by FeCl_3 within the dosage range of 9-18 mmol/L and the pH range of 5-9, respectively. NaOH and HCl solutions were prepared and used as pH regulators of the coagulation influent. The jar test procedure consisted of a 30 second premix (250 rpm), 1 min rapid mix (200 rpm), 15 min slow mix (30 rpm) and 20 min settling period. A small amount of sample was taken immediately to measure the zeta potential (Malvern, Zetasizer 2000, U.K.) after 30 s' rapid mix. After the settling period, supernatants were filtered through 0.45 μm membranes after which a series of further chemical analyses were conducted.

2.4. Chemical characterization and analysis of cassava distillery wastewater and coagulation effluent

Basic physiochemical parameters, including soluble COD, DOC, total nitrogen (TN), biochemical oxygen demand (BOD), conductivity and pH, were measured by DR2800 spectrophotometer with samples being digested by DRB200 digester (HACH, USA), TOC analyzer (TOC-V_{CPH}, Shimadzu, Japan), HQ30d conductivity meter (HACH, USA) and HQ11d pH monitors (HACH, USA), respectively. Color was determined by comparing the sample with platinum-cobalt standards (Sinopharm Chemical Reagent Co. Ltd., China) according to the Standard Methods for the Examination of Water and Wastewater (1998). One unit of color was that produced by 1 mg/L platinum in the form of chloroplatinate ion. The absorbance of the solution was measured at 480 nm on a UV/Vis 2800 spectrophotometer (UNICO, China). Color values beyond the

upper range of the instrument were measured at appropriate dilution.

High performance size exclusion chromatography (HPSEC) was employed to determine the apparent molecular weight distribution of DOM presented in water samples. A gel filtration chromatography analyzer was used which consisted of a TSK gel 4000SW column (TOSOH Corporation, Japan) and a liquid chromatography spectrometer (LC-10AD, Shimadzu, Japan). Polyethylene glycol (Merck Corporation, Germany) standards with different molecular weight were used for calibration. The elution at different time intervals was collected by an automatic fraction collector, and then analyzed automatically via a differential detector to obtain the molecular weight distribution.

Fluorescence excitation-emission matrixes (EEMs) were measured via an Aqualog Fluorescence Spectrophotometer (HORIBA Jobin Yvon, France). EEMs were generated over excitation wavelengths (λ_{ex}) of 240-600 nm at 3-nm increments and emission wavelengths (λ_{em}) of 212.61-618.96 nm at 3.37-nm intervals. Milli-Q water was used as blank. The inner-filter effect was corrected following the tutorial by Murphy et al. (2010). Raman peaks of Milli-Q water were computed by emission wavelengths of 380-420 nm at the excitation wavelength of 350 nm; and thus the normalization of fluorescence signals was carried out to make them comparable in different batches of measurement (Murphy et al. 2010). EEM spectrograms were subject to parallel factor analysis (PARAFAC) modeling, using MATLAB software with the DOMFluor toolbox (Stedmon and Bro, 2008). To avoid the impact of low accuracy data due to relatively low lamp output, the EEMs for PARAFAC modeling only covered part of the $\lambda_{\text{ex}}-\lambda_{\text{em}}$ range: λ_{ex} between 252-402 nm and λ_{em} between

291.99-618.96 nm were used. The PARAFAC models with two to seven components were computed, among which the three-component model was determined primarily based on split half analysis and core consistency. The fluorescence intensity of each component was represented by the maximum fluorescence ($F_{\max}$, Raman Unit, R.U.).

Fourier transform infrared (FT-IR) was performed from 4000 to 500 cm^{-1} with a Nicolet 5700 spectrophotometer. Freeze-dried samples of different wastewater were mixed with superior grade KBr to prepare the pellet for FT-IR. All measurements were carried out at room temperature.

The UV absorption measurement was performed on the three types of cassava distillery wastewater as well as the coagulation effluent using a UV-2550 UV-visible spectrophotometer (Shimadzu, Japan) in a 1 cm quartz cuvette; and UV spectra were recorded from 200 to 400 nm at the 1-nm interval.

3. Results and discussion

3.1. Characterization and evaluation of refractory DOM and colorants in cassava distillery wastewater and biological treatment effluents

The initial cassava distillery effluent, called raw distillery wastewater, was heavy in organic carbon, dark brown in color and acidic in pH. The anaerobically-aerobically combined processes were used as the primary treatment of the raw stillage, and results are given in Table 1. By means of anaerobic digestion, approximately 94% of soluble COD was reduced, which was tapped for the energy recovery and other bio-refinery products. Nevertheless, color only got a slight reduction of ~32% in this step. The bio-digested downstream was then subject to the aerobic biodegradation. The soluble

COD was further decreased from 2101±451 mg/L to 780±230 mg/L, whereas the residual BOD was only around 85 mg/L. Additionally, the effluent was still dark brown with a characteristic color of 588-960 in Pt-Co unit. Apparently, a non-negligible amount of unknown bio-refractory organic matter and colorants remained, which limited the direct discharge or reuse of the above biologically treated effluent. It is quite necessary to figure out the properties of DOM as well as its relationship with color, which will then benefit the advanced treatment. To begin with, crucial features, including molecular weight distribution, functional groups, and fluorescence components should be clarified; thereafter, the contribution of different components to color can be explored.

Table 1 Physiochemical characteristics of cassava-based distillery ethanol wastewater

Parameters	Raw stillage wastewater	Anaerobic digestion effluent	Aerobic treatment effluent
Soluble COD (mg/L)	33870-40400	1650-2552	550-1010
DOC (mg/L)	7600-14800	654-986	175-296
BOD (mg/L)	-	928-1422	80-90
Total nitrogen (TN, mg/L)	680-900	260-533	41-126
Color (Pt-Co unit)	2200-2950	1250-2245	588-960
Color	Dark brown	Dark brown	Brown
Conductivity (mS/cm)	6.3-6.7	5.4-5.9	2.3-2.4
pH	3.9-4.5	7.4-7.5	8.1-8.6

Note: -: data not available;

The data range was obtained based on 48 raw distiller wastewater samples, 48 anaerobic digestion effluent samples, and 48 aerobic treatment effluent samples, respectively.

3.1.1. Apparent molecular weight distribution of DOM and colorants

Results in Fig. 1 present the apparent molecular weight distribution of DOM in the raw distillery wastewater and effluents of biological treatment. Low molecular weight components (< 1 kDa) accounted for around 84.7% of DOM in the raw stillage

wastewater. Their ability to cross bacterial cell membranes endows them with high biological availability. Hence, the apparent molecular weight distribution was modified by the biological treatment: the DOM in the anaerobically digested effluent was mainly composed of molecules ranging from 1-50 kDa; thereafter, the aerobic treatment raised the proportion of molecules higher than 50 kDa. The low molecular weight portion was consumed and degraded in the anaerobic-aerobic process. Meanwhile, the increase of high molecular weight fraction might also be attributed to the re-polymerization of colored compounds during the multistage biological treatment. To be specific, melanoidins (> 10 kDa), lignins and their derivatives (typically 1-10 kDa) tended to bio-accumulate and were resistant to biological treatment (Arimi et al., 2015b; Leiviskä et al., 2008; Liang et al., 2009; Peña et al., 2003). The high molecular weight species left in the aerobic treatment effluent were probably those bio-recalcitrant fractions, such as melanoidins, polyphenols, lignins. More characterization and analysis is subsequently conducted to elaborate on the exact composition of residual DOM and colorants.

Fig. 1. Apparent molecular weight distribution in raw distillery wastewater, anaerobic digestion effluent and aerobic treatment effluent.

3.1.2. Featured functional groups and probably matched organic matter

The FT-IR spectroscopy was employed to analyze functional groups of organic matter in the cassava-based distillery wastewater. IR spectra in Fig. 2 were obtained from freeze-dried samples of the three types of wastewater mentioned in Section 3.1. Generally, the wide peaks of all the samples in the range of $3600\text{--}3000\text{ cm}^{-1}$ could be assigned to H-bond, NH groups, or OH stretching of carboxyl, phenol and alcohol (Liakos and Lazaridis, 2014). The absorptions in the $3000\text{--}2700\text{ cm}^{-1}$ range possibly reflected the C-H stretching in aliphatic structures. Relatively sharp bands at $1720\text{--}1550\text{ cm}^{-1}$ might result from either carbonyl groups in primary amide functions or the existence of C-O stretching of COO^- ketonic C-O and aromatic C-C conjugated with COO^- . The COO^- stretching vibration could then be indicated by the peaks at around 1400 cm^{-1} (Liakos and Lazaridis, 2014). Those bands at wavelength numbers

of 1300 cm^{-1} mainly corresponded to C-N bonds in aromatic amine functions, and the absorptions between 1150 cm^{-1} and 1000 cm^{-1} were reported to be sensitive to C-O bonds (possible alcohols) and/or minerals. The 950-833 cm^{-1} peaks might be related to the out-of-plane bending of aromatic CH (Yao et al., 2012). Moreover, broad bands (700-600 cm^{-1}) were indicative of the presence of carboxylate dimers, amine, and/or amides (Hatano et al., 2008). The three spectral profiles exhibited a strong similarity to the main absorbance bands of melanoidins (Hatano et al., 2008; Liakos and Lazaridis, 2014) and cooked cassava starch (Wu et al., 2014). Hence, the cassava-induced melanoidins are revealed as significant DOM and colorants in the currently studied wastewater. They might be the product of Maillard reaction between cassava-based carbohydrates and amino compounds, which happened in the distillery process. Apart from the characteristic melanoidin structure, the aromatic CH and phenolic functional groups recognized in IR spectra also revealed the possible existence of lignin fragments as colorant (Leiviskä et al., 2008).

Fig. 2. FT-IR spectra of dried samples derived from (a) raw stillage wastewater, (b) anaerobic digestion effluent, and (c) aerobic treatment effluent, respectively.

It is also worth noting that the chemical structures of those recognized colorants depended heavily on the reactions that they went through as well as the nature and concentration of the involved-reactants (Arimi et al., 2014; Pant and Adholeya, 2007). The characteristics of DOM and colorants could be explained by comparing FT-IR spectra of samples before and after the biological treatment. Peaks at 3000-2700 cm^{-1} , 1720-1550 cm^{-1} , 1300 cm^{-1} , 1150-1000 cm^{-1} and 950-833 cm^{-1} suffered red shifts or even disappeared after the anaerobic digestion and aerobic treatment. It was probably caused by the degradation of small hydrocarbon molecules and amines groups by microorganisms.

Additionally, in order to confirm the results obtained from the above FT-IR analysis, the UV absorption measurement was performed on the raw distillery wastewater, anaerobic digestion effluent and aerobic treatment effluent, respectively. Both UV254

and UV280 reflected the aromatic organic matter and thus could be used to evaluate the variation of related compounds (Li et al., 2016). The UV/Visible absorbance spectra in Fig. S1 revealed that the aromatic dissolved organic matter (such as lignin fragments, phenolic compounds) were removed from the cassava distillery wastewater through the anaerobic digestion, and subsequently were eliminated by the following aerobic process.

3.1.3. Fluorescence EEMs-based component estimation

To confirm the possibly existed melanoidins and phenolic compounds inferred by FT-IR and obtain the "fingerprint" for specific DOM, fluorescence EEMs was performed on the target wastewaters. Fig. S2 presents EEM spectra attained from the three types of cassava distillery wastewater as aforementioned. Overall, there were three fluorescence peaks (C, A and T) identified in the cassava-based wastewater. EEM peaks have been allocated to different compounds for various types of wastewater according to Carstea et al. (2016), and may offer some hints of possible DOM in this study. Accordingly, peak C ($\lambda_{Ex/Em} \sim 300\text{-}350/400\text{-}450$) and peak A ($\lambda_{Ex/Em} \sim 225/400\text{-}500$) might relate to fluorophores like lignins, humic acids, flavonoids, aromatic ketones, and other polycyclic aromatic fluorophores. Peak T ($\lambda_{Ex/Em} \sim 225(\sim 280)/\sim 350$) could be attributed to fluorophores containing a limited number of aromatic rings.

On the basis of the information obtained above, PARAFAC modeling was employed to separate the overlapping EEM spectra, categorize the independent fluorescence components and clarify their relationship with color. PARAFAC of 96 biologically treated effluent samples (48 anaerobic effluent samples and 48 aerobic effluent

samples) was conducted using the 3-component model, and thereby fluorescent components were suggested as C1, C2 and C3. The excitation and emission loadings are given in Fig. 3; origins and properties of corresponding components are depicted in Table S1. Different PARAFAC components were then pointed out: C1 and C2 with peaks of $\lambda_{\text{Ex/Em, 3C1}}=250/460$ nm and $\lambda_{\text{Ex/Em, 3C2}}=325/400$ nm, individually, could be associated with breakdown products of lignins (Carstea et al., 2016; Cawley et al., 2012; Wei et al., 2014) or humic acid-like substances (Shan et al., 2016); C3 with specific $\lambda_{\text{Ex/Em}}=275/340$ nm might originate from lignin phenols (Boyle et al., 2009; Cawley et al., 2012; Hernes et al., 2009; Walker et al., 2009). Lignins (aromatic polymers) and their derivatives have been reported to be the colored species in wastewater; thus, they could remain in the biologically treated wastewater due to the biodegradative persistence (Lewis et al., 2013). In addition, chemical features of melanoidins resemble humic substances, being acidic, polymeric and highly dispersed colloids which are negatively charged due to the dissociation of carboxylic and phenolic groups (Dwyer et al., 2009). In the current study, it is inferred that the humic acid-like component identified by PARAFAC might be melanoidins.

Fig. 3. 3-component PARAFAC model generated from samples of biologically treated wastewater: (a), (b) and (c) contour plots of the spectral shapes; (d), (e) and (f) line plots of the loadings. Relationship between fluorescence intensity and color of each component (■: Anaerobic digestion effluents, ☆: Aerobic treatment effluents): (g) C1, (h) C2 and (i) C3. (Results were obtained from samples with 50-fold dilution.)

To obtain the contribution of fluorescence components to the color variation, the relationship between the maximum fluorescence intensity ($F_{\max}$) of individual PARAFAC component and color was examined (see in Fig. 3(g)-(i)). It is worth emphasizing that a linear correlation between the $F_{\max}$ of C1 and color ($R^2=0.4238$, $p < 0.001$, Fig. 3(g)) was attained. This prominent correlation implied that C1 could be the proxy for color during the biological treatment. The $F_{\max}$ of the other two components (C2 and C3) was weakly correlated with color (data not shown) probably because of the effect of biological processes, especially the aerobic treatment. The average color of the 48 anaerobic digestion effluent samples was 2534 ± 197 while that of the 48 aerobically treated downstream samples decreased to 2059 ± 170 . Meanwhile, the $F_{\max}$ dropped with the color reducing as the biological treatment proceeded. the $F_{\max}$ of C1 and C2 went through a successive and relatively small reduction from 342 ± 9 to 314 ± 11 . In contrast, the $F_{\max}$ reduction of C3 was much more significant after the aerobic process, from 321 ± 27 to 85 ± 8 . The change of $F_{\max}$ and color revealed that the aerobic biodegradation of lignin phenols (C3) was relatively great, whereas the aerobic treatment was less efficient at removing colorants like lignin breakdown products or melanoidins (C1 and C2). Given to the bio-recalcitrant melanoidins and phenolic compounds after the biological processes, an advanced treatment other than biological processes should be adopted for the purpose of eliminating refractory colorants.

3.2. Coagulation removal of residual DOM and colorants from the aerobically treated wastewater

The aerobic treatment effluent of cassava distillery wastewater was low in BOD but high in DOC (Table 1). Results in Section 3.1 denoted that the pronounced amount of

residual DOM therein was highly likely to be the breakdown products of lignins (Carstea et al., 2016; Cawley et al., 2012; Wei et al., 2014), lignin phenols (Boyle et al., 2009; Cawley et al., 2012; Hernes et al., 2009; Walker et al., 2009), melanoidins and/or humic acid-like substances (Carstea et al., 2016; Wei et al., 2014). These compounds are reckoned as the target colorants and DOM in the tertiary treatment and even the possible inducements of aromatic DBPs in the final chlorine disinfection. Considering their resistant properties in the biological and oxidative processes, the classical physicochemical technique -- coagulation, was employed as a tertiary treatment following the currently-used aerobic process.

The coagulation behaviors at different coagulant dosages and influent pH values are shown in Fig. 4(a) and (b). The curve of color removal in Fig. 4(a) presents a typical trend in coagulation trials: the removal efficiency rose with the addition of FeCl_3 , reached the highest decolorization rate of 92.9-95.0% at the dosage of 10.5-13.5 mmol/L, and exhibited re-stabilization beyond that dosage range. The coagulation performance in DOC elimination was consistent with that in decolorization: the maximum DOC removal of 77.7-78.8% was attained when dosing FeCl_3 at 12.0-15.0 mmol/L. The optimum coagulant dosage of 13.5 mmol/L was defined when high coagulation efficiencies of both decolorization and DOM separation were achieved. The effect of influent pH value was investigated as well (see in Fig. 4(b)). Both of color and DOC removals achieved the maximum values at pH 7. The variation of zeta potential was also given to assess the electrostatic interaction between the coagulant and colloids in the coagulation suspension. FeCl_3 almost completely neutralized the negative surface charges of colloidal pollutants at the optimum coagulant dosage and influent pH value, resulting in ~0 mV zeta potential. It is also noteworthy that more

410 than 90.0% of color and around 70.0% of DOC were removed while the related zeta
411 potential of the coagulation suspension was lower than -10 mV, indicating an
412 incompletely destabilized system. This might be interpreted by the surface
413 complexation between Fe(III) and dissolved organic colorants, through which the
414 combination of ferric coagulant and colorants could occur even though the
415 electrostatic attraction was insufficient to lead to the formation of coagulation flocs.
416 The Fe(III)-colorant complexes could then turn to be insoluble, forming large
417 aggregates by further coagulation mechanisms such as adsorption and sweeping.

Fig. 4. Coagulation performance: removal of color and DOC varying with (a) coagulant dosages at the fixed initial pH of 7, and (b) initial pH values at the fixed FeCl_3 dosage of 13.5 mmol/L; (c) differential UV absorbance spectra of coagulation effluents; (d) $F_{\max}$ variation for the 3-component PARAFAC model.

Apart from the present investigation, a number of studies have been dedicated to the coagulation removal of bio-refractory DOM and colorants from distillery wastewater, but mainly focused on molasses-based wastewater (Arimi et al., 2015b; Inanc et al., 1999; Liakos and Lazaridis, 2014; Liang et al., 2009; Prajapati et al., 2015). Table 2 summarizes the typical coagulation results in the past twenty years. The initial organic content of the coagulation influent in this study was lower than that in others' work. Particularly comparing the current result with that of Arimi et al. (2015b), more equivalent FeCl_3 dosage was demanded to obtain the optimum treating efficiency. This might be attributed to the difference of organic colorants and DOM between the present work and other cases: it has been well accepted that melanoidins are the leading colorants in the molasses-based ethanol wastewater; however, when the feedstock of distillery was cassava, the composition of bio-recalcitrant DOM and colorants was complicated. With multiple colorants, including lignin breakdown products, lignin phenols, melanoidins and so forth, the biologically treated wastewater was supposed to exhibit quite different characteristics. Accordingly, the difficulty of the coagulation treatment was increased. In order to gain deeper insights into the coagulation behaviors, further analyses and investigation were made to explore the interaction between the aforementioned DOM/colorants and ferric coagulant used in this study.

441 **Table 2** Comparison of coagulation performance in treating different feedstock-based distillery wastewater

Feedstock	Type of distillery wastewater	Properties of influent		Optimum coagulation conditions			Coagulation performance		Reference
		Organic content	Color	Coagulant dosage	Coagulant dosage (mg/mg DOM)	pH	Organic carbon removal (%)	Color removal (%)	
Molasses	Anaerobically digested wastewater	450 mg/L DOC	0.8 Abs/cm ^a	1.6 g/L FeCl ₃	3.6 FeCl ₃ /DOC	5	63.3	92.7	Arimi et al., 2015b
Molasses	Aerobically treated wastewater	7000 mg/L COD	16800 ADMI ^b	100 mmol/L (16.2 g/L) FeCl ₃	2.3 FeCl ₃ /COD	5	90.0	90.0	Liakos and Lazaridis, 2014
Molasses	Aerobically treated wastewater	950-1000 mg/L COD	1.3-1.4 Abs/cm ^a	3.5 g/L FeCl ₃	3.5-3.7 FeCl ₃ /COD	8	86.0	96.0	Liang et al., 2009
Sugar beet molasses	Aerobically treated wastewater	2377 mg/L COD	3800 Pt-Co unit	20.0 g/L lime (CaO)	8.4 FeCl ₃ /COD	12.5	89.5	84.3	Inanc et al., 1999
Rice grain	Biodigester effluent	13600 mg/L COD	0.186 Abs/cm ^a	60 mmol/L (9.7 g/L) FeCl ₃	0.7 FeCl ₃ /COD	5	78.0	80.0	Prajapati et al., 2015
Cassava	Aerobically treated wastewater	236 mg/L DOC	774 Pt-Co unit	13.5 mmol/L (2.2 g/L) FeCl ₃	9.3 FeCl ₃ /DOC	7	78.8	94	The present study

442 ^a: Absorbance at 475 nm.

443 ^b: American Dye Manufacturers' Institute Color Index.

3.3. Interaction between bio-refractory DOM/colorants and ferric chloride in coagulation

The decolorization phenomenon could be immediately observed after FeCl_3 was added into the jar in the rapid-mix phase of coagulation. In order that the complex decolorization and coagulation mechanisms could be well understood, the change and fate of colorants and DOM in coagulation were primarily explored. The influence of coagulation on the molecular weight distribution was examined at the optimum FeCl_3 dosage. The proportion of high molecular weight components (> 50 kDa) was largely reduced in the coagulation effluent; and the fraction ratio of 1-50 kDa to < 1 kDa got decreased to some extent (Fig. S3). The high molecular weight components, including lignin compounds with aromatic structures and melanoidins, were thus suggested to preferably react with iron species, form the insoluble complexes of "colorants-ferric species", and then removed by coagulation-sedimentation. A small amount of refractory low molecular weight components were left, resulting in the residual DOC in the coagulation effluent (Fig. 4(a) and (b)).

The aforementioned lignin substances were particularly traced in the coagulation effluent. Since lignins are cross-linked phenolic polymers (Lebo et al., 2000) and melanoidins may also contain the aromatic functional groups. Thus, their coagulation performance can be evaluated by the reduction of UV/Visible absorbance in the coagulation treatment: the UV/Visible absorbance of the aerobic treatment wastewater was represented by UVA0 and that of the coagulation effluent was then represented by UVA (Fig. 4(c)). FeCl_3 -involved coagulation gave rise to a monotonic and significant decrease in the UV absorbance spectra over 240 nm. For all the coagulation effluents, the UV/Visible differentials increased at wavelength between 240 nm and 250 nm,

and then plateaus were reached. Herein, UV254 and UV280 were employed to evaluate the aromatic DOM (Li et al., 2016). Results in Fig. 4(c) indicated that the removal of aromatic DOM (lignin derivatives in the cassava distillery wastewater, such as lignin phenols) increased with FeCl_3 dosage from 9.0-13.5 mmol/L, whereas further increasing coagulant dosages resulted in less removal efficiencies due to the re-stabilization of the coagulation suspension.

Same fluorescence components before and after coagulation were found according to the results of Fluorescence EEMs and PARAFAC (Table S2 and Fig. S4). Red shifts were observed for each component, and it was likely that large molecules with more rings were removed by coagulant, leaving the aromatic components with simple structures. Furthermore, the F_{max} values for each component varying with the variation of coagulant addition were given in Fig. 4(d). Throughout the investigated FeCl_3 dosage range, C1 and C2 which indicated lignin breakdown products and/or melanoidins significantly dropped along with the FeCl_3 addition; Similarly, the decrease of lignin phenols were confirmed by the F_{max} reduction of C3. Those hydrophilic polymers were apt to form complex with metals (Caemmerer et al., 2012; Morales et al., 2005) and then be separated by coagulation. F_{max} of all the components almost unchanged beyond the optimum FeCl_3 dosage (> 13.5 mmol/L). Obviously, all the organic dissolved colorants were well removed by FeCl_3 coagulation. It should be noted that the variation of F_{max} was in good accordance with the change of color and DOC removals in Fig. 4(a).

Based on the coagulation behaviors and the above discussion, the FeCl_3 -involved coagulation process and the related mechanisms can be depicted as Fig. 5: the lignin

components (lignin breakdown products and lignin phenols) and melanoidins with high molecular weight might favorably chelate or bind with hydrolytic iron species. On the other hand, the polyanions resulting from the hydrolysis of colorant molecules could also interact with Fe(III) and its hydrolytes via the electrostatic attraction. Thus, the ferric coagulant converted the dissolved color fraction into the insoluble form through either surface complexation or charge neutralization, or both. Then, the insoluble coagulant-colorant aggregates then further conglomerated into larger flocs which could be easily separated from water through the classical flocculation mechanisms; the sweeping, adsorption, bridging and patch function were able to facilitate the subsequent sedimentation (Wu et al., 2007). A similar interaction between Fe(III) and organic pigments was also observed by Liang et al. (2009) in their study of coagulation removal of melanoidins from molasses wastewater.

Fig. 5. Proposed decolorization and DOC removal mechanisms in the Fe(III)-involved coagulation process (The scheme was drawn out of proportion).

4. Conclusions

In the present investigation, the multiple dissolved organic colorants, instead a single type, were found and evaluated in the cassava-based distillery wastewater. The highly possible bio-refractory colorants were the breakdown products of lignins, melanoidins and lignin phenols. They might also result in the formation of aromatic halogenated DBPs when the chlorine disinfection was adopted subsequently. Compared with lignin phenols, the former two types of colorants exhibited stronger bio-refractory activity and resulted in smaller color reduction after the aerobic treatment. It is worth noting that $F_{\max}$ might be the proxy of colored components according to the correlation of PARAFAC components with characteristic color value. The FeCl_3 -involved coagulation was proved to be an effective post-treatment of aerobically treated wastewater. The optimum removal of color and DOC, ~94.0% and ~78.3%, respectively, could be achieved at the FeCl_3 dosage of 13.5 mmol/L and the pH of 7. Essentially, the identified bio-recalcitrant organic colorants were hydrophilic and existed as polyanions in water; meanwhile, they were apt to form complexes with metals. Hence, the ferric coagulant was capable of converting those dissolved color fraction into insoluble form through either electric charge neutralization or surface complexation, or both. Via the coagulation process, aromatic compounds (such as lignin derivatives), melanoidins and other high molecules were well removed; and the variation of fluorescence components was in accordance with the specific removal of targeting DOM in the studied wastewater. In sum, those fundamental and profound results clarified the presence, characteristics and removal mechanisms of the mixed bio-resistant colorants, providing a significant indication into further investigations of decolorization and elimination of bio-refractory organics.

535

536 **Acknowledgements**

537 This research was supported by the National Science Foundation (No. 51378373 and
538 No. 51608373), the 59th China Postdoctoral Science Foundation (No. 2016M591713),
539 and the Foundation of State Key Laboratory of Pollution Control and Resource Reuse
540 (Tongji University), China, (No. CRRY15001). Prof. Qinghui Huang is acknowledged
541 for his help in the measurement of fluorescence EEMs.

542

543 **Appendix A. Supplementary material**

544 Supplementary material related to this article can be found at ...

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Characterization

Bio-refractory DOM and colorants

Coagulation-sedimentation

Interaction between
DOM (colorants) and
Fe(III)-coagulant