Treatment of Baker's Yeast Wastewater by Electrocoagulation and Evaluation of Molecular Weight Distribution with HPSEC

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Treatment of baker’s yeast wastewater by electrocoagulation and evaluation of molecular weight distribution with HPSEC

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Abstract

In the present paper, the molecular weight distributions (MWDs) of Baker’s yeast wastewater (BYW) during electrocoagulation (EC) are investigated by High Performance Size Exclusion Chromatography (HPSEC) with ultraviolet diode array (DAD) and refractive index detectors (RID). The results of this study show that using DAD and RID in HPSEC are quite useful in order to reveal changes in MWDs of all components by RID (whether UV-Vis absorption or not), and colored (strongly absorb UV-Vis radiation) by DAD. Molecular Weights (MW) of components are varied in a wide range of 92.0 Da - 2.1 × 10⁶ Da. The high molecular weight components (HMWCs) and low molecular weight components (LMWCs) are present in low concentrations but they contribute high amount to color intensity (total contribution of two fractions are about %80) whereas, the intermediate molecular weight components (IMWCs) have high concentration with low amount to color intensity. The optimum operating conditions for removal of color and COD are found as 86% and 43% at 80 A/m², pH 4 and 20 min in EC process with Al electrode. The EC process remove the HMWCs more efficiently, thus color removal efficiencies are high with respect to COD and TOC removal efficiencies during EC.
1. INTRODUCTION

Decolorization process of Baker’s yeast wastewater (BYW) is a necessary operation in order to meet discharge limits for the natural water environment. Effluents generated from Baker’s yeast manufacturing plants contain numerous organic matters as well as dark brown colored components (melanoidins). This dark brown color in BYW comes from molasses which is a by-product of the sugar industry and a cheap raw material often used in the Baker’s yeast manufacturing plants and other fermentation processes such as alcohol and acid production.

The BYW is characterized by extremely high chemical oxygen demand (COD; 50,000-100,000 mg/L) and biochemical oxygen demand (BOD; 20,000-50,000 mg/L), strong nitrogenous (total N of 0.5–1.5 g/L), sulfate-rich (2.0–10.0 g/L), apart from low pH (4-5), strong odor and dark brown color (1,2). Several treatment processes such as chemical oxidation, adsorption, membrane processes, chemical coagulation, biological oxidation such as anaerobic-aerobic and electrocoagulation (EC) have been used in decolorization of the BYW (3). The EC process for BYW using Al electrodes is applied for treatment of the anaerobic (AE) and aerobic treatment (AAE) stage effluents (3,4). Although our previous studies have been conducted on the decolorization of the BYW by EC (3,4). There aren’t any studies related to understand the effect of components’ chemical
structure on the color removal mechanism by determination of molecular weight
distribution (MWD). Thus, the effect of MWDs in BYW is investigated in this study to
evaluate color removal mechanism of EC processes.

Traditional approaches for determination of the molecular weight (MW) are
ultrafiltration, field-flow fractionation, vapor-pressure osmometry, small-angle X-ray
scattering, ultracentrifugation, and size exclusion chromatography (SEC). Advantages of
High Performance Size Exclusion Chromatography (HPSEC) to determine the MW over
other analytical techniques are small sample volumes (µL), minimal amount of pre-
treatment, ease and speed of analysis, availability of equipment, and the ability to
determine both number-average and weight-average MW. Several detectors have been
used with HPSEC including multi-angle light scattering, refractive index detector (RID)
and diode array detector (DAD) (5). In this study, HPSEC with RID and DAD is used to
evaluate the removal of MW fractions during EC. RID corresponds to the difference
between the refractive index of sample in the sample cell and the mobile phase in the
reference cell. RID is suitable for detecting all components because the change in
refractive index occurs for all analyte. Because of this advantage, RID is often used for
the detection by HPSEC. However RID is not as commonly used as DAD in wastewater
researches because RID has lower sensitivity than that of DAD. In addition, DAD is a
type of UV-Vis detectors and the UV and Vis detectors provide good sensitivity for light-
absorbing compounds especially for chromosphere groups. However DAD is not suitable
for detecting components which do not have UV-Vis absorption such as sugar, alcohol,
inorganic ions. In this study, both of detectors for characterization of MWD of BYW
during the treatment are used to combine the advantages of these detectors.

Some organic compounds commonly are found in water and wastewater, such as lignin, tannin, humic substances, and various aromatic compounds, absorb strongly UV-Vis radiation. UV detectors generally have been used in wastewater treatment process to characterize the organic carbon concentration, natural organic matter and aromaticity of components, and 254 nm has been used as the standard wavelength in UV region (6). Vis detectors are usually used for measurement of the wastewater color between 400 and 500 nm. The structures of BYW contain a range of chromophores with varying molar absorptivities at any given wavelength. Color of BYW is monitored at 475 nm by Vis detectors (3,7,8). In this study, monitoring of organic carbon content and aromaticity at 254 nm and monitoring of color and chromophores at 475 nm by DAD are analyzed in BYW.

There are a few studies in literature related to MWDs of BYW. Nakajima-Kambe et al. (7) uses various microorganisms for their ability to decolorize BYW under thermophilic and anaerobic conditions, and are determined for MWD by HPLC using a gel filtration column with DAD (AU475nm) and RID. They suggested that the decolorization of BYW by strain MD-32 is accompanied by a decrease in not only small molecules but also large ones. Mutlu et al. (9) developed a membrane-based treatment scheme to remove colorants from the effluent of BYW and used microfiltration, ultrafiltration and nanofiltration membranes with differing molecular weight cut-offs (MWCOs). They used Sephadex G-50 column for MWD determination by AU280nm. They suggested that there are very
fluctuations in the MWD of BYW and overall MWD shows a significant shift to lower MWs with a decrease in MWCOs of the membranes. Peña et al. (8) investigated for the color removal from biologically pre-treated molasses wastewater by means of chemical oxidation with ozone, and they determined MWDs by gel permeation chromatography (GPC) with two different Ultrahydrogel columns and DAD (AU475 nm and AU270 nm) in series with differential refractometer. They suggested that color removal is accompanied by a decrease in the concentration of chromophore groups but lower molecular weight compounds are not formed. Ozone oxidizes functional groups responsible for color but it only transforms chromophore groups, ozone do not break brown polymers to smaller compounds. Liang et al. (10,11) was applied coagulation process in BYW and monitored MWD by HPSEC with a Hewlett-Packard HPLC 1100-series system, equipped with a refractive index (RI) detector and a PL aquagel-OH column. They suggested that low MW fraction of melanoidins seems to preferentially react with hydrolytic iron species and is destabilized through the formation of insoluble melanoidins–Fe (III) complexes (11). At the same time they reported that the lowest MW fraction is obviously not amenable to removal by coagulation.

Yu et al. (12) used the internal electrolysis (IE) process as pretreatment to improve the biodegradability of BYW and they monitored MWD of BYW by an Agilent 1100 high performance liquid chromatography followed by a refractive index. They suggested that the compounds with large molecular weight in IE effluent are remarkably reduced. The medium molecular weight substances show little change and the low molecular weight substances increase. All the results demonstrate that some macromolecular organic
compounds change into small molecule substances during IE processes. As mentioned the above, there isn’t any studies in literature which deals with the role of component’s MWD in BYW on decolorization mechanism by the EC.

In this study, effect of parameters (current density, operating time and initial pH) and removal efficiency of COD, TOC and Color in BYW by the EC process are reported. Novelty of this study is that effects of the EC treatment and pH without the EC on MWDs of BYW are investigated for the first time by HPSEC using both RID and DAD to combine the advantages of these detectors.

2. MATERIALS AND METHODS

2.1. Wastewater Sample Preparation
Yeast wastewater samples are obtained from a Baker’s yeast-manufacturing factory in Kocaeli, Turkey. It has a full scale two-stage anaerobic and aerobic biological treatment plant. The wastewater samples collected from anaerobic influent, anaerobic effluent (AE) and anaerobic-aerobic effluents (AAE) are characterized (Table 1). As seen in Table 1, the anaerobic and anaerobic-aerobic stages from BYW are effective in removing of COD (92.2% and 98.0%) except for color (23.6% and 73.6%). In our previous study (13), we proved that EC isn’t efficient for treatment of anaerobic influent, so we apply EC to AE in this study.

2.2. Experimental Set-Up And Procedure
The EC treatment is carried out in a batch electrolytic reactor made from Plexiglas material with dimensions of 100 mm × 100 mm × 130 mm. Four aluminum electrodes (purity >99.5%) in the reactor are used as cathodes and anodes with effective dimensions of 80 mm×50 mm×3 mm. Total effective areas of electrodes are 240 cm². The electrodes are situated 5 mm apart from each other and connected to monopolar parallel connection mode.

The electrodes are placed in the reactor and 0.80 L sample are mixed at 300 rpm in the EC reactor. The current density \( (j) \) is adjusted by a digital DC power supply (TDK-Lambda Genesys model; 50 V-30A) operated at galvanostatic mode and the experiment is started (3). At the end of the experiments, color, COD and TOC of treated wastewater are measured. The samples are also filtered using a 0.45 µm Whatman filter before loading of sample to HPSEC. 20 µL sample is injected into the column and the analysis time takes 50 min. All experiments are performed at 25 °C.

2.3. Analytical Methods

The MWD is determined using HPSEC with a Hewlett-Packard HPLC 1100-series system equipped with a refractive index (RI) detector (G1362A, Agilent), a diode array detector (DAD, G1315A) and two ultrahydrogel (Waters, Product Number: WAT011535 and WAT011525) columns. RID measures change in refractive index and its unit called as “Refractive Index Units (nRIU)”. DAD is a type of UV-Vis detector and its unit called as absorbance unit (AU) at any wavelength. Deionized water at a flow rate of 1 mL/min is used as the mobile phase. Weight-average molecular weight \( (M_w) \) and number-
average molecular weight ($M_n$) are calculated from the data using the following equations (14) and these calculations are processed with ChemStation software.

\[
M_w = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i} \\
M_n = \frac{\sum_i n_i M_i}{\sum n_i}
\]

(1) \hspace{1cm} (2)

$M_i$ and $n_i$ are the molecular weight and the height of each i-th fraction eluted at the i-th volume in the chromatogram, respectively. The polydispersity index (PDI) or heterogeneity index, is a measure of the distribution of molecular mass in a given polymer sample. PDI is calculated from $M_w/M_n$. The results are calibrated using 1 g/L of polyethylene oxide with different molecular weights (25.3, 44.0, 78.3, 152.0 and 326.0kDa).

COD and TOC are measured according to standard methods (6). COD is measured by closed reflux titrimetric method and TOC levels are determined through combustion of the samples at 680 °C using a non-dispersive IR source (Tekmar Dohrmann Apollo 9000). Color contents are measured at 475 nm using a UV-Vis spectrophotometer (Perkin-Elmer 550 SE). pH of sample adjusted with 1N H$_2$SO$_4$ or 1N NaOH is measured by pH meter (WTW Inolab pH 720). Turbidity is directly read on a turbidity meter (Hach Lange, 2100Q IS) in nephelometric turbidity unit (NTU).

3. RESULTS AND DISCUSSION

3.1. The Effect Of EC Parameters On Removal Efficiency
In an EC process, the coagulating ions are produced from the sacrificial electrode such as iron (Fe) or aluminum (Al). When aluminum electrodes in the EC process are used as an anode and a cathode, the main reactions are as follows:

\[
\text{Al} \rightarrow \text{Al}^{3+} + 3e^- \quad \text{(at anode)}
\]

Hydrogen evolution takes place via the following reaction:

\[
3\text{H}_2\text{O} + 3e^- \rightarrow \frac{3}{2}\text{H}_2 + 3\text{OH}^- \quad \text{(at cathode)}
\]

It helps in floatation of the flocculated particles in the solution. \(\text{Al}^{3+}\) and hydroxyl ions generated by electrode reactions to form various monomeric-polymeric species, transformed initially into \(\text{Al(OH)}_3(s)\) and finally polymerized to \(\text{Al}_n\text{(OH)}_3n\) (Eq. 5) in the solution:

\[
n\text{Al(OH)}_3 \rightarrow \text{Al}_n\text{(OH)}_3n
\]

In EC process, the charge passed to the solution is directly proportional to the amount of electrode dissolved according to Faraday’s law (Eq. 6) and have important effect on pollutants removal.

\[
m = \frac{it_{EC}M_{\text{Al}}}{zF}
\]

where \(m\) is electrode consumption (g), \(t_{EC}\) is operating time (s), \(z\) is the number of electrons involved in oxidation/reduction reaction \((z = 3\ \text{for Al})\). \(M_{\text{Al}}\) is the atomic weight of anode material (26.98 g/mol) and \(F\) is the Faraday’s constant (96485 C/mol)(3,4,13).

Effects of the operating parameters such as pH, current density \((j)\) and \(t_{EC}\) on the EC process for AE are shown in Fig. 1. The optimum operating conditions for removal of color and COD are 86% and 43% for AE at 80 A/m², pH 4 and 20 min. Increasing
current density and time in the EC process (Fig. 1a and 1b) increases the amount of metal species formed by dissolution of the anode material (Eq. 6). The increased positively charged polymeric Al-species result in increment of melanoidins removal from BYW. As operation time and current density are further increased, the removal efficiencies aren’t increased at the same rate since pH of BYW increases during the EC process and 

\[ \text{Al(OH)}_4^- \] is dominant soluble aluminium species (pH>9) leading to lower the removal efficiencies [3].

\[
2\text{Al} + 6\text{H}_2\text{O} + 2\text{OH}^- \rightarrow \text{Al(OH)}_4^- + 3\text{H}_2(\text{g}) \tag{7}
\]

In the experiments, the electrochemical generated aluminum ions may form monomeric and polymeric Al- hydrolysis species such as \( \text{Al(OH)}^{2+}, \text{Al(OH)}^+_2, \text{Al(OH)}^-_4, \text{Al}_6(\text{OH})^{15+}, \text{Al}_7(\text{OH})^{4+}, \text{Al}_8(\text{OH})^{3+}, \text{Al}_{13}\text{O}_4(\text{OH})^{7+} \text{ and } \text{Al}_{13}(\text{OH})^{5+} \) between pH 4-6. Above pH>6, oversaturation and formation of amorphous hydroxide precipitate \( \text{Al(OH)}_{3(s)} \) enmeshes colloidal particles in a sweep flocs (15). Formation rates of the different species play an important role in the removal process. Four major interaction mechanisms in treatment of BYW are considered as co-precipitation, charge neutralization, adsorption and sweep coagulation, each one is related to the removal efficiencies at different pH ranges. Charge neutralization and co-precipitation between these hydrolysis products and hydrolyzed negatively charged organic molecules such as melanoidins in the BYW occur in pH 4-6. The EC in the low pH range is explained as co-precipitation while the higher pH >6 as adsorption and sweep coagulation (1,3,4,13,15).

3.2. The Mwds Of Biological Treated BYW
In HPSEC, larger molecules move rapidly through the column with eluent and leave column faster than smaller ones, so peak 1 (Fig. 2) has the highest MW fractions in the four peaks. As seen in Fig. 2, the dominant fractions of AE from the RID and DAD chromatograms are quite different. On the other hand, UV (AU_{254nm}) and Vis (AU_{475nm}) chromatograms obtained by DAD are very similar but UV chromatograms have higher absorbance values compared with that of the Vis. In RID chromatograms (Fig. 2a), intermediate molecular weight components (IMWCs; peak 3) have higher RIU values than that of both high molecular weight components (HMWCs; peaks 1 and 2) and low molecular weight components (LMWCs; peak 4). On the contrary, the most dominant fractions and colored components according to the DAD chromatogram (in Fig. 2b and 2c) are HMWCs and LMWCs. As seen in Table 2, total peak areas (TPA) for AE are 40.3% of TPA_{AE} at 475 nm in peak 1 for HMWCs, and 40.2% of TPA_{AE} at 475 nm in peak 4 for LMWCs. HMWCs and LMWCs correspond to about 80% of the color to AE from DAD chromatograms. It is clear that HMWCs and LMWCs in BYW are the major reason for color and aromaticity, however their concentrations are low in RID chromatograms. In RID chromatograms, the IMWCs are the dominant fraction and their concentration is high. It is suggested that relatively colorless fraction is IMWCs which causes high COD, and the colored fractions are HMWCs and LMWCs which cause low COD in BYW.

3.3. The Effect Of pH On MWD

The structural characterization of melanoidin molecules ranged from small molecules to extremely large polymers in BYW are complex polymers consisting of repeating units of
furans and/or pyrroles produced during the advanced stages of Maillard reaction and
remain linked by polycondensation reactions (16). In addition, Hofmann (17) also
reported that melanoidins are low molecular weight colored compounds, which crosslink
proteins via amino groups of lysine or arginine and polymerized through aldol-type
condensation reactions to produce high molecular weight of melanoidins. The chemical
structure of these melanoidins is mainly from sugar degradation products which largely
depend on the starting materials as well as reaction parameters (18,19). pH is one of the
most important reaction parameter which affects the polymerization degree of
melanoidins (3) and the performance of electrocoagulation process, as observed also by
other investigators (20–22). The color intensity and turbidity values of BYW behave
almost opposite to each other as a function of pH without EC (Fig. 3).

Firstly, the color intensity decreases between pH 1 and 2, increases sharply at pH 2-5, and
then gradually decreases with pH from 5 to 13. The turbidity results show fluctuations
between 76 NTU and 147 NTU at pH 1-8 and increase sharply from 123 NTU to 1644
NTU at pH 8-13. Liang et al. (10,11) reported that Zeta potential value of BYW is the
least near at pH 2 and increase gradually from -10 to -40 mV at pH 2-12. Zeta potential
value of BYW cause an auto-aggregation effect and leading to a rise in turbidity and a
decrease in color intensity near pH 2. Migo et al. (23) reported the similar results for
distillery wastewater containing natural melanoidins. They reported that melanoidin has a
net zero charge at pH 2.5. Therefore, the Zeta potential isn’t the reason for increasing of
turbidity at pH > 4. Probably the reason for the increase in turbidity and decreasing in
color intensity is the MWDs of BYW. Thus, AE with initial pH (pHi) 2.0–11.0 without

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the EC process were adjusted to examine their chromatogram (Fig. 4). The increasing pH causes forming of HMWCs from LMWCs and become more noticeable for MW of $2.3 \times 10^6 - 2.4 \times 10^5$ Da at pH 11 in Fig. 4a. It is clear that the forming of more HWMCs at pH > 4 causes great increase in turbidity and decrease in color intensity related to LMWCs turns to IMWCs and later IMWCs turns to HMWCs. The similar behaviors of forming HMWCs are also observed with DAD (Fig. 4b and 4c).

$M_n$ is more sensitive to low molecular weight components while $M_w$ is more sensitive to high molecular weight components. Table 3 shows that $M_w$ increases gradually between pH 2 and 11. $M_n$ values are very similar at pH 2, 7 and 11 but $M_n$ is remarkably higher than others at pH 4. BYW has the smallest PDI value at pH 4 and has the largest one at pH 11. The larger the polydispersity index, the broader the molecular weight. It is clear that BYW has the most homogeneity and narrow molecular distribution around pH 4 and this homogeneity and narrow molecular distribution are one of the main reasons which cause higher removal efficiency in chemical and electrochemical coagulation processes. For example, Liang et al. (11) reported that the final pH values at the optimum conditions are confined within narrow ranges for pH at 5.4-5.9 aluminum salts and pH at 4.3-4.6 for iron salts. On the other hand, the optimum operating conditions for removal efficiencies of color, COD and TOC are obtained at pH 4 for AE and at pH 5 for AAE with Al electrodes (3).

3.4. The Changing Of MWD During EC
HPSEC analysis is performed to investigate changes in the MWD of all compounds by RID and colored by DAD during the EC process. HPSEC analyses are conducted before treatment (AE_R; raw wastewater) and 4 min (AE_{4 min}), 12 min (AE_{12 min}) and 20 min (AE_{20 min}) intervals during the EC (Fig. 5). The HPSEC chromatograms of coagulated AE demonstrate multi-modal MW distribution. UV-Vis absorbance of AE decreases during EC because there is considerable removal or alteration of aromatic compounds at 254 nm and removal of color at 475 nm (Fig. 5a and 5b). The EC process is effective for all fractions because all peaks areas show decrease linearly with operating time and $\ln(Area)$. However Fig. 5c shows that the EC removes HMWCs more efficiently as compared to others. As mentioned the above, HWMCs has low concentration with high color intensity. Therefore, color removal efficiencies are higher than COD and TOC during EC.

On the other hand, the chromatograms at different operating times by RID (pH: 4.2 and $j$: 80 A/m²) and the differences in the chromatograms show that removal of components gradually increases with the operating time (Fig. 6). The positive RIU values in Fig. 6b show removal components during the EC and it is clear that the EC removes HMWCs primarily and more efficiently. On the contrary, negative RIU values show transformation of components. There may be two reasons for this transformation; (i) pH increases during the EC (3), causing re-polymerization of components and transformation of LMWCs to HMWCs as mentioned the above and (ii) oxidation process realizes during the EC especially at high current density and oxidation process leading to the transformation of HMWCs to LMWCs. Manka and Rebhum (24) attributed increasing
concentration of the LMWCs fraction to the formation of unidentifiable group in the lime treatment system.

The $M_n$, $M_w$ and PDI values of AE$_R$, AE$_{4\text{min}}$, AE$_{12\text{min}}$ and AE$_{20\text{min}}$ are determined by RID (Table 4). Increasing PDI values show that heterogeneity of wastewater increase during EC.

4. CONCLUSIONS

The following conclusions are obtained from the present study:

1) The optimum color and COD removal efficiencies are 86% and 43% at 80 A/m$^2$, pH $4$ and 20 min for AE. Co-precipitation is the predominant removal mechanism under the optimum operating conditions since the maximum decolorization is obtained at pH 4–6. The removal of higher percentage of colors obtained in the EC process, but the biological treatment process does not yield the same or a better removal rates.

2) RID and DAD together are quite useful in monitoring of the changes in the MWD of components whether they have UV absorption or not. RID is found to have lower sensitivity than that of DAD especially when monitoring colored components. Also, all MWDs are multi-modal distributions in BYW.

3) There are three main fractions of AE according to MWDs. IMWCs have high amount of COD and low color intensity (the contributions of IMWCs to color are 12.4% for AE) whereas HMWCs and LMWCs have low amount of COD and high color
intensity (the contributions of HMWCs and LMWCs to color are 40.2% and 40.3%, respectively).

4) The EC removes HMWCs more efficiently as compared to others and all peaks areas decrease linearly with operating time and \( \ln(\text{Area}) \). HWMCs have low concentration but they cause high color intensity. Thus, color removal efficiency is higher than COD and TOC during EC.

5) pH is one of the most important parameter which affects the polymerization degree of components in BYW. Increasing pH causes the forming of arise in MW of components. It is clear that the forming of new HWMCs at pH > 4 causes great increase in turbidity and decrease in color intensity. In addition, BYW has the most homogeneity around pH 4 and this homogeneity is the one of the main reasons which causes the higher removal efficiency in chemical coagulation and EC processes.

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REFERENCES


Table 1 Characterizations of raw and biologically treated BYW

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Anaerobic influent</th>
<th>Anaerobic treated effluent (AE)</th>
<th>Anaerobic-aerobic treated effluent (AAE)</th>
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<td>pH</td>
<td>5.6</td>
<td>7.2</td>
<td>7.7</td>
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<td>Temperature (°C)</td>
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<td>COD (mg/L)</td>
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<td>Re_{COD} (%)</td>
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<td>TOC (mg/L)</td>
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Re: Removal efficiency (%)
Table 2 Absorbance values of AE

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<th>Peak</th>
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<th>AU&lt;sub&gt;254 nm&lt;/sub&gt;</th>
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<td>No.</td>
<td>TPA&lt;sub&gt;AE&lt;/sub&gt; (AU × time)</td>
<td>TPA&lt;sub&gt;AE&lt;/sub&gt; (%)</td>
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<td>1</td>
<td>305</td>
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<td>3</td>
<td>94</td>
<td>12.4</td>
</tr>
<tr>
<td>4</td>
<td>304</td>
<td>40.2</td>
</tr>
<tr>
<td>Total</td>
<td>757</td>
<td>100</td>
</tr>
</tbody>
</table>

TPA: Total Peak Area calculated as integration of UV-Vis spectrum by software (AU × time)
Table 3 $M_n$ and $M_w$ results of AE at different pHs

<table>
<thead>
<tr>
<th></th>
<th>pH 2</th>
<th>pH 4</th>
<th>pH 7</th>
<th>pH 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mn (Da)</strong></td>
<td>130</td>
<td>1394</td>
<td>94</td>
<td>119</td>
</tr>
<tr>
<td><strong>Mw (Da)</strong></td>
<td>12820</td>
<td>21380</td>
<td>21880</td>
<td>52800</td>
</tr>
<tr>
<td><strong>PDI</strong></td>
<td>99</td>
<td>15</td>
<td>233</td>
<td>445</td>
</tr>
</tbody>
</table>
Table 4 $M_n$, $M_w$, and PDI results of AE during EC

<table>
<thead>
<tr>
<th></th>
<th>Before Treatment</th>
<th>Operating time, $t_{EC}$ (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(AE_R)</td>
<td>AE_{4.min}</td>
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<tr>
<td>$M_n$ (Da)</td>
<td>1394</td>
<td>760</td>
</tr>
<tr>
<td>$M_w$ (Da)</td>
<td>21380</td>
<td>20590</td>
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<tr>
<td>PDI</td>
<td>15</td>
<td>27</td>
</tr>
</tbody>
</table>
Figure 1. Effect of the operating parameters on EC process: (a) Current density (pH: 5, $t_{EC}$: 20 min), (b) Operating time (pH: 5, $j$: 65 A/m$^2$) and (c) pH ($t_{EC}$: 20 min $j$: 80 A/m$^2$).
Figure 2. The MWDs of biological treated BYW (a) the MWDs obtained by RID, (b) the MWDs obtained by DAD (AU\textsubscript{475 nm}), and (c) the MWDs obtained by DAD (AU\textsubscript{254 nm}).
Figure 3. Color and turbidity changes as functions of pH for AE.
Figure 4. HPSEC chromatograms of BYW as a function of pH, (a) the chromatograms by RID, (b) the chromatograms by DAD (AU$_{475}$ nm) and (c) the chromatograms by DAD (AU$_{245}$ nm).
Figure 5. The change of MWD for AE during the EC (pH: 4.2, $j$: 80 A/m²), (a) the chromatogram by DAD (AU$_{475\text{nm}}$), (b) the chromatogram by DAD (AU$_{254\text{nm}}$) and (c) the change of peak areas at 254 nm.
Figure 6. The change of MWD for AE during the EC (pH: 4.2, $j$: 80 A/m$^2$) (a) the chromatogram by RID at different operating time (b) the difference of the chromatogram by DAD (AU$_{254\text{nm}}$) at different operating time.