ABSTRACT

Melanoidins are high molecular weight polymers that contribute a dark brown colour and high organic load to some industrial effluents and result from reactions between sugars and amino acids, peptides or proteins (Maillard reaction). The objectives of this laboratory scale study were to investigate the feasibility of a range of processes, separately, or in combination as appropriate, for the removal of colour from a melanoidin-containing wastewater. The effectiveness of coagulation with a range of organic and inorganic coagulants, membrane filtration, ozonation and UVC/H$_2$O$_2$ treatment for colour removal was investigated.

INTRODUCTION

Melanoidin-containing wastewaters are produced by industrial fermentation processes which employ molasses, such as alcohol and baker’s yeast production. Melanoidins are high molecular weight polymers that contribute a dark brown colour and high organic load to effluents (González et al., 1999). The formation of melanoidins is via a series of consecutive and parallel chemical reactions between amino compounds and carbohydrates during the Maillard reaction (Kim et al., 1985; Cämmerer and Kroh, 1995). The chemical properties of melanoidin are similar to those of humic substances, being acidic, polymeric and highly dispersed colloids which are negatively charged due to the dissociation of carboxylic and phenolic groups (Migo et al., 1993). The colour, nitrogen content and carbon content are determined by the degree of polymerisation (Motai, 1974), aromaticity and saturation (Cämmerer and Kroh, 1995) that occurs as a result of the reaction conditions (i.e. temperature and formation time). Melanoidins are resistant to biodegradation (Zhou et al., 2008) thus the brown colour is not readily removed by conventional biological treatment and can even increase due to re-polymerisation (Peña et al., 2003). The structure and characteristics of the melanoidins are still not fully understood and this has consequently hindered the development of an appropriate process for their removal (Satyawali and Balkrishnan, 2008).

Methods which have been used to remove and/or decolourise humic-like matter and melanoidins include biological treatment with specific microorganisms, or consortia of microorganisms, and physico-chemical treatment. Biological treatment comprises both aerobic and anaerobic processes (Nandy et al., 2002; Jiménez et al., 2004). Physico-chemical treatment includes activated carbon adsorption (Mall and Kumar, 1997; Bernardo et al., 1997); coagulation and flocculation (Migo et al., 1993; Zhou et al., 2008); oxidation using ozone (Kim et al., 1985; Peña et al., 2003), UV/H$_2$O$_2$ or UV/H$_2$O$_2$/Fe (II) (Çatalkaya and Sengul, 2006) and membrane treatment (Mutlu et al., 2002). The original concentration of colour and the molecular size, and the net charge of the coloured molecules, plus the characteristics and concentration of the other species present, will greatly influence the effectiveness of the colour removal process. There has been very little consistency in the types of samples investigated and the methods used for measurement of colour concentration, and frequently the degree of colour removal is given in percentage terms with no initial values given. Consequently, no consistently effective method for melanoidin removal is apparent from examination of the literature.

The objectives of this laboratory scale study were to investigate the feasibility of and to compare coagulation, membrane filtration, ozonation and UVC/H$_2$O$_2$ oxidation separately or in combination as appropriate, for the removal of colour from an industrial wastewater stream containing melanoidins. This wastewater had very high colour and COD levels which have not been previously reported in the literature.

MATERIALS AND METHODS

Wastewater samples

Melanoidin-containing industrial wastewater samples were stored at 4°C on receipt. The samples were filtered (0.45 µm membrane) prior to the measurement of absorbance, DOC, true colour and fluorescence.

Chemicals

Alum (Al$_2$(SO$_4$)$_3$.16H$_2$O, AR), lime (CaO, LR) and FeCl$_3$ were supplied by BDH. ACH (aluminium...
chlorohydrate), sold as Megapac 23 (40% w/w), was supplied by Omega Chemicals. ACH dosages are reported in terms of pure ACH. Poly(diallyldimethylammonium chloride) (polyDADMAC) was supplied as Magnafloc LT410, by Ciba Specialty Chemicals Pty, Ltd.

Coagulation tests
Coagulation tests were conducted using a laboratory jar tester (Phipps and Bird, PB-700) with rapid mixing for 2 minutes at 250 rpm followed by slow mixing for 20 minutes at 30 rpm. The coagulated water was settled for 2 hours and the supernatant was then collected for measuring colour.

For ACH/polyDADMAC LT410 treatment, ACH was added to the wastewater and mixed at 250 rpm for 2 minutes. PolyDADMAC was then added to the solution and mixed at 250 rpm for a further minute, followed by slow mixing at 30 rpm for 20 minutes.

Ultrafiltration
Ultrafiltration was conducted using 100 kDa (PES, Amicon PBHK) or 30 kDa MWCO (PVDF, GE Sepa CF) disc membranes in a stirred-cell (Amicon 8050) at a trans-membrane pressure of 220 kPa and stirring speed of 430 rpm.

Ozonation
Ozone treatment of the wastewater was carried out in a 250 mL glass column using a lab-scale ozone generator (Ozomatic LAB 802) at room temperature (~20°C) in a fume cupboard. Ozone gas was produced from pure oxygen and the inlet gas flow rate was 0.33 L min⁻¹. The volume of wastewater was 100 mL and the ozone concentration was 7 mg L⁻¹. The reaction time was 45 minutes for all samples. Two ozone traps containing 2% potassium iodide solution were connected in series with the reactor in order to collect unreacted ozone.

AOP treatment of the coagulated wastewater samples
The wastewater was coagulated with ACH/polyDADMAC LT410 and was settled for 2 hours, the supernatant was then filtered (filter paper 5 µm; Advantec No.2). UVC/H₂O₂ treatment of the filtered coagulated wastewater samples was then conducted in the annular reactor detailed by Thomson et al. (2004). The average fluence value of the UV-lamp was 12.95 mJ s⁻¹ and the H₂O₂ dosage was 5.3 g L⁻¹. The dose of hydrogen peroxide was chosen after consideration of the colour degradation effect reported in the literature, the residual mass and the cost of the peroxide (Dwyer et al., 2008).

Analytical methods
UV Absorbance
The UV absorbance at 254 nm was determined using a double beam scanning spectrophotometer (Unicam UV2, 1 cm pathlength).

Colour
The true colour (ie., after filtration, 0.45 µm) of the samples was measured in Pt-Co units at 455 nm using a Hach DR/4000U spectrophotometer. Error is cited as ±5%.

Dissolved Organic Carbon (DOC)
DOC was measured with a total organic carbon analyser (Sievers 820).

pH
pH was measured with a Hach Sension 156 pH/conductivity meter.

Chemical oxygen demand (COD)
COD was measured using Hach method 8000 and a Hach DR/4000U spectrophotometer.

Excitation Emission Matrices (EEMs)
Fluorescent three-dimensional excitation emission matrices EEMs were obtained with a Perkin Elmer Luminescence Spectrometer LS 55. Excitation and emission wavelength ranges were 200-550 nm.

RESULTS AND DISCUSSION

Characteristics of the raw wastewater
The water quality characteristics of the wastewater in terms of pH, colour, DOC, and SUVA are shown in Table 1. The wastewater had a very high colour (30,250-37,500 Pt-Co units) and there was marked variation in the colour concentrations of samples for different batches. Over 90% of the colour passed through a 0.45µm filter and so was in dissolved and colloidal form.

Table 1: Characteristics of the raw wastewater

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.7-5.9</td>
</tr>
<tr>
<td>Colour (Pt-Co units)</td>
<td>30,250-37,500</td>
</tr>
<tr>
<td>DOC (mg L⁻¹)</td>
<td>8.250-10,800</td>
</tr>
<tr>
<td>UV absorbance A₂54 (cm⁻¹)</td>
<td>0.109-0.160</td>
</tr>
<tr>
<td>SUVA (L m⁻¹ mg⁻¹)</td>
<td>1.38-1.40</td>
</tr>
</tbody>
</table>

(*) for diluted solution 1:1000

Coagulation
The colour removal by various coagulation treatments of the wastewater sample is shown in Figure 1.

The best result was obtained from lime treatment at the dosage of 20 g CaO L⁻¹ (60% colour removal), however, a significant amount of sludge was produced and the pH increased markedly from 5 to more than 12 which would necessitate adjustment prior to discharge as Trade Waste.

At the same dosage of 200 mg Al³⁺ L⁻¹, colour removal with alum was better than with ACH,
however, the amount of sludge produced after treatment by alum was higher than for ACH treatment.

Treatment of the wastewater stream with low molecular weight polyDADMAC (LT410) at the optimum dosage of 1.2 g L\(^{-1}\) resulted in 30% colour removal. Sequential treatment by alum and LT410 did not increase colour removal although sequential treatment by ACH and LT410 removed a further 10% (overall colour removal of 40%).

Coagulation with FeCl\(_3\) removed 20% of colour, however the pH decreased markedly, from 5.9 to 2.7, and the wet volume of the sludge in the FeCl\(_3\) treated water was about 6-7 times higher than that for the ACH/PolyDADMAC LT410 treated wastewater. The results indicate that the sequence ACH/polyDADMAC may be a suitable coagulation treatment for the wastewater.

**Ultrafiltration**

Colour removal from the wastewater by ultrafiltration using 30 and 100 kDa membranes is shown in Table 2. Although the colour removal was higher than for coagulation treatments, the permeate flux was very low due to severe membrane fouling. The flux for the 30 kDa membrane was markedly improved by pre-treatment of the sample with ACH/LT410 at the optimum dosages, although overall colour removal was not improved, and membrane fouling was still significant due to the high level of organic matter in the sample. Furthermore, there would be potential for biofouling of the membrane in long term use.

**Table 2: Colour removal from wastewater sample by ultrafiltration**

<table>
<thead>
<tr>
<th>Molecular weight cut off (MWCO)</th>
<th>Colour removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 kDa</td>
<td>52</td>
</tr>
<tr>
<td>30 kDa</td>
<td>66</td>
</tr>
</tbody>
</table>

**Oxidation**

Colour removal by coagulation with ACH/LT410, and coagulation followed by UVC/H\(_2\)O\(_2\) treatment, of the wastewater sample is shown in Figure 2.

**Figure 2: Colour removal by coagulation and UVC/H\(_2\)O\(_2\) treatment of wastewater**

At the optimum dosage of 0.96 g L\(^{-1}\) for ACH and 1.2 g L\(^{-1}\) for LT410, coagulation removed only 40% of the colour. Subsequent treatment of the coagulated wastewater with UVC/H\(_2\)O\(_2\) for 60 minutes removed further colour to give an overall removal of 59%, treatment for 120 minutes led to little further colour removal. Although this suggests that the peroxide concentration may have become limiting for hydroxyl radical production and thus oxidation of the melanoidins, measurement of residual peroxide after 2 h irradiation gave concentrations in excess of 250 mg L\(^{-1}\). The peroxide dose was not optimised for this waste as uneconomically large doses were indicated.

Although treatment of melanoidin-containing water and wastewater with UVC/H\(_2\)O\(_2\) has been shown to be feasible by Çatalkaya and Sengül (2006), Dwyer et al. (2008) and Dwyer and Lant (2008), it was not so for this highly coloured sample. As anticipated, the high colour concentration (leading to “inner filter” effects, i.e., the high concentration of chromophores absorbs the incident radiation and so reduces the amount of radiation available to be absorbed by the target molecules and consequent photooxidation) and the high DOC level of the waste meant that colour removal was limited. Taken overall, the extent of colour removal, the high doses of peroxide and the high energy requirements mitigate against the use of this process for this application.

**Ozone treatment**

Ozonation gave the greatest reductions in colour, DOC and COD (Table 3). However, coagulation with ACH/LT410 prior to ozone treatment only increased reduction in \(A_{254}\) (absorbance at 254 nm).
Coagulation decreased the $A_{254}$ of the wastewater by 30%, while ozonation decreased it by 28%, and the sequence of coagulation and ozonation decreased it by 43%.

Table 3: Reductions in colour, DOC, COD and $A_{254}$ after coagulation and ozone treatments of the wastewater.

<table>
<thead>
<tr>
<th></th>
<th>Colour removal (%)</th>
<th>DOC removal (%)</th>
<th>COD removal (%)</th>
<th>Decrease in $A_{254}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After coagulation</td>
<td>40</td>
<td>12</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>After ozone treatment</td>
<td>86</td>
<td>71</td>
<td>51</td>
<td>28</td>
</tr>
<tr>
<td>After coagulation + ozone treatment</td>
<td>87</td>
<td>72</td>
<td>51</td>
<td>43</td>
</tr>
</tbody>
</table>

The characteristics of the organic matter in the untreated and treated wastewater were investigated using three-dimensional excitation and emission matrices (EEMs). EEMs provide 3-dimensional plots of the intensity of molecular excitation and emission in the UV-visible wavelength range that can be used for providing a “fingerprint” of fluorescent dissolved organic compounds in water. The EEM spectra were divided into five regions: regions I and II contain peaks at shorter excitation and emission wavelengths which are related to simple aromatic proteins (Determann et al., 1994). Region III comprises peaks which are associated with fulvic acid-like materials (Nguyen et al., 2005). Region IV consists of peaks which are related to soluble microbial product (SMP) materials (Sheng and Wu, 2006). Region V includes peaks which are associated with humic acid-like organics (Mounier et al., 1999).

The spectra show that coagulation with ACH/LT410 removed only a small proportion of the fulvic acid-like (region III) and humic acid-like materials (region V) from the wastewater. Ozonation almost completely degraded the SMPs (region IV) and most fulvic acid-like (region III) and humic acid-like (region V) materials. Ozonation after coagulation led to almost complete destruction of all the fluorophores. These data are consistent with the reductions in colour in Table 3.

These results demonstrate that ozonation is very effective for breaking down the chromophores and thus reducing the colour of the wastewater. Hence ozonation appears to be a promising treatment for the removal of colour from this coloured industrial effluent.

CONCLUSIONS

Of all the treatments tested, ozonation was found to be the most effective treatment system for removal of colour due to melanoidins. However, other treatment methods also achieved significant reductions.

The most effective coagulants were lime (20 g L$^{-1}$) which gave 60% of colour removal and ACH/LT410 (0.96 g L$^{-1}$/1.2 g L$^{-1}$) which removed 40% of colour from the wastewater. Of these, the latter option would be favoured due to the lower coagulant requirement and consequential lower sludge production, and minor, if any, pH adjustment necessary prior to release of the treated water.

Ultrafiltration with 30 kDa MWCO membrane gave 66% removal of colour, however, the flux was extremely low. Although pre-treatment with ACH/LT410 greatly improved the flux, it was still low and the overall colour removal was not increased. Use of a cross-flow membrane system
would probably further improve the flux, although membrane fouling would be likely due to the high concentration of organic matter remaining after coagulation.

UVC/H₂O₂ treatment after coagulation with ACH/LT410 increased colour removal to give an overall colour removal of 60%. The extent of colour removal, the high doses of peroxide and the high energy requirements mitigate against the use of this process for this application.

Ozone treatment gave a colour removal of 86% and significant reductions in DOC (71%) and COD (58%) for the wastewater. Thus biological pre-treatment and optimisation of the ozonation system will be the subject of future investigation for the removal of melanoids.

ACKNOWLEDGEMENT

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