African Journal of Biotechnology Vol. 11(22), pp. 6083-6090, 15 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3360
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Simultaneous adsorption and biodegradation of synthetic melanoidin

Maurice S. Onyango[1]*, Vincent O. Ojijo[1], Aoyi Ochieng[2], Jacob O. Kittinya[1] and Fred O. Otieno[3]

[1]Department of Chemical and Metallurgical Engineering, Tshwane University of Technology, Private Bag X680, Pretoria, South Africa.
[2]Department of Chemical Engineering, Vaal University of Technology, Private Bag X021, Vanderbijlpark, South Africa.
[3]Durban University of Technology, P.O. Box 1334, Durban, South Africa.

Accepted 16, January 2012

Molasses spent wash contains melanoidin, a dark brown recalcitrant compound. It is not easily biodegraded and causes a number of problems such as reduction in photosynthetic activities and dissolved oxygen when discharged to aqueous environment. Being an antioxidant, melanoidin removal through purely biodegradation has been inadequate. Consequently, in the current study, simultaneous adsorption and biodegradation (SAB) was employed in a stirred tank system to remove melanoidin from synthetic wastewater. Mixed microbial consortium was immobilized onto 200 g of activated carbon and used to degrade 3.5 L of melanoidin solutions with varying chemical oxygen demand (COD) concentrations. The effects of the initial COD level, pH and temperature on COD removal were then studied. Ultimately, the SAB performance was compared to that of batch adsorption or biodegradation carried out independently. After 48 h of operation, the SAB process yielded the best COD removal efficiency of 75% as compared to 49.3 and 51.9% for adsorption and biodegradation, respectively, for the initial COD value of 10800 mg/ L at a temperature of 296 K and pH 6.97. This therefore showed that the SAB process can successfully be applied to enhance the removal of melanoidin from wastewater.

Key words: Adsorption, biodegradation, melanoidin, SAB, wastewater.

INTRODUCTION

Melanoidins are dark brown complex bio-polymers formed through the non-enzymatic browning reaction (also called Maillard reaction), between amino acids and carbonyl groups in organic substances (Martins et al., 2001; Wedzicha and Kaputo, 1992). In the Maillard reaction, melanoidins are the main end products of the reaction whose 3-stage pathway includes cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations (Martins et al., 2001; Coca et al., 2004). They are widely found in nature and discharged by various agro-based industries; especially the sugar cane molasses based fermentation industry. Cane molasses for instance, contains around 2% of the melanoidin that imparts colour to the distillery spent wash (Kalavathi et al., 2001).

Like other Maillard reaction products, melanoidins have antioxidant properties that render them toxic to aquatic micro- and macro-organisms (Jing and Kitts, 2004; Fitzgibbon et al., 1995; Wang et al., 2008). Apart from this, when molasses spent wash containing melanoidin is discharged into a water body the dark brown colour impedes sunlight penetration, thereby reducing photosynthetic activities. Also, due to the high chemical oxygen demand (COD) values of about 85,170 mg/ L from such wastewater, dissolved oxygen is depleted and hence severely affecting the aquatic life (Fitzgibbon et al., 1995). Moreover, they are phyto-toxic at various concentrations when discharged on land, with seed germination being hindered (Kannan and Upreti, 2008). Furthermore, soil alkalinity and manganese availability have reportedly been reduced by molasses spent wash (Chandra et al.,

*Corresponding author. E-mail: OnyangoMS@tut.ac.za. Tel: +27-12-382-3533. Fax: +27-12-382-3532.

2008). To mitigate these negative effects, various treatment techniques have been attempted in the past, from which varying degree of success has been reported (Agarwal et al., 2010).

For instance, electrochemical method attained a decolorization efficiency of 88.3% and COD reduction of 39.7% for a distillery spent wash containing melanoidin (Prasad and Srivastava, 2009). Even though this method guarantees high decolorization efficiency, its effectiveness depends on the type of electrodes, the construction of electro-coagulators and the condition under which the process is run. Moreover, electrochemical method can also be expensive since the cost of replacement of electrodes such as the titanium substrate insoluble anode might be high. Coagulation based treatment using *Moringa oleifera* seeds resulted in a decolorization efficiency of about 53% for molasses spent wash (Prasad, 2009). But due to its antimicrobial activity, it might be counterproductive in cases where treatment process is inclusive of biodegradation. Other coagulants such as alum have been shown to cause Alzheimer's disease and might not be so attractive to the treatment of melanoidin-containing water streams. In exploring melanoidin treatment using chemical (H_2O_2) oxidation followed by 7 days of aerobic biodegradation, Dwyer and Lant (2008) observed an 82% dissolved organic carbon removal from a synthetic melanoidin solution. This method, however, might be expensive as well since the running cost of the chemicals could be high, apart from the fact that highly trained personnel is needed for the operation of an advanced oxidation process (Peña et al., 2003).

Another technique attempted with moderate success is membrane treatment coupled with biodegradation, in which it was reported that only 41% of COD reduction was achievable from molasses spent wash. It was postulated that most of the removed COD consisted of low molecular weight compounds while the high molecular weight compounds such as melanoidin remained unaffected (Satyawali and Balakrishnan, 2008). Biodegradation is a low cost method of wastewater treatment but due to the recalcitrance of the melanoidin, relatively poor results (39 to 67% COD reduction) have been achieved (Harada et al., 1996). Adsorption on the other hand has been shown to be very robust and sorbents like activated carbon are known to have high affinity for organics (Moreno-Castilla, 2004; Figaro et al, 2006). However, the cost might be an impediment in its application, especially for commercially procured activated carbon. Nevertheless, a combined process of adsorption and biodegradation should result in synergies during the treatment of molasses spent wash with adsorption of the toxic substances onto the adsorbent expected to reduce inhibitory effect on microbial growth. Another benefit expected from this hybrid system is the increase in the adsorption capacity of the adsorbent due to bio-regeneration (Aktaş and Çeçen, 2007; Lim et al., 2002).

Therefore, the objective of this study was to investigate the applicability of simultaneous adsorption and biodegradation (SAB) in the removal of melanoidin. For comparative reasons only, biodegradation and adsorption studies are also done independently. In the current study, synthetic melanoidin was used since both natural and synthetic melanoidins have similar elemental (CHON) compositions, spectroscopic properties and electrophoretic mobilities at various pH values (Migo et al., 1997).

MATERIALS AND METHODS

Preparation of synthetic melanoidin

Synthetic melanoidin was prepared by mixing 4.5 g of glucose (G8270 D-(+), Sigma-Aldrich), 1.88 g of glycine (G7126, reagentplus TM>=99%, Sigma-Aldrich) and 0.42 g of sodium bicarbonate with 100 ml of distilled water and then heated for 7 h at 368 K. After heating, 100 ml of water was added (Bernado et al., 1997). The prepared solution had a COD value of 29,160 mg/L from which dilute solutions of melanoidin were prepared. For pH adjustment, 0.1 M NaOH and 0.1 M HCl were used.

Sorbent

Commercial granular activated carbon- GAC, (ENVIRONCARB^{TM} 207C 4 X 8 from Chemviron Carbon) was used for the sorption of melanoidin. It had a mean particle size of 3.5 mm, iodine number of 1100, molasses number of 450, bed density of 510 kg/m^3 and a hardness number of 97%. Specific surface area (S_{BET}) was determined from the adsorption-desorption isotherm of nitrogen at 77K using the Micrometrics (TriStar 3000) Surface Area and Porosity Analyzer and found to be 967 m^2/g. Before melanoidin sorption experiments, the GAC was washed several times with distilled water and dried at 373 K for 24 h prior to use.

Batch adsorption studies

Batch adsorption experiments were carried out for comparison purposes only. Briefly, 200 g of washed and dried carbon was contacted with 3.5 L of melanoidin solution of varying concentrations. This was then stirred at 400 rpm in a 5 L container for 48 h while samples were taken at predetermined time for COD analysis. The initial pH of the solution was adjusted to 6.97, a value found to be optimal in the preliminary experiments for adsorption. The initial COD value, temperature and residence time were the factors whose effect on COD removal efficiency were studied. The COD reduction efficiency was evaluated by using the formula:

$$\text{COD Reduction (\%)} = \frac{\left(COD_i - COD_f\right)}{COD_i} \times 100 \quad (1)$$

Where COD_i is the initial COD value and COD_f is the final COD.

Aerobic biodegradation experiments

Similar to adsorption experiments, biodegradation studies were carried out for comparison purposes only. Prior to the start of

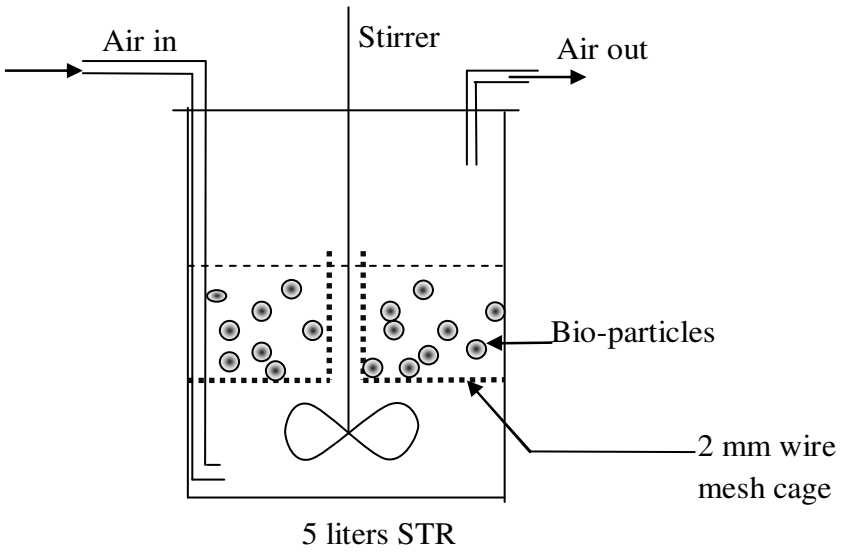

Figure 1. Stirred tank reactor set up for SAB process.

biodegradation experiments, a mixed culture of microbes obtained from a municipal wastewater treatment facility was allowed to acclimatize to melanoidin for a period of about 9 months. This was done by weekly addition of fresh melanoidin solution of approximately 9000 mg COD/L. This consortium of microbes was then used for biodegradation studies. The inoculum was added to the synthetic wastewater solution in the volume ratio of 1:10 (Tondee and Sirianuntapiboon, 2008). The COD: N: P ratio was set at 125: 2.5: 1, which was found to be optimum from the preliminary experiment. This represents a higher ratio of COD to N than that reported in a previous study of the biodegradation brewery and petroleum wastewater (Ochieng et al., 2002) in a similar reactor. The compositions of other nutrients were: NaCl (203.3 mg/L); $MgCl_2.4H_2O$ (0.8 mg/L); $FeSO_4.7H_2O$ (0.4 mg/L) and $CaCl_2.6H_2O$ (0.4 mg/L). For phosphorous source, Na_2HPO_4 and KH_2PO_4 were used.

The same experimental set up such as the one used in SAB process (Figure 1), but without microbial support was used. A sample of 3.5 L of melanoidin solution of different initial COD values were degraded over a period of 48 h in the described reactor, with air being supplied from a compressor and overhead stirrer speed kept at 400 rpm. Samples were taken periodically and COD values determined.

Simultaneous adsorption and biodegradation (SAB)

Experimental setup

The experimental set up as shown in Figure 1 comprised a 5 L stirred tank reactor (STR), a 2 mm wire mesh cage which housed the bio-particles, air inlet from a compressor and an overhead stirrer whose speed was kept at 400 rpm. The bio-particles were GAC particles with microbes immobilized on them.

Bio-particle development

The initial phase of the experiment involved growing biofilm on the particle surface and letting the bio-particle get acclimatized to the reactor condition. A fixed amount of carbon (200 g) was put into the wire mesh cage and contacted with 3.5 L of melanoidin solution and the nutrients in the reactor, while the stirrer speed was maintained at 400 rpm. This was carried out for a period of 36 days during which time fresh melanoidin solutions of varying concentrations (4900 to 14580 mg/L) were introduced to the reactor at various intervals. Residual COD level, turbidity, temperature, and dissolved oxygen of the system were also monitored.

The surface morphology of free activated carbon and that with microbes attached to it was investigated using Field Emission Scanning Electron Microscope (JEOL JSM-7500F). The samples were mounted on carbon tape and imaged at 2 kV accelerating voltage. Micrographs were then taken at a magnification of ×1 000.

SAB experimental runs

After the initial phase, the focus shifted to studying the effect of temperature, pH, initial concentration and residence time on COD removal efficiency. Temperature was varied from 295 to 318 K, while pH was varied from pH 4 to 9 and their effect on COD reduction monitored. Likewise, the initial concentration was varied from 4600 to 16800 mg/L, while the residence time effect was explored from 2 to 48 h. Comparison of performance of the three systems: adsorption alone, biodegradation alone and coupled adsorption and biodegradation were then evaluated.

Analyses

Throughout the biodegradation and SAB experiments, dissolved oxygen (DO), COD, turbidity, pH and temperature were monitored. Before samples were analyzed for COD, a supernatant from the sample was obtained by centrifugation at 4000 rpm for a period of 10 min using ROTOFIX 32A (Andreas Hettich GmbH & Co. KG, Germany) centrifuge. The COD was determined using Hanna C214 Multiparameter Bench Photometer, employing an adaptation of the US Environmental Protection Agency (USEPA) 410.4 approved method. A 0.2 ml sample solution was put into test vials (HI93754C) for COD analysis in the concentration range of 0 to 15,000 mg/L. These vials were then inserted into a digester (Hanna HI839800 COD reactor) and preheated to 150°C for 2 h. The reactor was then switched off and the vial allowed to cool to 120°C for 20 min. The

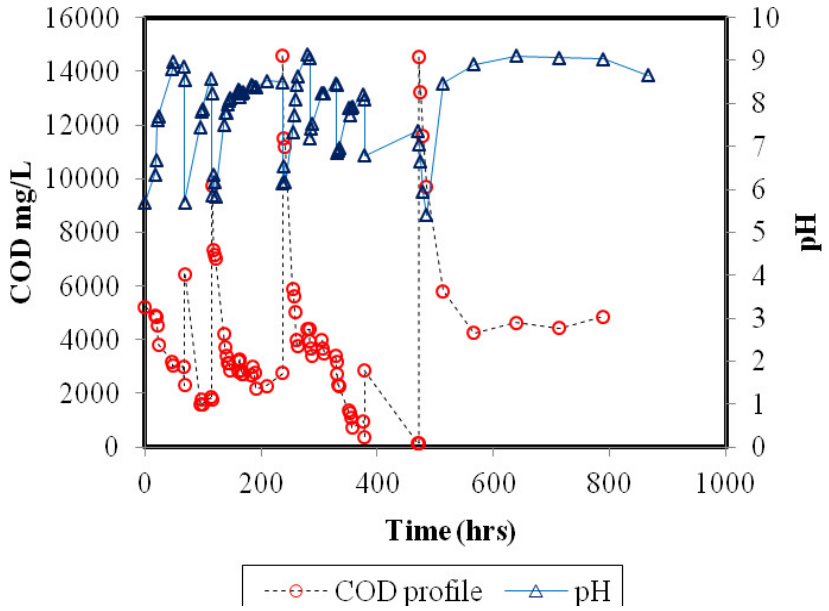

Figure 2. COD profile during biofilm development stage.

COD readings were then taken directly from the photometer.

The turbidity of the wastewater was measured in nephelometric turbidity units (NTU) using turbidity meter (model TN-100, Eutech Instruments, Singapore). The meter was calibrated with solutions of 0.02, 20, 100 and 800 NTU. The measurements were done immediately after sampling in order to avoid particle flocculation and sedimentation. At the same time, pH and temperature were measured by Ecoscan pH/mV/°C meter from Eutech Instruments, Singapore. Lastly, dissolved oxygen (DO) was determined by WinLab Data Line Oxygen-Meter from Windaus Labortechnik, Germany.

RESULTS AND DISCUSSION

Acclimatization and biofilm development on the carbon support

Formation and development of biofilm on the carbon surface was carried out for a period of 36 days. During this phase, melanoidin solutions with different COD values were introduced batch-wise into the stirred tank reactor (STR). Figure 2 shows the residual COD and pH profiles during biofilm development in the STR. In all batches, the solution reached a maximum of around pH 9, which coincided with the maximum removal of COD. Figure 3 presents the SEM maps of carbon before (A) and after (B) biofilm formation, showing a morphological change in the carbon particles indicating biofilm formation. After 36 days, the developed carbon bio-particles were used to degrade melanoidin.

Effect of initial COD value

The kinetics of COD reduction is shown in Figure 4. Results reveal that COD removal efficiency increased with time. After 48 h, the COD removal efficiency increased from 61.7 to 74.9% as the initial concentration increased from 3240 to 10800 mg/L. However, when the initial concentration was increased beyond 10, 800 mg/L, there was drastic reduction in COD removal efficiency. An increase of initial COD from 10, 800 mg/L to 16, 830 mg/L resulted in a decrease in COD removal efficiency from 74.9 to 52.5%. The reason for this could be due to substrate limitation below 10 800 mg/L, whereas above this concentration, microbial growth and hence biodegradation was suppressed increasingly due to substrate inhibition.

Back to the kinetics, the initial rate and attainment of optimal COD reduction was enhanced with a reduction in initial concentration. The system with an initial COD value of 16830 mg/L took about 40 h to attain optimal COD reduction efficiency, while that of 3240 mg/L took only about 12 h. However, the initial rate of degradation of melanoidin was not as fast as that experienced during adsorption process, even though it was faster than that of biodegradation alone. Figure 5 (A to E) shows a comparison of COD removal efficiencies of SAB process against those of biodegradation and adsorption at different initial COD values: 4840, 7900, 10800, 13920 and 16830 mg/L. In all instances, SAB was better than both biodegradation and adsorption processes alone. Adsorption process was, however, the most rapid one followed by SAB and lastly, biodegradation as indicated in Figure 5. Biodegradation was the slowest due to delayed growth of microbes in the presence of melanoidin. The removal efficiency exhibits two phases: a rapid initial phase followed by a slow phase thereafter.

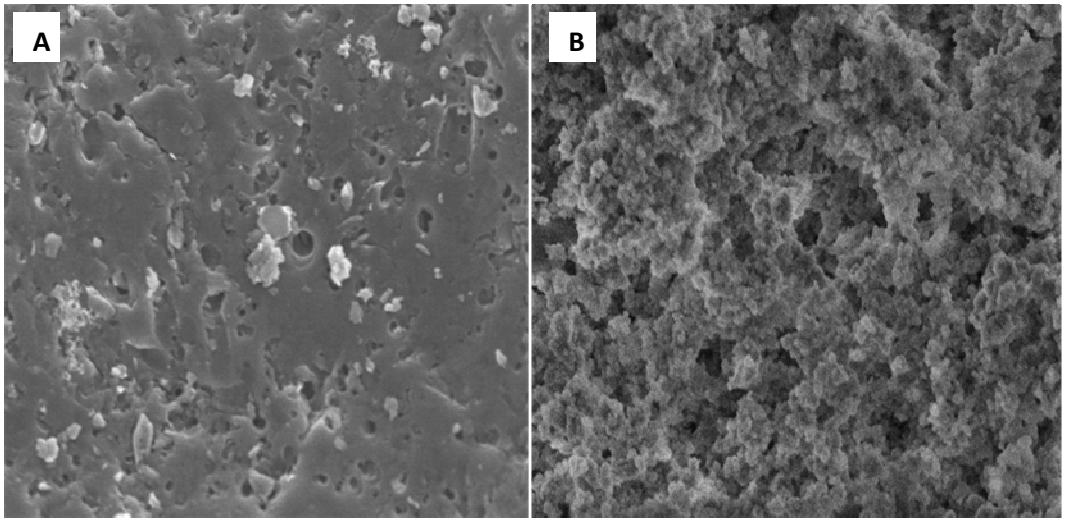

Figure 3. SEM maps of (A) carbon alone and (B) Bio-particle.

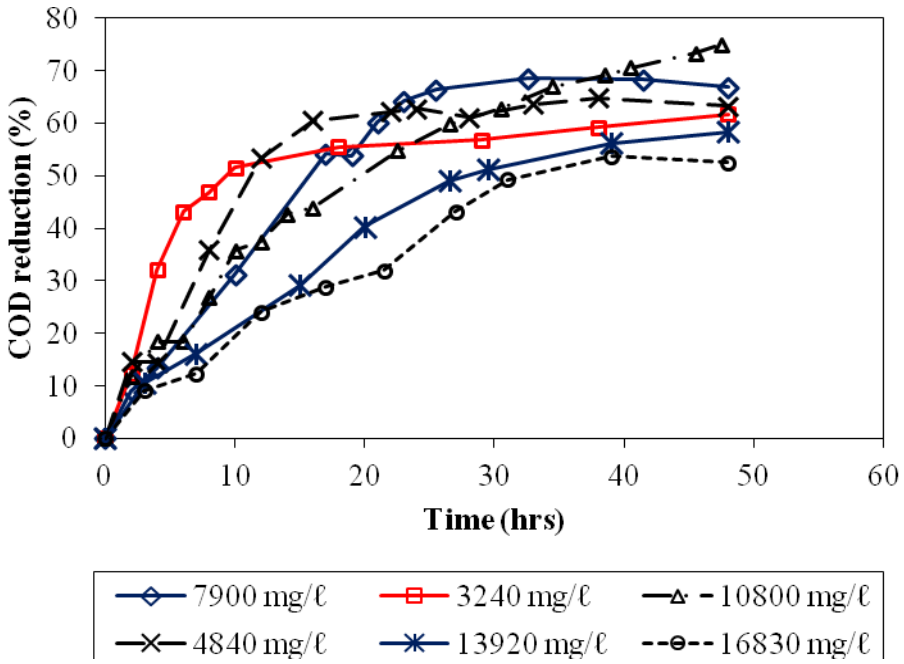

Figure 4. Effect of initial concentration on COD reduction (dry biomass support: 200 g; temp: 298 K; pH: 6.97).

Such a two-phase phenomenon was also found when metal cyanide bearing wastewater was treated using *Pseudomonas fluorescens* immobilized on granular activated carbon in a SAB process (Dash et al., 2008). For initial COD value of 10800 mg/L, the SAB process resulted in the highest COD removal efficiency of 75% against 51% for biodegradation and 49% for adsorption at a temperature of 293 K.

For solutions with lower initial COD value, adsorption resulted in better removal efficiency as compared to that of biodegradation as shown in Figure 5 A and B. However, as the initial COD is increased beyond 10800 mg/L, adsorption performance either becomes equal (Figure 5D) or less than biodegradation (Figure 5 E). The high concentration may have led to saturation of adsorption sites necessary for melanoidin adsorption. On the other hand, the SAB process resulted in the highest COD removal efficiency at any initial COD value. It is however worth noting that the synergy realized in the SAB process over adsorption or biodegradation process

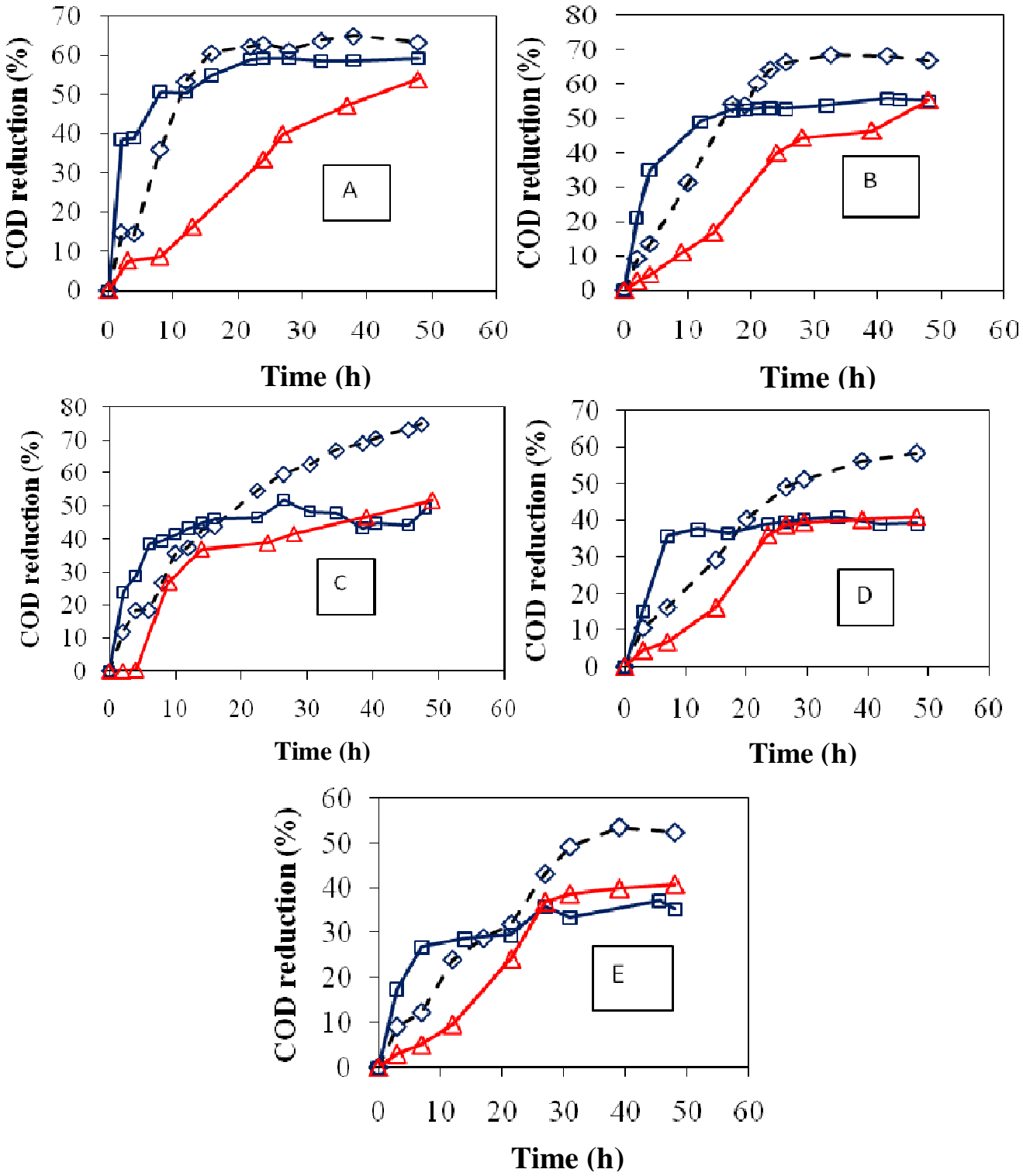

Figure 5. Comparison of COD reduction efficiencies of SAB to biodegradation and adsorption (initial COD: - (A): 4840 mg/L; (B): 7900 mg/L; (C): 10800 mg/L; (D): 13920 mg/L; (E): 16830 mg/L; temp: 296 K; pH: 6.97). Red line, biodegradation; blue, adsorption; Dotted, SAB.

carried out independently was not effective. This is primarily because mass transfer is impeded by the biofilm formed on the activated carbon; hence in the SAB process adsorption cannot proceed to cover all sorption

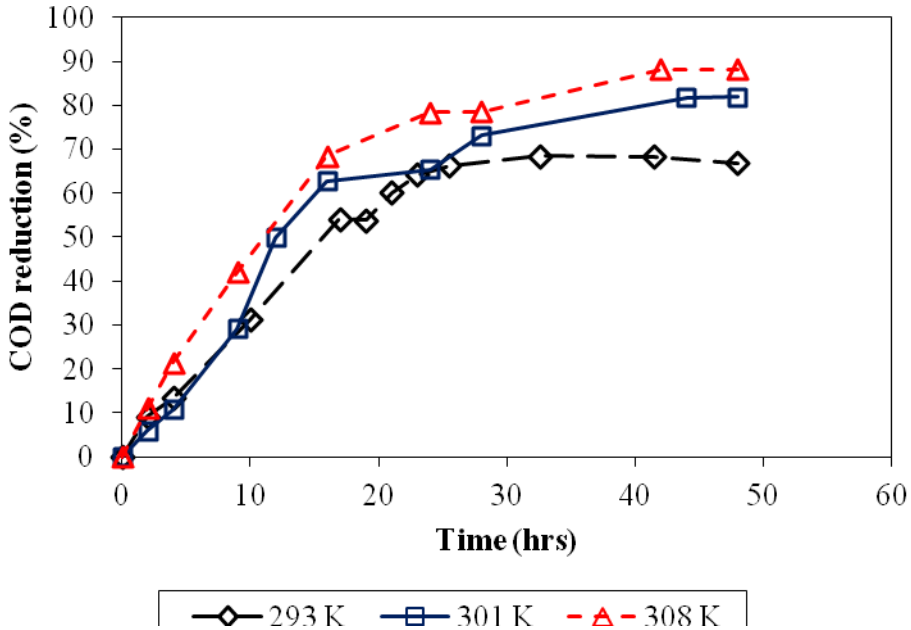

Figure 6. Effect of temperature on COD reduction (pH: 6.97; initial COD 7900 mg/L).

sites. In the presence of microbial film, the removal of melanoidin is mechanistically complex involving (i) transport of substances from the bulk liquid to the surface of microbial film, (ii) simultaneous mass transfer, adsorption and biochemical reaction within microbial film, and (iii) simultaneous mass transfer and adsorption within adsorbent (Mondal and Balomajumder, 2007). The complexity increases due to the dynamic nature of the microbial film. As biochemical reaction of substances may occur on the adsorbent and in bulk suspension, the presence of biomass in SAB process could also be expected, to some extent, to regenerate the adsorbent and thus guarantee longer operation of the process.

Activated carbon has been found to be partially regenerated by microorganisms while the carbon bed is in operation (Aktaş and Çeçen, 2007). In the SAB process, the presence of activated carbon increases the liquid–solid surfaces on which microbial cells, enzymes, organic materials and oxygen are adsorbed providing an enriched environment for microbial metabolism (Mondal and Balomajumder, 2007). The SAB process was therefore justifiably found to be more effective than other processes carried out independently.

Effect of initial pH of solution

The effect of pH on the performance of SAB process was explored in terms of COD removal efficiency in the pH range of 4 to 9. Results obtained after 48 h of operation showed that pH did not have significant effect. The COD removal efficiency was 61% at pH 4.38, 66.8% at pH 6.97 and 65% at pH 8.97. However, the initial rate of melanoidin removal (COD reduction) was highest for acidic medium (pH 4.38), while it was lowest for basic media (pH 8.97). This was primarily due to the fact that the solubility of melanoidin is higher in basic media than in acidic media (Miranda et al., 1996), thus resulting in a rapid adsorption, which is the favored in the initial step in SAB process. Near-neutral pH was therefore considered optimal for SAB process. Similar pH value was reported to be optimal for a purely biodegradation system involving decolorization and bioremediation of melanoidin containing molasses spent wash (Singh et al., 2007) and decolorization of anaerobically treated distillery spent wash by bacterial consortium (Mohana et al., 2007).

Effect of temperature

Temperature increase from 293 to 308 K did increase COD removal efficiency from 66.8 to 88% as shown in Figure 6. This could be explained by increased enzymatic activity with enhanced temperature, as well as solid-solute interaction due to increased kinetic energy of melanoidin molecules, leading to better biodegradation and adsorption, respectively. It was however not necessary to go beyond a temperature of 308 K because previous studies on biodegradation revealed that microbial death occurred at temperatures above 310 K. Hence, a temperature of 308 was considered optimum for the removal of melanoidin through the SAB process.

Conclusion

Wastewater containing melanoidin was treated using

anaerobic biodegradation and adsorption in a batch reactor. The application of a combined process of biodegradation and adsorption has been shown to be a better way for treating melanoidin containing wastewater such as molasses spent wash than either of them carried out independently. Furthermore, its performance was slightly dependent on pH, underscoring its superior ability to absorb shocks in the system. Moreover, since post biomethanated molasses spent wash normally still contains the recalcitrant melanoidin, it is suggested that the SAB process be used for its further treatment.

ACKNOWLEDGEMENTS

The authors would like to thank the International Foundation for Science (IFS) for financial support. V. O. Ojijo would also like to thank Tshwane University of Technology for granting him a scholarship to pursue postgraduate studies.

REFERENCES

Agarwal R, Lata S, Gupta M, Singh P (2010). Removal of melanoidin present in distillery effluent as a major colorant: a review. J. Environ. Biol. 31(4): 521-528.

Aktaş Ö, Çeçen F (2007). Adsorption, desorption and bioregeneration in the treatment of 2-chlorophenol with activated carbon. J. Hazard. Mater. 141: 769-777.

Bernado EC, Egashira R, Kawasaki J (1997). Decolorization of molasses' wastewater using activated carbon prepared from cane bagasse. Carbon, 9: 1217-1221.

Chandra R, Bharagava RN, Rai V (2008). Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation. Bioresour. Technol. 99: 4648-4660.

Coca M, Teresa-García M, González G, Peña M, García JA (2004). Study of coloured components formed in sugar beet processing. Food Chem. 86: 421-433.

Dash RR, Balomajumder C, Kumar A (2008). Treatment of metal cyanide bearing wastewater by simultaneous adsorption and biodegradation (SAB). J. Hazard. Mater. 152: 387-396.

Dwyer J, Lant P (2008). Biodegradability of DOC and DON for UV/H2O2 pre-treated melanoidin based wastewater. Biochem. Eng. J. 42: 47-54.

Figaro S, Louisy-Louis S, Lambert J, Ehrhardt JJ, Ouensanga A, Gaspard S (2006). Adsorption studies of recalcitrant compounds of molasses spentwash on activated carbons. Water Res. 40: 3456-3466.

Fitzgibbon FJ, Nigam P, Singh D, Marchant R (1995). Biological treatment of distillery waste for pollution-remediation. J. Basic Microbiol. 35: 293-301.

Harada H, Uemura S, Chen AC, Jayadevan J (1996). Anaerobic treatment of a recalcitrant distillery wastewater by a thermophilic UASB reactor. Bioresour. Technol. 55: 215-221.

Jing H, Kitts DD (2004). Antioxidant activity of sugar-lysine Maillard reaction products in cell free and cell culture systems. Arch. Biochem. Biophys. 429: 154-163.

Kalavathi DF, Uma L, Subramanian G (2001). Degradation and metabolization of the pigment-melanoidin in a distillery effluent by the marine cyanobacterium Oscillatoria boryana BDU 92181. Enzyme Microb. Technol. 29: 246-251.

Kannan A, Upreti RK (2008). Influence of distillery effluent on germination and growth of mung bean (Vigna radiata) seeds. J. Hazard. Mater. 153: 609-615.

Lim PE, Ong SA, Seng CE (2002). Simultaneous adsorption and biodegradation processes in sequencing batch reactor (SBR) for treating copper and cadmium-containing wastewater. Water Res. 36: 667-675.

Martins SIFS, Jongen WMF, Van Boekel MAJS (2001). A review of Maillard reaction in food and implications to kinetic modeling. Trends Food Sci. Technol. 11: 364-373.

Migo VP, Del Rosario EJ, Matsumura M (1997). Flocculation of melanoidins induced by inorganic ions. J. Ferment. Bioeng. 83: 287-291.

Miranda MP, Benito GG, Cristobal NS, Nieto CH (1996). Color elimination from molasses wastewater by Aspergillus niger. Bioresour. Technol. 57: 229-235.

Mohana S, Desai C, Madamwar D (2007). Biodegradation and decolourization of anaerobically treated distillery spent wash by a novel bacterial consortium. Bioresour. Technol. 98: 333-339.

Mondal P, Balomajumder C (2007). Treatment of resorcinol and phenol bearing waste water by simultaneous adsorption biodegradation (SAB): optimization of process parameters. International J. Chem. Reactor Eng. 5: S1.

Moreno-Castilla C (2004). Adsorption of organic molecules from aqueous solutions on carbon materials. Carbon, 42: 83-94.

Ochieng A, Ogada T, Sisenda W, Wambua P (2002). Brewery wastewater treatment in a fluidised bed bioreactor, J. Hazard. Mater. 90(3): 311-321.

Peña M, Coca M, González R, Rioja R, García MT (2003). Chemical oxidation of wastewater from molasses fermentation with ozone. Chemosphere, 51: 893-900.

Prasad RK, Srivastava SN (2009). Electrochemical degradation of distillery spent wash using catalytic anode: Factorial design of experiments. Chem. Eng. J. 146: 22-29.

Prasad RK (2009). Color removal from distillery spent wash through coagulation using Moringa oleifera seeds: Use of optimum response surface methodology. J. Hazard. Mater. 165: 804-811.

Satyawali Y, Balakrishnan M (2008). Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: A review. J. Environ. Manage. 86: 481-497.

Singh KD, Sharma S, Dwivedi A, Pandey P, Thakur RL, Kumar V (2007). Microbial decolorization and bioremediation of melanoidin containing molasses spent wash. J. Environ. Biol. 28(3): 675-677.

Tondee T, Sirianuntapiboon S (2008). Decolorization of molasses wastewater by *Lactobacillus plantarum* No. PV71-1861, Bioresour. Tech. 99: 6258-6265.

Wang BS, Li BS, Zeng QX, Liu HX (2008). Antioxidant and free radical scavenging activities of pigments extracted from molasses alcohol wastewater. Food Chem. 107: 1198-1204.

Wedzicha BL, Kaputo MT (1992). Melanoidins from glucose and glycine: composition, characteristics and reactivity towards sulphite ion. Food Chem. 43: 359-367.