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Effects of Experimental Conditions on the Decolorisation of Anaerobically Treated Wastewater from Molasses Based Ethanol Production by a Modified Chitosan

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***Abstract*:** *The effluent from an anaerobic wastewater treatment system of an ethanol production using molasses as the raw material, biomethanated WMB, contains very dark brown colour with high COD. This brown colour is mainly caused by melanoidin, a recalcitrant molecule with net negative charges. A modified chitosan, N-[(2hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) was obtained from a chemical modification in order to increase the positive charges to the molecule and was used as a flocculant. In this work, the effects of several experimental condition were investigated. It was found that the agitation by a reciprocal shaker at 100 osc/min and an overhead stirrer at 500 rpm was required no longer than 5 and 3 min, respectively. The zeta potential values of HTCC at pH 4-9 were positive and it was decreased when the pH was increased. The extent of decolorisation when the pH of biomethanated WMB was in the range of 6-10 were not significantly different.*

Keywords: *flocculation, N-[(2hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC), pH, agitation*

1. Introduction

An anaerobic biological treatment system is used commonly to treat the wastewater from molasses based ethanol production (WMB) and it produces biogas as a valuable by-product. However, after the treatment with this system, the biomethanated effluent still contains high COD and an even more intense dark brown colour [3]. It is not possible to release this biomethanated effluent into natural water courses. The very strong colour inhibits sunlight penetrating into rivers resulting in a decrease in photosynthesis which affects aquatic life. It has a high pollution load which results in eutrophication and an obnoxious smell [6], [10].

The brown pigments in the biomethanated water are not extensively biodegraded. These pigments are mainly melanoidin which are net negatively charged [8], produced by Maillard reactions, and smaller amounts of phenolic compounds [3], [6], [10].

Flocculation is a process for removing colloidal particles from wastewater by charge attractions leading to precipitation of flocs. A well-known organic flocculant is chitosan. Its flocculation ability can be increased by increasing the positive charge on the molecule resulting in stronger binding ability of negatively charged colloidal particles [11].

The chemical modification of chitosan by glycidyltrimethylammonium chloride (GTMAC) generates 2-hydroxypropyl-3-trimethylammonium chitosan chloride (HTCC). It is a quaternised chitosan with strong positive charges [8]. This substance has the potential to be a superior flocculant for the removal of melanoidin from biomethanated WMB.

In this work, the effects of several experimental conditions on the decolorisation of biomethanated WMB by HTCC were examined.

2. Materials and Methods

2.1. Biomethanated WMB

Anaerobically treated biomethanated WMB generated during the production of ethanol was obtained from a company located in the central region of Thailand. Its pH was 8.0 and it was diluted with distilled water to adjust its absorbance at 475 nm to 1.0 and stored at 4 ^{o}C .

2.2. HTCC Synthesis

HTCC was synthesised according to [9]. Chitosan (5 g, 85% of deaceylation minimum) was dissolved completely in 191 ml of 2% acetic acid. The dissolved solution was added 27.6 ml GTMAC and the mixture was stirred with a magnetic stirrer at 50 ^{0}C, 350 rpm for 18 hours under N_2. HTCC was precipitated by pouring the mixture into chilled acetone. The precipitated HTCC was washed with methanol to remove the excess of GTMAC and was left to dry at room temperature for 4 hours and then further dried in a vacuum oven at 60 ^{0}C for 2 days. After drying, the HTCC was powdered and stored at room temperature.

2.3. Optimization of Agitation Time

Mixtures containing 7.5% (v/v) of 1% HTCC solution and 20 ml of diluted biomethanated WMB were stirred for 5-25 minutes at 100 osc/min on a reciprocal shaker. Another set of the same mixtures was stirred rapidly by using an overhead stirrer at 500 rpm for 3 min followed by gentle stirring at 60 rpm for 30 min. All experiments was done in triplicate. The concentration of colour after centrifugation at 14,087 g was measured by a spectrophotometer at 475 nm.

2.4. Measurement of Zeta Potential

1% (w/v) HTCC solution was prepared in distilled water using an overhead stirrer (500 rpm). The pH was adjusted to 4-9 by 3 M HCl or NaOH. The solution that had been stored for 12 hr at 4 ^{o}C in air-tight containers were used to measure the zeta potential of HTCC. The refractive indices of chitosan and water at 25^{o}C were 1.523 and 1.330, respectively [1], [2]. The measurement was performed at 25 ^{o}C and 173^{o} angle by a Zetasizer Nano ZS series, (Malnern instruments Ltd., UK).

2.5. Effect of pH of Biomethanated WMB on the Decolorisation

The pH of biomethanated WMB was adjusted by adding 3 M HCl or NaOH to give pHs from 6 to 10 and 7.5% (v/v) portions of 1% HTCC solution were added to 20 ml volumes of pH adjusted biomethanated WMB. The mixtures were stirred on a reciprocal shaker at 100 osc/min for 5 min at ambient temperature (25^{o}C). The colours of biomethanated WMB before and after shaking were determined spectrophotometrically at 475 nm.

3. Results and Discussions

3.1. The Effect of Agitation Time for the Flocculation Process

There was no significant difference in the extents of decolorisation when the solutions were agitated using a reciprocal shaker at 100 osc/min) over periods from 5 to 25 min (Fig 1) even although the size of flocs became visibly larger with increasing agitating time. When the HTCC solution was mixed with biomethanated WMB using an overhead stirrer, it was observed that the extent of decolourisation caused by a slow mixing at 60 rpm for 30 min did not increase following a rapid mixing at 500 rpm for 3 min (Fig 2). These results indicate that melanoidin can be removed by HTCC solution using a direct flocculation method and this is a simpler method than coagulation-flocculation; only one floccculant is needed while a coagulant is not [4]

3.2. Zeta Potential

The ionic charge at the surface of HTCC particles at the boundary between the HTCC molecules and disperse medium is called the zeta potential. The zeta potential determines the electrophoretic mobility of the particles and influences the stability of colloidal systems [12]

At pH 4-9, HTCC had a positive zeta potential that decreased as pH was increased (Fig 3). The lowest zeta potential of HTCC was recorded at pH 8 (+28.35 mV). Theoretically, zeta potentials higher than +30 mV and lower than -30 mV confer stability on colloidal dispersions [7] and it is possible that HTCC particles in a dispersion at pH 8 might coagulate loosely to form a weak gel. The positive values of the zeta potential of HTCC in this pH range (Fig 3) confirm that HTCC is net positively charged [7].

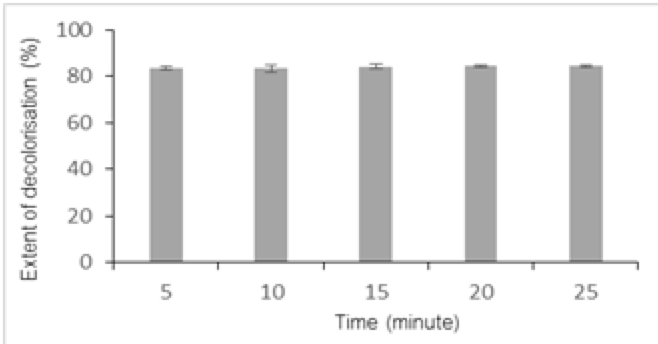

Fig. 1: Decolorisation of biomethanated WMB by addition of 1% HTCC solution (final concentration of 0.07%) after various times of agitation at 100 osc/min. The means of percentages of decolorisation recorded in three separate tests are shown and the error bars represent the standard deviations.

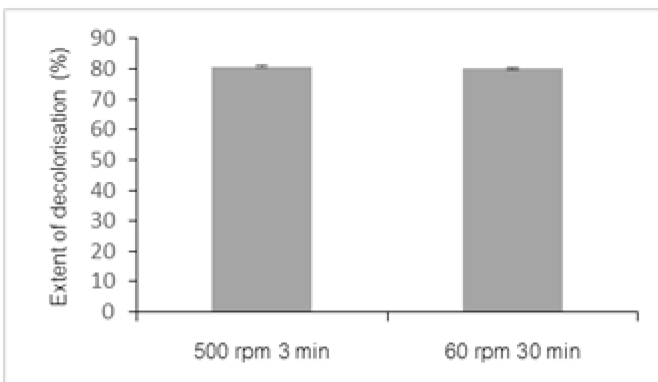

Fig. 2: Decolorisation of biomathanated WMB by addition of 1% HTCC solution (final concentration of 0.07%) using an overhead stirrer with a rapid mixing for a short period followed by slower mixing for a longer period (see also the text). The means of the percentages of the reduction of colour recorded in three separate tests are shown and the error bars represent the standard deviations.

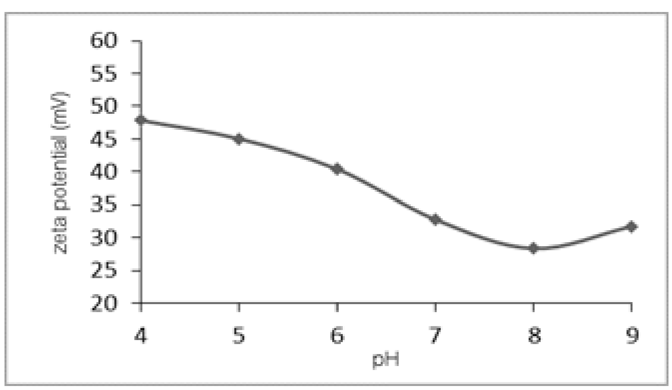

Fig. 3: The zeta potential of HTCC at pHs from 4 to 9

3.3. Effects of pH of Biomethanated WMB on the Decolorisation of Biomathanated WMB by HTCC

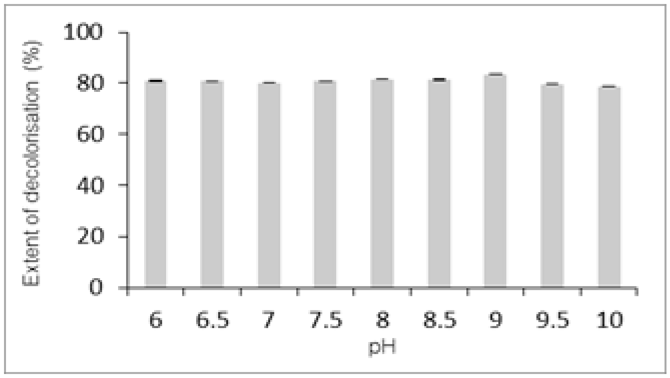

Fig. 4: Decolorisation of biomethanated WMB by 1% HTCC solution (final concentration of 0.07%) at various values of pH. The means of the percentages of the reduction of colour recorded in three separate tests are shown and the error bars represent the standard deviations

Variation of the pH of the biomethanated WMB from 6 -10 did not have any effect on the decolorisation by HTCC. The extent of decolorisation was consistently about 80% (Fig 4). However, larger flocs were observed at the lower pHs in this range. The zeta potential of HTCC varied in a narrow range of 30-40 mV at pHs between 6 and 9 (Fig 3) and these small changes of zeta potential were not sufficient to have caused any significant difference in the extents of decolorisation. [6] reported that the efficiency of removal of tannins by HTCC was increased as pH was increased from 1 to 4.5 and decreased as pH was raised from 4.5 to 9. [5] reported a decrease in the capacity of HTCC to flocculate low-methoxyl pectin at pH 4–5 compared with at pH 2-4. The pH of biomethanated WMB is usually slightly alkaline if the anaerobic wastewater treatment system operates effectively. The results shown in Fig 4 indicate clearly that HTCC can be used as an effective flocculant to remove the colour from slightly alkaline biomethanated WMB.

From these results, it can be seen that the pH of biomethanated WMB in the range of 6–10 did not influence the ability of HTCC solution (final concentration of 0.07%) to decolorise. Therefore, HTCC solution can be used for the flocculation of biomethanated WMB over a rather wide range of slightly alkaline pH.

4. Conclusions

HTCC could reduce the colour of the biomethanated WMB up to 84%. The use of HTCC in a form of a solution gave higher flocculation efficiency. Agitation was needed no longer than 5 min to complete the flocculation by HTCC. The pH of the water from 6-10 did not have any effect on the extent of decolorisation.

5. References

[1] Amy, H. 2005. **Index of refraction of water**. Available Source: https://hypertextbook.com/facts/2005/AmyHo.shtml, 7 Nov.

[2] Bismita, N., K.P. Pravat, R. Pradipta, R. Pratima and K.P. Amulya. 2011. Chitosan micro and nanoparticles for delivery of drugs and therapeutic proteins, p. *In* **International World Conference, WCN 2011, Second world conference on nanomedicine and drug delivery.** Kottayam, Kerala , India.

[3] Jiranuntipon, S. 2009. **Decolorization of molasses wastewater from distilleries using bacterial consortium.** Master, University de toulouse.

[4] Jorgensen, S.K. 1979. Industrial wastewater management. **Studies in** Company, Amsterdam.

[5] Liu, J., Y. Tian, X. An, G. Li and Y. Kang. 2015. Flocculation performance and mechanism of removing pectin by n-hydroxypropyl trimethyl ammonium chloride chitosan. **Journal of Dispersion Science and Technology**. 36: 1612-1620.

[6] Pant, D. and A. Adholeya. 2007. Biological approaches for treatment of distillery wastewater: A review. **Bioresour Technol**. 98: 2321-2334.

[7] Qin, L., J. Liu, G. Li and Y. Kang. 2015. Removal of tannic acid by chitosan andn-hydroxypropyl trimethyl ammonium chloride chitosan: Flocculation mechanism and performance. **Journal of Dispersion Science and Technology**. 36: 695-702.

[8] Rubinsztain, P., P. Ioselis, R. Ikanand and Z. Aizenshtat. 1984. Investigations on the structural units of melanoidins. **Organic Geochemistry**. 6: 791-804.

[9] Rwei, S.P., Y.M. Chen, W.Y. Lin and W.Y. Chiang. 2014. Synthesis and rheological characterization of water-soluble glycidyltrimethylammonium-chitosan. **Mar Drugs**. 12: 5547-5562.

[10] Satyawali, Y. and M. Balakrishnan. 2008. Wastewater treatment in molasses-based alcohol distilleries for cod and color removal: A review. **J Environ Manage**. 86: 481-497.

[11] Sayed, S. and A. Jardine. 2015. Chitosan derivatives as important biorefinery intermediates. Quaternary tetraalkylammonium chitosan derivatives utilized in anion exchange chromatography for perchlorate removal. **Int J Mol Sci**. 16: 9064-9077.

[12] Schramm, L.L. 2005. **Emulsions, Foams, and Suspensions: Fundamentals and Applications.** Wiley-VCH: Weinheim.