

## EFFECT OF ISOTHIAZOLONE BIOCIDES ON THE PERFORMANCE OF A LABORATORY SCALE ROTATING BIOLOGICAL CONTACTOR AND BIOCIDES EFFICACY

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The effect of isothiazolones, a commercial biocide widely used in paper and pulp industry, on the performance of laboratory-scale rotating biological contactors (RBCs) as well as biocide efficacy was studied. Biofilms were established on the RBCs and then exposed to the synthetic wastewater containing 0 (control), 3, 6 and 12 ppm isothiazolones at a flow rate of 2.5 litres h<sup>-1</sup> for 14 days. The results showed that COD removals at steady state of the control, 3 and 6 ppm RBC units were 68.35 ± 4.40, 57.47 ± 1.75 and 15.27 ± 2.67 % respectively, while COD removal of the 12 ppm RBC unit was totally inhibited after day 12 of the treatment. Isothiazolones could be removed 77% by the RBC unit receiving 3 ppm isothiazolones. At the higher concentrations, however no significant isothiazolone removal occurred. Isothiazolone concentrations up to 3 ppm had no significant effect on viable populations of biofilms and planktonic cells in the RBCs. However, 6 and 12 ppm isothiazolones caused approximately 10<sup>4</sup>-fold and 10<sup>5</sup>-fold reductions of the numbers of colony-forming units in the biofilms. Moreover, when 12 ppm isothiazolones was applied to the system no viable planktonic cells could be detected.

Keywords: biocide, rotating biological contactor (RBC), isothiazolone compounds

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**Introduction :** Isothiazolone compounds (5-chloro-2-methyl-4-isothiazoline-3-one and 2-methyl-4-isothiazoline-3-one) are biocides which achieve their biocidal activity by reaction with thiol-containing enzymes (Collier et al. 1990). They are widely used as antimicrobial agents in a variety of applications, such as cooling water, paper, cosmetics and textiles. The use of large amounts of biocide in industry may cause environmental, ecological and toxicological problems when water contaminated with the biocide is discharged directly to natural water or to municipal effluent treatment plants (Wyndham and Kennedy, 1995). In this study the effects of isothiazolones, a commercial biocide widely used in paper and pulp industry, on the performance of a laboratory-scale rotating biological contactor (RBC) unit, a secondary wastewater treatment system with fixed film process, in terms of the reduction in chemical oxygen demand (COD) and biocide degradation as well as biocide efficacy were investigated.

### **Materials and Methods :**

*The single-stage (3-disc) lab-scale RBC unit:* The single-stage laboratory-scale RBC unit consisted

of an influent chamber, a disc stage and a settling tank as described in Laopaiboon et al. (2006).

*The synthetic wastewater:* The standard synthetic wastewater consisted of (mg l<sup>-1</sup>): lab-lemco broth, 90; NH<sub>4</sub>Cl, 54; K<sub>2</sub>HPO<sub>4</sub>, 28; NaCl, 7; CaCl<sub>2</sub>·2H<sub>2</sub>O, 4; and MgSO<sub>4</sub>·7H<sub>2</sub>O, 2.

*Biocide removal from the RBC unit without biofilms on discs:* Isothiazolones at 6 ppm was prepared in tap water and in the synthetic wastewater and then added into separate clean RBC units with discs (without biofilm) rotating at 16 rpm. Samples were taken over 3 h to determine biocide concentration.

*Establishment of biofilms and biocide treatment:* Recycled sludge was added in to the disc stage of the units filled with the wastewater. The flow of the wastewater was introduced to the units at a rate of 2.5 litres h<sup>-1</sup>. After one week the surface of the discs was covered by a thin homogenous biofilm. Then a commercial biocide, isothiazolones, was added to the wastewater to give final concentrations of 0, 3, 6 and 12 ppm.

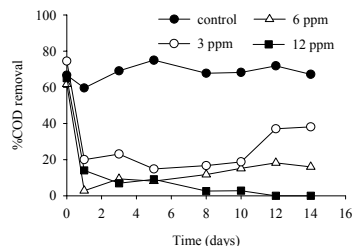
*Analytical methods:* COD and isothiazolone concentrations of the filtered influent and effluent samples of the RBC units were determined by the

modified closed reflux, titrimetric method (APHA AWWA and WPCE, 1995) and HPLC (Sible 1996) respectively. Viable populations of biofilm and planktonic bacteria were determined as described in Laopaiboon et al. (2006).

**Results and Discussion :**

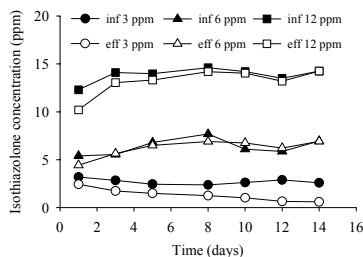
*Biocide removal from the RBC unit by non-biological means:* When the biocide was prepared in tap water and in the synthetic wastewater, it was found that biocide concentration was relatively constant during 3 h of the experiment (data not shown). This indicated that no adsorption of the biocide onto the discs and tank material (fiber glass) as well as by evaporation occurred.

*COD and biocide removal by the RBCs:* Isothiazolones have been shown to significantly reduce the treatment efficiency of the RBCs when applied at 3 ppm or greater (Fig. 1). COD removal of the untreated control unit was  $65.99 \pm 3.27\%$ . When 3 ppm isothiazolones was applied, COD removal decreased to around 20 % at day 1 but then subsequently recovered to around 40 % on day 12. When applied at the higher concentrations, isothiazolones caused increasing reductions in COD removal with total inhibition occurring at 12 ppm isothiazolones after day 12 of the treatment. Fig. 2 showed that isothiazolones could be removed 77% by the RBC unit receiving 3 ppm biocide. The loss of the biocide (by adsorption and evaporation) in the RBC unit without biofilm rarely occurred. Thus the removal of the biocide may be attributed to biological action. At the higher concentrations, however no significant biocide removal occurred (Fig. 2).

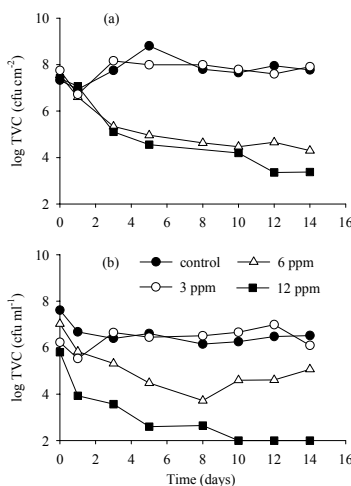


**Fig. 1** Effect of isothiazolones on COD removal in the RBCs.

*Viable population :* Fig. 3 showed that biocide concentrations up to 3 ppm had no significant effect on viable populations of biofilms and planktonic cells in the RBCs. However, 6 and 12 ppm isothiazolones caused approximately 10<sup>4</sup>-fold and 10<sup>5</sup>-fold reductions of the numbers of colony-forming units in the biofilms (Fig. 3a). Moreover, when 12 ppm isothiazolones was applied to the systems no viable planktonic cells could be detected (less than 10<sup>2</sup> cfu ml<sup>-1</sup>)(Fig. 3b).



**Fig. 2** Isothiazolone removal in the RBCs: inf = influent and eff = effluent.



**Fig. 3** Effect of isothiazolones on viability of biofilm (a) and planktonic cells (b) in the RBCs.

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**References :**

- (1) APHA AWWA & WPCE (1995) Eaton, A.D., Clesceri, L.S. and Greenberg, A.E., (Eds.) Standard Methods for the Examination of Water and Wastewater, 19 edn. Washington DC: American Public Health Association.
- (2) Collier, P.J., Ramsey, A., Waigh, R.D., Douglas, K.T., Austin, P. and Gilbert, P. (1990) Chemical reactivity of some isothiazolone biocides. J. Appl. Bacteriol. 69: 578-584.
- (3) Laopaiboon, L., Phukoetphim, N. and Laopaiboon, P. (2006) Effect of glutaraldehyde biocide on laboratory-scale rotating biological contactors. Electron. J. Biotechnol. 9 (4): 1-12, July 15.
- (4) Sible, V.S. (1996) Quantitative analysis of a biocide in silicone emulsions using high performance liquid chromatography. J. Liq. Chromatogr. R. T. 19: 1353-1367.
- (5) Wyndham, R.C. and Kennedy, K.J. (1995) Microbial consortia in industrial wastewater treatment. In: Lappin-Scott, H.M. and Costerton, J.W., (Eds.) Microbial Biofilms, pp. 183-195. Cambridge: Cambridge University Press.