BIODEGRADATION OF ISOTHIAZOLONE BIOCIDE BY LABORATORY-SCALE ROTATING BIOLOGICAL CONTACTORS

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Abstract

The aim of this study was to investigate the effects of operating conditions (organic loading rate, OLR; hydraulic loading rate, HLR and hydraulic retention time, HRT) as well as the presence of other carbon sources (lab-lemco broth) in the synthetic wastewater contaminated with isothiazolone on COD removal and biocide degradation by the RBCs. Biofilms had been established on the RBCs and then exposed to the wastewater containing 6 ppm isothiazolone under various operating conditions. The results showed that a period of cell acclimatization under biocide stress, the amount of growth substrate and the operating conditions were important parameters to increase the treatment efficiency of the system. When the normal synthetic wastewater containing 6 ppm isothiazolone was introduced to the units at HRT of 36 min, after acclimatization period COD removal was 16.49 ± 1.55% and biocide removal was rarely occurred. Isothiazolone degradation increased with increasing OLR of a growth substrate and/or HRT. Acclimatized biofilms could degrade isothiazolone via co-metabolism using lab-lemco broth as the growth substrate. At OLR (of lab-lemco broth) of 17 to 20 g COD m^{-2} d^{-1}, the acclimatized biofilms degraded approximately 73.11 ± 5.01 to 77.38 ± 6.66% and 45.76 ± 5.01% of 6 ppm isothiazolone at HRT of 72 and 36 min, respectively. When the OLR of the growth substrate was doubled, isothiazolone degradation increased to 59.33 ± 5.58% at an HRT of 36 min. The results also showed that biodegradability of isothiazolone did not totally relate with the susceptibility of cells to the biocide indicating by minimum lethal concentration (MLC) values. The resistant bacterium to isothiazolone was tentatively identified as predominantly of the species *Burkholderia cepacia*.

Keywords: biocide, biofilm, biodegradation, isothiazolone compounds, RBC, wastewater treatment

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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^{-1}): lab-lemco broth, 90; NH_4Cl, 54; K_2HPO_4, 28; NaCl, 7; $CaCl_2.2H_2O$, 4; and $MgSO_4.7H_2O$, 2.

Biofilm establishment and biocide treatments: A volume of 350 ml of recycled sludge from the wastewater treatment system of Phoenix Pulp & Paper Public Company Ltd., Khon Kaen, Thailand, was added into the disc stage of the RBC units filled with the synthetic wastewater. The units were operated as a batch system for 24 hours before the flow of the synthetic wastewater was introduced to the units to provide a medium for microbial growth at a rate of 2.5 litres h^{-1} for 2 weeks. After one week the surface of the discs was covered by a thin homogeneous biofilm as observed on operational RBCs. When the system was in steady state (no change in COD removal), a commercial isothiazolone was then added to the synthetic wastewater to give the final concentrations of 0 (control unit) and 6 ppm (active ingredients).

Effects of operating conditions on the treatment efficiency and isothiazolone biodegradation in the RBCs: Seven experimental runs; Runs 1 to 7, were performed. Each run lasted for about one week of stable operation as indicated by constant effluent COD and isothiazolone concentration. Untreated control units were also performed under the same conditions of Runs 1, 2 and Run 4 but without addition of isothiazolone. During various operating conditions, influent and effluent of the RBC units were collected for analysis. To study the decreased susceptibility of isothiazolone on acclimatized cells, minimum lethal concentration (MLC) of the biocide on non-acclimatized planktonic cells from the control RBC and acclimatized planktonic cells from the RBC receiving 6 ppm in Run 6 were determined. At the end of the experiment, single colonies of bacterial cells in the acclimatized biofilms were isolated on tryptone soya agar (TSA) plate by spread plate technique. The colonies were tentatively identified by the API 20 NE strip test (BioMérieux sa, France) (Laopaiboon et al. 2006).

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 :

Effects of operating conditions on treatment efficiency of RBCs receiving 6 ppm isothiazolone and susceptibility changes of acclimatized cells to biocide: COD removal and characteristics of the control RBC (without biocide) and the treated RBC under different operating conditions are shown in Figure 1, Figure 2 and Table 1. From Table 1, COD removal of the treated RBC in Run 1 to 7 were 16.49 ± 1.55, 44.05 ± 0.44, 0, 22.12 ± 4.57, 38.29 ± 3.61, 23.02 ± 3.49 and 29.31 ± 3.68% respectively. Isothiazolone removal of Run 1 to 7 from Table 1 were 0.90 ± 1.62, 77.38 ± 6.66, 0, 27.99 ± 4.74, 73.11 ± 5.01, 45.76 ± 5.01, 59.33 ± 5.58% respectively. The results from Run 1 to Run 7 showed that acclimatized cells can not utilize isothiazolone as a sole carbon or energy source. Isothiazolone was degraded biologically through cometabolism; metabolism of a compound (cosubstrate) in the presence of a second organic compound (growth substrate) which is used as the primary energy source or carbon source. In order to degrade a cosubstrate, the

microorganisms need enzymes and reducing equivalents or energy sources which are induced by the primary substrate (growth substrate) (Strand et al., 1990; Bae and Rittmann, 1995). In this study, in order to degrade isothiazolone the cells need an energy source which is generated during the transformation of the lab-lemco broth (primary or growth substrate) and enzymes which are induced by lab-lemco broth and are able to breakdown isothiazolone. When OLR of growth substrate was constant (Run 1 & Run 2), increasing HRT resulted in an increase in growth substrate removal. Consequently, more enzymes and the reducing equivalents or energy sources are generated during the degradation of the growth substrate. As a result, more degradation of isothiazolone was observed (in Run 2). When lab-lemco broth was removed from the influent wastewater (Run 3), the enzymes and reducing power or storage compounds which were still present in the system which can be used to degrade isothiazolone before a new steady state was reached after 5 days. When the hydraulic loading rate (HLR) was constant (Run 2 & Run 4 and Run 6 & Run 7), isothiazolone degradation increased with increasing primary substrate loading rate. This was probably due to the fact that with lab-lemco broth supplementation, the metabolic enzyme system of the cells can be increasingly induced for the degradation of isothiazolone. The dramatic increase in treatment efficiency in terms of both total COD and isothiazolone removals under the same operating conditions (Run 1 & Run 6) implied that a period of cell acclimatization under biocide stress together with suitable operating conditions were very important to increase in bio-oxidation and biodegradation of isothiazolone.

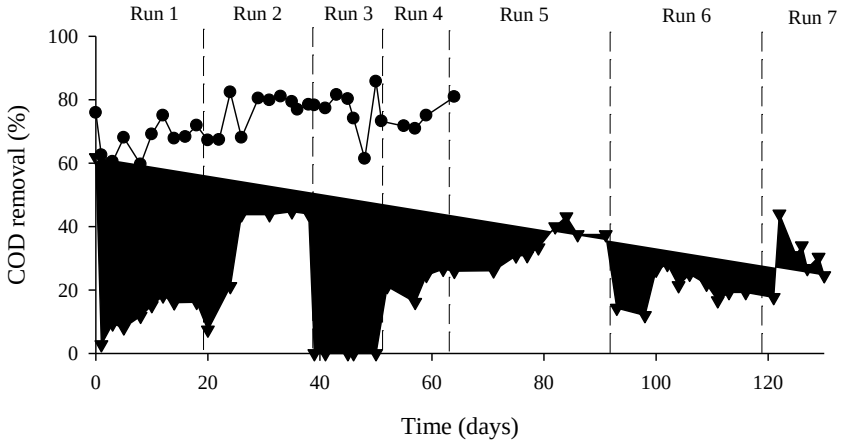

Fig. 1 Effect of isothiazolone on COD removal in the RBCs: control (●) and 6 ppm (▼). (The operating conditions in Run 3 and Run 4 of the control RBC were identical).

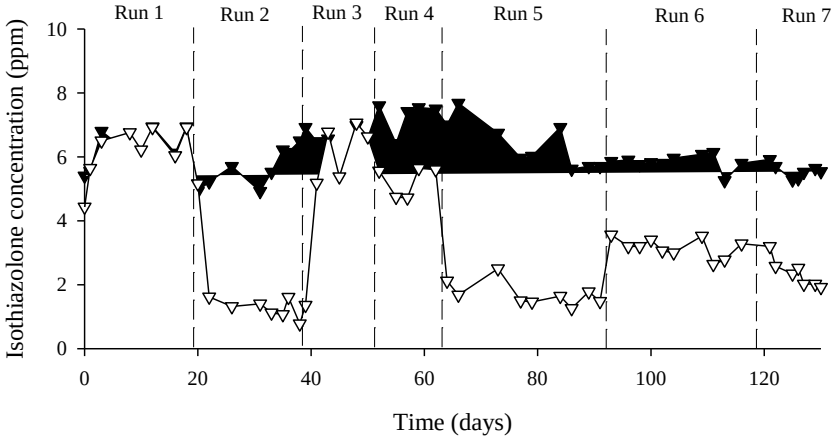

Figure 2 Isothiazolone removal in the RBCs**:** influent (▼), effluent (∇).

Table 1 Characteristics of the RBC receiving 6 ppm isothiazolone under different operating conditions

Run	Flow rate (litres h^{-1})	HRT (h)	Organic loading rate (g COD m^{-2} d^{-1})	COD removal		Isothiazolone removal	
				%	mg l^{-1}	%	ppm
1	2.5	0.62	23.64	16.49 ± 1.55	14.50 ± 6.74	0.90 ± 1.62	0.06 ± 0.11
2	1.25	1.25	17.37	44.05 ± 0.44	85.00 ± 2.00	77.38 ± 6.66	4.47 ± 0.79
3	1.25	1.25	2.74	0	0	0	0
4	1.25	1.25	10.94	22.12 ± 4.57	27.00 ± 7.02	27.99 ± 4.74	2.04 ± 0.39
5	1.25	1.25	17.64	38.29 ± 3.61	75.20 ± 9.12	73.11 ± 5.01	4.66 ± 0.64
6	2.5	0.62	20.78	23.02 ± 3.49	26.86 ± 5.98	45.76 ± 5.01	2.67 ± 0.34
7	2.5	0.62	39.88	29.31 ± 3.68	64.80 ± 6.57	59.33 ± 5.58	3.27 ± 0.36

Decreased susceptibility of acclimatized cells to biocide: From Table 2, the susceptibility changes of cells to the bactericidal activity of isothiazolone can be assessed by determination of MLC. The higher MLC the lower susceptibility. MLC of the non-acclimatized planktonic cells from the control RBC and the acclimatized planktonic cells from the treated RBC in Run 6 are shown in Table 4.6. The results showed that susceptibility of the non-acclimatized and acclimatized cells on isothiazolone in terms of MLC was similar with the MLC being 10-12 ppm. The results also imply that biodegradability of isothiazolone may not totally relate with the susceptibility of cells to the biocide. Isothiazolone removal by the non-acclimatized biofilms in Run 1 and the acclimatized biofilms in Run 6 was significantly different with the value of 0.90 ± 1.62% and 45.76 ± 5.01% respectively (Table 1), even though MLC of isothiazolone to the planktonic cells detaching from those biofilms was similar at 10-12 ppm.

Table 2 MLC of isothiazolone on the non-acclimatized planktonic cells and acclimatized planktonic cells

Inoculum	MLC [a] (ppm)	Tested cell concentration [a] (cfu ml^{-1})
non-acclimatized planktonic cells-1	12	3.90×10^6
non-acclimatized planktonic cells-2	10	1.96×10^6
acclimatized planktonic cells	10	5.40×10^5

[a] The values are expressed as mean ± SD of triplicate experiments.

Tentative identification of acclimatized cells: There were many distinct colonies from the non-acclimatized biofilm of the control RBC. The isolates were tentatively identified as the species *Burkholderia cepacia*, *Sphingomonas paucimobilis*, *Chryseobacterium indologenes*, *Aeromonas hydrophila* and *Aeromonas salmonicida*. Less diversity of biofilm bacteria was observed in the RBCs receiving the wastewaster containing isothiazolone. At the end of the experiments in Run 2, bacterial cells from the biofilms were isolated. All tested isolates were Gram-negative rods and oxidase positive. The resistant bacterium to isothiazolone was tentatively identified as predominantly of the species *Burkhol. cepacia*. *Burkhol. cepacia* is a group of catalase-producing, non-lactose fermenting Gram-negative bacteria (Wikipedia, 2007). The results were similar to Bae and Rittmann (1995) who found that the dominant species in continuous-flow reactor of mixed phenolic compounds were *Burkhol. cepacia* and *Pseudomonas testosteroni*.

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