SUSCEPTIBILITY OF MIXED POPULATIONS IN ROTATING BIOLOGICAL CONTACTORS TO ISOTHIAZOLONE BIOCIDE AND TREATMENT EFFICIENCY OF THE SYSTEM

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Abstract

The effects of isothiazolone (IT) on treatment efficiency of laboratory-scale rotating biological contactors (RBCs) as well as biocide efficacy were studied. Biofilms were established on the RBCs and then exposed to the synthetic wastewater containing 0 (control) and 6 ppm IT. The results showed that the degree of chemical oxygen demand (COD) removal and biocide degradation of the system depended on hydraulic loading rate (HLR) and hydraulic retention time (HRT). Acclimatized biofilms could degrade IT via co-metabolism using lab-lemco broth as a growth substrate. Biofilms on the discs could retain their microbial activities in the presence of 6 ppm IT, while bio-oxidation of the planktonic cells in the RBCs expressed as biochemical oxygen demand (BOD_5) was almost totally inhibited in the presence of 0.6 ppm IT. The planktonic cells sloughed from the acclimatized biofilms on IT showed less susceptibility to IT than that of the planktonic cells sloughed from the non-acclimatized biofilms. However, biodegradability of IT did not totally relate with the susceptibility of cells to the biocide indicating by minimum lethal concentration (MLC) values. The resistant bacterium to IT was tentatively identified as predominantly of the species *Burkholderia cepacia*.

Key words: isothaiazolone compounds, rotating biological contactors (RBCs), biocide efficacy, treatment efficiency

1. Introduction

Isothiazolone compounds (IT, 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 [2]. 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 [5]. The aim of this study was to investigate the susceptibility changes of mixed populations under IT biocide stress after a period of acclimation in a laboratory-scale rotating biological contactors (RBC), a secondary wastewater treatment system with fixed film process. The treatment efficiencies of the system in terms of COD removal and biocide degradation were also studied.

2. Materials and methods

2.1 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. [3].

2.2 The synthetic wastewater : The standard synthetic wastewater consisted of (mg l^{-1}): lab-lemco broth (LLB), 90; NH_4Cl, 54; K_2HPO_4, 28; NaCl, 7; $CaCl_2.2H_2O$, 4; and $MgSO_4.7H_2O$, 2.

2.3 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^{-1}. 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 (control) and 6 ppm.

2.4 Effects of operating conditions on the treatment efficiency and biocide degradation in the RBCs : Three experimental runs; Runs 1 to 3, were performed. Each run lasted for about one week of stable operation as indicated by constant effluent COD and IT concentration. The details of each run are summarized in Table 1. During various operating conditions, influent and effluent of the RBC units were collected for analysis.

Table 1 The experimental runs with the synthetic wastewater containing 6 ppm IT.

Run	Lab-lemco broth (mg l^{-1})	Flow rate of the wastewater (litres h^{-1})
1	90	2.5
2	180	1.25
3	-	1.25

2.5 Survival of planktonic bacteria : The survival of planktonic bacteria in the RBC units receiving 6 ppm IT for 3 weeks and non acclimated planktonic bacteria was further observed by sampling 50 ml of the planktonic phase into sterile 250-ml Erlenmeyer flasks. IT was added into the flasks to give final concentrations of 3, 6 and 12 ppm. The flasks were then incubated at ambient temperature. Aliquots (1 ml) of the sample were taken every 2 hours and serially diluted in quarter strength Ringer solution and plate onto strength tryptone soya agar (TSA, Oxoid). Numbers of colony forming units (cfu's) were determined after incubation at 37 °C for 48 hours.

2.6 Minimum lethal concentration (MLC) of isothiazolone : One ml of the planktonic phase from the control RBC or the treated RBC unit was inoculated into a series of sterile vials containing 9 ml of the synthetic wastewater and the desired concentration of isothiazolone. The vials were incubated at 37°C for 48 h. The MLC was determined by adding 1 ml of the 48-h samples above into sterile vials containing 5 ml of double strength tryptone soya broth (TSB, Oxoid) and 4 ml of sterile distilled waster. The vials were incubated at 37°C for 48 h. The lowest concentration of biocide showing absence of growth by turbidity was taken to be MLC.

2.7 Tentative identification of bacterial cells : 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).

2.8 Analytical methods : COD and BOD were determined as described by Laopaiboon et al. [3]. IT concentration was analysed by HPLC [4]. Total viable count (TVC) of planktonic bacteria was determined as described by Laopaiboon et al. [3].

3. Results and discussion

3.1 Treatment efficiency by the RBCs : COD and IT removal of the control RBC (without biocide) and the treated RBCs under different operating conditions are shown in Fig. 1 and Table 1. The results showed that the degree of COD removal and biocide degradation of the system depended on HLR and HRT. When the normal synthetic wastewater containing 6 ppm IT 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. IT degradation increased with increasing HRT. Acclimatized biofilms could degrade IT via co-metabolism using lab-lemco broth as the growth substrate.

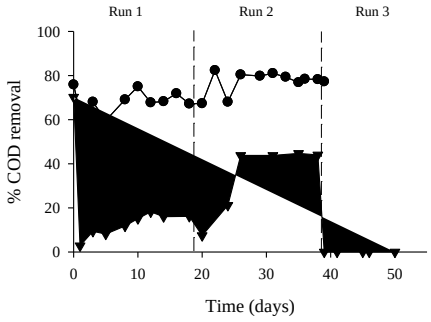

Fig. 1 Effect of IT on COD removalin the RBCs: control (●) and 6 ppm (▼).

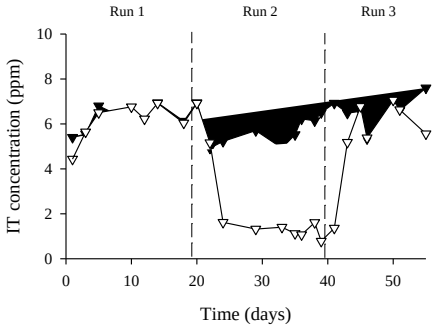

Fig. 2 IT removal in the RBCs**:** influent (▼), effluent (∇).

Table 1 Treatment efficiencies of the control RBC and the RBC receiving 6 ppm IT (treated RBC) under various operating conditions

RBC	HLR ($m^3\ m^{-2}\ d^{-1}$)	Lab-lemco broth (carbon source)	HRT (min)	COD removal (%)	IT removal (%)
Control RBC, Run 1	0.18	+	36	69.64 ± 5.92	-
Control RBC, Run 2	0.09	+	72	78.27 ± 3.72	-
Treat RBC, Run 1	0.18	+	36	16.49 ± 1.55	0.90 ± 1.62
Treat RBC, Run 2	0.09	+	72	44.05 ± 0.44	77.38 ± 6.66
Treat RBC, Run 3	0.09	-	72	0	0

3.2 Five-day biochemical oxygen demand (BOD_5) : The results showed that in the influent sample was diluted in 10-fold prior to the BOD test, the concentration of IT in the BOD bottle would have been only 0.6 ppm in the influent samples containing 6 ppm IT. Nevertheless this was sufficient to decrease metabolic activity of the planktonic bacteria present in the BOD_5 test at approximately 97%. The results obtained indicate that the BOD_5 test is not an appropriate analytical method for investigating the efficiency of wastewater treatment units when the influent contains biocides or other toxic/inhibitory materials due to the inhibition of bio-oxidation.

3.3 Survival of planktonic bacteria : Susceptibility changes of planktonic microorganisms sloughed from non-acclimatized biofilms and acclimatized biofilms receiving 6 ppm IT. When the planktonic cells of the control RBC were exposed to various IT. The results showed that IT at 3, 6 and 12 ppm caused reductions of the numbers of colony-forming units of the planktonic cells at the end of the experiments. While survival of the planktonic cells of the treated RBC (containing 6 ppm IT) was slightly decreased in the first 30 min but afterwards the cell number were approximately constant throughout remainder of the experiment. The results obtained from this stuty clearly showed that in acclimatized biofilms, planktonic cells sloughed from the biofilms showed significantly less susceptibility to IT (with higher cell survival) compared to that of non-acclimatized cells.

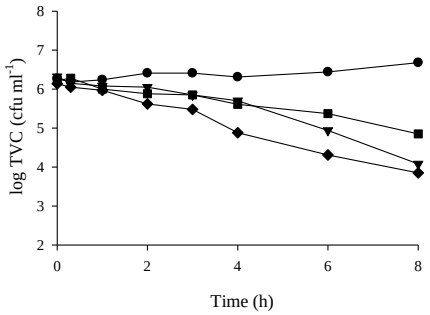

Fig. 3 Survival of planktonic bacteria of the control RBC unit in the presence of various IT concentrations at the initial cell concentration of 10^6 cell ml^{-1} : control (●), 3 ppm (■), 6 ppm (▼) and 12 ppm (♦).

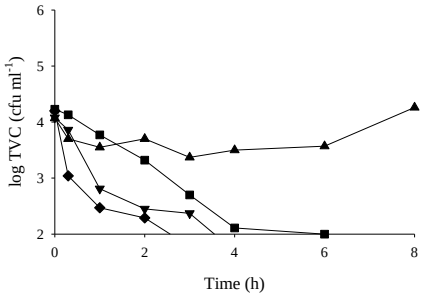

Fig. 4 Survival of control planktonic cells and acclimatized cells in the presence of various IT concentrations at the initial cell concentration of 10^4 cell ml^{-1} : control planktonic cells receiving 3 ppm (■), 6 ppm (▼) and 12 ppm (♦) and acclimatized cells at 6 ppm isothiazolone (▲).

3.4 Minimum lethal concentration (MLC) of isothiazolone : The susceptibility changes of cells to the bactericidal activity of isothiazolone can be assessed by determination of MLC. The results showed that susceptibility of the non-acclimatized and acclimatized cells on IT in terms of MLC was similar with the MLC being 10-12 ppm. The results also imply that biodegradability of IT 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 was significantly different with the value of 0.90 ± 1.62% and 77.38 ± 6.66% respectively, even though MLC of isothiazolone to the planktonic cells detaching from those biofilms was similar at 10-12 ppm.

3.5 Tentative identification of bacterial cells : 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. The results were similar to Bae and Rittmann [1] who found that the dominant species in continuous-flow reactor of mixed phenolic compounds were *Burkhol. cepacia* and *Pseudomonas testosteroni*.

4. Conclusions

The RBC units were successfully used to simulate and predict treatment efficiency in terms of COD removal and IT degradation of a full-scale effluent treatment plant in the presence of IT biocide. Degradation of IT under the aerobic condition occurred via co-metabolism using lab-lemco broth as a growth substrate. A period of cell acclimatization under biocide stress, the presence of growth substrates and operating conditions (HLR and HRT) were important parameters to increase the treatment efficiency of the system. Due to the severe inhibition of bio-oxidation by the biocide, BOD test was not an appropriate analytical procedure for investigating the efficiency of wastewater treatment units contaminated with the biocide even though adapted seed was used and/or the incubation time was prolonged.

5. Acknowledgements

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