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Effects of temperature and pH on the biokinetic properties of thiocyanate biodegradation under autotrophic conditions

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ABSTRACT

The simultaneous effects of temperature and pH on the biokinetic properties of thiocyanate biodegradation under mixed-culture, autotrophic conditions were investigated using response surface analysis (RSA) combined with biokinetic modeling. A partial cubic model, based on substrate inhibition biokinetics, was constructed for each kinetic coefficient in Andrew model (i.e., maximum specific growth rate (μ_m), saturation coefficient (K_S), and substrate inhibition coefficient (K_{SI})). Each model proved statistically reliable to approximate the responses of the kinetic coefficients to temperature and pH changes ($r^2 > 0.8$, $p < 0.05$). The response surface plots demonstrated that the biokinetic coefficients change with respect to temperature and pH significantly and in different ways. The model response surfaces were substantially different to each other, indicating distinct correlations between the independent (temperature and pH) and dependent (model response) variables in the models. Based on the estimated response surface models, temperature was shown to have significant effects on all biokinetic coefficients tested. A dominant influence of temperature on μ_m response was observed while the interdependence of temperature and pH was apparent in the K_S and K_{SI} models. Specific growth rate (μ) versus substrate (i.e., thiocyanate) concentration plots simulating using the obtained response surface models confirmed the significant effects of temperature and pH on the microbial growth rate and therefore on the thiocyanate degradation rate. Overall, the response surface models able to describe the biokinetic effects of temperature and pH on thiocyanate biodegradation within the explored region (20–30 °C and pH 6.0–9.0) were successfully constructed and validated, providing fundamental information for better process control in thiocyanate treatment.

1. Introduction

Thiocyanate (SCN^-), a hazardous compound, is often found in industrial waste streams as it is used or generated in various industries including photofinishing, dyeing, coking, metal separation, and electroplating (Ahn et al., 2004). Thiocyanate is toxic at low concentrations (1–2 mM) to higher animals including humans. It binds to proteins and inhibits enzymatic reactions (Wood et al., 1998), leading to damage to human central nervous system and thus severe clinical problems

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such as nervousness, hallucination, psychosis, and convulsions (Lewis, 1992). Effective removal of thiocyanate is therefore of particular concern when treating wastewaters from the industries mentioned above.

Biological treatment of thiocyanate has extensively been studied and several thiocyanate-degrading bacteria, e.g., *Arthrobacter*, *Bacillus*, *Escherichia*, *Klebsiella*, *Methylobacterium*, *Pseudomonas*, and *Thiobacillus*, were identified from various sources (Ebbs, 2004; Lee et al., 2003). These bacteria degrade and utilize thiocyanate as an energy and/or nutrient source through two distinct metabolic pathways: carbonyl and cyanate pathways (Bezsudnova et al., 2007; Ebbs, 2004). In the former, thiocyanate is first hydrolyzed to ammonia and carbonyl sulfide (COS) by thiocyanate hydrolase, and then COS breaks into H_2S and CO_2. In the latter, thiocyanate is initially degraded to cyanate (CNO^-) and H_2S, and then CNO^- is hydrolyzed to ammonia and CO_2 by cyanase. The sulfide and ammonia released can be utilized as electron donor, nitrogen source, or sulfur source for microbial growth.

Like other biological processes, activities of the microorganisms involved in thiocyanate biodegradation are to be affected by environmental factors. Understanding such influence is helpful in devising methods for enhancing microbial activities and thus process performance. Among many environmental factors, temperature and pH are universally known as the major factors affecting microbial growth (Madigan et al., 2009; Rosso et al., 1995). The temperature and pH effects on microbial activities are well documented in literature (Laidler, 1984; Madigan et al., 2009; Nedwell, 1999; Tan et al., 1998). A few studies have investigated the effects of temperature and pH in biological thiocyanate treatment processes (Kim and Katayama, 2000; Lay-Son and Drakides, 2008; Vazquez et al., 2006). However, previous studies have examined only the responses of processes to such environmental factors but, to our knowledge, not their effects on the biokinetic properties of thiocyanate degrading communities. Biokinetics mathematizes the relationship between microbial growth and substrate consumption using a combination of coefficients, helping describe and predict process performance. From an engineering point of view, biokinetic modeling is a useful tool for understanding the basic mechanism resulting in microbial growth with pollutant removal under a certain condition. It is therefore plain that an understanding of how environmental factors affect the biokinetics of a biological process will provide fundamental information that helps better describe how and why the system changes at the process level. Accumulation of such information will form a basis for better design and operation of biological processes.

In this study, we aimed to investigate the effects of temperature and pH on the biokinetic properties (i.e., biokinetic coefficients) of thiocyanate biodegradation under a mixed-culture, autotrophic condition. Taking into account the two factors that vary simultaneously, response surface analysis (RSA), an effective statistical technique for evaluating simultaneous effects of multiple variables (Montgomery, 1997), was employed. RSA was used to design the experimental runs to test and build the response models for biokinetic coefficients. This study provides a biokinetic insight into the biodegradation process of thiocyanate.

2. Materials and methods

2.1. Microbial source and culture medium

Activated sludge from a local sewage treatment plant (Pohang, Korea) was cultivated in a continuously stirred tank reactor (CSTR) with a working volume of 7 L to produce consistent inoculum for subsequent experiments. Thiocyanate-degrading microorganisms were enriched using a synthetic wastewater containing the following components in 1 L (Hung and Pavlostathis, 1997): 500 mg SCN^-, 838 mg KSCN, 500 mg $NaHCO_3$, 385 mg K_2HPO_4, 129 mg KH_2PO_4, 50 mg $MgSO_4 \cdot 7H_2O$, 7 mg KCl, 5 mg $CaCl_2 \cdot 2H_2O$, 5 mg $FeSO_4 \cdot 7H_2O$, and 5 mg $MnSO_4 \cdot H_2O$. The inoculum system was operated at 7.2-day hydraulic retention time (HRT), 30 °C, and pH 8.5. After over 10 turnovers of steady-state operation (>99% SCN^- removal efficiency), the effluent from this system was used as seed inoculum for the microcultivation tests for biokinetic studies.

2.2. Response surface analysis design

Response surface analysis (RSA) was employed to evaluate the simultaneous effects of temperature and pH on the biodegradation kinetics of thiocyanate with minimum number of experimental trials (Montgomery, 1997). A face-centered design (FCD) with extra trials added to augment the response surface model (Table 1) was applied to evaluate the biokinetic responses to changes in temperature and pH (i.e., independent variables). Based on literature values, the exploring ranges (center point ± variance) of temperature and pH were determined to be 30 ± 10 °C and 7.5 ± 1.5, respectively (Ahn et al., 2004; Hung and Pavlostathis, 1997; Kim and Katayama, 2000; Lee et al., 2003). RSA was performed by a sequential procedure of collecting experimental data from each trial, estimating polynomials, and checking model adequacy (Lee et al., 2011),

Table 1 – Experimental design and observed results of the response surface analysis.

Trials	Conditions		Observations		
	T (°C)	pH	μ_m (d^{-1})	K_S (mg SCN^-/L)	K_{SI} (mg SCN^-/L)
Original face-centered design					
1	20	6.0	0.380	140.9	197.0
2	20	9.0	0.326	106.5	216.1
3	40	6.0	0.422	143.4	261.4
4	40	9.0	0.290	74.9	461.2
5	30	6.0	0.428	156.2	212.1
6	30	9.0	0.495	147.4	241.1
7	20	7.5	0.335	122.8	244.7
8	40	7.5	0.412	146.8	272.2
9[a]	30	7.5	0.497 (0.036)	148.1 (18.9)	205.0 (26.3)
Augmented experimental points					
10	35	6.0	0.471	171.1	206.4
11	20	7.0	0.356	134.1	230.1
12	35	7.0	0.446	149.6	250.1
13	40	8.5	0.348	112.1	356.4

a Center point. Experiment was triplicated and the responses are presented as average values (standard deviation).