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Biogenic substrate benefits activated sludge in acclimation to a xenobiotic

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ABSTRACT

Activated sludge that originated from a biogenic fed-batch reactor under steady-state was re-cultivated with the same biogenic substrates to test the changes in the sludge's performance in acclimation and degradation of a xenobiotic. Re-cultivations with varying biogenic concentrations were conducted at time points ranging from 16 d before to 4 d after the acclimation reactions. Biogenic re-cultivation energizes sludge cells thereby benefiting the re-cultivated biomass by shortening its acclimation lag time. Lag time increases on both sides of the re-cultivation time where lag has been shortened the most: (1) in short re-cultivation times before and after acclimation reactions, high concentrations of new or unfinished biogenic substrates cause diauxic growth that delays acclimation; (2) in long re-cultivation times, the re-cultivated biomass loses its energy-rich advantage. Both these lag lengthening situations have their worst cases in which acclimation lag times become longer than that of the original sludge, thus counterbalancing the benefits.

1. Introduction

Xenobiotic organic chemicals, including phenoxy acid herbicides, are so defined because they are foreign to natural (indigenous) microorganisms. Although xenobiotics containing wastewaters can be suitably treated using biological methods (Chin et al., 2005; Ettala et al., 1992; Hill et al., 1986; Meric et al., 2003), the xenobiotic nature of the pollutant requires the treatment plant microorganisms (typically activated sludge) to go through an acclimation phase before the microorganisms evolve the degradation capability for treating the influent xenobiotics. The doubt and concern about successful biological treatment of xenobiotics is twofold: a prolonged acclimation phase (length of lag time) before the start of degradation, and slow degradation kinetics after the lag. Between these two concerns, the more important one is often the acclimation lag time, rather than the treatment kinetics, which may be met with the adjustment of operating conditions within a reasonable range, especially for those mildly persistent xenobiotics.

Activated sludge biomass grown on the feed of biogenic substrate must be in a healthy physiological condition. These healthy conditions should be favorable to the microorganisms when they must go through the energy-expensive xenobiotic acclimation process. However, inconsistent results are found in literature about the effects of biogenic organics on degradation of man-made xenobiotics or hydrocarbons. There have been some study cases of both beneficial and adverse effects of biogenic organics on xenobiotic degradation. The beneficial cases include: citrate on toluene (Harrison and Barker, 1987); natural amino acids on mono-substituted phenol (Shimp and Pfeander, 1985); natural organics such as manure on two chloro- and a nitro-herbicides (Moorman et al., 2001); fatty acids on soil hydrocarbon (Nelson et al., 1996); pyruvate on naphthalene (Lee, 2003). Conversely, adverse cases are also found: glucose or amino acids on xylene and toluene (Swindoll et al., 1988); ethanol on benzene, toluene and xylene (Corseuil et al., 1998); glycolic acid and glucose on *p*-cresol (Lewis et al., 1986); yeast extract and milk on 3-nitrobenzoate, 4-chlorobenzoate, 4-chlorophenol (Hu et al., 2005).

The microbiological aspects of xenobiotic degradation are examined here in order to diagnose the causes of the inconsistent results listed above about the effects of biogenic on xenobiotic degradation: (1) the involvement of symbiotic functions or co-metabolisms needed for the microbial communities to succeed in degrading the target xenobiotics. For the degradation of those xenobiotics in the examples listed above, the requirement of ecological cooperation of the microbial communities should not be overly demanding; the microbial diversity that activated sludge contains can well satisfy the ecological needs for degradation of a xenobiotic solely (Sanapareddy et al., 2009; Wagner et al., 2002). This ecological factor can slightly affect xenobiotic acclimation in either the advantageous or the disadvantageous ways, if there is any effect at all. Therefore, the ecological factor can have a neutral or unimportant effect on the efficiency of activated sludge in its

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acclimation to a xenobiotic. (2) The effect that activated sludge use a biogenic substrate preferentially to xenobiotic substrate (diauxic growth) (Basu et al., 2006; Chong and Chiou, 2010; Harder and Dijkhuizen, 1982). This factor can slow down xenobiotics acclimation and thus affect xenobiotic degradation disadvantageously. (3) Biogenic substrate enhancement of the elements needed for mediating xenobiotic degradation. Such elements include enzyme activities (Margesin et al., 2000; Taylor et al., 2002) and energy (ATP) contents in the microbial cells (Wilson et al., 1986). The production of catabolic enzymes means that the microorganisms are successful in evolution of their degradation capability. To drive these chemical reactions, including the production of enzymes and the subsequent catabolism of the xenobiotics, the microbial cells must possess a rich energy reserve. Consequently, enhancing the cells' energy-richness can be advantageous for the cells to break down the stable structure of the target xenobiotic. In summary, diauxic growth and energy richness are the major factors requiring systematic examination to resolve the inconsistencies noted above.

The purpose of this study, therefore, was to investigate the effects of nourishing activated sludge with biogenic substrates on the sludge's performance or efficacy in acclimation and degradation of a xenobiotic. In addition to seeking a unified explanation for the inconsistent results mentioned above, this study also intended to seek indications about the xenobiotic treatment potency of activated sludge when a xenobiotic appears periodically in the influent of the activated sludge treatment plant. To fulfill the purpose of this study, experiments were conducted with which a biomass of steady-state growing activated sludge was separately re-cultivated with a feed of biogenic nature (containing sucrose and peptone). The times at which re-cultivation of the sludge started were before, after and concurrently with the sludge's acclimation and degradation of a model xenobiotic 2,4-dichlorophenoxyacetic acid (2,4-D). The test variables included (1) the points of time when re-cultivations started; and (2) the biogenic substrate concentrations in the re-cultivation feed. The lengths of lag time during acclimation and the kinetics of the succeeding degradation were quantified using a mathematical acclimation model developed by Chong (2009). The advantages or disadvantages of biogenic substrates for the sludge biomass' xenobiotic acclimation and degradation were examined using the model parameters that describe the efficiency of an acclimation process. The major factors studied were the sludge cells' energy contents and biogenic interference on the sludge biomass' performance in acclimation and degradation of the xenobiotic.

2. Methods

2.1. Target xenobiotic and activated sludge

The target xenobiotic was the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D).

The initial activated sludge seeds were obtained from a soil that did not have any record of 2,4-D nor metal (slag) contamination. The mixed culture from soil was grown to a suitable amount on Nutrient Broth (NB Difco 234000) for a number of subcultures in shake-flasks before the culture was seeded to a long-term cultivation reactor that was operated in a fed-batch mode. The fed-batch reactor was fed once everyday with biogenic substrates (100 mg/l sucrose and 25 mg/l peptone). The feed also contained minerals: $FeCl_3$ 1.0 mg/l, NH_4Cl 30.0 mg/l, K_2HPO_4 200.0 mg/l, KH_2PO_4 156.0 mg/l, $MgSO_4$ 31.0 mg/l. Reactor suspension, one-tenth (1/10) of the liquid volume in the reactor tank, was wasted everyday so that a mean cell resident time (θc) of 10 d was achieved. The fed-batch reactor was operated uninterrupted for a prolonged period to reach a pseudo-steady state. The sludge obtained from this fed-batch reactor was referred to as the original sludge, which resembled the common activated sludge biomass produced from a continuous activated sludge treatment plant.

2.2. Re-cultivations of activated sludge

This study employed a major experimental design referred to as the re-cultivation of the activated sludge biomass. The details of re-cultivation are as follow: (1) the biomass re-cultivated was harvested the fed-batch reactor; (2) the re-cultivating feed was biogenic in nature, consisting of sucrose and peptone at the proportion of 4:1 on the weight to weight basis (w/w). This ratio was kept constant when the biogenic feed concentration was changed; (3) the re -cultivation times (the times at which the feed and biomass started reaction) were divided into three time segments relative to the start-time of a 2,4-D acclimation and degradation reaction: before (pre-cultivation), concurrent, and after (post-cultivation). The biogenic re-cultivation scheme, with variations of re-cultivation time and biogenic substrate feed concentration, is listed in Table 1 (with designations of the tests).

The re-cultivation media contained minerals listed above, different concentrations of biogenic substrates (Table 1), and activated sludge thickened from the fed-batch reactor suspension with minimum carry-over of supernatant (sludge suspension settled for longer than 30 min and approximately 70% of supernatant discarded). The re-cultivation reactors were operated in shake-flasks (300 ml conical flasks containing 150 ml liquid). The activated sludge concentration (measured as suspended solids, SS) initially added to the re-cultivation reactors was approximately 100 mg-SS/l. For the pre-cultivation reactors, biogenic feeds were administered once and the reactors were then let idle (shaking was maintained) until it was time the sludge biomass was re-harvested and used in new reactors for the 2,4-D acclimation and degradation tests. For the concurrent (0 h) and post-cultivation tests, biogenic substrates were added, at the intended times, directly to the 2,4-D acclimation reactors. Concentrated biogenic substrates were spiked-fed to the post-cultivation reactors to avoid excessive change in liquid volume.

2.3. Acclimation and degradation experiments

The activated sludge biomass used for acclimation and degradation of 2,4-D was harvested from the composite of multiple pre-cultivation reactors. Sludge concentrations (SS) were measured to calculate the amounts of sludge to be transferred to the

Table 1
Biogenic substrate concentration and culture time applied to the re-cultivation of activated sludge biomass used for 2,4-D acclimation and degradation.[a]

Culture time[c]	Biogenic conc.[b] (mg/l)			
	20	50	100	200
	Designations[c]			
Post-cultivation	−1 d		−1 d100 S	
	−2 d		−2 d100 S	
	−4vd		−4 d100 S	
Concurrent Pre-cultivation	0 h	0 h20 S	0 h50 S	0 h100 S	0 h200 S
	1 h	1 h20 S	1 h50 S	1 h100 S	1 h200 S
	8 h			8 h100 S	
	2 d	2 d20 S	2 d50 S	2 d100 S	2 d200 S
	3 d	3 d20 S	3 d50 S	3 d100 S	3 d200 S

[a] Initial sludge concentration was 50 mg/l (SS) in all 2,4-D acclimation and degradation tests.

[b] Biogenic substrates consisted of sugar (sucrose) and peptone at the ratio of 4:1 w/w. The concentrations listed denoted sugar concentration only.

[c] Designation rules: n_1 *T* n_2S. n_1 stands for re-cultivation time relative to the start of acclimation test; *T* stands for time (in hour or day); n_2S stands for concentration of biogenic feed (sugar). n_1-*T* and n_2S are used independently when re-cultivation time (row of the table), and re-cultivation feed concentration (column), respectively, are described in the text and figures.