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Initial Energy Content of Cells Improve Efficiency of Activated Sludge Acclimation and Degradation of a Xenobiotic Compound

Nyuk-Min Chong[1], Lan Huong Nguyen[1,2]
[1]Department of Environmental Engineering, Da-Yeh University
168 University Road, Dacun, Changhua 51591, Taiwan, ROC
chong@mail.dyu.edu.tw
[2]Faculty of the Environment and Labour Safety
19 Nguyen Huu Tho Street, Tan Phong Ward, District 7, Ho Chi Minh City, Vietnam.
huongnl@tdt.edu.vn

Abstract - Shortening of lag phase and enhancement of degradation rate are important goals concerning the treatment of xenobiotic compounds by activated sludge. In this study, activated sludge from a steady-state fed-batch reactor was used as-is (raw) and enriched with biogenic substrates in tests to determine sludge's performance in acclimation and degradation of a model xenobiotic compound. Xenobiotic concentrations, growth of sludge, and sludge cells' ATP along the courses of xenobiotic acclimation and degradation were measured. Test results show that the higher amount of energy initially contained in the enriched sludge was a factor that is instrumental to bring a shortening of acclimation lag time compared with that of raw sludge. Energy invested into activated sludge cells externally with easy substrates was found to improve activated sludge acclimation to a xenobiotic by sustaining the energy needs for the difficult metabolism of the xenobiotic.

Keywords**:** Xenobiotic, Biodegradation, Activated sludge, Acclimation, ATP

1. Introduction

Xenobiotic organic are compounds mostly comprised of chlorinated ring structures that are difficult for indigenous microorganisms to find an initial attack point of catabolism [1]. Through the acclimation process in which novel enzymes may have evolved, activated sludge can become xenobiotic degradative [2]. The common weakness for activated sludge acclimation is the prolonged lag time, and even after the degradability acquirement from acclimation, xenobiotic organic compounds are hard to break-down due to their stable structure. In addition to the energy consumptive stable structure breaking processes, some oxidative xenobiotic metabolism steps also consume reducing power [3] that further reduces energy generation. As a result, energy reserve contained in sludge cells is often found deficit after xenobiotic degradation, which is disadvantageous for activated sludge in its treatment of xenobiotic pollutants. In hope to compensate such energy deficit, external biogenic substrates were fed to sludge so that metabolism of biogenic substrate would enrich the cells' energy reserve before the sludge starts to tackle the xenobiotic target. Previous studies using glucose and peptone [4], and propionate [5], were found favorable for pentachlorophenol and propanil degradation, respectively. Nutrients nitrogen, phosphate and minerals were also found beneficial for xenobiotic degradation [6]. However, reasons for such enhancements were seldom related to the energy aspect of the degrader cells.

It is unknown if energy added externally to the activated sludge cells from consuming biogenic substrates is beneficial in shortening lag and/or enhancing degradation rate. The purpose of this study, therefore, was to investigate the possibility of a higher initial energy content in activated sludge to remedy energy deficit, to increase cell yield, and thereby shorten acclimation lag phase and enhance degradation rate for a model xenobiotic 2,4-dichlorophenoxyacetic acid (2,4-D). To fulfill this purpose, raw activated sludge from a steady-state growing reactor, and such sludge re-cultivated with a feed of biogenic substrates to enrich its energy content, were tested in parallel to determine the sludge's acclimation and degradation performance for 2,4-D. Time course of acclimation and degradation of 2,4-D, sludge growth, and ATP contents of raw and enriched activated sludge were measured and compared to find answers to the above unknowns.

2. Materials and Methods

The target xenobiotic was the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Indigenous activated sludge seed was grown in a fed-batch reactor that was re-fed once daily with a fresh medium containing biogenic substrates (120 mg/L sucrose and 50 mg/L peptone) and 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, $MgSO_4.7H_2O$ 65.0 mg/L. The fed-batch sludge (as the 'raw sludge') was grown at a mean cell residence time (θ_c) of 10 days, which must have maintained the microorganisms in the late stationary phase of grow.

Some raw sludge was individually re-cultivated with biogenic substrates of sucrose (100 mg/L) and of peptone (25 mg/L) in shake-lfask ; this added biogenic feed was to maintain higher cell ATP content than that of raw sludge, at a time point 1 day prior to acclimation tests. The fast growing sludge was referred to as the enriched sludge.

Both the raw and enriched sludge were tested for acclimation and degradation to 2,4-D in reactions conducted in batch shake-lfasks shaken at 100 rpm orbital, under room temperature (25 ± 2°C). The acclimation reactors contained 100 mg-/L (SS) of the respective sludge, 100 mg/L of 2,4-D and minerals listed above. Measurements were made daily for 2,4-D concentrations remaining in solution, sludge concentrations that indicated sludge growth, and ATP contained in the cells. Acclimation and degradation experiments were conducted in triplicate.

Soluble 2,4-D concentrations were measured with HPLC. Activated sludge concentrations were measured as the dried weight (SS) following the standard method [7] of SM2540-D (ifl er and dried at 103-105°C). COD of selected samples were measured with the standard method of SM5220-C. Detailed method of ATP measurements can be found in Nguyen and Chong [8].

3. Results and Discussion

Figs. 1a and b show the courses of 2,4-D degradation, the growth of sludge (SS) and ATP contents of the raw activated sludge cells that were harvested from a slowing growing reactor; Figs 1c and d show those curves for sludge that had been re-cultivated and energy enriched. Fig. 1a indicates that the lag of raw sludge acclimation to 2,4-D was approximately 4 days and was followed with a fast degradation rate that is typical of activated sludge degradation of 2,4-D. SS growth produced a yield of 0.25 mg SS/g COD from 2,4-D, which is a low yield compared to that from sludge growth on common biogenic substrates. A low yield of activated sludge in metabolizing a xenobiotic is due to diversion of available energy of metabolism to satisfy the energy requirement for breaking (catabolism) the stable xenobiotic structure.

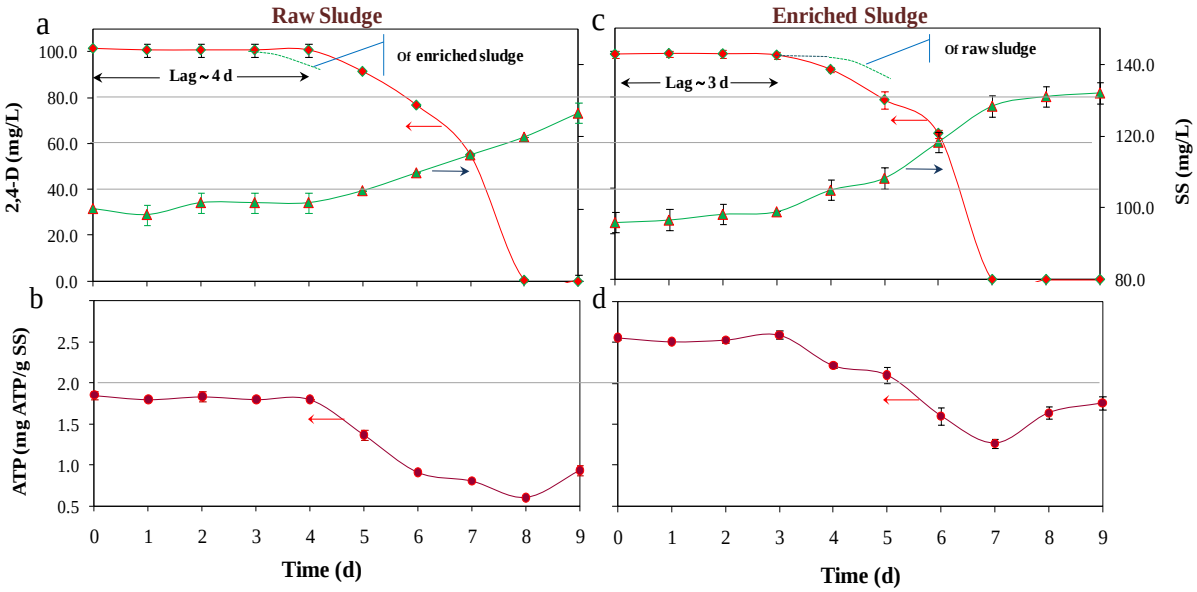

Fig. 1: Courses of 2,4-D, SS and ATP during acclimation and degradation of 2,4-D. (a,b) raw sludge, (c,d) enriched sludge. Error bars indicate 1 standard deviation of triplicate readings.

To dechlorinate a xenobiotic such as 2,4-D, reducing power (NADH) is spent and thus ATP production is further reduced. Fig. 1b shows that for raw activated sludge in acclimation and degradation of 2,4-D, ATP in unit of cell mass was

essentially constant in the acclimation phase. Evolution of degradative enzyme is shown to be mildly energy expensive. During the degradation phase, however, ATP was lowered noticeably until 2,4-D was dissipated. Unlike the healthy growth on biogenic substrates, growth on xenobiotic could results only in low cell yield, yet each cell suffers a decrease in its ATP content. Microbial populations must reproduce new cells in metabolism, but the cost of xenobiotic catabolism energy is deducted from the ATP reserve of the cells. This metabolism strategy indicates how microorganism could set the priority for survival: new cell production is more important over energy richness in the cells. After surviving this growth condition, energy budget management must be readjusted to suit the subsequent growth condition, as is evidenced from the reversal of ATP per cell in the last legs of ATP curves in Fig 1.

Fig. 1c shows that the enriched sludge, which had its initial energy content increased, would acclimate to 2,4-D with a lag time shortened by approximately 1 day compared to that of raw sludge. Although the acclimation phase was apparently not ATP intensive, how a higher ATP shortens acclimation lag can be viewed as an earlier start of degradation (degradation curvature comparison, Figs. 1a and c). The energy enriched sludge was more readily to meet the energy demand for xenobiotic degradation which must start as soon as acclimation is completed.

The yield of enriched sludge growing on 2,4-D was found slightly higher than that of raw sludge (0.34 and 0.25 g cell/g COD, respectively). A higher cell yield for the energy enriched cells can be most reasonably explained by the provision of more energy for cell yield. Shown in Fig 2, slightly more ATP was consumed of the cells of the enriched sludge. It is logical to have higher energy expenditure to produce a higher cell mass yield. This finding is also indicative of the efficiency of microbial cells in allocating available energy in balancing yield and ATP reserve. The maximum ATP deficit for the raw sludge may have been bounced by the lowest limit of ATP content in the cell. The enriched sludge, on the other hand, still had not spent ATP to this limit so that some more energy can be channeled to cell mass yield.

4. Conclusion

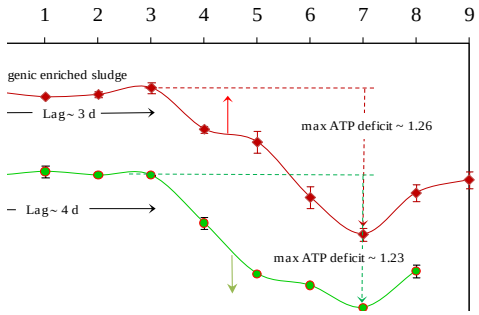

Fig. 2: Differences between ATP contents in raw and enriched activated sludge during sludge acclimation and degradation of 2,4-D.

Activated sludge degradation of a xenobiotic, exemplified with 2,4-D, consumed a large amount of energy that caused an energy deficit in activated sludge cells after degrading the xenobiotic. By adding biogenic substrates at a time point prior to xenobiotic degradation, extra ATP was invested into the cells and this energy was useful in increasing or compensating part of the ATP that may be spent in metabolism of the xenobiotic. The overall benefit of this energy enrichment was the shortening of lag and thus the shortening of overall time for complete xenobiotic degradation. The addition of biogenic substrates for energy enrichment is a feasible way to improve activated sludge in the treatment of xenobiotic pollutants.

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