EFFECTS OF BIOGENIC SUPPLEMENTATION ON XENOBIOTIC DEGRADATION

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SUMMARY

Activated sludge acclimated to 2,4-D was used to degrade 2,4-D in batch reactions. Biogenic substrates of sucrose and peptone, in varying concentrations, separately and combined, were supplemented to the degradation reactions to find if, how and why biogenic supplementations were beneficial to the degradation of the xenobiotic. With the biogenic supplement as a variable and other reaction conditions, optimal constant supplementation schemes were found which suit as feasible ways for enhancing the degradation rate of 2,4-D. Among the number of supplementation combinations, the ones having optimal advantage were 50 mg/L of sucrose, 80 mg/L of peptone, each separately, and 20 mg/L sucrose and 40 mg/L peptone combined.

Keywords: activated sludge, acclimation, biodegradation, 2,4-D, degradation kinetics.

1 INTRODUCTION

Difficulties are frequently observed for activated sludge to efficiently treat a xenobiotic pollutant. The most common cause of this drawback is that the molecular structures of xenobiotics are different from natural microbial substrates or their metabolic intermediates. The non-degradability is most noticeable with activated sludge containing indigenous microbial populations that encountered a xenobiotic compound for the first time. Given the right conditions both physical and biochemical, the microbial metabolic ability is sufficient for the total conversion of most xenobiotic organics to mineral states [1]. Acclimating activated sludge to the target xenobiotic is a feasible method that would establish indigenous sludge with a degradation capability of sufficient strength. The rate of degradation of the acclimated sludge, however, may still be subjected to limitations, leading to some thoughts that enriching biomass growth may become a beneficial way to improving the activated sludge's xenobiotic degradation rate. Biogenic substrates were found beneficial in this application [2, 3, 4, 5]. The exact mechanisms of this benefit were somewhat postulated; whether the advantages are due to the enlargement of biomass, enrichment of energy (ATP), or the increment of metabolic cofactors such as NADH, are subjects of discussion.

The purpose of this study was to find the ways that are beneficial to the degradation of target organic xenobiotic by activated sludge. Supplementing the biogenic substrate(s) to xenobiotic degradation reactions was the chosen option. The experiments were conducted on the degradation of a target xenobiotic 2,4-dichlorophenoxyacetic acid (2,4-D) using the activated sludge that was fully acclimated to 2,4-D immediately prior to the degradation tests. Two selected biogenic substrates, namely sucrose and peptone were supplemented separately and combined in varying concentrations to the degradation of 2,4-D. The best enhancement cases were located and the enhancing phenomena were theoretically explored.

2 MATERIALS AND METHODS

Xenobiotic and Activated Sludge

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was the target xenobiotic. The initial activated sludge seeds were obtained from a soil not having any record of 2,4-D nor metal contamination. This indigenous activated sludge seeds were grown in a fed-batch reactor that was re-fed once daily with a fresh medium containing biogenic substrates (120 mg/L of sucrose and 50 mg/L of 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 acclimated sludge was cultivated, each time before its degradation, by subjecting the fed-batch indigenous sludge to reactions with 2,4-D. The acclimation of the activated sludge was achieved after the sludge had completely degraded 2,4-D three (3) times consecutively. The lag time for degrading 2,4-D was eliminated after the third-time acclimation reaction and full degradation power was invested to the sludge.

Degradation Tests

The degradation of 2,4-D was conducted in shake-flasks under 100 rpm orbital shaking, at room temperature (25 ± 2°C). The starting acclimated activated sludge concentration was targeted at 100 mg/L. The media in the degradation reactors contained 100 mg/L of 2,4-D, the minerals listed above, and in the supplementation cases, biogenic substrates in varying concentrations. The supplementation tests were conducted with the addition of sucrose (technical grade) and/or peptone (technical grade) to the 2,4-D degradation reactors at the start of reaction. Sucrose or peptone were supplemented separately and also combined to the degradation reactions.

The supplementation schemes were tried each with a different biogenic concentration. The ranges of trial concentrations of sucrose and peptone were, 30 to 200 mg/L and 50 to 200 mg/L, respectively. The sucrose and peptone combination was supplemented with organic contents (as COD) similar to those of separate sucrose or peptone.

From the degradation test, a 2,4-D disappearance course and a biomass growth course were obtained from the measurements of reactor suspensions taken at regular intervals (typically hourly). All tests were performed at least in duplicates.

Measurements

The soluble 2,4-D concentrations in the degradation reactor suspensions were measured with HPLC (Shimadzu 20AT). The concentrations of activated sludge were measured as the dried weight (SS) of the sludge biomasses from the filterable (Whatman GF/C) portion of the reactor suspensions. The biogenic substrate was measured using COD for selected tests. The SS and COD measurements were performed, respectively, following the standard methods of SM2540-D (filtered and dried at 103-105°C) and SM5220-C (closed refllux, titrimetric) [6].

Kinetics Examination

Degradation kinetics is most conveniently represented with the parameters of a microbial growth model. Because of its inhibitory nature, the degradation of 2,4-D was considered to follow the Haldane kinetics, which has the initial slow rate at a high 2,4-D concentration, turning to a maximum, and followed by a gradual decrease in the rate when 2,4-D was lowered. The model fits only to the xenobiotic degradation course using the integrated form of the Haldane kinetic equation:

$$K_S ln\frac{S_0}{S} + (S_0 - S) + \frac{1}{2K_I}(S_0^2 - S^2) = q_{gm}.t$$

To calculate the Haldane parameters from a batch type growth reaction, simultaneous equations governing the biomass growth and substrate depletion must be available. However, with the added biogenic substrates in most cases in this study, the biomass growth was complicated reflecting not only the growth of degraders on their xenobiotic substrate. In other words, the joint yields from biogenic and xenobiotic substrates were not easily separated. Furthermore, actual mathematical fit only to the experimental data on the substrate degradation course using the Haldane kinetic model is difficult, owing to the fact that the solution for the time-derivative equation for the substrate S (Haldane growth divided by yield) has a non-close-form and is implicit [7]. To express the degradation kinetics possibly calculated from the degradation curve, the linear slopes of three time points at the first hour through the third hour were calculated. The gross degradation rates were determined; the degradation rates specific to the total biomass SS (SDR) were subsequently calculated by dividing the gross rate by the initial (the zero hour-H0) SS used in the respective degradation tests.

3 RESULTS AND DISCUSSION

Beneficial Biogenic Dosages

Figure1 shows typical examples of the courses of the activated sludge degradation of 2,4-D with or without the supplements of biogenic substrates. Similar shapes of the 2,4-D degradation courses under the supplementation of sucrose, peptone, and the combined sucrose and peptone, were observed. The effects of biogenic supplementation on the 2,4-D degradation were shown by the differences in the slopes (rates) of the curves. While other experimental conditions were kept constant, the changes in the 2,4-D degradation rates were suitably reasoned to be due to the only variable of the supplemental biogenic concentration.

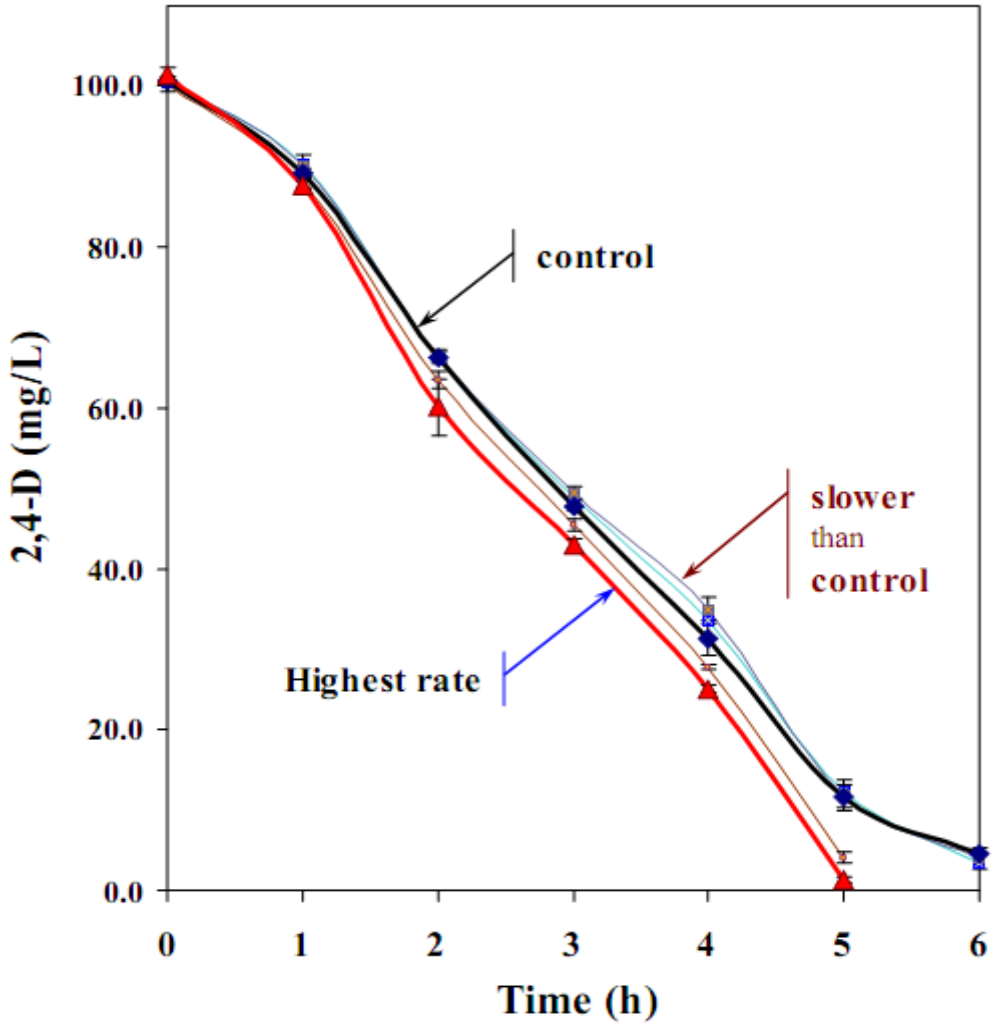

Figure 1: **Time courses of 2,4-D degradation with and without biogenic substrates supplementation. Degradation rates higher and lower than the control were shown with the variations of supplementing biogenic concentrations**

From the considerable number of tests with the series of biogenic substrate supplementations to 2,4-D degradation reactions, each of the degradation course obtained, when compared with that of the control (sole 2,4-D feed), could show a condition that was beneficial to the 2,4-D degradation. Different dosages of sucrose, peptone, and the combined sucrose and peptone had different effects on the 2,4-D degradation rate. It is observed from *Figure 1* that there were cases where biogenic supplements decreased the 2,4-D degradation rate, and cases where the substrate degradation rates were enhanced. From these variations, the conditions that made the optima benefits (the highest increase in 2,4-D degradation rates) were found for each of the three biogenic supplementation schemes. The optimal supplementation concentrations were 50 mg/L of sucrose and 80 mg/L of peptone separately, and 20 and 40 mg/L of sucrose and peptone combined, respectively.

The ranges, in which the degradation rate varied around the optima, were narrow so careful experimental designs, or a large number of trials, were needed to locate the optima. Although the differences shown for the increases in the 2,4-D degradation rates by the biogenic supplements may be slight, the benefits were encouraging. Furthermore, any benefit will be appreciated for the treatment of xenobiotic, especially when the benefit was resulted from a method using inexpensive materials.

Kinetics of degradation

The performances of biogenic supplementations compared to that of the control were further confirmed with kinetics calculations. The degradation rates (SDR) of those degradation curves selected around the optima are shown in Figure 2. The kinetics picture shows there were obvious optima of supplement dosages for the separate and combined supplementation cases. It was found that peptone needs a higher concentration to achieve the optimal benefit compared to sugar. For the combined cases, both sucrose and peptone concentrations at the optimum were lower than those when the two substrates were supplemented separately.

An observation using COD as an indicator of the concentration of supplemented biogenic is also included in Figure 2. Approximately equal biogenic materials, expressed as COD concentrations, were required for the best advantage of the 2,4-D degradation. The degradation rates (SDRs) specific to the respective initial activated concentrations shown in Figure 2 were found to be 0.16 for the control (solely 2,4-D), and 0.183, 0.181, and 0.176 mg-2,4-D/mg-SS/hr, respectively, for the optima achieved by supplementing 50 mg/L of sucrose, 80 mg/L of peptone, and 20 and 40 mg/L of sugar and peptone combined. Numerical data supported graphical observations about the supplement schemes that brought the highest increases in the degradation rates of 2,4-D when compared to that of the control. Favourable degradation conditions were hard to find as it is not easily understood how a supplemental biogenic substrate functions in the degradation biological system. That is why painstaking efforts to find the favourable conditions are often required. The activated sludge growth (measured as SS, data not shown) in the xenobiotic degradation systems was enhanced by the additions of the easy-to-use biogenic substrates. However, the degradation of the target xenobiotic substrate did not follow the trend of the increase of the total SS. The previous finding shows that not all the acclimated sludge was 2,4-D degraders [8]. The actual amount of xenobiotic degraders in the mixed-feed system puts forward a complicated picture. The simple way of increasing the biomass growth for the hope of a positive effect on the target xenobiotic degradation does not always come true. On the contrary, unwanted effects on the xenobiotic degradation may surface when all microbial cells chose to grow on the biogenic substrate in the feed. This is the most suitable reason to explain why contradicting results were often reported for biogenic substrate's presence in xenobiotic degradation. Examples of contradicting findings include, for the positive, those reported by Fakhruddin and Quilty [3] and Lob and Tan [4], and for the negative, those reported by Kulkarnia and Chaudhari [9] and Swindoll et al. [10].

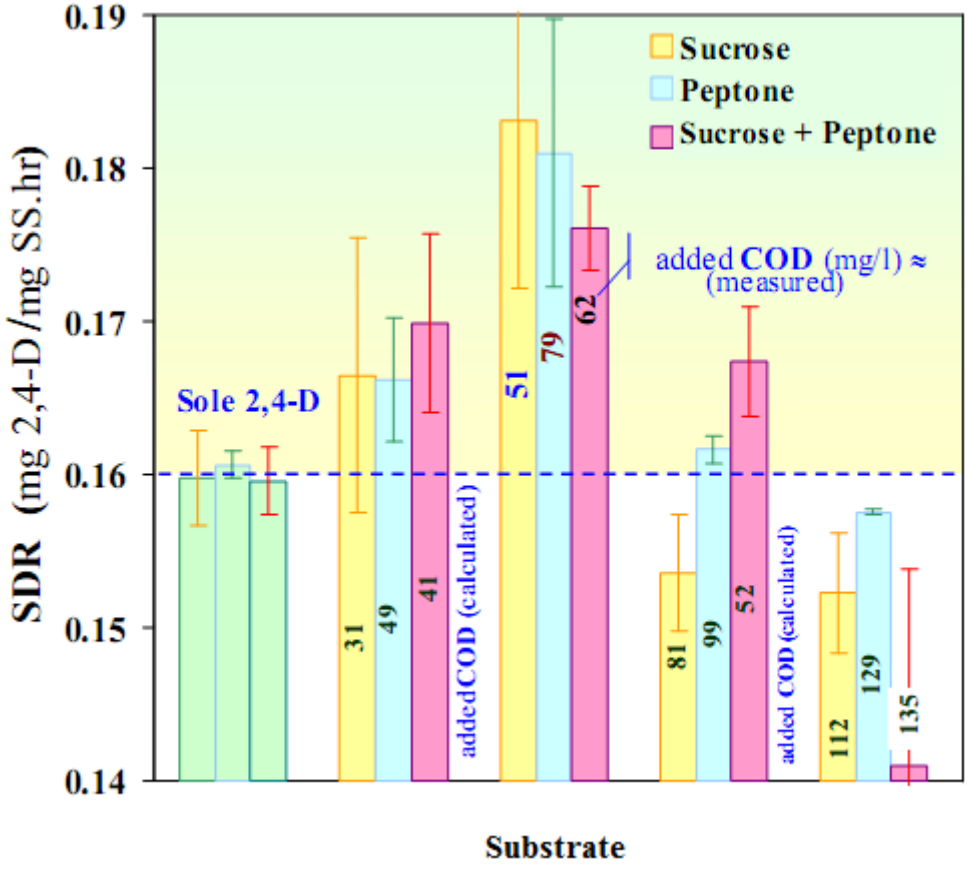

Figure 2: **Rates of 2,4-D degradation for selection biogenic supplementations around the optima. COD of the added biogenic were measured (or calculated) to shown the effect of degradation rate based on COD**

The internal complication that happens in the simultaneous presence of biogenic and xenobiotic substrate with the degradation of xenobiotic may be substantiated by many scientific facts, each and every one of which was not easy to explain, and definitive experimental evidences are not easy to obtain. A few of the explanations are explored here: in the negative ways, 1) the preference growth of prominent xenobiotic degradation biogenic substrate(s) delays or hinders the use of the xenobiotic; 2) the dilution of degraders' xenobiotic metabolism mechanisms resulting from their growth on the biogenic for a number of generations. Degraders' overall degradative power is weakened by the dilution. In the positive way, 1) the degraders grown on biogenic substrates have offspring that has inherited xenobiotic degradative mechanisms. The total degrader amount can

be larger than that grown from the xenobiotic when it is the sole substrate; 2) a healthy growth of the degraders on biogenic substrate enriches degraders' energy profile, thus enabling the degraders to more efficiently tackle the energy expensive degradation of the stable xenobiotic.

Behaviour of Biogenic Substrates during Xenobiotic Degradation

Some of the items discussed above may be visualized with further experimental findings. Figure 3 shows examples of the time sequences of the biogenic substrates of sucrose, peptone and their blends, for being utilized by the 2,4-D degrading sludge, with comparisons against the time required for the degradation of the sole 2,4-D in the feed.

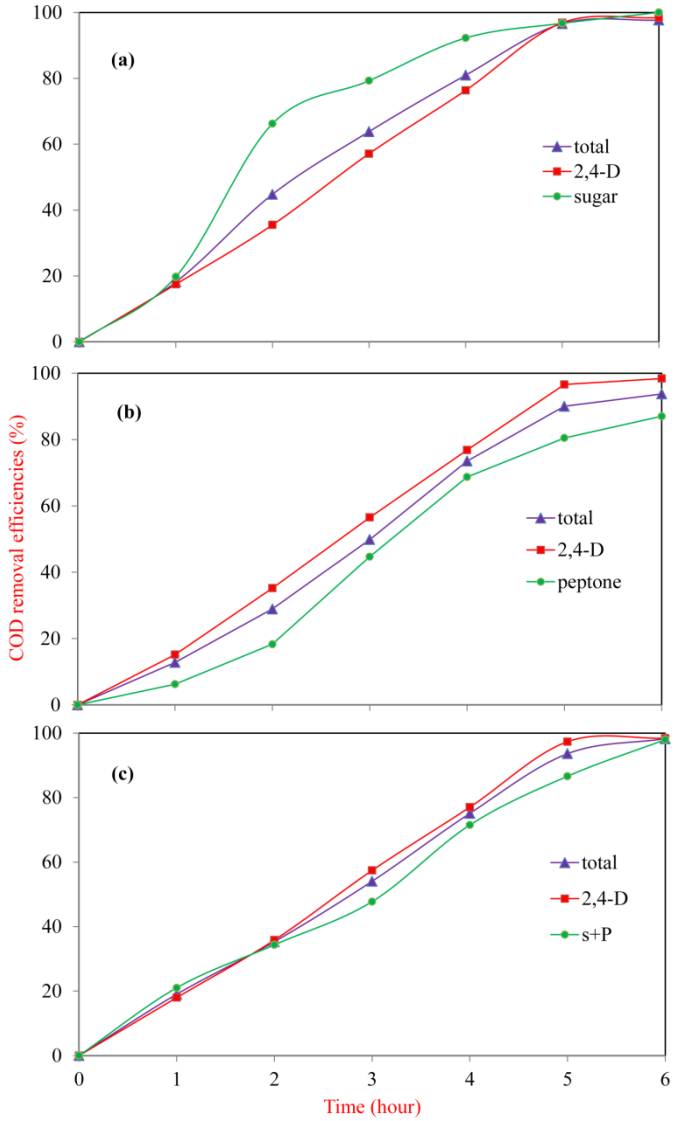

Figure 3: **Time course of COD removal efficiency (%)**

1) Sucrose: sucrose was utilized faster and depleted earlier than 2,4-D (Fig 3a). Sucrose is obviously a substrate easily used by all cells and is certainly being consumed quickly. With sucrose being preferentially utilized, the degradation of the xenobiotic, the extent of which may depend on the abundance of sucrose, will be neglected or delayed. The benefit of the sucrose utilization, on the other hand, may result from enriching energy contents of the degraders so that the degraders are in a healthy state during 2,4-D degradation;

2) peptone: peptone was shown to be more difficult and slower to utilize than 2,4-D (Fig 3b). A peptone concentration higher than that of sucrose was needed to bring a beneficial effect. While not preferentially utilized, peptone may not delay the 2,4-D degradation. The enhanced degradation rate by peptone can still be due to energy enrichment provided by peptone. A higher concentration of peptone was needed to have a rate enhancement due to the smaller amount of energy biochemically obtainable from a unit of peptone mass, compared with that of sucrose;

3) the combination of sucrose and peptone. The total COD of the combined supplement at its optimum was approximately equal to that of the separate supplement of sucrose or peptone. Shown from the COD course of sucrose and peptone combined (Fig. 3c), the combined substrates were together degraded at approximately the same time as 2,4-D. This combined supplement indeed had an about-equal SDR enhancement. Combining sugar and peptone provided degraders with energy enrichment, while the effect of the preferential growth on sucrose was neutralized by peptone and became less important than that which could happen with supplementing sucrose alone. If there was a detail kinetics analysis, the resulting K_s value may show the degraders' affinity for their substrate. On the other hand, the K_I coefficient may indicate the scale of the inhibitory effect of 2,4-D on the degraders.

The acclimated activated sludge used in all tests must contain degraders that have a unified degradative characteristic initially. The variations of degradation rates caused by biogenic addition may also be due to the extent that each substrate may assist in counteracting the inhibition exerted by the xenobiotic on the activated sludge microorganisms, degraders or non-degraders together. The overall beneficial effect of biogenic supplements can be seen as enhancing the degrader growth and providing the degraders with additional energy. In any cases, the ways that could help xenobiotic degradation were found.

4 CONCLUSION

From the trial tests involving a large number of combinations of biogenic substrate supplementation into the xenobiotic degradation reaction, the ways that could enhance the xenobiotic degradation and thus its treatment were found. A narrow range around optimal supplementation concentrations was detected, suggesting the delicate ways that may influent the activated sludge degradation of organic xenobiotic. Performing such enhancement methods can be formulated into guidelines for the application in engineering practices.

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