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THE INFLUENCE OF HIGH PHENOL CONCENTRATION ON MICROBIAL GROWTH

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ABSTRACT

This investigation looked at the influence of high phenol concentrations (1000-1500 mg/l) on the growth yield of phenol degrading organisms in batch culture. The yield coefficient varied from 0.16 to 0.27. These values are considerably lower than those determined by others at lower phenol substrate concentrations. Although the conversion efficiency to biomass was low, the removal of phenol in terms of COD in the batch cultures was high (93.6% average). Present results did not show a relationship between yield and specific growth rate over the range 1000-1500 mg/l phenol. More work is required over a wider range of substrate concentration. With increasing phenol concentrations, the specific growth rate declined, consistent with Haldane inhibition kinetics. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Yield; maintenance coefficient; inhibitory substrate; specific growth rate.

INTRODUCTION

The *growth yield* (Y) is a key parameter used in modelling the performance of activated sludge biodegradation processes. One of its primary uses is in the prediction of the amount of sludge formed in the process. It expresses the quantitative substrate/nutrient requirements of the microorganism and, as well, is a measure of the cell efficiency in converting substrate into biomass. This investigation looked at the influence of high phenol concentrations (1000-1500 mg/l) on the growth yield. Considerable work has been done by others on phenol as the sole carbon source in biodegradation studies. However, most work relates to phenol concentrations from 500-800 mg/l (Rozich *et al.*, 1983; Nakhla and Harazin, 1993). A number of industrial wastewaters, however, contain high concentrations of phenolic compounds, e.g. from coke processing and oil refinery plants. The design and operation of wastewater treatment plants to handle these wastes require a knowledge of the key parameters of the system (e.g. growth yield, Y, and maximum specific growth rate, μ_{max}) for efficient treatment.

The yield constant was originally defined by Monod (1949) in terms of mass units and is often presented as the yield of cells on the basis of carbon substrate consumed. The growth yield (Y) at any point in a culture

may be defined as the incremental increase in biomass resulting from metabolism of an incremental amount of substrate, as follows:

$$Y = -\frac{dX/dt}{dS/dt} = \frac{\mu}{q} \tag{1}$$

where q is the specific utilisation rate, T^{-1}, and μ is the specific growth rate, T^{-1}.

The yield coefficient used here is expressed in terms of the quantity of cell biomass-COD produced by the corresponding amount of substrate utilised in terms of COD. With both terms being expressed as COD, the yield is a standardised dimensionless number. This method requires the initial determination of the cell biomass-COD relationship. The substrate utilised is easily obtained by subtracting the residual COD filtrate in a sample from the initial substrate COD value (ie. S_o).

The expression in equation (1) for growth yield represents the observed yield coefficient, which is sometimes written as Y_{obs}. The equation for the determination of the observed growth yield appears simple but its value is dependent on two fundamental metabolic processes. The growth-limiting substrate is utilised by the microorganisms for the production of new biomass as well as for the generation of energy to drive a range of metabolic reactions. These metabolic reactions include maintenance of solute gradients, motility, internal pH maintenance, turnover of macromolecules, etc, which are collectively known as the maintenance functions (Lynch and Hobbie, 1988). Clearly, if the dissimilation of substrate to provide energy for these maintenance functions_is small, then more substrate is available for biomass production.

This partitioning may be expressed as (Lynch & Hobbie, 1988):

$$\frac{\mu}{Y} X = \frac{\mu}{Y_G} X + m X \tag{2}$$

where Y_G is the true growth yield, X is the cell biomass, and
m is the maintenance coefficient

From equation (2) the maintenance coefficient m, is defined as the amount of substrate used for maintenance purposes per unit amount of biomass per unit time. As both substrate and biomass are measured in terms of COD mg/l, the maintenance coefficient has the unit T^{-1} (ie. h^{-1}).

Re-arranging equation (2) leads to:

$$\frac{1}{Y} = \frac{1}{Y_G} + \frac{m}{\mu} \tag{3}$$

By measuring Y and μ in either batch or continuous culture at varying substrate levels, it is possible to determine the true growth yield, Y_G and the maintenance coefficient, m.

MATERIALS AND METHODS

Batch/Continuous-flow reactor. The reactor comprised a perspex cylinder and detachable base-plate secured to one end with a o-ring seal. The top of the cylinder was enclosed with a removable cover in two-halves. Aeration was provided at the base of the cylinder through two air-stones at opposite ends while air was supplied through a rotameter at the rate of 2 l/min. A magnetic stir-bar ensured complete mixing in the reactor. It was possible to operate the reactor either in batch mode or in continuous flow mode using two

peristaltic pumps. The arrangement also permitted variable operating volumes from 1 l to 4 l. Thus, the dilution rate could be easily varied through changing the pumping rates or the operating volume.

Inoculum. Initial work involved adaptation to the phenol substrate using biomass from a local activated sludge plant. The heterogeneous microorganisms were gradually acclimated to incremental phenol concentrations (Yoong and Edgehill, 1993). Seed for batch growth was obtained from an acclimated culture maintained in a nutrient medium with 1300 mg/l phenol.

Biomass growth. This was determined using the *Standard Methods for the Examination of Water and Wastewater*, 18th edition, APHA 1992, Method No. 2540 D for total suspended solids, but modified using 47 mm diameter 0.2 μm pore size cellulose nitrate membrane filter in a Naglene reusable filter holder. Biomass was also recorded as optical density absorbance at 540 nm using a Novaspec II visible spectrophotometer. A correlation curve was plotted for biomass and absorbance at 540 nm.

Substrate utilisation. Substrate utilisation was measured as COD remaining at various stages of growth in batch, and in continuous-flow using the closed reflux titrimetric method (*Standard Methods for the Examination of Water and Wastewater*, 18th edition, APHA 1992, Method 5220 C, pp. 5.8-5.9).

Nutrient medium. The growth medium which provided the microorganisms with required nutrients during the continuous, and batch modes consisted of components listed in Table 1, per 500 mg/l phenol. A 0.2124M phenol stock solution was prepared separately and mixed with the inorganic salts medium as the sole carbon source to achieve the desired concentration.

Table 1. Inorganic salts medium

Ammonium sulphate, $(NH_4)_2SO_4$, g/l	0.25
Ferric chloride, $FeCl_3.6H_2O$, g/l	0.0015
Manganous sulphate, $MnSO_2.H_2O$, g/l	0.0065
Magnesium sulphate, $MgSO_4.7H_2O$, g/l	0,05
Calcium chloride, $CaCl_2$, g/l	0.007
2M Phosphate buffer, ml/l	10

RESULTS AND DISCUSSION

Determination of Biomass-COD. Biomass-COD data were obtained from samples collected from the batch reactor. From each sample the cell biomass dried weight, COD of the cell biomass and filtrate were obtained. Because substrate was synthetic, it may be reasonably assumed biomass consisted only of cells.

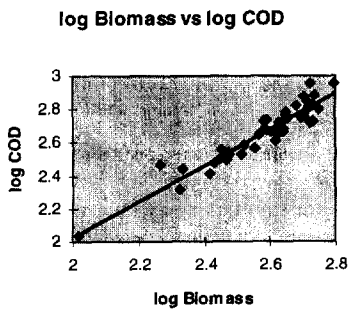

Figure 1. Biomass COD curve (r = 0.947, n = 45).

This was confirmed through microscope observation. The data conformed well to the power relation, COD = 0.7052 x (Biomass)$^{1.0899}$ as shown in Fig. 1. The linearised form returned a correlation coefficient of r = 0.947, n = 45. The Biomass-COD factor was not constant. At low biomass values the ratio between Biomass-COD and VSS (biomass) was low and high at high biomass value. The relationship was in good agreement with work by Bullock *et al.* (1996) which showed that the ratio between COD and VSS for biomass was approx. 1.2-1.6 mg COD/mg VSS. Porges *et al.* (1956) assumed the COD of cells during growth to be fairly constant and used an empirical formula to determine a value of 1.42 mg O_2/mg biomass.

Yield coefficient. This was determined in terms of Biomass-COD produced per unit substrate COD utilised. Data were collected from a series of batch experiments over a period of five months. A typical batch curve is shown in Figure 2. The observed yield was not constant but varied from 0.16-0.27 (0.22 average, 0.03 std. dev.); all values were lower than those reported in the literature for lower phenol concentrations. This is consistent with the known effects of alcohols on the cell membrane, whereby the biosynthetic reactions are adversely affected leading to lower cell yields. The yield coefficient reported by Rozich *et al.* (1983) is 0.46. Although the growth yield was low, the percentage removal of substrate in batch cultures was high (93.6% average, 8.4 std. dev.). A plot of $1/Y$ against $1/\mu$ (Figure 3) showed no clear trend and the true growth yield, Y_G, could not be determined for phenol concentrations from 1000-1500 mg/l. The range of observed yield suggests some uncertainty of the measurements. One possible factor is the extent of active and inert biomass in the volatile suspended solids; a study of which is being made.

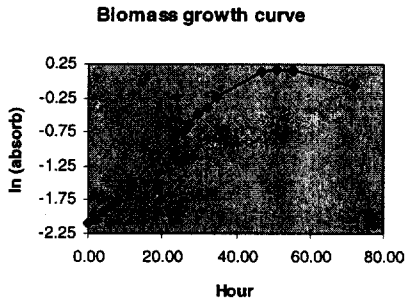

Figure 2. A typical batch assay.

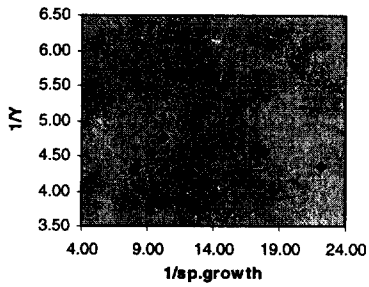

Figure 3. Evaluation of true yield.

Specific growth rate, μ. In each batch assay the exponential growth phase represents the specific growth rate corresponding to the initial substrate concentration (S_o). Linear regression analyses showed clear delineation of this log-growth period, with each coefficient of determination (r^2) exceeding 0.95. The relationship between μ and S_o indicated a Haldane inhibition kinetics. At high substrate concentration, the specific growth rate declined because of this inhibition.

CONCLUSIONS

The observed yield coefficient was not constant but varied from 0.16-0.27 (0.22 average, 0.03 std. dev.) which were considerably less than that determined by others at lower phenol substrate concentrations. It was likely that inhibitory effects of high phenol concentration contributed to this low yield coefficient. However, in batch cultures the removal rate of substrate was high (93.6% average, 8.4 std. dev.). Present data did not show a sufficiently strong trend to enable the true yield to be determined for phenol concentrations in the range 1000-1500 mg/l. More work is required over a wider range of substrate concentrations.

At high substrate concentrations the specific growth rates declined, consistent with Haldane kinetics.

REFERENCES

Bullock, C. M., Bicho, P. A., Zhang, Y. and Saddler, J. N. (1996). A solid chemical oxygen demand (COD) method for determining biomass in waste waters. *Wat. Res.*, **30**, 1280-1284.

Lynch, J. M. and Hobbie, J. E. (eds). (1988). *Micro-organisms in Action: Concepts and Application in Microbial Ecology*. Blackwells Scientific Publication, Oxford.

Monod, J. (1949). The growth of bacterial cultures. *Annual Review of Microbiology*. **3**, 371-393.

Nakhla, G. F. and Al-Harazin, I. M. (1993). Simplified analysis of biodegradation kinetics of phenolic compounds by heterogeneous cultures. *Environmental Technology*, **14**, 751-760.

Porges, N., Jascewicz, L. and Hoover, S. (1956). Principles of biological oxidation. In: *Biological Treatment of Sewage and Industrial Wastes*, McCabe, B. J. and Eckenfelder, W. W. (eds), Reinhold, New York, pp. 25-48.

Rozich, A. F., Gaudy, A. F. Jr. and D'Adamo, P. C. (1983). Predictive model for treatment of phenolic wastes by activated sludge. *Wat. Res.*, **17**, 1453-1466.

Standard Methods for the Examination of Water and Wastewater (1992). 18th Ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Yoong, E. and Edgehill, R. U. (1993). Inhibitory substrate biodegradation using acclimated municipal activated sludge. *The 15th Federal Convention AWWA*, Gold Coast April 18-23, 1993, pp. 635-639.