Biomass Yield Dependence
on
Inhibitory Substrate Concentration

A thesis presented as a requirement
for the degree of Master of Technology
in Biotechnology at Massey University

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1992

Abstract

Variations of growth yield coefficient with substrate concentration of a mixed microbial population was studied. Substrates used for growth were 2,4-dichlorophenoxyacetic acid (2,4-D) and *para*-chloro-*ortho*-cresol (PCOC). The synthetic medium used was designed so that substrate was the limiting-nutrient. The microbial culture was obtained from an activated sludge system treating effluent containing 2,4-D and PCOC. This was acclimated to the particular substrate metabolised in the investigations. Growth was conducted in batch and chemostat configurations.

Experimental data obtained indicated variations in growth yield did occur and were dependent on substrate concentration. Growth yield and specific growth rate biokinetics were directly calculated from the data obtained. Analysis of specific growth rate help in understanding culture-substrate systems. Specific growth rate increased to a maximum then decreased with increasing substrate concentration in batch configuration. Decrease in growth rate began above 500 mg/l for 2,4-D and above 60 mg/l for PCOC. This is an indication of substrate inhibition. The design of the chemostat maintained a constant specific growth rate.

Growth yield decreased with increasing substrate concentration with growth on either 2,4-D and PCOC in batch and chemostat configurations. A review of the literature indicated maintenance coefficient is a key parameter in explaining variations in observed growth yield. Data analysis for determination of the biokinetic constants of maintenance coefficient, m_e, and half-saturation constant, K_s, was performed. Analysis techniques for these constants are traditionally derived from Monod kinetics. Monod kinetics adequately explains growth on innocuous substrates. However analysis of specific growth rate had indicated the substrates used were inhibitory. Determination of maintenance coefficient and half-saturation constant by Monod derived techniques was unsatisfactory.

The effect of maintenance coefficient on growth yield was considered. The literature indicated maintenance coefficient is constant for growth on innocuous substrates. The substrates used in the investigations have phenolic structures. Phenolic compounds are recognised to be destructive to cell membranes. It was proposed that maintenance coefficient increased with increasing inhibitory substrate concentration as a result of increasing cell damage. An explanation for the decreasing growth yield with substrate concentration is adequately given by considering the

variable maintenance coefficient. Substrate used for maintenance is substrate that is not available for growth.

The relationship between growth yield and substrate concentration is essentially linear. Linear regression of yield on substrate was performed for growth on 2,4-D and PCOC in batch configuration and PCOC in chemostat configuration. Fitting of the linear functional form was considered appropriate. Analysis of the linear models for the three biosystems indicated yield variations with substrate concentration are significant. The models for the three biosystems have been given:

For growth on 2,4-D in batch configuration

$$Y(s) = 0.334 - (2.8\times10^{-4})s$$

For growth on PCOC in batch configuration

$$Y(s) = 1.03 - (5.6\times10^{-3})s$$

For growth on PCOC in chemostat configuration

$$Y(s) = 0.799 - (6.0\times10^{-3})s$$

Growth yield models may be incorporated into an overall growth model when similar biosystem configuration and substrates are studied. It is considered that this will give a growth model with greater accuracy in design and operation of biological treatment plants.

Acknowledgments

Thanks are due to a number of people for their assistance during this study:

My supervisors Associate Professor Rao Bhamidimarri and Associate Professor Bob Chong for their support and constructive criticism. A special thanks to Associate Professor Rao Bhamidimarri for his part in planning during all stages and the prompt consideration given to the manuscript.

Thanks are also extended to John Alger, Bruce Collins and Wayne Mallet, the workshop staff in the Biotechnology Department. They did an excellent job in designing and maintaining the required equipment. Dr Graham Manderson gave advice on microbiological aspects. The laboratory staff and administrators, Mike Stevens, Anne-Marie Jackson, Janice Tuncliffe, John Sykes and Judy Collins assisted in obtaining chemicals and operating laboratory equipment.

The assistance of DowElanco staff in providing information and chemicals for the duration of the project was appreciated.

A special thanks to post-graduates Sirinda Yunchalard and Sridhar Susarla. They provided valuable advice and support during the day to day routines of investigation.

This study was financially supported by a grant from Massey University.

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Chapter 1

Background and Introduction

Hazardous waste management is an evolving practice throughout the world. It involves continually developing policies and regulatory approaches to the problems posed by hazardous waste production and disposal. Many countries have developed a wide variety of technologies for dealing with hazardous waste problems. Significant research and development efforts have been sponsored in this field.

Hazardous wastes are defined as hazardous substances that have no further safe and/or economic use. They may be chemically reactive, explosive, flammable, corrosive, toxic, disease-causing, persistent, or may accumulate in the environment. Because of these characteristics, they pose a present or potential treat to the public or environmental health. Unwanted pesticides represent an example of a hazardous waste.

In New Zealand pesticide use has been practised for over forty years (Harris *et al.*, 1992). *Pesticides* also refer to herbicides and other biocides. Disposal options have been limited and in some instances non-existent. Inappropriate disposal methods have lead to wide spread public concern in recent years for public health and the environment. As a result policies and action plans have and are being developed to provide for the safe use and disposal of pesticides.

Many treatment processes have been applied to cleaning up hazardous wastes. Treatment processes may be categorised as physical (eg centrifugation, evaporation), chemical (eg neutralisation, ozonation), or biological (eg activated sludge, anaerobic digestion) (Biosystems Technology Development Program, 1990). The key factors considered in assessing the applicability of a particular technology are listed below:

Function	- the purpose and applicability
Description	- theoretical operating principles and design features.
Performance	- examples of demonstrated clean-up performance.
Limitations	- physical/chemical characteristics that limit applicability.
Economics	- capital, operating and maintenance costs.
Status	- current development status, availability, and research plans.

Some of the most promising of the new technologies for handling hazardous wastes are biological treatments. These appear to provide solutions where other technologies are expensive, inappropriate for the site, or ineffective.

Biological treatment uses microorganisms, such as bacteria or fungi, to transform harmful chemicals into less toxic or non-toxic compounds. Pollutants serve as an energy source for the microorganisms as they are broken down. These organisms have a wide range of abilities to metabolize different chemicals. Organisms that can break down a particular pollutant can be selected for use in a treatment system. Often technologies are developed utilizing the native microorganisms demonstrated to be actively metabolising pollutants at a contaminated site. The biosystems developed often allow for the addition of nutrients or other amendments promoting activity of the microorganisms. The processes are carefully monitored to reduce the possibility of a product of the process being more toxic than the original pollutant.

A biosystem for clean-up of a contaminated site may be based on a number of media or process types:

Liquid bioreactors
Ground-water treatment
Soil/sediment treatment

The process type is chosen to match the site's environment (Biosystems Technology Development Program, 1990). Liquid reactors have been particularly successful in bringing hazardous pollutants into contact with microorganisms for accelerated degradation. Landfill leachates are particularly amenable to liquid reactor treatment. Other treatments have been used with varying degrees of success.

Biodegradation is an attractive option because it is natural, and the products from the processes are usually harmlessly utilized in the biosphere.

Background

The disposal of significant amounts of toxic wastes to unsecured landfills has been practiced in many countries. With time leachate from these sites may migrate and pollute their surrounding

environment. In New Zealand there exits a small number of unsecured industrial hazardous waste landfill sites.

One such site containing industrial waste herbicides was brought to the publics attention in December 1982. Chemical odours were noticed on a popular foreshore. The dumpsite was located nearby. A study in 1984 determined that migration of hazardous components from the site was not significant (Collier and Oldham, 1986). However the nature of the unsecured site could not guarantee that migration of hazardous components would not occur further than the boundary of the landfill area. Containment of the existing site was not considered feasible. It was decided to construct a new secure landfill to contain the contents of the old site (Collier and Oldham, 1986).

The secure landfill was constructed following the guidelines of the Resource Conservation and Recovery Act (USA). Containment was ensured by a double liner and secondary leakage detection system. Water injection and primary leachate collection systems were installed. The contents at the old site were transferred to the new landfill. Leachate is collected from the landfill for biological treatment.

An analysis of the leachate showed it to contain significant quantities of phenoxyacetic acids, associated chlorophenols, and a number of alcohols. The significant specific compounds identified in these categories were: (phenoxies) 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T); (chlorophenols) 2,4-dichlorophenol (2,4-DCP), *para*-chloro-*ortho*-cresol (PCOC) and a trace quantity of 2,4,5-trichlorophenol (2,4,5-TCP); (alcohols) methanol, butan-1-ol and butan-2-ol.

In 1990 a study was completed at Massey University (McAlister, 1990) for describing a suitable process for the treatment of the leachate. A mathematical model was developed to describe the rate of biological growth. Preliminary experiments justified microbial growth to be described according to each of the three substrate categories: phenoxies, chlorophenols, or alcohols. As a result an interactive three-substrate model was developed. Mathematical models are useful for design and operation of biological treatment facilities. Applicability of the models is often dependent on the accuracy of the biokinetic growth constants used.

Microbial growth on inhibitory (tending to mild biotoxicity) carbon sources offers interesting challenges. Difficulties and uncertainties result from the nature of the substrate and from the

heterogeneity of the microbial populations. Consequently difficulties occur in obtaining and analyzing experimental data for the purpose of determining biokinetic constants. As a result there are reports of deficiencies in a representative data base for a range of numerical values for these constants (D'Adamo *et al.*, 1983). Investigators dependent upon these constants have often had to make assumptions and accept the resulting inaccuracies. A common assumption, and one used by McAlister (1990), involved the invariability of the growth yield coefficient. This was assumed for each of the three substrate categories. Growth yield is one of the biokinetic constants.

The growth yield coefficient is defined as the amount of biomass produced from a given amount of substrate metabolised. This parameter has significant importance in the design of biological treatment facilities. Growth yield is one of the parameters employed in kinetic models and in mass and energy balance equations. These are used to describe and predict the operational, and design characteristics of the treatment process. It also represents a large portion of the *sludge* which must be disposed of as a byproduct of the process.

Studies have indicated a variability of growth yield with changes in substrate concentrations and specific growth rates (Stouthamer, 1976). Experimental data suggests that continuous bioreactors can exhibit periodic oscillations in cell-substrate concentrations (Curds, 1971; Tsuchiya *et al.*, 1972). It was shown numerically that when the yield term is allowed to depend on the substrate concentration in the bioreactor, cell-substrate concentration oscillations can exist (Crooke and Tanner, 1982). No experimentally determined quantitative values describing growth yield variability have been cited in the literature.

Investigation

An investigation was conducted into the growth yield coefficient of an activated sludge biosystem defined and modelled by McAlister (1990). The investigation was performed under similar conditions used by McAlister (1990) to give compatibility of kinetic parameters.

Experiments performed used the pure substrates 2,4-D, a phenoxy, and PCOC, a chlorophenol. These were chosen to represent two of the three substrate categories present in the leachate media used by McAlister (1990). Leachate could not be used in the current investigation because of the pure substrate requirement. A synthetic medium with similar composition to the

leachate, without the assortment of carbon substrates, was developed. The pure substrates were added to the synthetic medium.

The activated sludge system developed by McAlister (1990) was maintained throughout the following investigations. This provided a compatible culture to work with. For the experimental purposes this culture was acclimated to the pure substrate media. Batch and chemostat operating configurations were used. It was considered analysis of growth under the different configurations would give results greater scope for application. The chemostat configuration is compatible with the original activated sludge system.

This thesis completes a one and a half year experimental study on aspects of the growth yield coefficient. Growth yield variability with changing environment, particularly substrate concentrations, were investigated. Values are given for the yield coefficient at a variety of substrate concentrations. A mathematical equation has been given describing the relationship of yield and substrate for the substrates used.