BIOSORPTION OF HAZARDOUS ORGANIC POLLUTANTS

By

© JOHN PAUL BELL, B.Sc., M.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

For the Degree

Doctor of Philosophy

McMaster University

December 1986

BIOSORPTION OF HAZARDOUS ORGANIC POLLUTANTS

DOCTOR OF PHILOSOPHY (1986)
(Chemical Engineering)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Biosorption of Hazardous Organic Pollutants

AUTHOR: John Paul Bell, B.Sc. (United States Military Academy)
M.Sc. (Case Western Reserve University)

SUPERVISOR: Dr. Marios Tsezos

NUMBER OF PAGES: xix, 242

ABSTRACT

A study of the sorption of hazardous organic pollutants by live and dead microbial biomass (biosorption) has been made. Biosorption of lindane, pentachlorophenol, diazinon, 2-chlorobiphenyl, and malathion by *Rhizopus arrhizus* and activated sludge were investigated. Malathion was found to be removed by a chemical decomposition process when contacted with dead biomass. The other compounds were observed to be sorbed by the biomass, and the sorption process was found to be reversible. The biosorption isotherms could be represented by the Freundlich equation and were found to be nearly linear over the range of concentrations examined. The biosorptive uptake is positively correlated with the octanol/water partition coefficient for the compounds. Heats of sorption were estimated and indicate that the biosorption process invloves a physical rather than a chemical mechanism. The biosorption phenomenon appears to involve both surface adsorption and absorption into the cell interior. Biosorptive uptake generally appears not to be strongly affected by competition from other sorbing compounds. The kinetics of biosorption of lindane are characterized by a rapid initial uptake followed by a slower accumulation process. In general, live and dead biomass were found to exhibit a different level of biosorptive uptake, however no generalizations could be made concerning the direction or magnitude of the differences. The order of magnitude of removal of non-biodegradable hazardous compounds in biological treatment plants can be predicted from the biosorption isotherms.

ACKNOWLEDGEMENTS

I would like to express my appreciation to all those who helped make this work possible. I am particularly grateful to:

My supervisor Dr. M. Tsezos for his guidance, encouragement, and financial support.

The members of my supervisory committee, Dr. J.L. Brash, Dr. J.M. Dickson, and Dr. G.G Patry for their helpful suggestions.

Dr. R.B. Anderson for his helpful discussions and suggestions.

McMaster University, the Department of Chemical Engineering, the Natural Sciences and Engineering Research Council of Canada, Shell Canada, and the Canadian Water Resources Association for scholarships.

My fellow graduate students, Dr. R.M. Narbaitz, Dr. S.H. Noh, and W. Seto for their good advice and counsel.

Ms. C. Walker for help with growing and processing biomass and with other experimental work.

Dr. M.W.C. Hatton and L. Berry of the Department of Pathology for assistance with carbon-14 analysis.

Dr. J.N.A. Lott of the Department of Biology for assistance with preparation of cell walls.

The staff of the Department of Chemical Engineering for their technical and administrative assistance.

The staff of the Word Processing Centre for their help with preparation of the manuscript.

My parents for their early guidance and encouragement.

Finally and most of all, my wife Ellen and my daughter Betsy for their sacrifices and constant support and encouragement throughout this work.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

CHAPTER ONE

INTRODUCTION

1.1 THE PROBLEM

Many hazardous organic pollutants have been identified in the influents, effluents, and sludges of publicly owned wastewater treatment facilities in North America (Bishop, 1982; Bridle, 1982; Burns and Roe Industrial Services Corp., 1982; Clevenger, 1983; Lue-Hing et al., 1980). These pollutants, many of which are not readily biodegradable, may come from discharges from industrial plants or from contaminated runoff which enters the sewer systems. It has been estimated that there are 50,000 different chemicals in industrial use in North America, and that more than 700 new chemicals are introduced each year (Jackson and Weller, 1982). Even chemicals which are no longer manufactured, such as polychlorinated biphenyls (PCB's), continue to find their way into wastewater treatment systems. Environment Canada has developed a working list of "Suspect/Priority Toxic Chemicals" containing 150 compounds or groups of compounds deemed to be of prime importance environmentally (Bridle, 1982). In the United States the Environmental Protection Agency has established a list of "Priority Pollutants" containing 129 compounds, including 114 organics (Bishop, 1982). These lists contain only a fraction of the potentially hazardous chemicals likely to be present in wastewater discharges.

The fate of toxic organic pollutants entering biological wastewater treatment plants is not well understood. Volatile compounds may be partially removed by air stripping. Biodegradation may be a viable process for some compounds, but the products of biodegradation may be other hazardous compounds. Another possible removal mechanism is sorption of the chemicals by the microbial biomass or other particulate matter. The uptake or

accumulation of chemicals by organisms has been termed biosorption. The term biosorption will be used to describe this process in the present study. The mechanisms involved in the accumulation may involve surface adsorption phenomena or absorption into various components of the cells. Accumulation of hazardous pollutants in the sludges results in their removal from the wastewater stream, but ultimate disposal of the waste sludges then poses a potential environmental hazard, particularly if the pollutants are not irreversibly bound to the sludge. A better understanding of the processes and mechanisms which control the fate of hazardous pollutants in biological wastewater treatment systems is needed if we are ultimately to be able to predict their behaviour and design for their effective removal.

1.2 OBJECTIVES AND SCOPE OF THE PRESENT STUDY

The overall aim of the present work is to develop a better understanding of the mechanism of the biosorption process. The following are the specific objectives of the present work:

1. To develop equilibrium biosorption information for selected hazardous organic compounds with representative types of microbial biomass and to model the data.
2. To determine the effect of temperature on biosorption equilibria and to estimate basic thermodynamic parameters related to the mechanism of biosorption.
3. To examine the reversibility of biosorption.
4. To resolve the contribution of cellular components (cell walls) in the biosorption process.
5. To examine the kinetics of the biosorption process.
6. To examine the effects of competing sorbates on biosorption equilibria.
7. To formulate a preliminary mechanism hypothesis for the biosorption of the selected hazardous pollutants.

Five organic compounds were selected for study in the present work. The compounds were picked from Environment Canada's NAQUADAT list of pesticides and the U.S. EPA list of priority pollutants (McNeely et al., 1979; Bishop, 1982). The selected compounds represent two significant families of pesticides, organochlorine pesticides and organophosphorus pesticides, and the family of polychlorinated biphenyls. The specific compounds were chosen because they possess the characteristics of reasonably high water solubility, low volatility, and high environmental hazard, and are currently, or were formerly, in widespread use. Relatively water soluble compounds were selected because they have the potential to exist in relatively high concentrations in wastewater discharges and their biosorptive behaviour could be observed over a wider concentration range. Compounds of relatively low volatility were chosen to minimize the losses of compound by evaporation during sorption studies. Also, nonvolatile compounds would tend not to be removed by air stripping in biological treatment plants, and therefore the biosorption process would be a more important removal mechanism for these compounds. The five compounds selected are lindane, pentachlorophenol, diazinon, malathion, and 2-chlorobiphenyl. Lindane (gamma-hexachlorocyclohexane) is a widely used organochlorine insecticide. Pentachlorophenol is a common wood preserving chemical. Diazinon (O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) and malathion (O,O-dimethyl S-1,2 di(ethoxycarbonyl)ethyl phosphorothioate) are organophosphorus insecticides in wide use. The 2-chlorobiphenyl is representative of the PCB family of compounds. Although no longer manufactured in North America, PCB's were formerly in widespread use as dielectric fluids in electrical equipment and in a variety of other applications and are still found in the environment. Figure 3.2.1 shows the chemical structure of the five compounds.

Two types of microbial biomass were chosen for investigation of the biosorption phenomenon. The selected biomass types represent two important classes of microorganisms,

fungi and bacteria. A pure strain, *Rhizopus arrhizus*, a fungus used in industrial fermentations, was selected to represent the fungi. *R. arrhizus* was selected because: (1) It has a well known structure; (2) It has been extensively studied in relation to biosorption of ions (Tsezos, 1980, 1983, 1984; Tsezos and Keller, 1983; Tsezos and Volesky, 1981, 1982a, 1982b); (3) It has been shown to exhibit relatively high uptake of chloroethanes (Tsezos and Seto, 1986); and (4) It has a relatively large cell size which might facilitate mechanistic studies. The other type of biomass, chosen to represent the bacterial class of microorganisms, was activated sludge from a biological wastewater treatment plant. Activated sludge is a mixed culture of microorganisms, consisting mainly of bacteria. Activated sludge was selected because it is representative of the microbial population in the most commonly used biological wastewater treatment process. The use of activated sludge also provides for a more direct observation of the behaviour of the selected pollutants in conventional wastewater treatment plants. Dead biomass was used in most of the study because it is a stable sorbent and eliminates the potential for biodegradation that would limit the mechanistic usefulness of the experiments. Comparative studies were done with live biomass using conditions that minimized potential biodegradation as explained in detail in Sections 3.8 and 5.7.

During the experimental work it was discovered that malathion appeared to be removed by a process other than, or in addition to, sorption. This finding was followed up by additional experimental work carried out to confirm the hypothesis that malathion was being removed by a chemical decomposition reaction in addition to sorption.

CHAPTER TWO

BACKGROUND AND LITERATURE REVIEW

2.1 INTRODUCTION

Previous investigations related to the present work are primarily found in two areas of study. The first area concerns the accumulation of hazardous organic pollutants in aquatic organisms. The second area is concerned with the fate of these pollutants when they enter biological wastewater treatment systems. There appears to be little or no work linking these areas together. Investigations in the first area are mainly aimed at determining the fate of pollutants in natural aquatic systems with the emphasis on studying the movement and concentration of pollutants in the food chain. Many of these studies have been concerned with higher organisms such as fish, and only a relatively small number have dealt with microorganisms. This work has been reviewed by Kenaga (1972), Baughman and Paris (1981), and Lal and Saxena (1982). A comprehensive review is given in the following sections. The most comprehensive examination of the fate of hazardous pollutants in biological wastewater treatment systems has been done by the U.S. Environmental Protection Agency (EPA) (Petrasek et al., 1983). The EPA has conducted pilot plant studies to determine what happens to a large number of priority pollutants in an activated sludge process. Environment Canada has undertaken similar studies using a bench-scale activated sludge system (Melcer and Bedford, 1986). A few other studies have been done to examine the fate of hazardous pollutants in full size municipal wastewater treatment plants (Burns and Roe Industrial Services Corp., 1982; Clevenger et al., 1983; Lue-Hing et al., 1980).

Some of the investigations into microbial accumulation of pollutants have examined desorption or elimination of pollutants by the microorganisms. In many cases the

desorption studies were done by washing the biomass one or more times with water and observing the quantity of compound desorbed. Generally equilibrium desorption isotherms were not produced so it is difficult to make general conclusions concerning reversibility of the biosorption process from these data. Some studies of the kinetics of the accumulation and desorption processes have been made, however most reports do not contain sufficient information to determine whether the observed rates are intrinsic sorption rates, independent of bulk phase mass transfer, or are dependent on the particular experimental system used. The difference in uptake of pollutants by live and dead microorganisms has also been reported in several cases. Most investigators have modelled uptake data using a bioconcentration factor, which is a proportionality constant characterizing a linear relationship between the concentration of the pollutant on the biomass and the concentration in the liquid phase. Other investigators have modelled their data using a conventional adsorption model such as the Freundlich equation. In some cases the uptake data has been correlated with the water solubility of the pollutant or with its octanol/water partition coefficient. Very little data has been published concerning competitive accumulation of pollutants or on the thermodynamics of the accumulation process.

There appears to be no consensus in the literature concerning the mechanism of the accumulation of pollutants by microorganisms. Many of the published reports do not speculate on the mechanism. Some authors describe the mechanism as surface adsorption, while others refer to a process of absorption into the lipid fraction of the cells or in some cases to a combination of these mechanisms. The terms bioaccumulation, bioconcentration, and biosorption have been used to describe the process of accumulation of pollutants by microorganisms. The process will be termed biosorption in the present study and will be taken to include any and all mechanisms through which pollutants are accumulated by living or dead microbial biomass.

2.2 BIOSORPTION OF HAZARDOUS ORGANIC COMPOUNDS BY MICROORGANISMS

2.2.1 Mechanism of Biosorption

The subject of accumulation of hazardous organic pollutants by microorganisms has been reviewed by Kenaga (1972), Baughman and Paris (1981), and Lal and Saxena (1982). Kenaga (1972) characterized the accumulation process as one involving a temporary equilibrium by adsorption followed by redistribution of the pollutant by ingestion, absorption, metabolism, partitioning, storage, and elimination. Baughman and Paris (1981) conclude that it is not clear whether sorbed compounds reside in or on the cells or cell membranes. Lal and Saxena (1982) also conclude from their review that the mechanism of biosorption is not clearly understood. Lieb and Stein (1969) proposed that the permeability of hydrophobic compounds into the cell membrane can be treated like diffusion through a soft, non-porous polymer. The permeability is proportional to the membrane/water partition coefficient and inversely proportional to a fractional power of the molecular weight. If this is the case, we would expect that hydrophobic pollutants could accumulate in the cell membrane or even in the cell interior. Sugiura et al. (1975) postulate a two step process for the biosorption of lindane and other isomers of hexachlorocyclohexane (HCH) on five types of unidentified bacteria. The first step is rapid surface adsorption followed by diffusive penetration in the second step. Wedemeyer (1966) also proposes a two step process for uptake of 2,4-D by *Pseudomonas fluorescens*: sorption unto the cell wall followed by passive diffusion into the cytoplasm. Sodergren (1968) suggested that the uptake of DDT by algae involved an absorption rather than an adsorption mechanism in that lipid-soluble materials are known to pass through the cell wall. He states, however, that because of the affinity of DDT for interfaces, adsorption would seem to be the primary mechanism, but adsorption is discounted

because no desorption was observed in the experiments. Canton et al. (1975) found that α-HCH seems to prefer to accumulate in the cytoplasma rather than the cell walls of algae. Canton et al. (1977) found that 13% of the accumulated α-HCH was in the cell walls of a marine alga with the remainder in the cell content. Ten percent was in the lipid fraction of the cell wall and 76% was in the lipid fraction of the cell contents. Two types of algae with different levels of accumulation showed nearly the same accumulation when normalized based on their lipid content. Reinert (1972) concluded that uptake of dieldrin by *Daphnia magna* and guppies was by absorption but could not determine whether the accumulation by algae was by absorption or adsorption. Clayton et al. (1977) concluded that accumulation of PCB's in marine zooplankton was predominantly controlled by equilibrium partitioning between the internal lipid pools of the biota and the water. Johnson and Kennedy (1973) found that the accumulation of DDT by two bacterial species with approximately the same lipid content was the same although, the uptake of methoxychlor differed by a factor of two. The uptake did not seem to be related to surface area, as the surface areas of the bacteria differed by a factor of ten. Ellgehausen et al. (1980) also concluded that the total mass and not the surface area controls the extent of biosorption. Tsezos and Seto (1986) found large differences in uptake of trichloroethane and tetrachloroethane by dead bacteria and fungi with similar lipid content. They hypothesized that the differences in uptake could be partly due to differences in the numbers of ruptured cells which would have exposed more adsorption sites. They observed that the uptake was positively correlated with the soluble organics leached from the dead biomass, which they concluded came from the cell cytoplasm and thus was correlated with the extent of cell rupture.

The view of the biosorption process as a partitioning between the cell lipids and the ambient water has lead to the use of the octanol/water partition coefficient as a predictor of biosorption potential (Baughman and Paris, 1981). Casserly et al. (1983) and Ellgehausen et

al. (1980) correlated uptake of organic compounds on algae with the octanol/water partition coefficient. Dobbs et al. (1986) correlated the biosorption of toxic organics by activated sludge with the octanol/water partition coefficient. Accumulation of seven polynuclear aromatic hydrocarbons on a zooplankter was also correlated with the partition coefficient (Southworth et al., 1978). Steen and Karickhoff (1981) correlated the biosorption of pyrene and phenanthrene by 14 different mixed microbial populations with the octanol/water partition coefficient and also with a combination of water solubility and melting point. While these correlations would tend to imply that a lipid absorption mechanism is dominant, this is not necessarily the case. The octanol/water partition coefficient is generally correlated with water solubility which is correlated with adsorption potential. It would, therefore, be expected that adsorption would also be correlated with the partition coefficient. Grimes and Morrison (1975) reported that the uptake of five organic compounds by 13 bacterial species was inversely correlated with the water solubilities of the compounds. Unfortunately, these correlations do not necessarily lead to a better understanding of the biosorption mechanism.

When investigating the mechanistic aspects of biosorption the question of whether an active or passive mechanism is involved must be addressed. In general, this question has been examined by observing the difference in uptake by live and dead biomass. In almost all cases reported, the uptake by dead biomass was equal to or greater than that of live biomass (Baughman and Paris, 1981; Chacko and Lockwood, 1967; Johnson and Kennedy, 1973; Lederman and Rhee, 1982; MacRae, 1985; Paris et al., 1977; Paris and Lewis, 1976; Sugiura et al., 1975; Voerman and Tammes, 1969;). Werner and Morschel (1978), however, reported that dead cells sorbed slightly less than live cells in their experiments. The reported comparisons of live and dead cell uptake seem to indicate that the biosorption process is passive and does not involve active transport by the live microorganisms. The fact that dead cells frequently accumulate greater quantities of pollutants than live cells may be caused by

the generally greater permeability of the cell membrane in dead cells (Davson and Danielli, 1952). Johnson and Kennedy (1973) proposed the hypothesis that changes in the cellular membrane occur in autoclaving bacteria which increases the uptake sites.

From this survey of the literature it appears that firm, general conclusions cannot be reached concerning the mechanism of biosorption. Indeed, the mechanisms may be specific to the particular system of chemicals and biomass. It seems likely that a combination of adsorption and absorption may be responsible for the phenomenon of biosorption and that the importance of each mechanism may vary from system to system.

2.2.2 Desorption

Desorption of accumulated pollutants has been studied by a number of investigators. Voerman and Tammes (1969) observed desorption of lindane and dieldrin from yeast but they did not produce equilibrium desorption isotherms so that it is not possible to determine if the biosorption is completely reversible. Johnson and Kennedy (1973) observed the desorption of DDT and methoxychlor from bacteria during an initial wash but saw no desorption in subsequent washes. Desorption equilibrium data were not reported. Lederman and Rhee (1982) found desorption rates to be lower than sorption rates and observed incomplete reversibility. Paris et al. (1977) observed that the rate of biosorption and desorption of toxaphene on bacteria, fungi, and algae were the same and that the same distribution coefficients resulted from sorption and desorption. Paris and Lewis (1976) found the biosorption of methoxychlor by bacteria, fungi, and algae to be reversible. Canton et al. (1977) reported the sorption of α-HCH by marine algae to be completely reversible. It appears from this review of the literature that biosorptive phenomena are often reversible. In some cases the reversibility seems to be complete and in other cases there appears to be some fraction which is irreversibly sorbed. The reported incomplete reversibility may be caused by

too short a contact time to achieve equilibrium in cases where the desorption rate is slower than the uptake rate. It is not possible to determine this from the reported results. In practical terms, if the desorption rate is very much slower than the sorption rate the effect is virtually the same as if the process were irreversible. The reversibility of the biosorption process is probably dependent on the mechanism or mechanisms involved and may vary with different chemicals and types of biomass.

2.2.3 Kinetics of Biosorption

The rates of biosorption and desorption have been investigated by a number of workers. The time to reach equilibrium has been reported to be from a few seconds to several hours. None of the studies found in the present literature survey contained sufficient information to determine whether the reported rates were intrinsic uptake or desorption rates or overall rates characteristic of the particular experimental system. Chacko and Lockwood (1978) found that the accumulation of dieldrin on soil microorganisms reached equilibrium in about 15 minutes while the equilibrium time for the less water soluble compound, DDT, was more than 24 hours. This is inconsistent with early work on cell permeability which found permeability to be proportional to the octanol/water partition coefficient which is inversely related to water solubility (Baughman and Paris, 1981). Baughman and Paris (1981) give as possible explanations for this the possibility that the equilibrium time is system specific or that a different mechanism is involved since the early studies used more soluble compounds. Ellgenhausen et al. (1980) concluded that sorption rates were greater for more lipophilic compounds, which is not consistent with the findings of Chacko and Lockwood (1967). They also found that desorption rates decreased with increasing lipophilicity.

Most studies have found that biosorption by microorganisms is a relatively rapid process, reaching equilibrium in less than an hour. Generally, desorption is also reported to

be relatively fast. Weber et al. (1987) reported that equilibrium was achieved in less than 15 minutes for biosorption of lindane by live activated sludge. Sodergren (1968) reported that equilibrium was established within 15 seconds for sorption of DDT by algae and that the sorption rate was the same as the diffusion rate of DDT in the water. Herbes (1977) found that equilibrium was achieved within one minute for sorption and desorption of anthracene by autoclaved yeast cells. Mac Rae (1985) determined that equilibrium was reached within one minute for sorption of lindane by bacteria adsorbed on magnetite. Lower biomass concentrations, however, required longer times to reach equilibrium. Canton et al. (1975) and Canton et al. (1977) reported equilibrium times for sorption and desorption of α-HCH by algae to be within 15 minutes and 30 minutes, respectively. Sorption of five organic compounds on 13 bacterial species was found to reach equilibrium within 15 minutes (Grimes and Morrison, 1975). Accumulation of 2,4-D by bacteria reached equilibrium within 20 minutes (Wedemeyer, 1966).

Some studies have shown that algae and bacteria exhibit faster uptake and desorption than do fungi. For example, Paris et al. (1977) reported equilibrium times for sorption of toxaphene by algae, bacteria, and fungi as 10 minutes, 30 minutes, and two hours, respectively. Paris and Lewis (1976) found that algae and bacteria sorbed and desorbed methoxychlor to equilibrium levels within 30 minutes, while 16 hours was required by fungi.

Slower biosorption rates for ostensibly similar systems have been reported. Johnson and Kennedy (1973) found that 80-90% of the 24 hour uptake of DDT and methoxychlor was attained within 30 minutes, but their data show continued uptake at a slow rate after 30 minutes. Reinert (1972) reported that 24 to 36 hours was required to reach equilibrium for sorption of dieldrin by algae. Sorption of hexachlorobiphenyl by algae required 12 to 24 hours to achieve equilibrium (Lederman and Rhee, 1982). Sugiura et al. (1975) found that the sorption of lindane reached equilibrium within one hour, but other HCH

isomers required much longer times. Werner and Morschel (1978) reported the attainment of equilibrium within one hour for sorption of dieldrin by two strains of diatoms but over 24 hours for some strains of algae.

Few attempts to model kinetic data for biosorption by microorganisms have been reported. Paris and Lewis (1976) found their data to fit a first order rate model. Ellgehausen et al. (1980) reported a second order kinetic model best described their data.

From the biosorption kinetics information reported in the literature it may be concluded that uptake and desorption of hydrophobic organic compounds by microorganisms are generally rapid processes. Some exceptions to this conclusion, for certain systems, appear to exist. As would be expected, the sorption and desorption rates depend on the types of biomass and chemical compounds used. Algae and bacteria appear to exhibit higher rates than fungi. There is conflicting evidence concerning the relation of lipophilicity to sorption and desorption rates. No general models of microbial biosorption are evident in the literature. The kinetic studies reviewed do not appear to cast any light on the mechanisms of biosorption.

2.2.4. Thermodynamics of Biosorption

The only previous investigation into the thermodynamics of microbial biosorption found in the survey of the literature was that of Herbes (1977) who studied biosorption of anthracene by autoclaved yeast. The heat of adsorption for this system was determined from sorption isotherms at two different temperatures to be -5.2 kcal/mol. The magnitude of the heat of adsorption suggests a physical rather than a chemical mechanism.

2.2.5 Competitive Biosorption

Only two previous studies of the effects of competition by two or more sorbing organic chemicals on microbial biosorption were found. It might be expected that competition between two or more chemicals would result in reduced uptake of each chemical. Tsezos and Seto (1986) reported a reduction in the individual uptake of trichloroethane and tetrachloroethane by dead biomass when the two compounds were sorbed from a common solution over that observed when each compound was sorbed from a single solute system. On the other hand, Voerman and Tammes (1969) concluded that there was no difference in the individual uptake of lindane and dieldrin by yeast whether the compounds were sorbed from single solute solutions or from combined solutions.

2.3 FATE OF HAZARDOUS ORGANIC COMPOUNDS IN WASTEWATER TREATMENT PLANTS

Many hazardous organic pollutants have been found in the influents, effluents, and sludges of publicly owned wastewater treatment plants (Bishop, 1982; Bridle, 1982; Burns and Roe Industrial Services Corp., 1982; Clevenger et al., 1983; Lue-Hing et al., 1980). In Canada, the Environmental Protection Service identified 57 toxic organics in sludges from 13 sewage treatment plants (Bridle, 1982). In the United States the Environmental Protection Agency found numerous hazardous organic pollutants in sludges from 40 publicly owned treatment facilities (Burns and Roe Industrial Services Corp., 1982). Clevenger et al. (1983) found PCB's, lindane, and other toxic organics in the sludges of 74 municipal treatment plants in Missouri. Hazardous organics were discovered in the influents, effluents, waste activated sludges, and digested sludges of three large treatment facilities in the Chicago area (Lue-Hing et al., 1980). Maximum concentrations found in the plant influents range from less than one µg/L to several thousand µg/L. Concentrations of hazardous organics in the sludges

have been found in the range of a thousand µg/g of dry solids to less than one µg/g of dry solids. It is apparent from these studies that many hazardous organics are removed from wastewater entering biological treatment systems by partitioning onto the sludges. Many compounds appear to remain on the sludge through the sludge digestion process (Bridle, 1982; Lue-Hing et al., 1980).

The United States Environmental Protection Agency has conducted a pilot plant study to determine the fate of 22 toxic organic compounds discharged into an activated sludge pilot plant (Petrasek et al., 1983). Many compounds were found to accumulate in the primary and secondary sludge. In general, higher concentrations were found in the primary sludge than in the secondary sludge. The fraction of the compounds removed by the primary sludge was reasonably well correlated with the octanol/water partition coefficient of the compounds, however the fraction removed by the secondary sludge was not well correlated. Two of the compounds examined in the present study were included in the EPA study. Twelve percent of the lindane entering the plant was reported to be removed by the primary sludge, 8% by the activated sludge, and 55% was discharged with the effluent. The remaining 25% was assumed to have been biodegraded. Weber et al. (1987) reported 7% removal of lindane by biosorption in a completely mixed activated sludge flow reactor and found no evidence of biodegradation. In the case of pentachlorophenol, 26% was removed in the primary sludge, 5% in the activated sludge, and 81% was found in the final effluent. Blackburn et al. (1984) in a lab scale activated sludge process study reported that approximately 7% of the influent pentachlorophenol was sorbed on the waste sludge. These studies confirm that sorption of biorefractory organic pollutants by microorganisms and other solids is an important removal mechanism in biological waste treatment plants.

CHAPTER THREE

EXPERIMENTAL MATERIALS AND METHODS

3.1 PREPARATION OF INACTIVE BIOMASS

3.1.1 Preparation of Activated Sludge Biomass

The inactive activated sludge used in the experiments was prepared by processing return activated sludge obtained from the Hamilton, Ontario municipal wastewater treatment plant. All experimental work involving inactive activated sludge was done with a single batch of biomass. The live sludge was washed repeatedly with tap water by allowing the sludge to settle, decanting the supernatant water, and replacing it with fresh water. After washing, the sludge was concentrated by centrifugation in a laboratory centrifuge at approximately 3000 rpm for 5 min. The thickened sludge was spread in thin layers on stainless steel trays and dried at 115°C. The dry sludge was carefully ground using a mortar and pestle to pass a 50 mesh screen. The powdered sludge was washed with tap water followed by distilled water. The sludge was separated by centrifugation and was dried at 115°C. It was again ground using a mortar and pestle to pass a 50 mesh screen. The sludge was stored in a jar at room temperature for use in the experiments.

3.1.2 Preparation of R. arrhizus Biomass

The inactive *R. arrhizus* used in the experiments was grown in a New Brunswick Scientific Co. Microferm bench top fermentor. The original culture of *R. arrhizus* was obtained from Canada Packers. Sterile procedures were used to insure the production of a pure strain of *R. arrhizus*. The growth medium contained glucose water solution along with other nutrients and pH control chemicals. The *R. arrhizus* was separated by filtering the

fermentor contents through cheese cloth. The biomass was washed thoroughly with tap water, dewatered by squeezing the excess water off, and autoclaved at 103 kPa for 30 min. The biomass was then vacuum freeze dried in a Virtis lyophilizer. The dry biomass was carefully ground using a mortar and pestle to pass a 50 mesh screen and then further dried at 35°C. The powdered biomass was stored in jars at room temperature for use in the experiments. A single batch of inactive *R. arrhizus* was used for all experiments involving inactive biomass.

3.1.3 Preparation of Cell Walls

Both activated sludge and *R. arrhizus* cell walls were prepared using the same procedure. The cell wall preparation procedure was similar to the procedure reported by Mahadevan and Tatum (1965). Four grams of biomass was mixed with approximately 100 mL of distilled water and was homogenized in a Virtus 45 Homogenizer for 5 min. at 45,000 rpm. The mixture was centrifuged at 12,000 x g for 5 min. and the water was decanted. The separated solids were washed with a 1% solution of sodium dodecyl sulphate in distilled water and again centrifuged for 5 min. at 12,000 x g. This washing was repeated two more times, each time followed by centrifugation under the same conditions to separate the solids. The solids were then washed six times with distilled water and three times with a 95% ethanol in water solution with separation by centrifugation between each washing. Finally, the separated cell walls were dried at 105°C and were ground using a mortar and pestal. The *R. arrhizus* cell wall preparation had the appearance of a light tan powder, the colour being much lighter than that of the whole cell preparation. The activated sludge cell wall preparation was medium brown in colour and was lighter than the whole cell preparation from which it was prepared.

3.2 CHEMICALS

3.2.1 Distilled Water

All water used in the experiments, except where tap water is specifically referred to, was prepared in the laboratory by distillation in a stainless steel laboratory still followed by deionization in a mixed bed ion exchange column. Periodic gas chromatographic analysis of the distilled water showed no traces of extractable organic contaminants.

3.2.2 Extraction Solvents

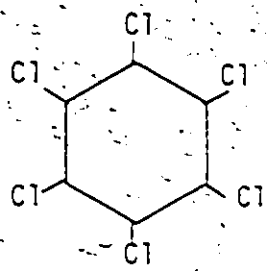
Hexane and iso-octane used as extraction solvents and for preparation of standards were pesticide grade chemicals purchased from Fisher Scientific Co.

3.2.3 Organic Chemicals

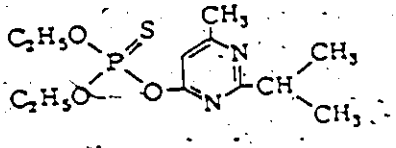
The lindane, pentachlorophenol (PCP), diazinon, malathion, and 2-chlorobiphenyl (2-PCB) used in the experiments were of 99% or greater purity and were purchased from Crescent Chemical Co. The carbon-14 labelled malathion was purchased from Amersham Corp. It had a specific activity of 1.37 GBq/mmol and was dissolved in toluene. The structure of the five organic chemicals is shown in Figure 3.2.1. Table 3.1 lists important properties of the compounds.

3.2.4 Analytical Standards

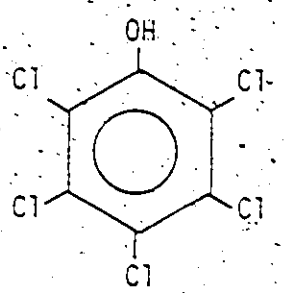
Primary analytical standards for lindane, diazinon, and malathion consisted of solutions of the compounds in benzene and were purchased from Chromatographic Specialties Ltd. Analytical standards for pentachlorophenol and 2-chlorobiphenyl were prepared using the pure chemicals. Water solution standards were prepared by dissolving the pure



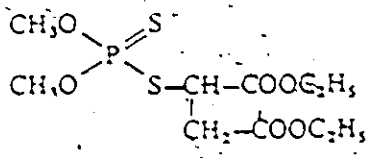
Lindane



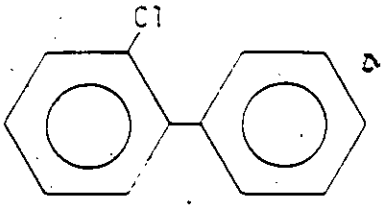
Diazinon



Pentachlorophenol



Malathion



2-Chlorobiphenyl

Figure 3.2.1 Structure of the organic compounds

compounds in distilled water and determining the concentrations by analysis in comparison with the primary standards. Appendix A gives details covering preparation of standards.

Table 3.1 Properties of the Organic Compounds

Property	Lindane	PCP	Diazinon	2-PCB	Malathion
Empirical Formula	$C_6H_6Cl_6$	C_6Cl_5OH	$C_{12}H_{21}N_2O_3PS$	$C_{12}H_9Cl$	$C_{10}H_{19}O_6PS_2$
Molecular Weight	290.83	266.34	304.35	188.66	330.36
Form at 20°C	Solid	Solid	Liquid	Solid	Liquid
Specific Gravity	1.87[1]	1.978[1]	1.1129[2,3]	1.1499[3]	1.2315[1]
Water Solubility (mg/L)	10[4]	14[5]	40[4]	5.96[6]	150[4]
Log Octanol/Water Partition Coefficient	3.72[7]	4.65[7]*	3.14[8]	4.87[7]**	2.89[7]

* Average of reported values
** Estimated by fragment method

References:

1. Hawley (1971)
2. Buckingham (1982)
3. Weast and Astle (1985)
4. McNeely et al. (1979)
5. Verschueren (1977)
6. Hutzinger et al. (1974)
7. Hansch and Leo (1979)
8. Zaroogian et al. (1985)

3.2.5 Scintillation Fluid

The scintillation fluid used for carbon-14 analysis was Amersham Aqueous Counting Scintillant.

3.3 GLASSWARE CLEANING PROCEDURES

All glassware used for water solutions was thoroughly rinsed with tap water, washed with a laboratory glass cleaning detergent (Fisher Sparkleen), rinsed thoroughly

with hot tap water, rinsed at least three times with distilled water, and dried at 105°C. Extraction vials were rinsed four times with HPLC grade acetone, washed and dried as described above, rinsed three times with pesticide grade hexane and redried at 105°C. Periodic analysis of blank water and solvent solutions contacted with cleaned glassware showed no evidence of contamination.

3.4 ANALYTICAL METHODS

3.4.1 Organic Chemical Analysis

Analysis of organic compounds in water solution was accomplished by gas chromatography. Details of the analytical procedure are given in Appendix A. The organic chemicals were extracted from water solution by contacting a known quantity of the water solution with a known quantity of hexane or iso-octane. The extractions were done in Teflon sealed, glass septum vials agitated by a wrist-action shaker for 30 min. The solvent extracts were analyzed using a Hewlett-Packard Model 5830A gas chromatograph with an electron capture detector and a digital integrator. Water solution standards of the organic compounds were analyzed along with the unknown samples. The standards were extracted in the same way as the unknowns so that it was not necessary to know the extraction efficiency since the same efficiency could be assumed for standards and unknowns which are close in concentration. Concentration of each unknown was determined by linear interpolation between adjacent lower and higher standards and was based on peak areas computed by the integrator. The analytical precision was generally within 2%. The overall accuracy of the concentration measurements was estimated to be ±10%.

3.4.2 Carbon-14 Analysis

A Beckman Model LS-230 Liquid Scintillation System was used to analyze samples for C-14. Liquid samples were prepared by adding 0.2 mL of the unknown sample, 1.8 mL of

distilled water, and 15.0 mL of scintillation fluid to a plastic scintillation vial and mixing with a vortex shaker. Filter and biomass samples were placed in the scintillation vials and 2.0 mL of distilled water and 15.0 mL of scintillation fluid were added to each vial. The contents of the vials were mixed with the vortex shaker. Spiked samples used to evaluate quenching were made by adding 0.05 mL portions of a C-14 labelled citric acid solution to prepared samples. The quantity of C-14 added by the spikes was determined by counting similarly spiked distilled water samples. All samples were counted for 10 min.

3.5 BIOSORPTION EQUILIBRIUM EXPERIMENTS WITH INACTIVE BIOMASS

Adsorption equilibrium experiments involved the contact of a quantity of biomass with a water solution of the organic compound or compounds to be studied for sufficient time to allow the system to reach equilibrium. The biomass concentrations and initial solution concentrations were varied to cover a range of equilibrium concentration. The biomass was added to an aluminum weighing dish placed on the pan of an analytical balance. The total weight of the dish and the biomass was recorded. The biomass was carefully poured into a 250 mL screw-top flask. The empty dish was weighed and the weight was recorded. The difference between the full and empty weights of the dish was taken as the weight of the biomass added to the flask. The flask was next filled with the solution of the compound or compounds to be tested. Solutions were prepared by diluting a stock solution made up by dissolving a quantity of the pure chemical in distilled water. The solution was added to the flask which was tared on a digital laboratory balance. The weight of solution added was recorded and the flask was sealed with a screw cap with a Teflon seal. Control flasks, containing only the solution and no biomass, were also prepared. For the initial experiments, blanks containing only distilled water or distilled water and biomass were also run. When all

flasks were prepared they were placed on an orbital shaker and agitated at 250 rpm in a constant temperature room for three days.

After the three day contact period the solutions were filtered to separate the biomass. The filtrations were done in all glass filter apparatus using Millipore 0.45 μm membrane filters. The filters were first rinsed to remove any leachable materials by filtering 300 mL of distilled water. Approximately half of the solution to be separated (50-75 mL) was filtered and the filtrate was discarded. This was done to saturate the filter and glassware with the adsorbate to prevent further adsorption. The remainder of the solution was then filtered and the filtrate was recovered. Control solutions containing no biomass were filtered in the same manner. The solute concentration in the filtrate from the control was taken as the initial concentration to account for any losses due to adsorption on the glassware or filter or other losses. The collected filtrates were analyzed as described in Section 3.4.1. The solid phase adsorbate concentration as computed by a mass balance. As the compounds were nonvolatile and dead biomass was predominately used, the mass balance technique is considered appropriate.

3.6 DESORPTION EQUILIBRIUM EXPERIMENTS WITH INACTIVE BIOMASS

Desorption experiments were begun by setting up an adsorption experiment as described in the previous section. At the end of the three day contact period, when the solutions and biomass were in equilibrium, the shaker was stopped and the biomass was allowed to settle. After settling, the supernatant solution was decanted and replaced by distilled water. The weights of the solution decanted and the distilled water added were recorded. The flasks were returned to the shaker and agitated for three days in the constant temperature room. The decantate was filtered as described in Section 3.5 and the filtrate was

analyzed. Preweighed filters were used to filter the decantate, and the filters were dried and reweighed after filtration to determine the weight of biomass in the decantate. At the end of the three day desorption period the solutions were filtered and the filtrates were analyzed. The concentration of adsorbate on the biomass after desorption was computed by a mass balance.

3.7 KINETICS EXPERIMENTS

3.7.1 Experiments Using Contact Flasks

Kinetics experiments with diazinon and malathion were done by setting up adsorption experiments using contact flasks as described above. Each flask contained identical biomass and organic solute concentrations. The flasks were removed from the shaker and the solutions were filtered at different times to determine the rate of adsorption.

3.7.2 Experiments Using Stirred Batch Reactor

Kinetics experiments with lindane were done in a stirred batch reactor. The reactor was a cylindrical plexiglass chamber with baffles to promote good mixing. A bottom outlet was provided with a membrane filter through which solution could be removed while retaining the biomass. A stainless steel mixer, powered by a variable speed air motor, provided agitation. The solution was prevented from flowing through the filtered outlet by maintaining the reactor under a slight vacuum. A filtered sample of the solution in the reactor could be drawn by applying a slight pressure to the reactor with nitrogen gas.

The reactor was first filled with a known weight of lindane solution. The mixer was started and the mixer speed was measured using a strobe light tachometer. A period of 30 min. was allowed for the reactor to come to sorption equilibrium with the solution. A solution sample was taken from the reactor at the end of the equilibration period. This sample

represented the initial concentration in the reactor. All samples were collected in preweighed bottles so that sample weights could be determined. A weighed quantity of biomass was added to the reactor and a timer was started. Samples of the solution in the reactor were taken at various time intervals. All sample bottles were weighed to determine the quantity of sample collected each time. Since the removal of samples affected the mass balance in the reactor, the biomass loading was corrected to account for sample removal. The collected samples were analyzed using the procedure described in Section 3.4.1.

3.8 LIVE BIOMASS EXPERIMENTS

Adsorption and desorption experiments with live biomass were performed in essentially the same way as experiments with inactive biomass as described in Sections 3.5 and 3.6. The contact time for adsorption and desorption was, however, shortened to one day except for one flask in each experiment which was allowed a three day contact period for adsorption and desorption. The contact time was reduced to one day for the live biomass to reduce the potential for biodegradation. The three day samples were used to investigate the effects of longer contact time and to observe any biodegradation that might have taken place.

Live activated sludge was obtained by collecting return activated sludge from the Hamilton, Ontario municipal wastewater treatment plant. The sludge was washed thoroughly with tap water and then distilled water and dewatered by vacuum filtration. Four samples of the filtered sludge were placed in preweighed weighing dishes which were then weighed. The dishes were placed in an oven for drying at 105°C. After the samples were dry they were reweighed and the percentage of dry weight in the wet filtered sludge was determined by taking the average of the four samples. This was the basis for determining the dry solids concentration in each adsorption flask upon which the biomass loading was based. Immediately after the filtered sludge samples were taken and weighed, quantities of the

biomass were added to contact flasks. The biomass was added to flasks which were tared on a digital laboratory balance and the wet biomass weights added were recorded. The remainder of the experiments proceeded as for inactive biomass.

Live *R. arrhizus* was grown as described in Section 3.1.2. The biomass was harvested by filtering the fermentor contents through a cloth bag. The biomass was washed thoroughly with tap water and then distilled water and then squeezed dry. The remainder of the experiment was conducted as described above for activated sludge.

Kinetics experiments with malathion were done in the same manner as the experiments with inactive biomass, using individual contact flasks starting with equal solution and biomass concentrations.

3.9 CARBON-14 LABELED MALATHION EXPERIMENT

A stock solution of labeled malathion was prepared by first evaporating the toluene solvent from the malathion solution with a stream of nitrogen gas in a glass flask and then adding distilled water. The contents of the flask were mixed overnight to insure dissolution of the malathion. The remainder of the experiment was conducted in the same way as the adsorption experiments except that a contact time of approximately eight days was allowed to insure that significant removal of malathion would occur. Analysis of the solutions and the filters with biomass were done as described in Section 3.4.2.

CHAPTER FOUR

EXPERIMENTAL RESULTS

4.1 SINGLE SOLUTE BIOSORPTION BY INACTIVE BIOMASS

Biosorption experiments were performed to develop sorption isotherms covering approximately three orders of magnitude of liquid phase concentration. Figures 4.1.1 through 4.1.10 are plots of equilibrium solid phase concentration of the organic chemicals versus the corresponding liquid phase concentration at 20°C. Apparent sorption data are given for malathion, although experimental evidence suggets that malathion was removed primarily by chemical decomposition. The behaviour of malathion is discussed in Chapter Five. Experimental data for the isotherms are given in Appendix B1. The lines shown on the graphs represent the Freundlich equation fit to the experimental data. The Freundlich equation was selected because it generally appeared to provide the best fit to the experimental data of the commonly used sorption models. A more detailed discussion of the different sorption models is given in Section 5.1.1.

The Freundlich equation has the form

$$q = K_F C^{1/n}$$

where

q = equilibrium concentration of sorbate in biomass, µg/g,

C = equilibrium concentration of sorbate in solution, µg/L,

K_F = adjustable parameter,

n = adjustable parameter.

In the present study, the variable q, which is often referred to as loading or uptake, was determined by the mass balance equation

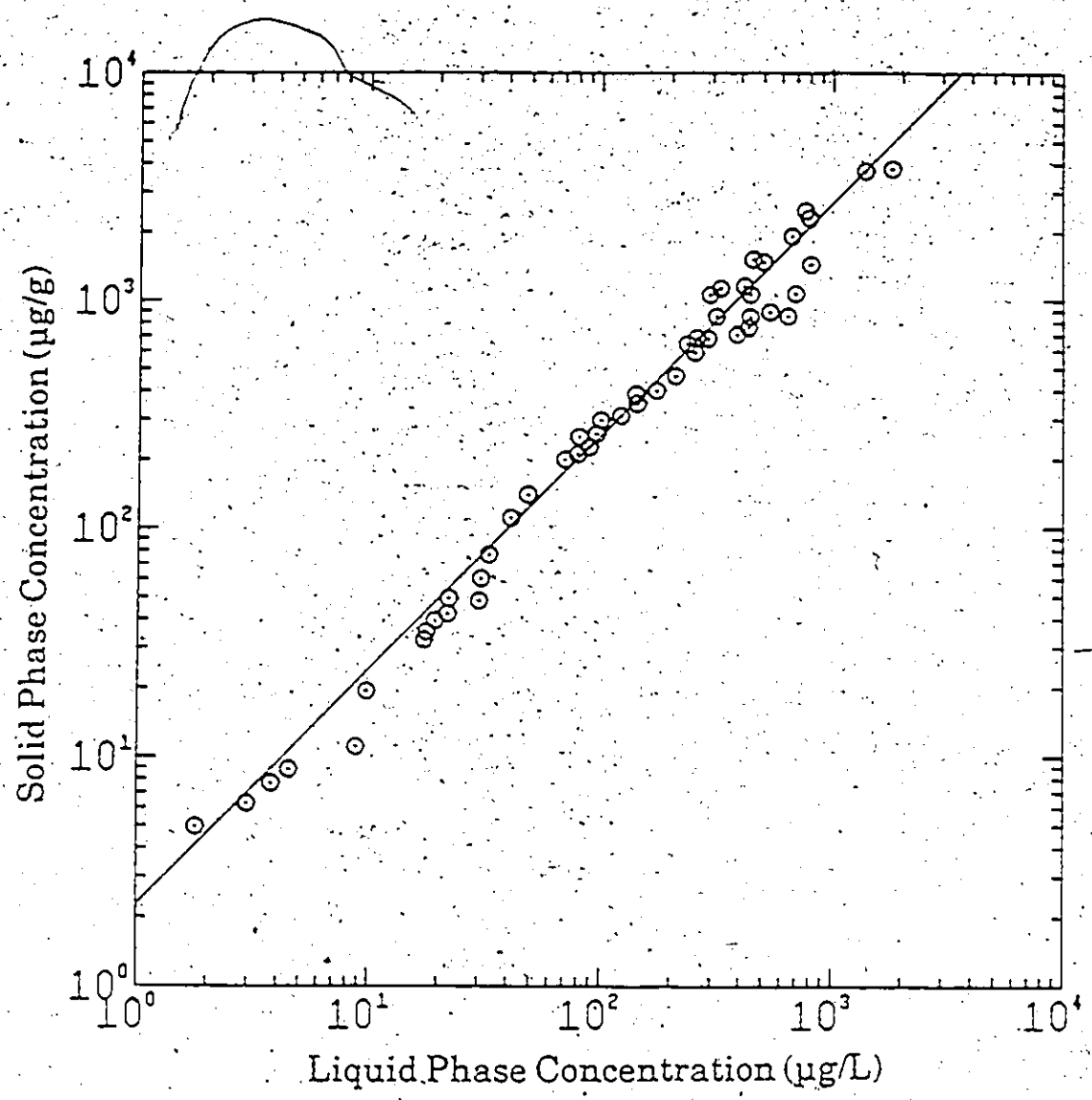


Figure 4.1.1 Biosorption of lindane by *R. arrhizus*.

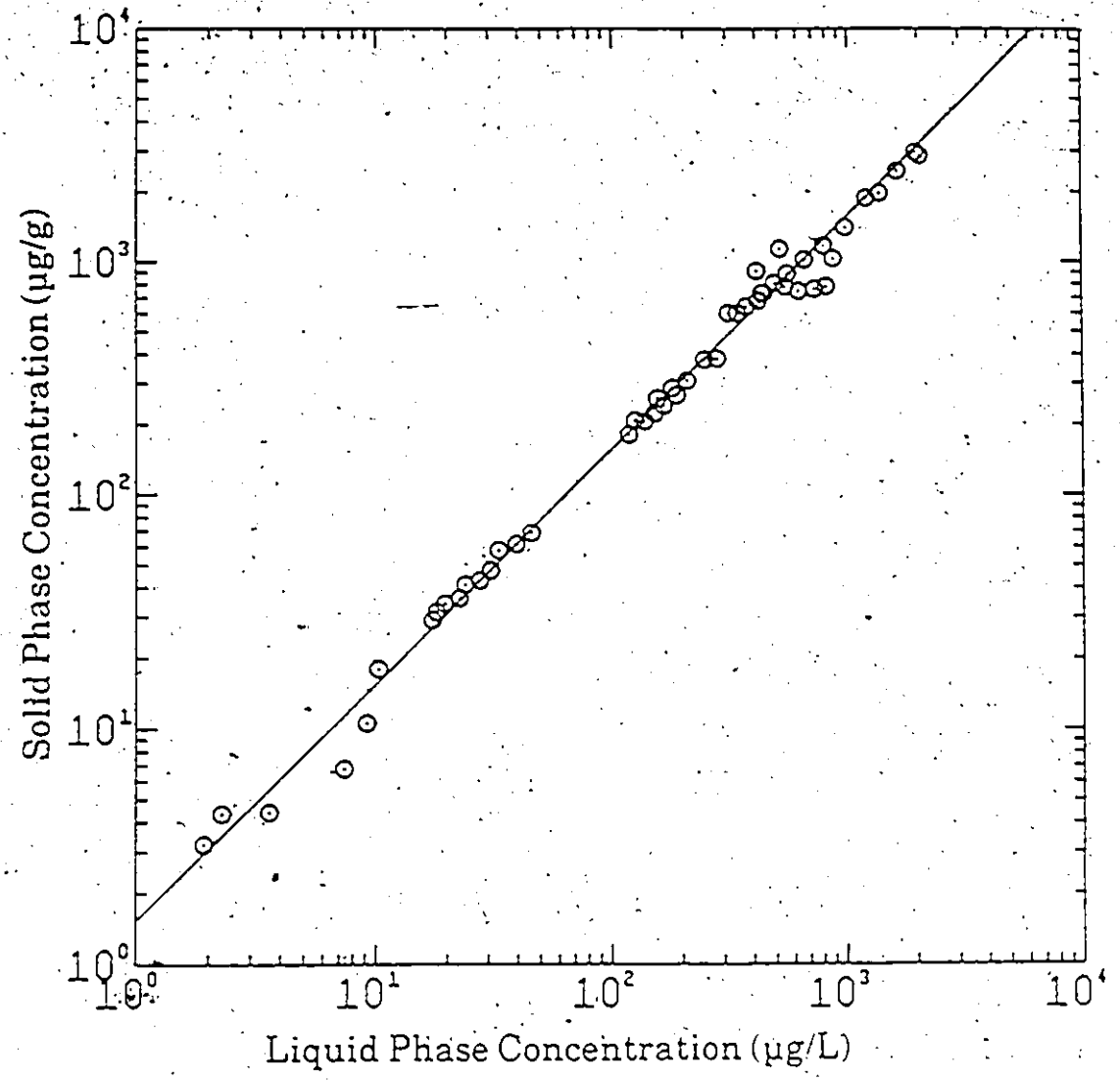


Figure 4.1.2 Biosorption of lindane by activated sludge.

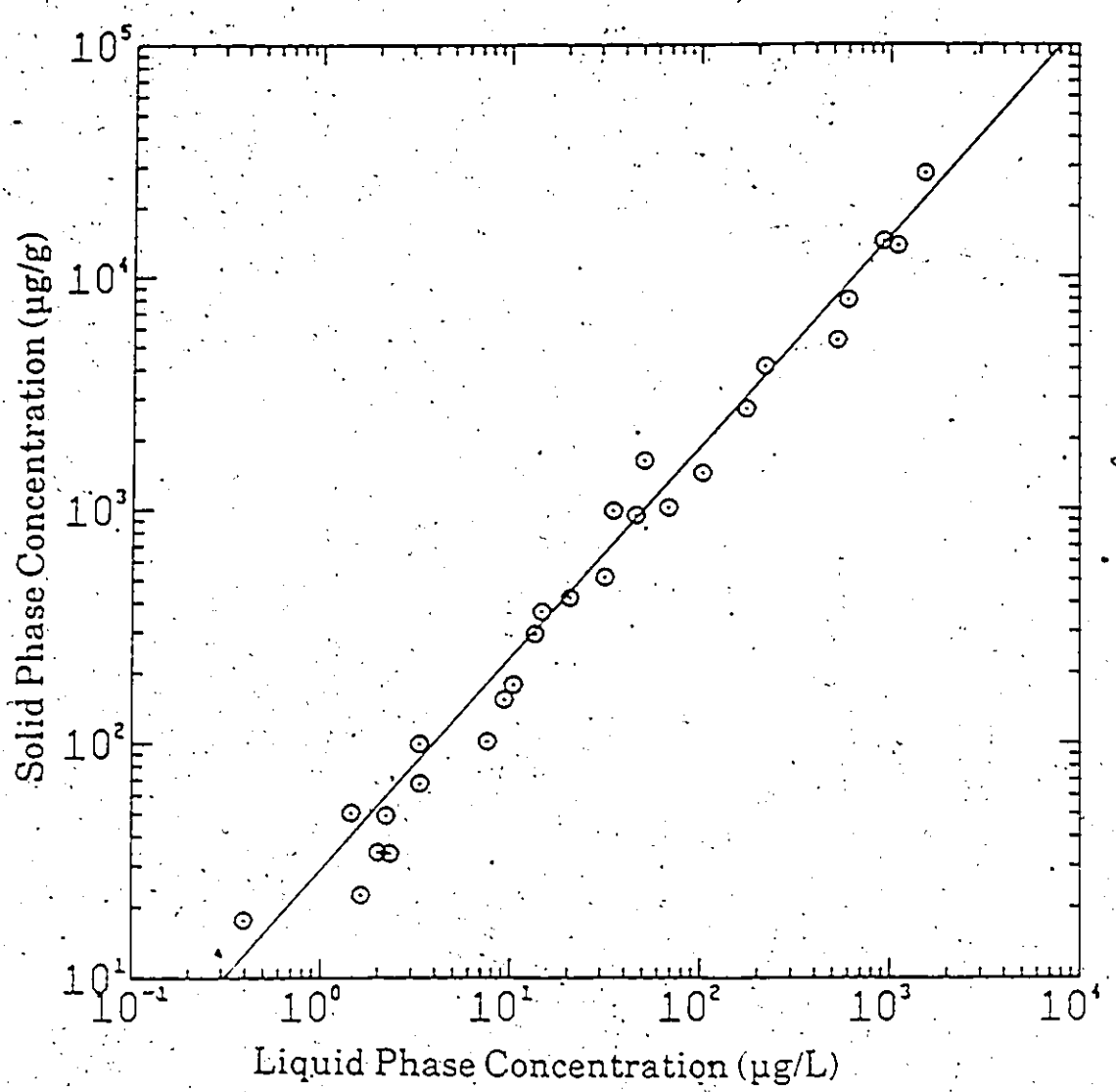


Figure 4.1.3 Biosorption of pentachlorophenol by *R. arrhizus*.

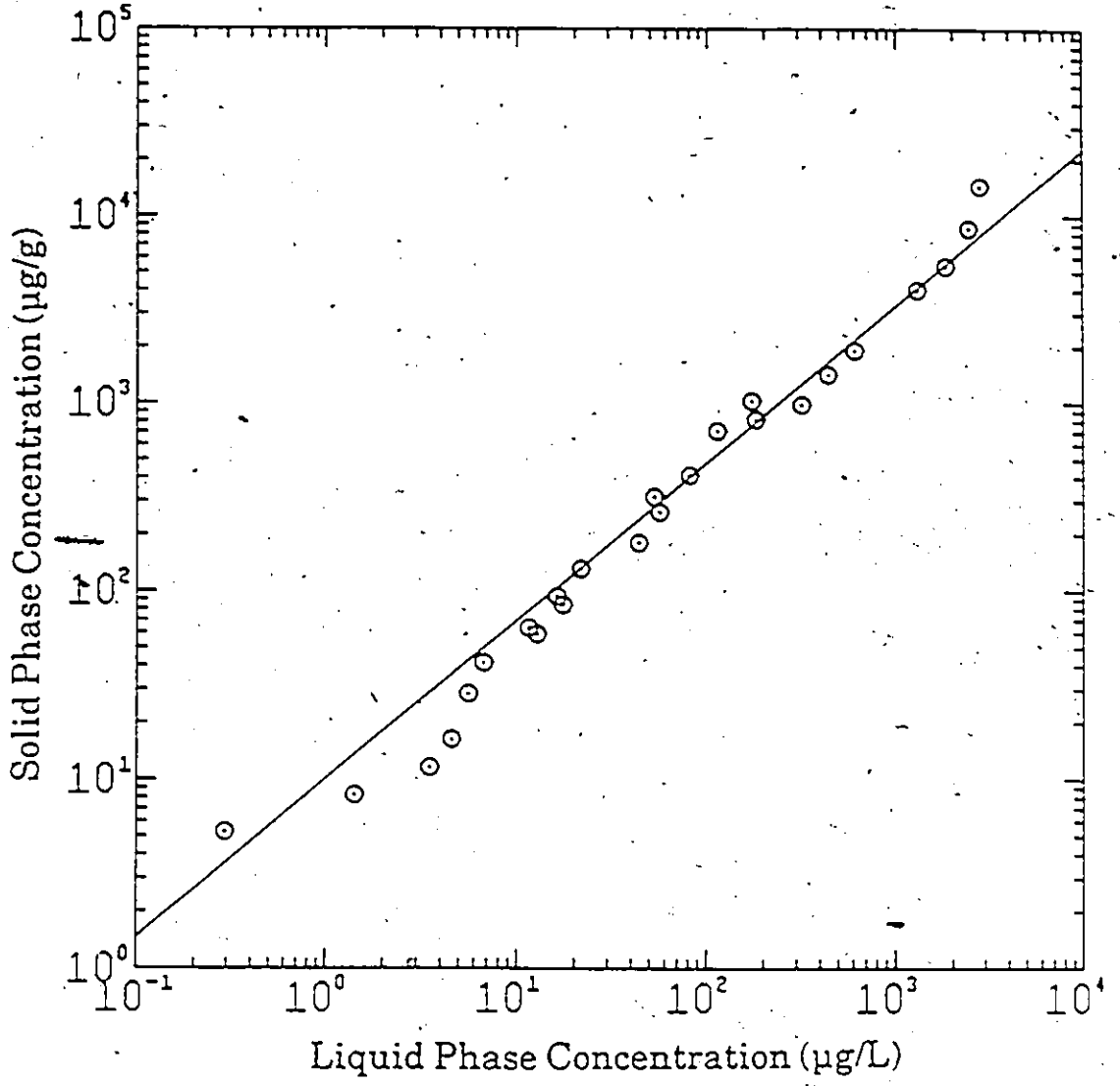


Figure 4.1.4 Biosorption of pentachlorophenol by activated sludge.

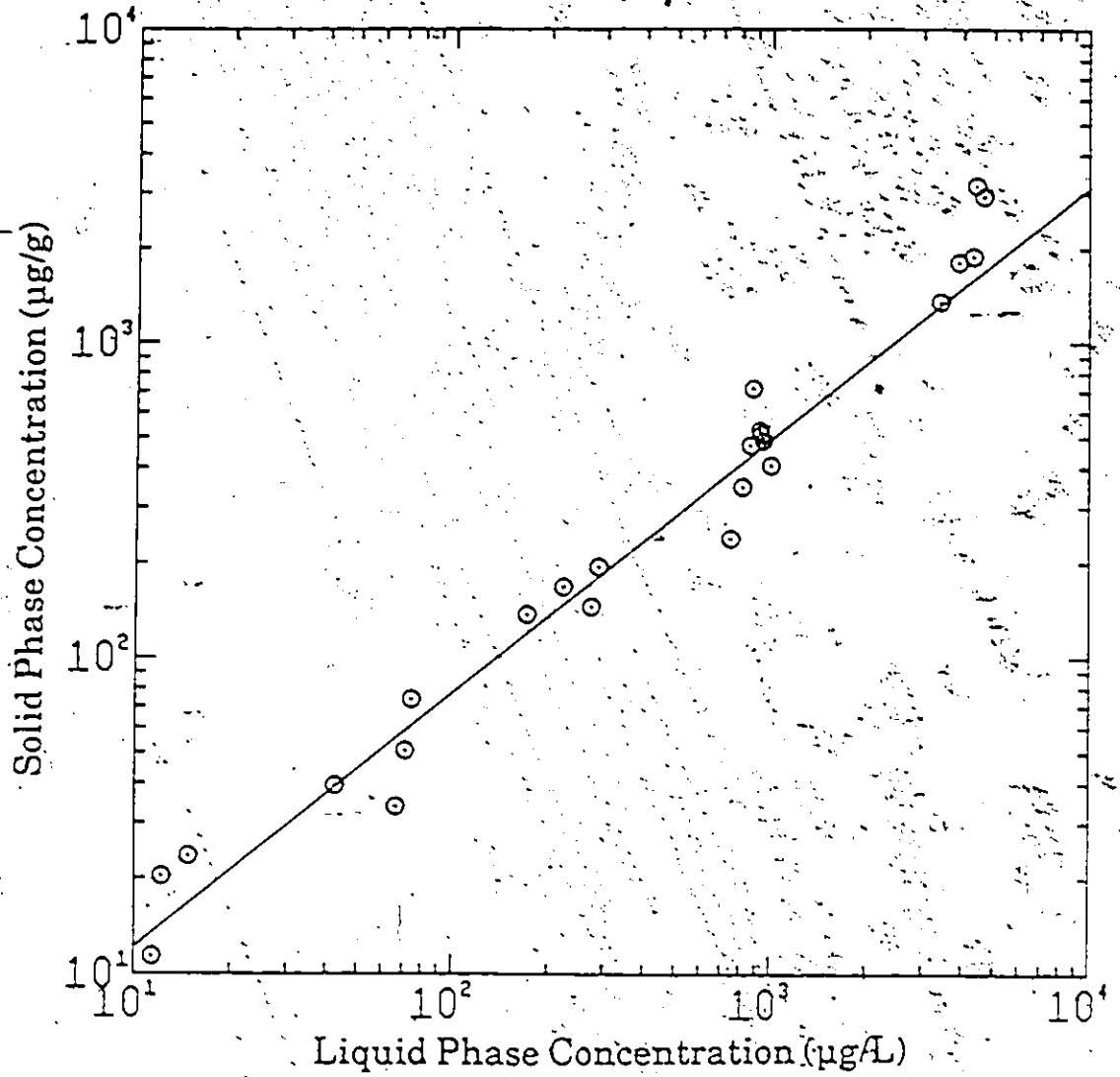


Figure 4.1.5 Biosorption of diazinon by *R. arrhizus*.

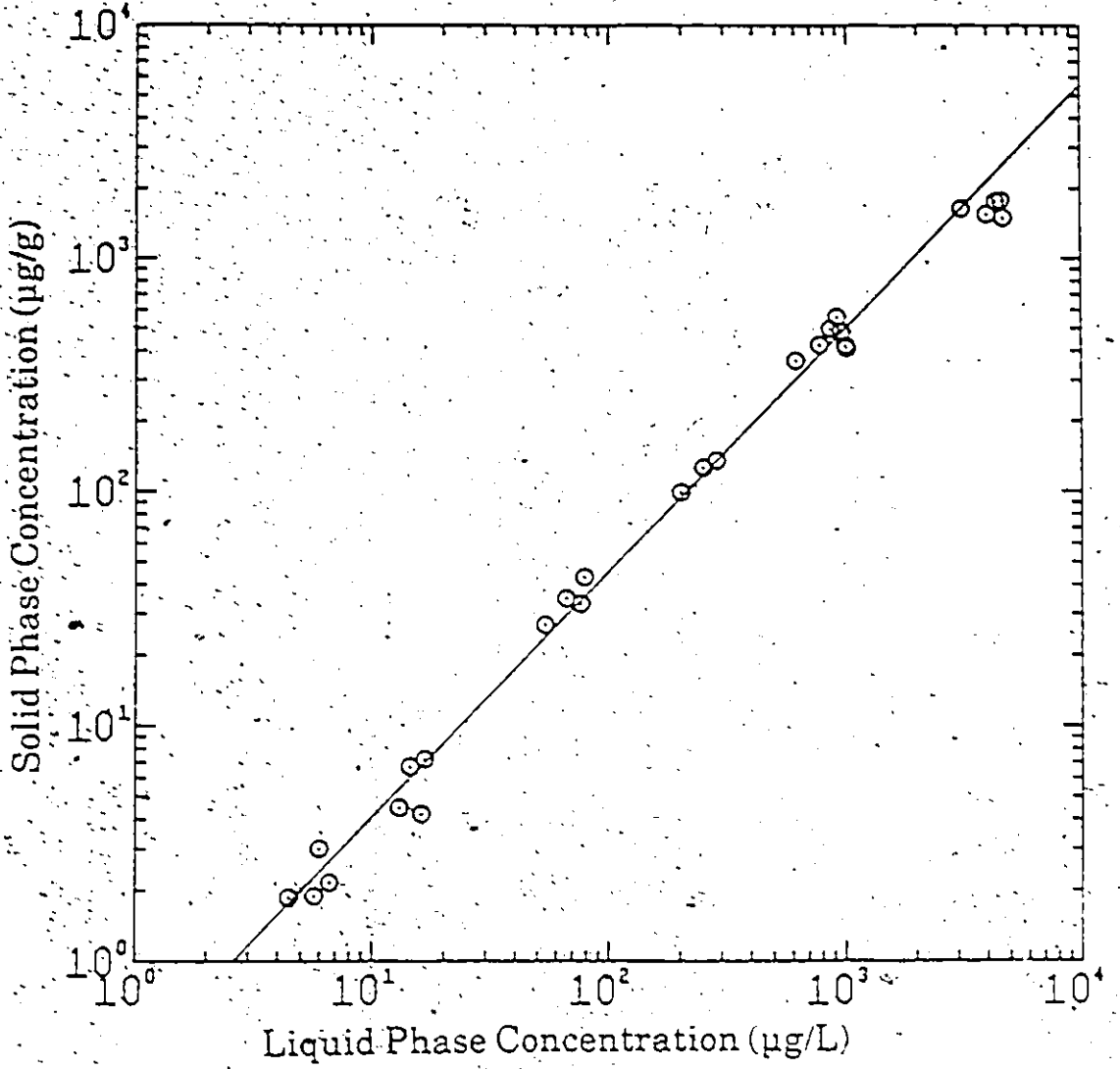


Figure 4.1.6 Biosorption of diazinon by activated sludge.

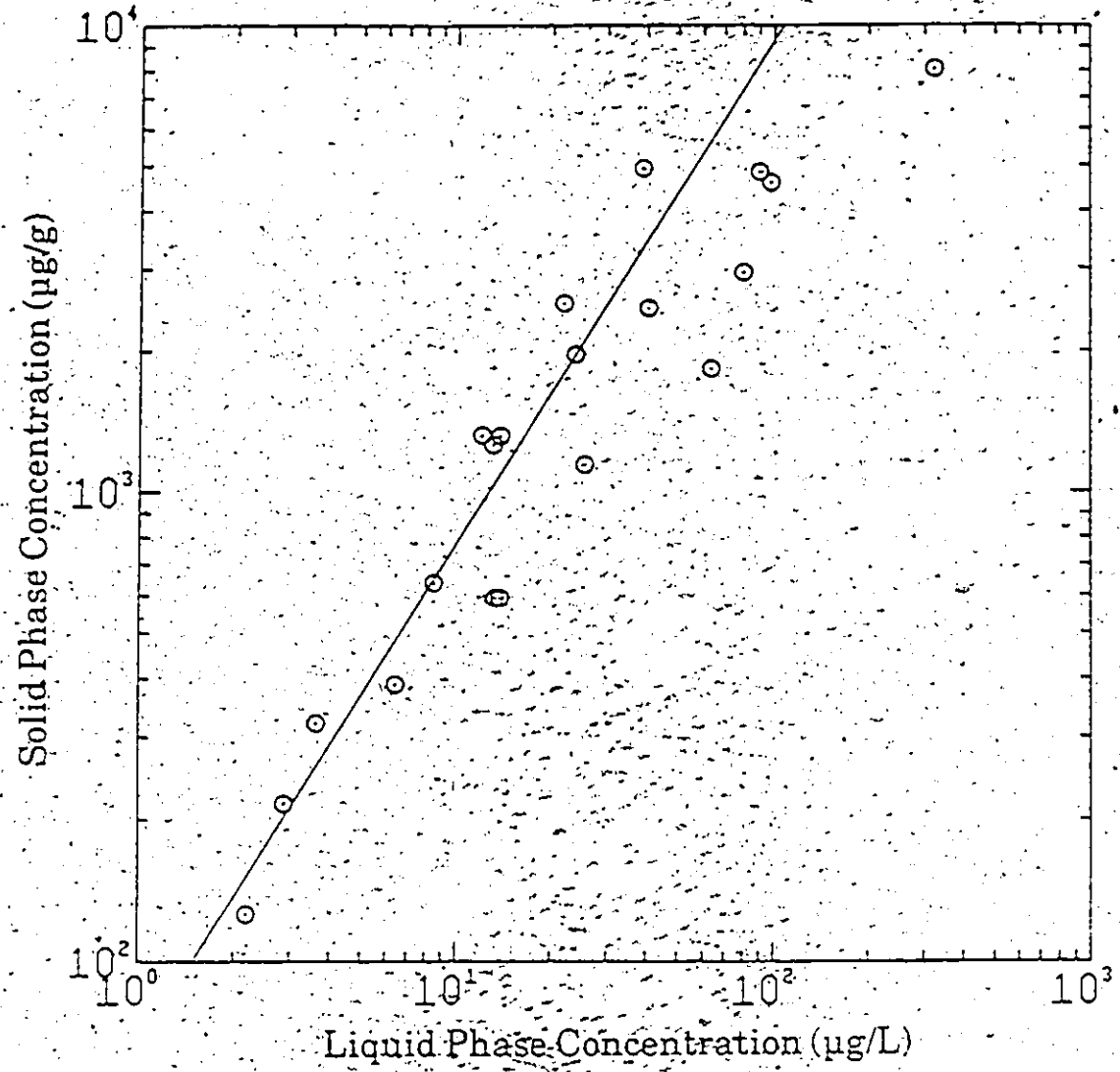


Figure 4.1.7 Biosorption of 2-chlorobiphenyl by *R. arrhizus*.

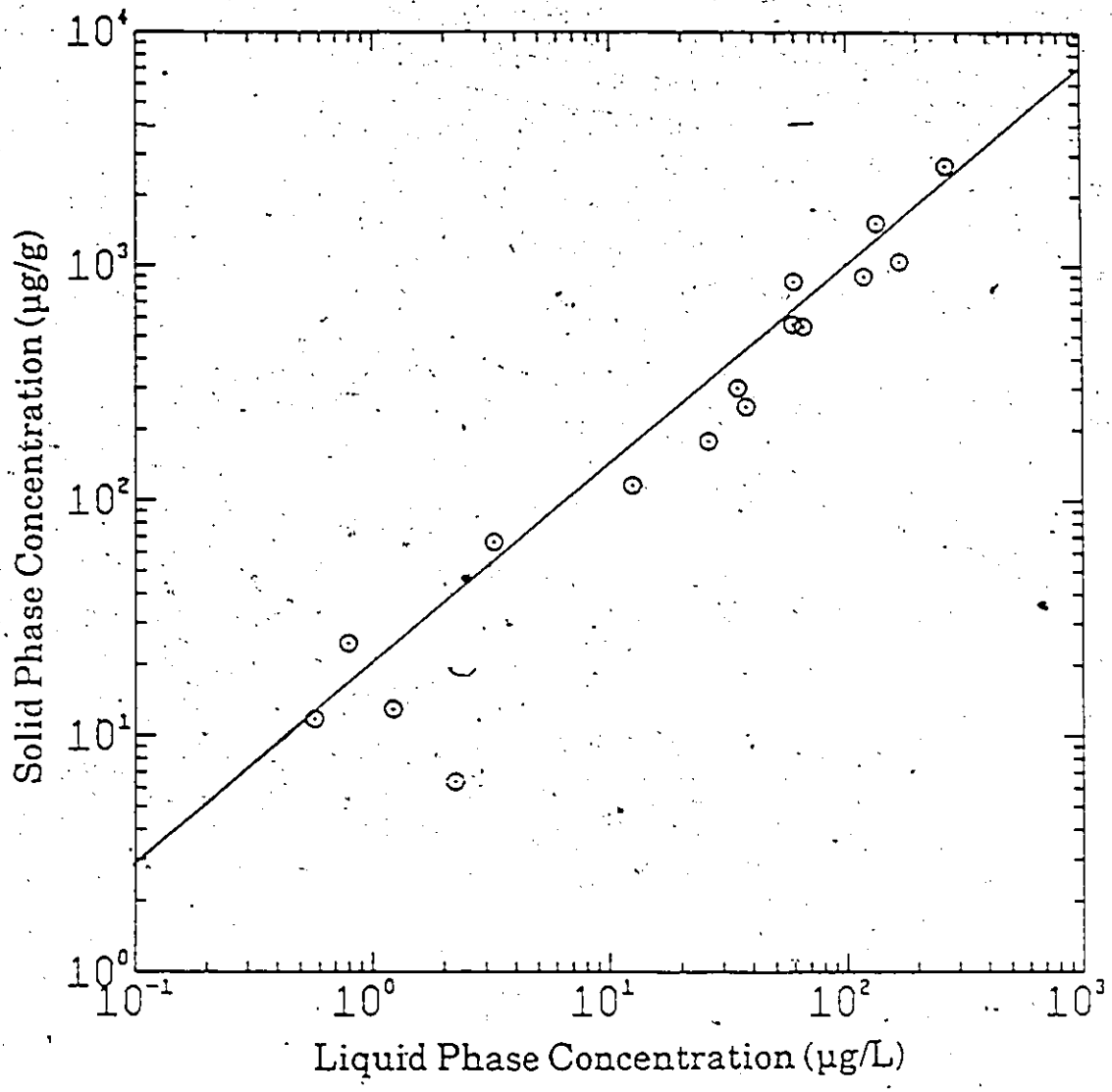


Figure 4.1.8 Biosorption of 2-chlorobiphenyl by activated sludge.

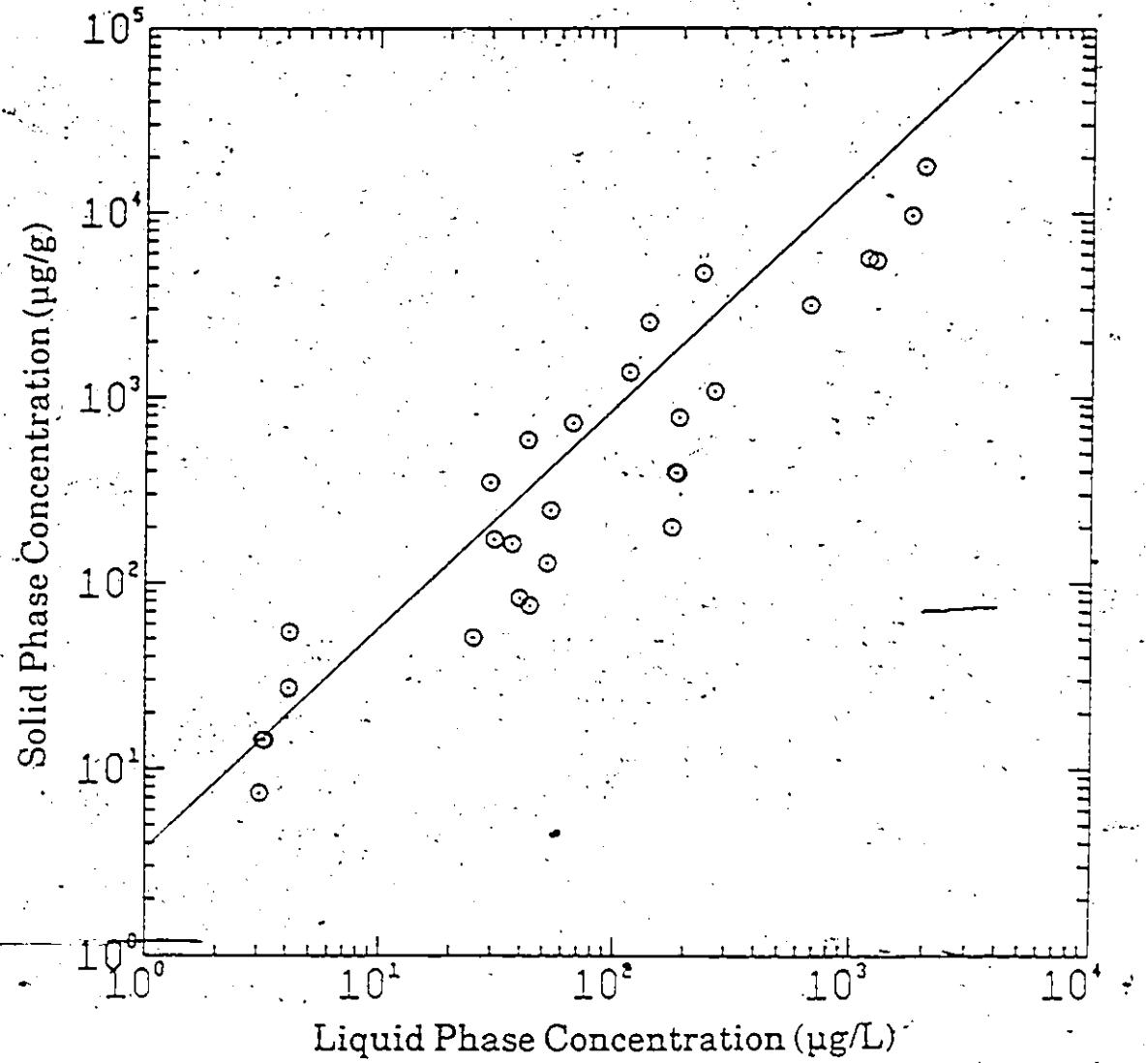


Figure 4.1.9 Apparent biosorption of malathion by *R. arrhizus*.

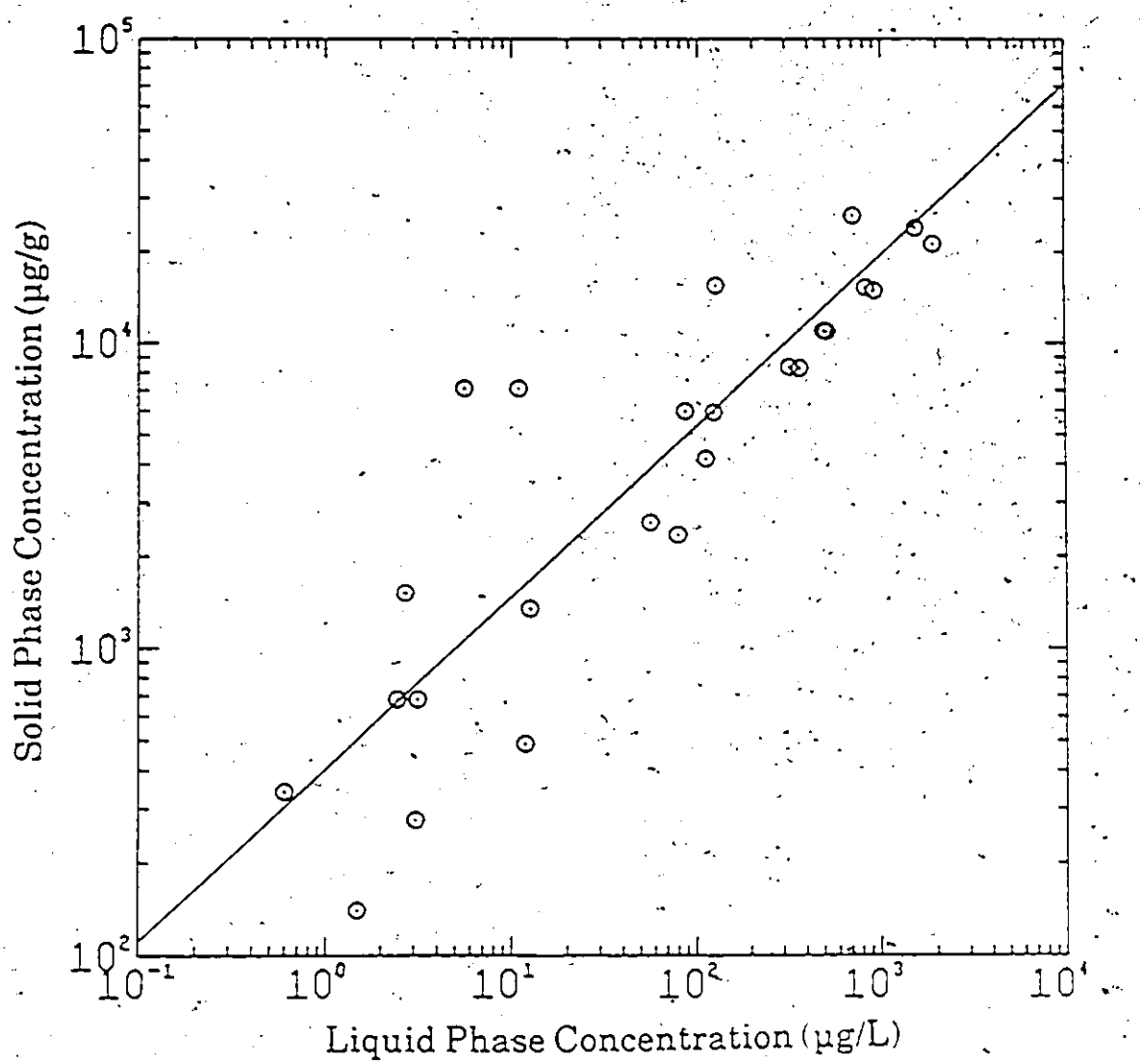


Figure 4.1.10 Apparent biosorption of malathion by activated sludge.

$$q = \frac{C_o - C}{B}$$

where

C_o = initial concentration of sorbate in solution, μg/L,

B = biomass concentration, g/L.

Typically, the Freundlich equation parameters are determined by applying linear regression to the logarithmic form of the equation, with log q taken as the dependent variable and log C taken as the independent variable. In the biosorption experiments, however, the true dependent variable is the equilibrium concentration, C, and the independent variables are the initial concentration, C_o, and the biomass concentration, B. In the present work the Freundlich parameters, K_F and n were determined, using the true dependent and independent variables, by a nonlinear least squares routine. Because the data cover a wide concentration range, a reduced least squares technique was used to avoid giving greater weight to the higher concentration values. The parameters which minimize

$$\sum \left(1 - \frac{C_{mod}}{C_{obs}}\right)^2$$

were determined, where

C_{mod} = values of C predicted by the model,

C_{obs} = observed values of C.

The Freundlich parameters for the biosorption isotherms at 20°C are given in Table 4.1.

Table 4.1 Freundlich parameters for biosorption isotherms at 20°C.

Compound	Biomass	K_F	1/n
Lindane	*R. arrhizus*	2.3	1.0
	Activated sludge	1.5	1.0
Pentachlorophenol	*R. arrhizus*	28.8	0.9
	Activated sludge	10.1	0.8
Diazinon	*R.arrhizus*	1.9	0.8
	Activated sludge	0.4	1.0
2-chlorobiphenyl	*R. arrhizus*	62.6	1.1
	Activated sludge	20.5	0.8
Malathion*	*R. arrhizus*	(3.7)	(1.2)
	Activated sludge	(402.9)	(0.6)

* Data assuming sorption mechanism only.

Table 4.2 gives the equilibrium biosorption loading of the biomass at various liquid phase concentrations, calculated from the Freundlich equation.

Table 4.2 Equilibrium biosorption loading at 20°C.

Compound	Biomass	Biosorption Loading at Stated Concentration (µg/g) 10 µg/L	100 µg/L	1000 µg/L
Lindane	*R. arrhizus*	24	254	2690
	Activated sludge	15	156	1570
Pentachlorophenol	*R.arrhizus*	231	1860	14900
	Activated sludge	69	478	3300
Diazinon	*R. arrhizus*	12	77	491
	Activated sludge	4	45	501
2-chlorobiphenyl	*R. arrhizus*	773	9250	111000
	Activated sludge	128	759	4491
Malathion*	*R. arrhizus*	(57)	(863)	(13200)
	Activated sludge	(1470)	(5340)	(19400)

* Data assuming sorption mechanism only

Apparent biosorption data for malathion are shown for reference although later experiments confirmed that at 20°C the primary mechanism for removal of malathion was chemical decomposition rather than biosorption.

4.2 TEMPERATURE EFFECTS ON BIOSORPTION

The effect of temperature on biosorptive uptake was examined by running biosorption experiments for selected compounds at different temperatures. Isotherms for lindane with both types of biomass were done at temperatures of 5°C and 34.5°C. Isotherms for diazinon and malathion were determined at 5°C for both types of biomass. Assessment of the temperature effects made possible the determination of some thermodynamic parameters of the biosorption process. Isotherms developed under different temperature conditions are shown in Figures 4.2.1 through 4.2.8. Experimental data for these isotherms are given in Appendix B2. Table 4.3 gives the Freundlich parameters for biosorption isotherms at 5°C and 34.5°C.

Table 4.3 Freundlich parameters for biosorption at 5°C and 34.5°C.

Compound	Biomass	Temperature, °C	K_F	1/n
Lindane	*R. arrhizus*	34.5	1.64	1.0
	Activated sludge	34.5	0.71	1.1
	R. arrhizus	5.0	3.18	1.0
	Activated sludge	5.0	1.82	1.0
Diazinon	*R. arrhizus*	5.0	0.08	1.3
	Activated sludge	5.0	0.13	1.2
Malathion*	*R. arrhizus*	5.0	(0.39)	(1.2)
	Activated sludge	5.0	(0.54)	(0.9)

* Data assuming sorption mechanism only.

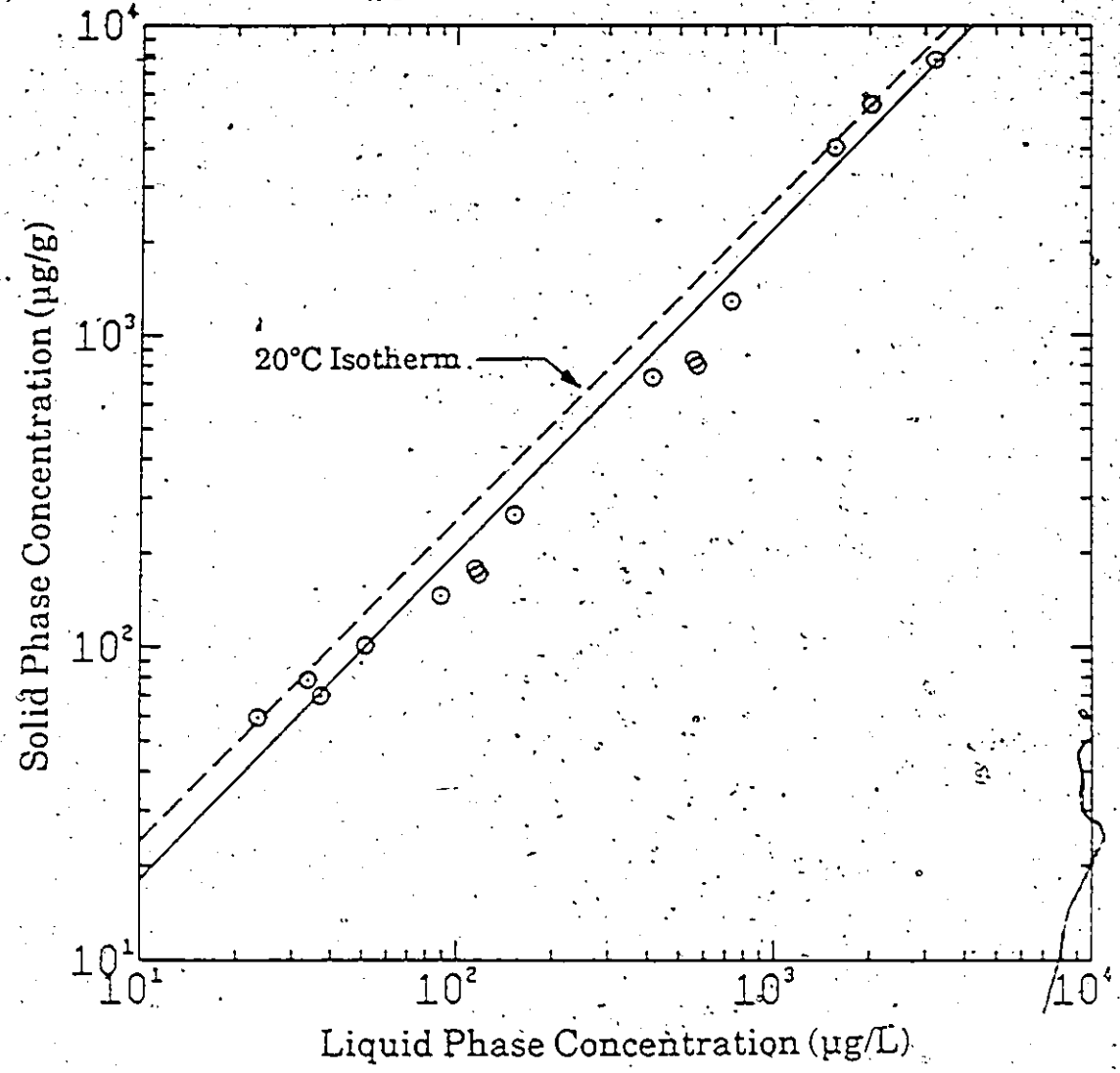


Figure 4.2.1 Biosorption of lindane by *R. arrhizus* at 34.5°C.

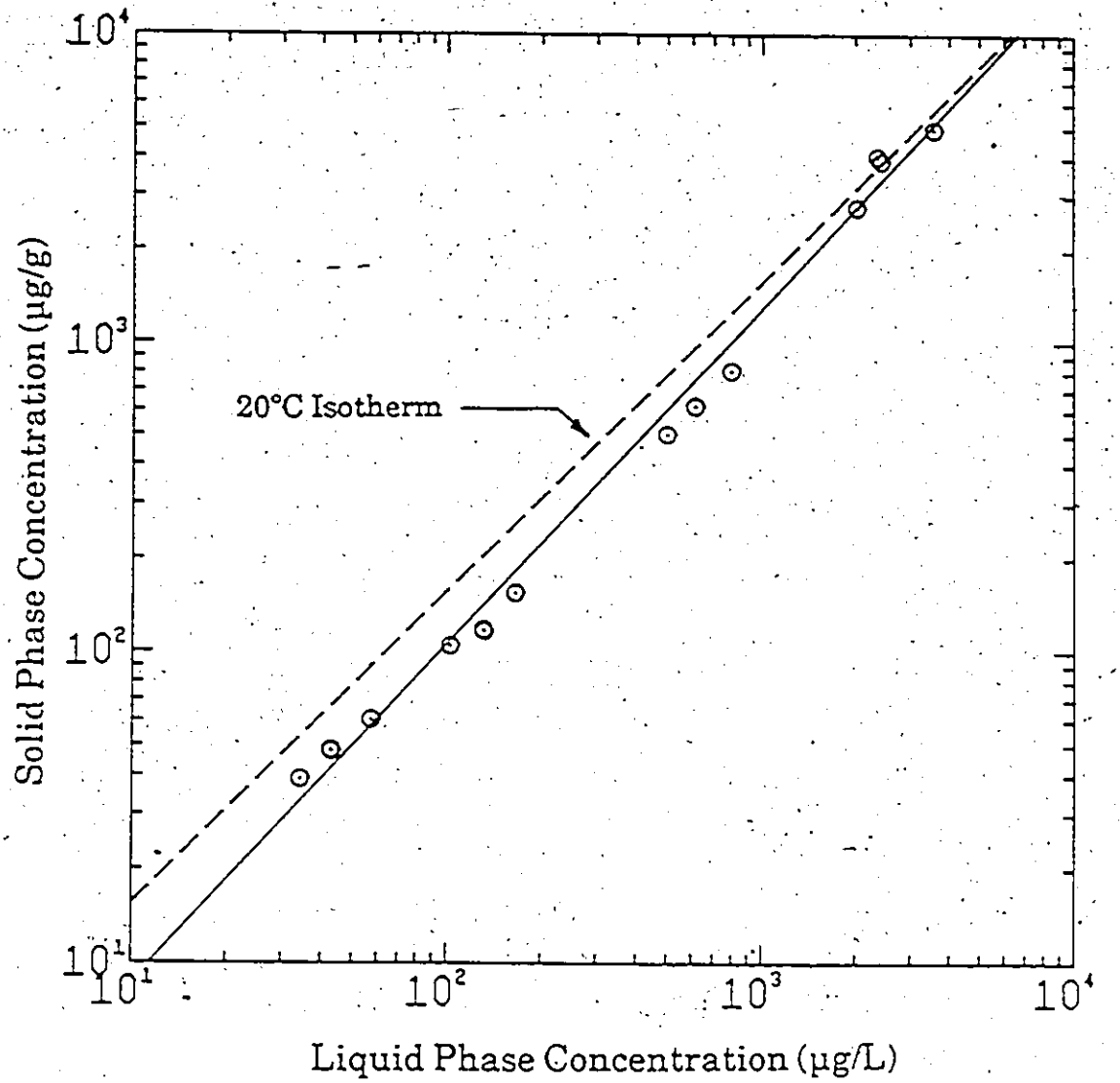


Figure 4.2.2 Biosorption of lindane by activated sludge at 34.5°C.

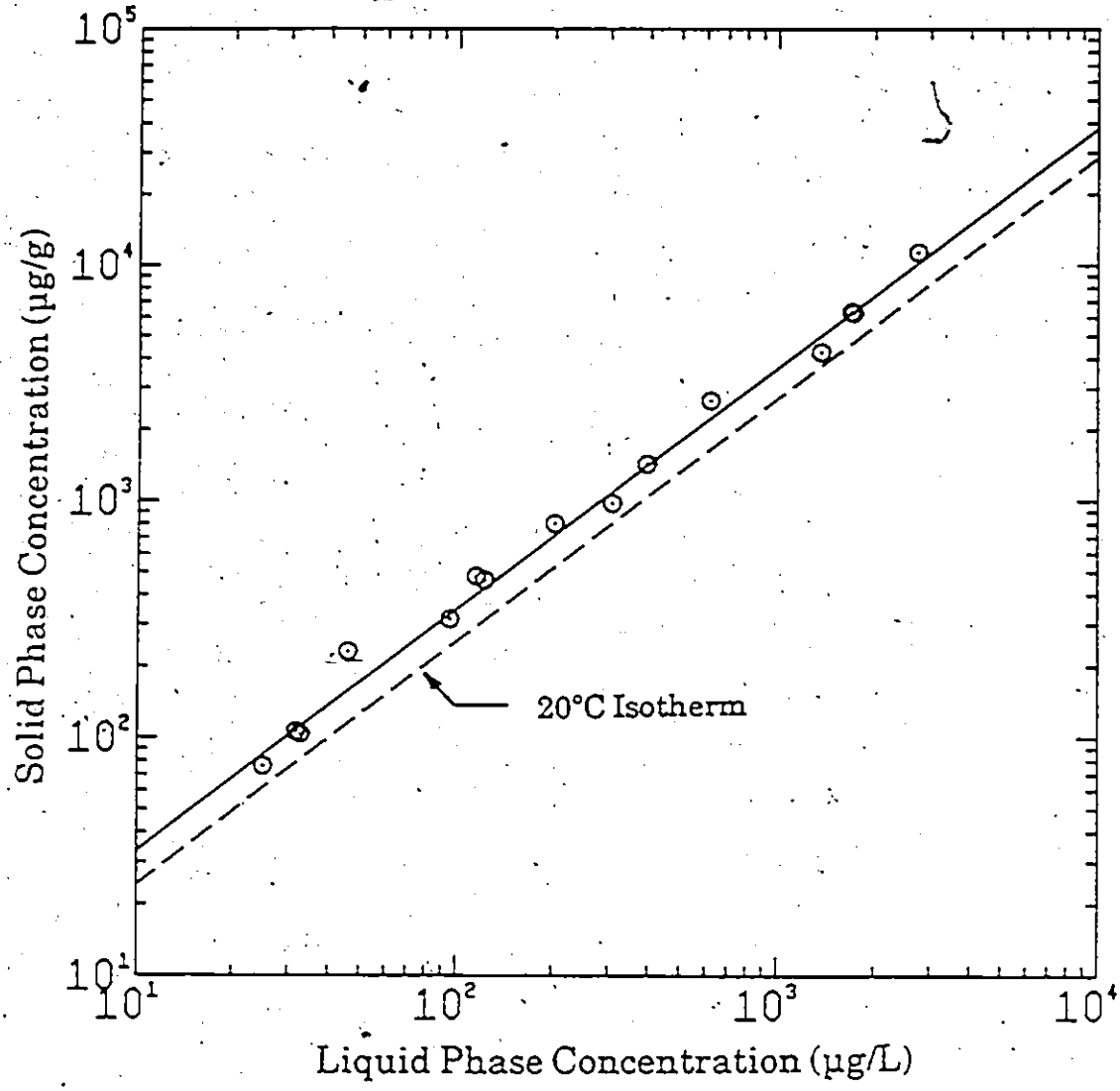


Figure 4.2.3 Biosorption of lindane by *R. arrhizus* at 5°C.

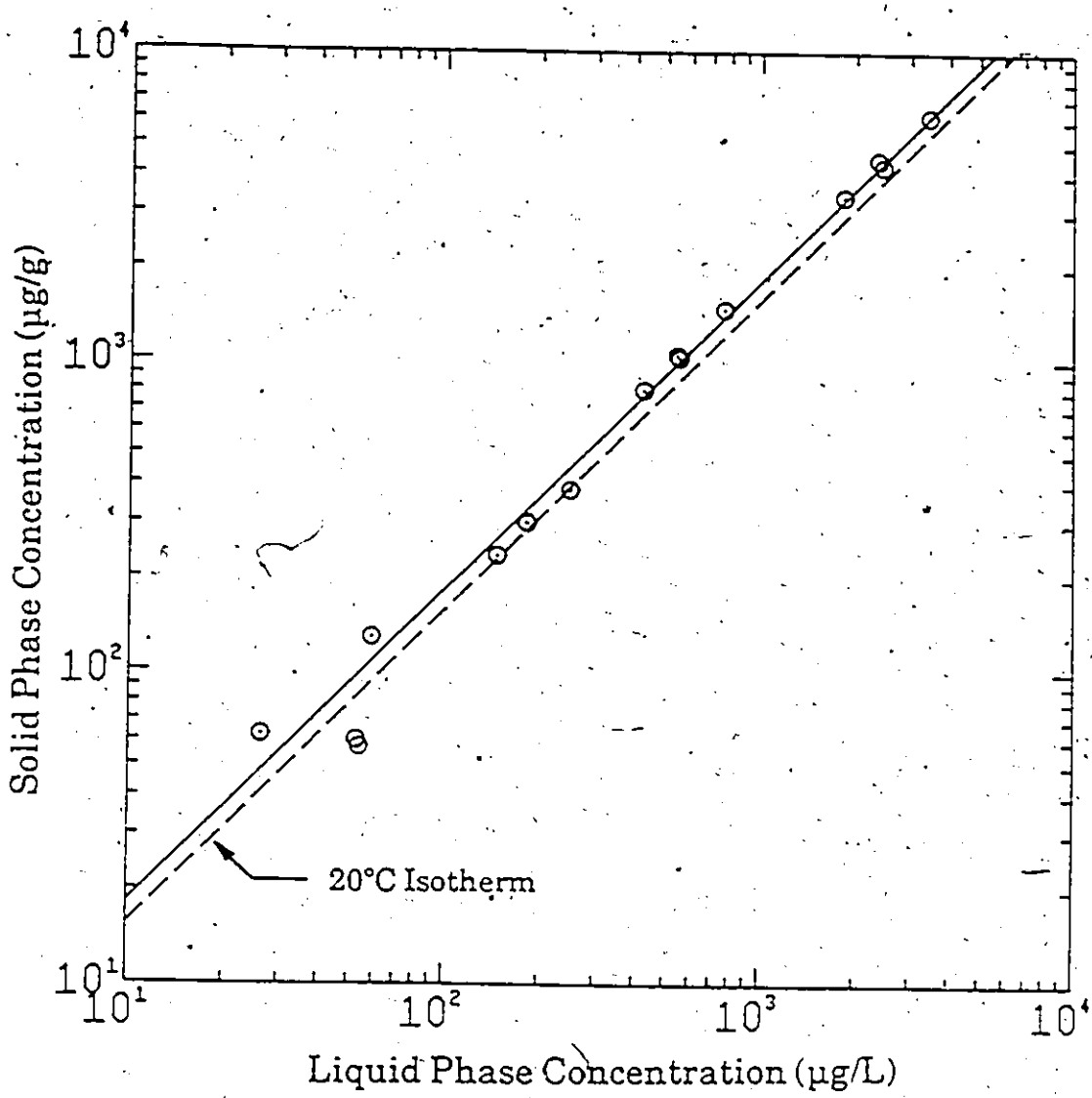


Figure 4.2.4 Biosorption of lindane by activated sludge at 5°C.

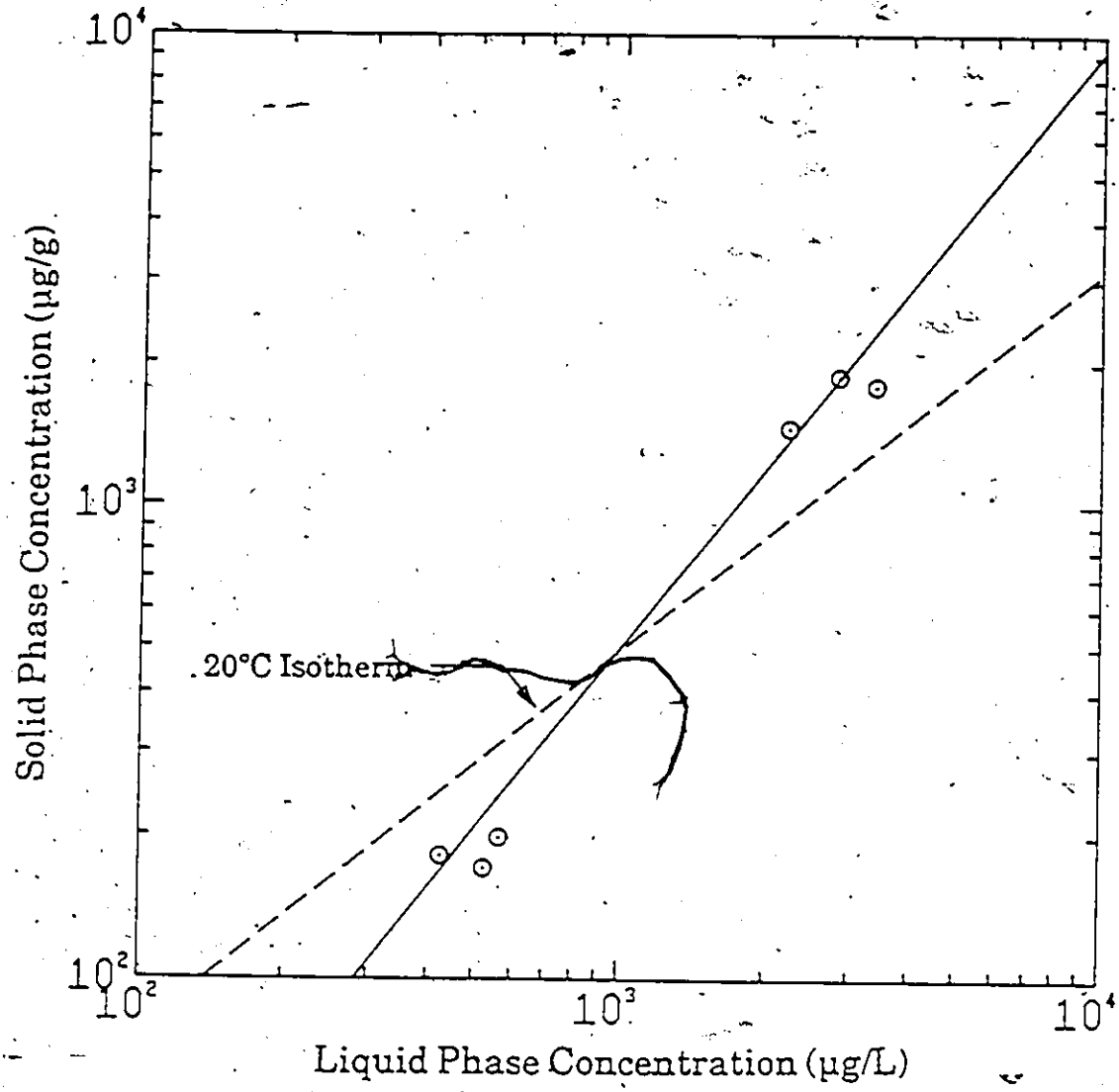


Figure 4.2.5 Biosorption of diazinon by *R. arrhizus* at 5°C.

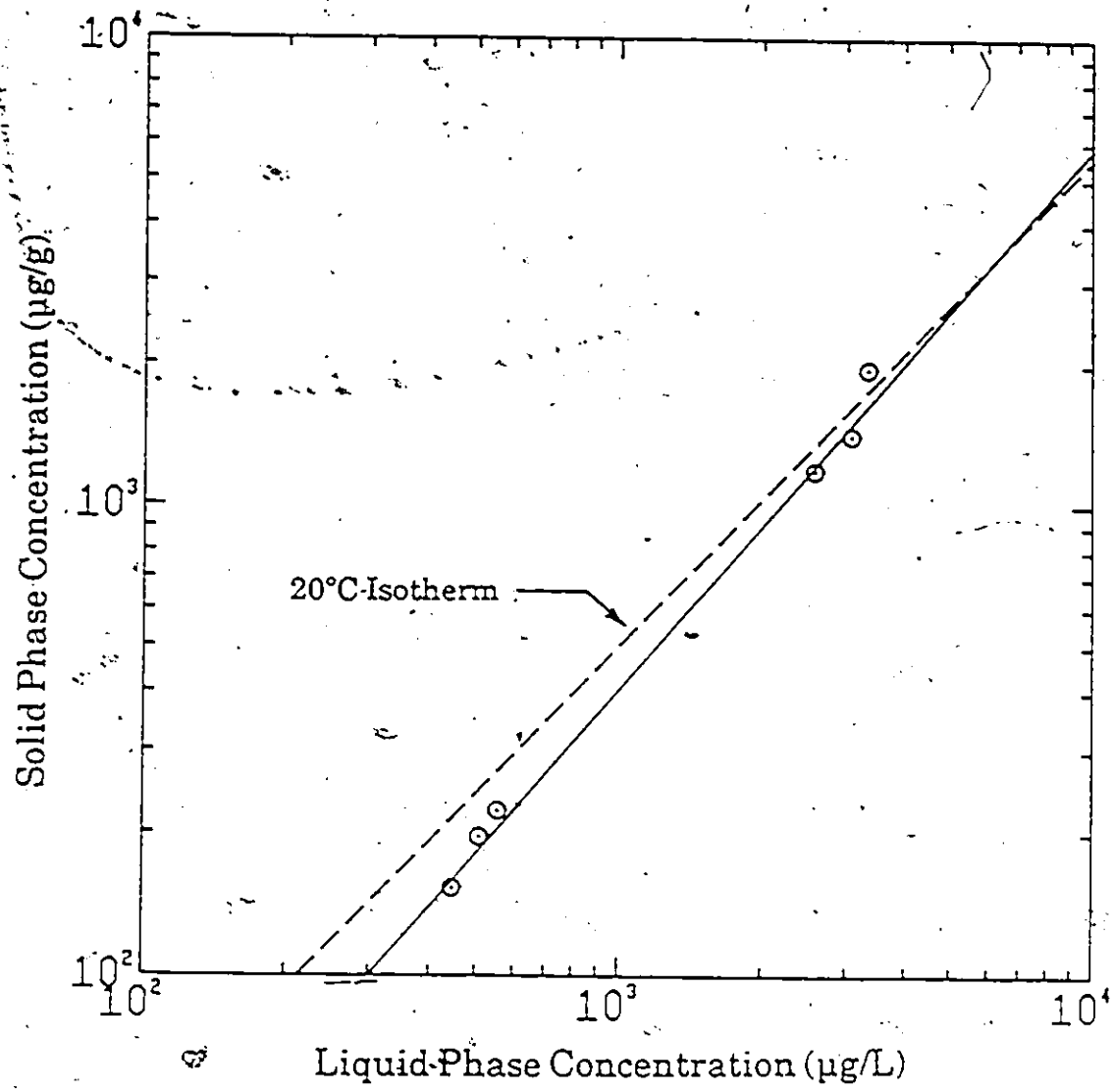


Figure 4.2.6 Biosorption of diazinon by activated sludge at 5°C.

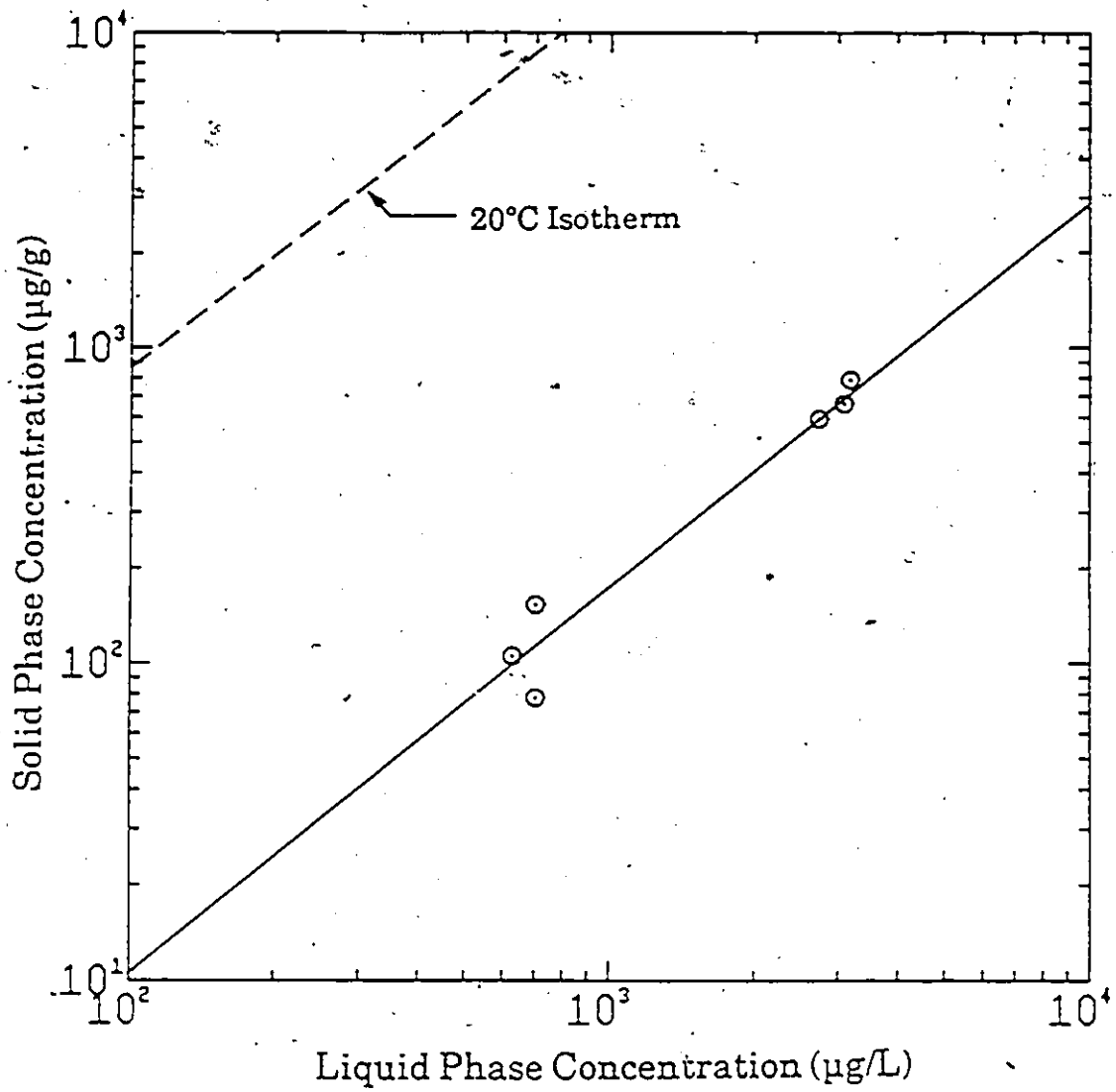


Figure 4.2.7 Apparent biosorption of malathion by *R. arrhizus* at 5°C.

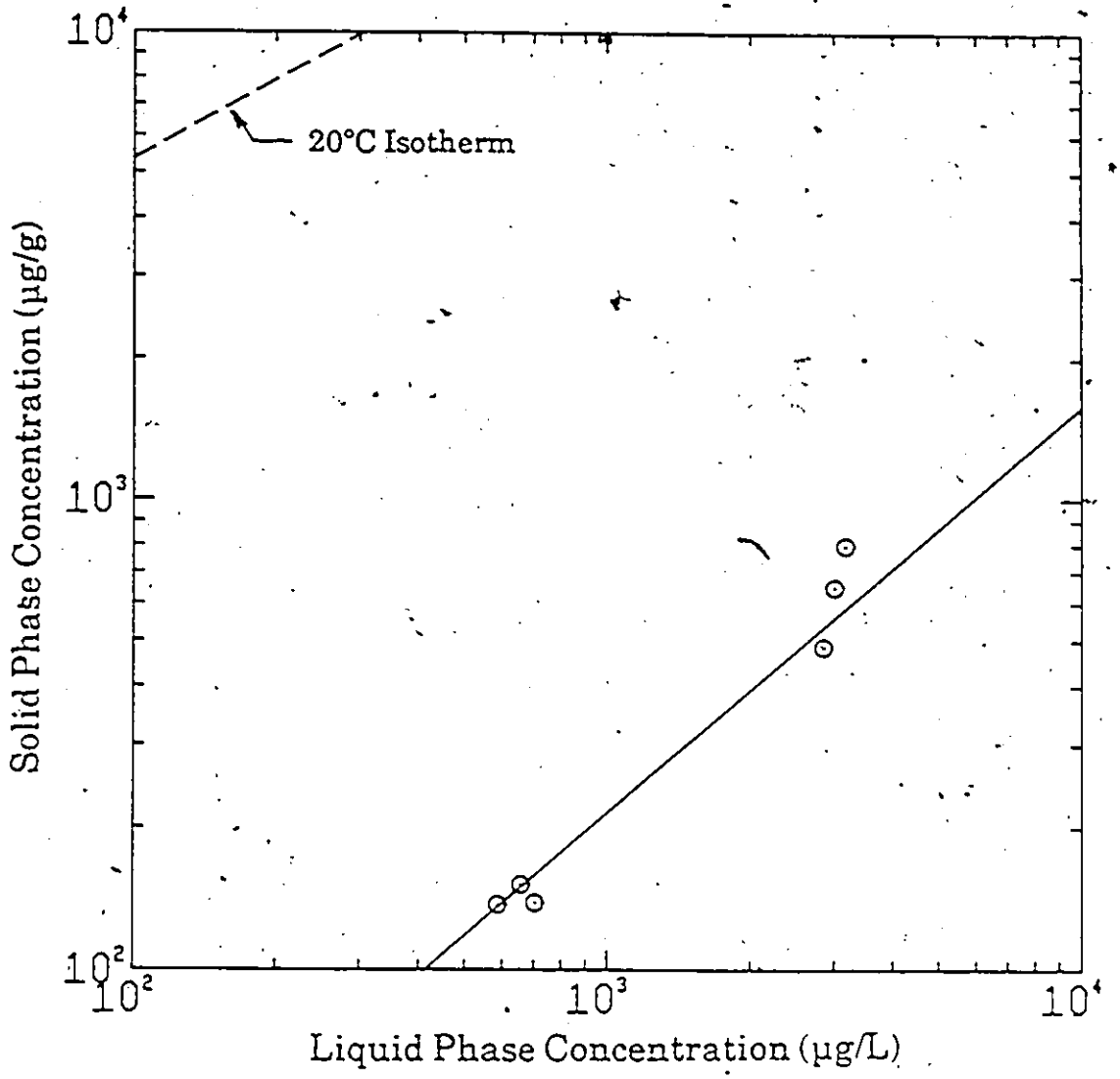


Figure 4.2.8 Apparent biosorption of malathion by activated sludge at 5°C.

4.3 DESORPTION

Desorption isotherms were developed for lindane, diazinon, 2-chlorobiphenyl, and malathion, for both types of biomass. Figures 4.3.1 through 4.3.10 show the desorption data points together with the sorption isotherm lines which are shown for comparison. Desorption data are given in Appendix B3. Negative values of loading shown in Tables B3.3 and B3.7 are the result of uncertainty in the determination of the equilibrium solution concentrations (see Sections 3.4.1 and 3.6).

4.4 SORPTION ON CELL WALLS

Biosorption experiments were conducted with lindane, diazinon, and malathion on *R. arrhizus* and activated sludge cell wall preparations. Figures 4.4.1 through 4.4.6 show the sorption data for cell walls with the isotherm lines for whole cells given for comparison. Cell wall sorption data are given in Appendix B4.

4.5 KINETICS OF BIOSORPTION

The kinetics of biosorption of lindane by *R. arrhizus* and activated sludge were determined using a stirred tank batch reactor. In each case the mixing rate was varied to determine whether the rate of mass transfer was affected by mixing in the bulk solution or if the intrinsic sorption rate was controlling. Figures 4.5.1 and 4.5.2 show reduced liquid phase concentration (concentration/initial concentration) as a function of time for lindane sorption by *R. arrhizus* and activated sludge. Data for two different mixing speeds are shown in each case. Since there is no significant difference in the uptake rate with the higher mixing speed it can be concluded that the rate being measured is the intrinsic biosorption rate, independent of the rate of mass transfer in the bulk solution. Figures 4.5.3 and 4.5.4 show the solid phase concentration versus time. The lines through the data are hand drawn to aid in visualization

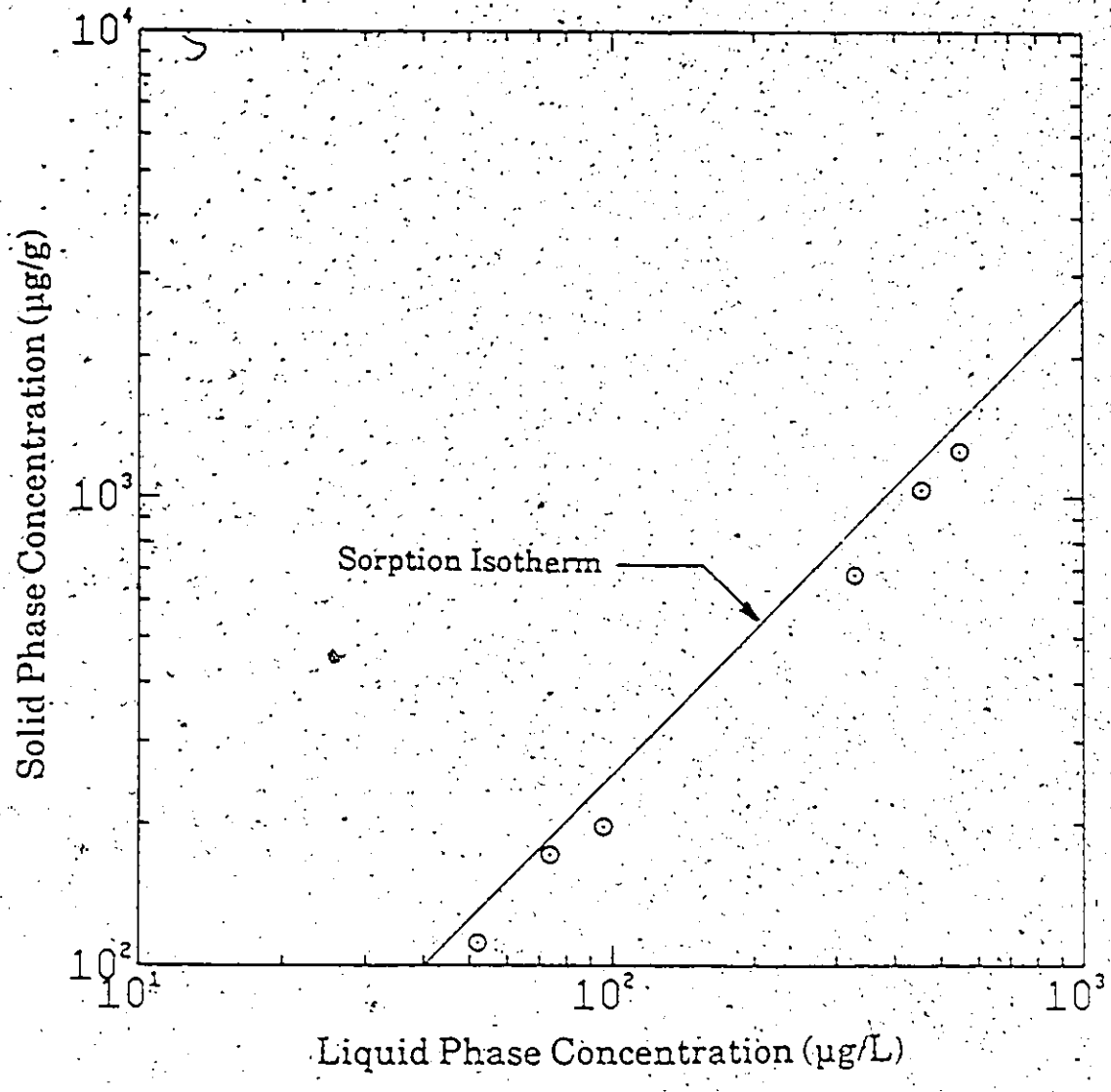


Figure 4.3.1 Desorption of lindane by *R. arrhizus*.

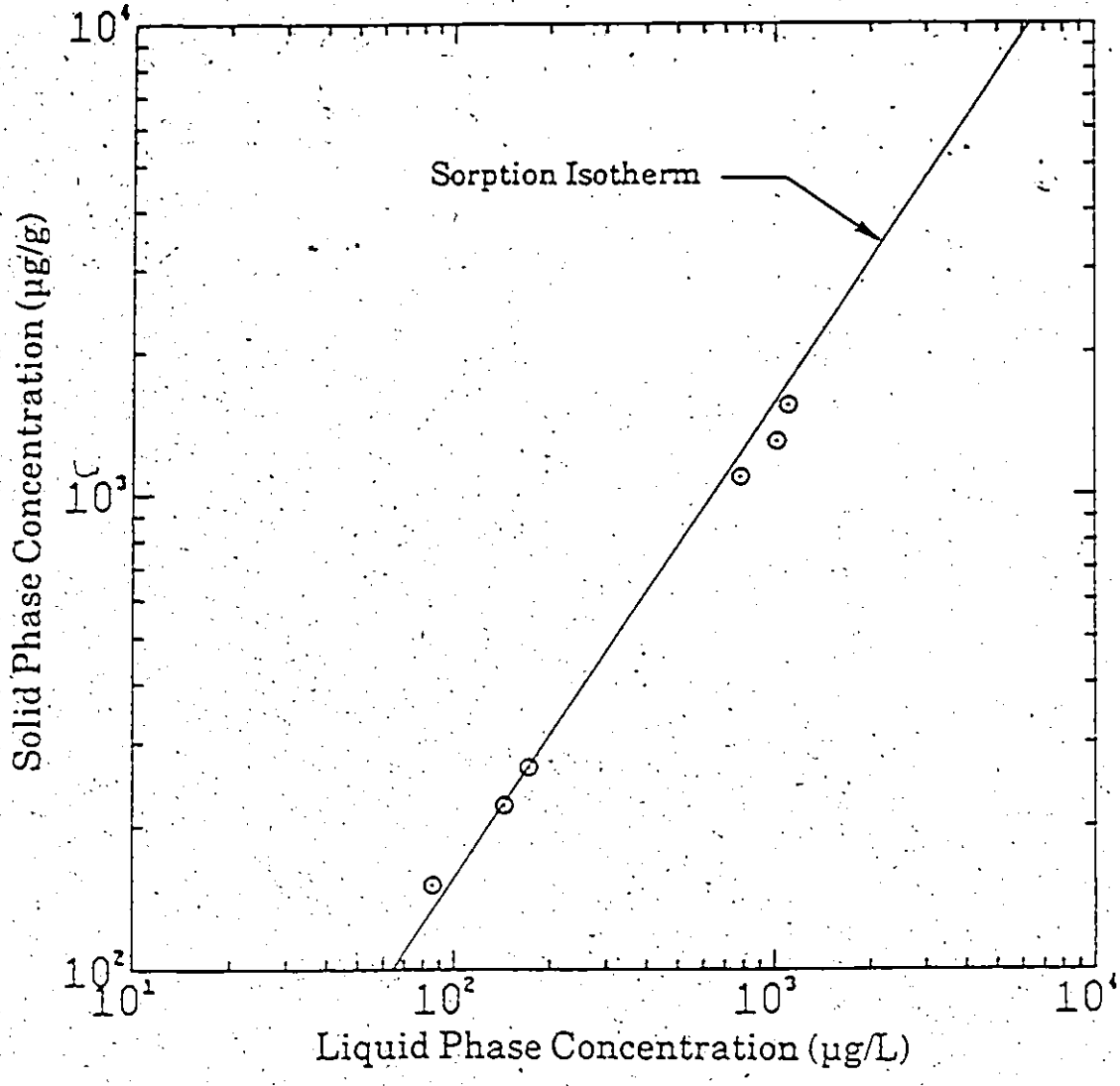


Figure 4.3.2. Desorption of lindane by activated sludge.

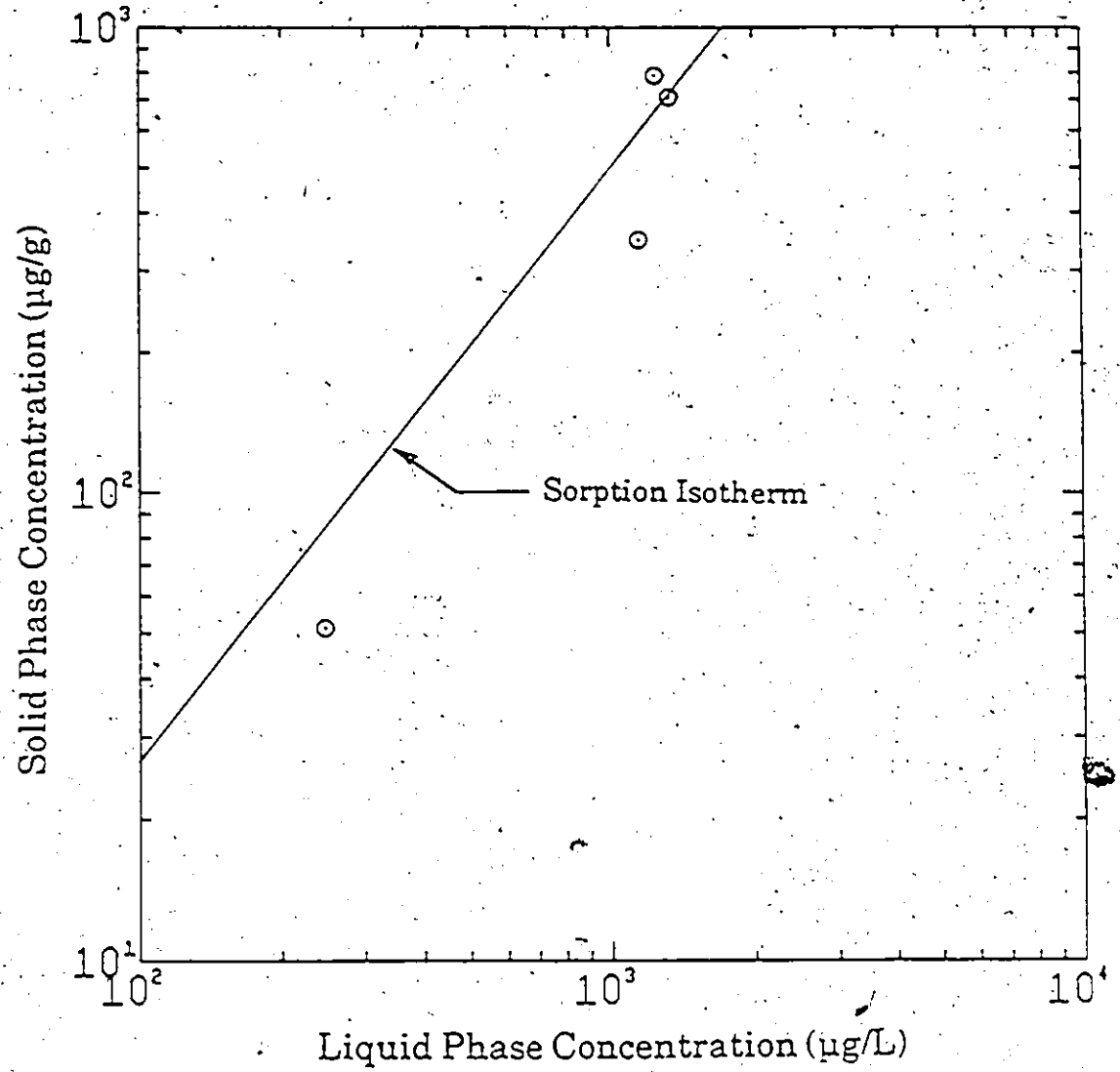


Figure 4.3.3 Desorption of diazinon by *R. arrhizus* at 5°C.

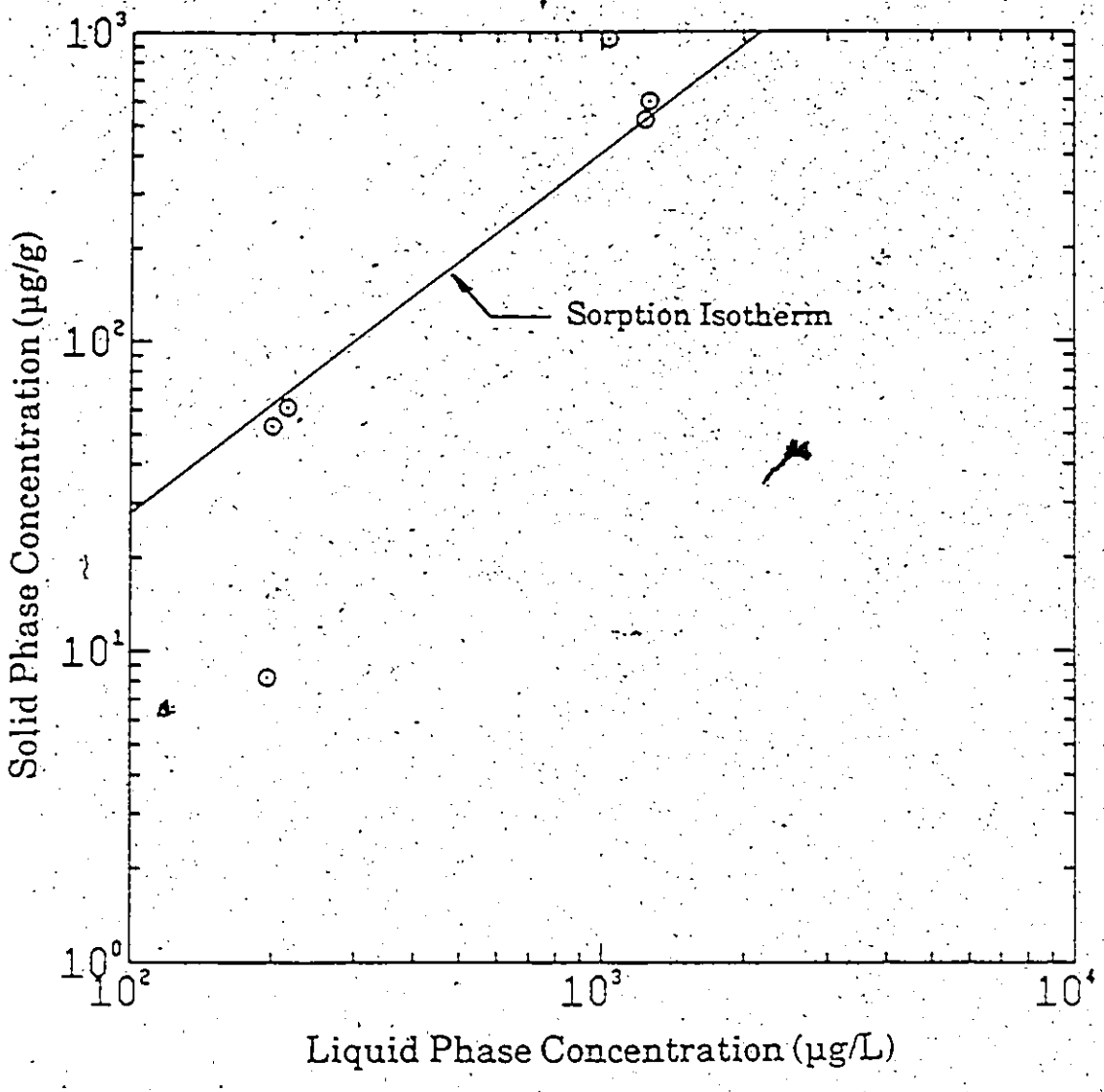


Figure 4.3.4 Desorption of diazinon by activated sludge at 5°C.

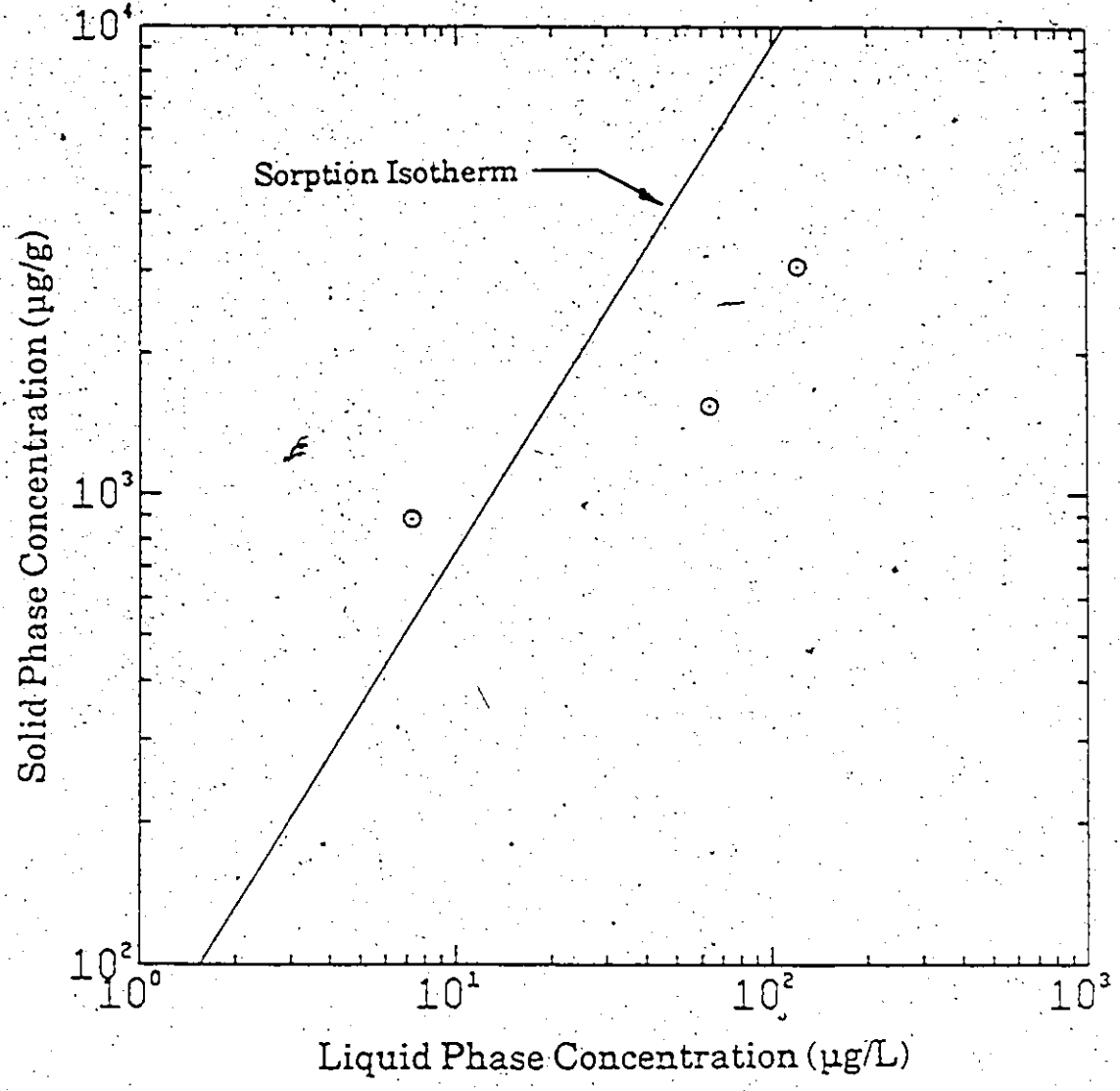


Figure 4.3.5 Desorption of 2-chlorobiphenyl by *R. arrhizus*.

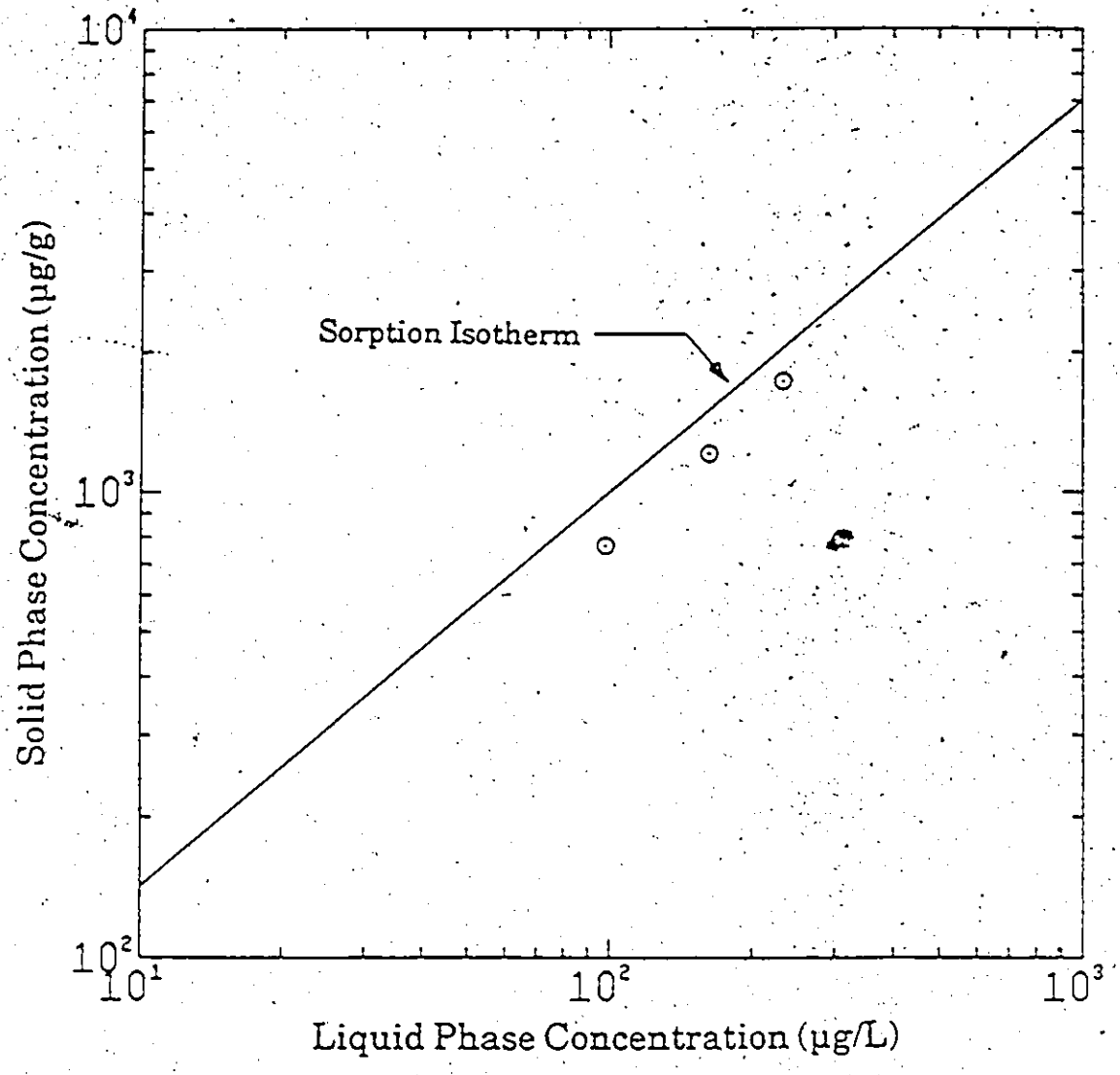


Figure 4.3.6 Desorption of 2-chlorobiphenyl by activated sludge.

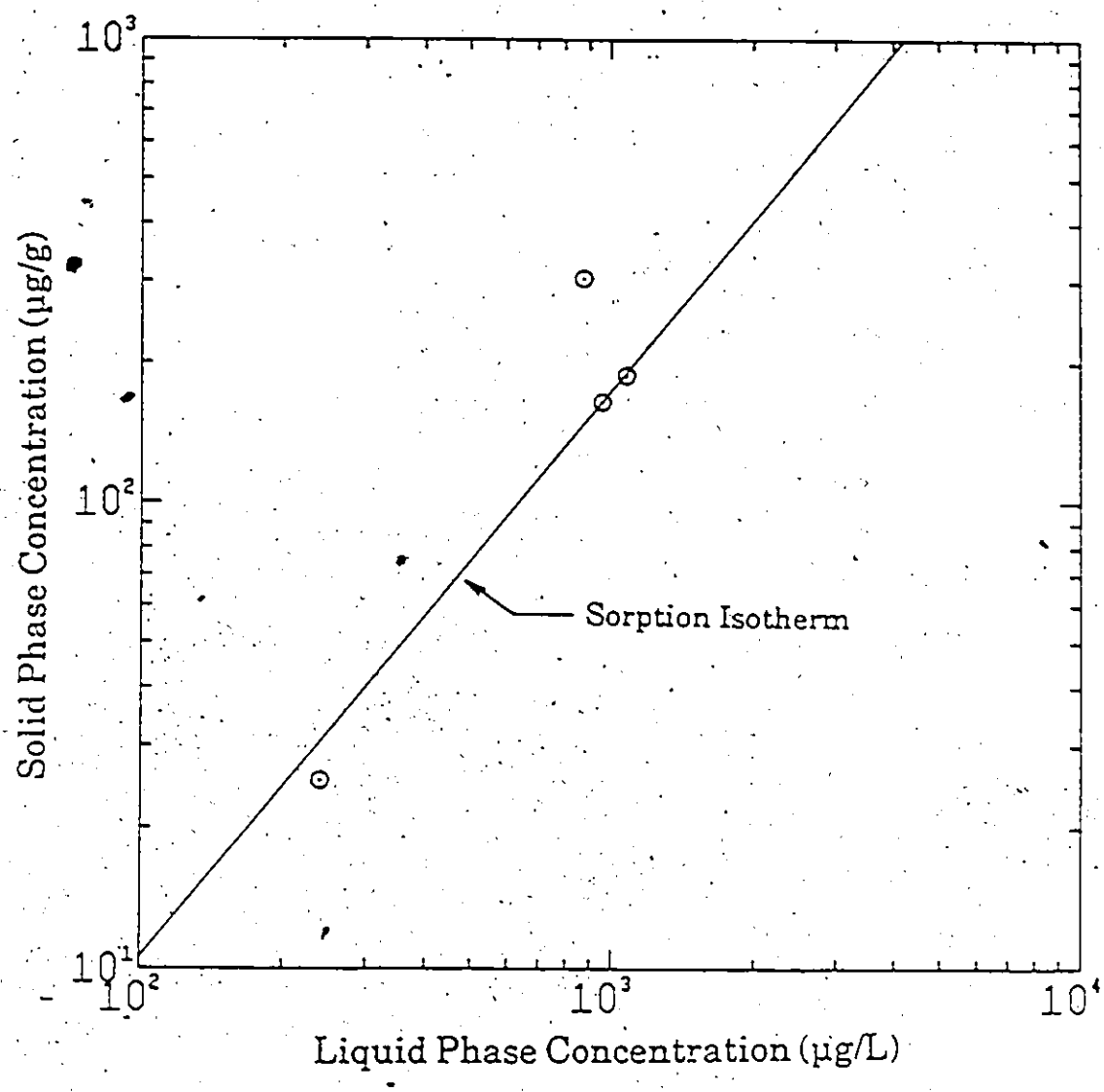


Figure 4.3.7 Apparent desorption of malathion by *R. arrhizus* at 5°C.

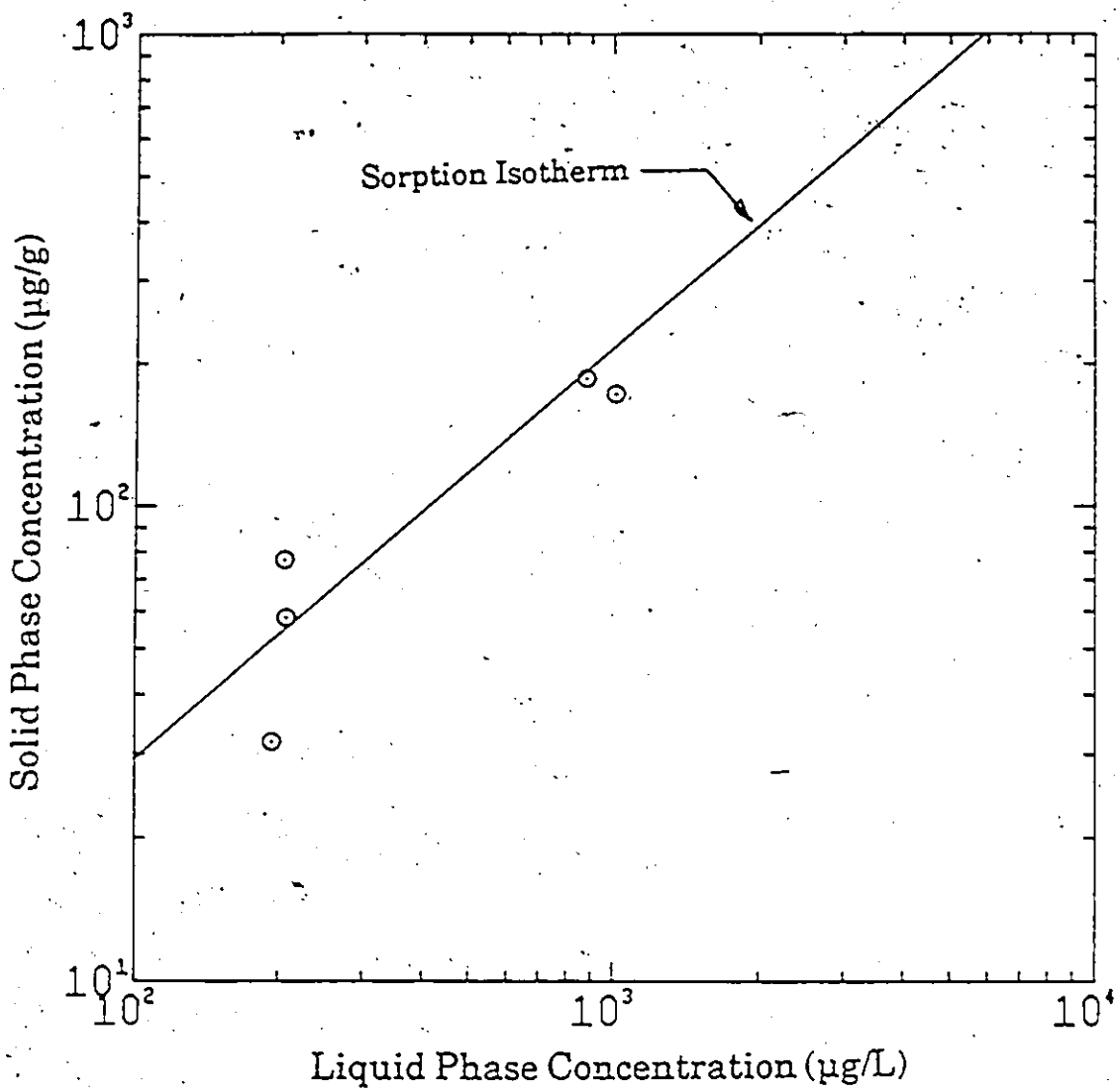


Figure 4.3.8 Apparent desorption of malathion by activated sludge at 5°C.

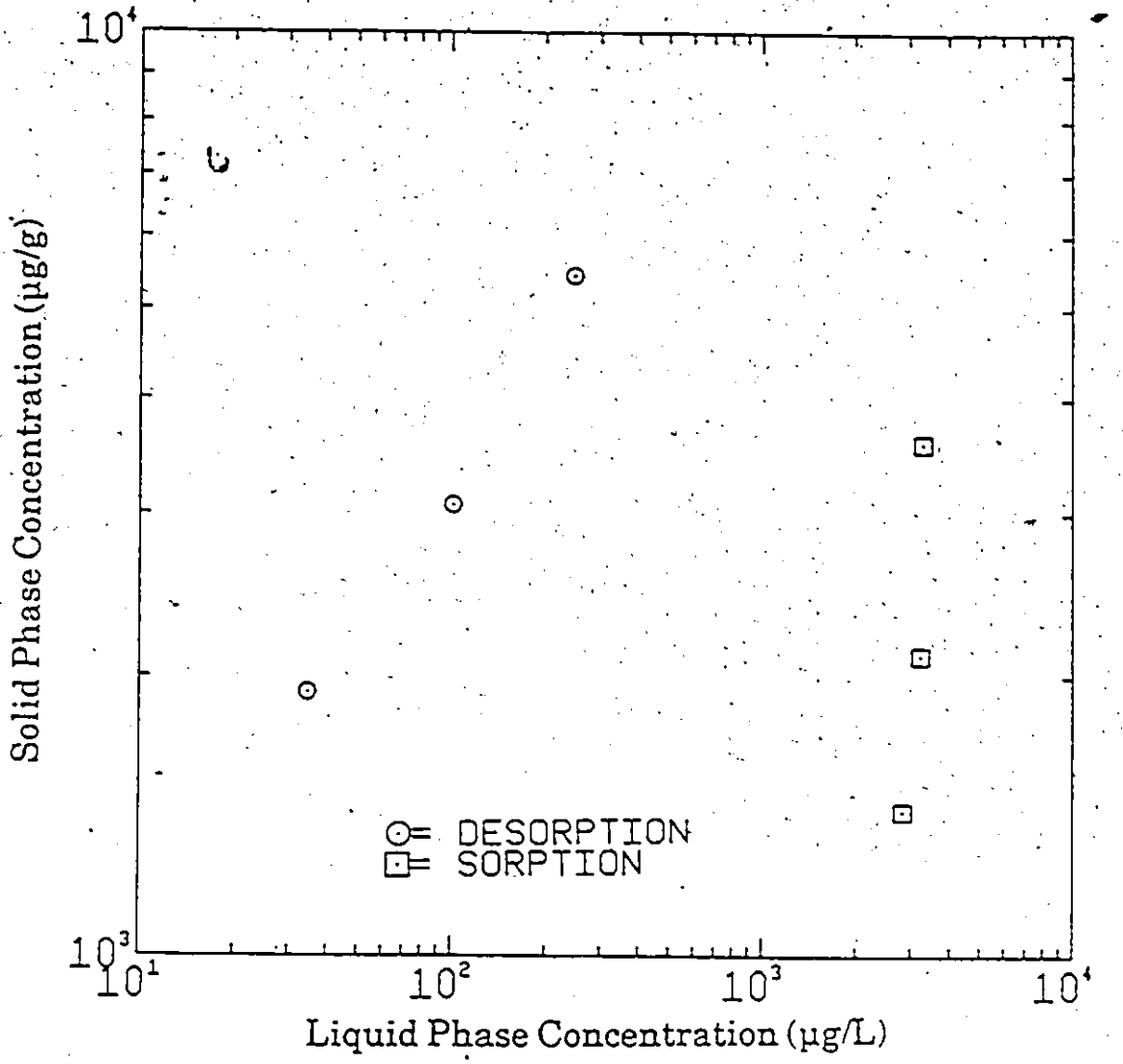


Figure 4.3.9 Apparent desorption of malathion by *R. arrhizus* at 20°C.

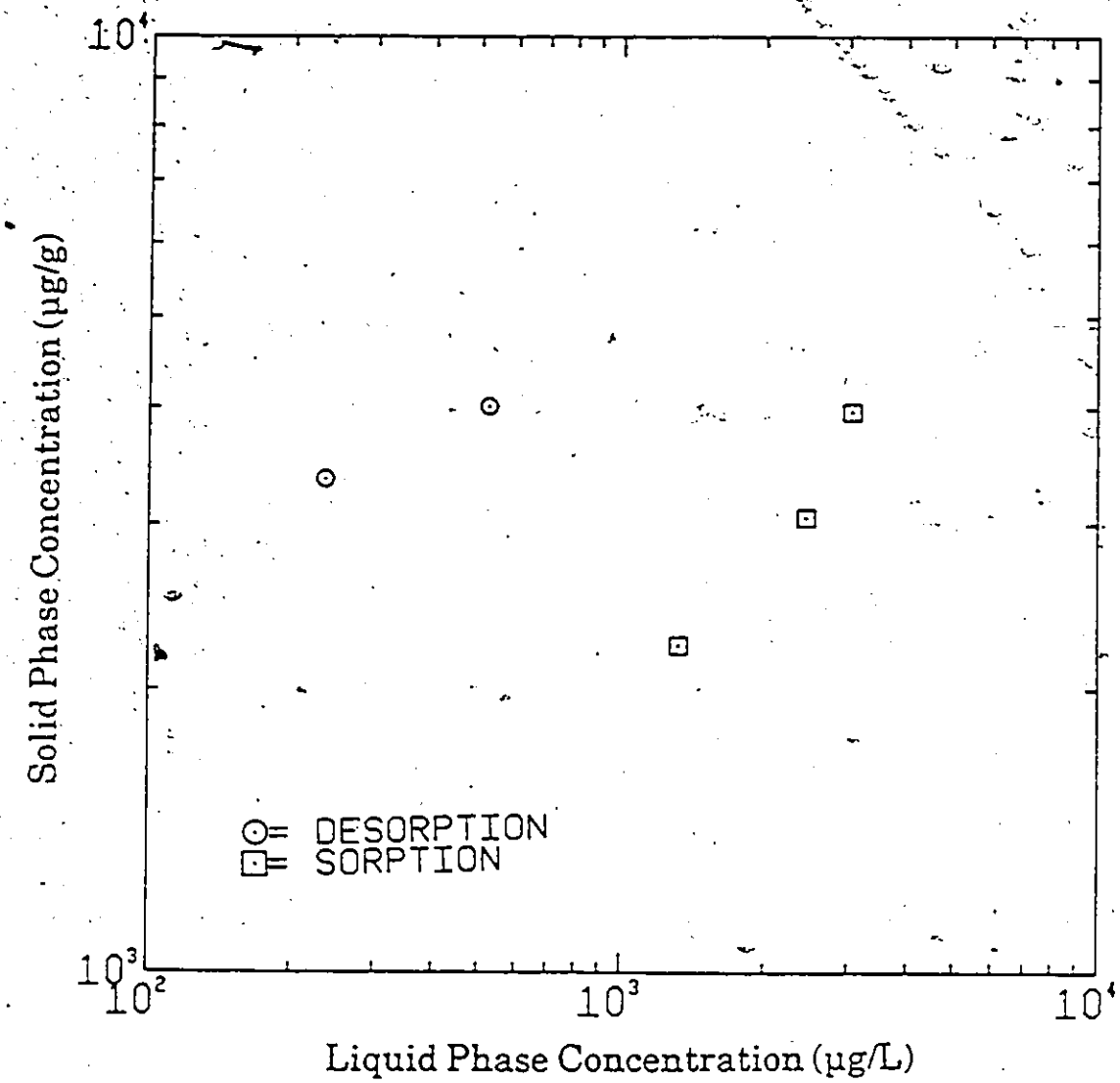


Figure 4.3.10 Apparent desorption of malathion by activated sludge at 20°C.

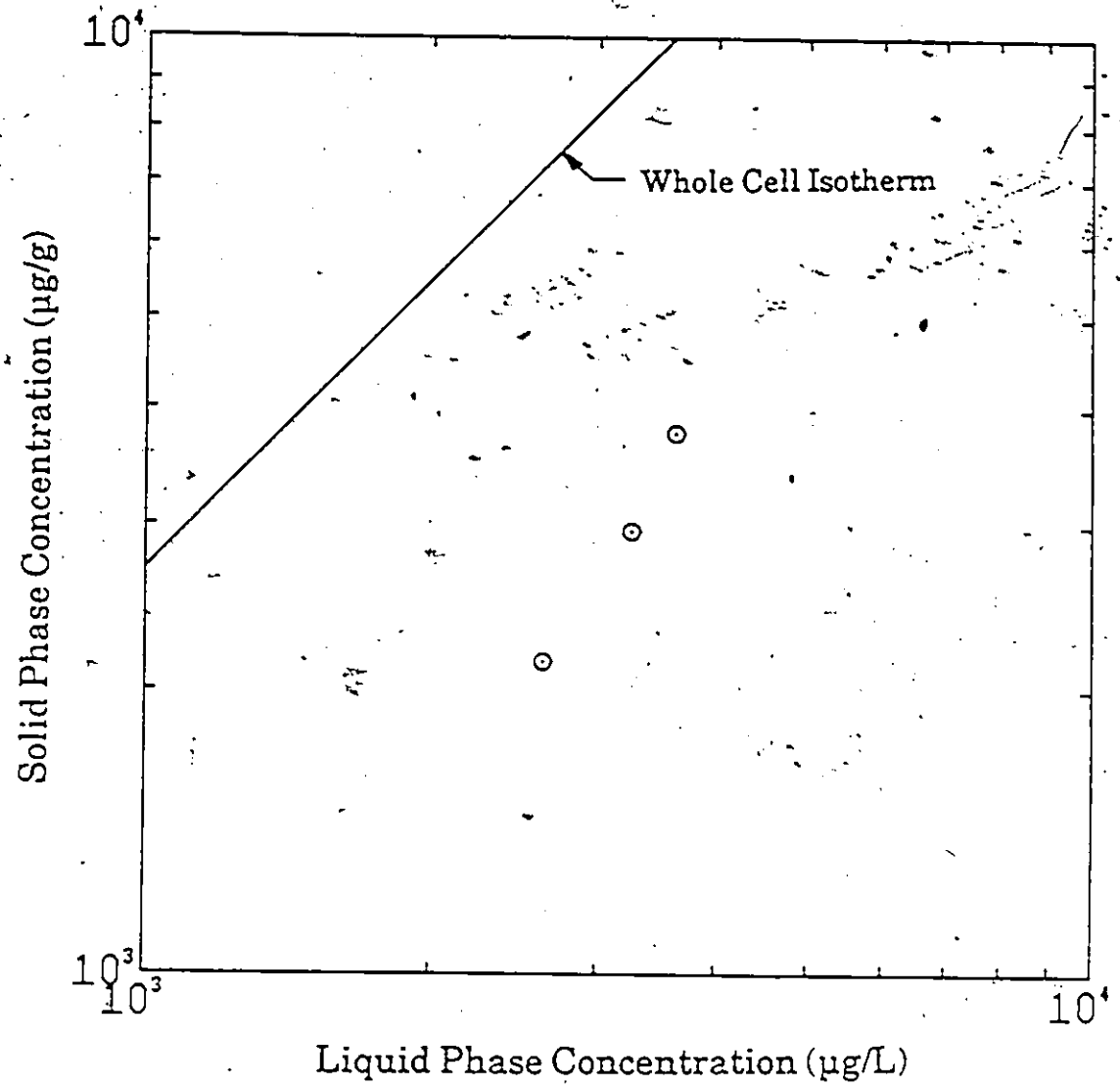


Figure 4.4.1 Biosorption of lindane by *R. arrhizus* cell walls.

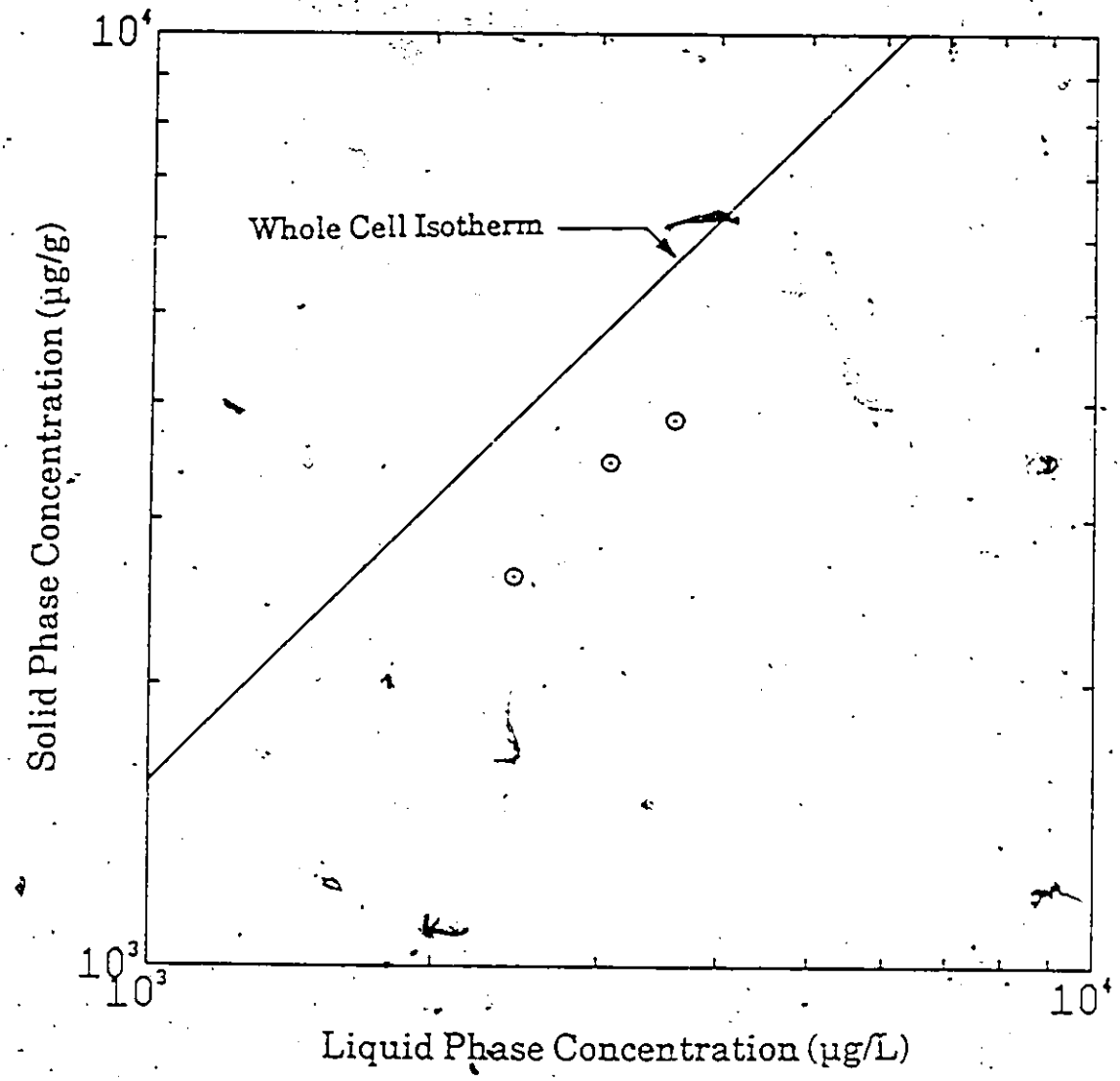


Figure 4.4.2 Biosorption of lindane by activated sludge cell walls.

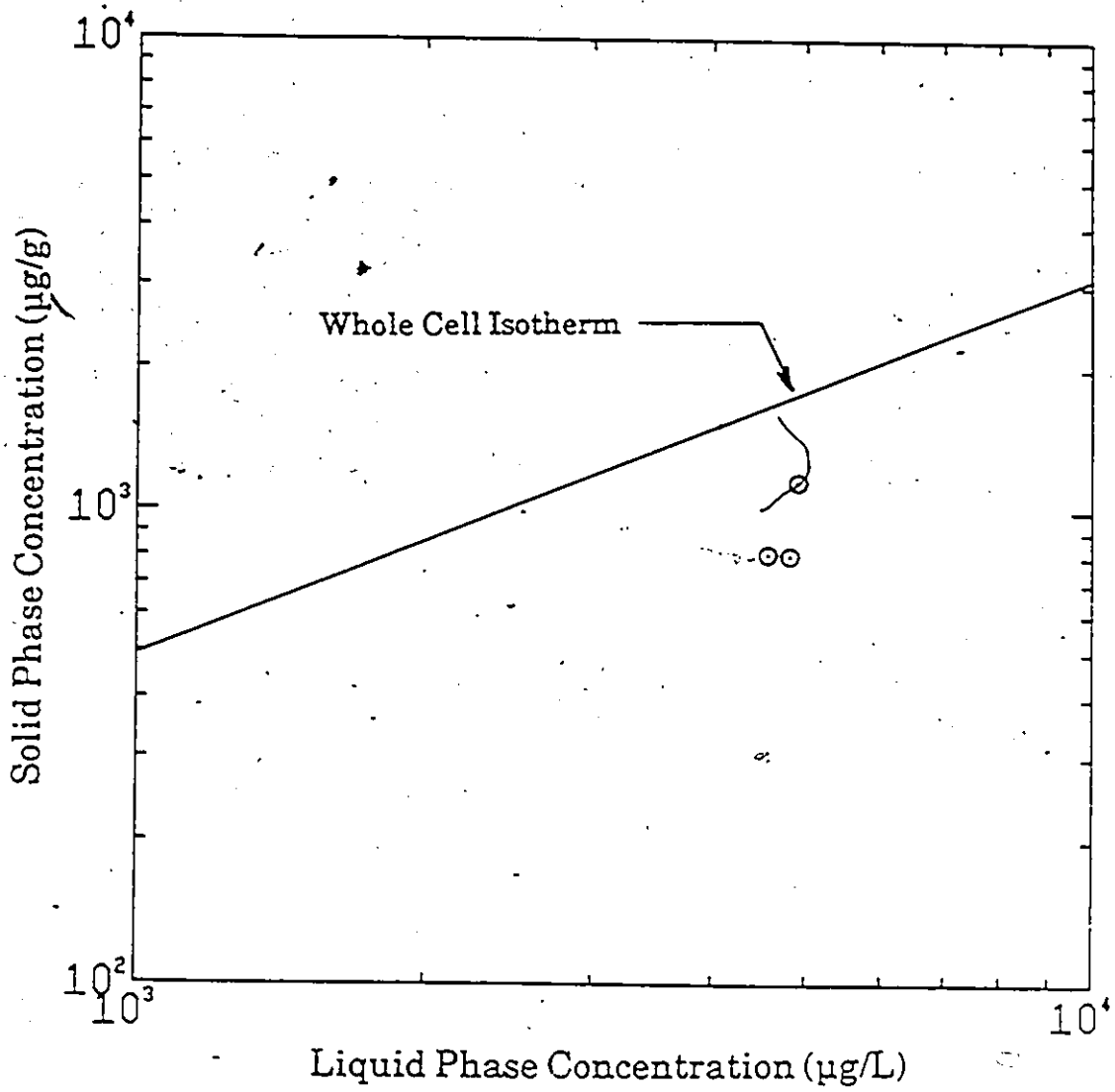


Figure 4.4.3 Biosorption of diazinon by *R. arrhizus* cell walls.

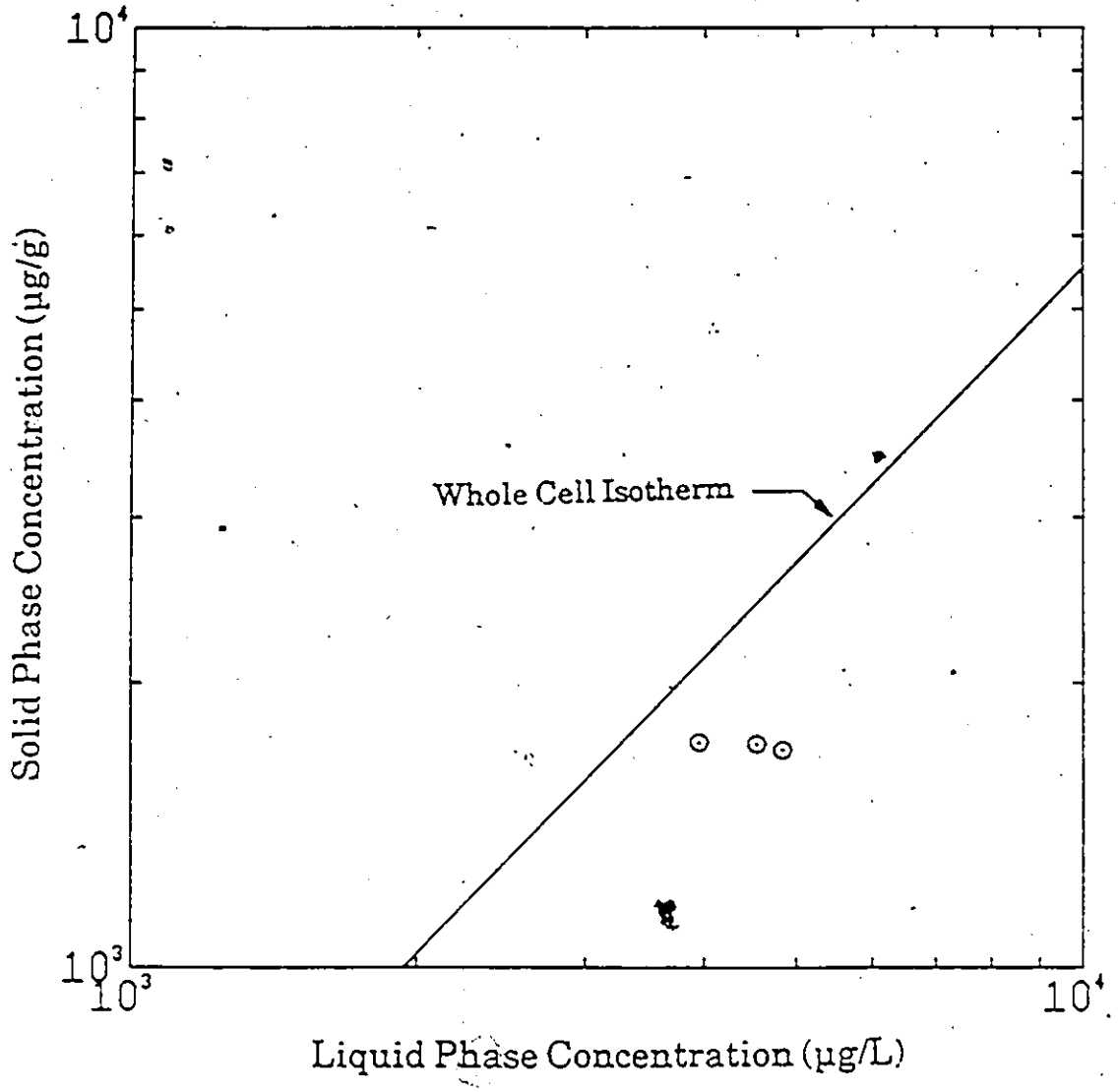


Figure 4.4.4 Biosorption of diazinon by activated sludge cell walls.

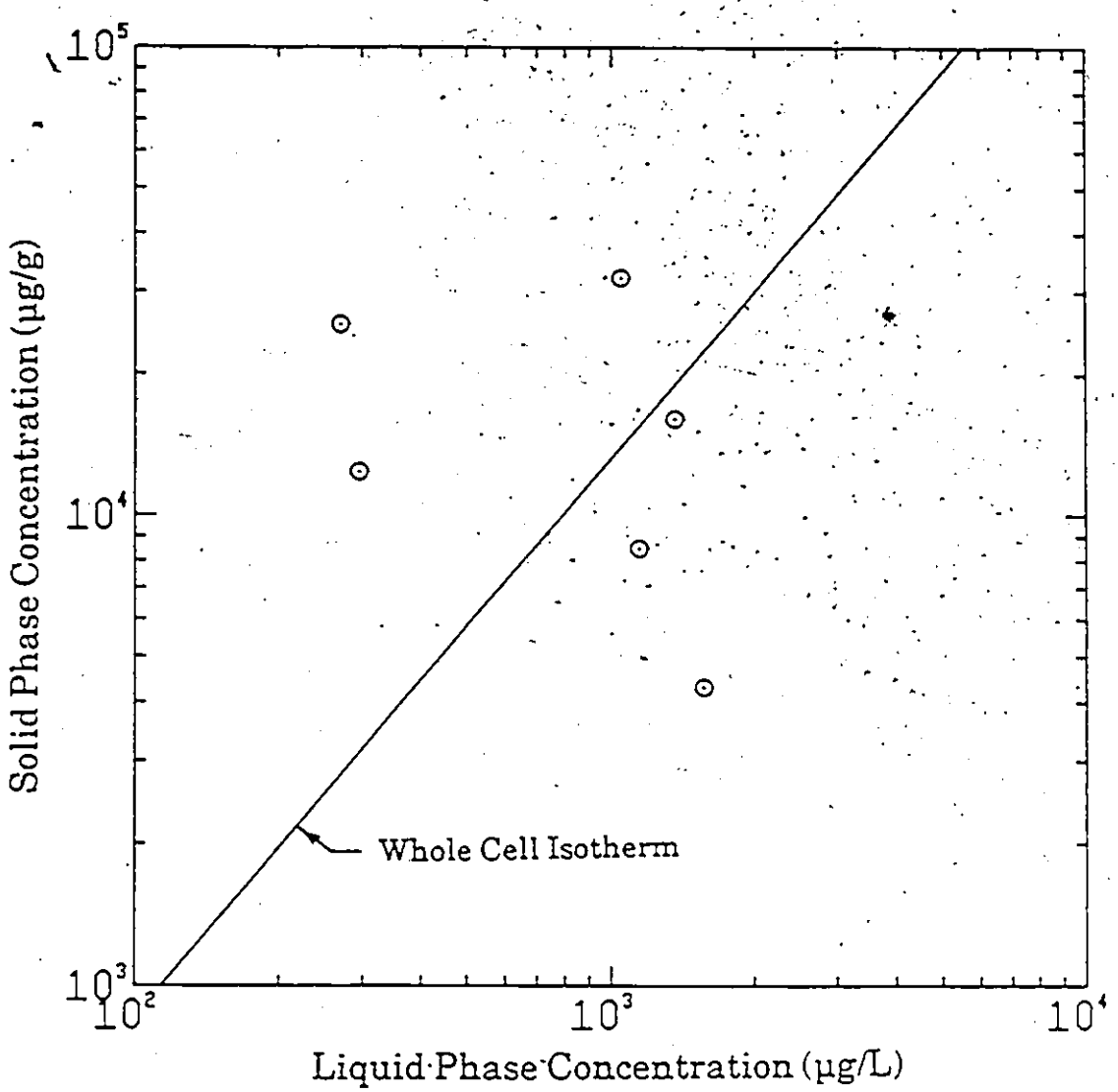


Figure 4.4.5 Apparent biosorption of malathion by *R. arrhizus* cell walls.

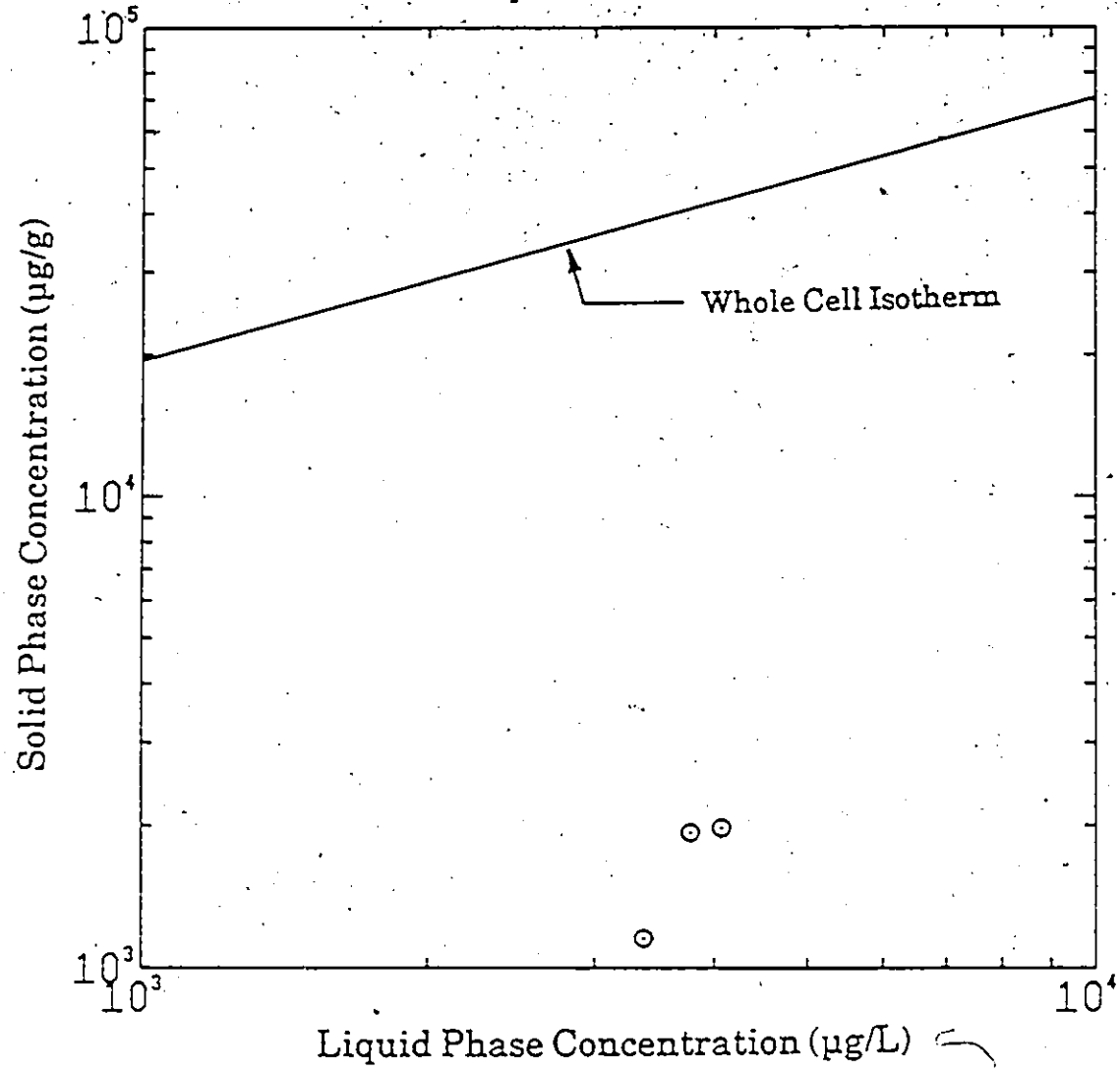


Figure 4.4.6 Apparent biosorption of malathion by activated sludge cell walls.

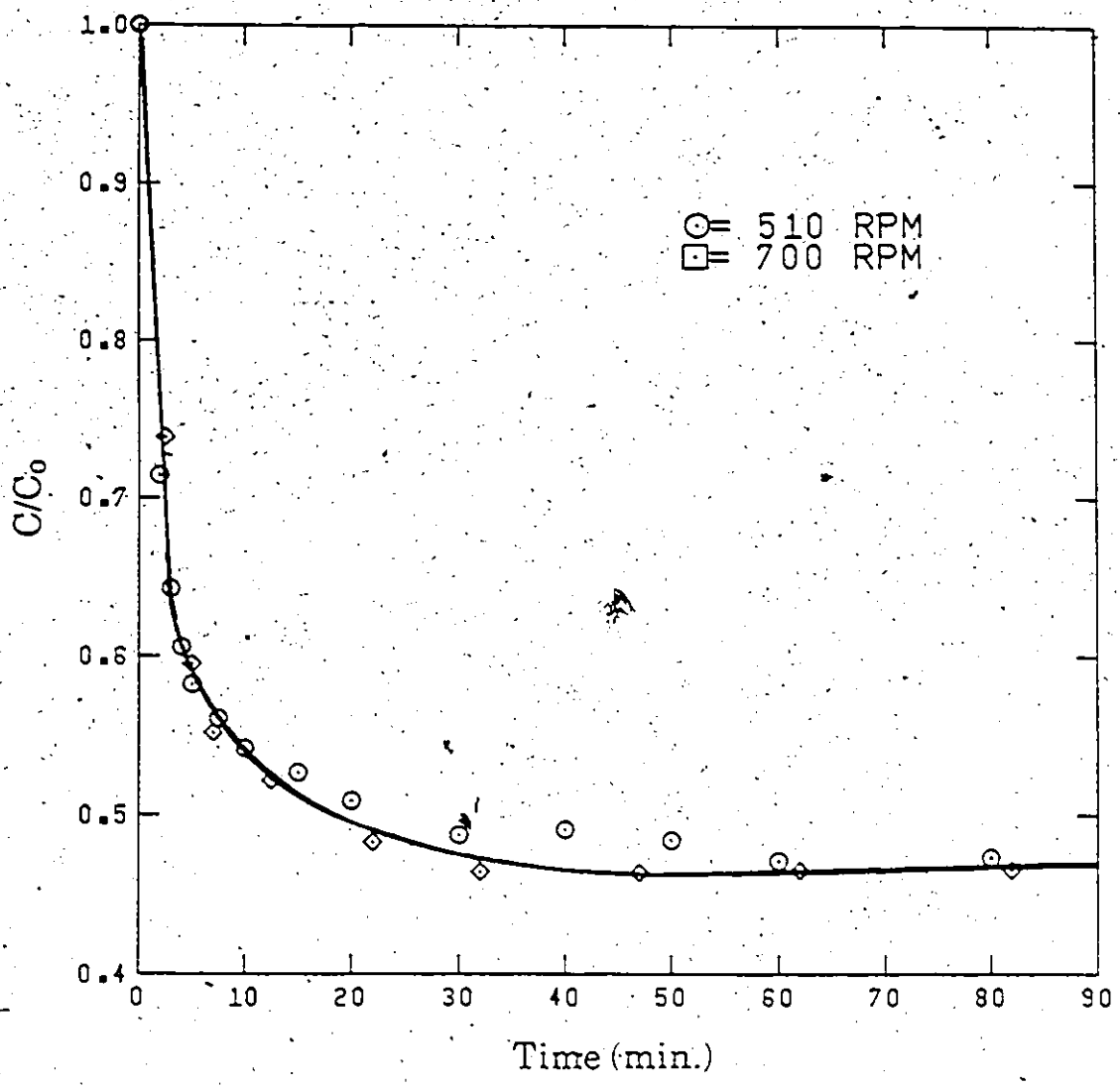


Figure 4.5.1 Kinetics of biosorption of lindane by *R. arrhizus*.

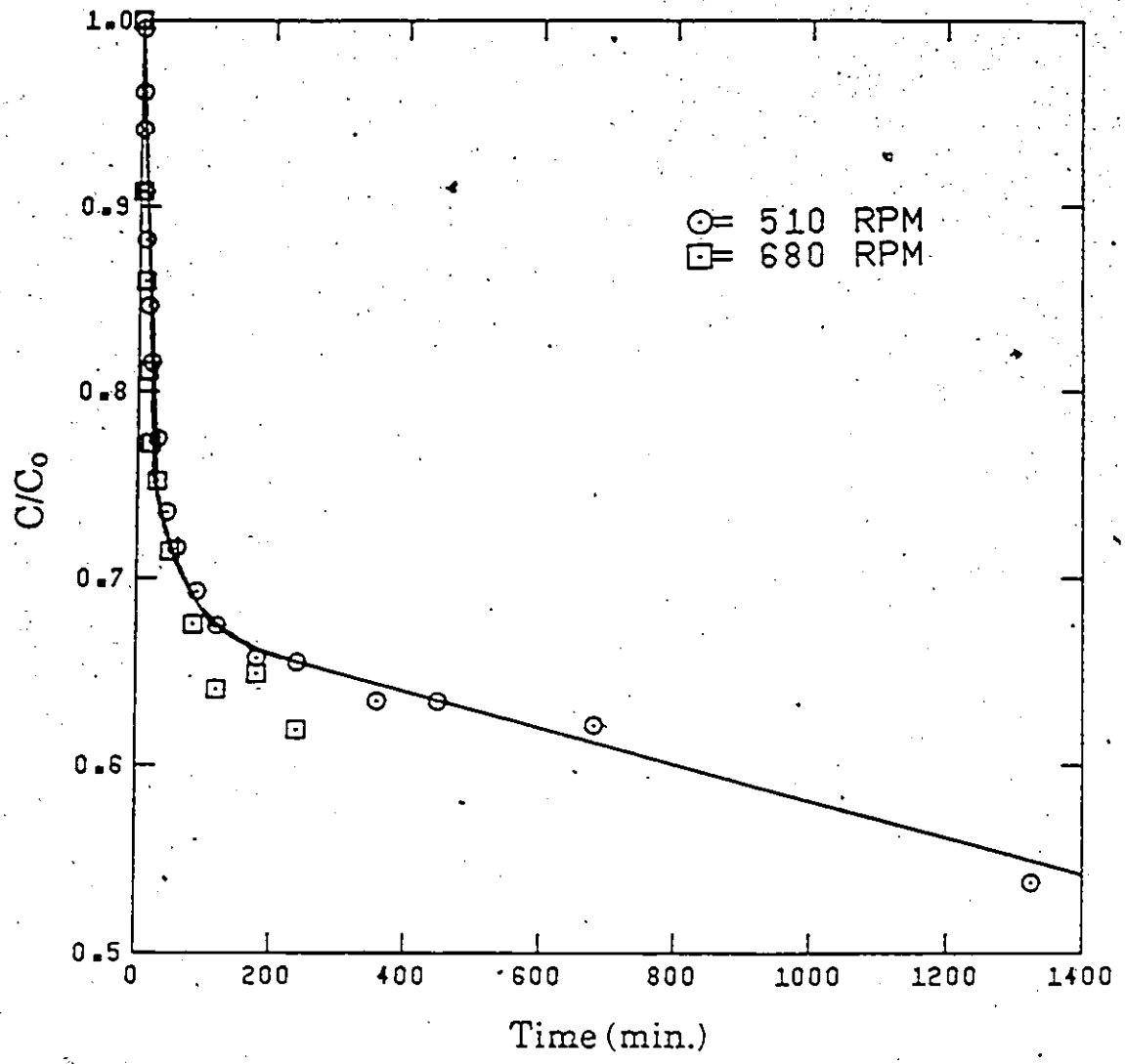


Figure 4.5.2 Kinetics of biosorption of lindane by activated sludge

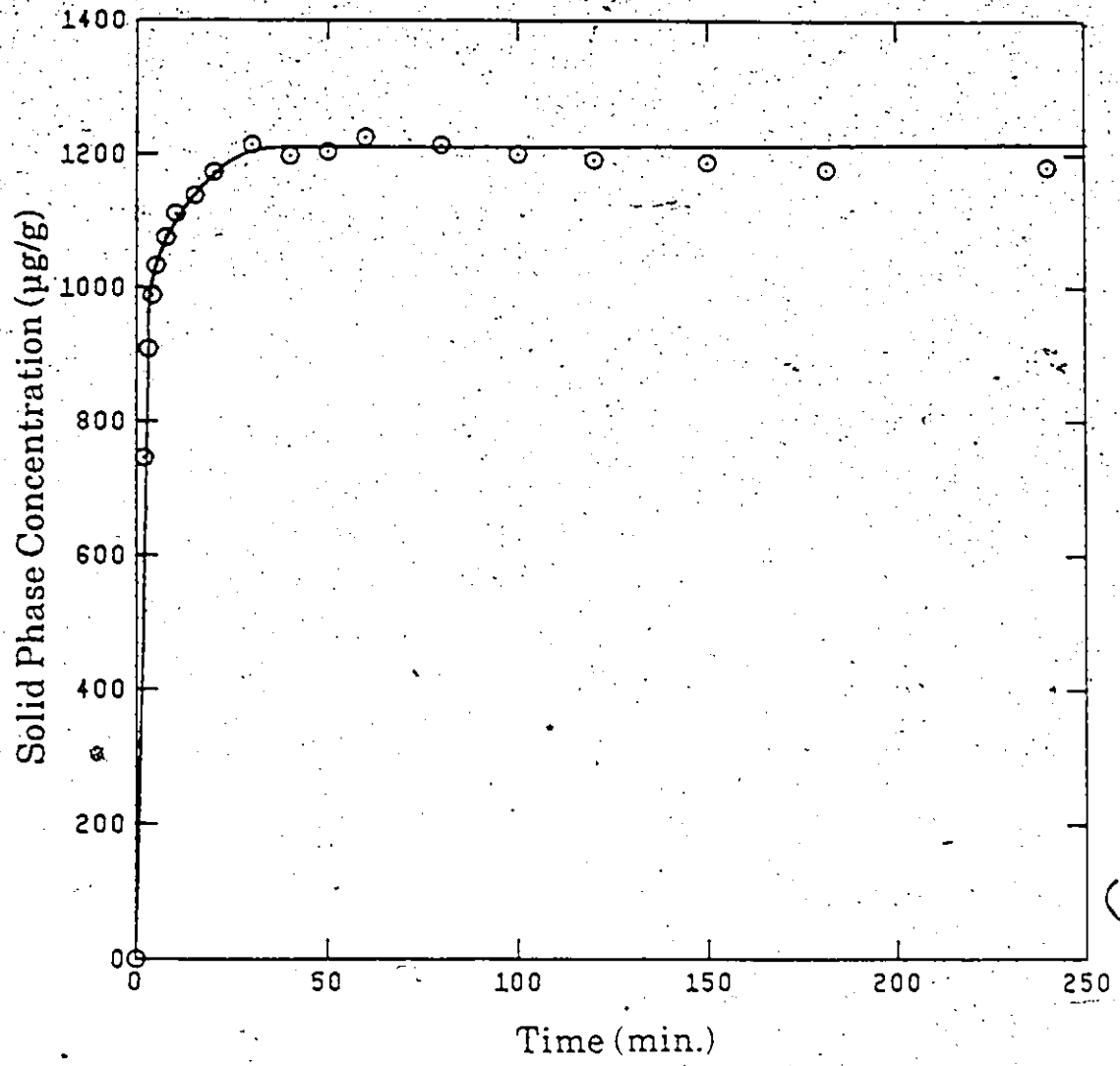


Figure 4.5.3 Kinetics of biosorption of lindane by *R. arrhizus*.

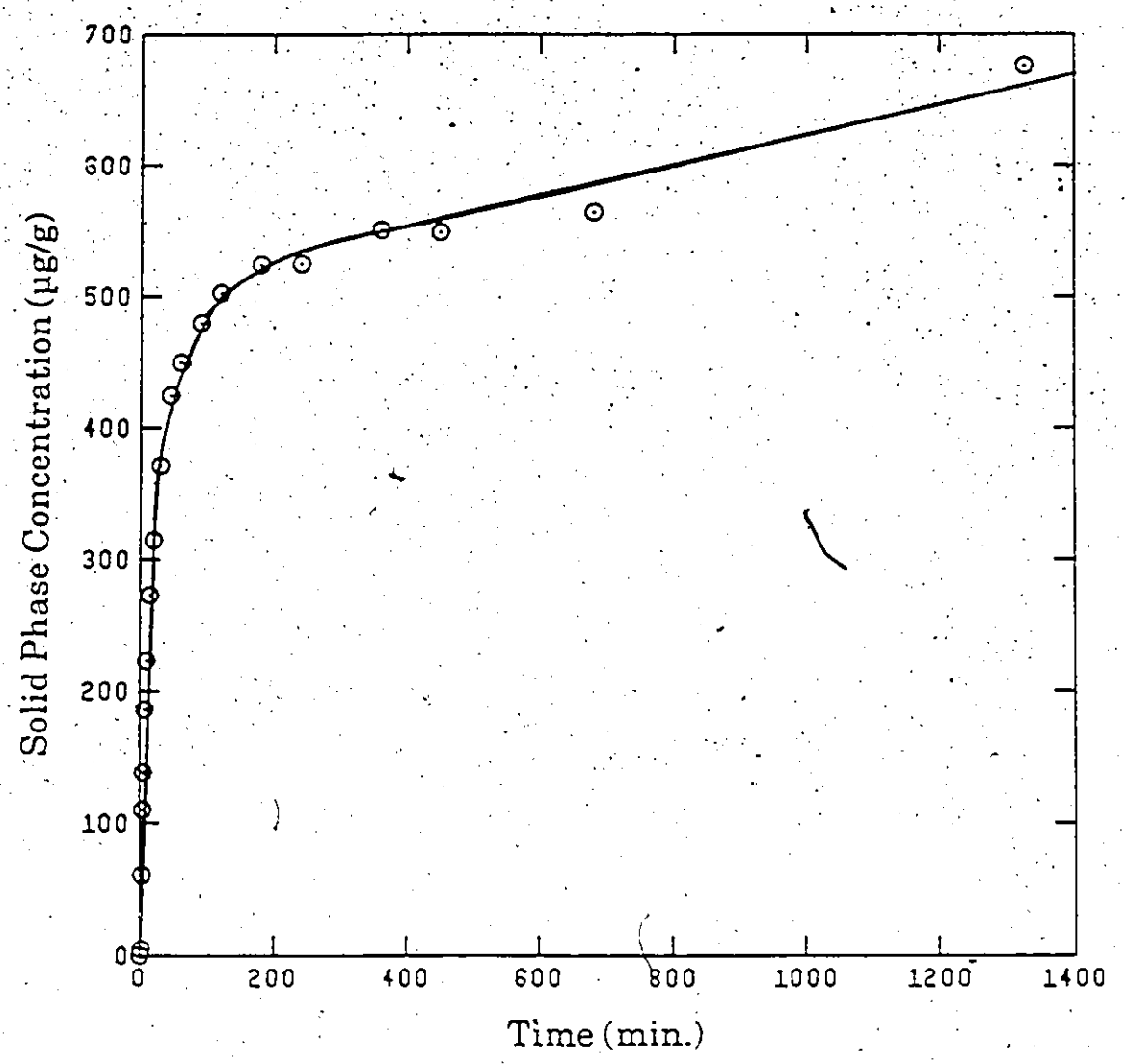


Figure 4.5.4 Kinetics of biosorption of lindane by activated sludge.

of the trend of the data. Solid phase concentrations were calculated using a mass balance with a correction for the removal of lindane with each sample removed from the reactor. Experimental data is given in Appendix B5.

The kinetics of diazinon sorption by both types of biomass were investigated using individual contact flasks containing equal initial solution and biomass concentrations. The flasks were agitated on an orbital shaker and were removed and analyzed after different contact periods. Figures 4.5.5 and 4.5.6 show the results of these experiments. Experimental data are given in Appendix B5.

The kinetics of malathion removal by inactive biomass at 20°C and 5°C were investigated together with the kinetics of removal by live *R. arrhizus*. Since malathion is known to be subject to hydrolysis (Muhlmann and Schrader, 1957; Freed et al., 1979), the rate of its disappearance with no biomass present was assessed. These experiments were carried out in individual contact flasks as for diazinon. Experimental data is given in Appendix B5 and is shown graphically in Figures 4.5.7 through 4.5.9.

4.6 COMPETITIVE BIOSORPTION

Biosorption experiments wherein the biomass was contacted with solutions containing two or more solutes were performed to evaluate the effects of competition among different chemicals. Combinations of lindane and pentachlorophenol; lindane and diazinon; diazinon and malathion; and lindane, pentachlorophenol, diazinon, and malathion were tested with both types of biomass. Initial concentrations of the compounds were approximately equimolar in each solution. Experimental data are given in Appendix B6. Figures 4.6.1 through 4.6.18 show the competitive biosorption data compared with the single solute isotherm lines.

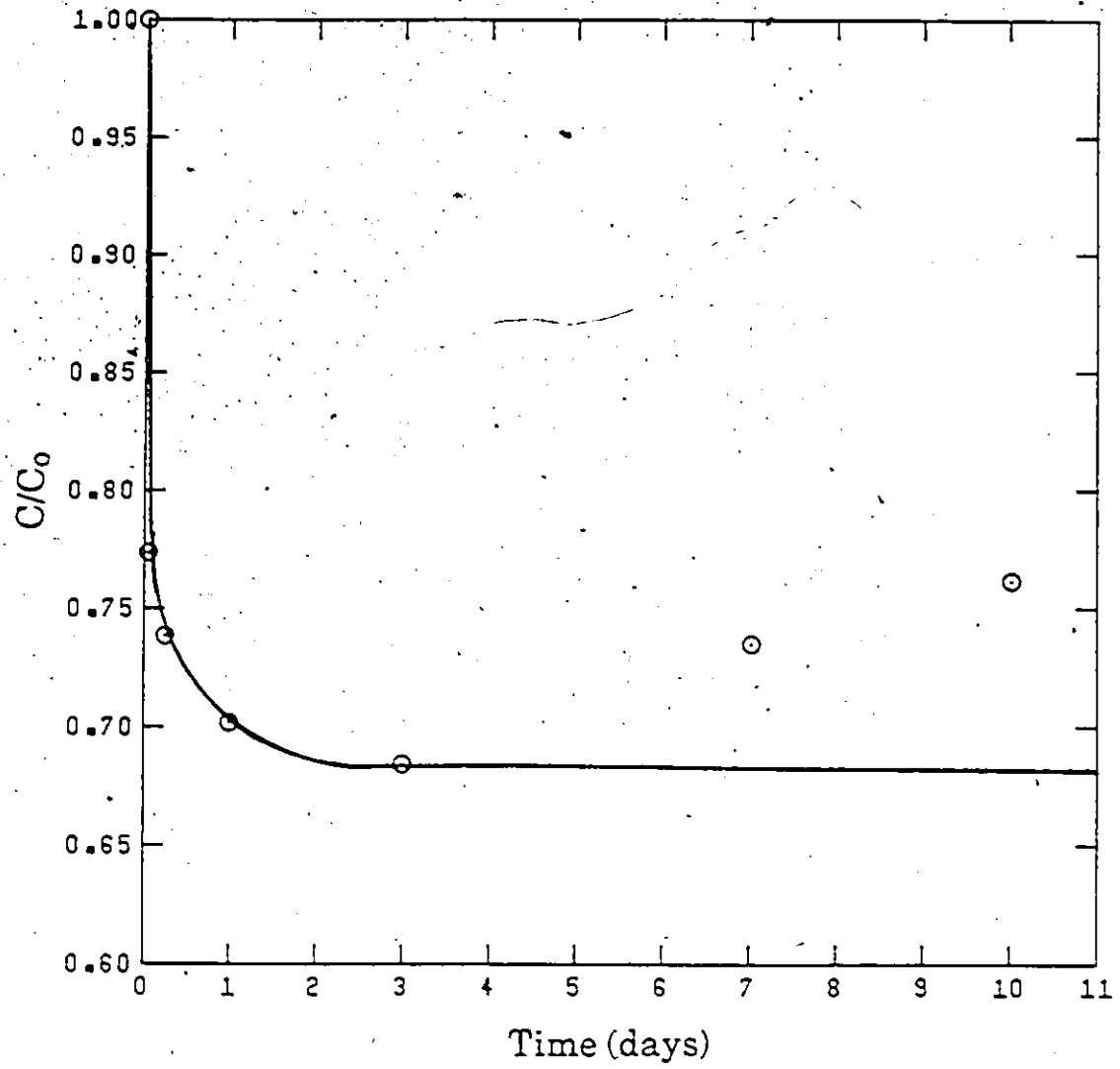


Figure 4.5.5 Kinetics of biosorption of diazinon by *R. arrhizus*.

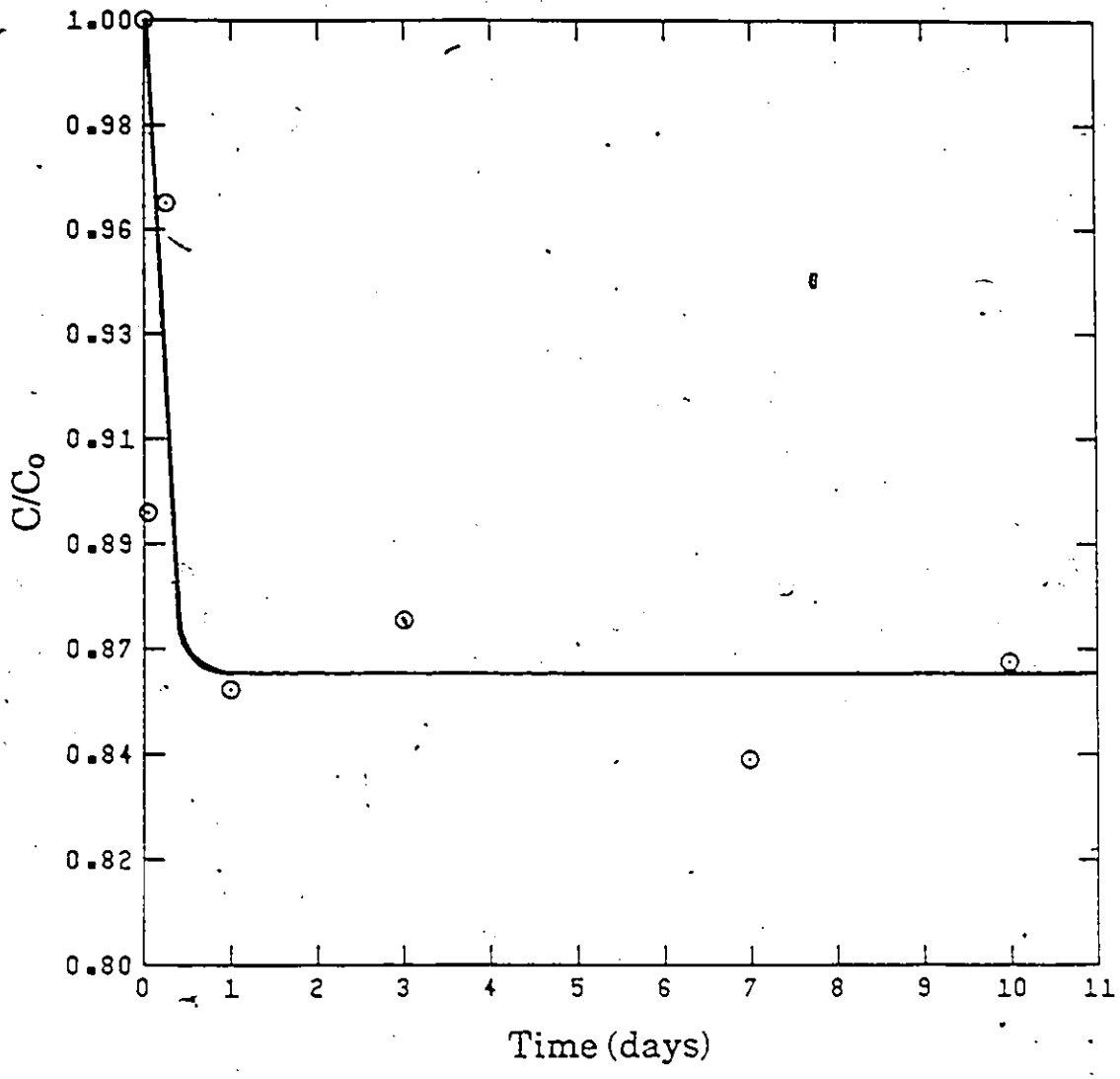


Figure 4.5.6 Kinetics of biosorption of diazinon by activated sludge.

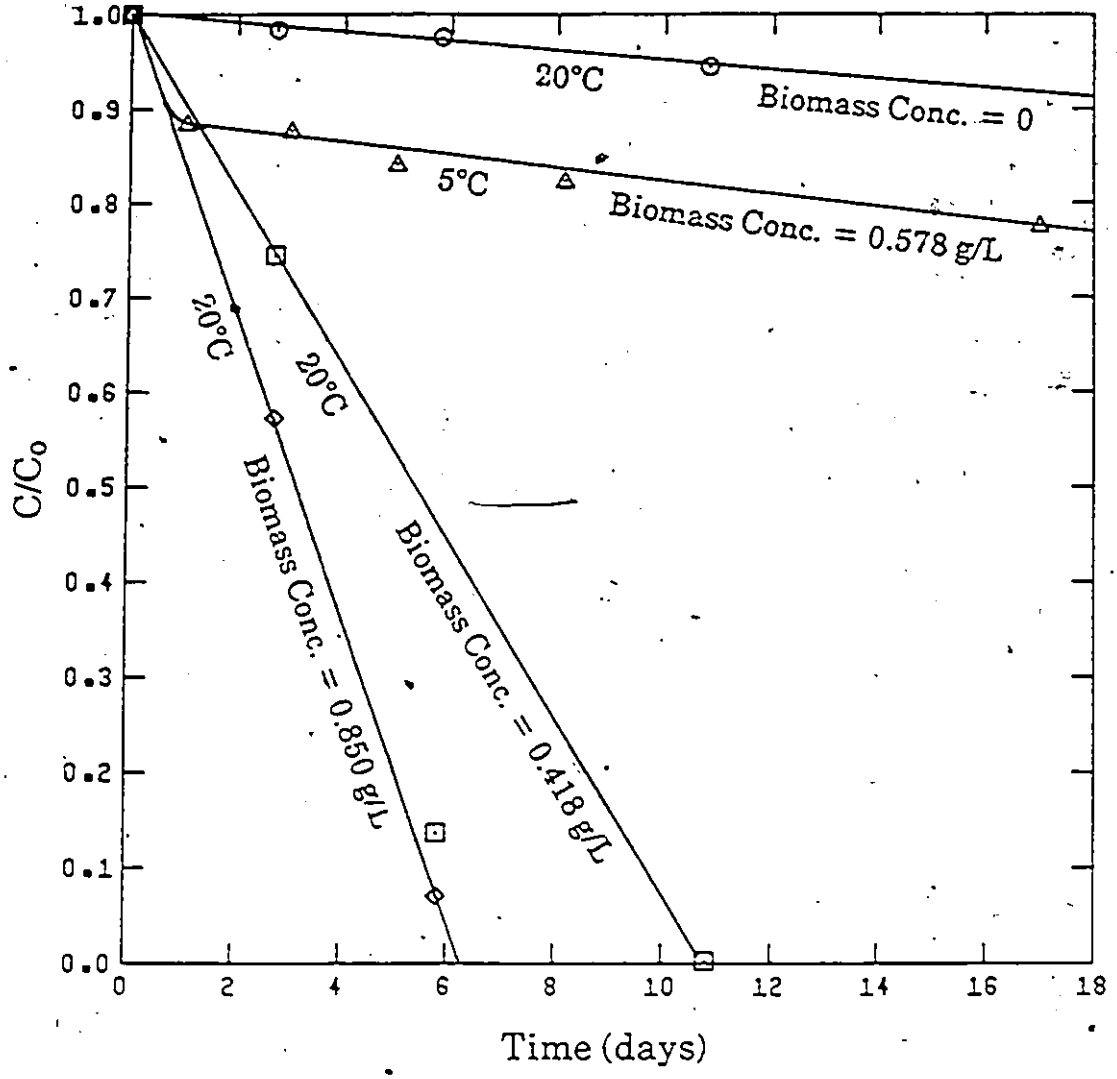


Figure 4.5.7 Kinetics of malathion removal by *R. arrhizus*.

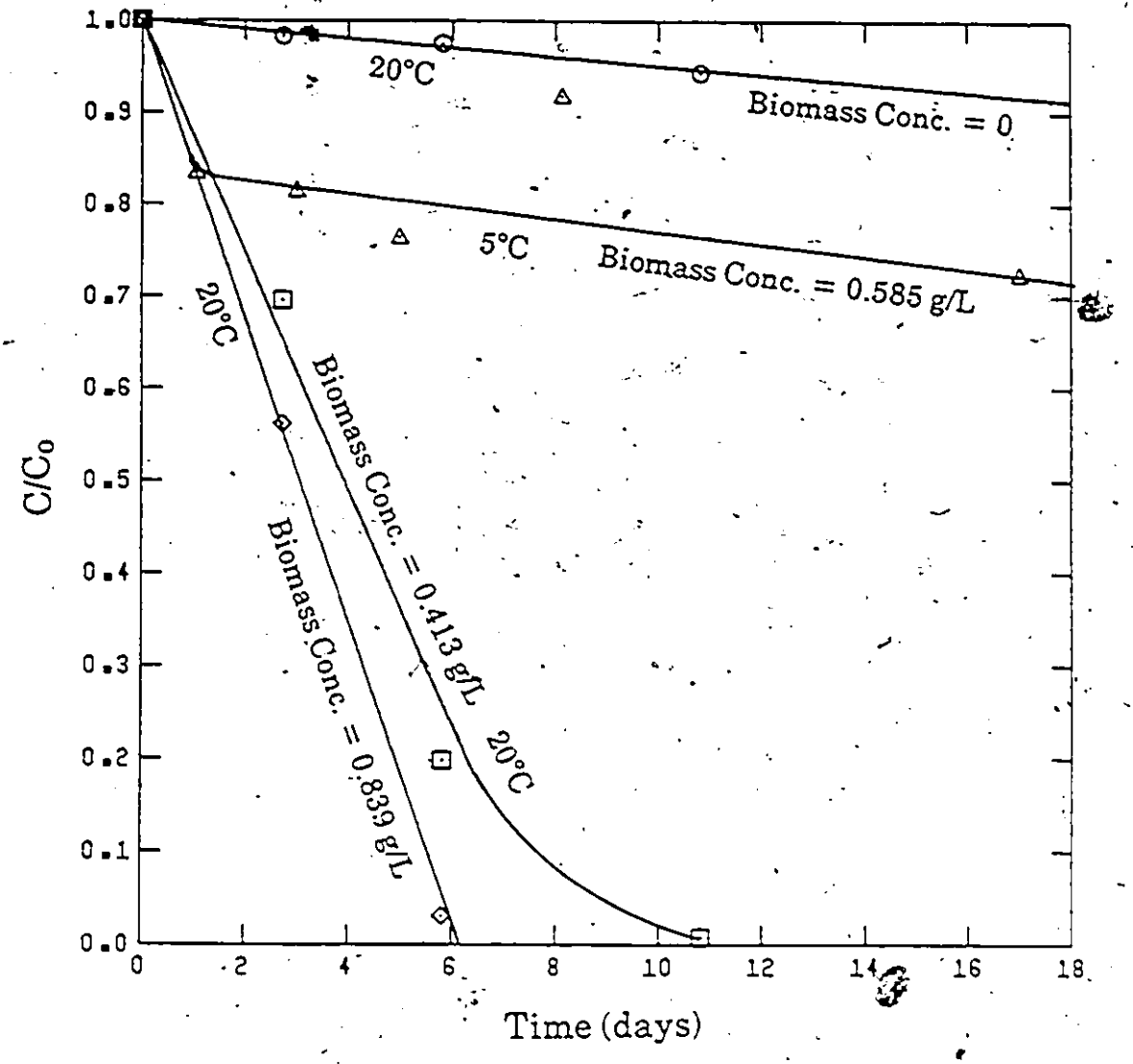


Figure 4.5.8 Kinetics of malathion removal by activated sludge.

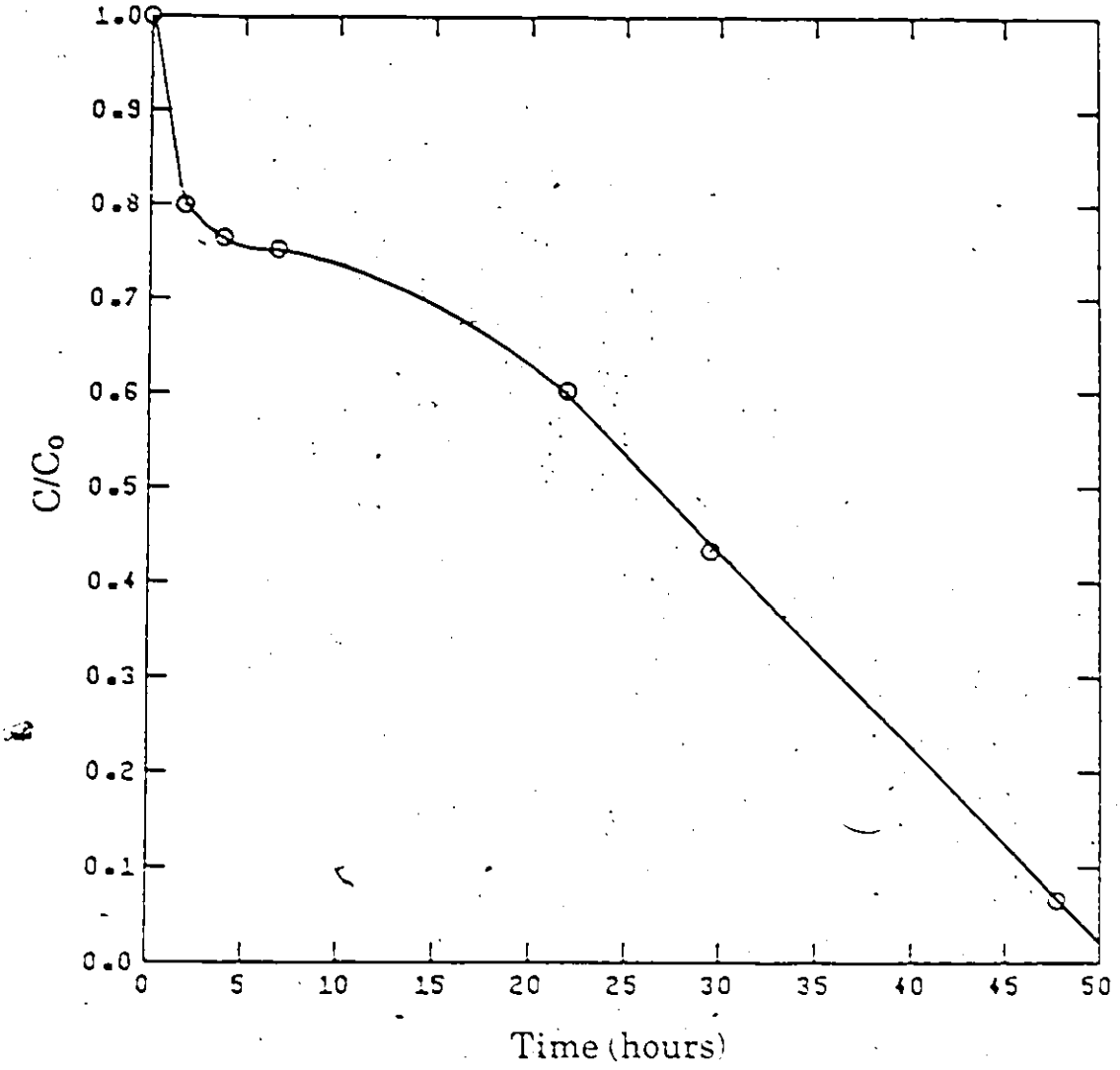


Figure 4.5.9. Kinetics of malathion removal by live *R. arrhizus*

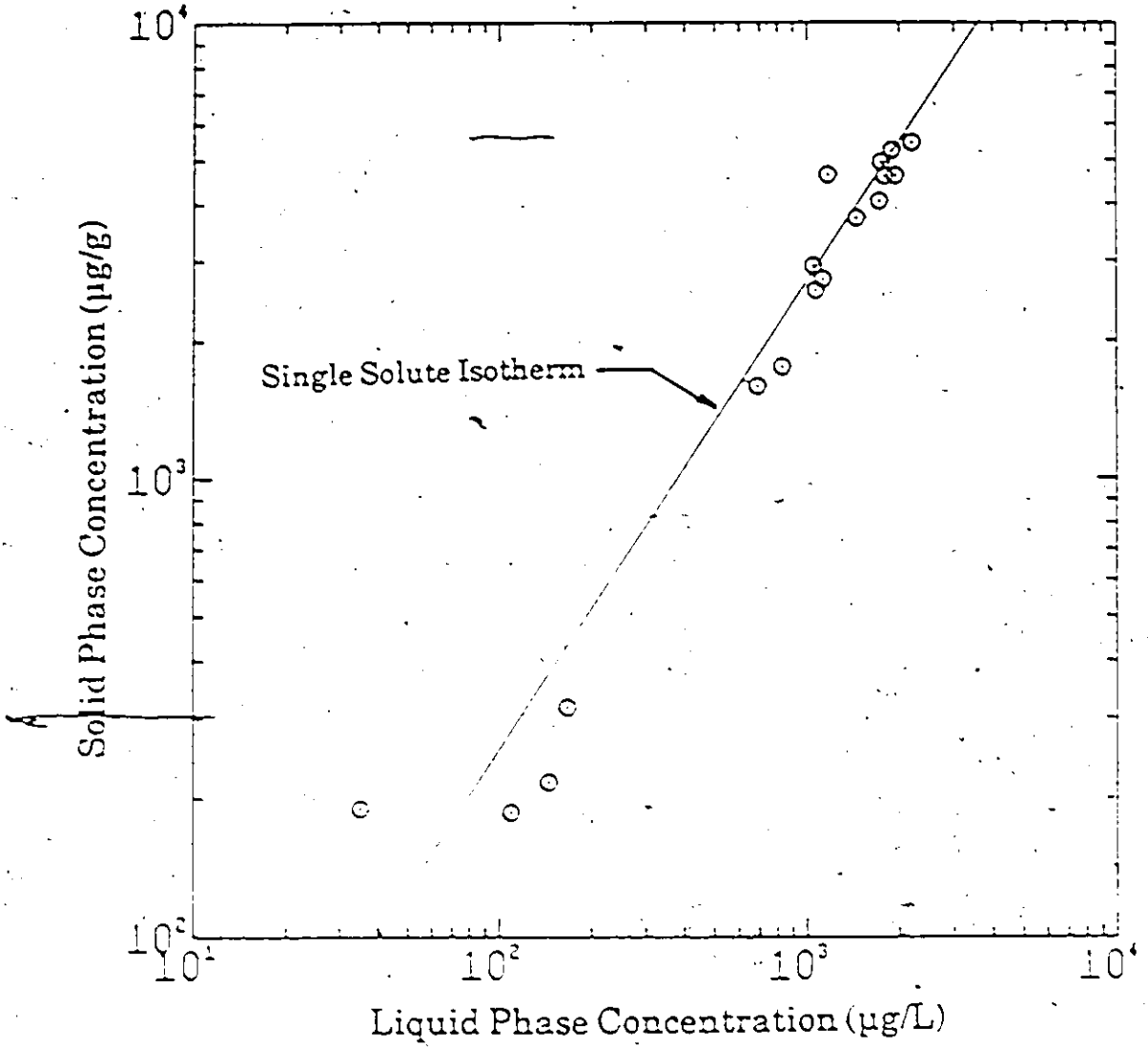


Figure 4.6.1 Biosorption of lindane by *R. arrhizus* in competition with pentachlorophenol.

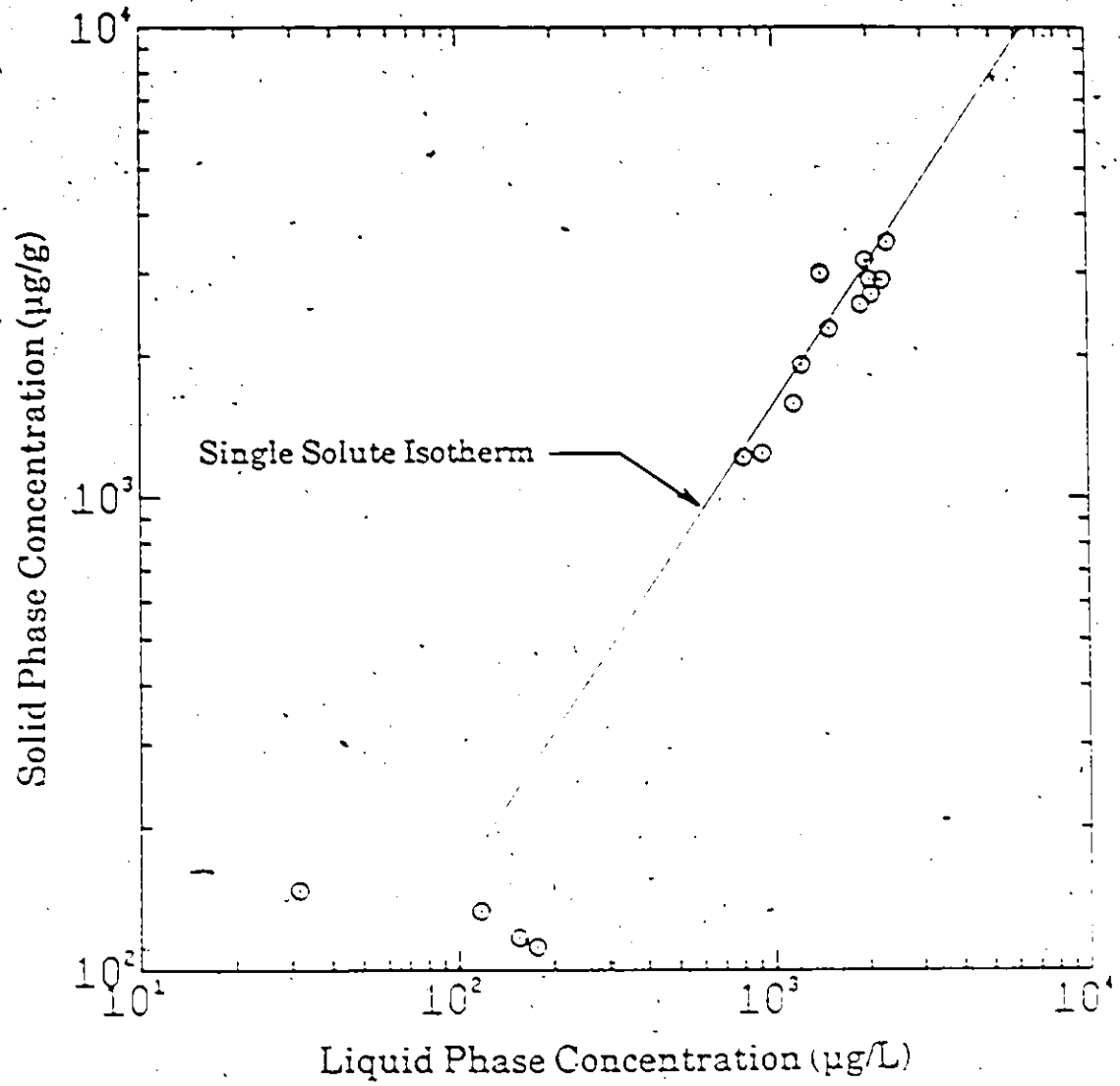


Figure 4.6.2 Biosorption of lindane by activated sludge in competition with pentachlorophenol.

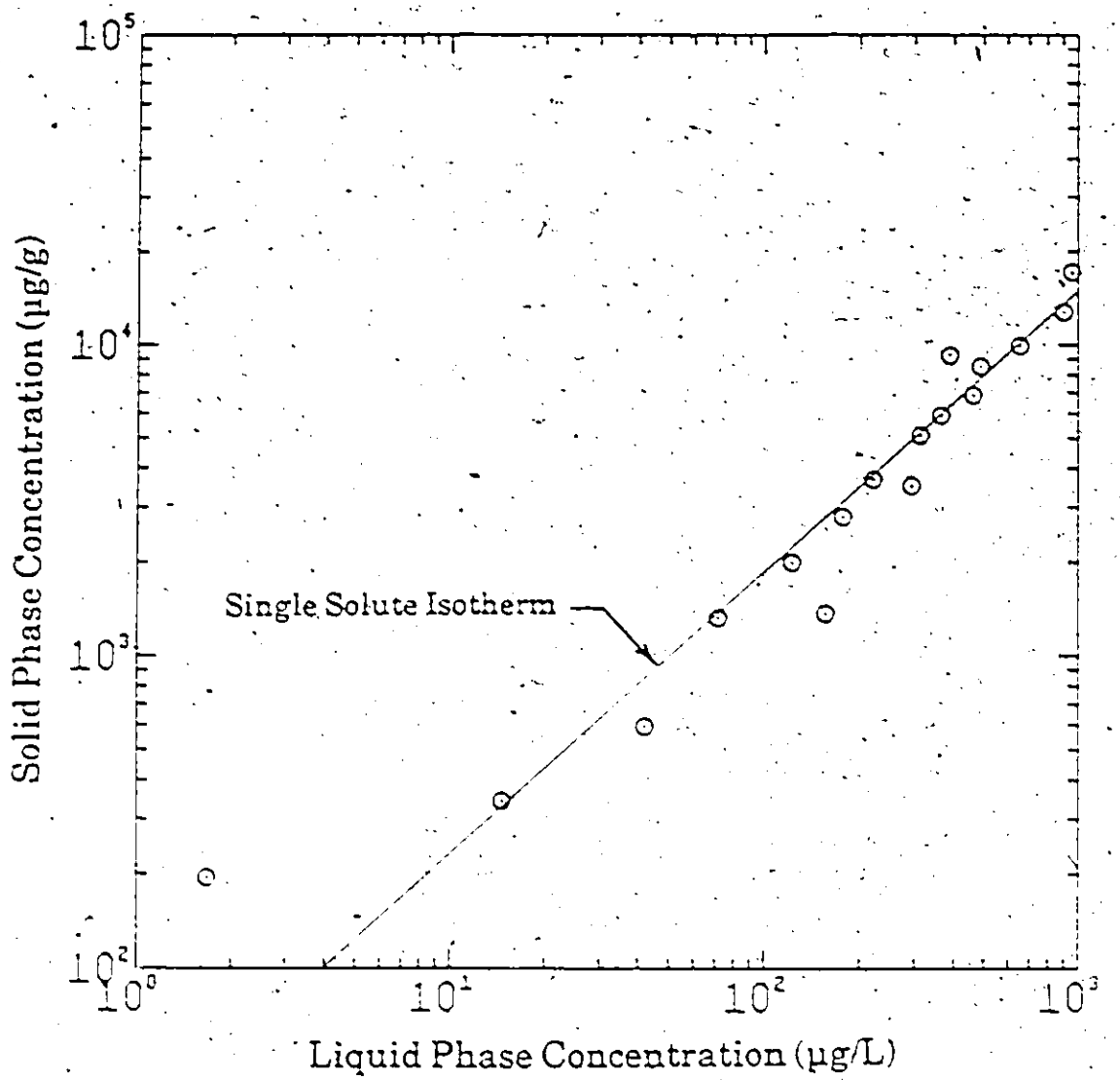


Figure 4.6.3 — Biosorption of pentachlorophenol by *R. arrhizus* in competition with lindane.

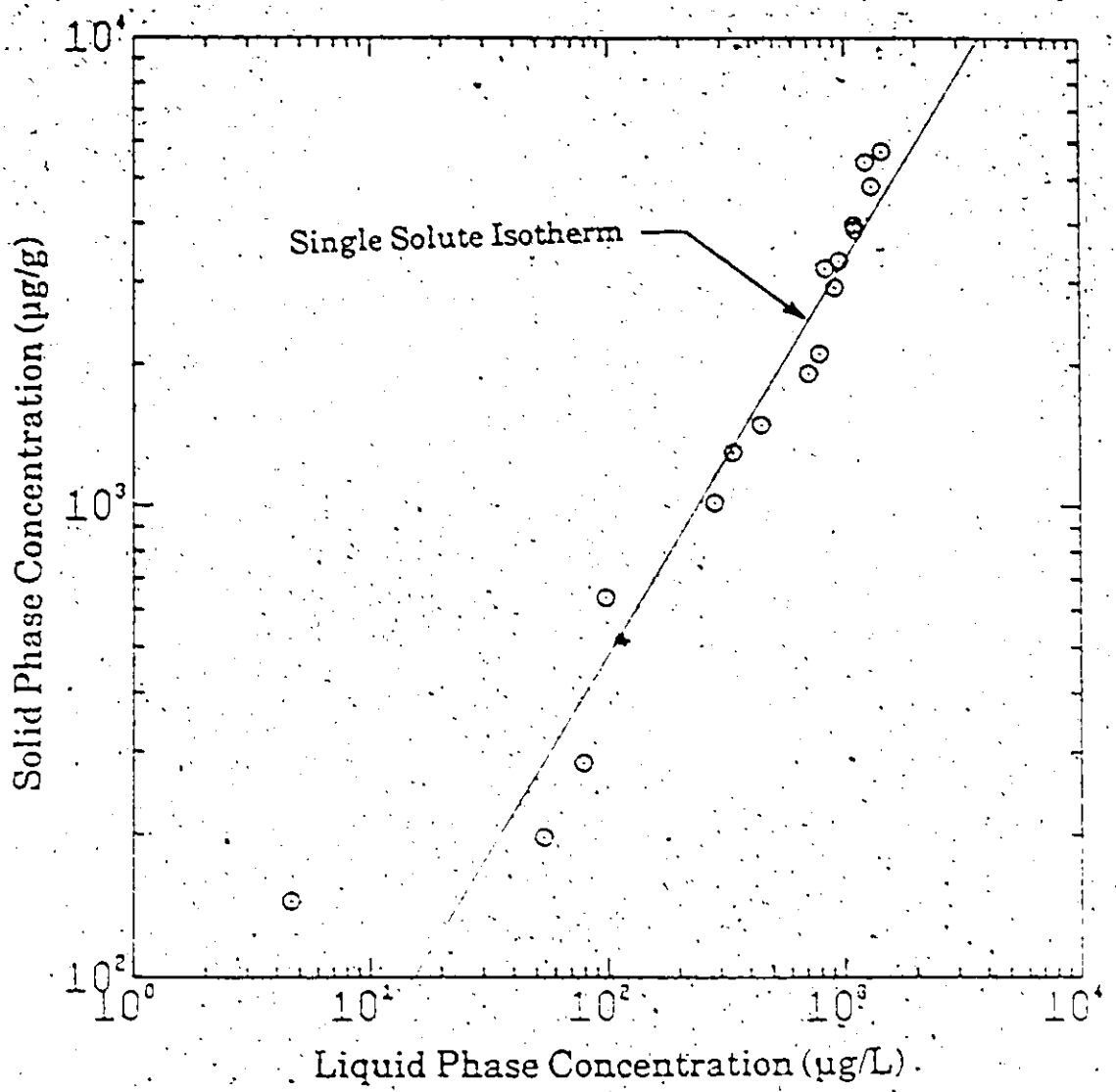


Figure 4.6.4 Biosorption of pentachlorophenol by activated sludge in competition with lindane.

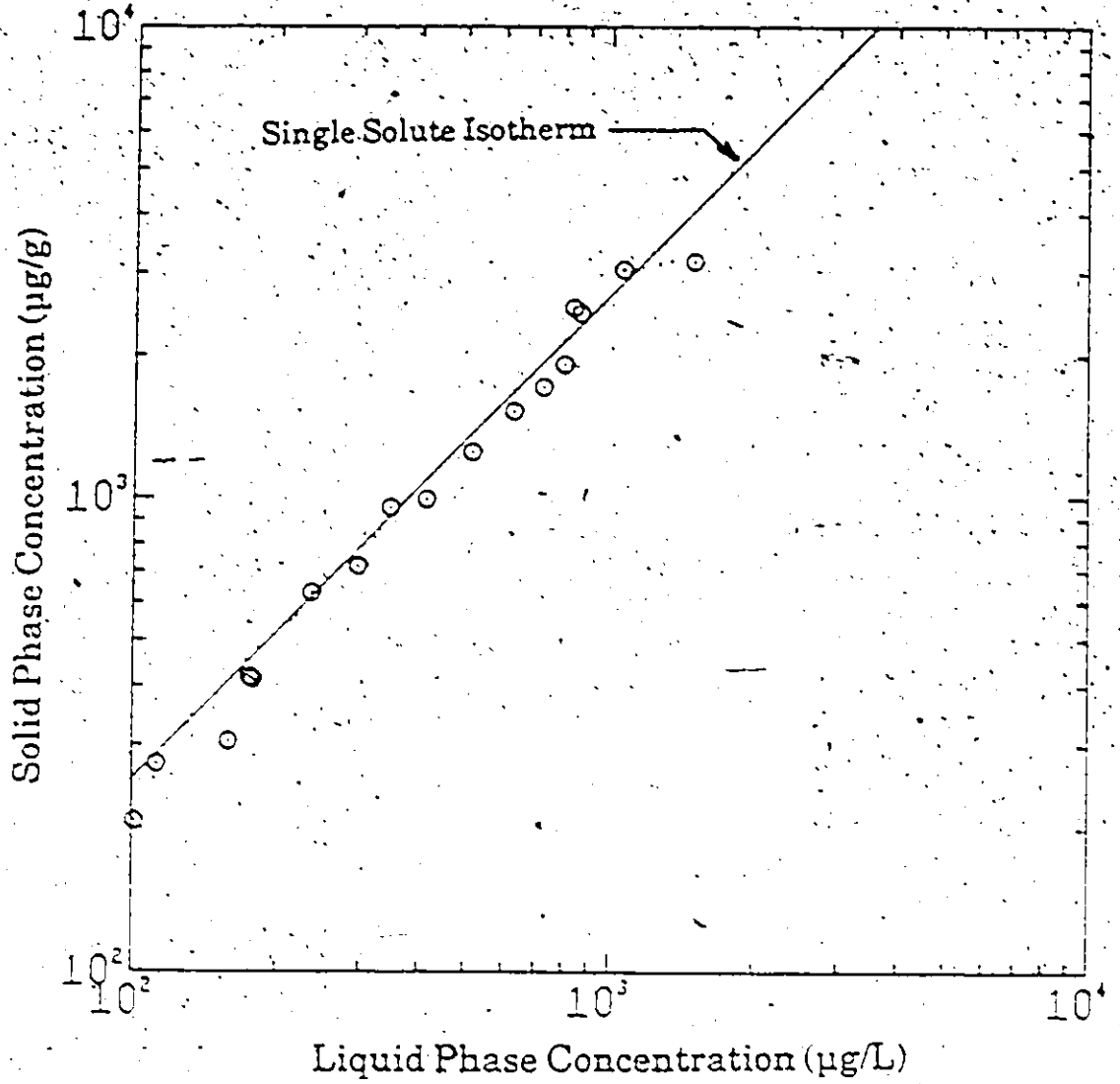


Figure 4.6.5 Biosorption of lindane by *R. arrhizus* in competition with diazinon.

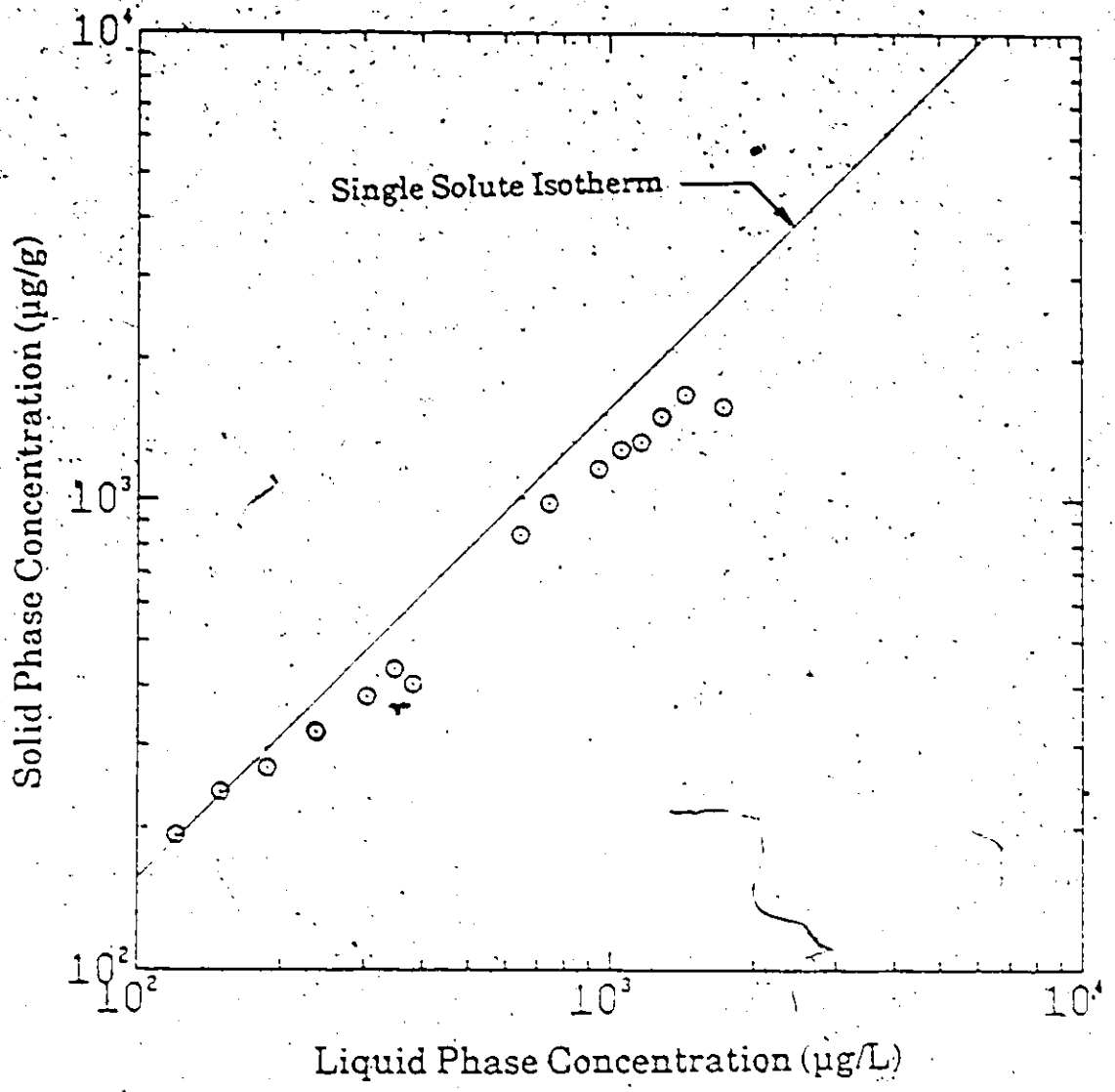


Figure 4.6.6 Biosorption of lindane by activated sludge in competition with diazinon

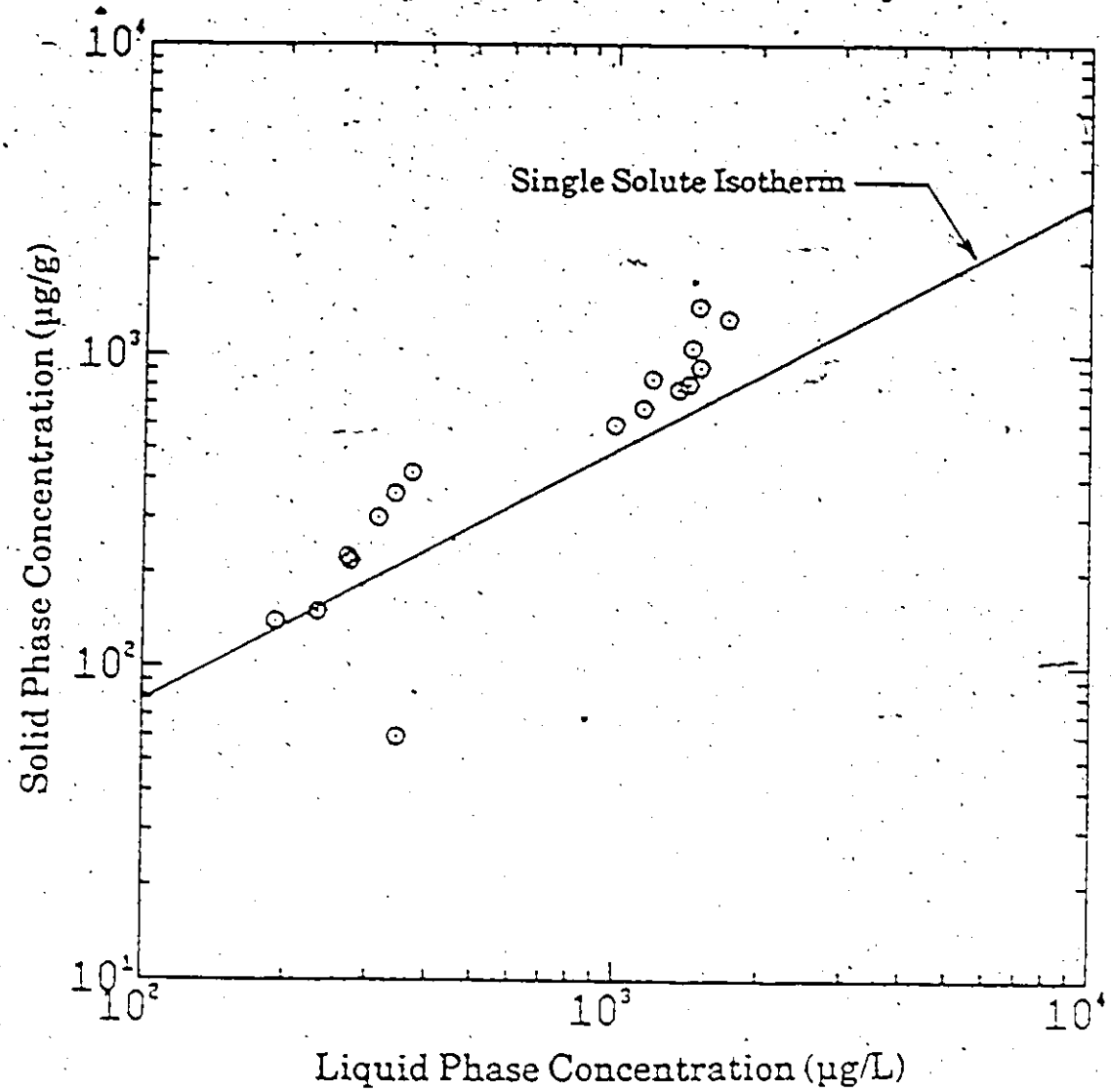


Figure 4.6.7 Biosorption of diazinon by *R. arrhizus* in competition with lindane.

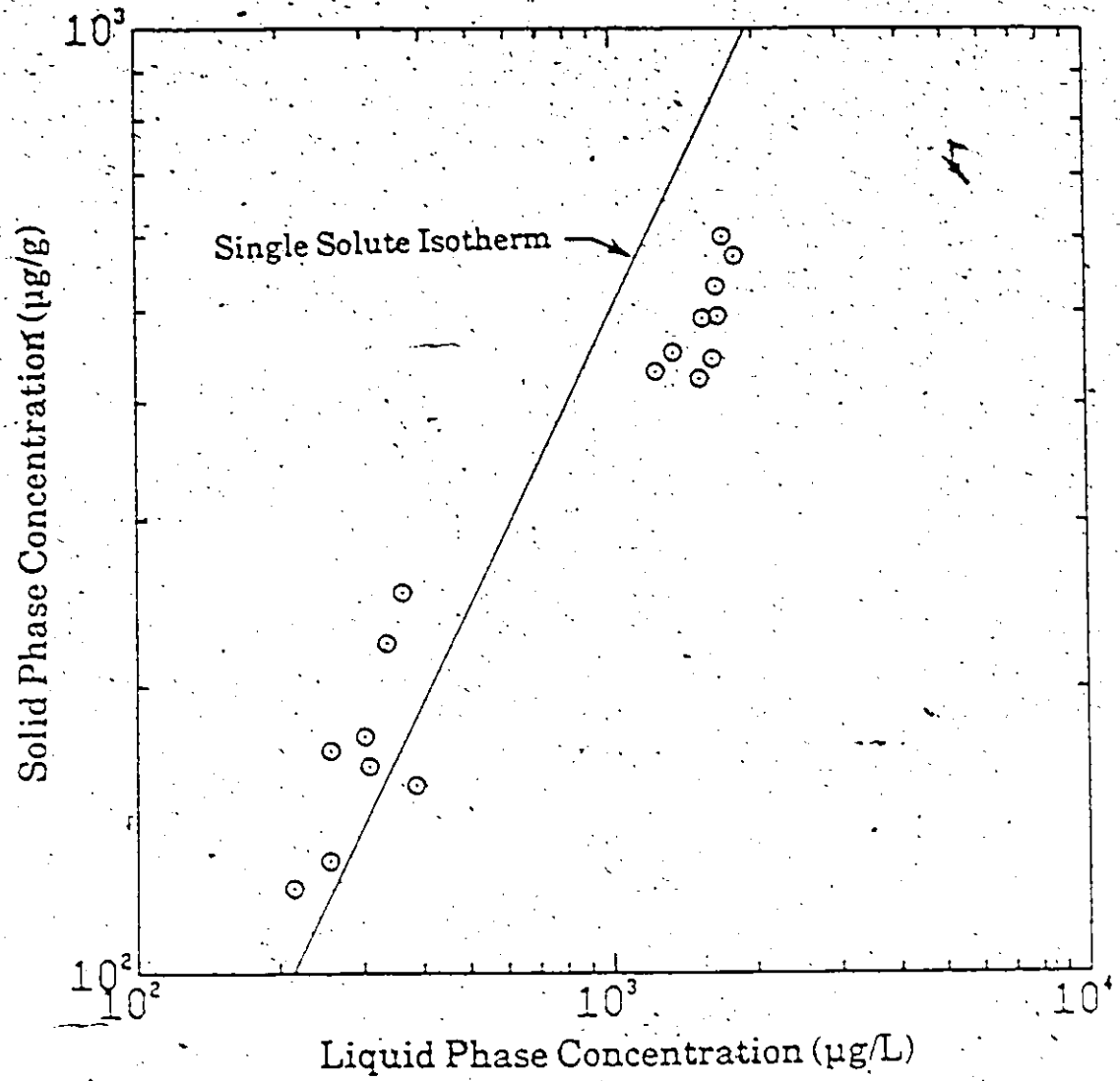


Figure 4.6.8 Biosorption of diazinon by activated sludge in competition with lindane

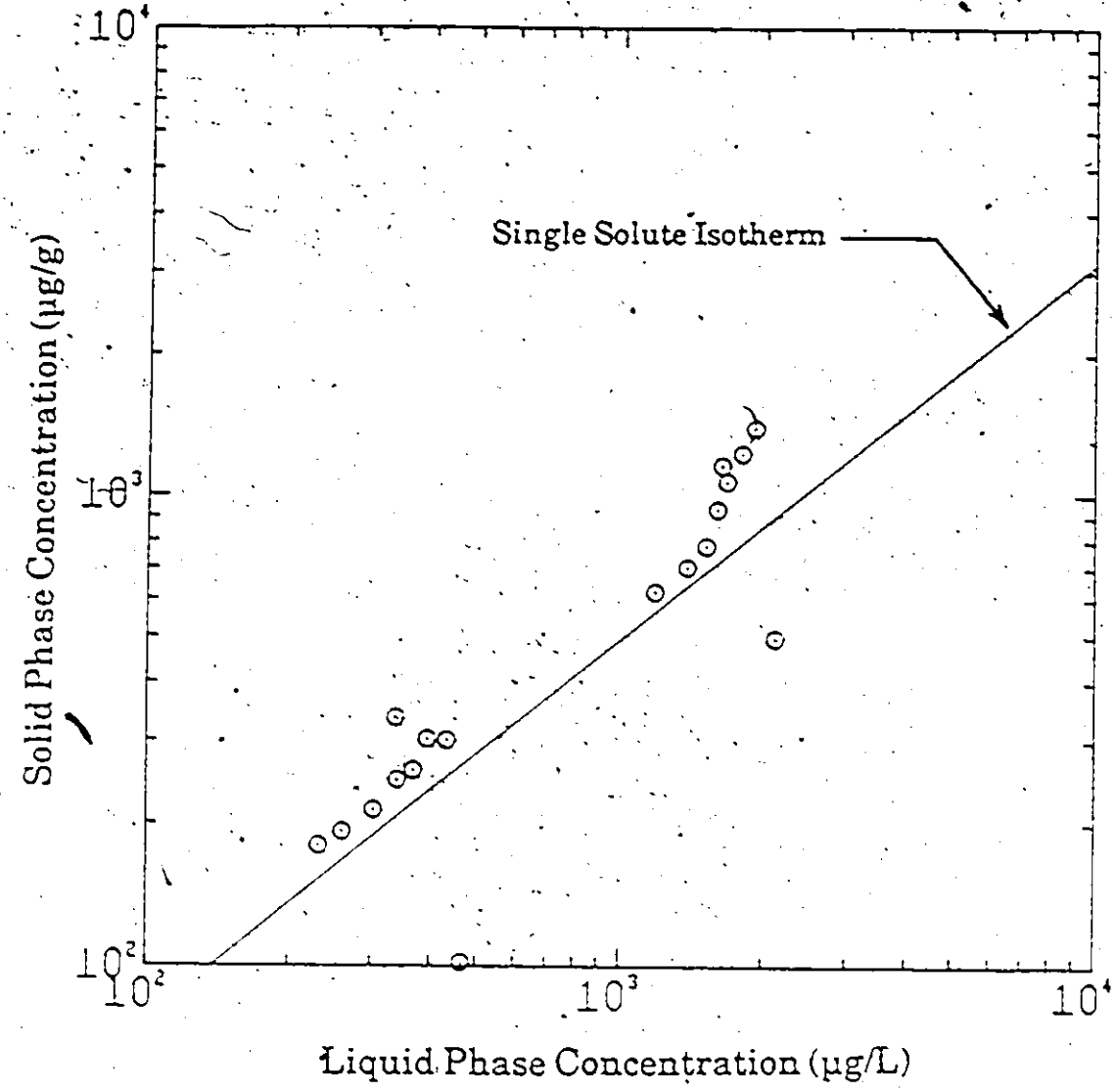


Figure 4.6.9 Biosorption of diazinon by *R. arrhizus* in competition with malathion.

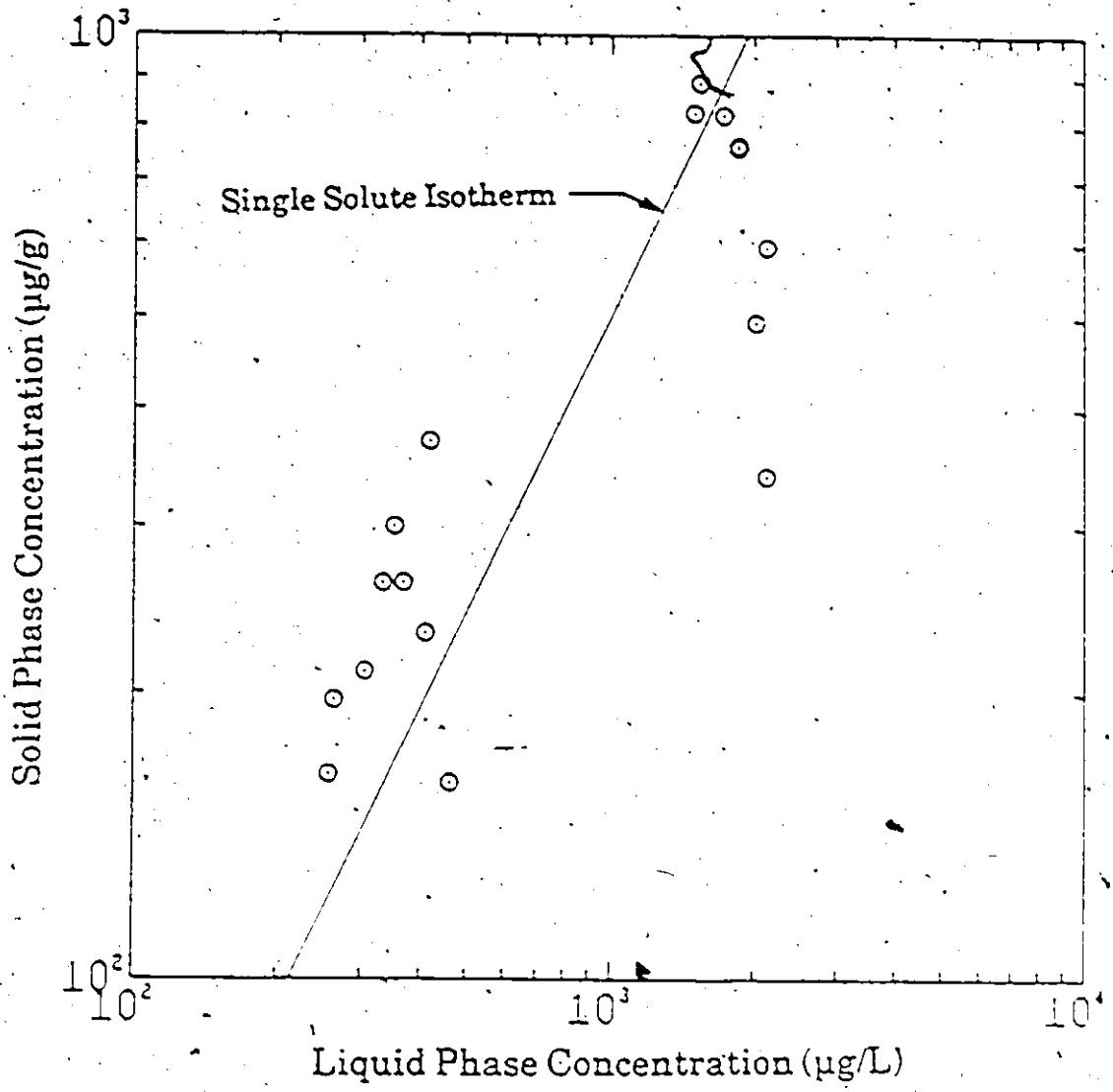


Figure 4.6.10 Biosorption of diazinon by activated sludge in competition with malathion

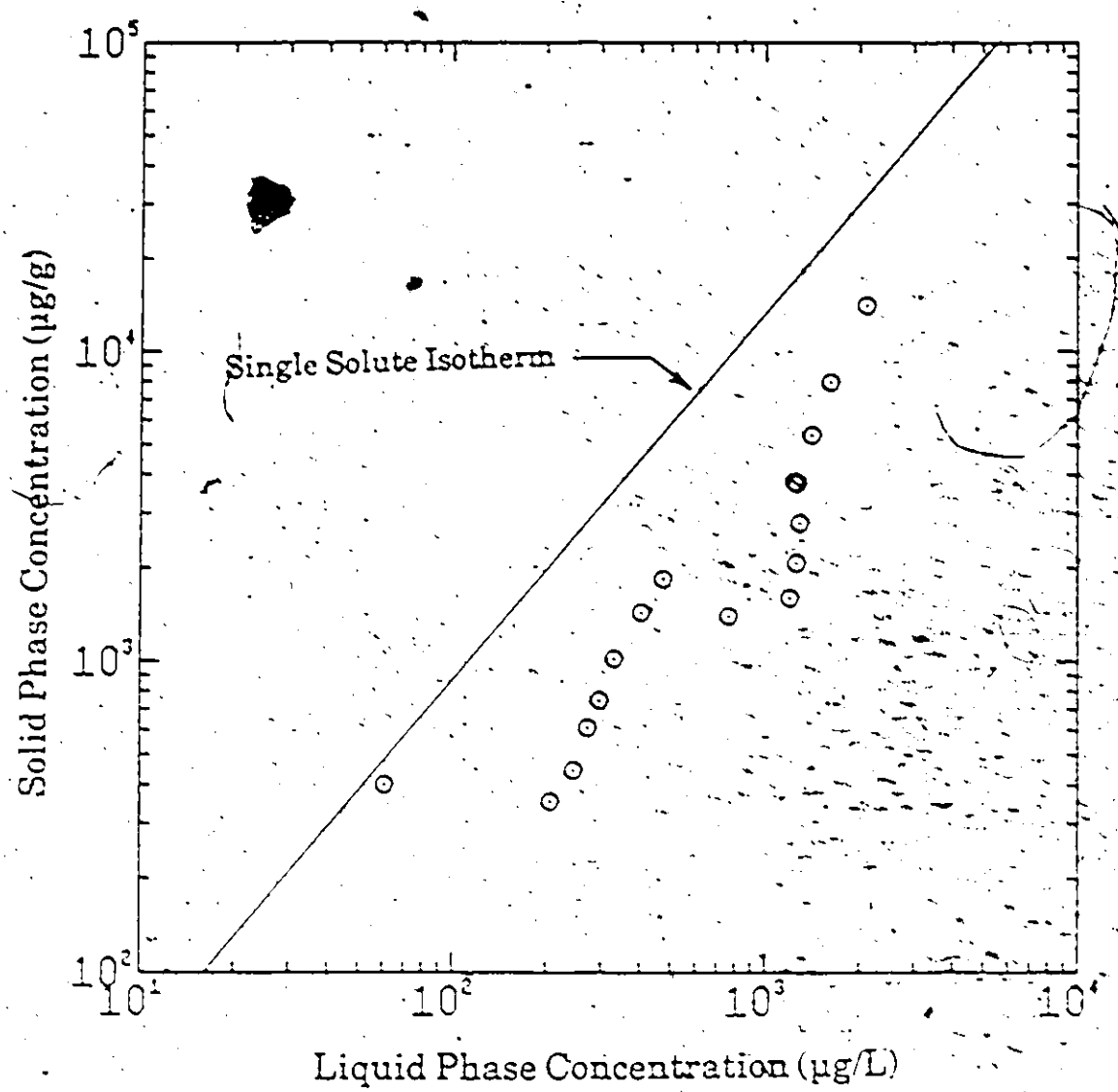


Figure 4.6.11 Apparent biosorption of malathion by *R. arrhizus* in competition with diazinon.

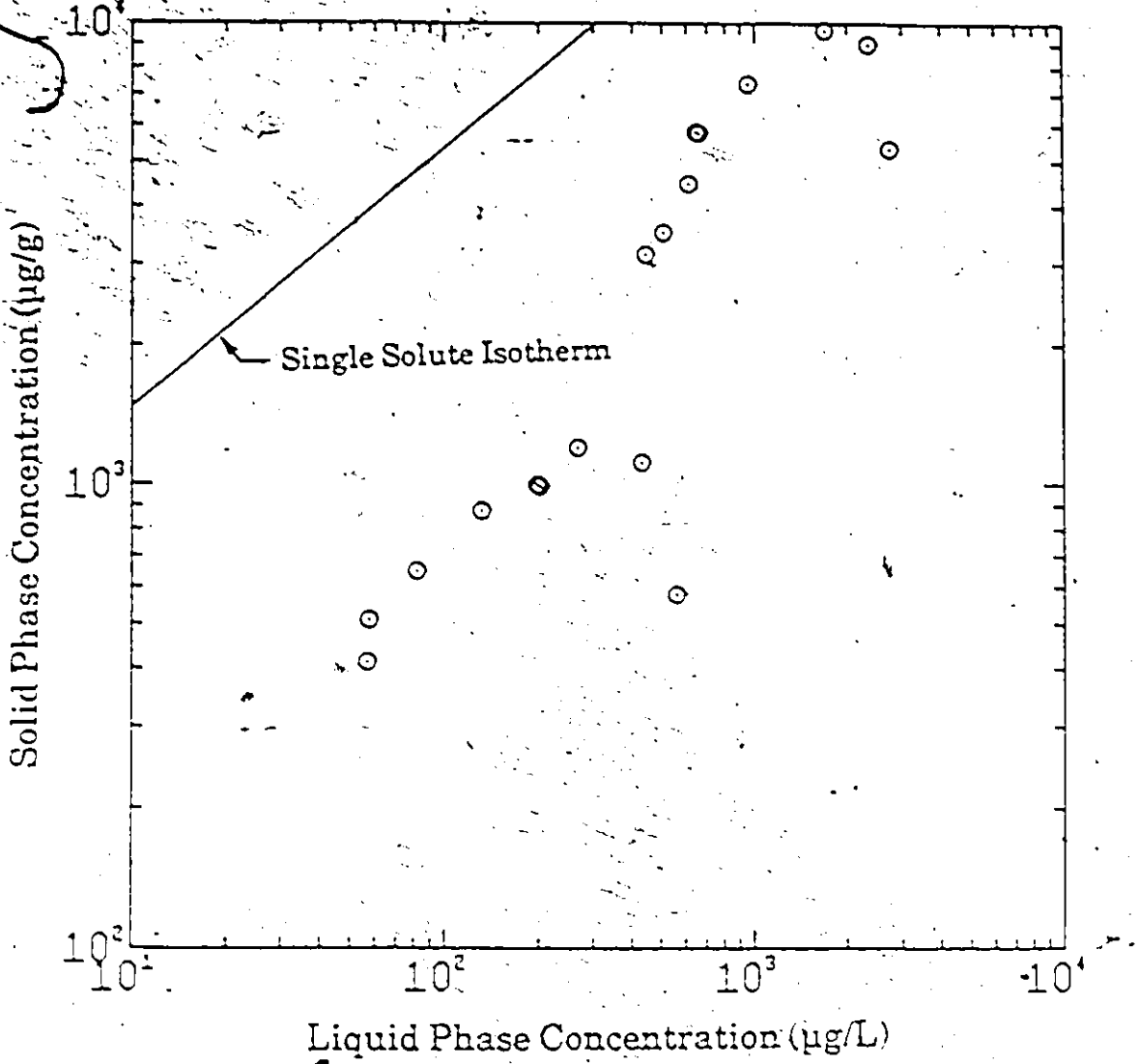


Figure 4.6.12 Apparent biosorption of malathion by activated sludge in competition with diazinon.

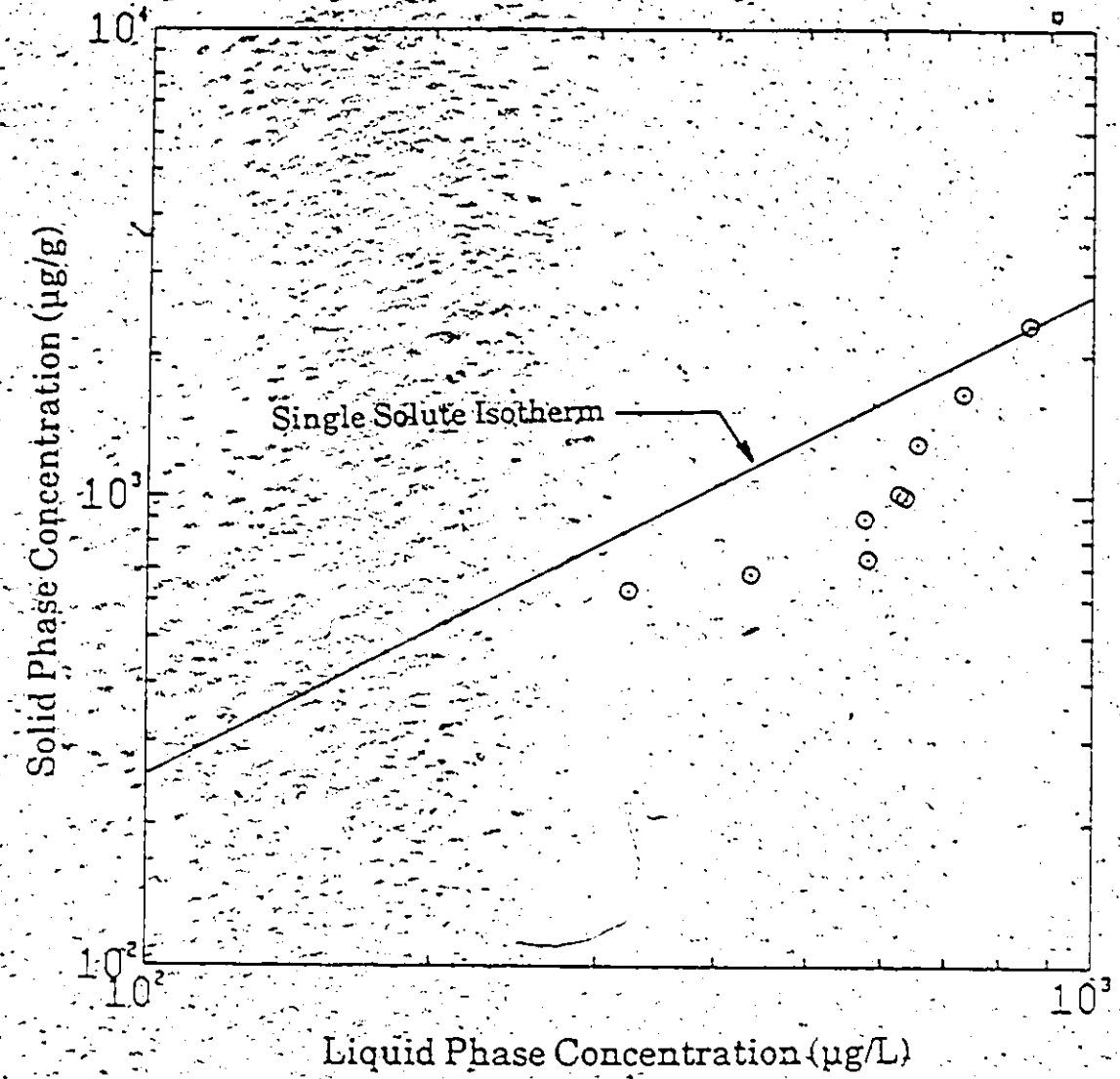


Figure 4.6.13 Biosorption of lindane by *R. arrhizus* in competition with diazinon, malathion, and pentachlorophenol.

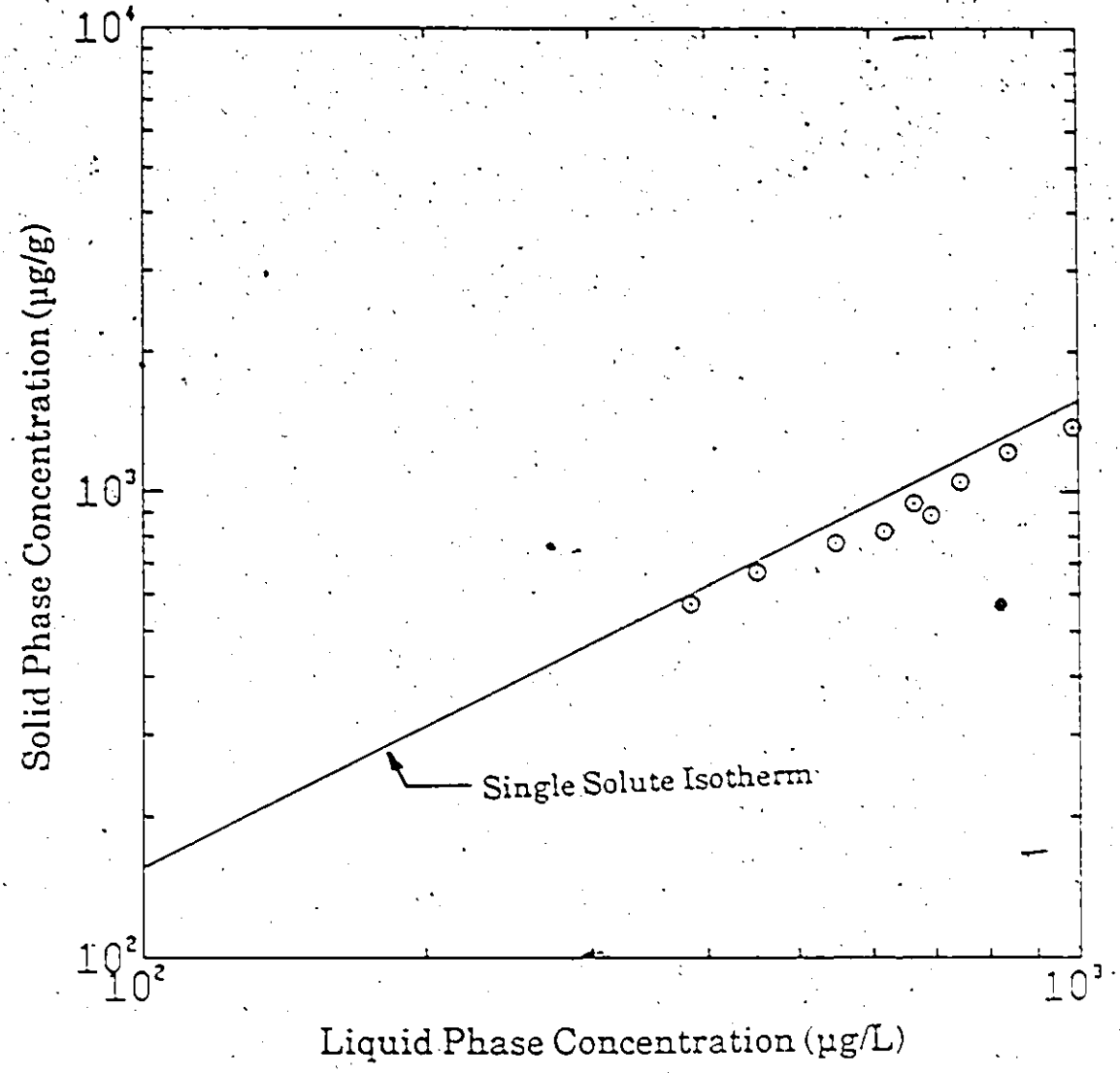


Figure 4.6.14 Biosorption of lindane by activated sludge in competition with diazinon, malathion, and pentachlorophenol.

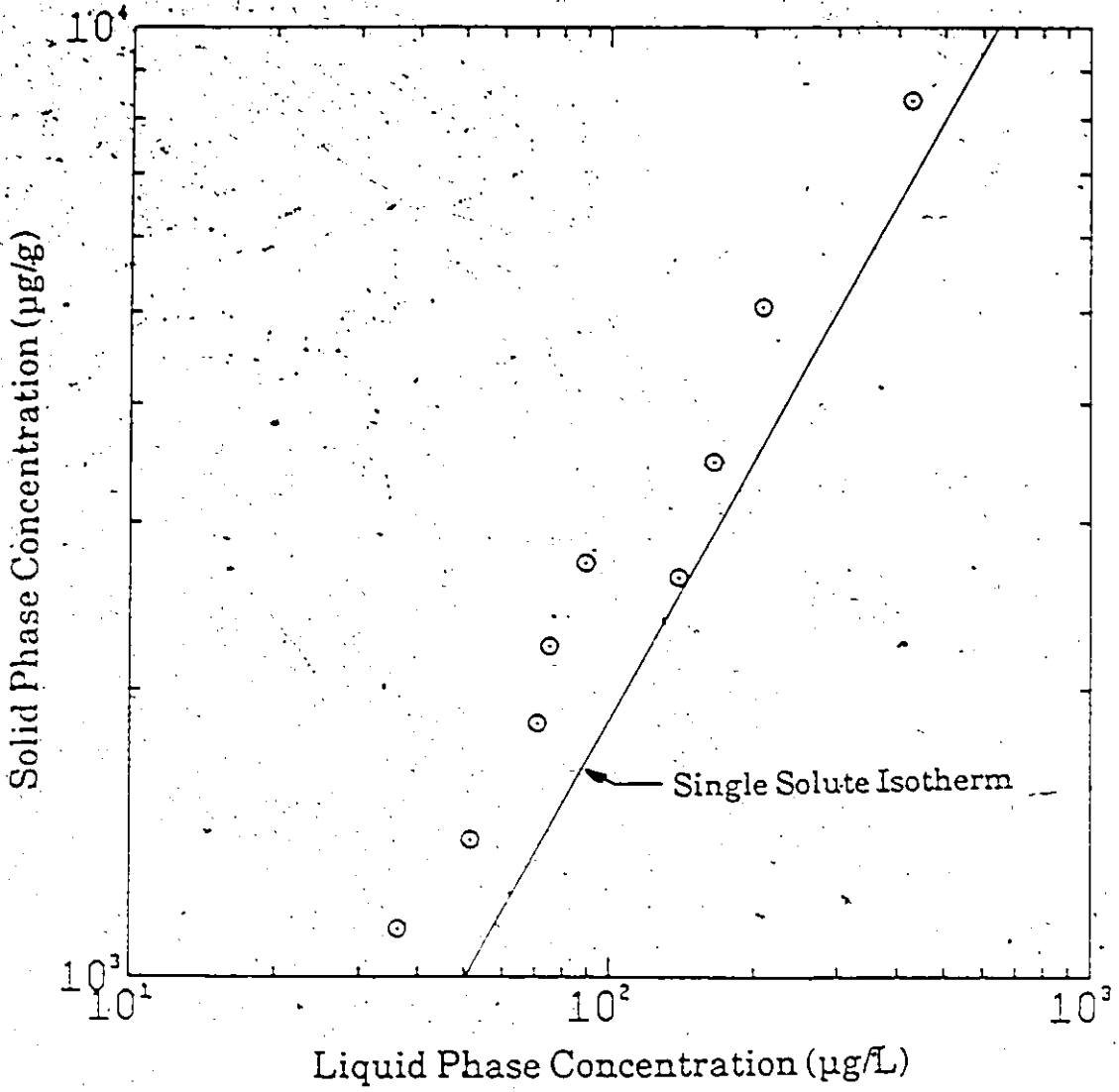


Figure 4.6.15 Biosorption of pentachlorophenol by *R. arrhizus* in competition with diazinon, lindane, and malathion.

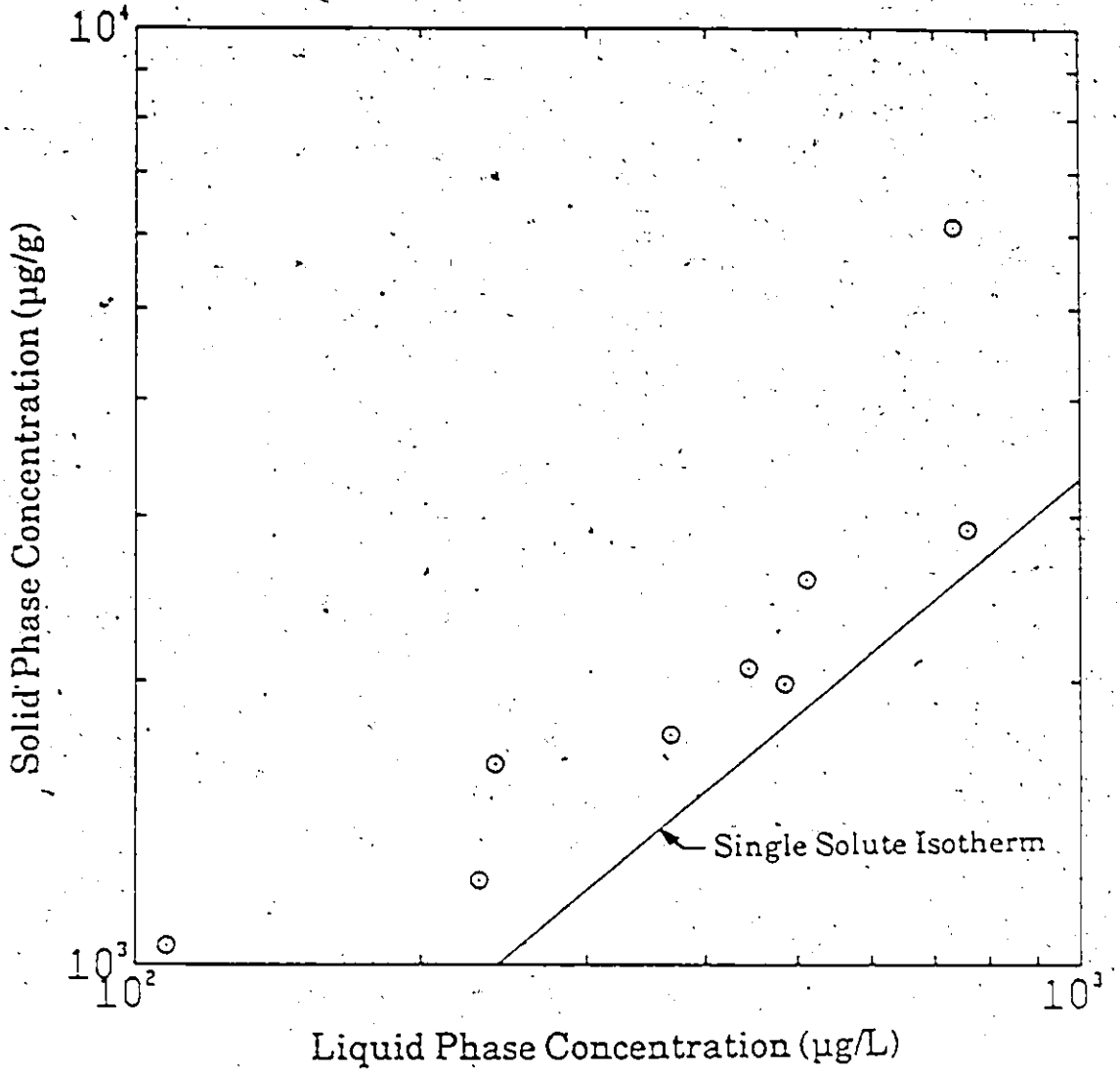


Figure 4.6.16 Biosorption of pentachlorophenol by activated sludge in competition with diazinon, lindane, and malathion.

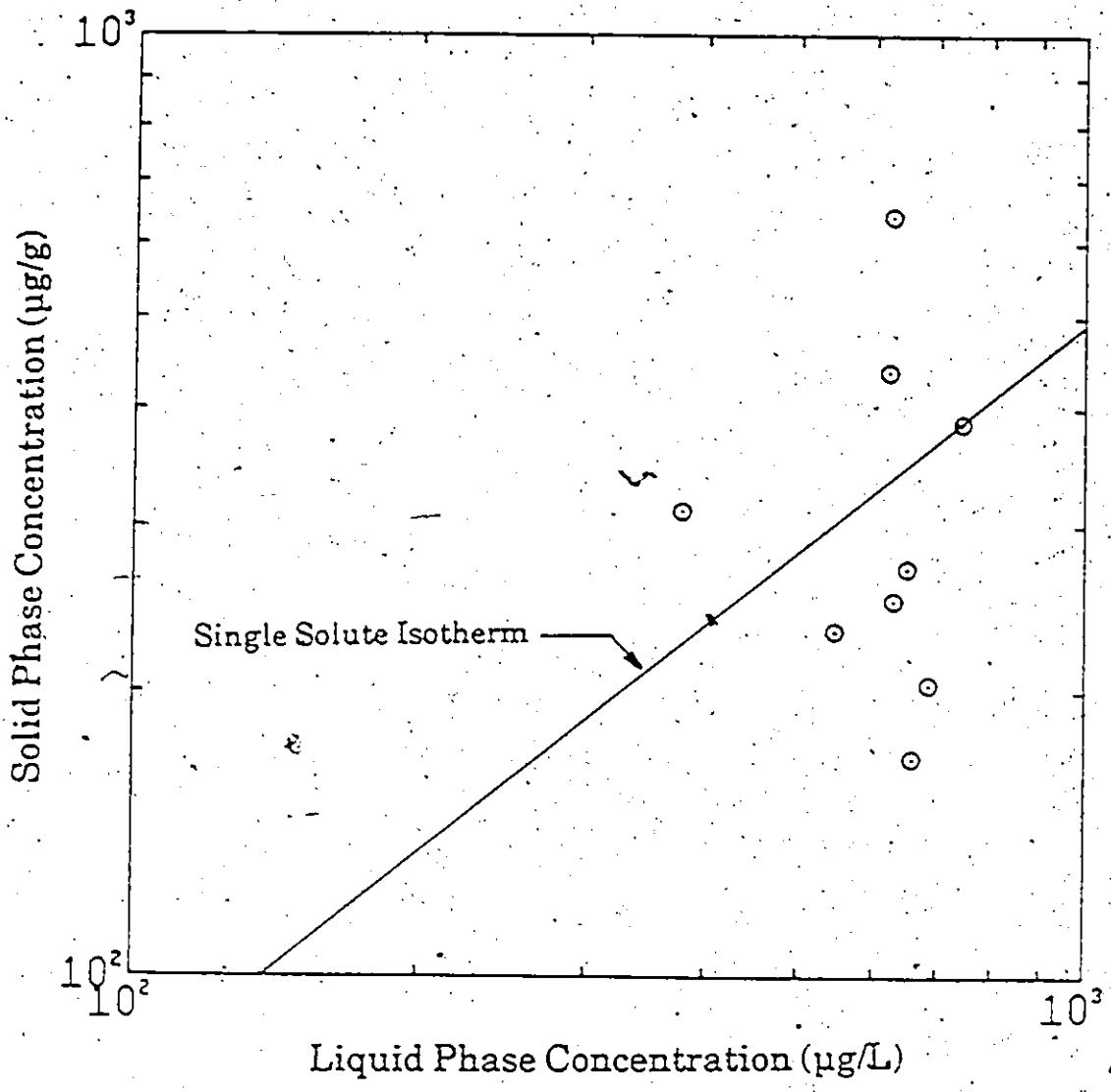


Figure 4.6.17 Biosorption of diazinon by *R. arrhizus* in competition with lindane, malathion, and pentachlorophenol.

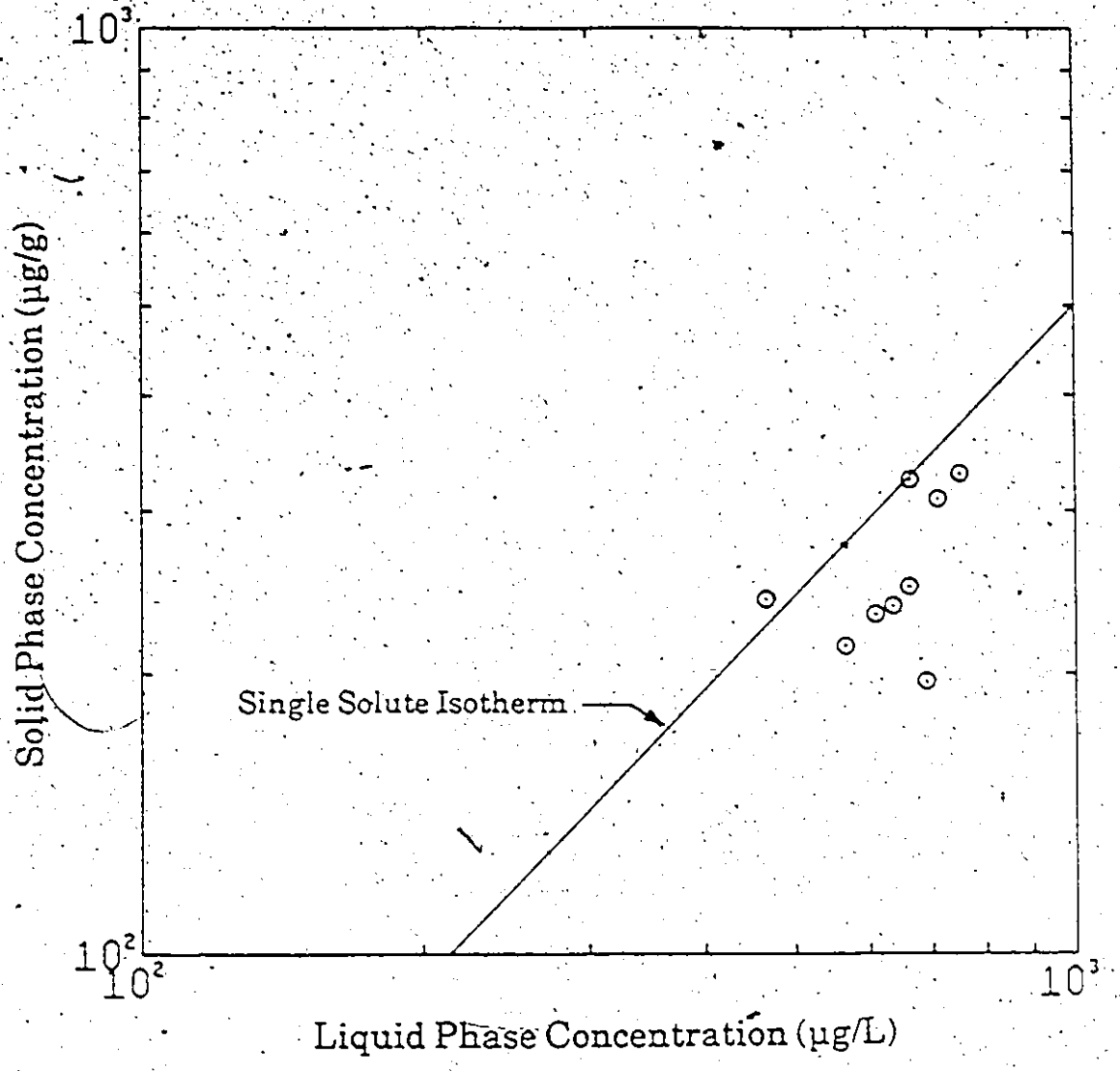


Figure 4.6.18 Biosorption of diazinon by activated sludge in competition with lindane, malathion, and pentachlorophenol.

4.7 SORPTION AND DESORPTION BY LIVE BIOMASS

Biosorption and desorption experiments were performed with live *R. arrhizus* and live activated sludge for all compounds except malathion. Since malathion appeared to be removed primarily by degradation rather than sorption and is known to be biodegradable (Paris et al., 1975), kinetics rather than equilibrium experiments were selected. Kinetics of degradation of malathion by live *R. arrhizus* were reported in Section 4.5 with the results of the dead biomass kinetics experiments. All experiments were conducted at a temperature of 20°C, and, in addition, experiments with lindane were done at 5°C. One sorption and one desorption datum point in each experiment was obtained using a contact time of three days for sorption and three days for desorption. All other data were obtained using a contact time of one day for sorption and one day for desorption to minimize the opportunity for biodegradation. Biosorption and desorption data are shown on Figures 4.7.1 through 4.7.20. Experimental data for sorption by live biomass are listed in Appendix B7 and corresponding data for desorption are given in Appendix B8.

4.8 CARBON-14 LABELED MALATHION EXPERIMENT

Carbon-14 labeled malathion in solutions of known concentration was contacted with inactive biomass and cell walls for a period of seven days to promote removal of the malathion from solution. The contact solutions were then filtered to separate the biomass. The filtrates were analyzed for residual malathion to determine the quantity of malathion which had been removed from solution. The filtrates were also analyzed for C-14 to determine the quantity of C-14 remaining in solution. If a large percentage of the malathion was found to have been removed from solution, while most of the original C-14 remained in solution it would indicate that the malathion had been decomposed and the decomposition products had remained in solution. The filters with the collected biomass were also analyzed for C-14 to

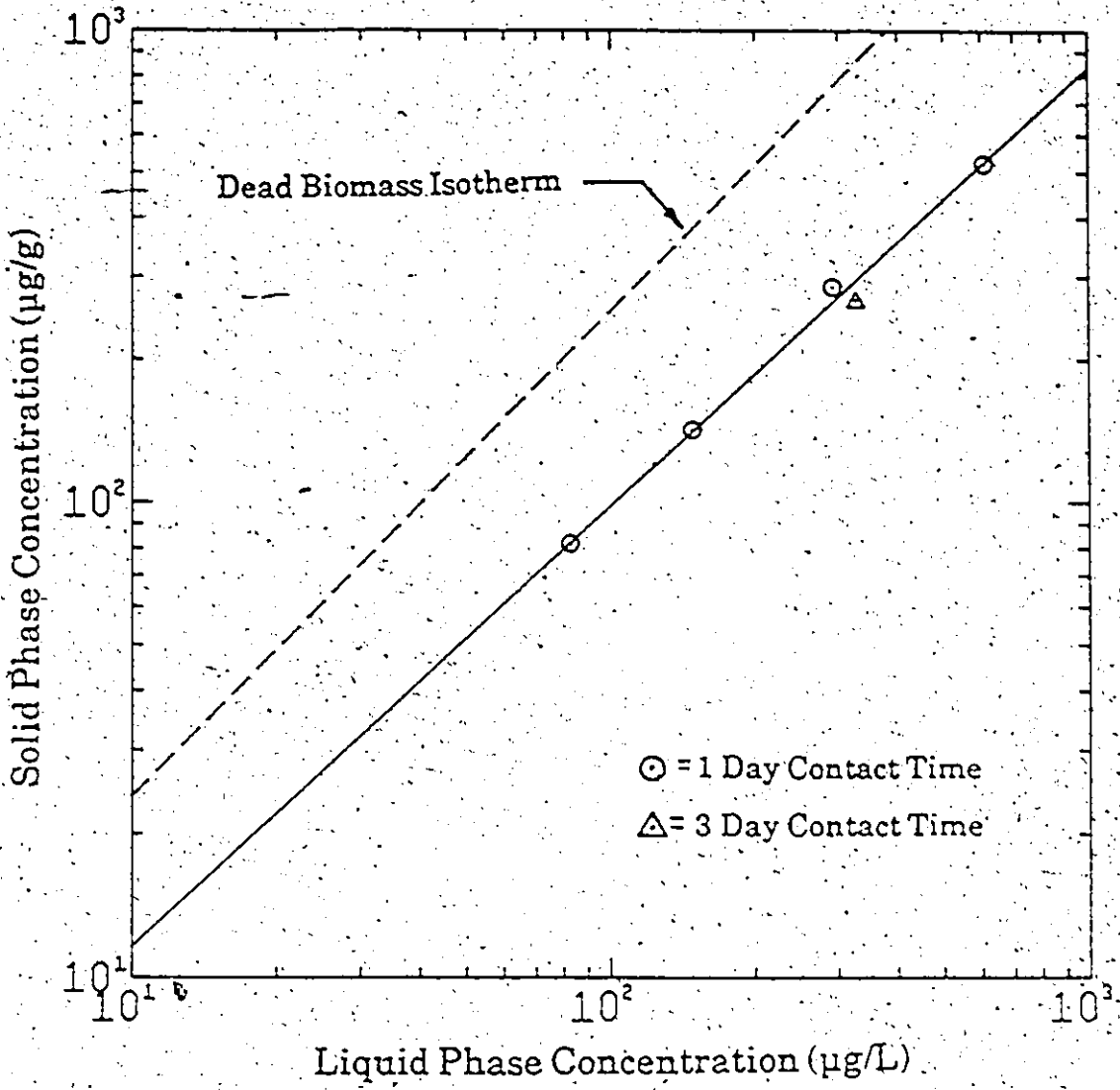


Figure 4.7.1 - Biosorption of lindane by live *R. arrhizus*.

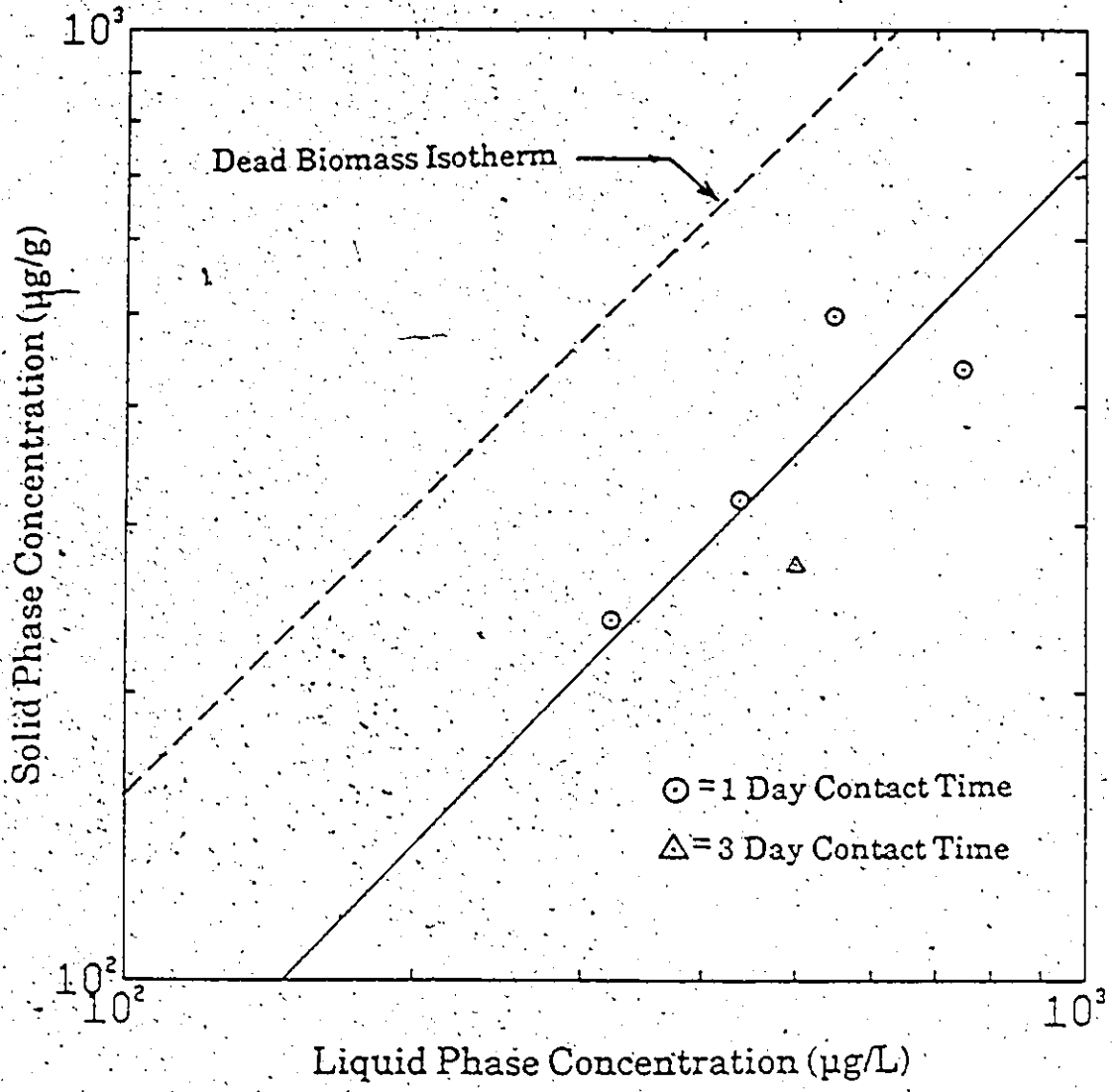


Figure 4.7.2 Biosorption of lindane by live activated sludge.

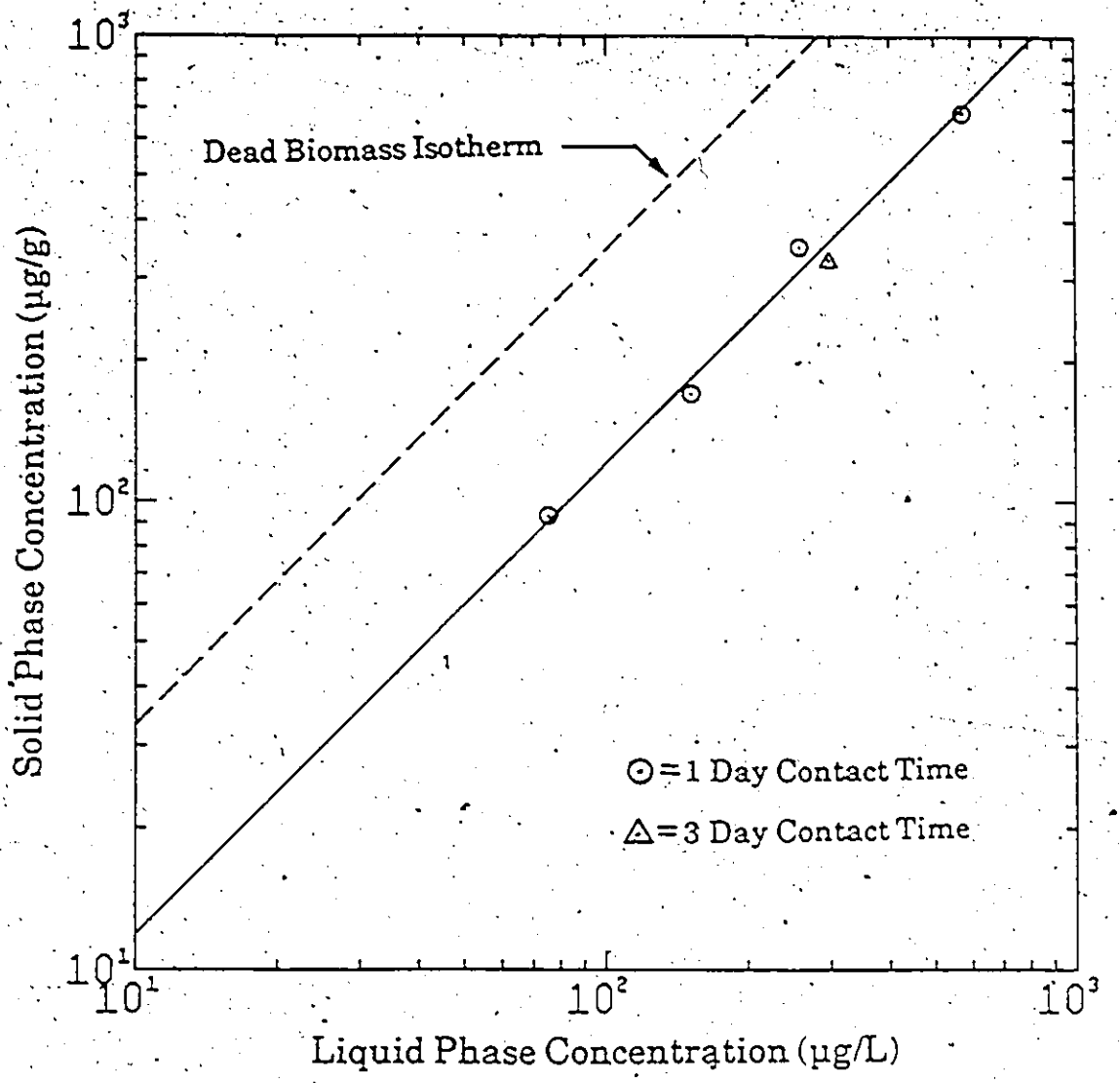


Figure 4.7.3 Biosorption of lindane by live *R. arrhizus* at 5°C.

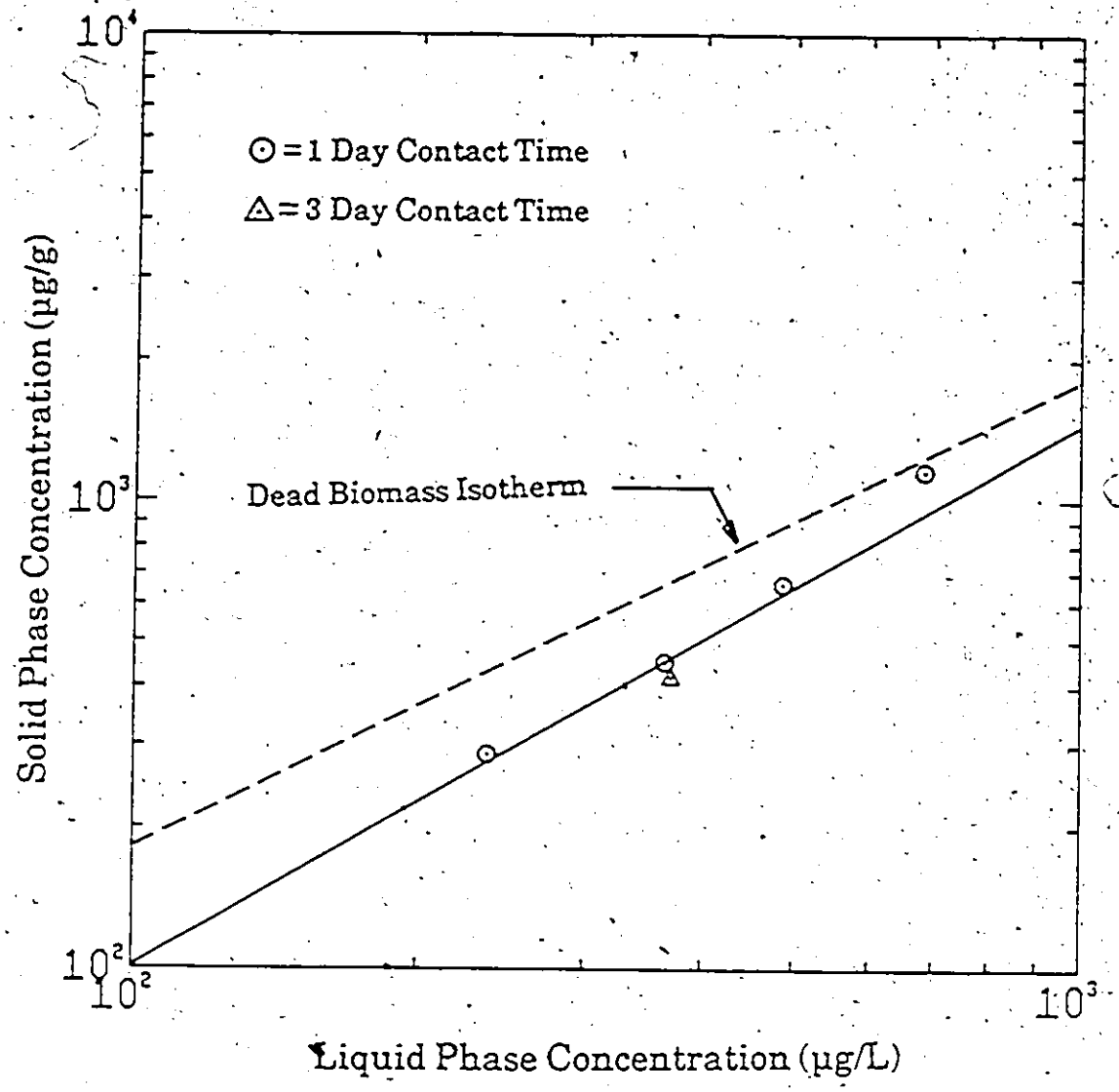


Figure 4.7.4 Biosorption of lindane by live activated sludge at 5°C.

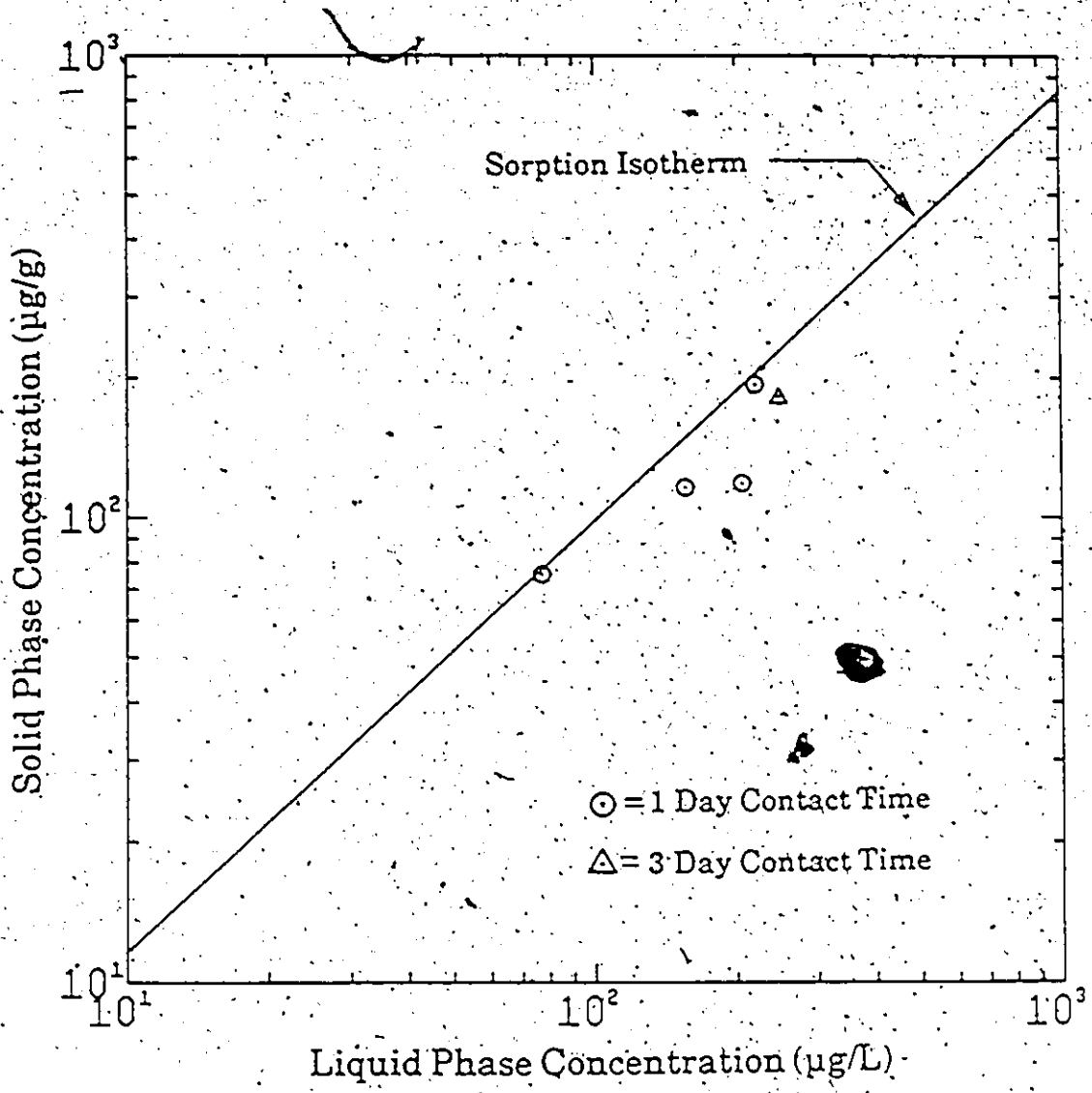


Figure 4.7.5 Desorption of lindane by live *R. arrhizus*.

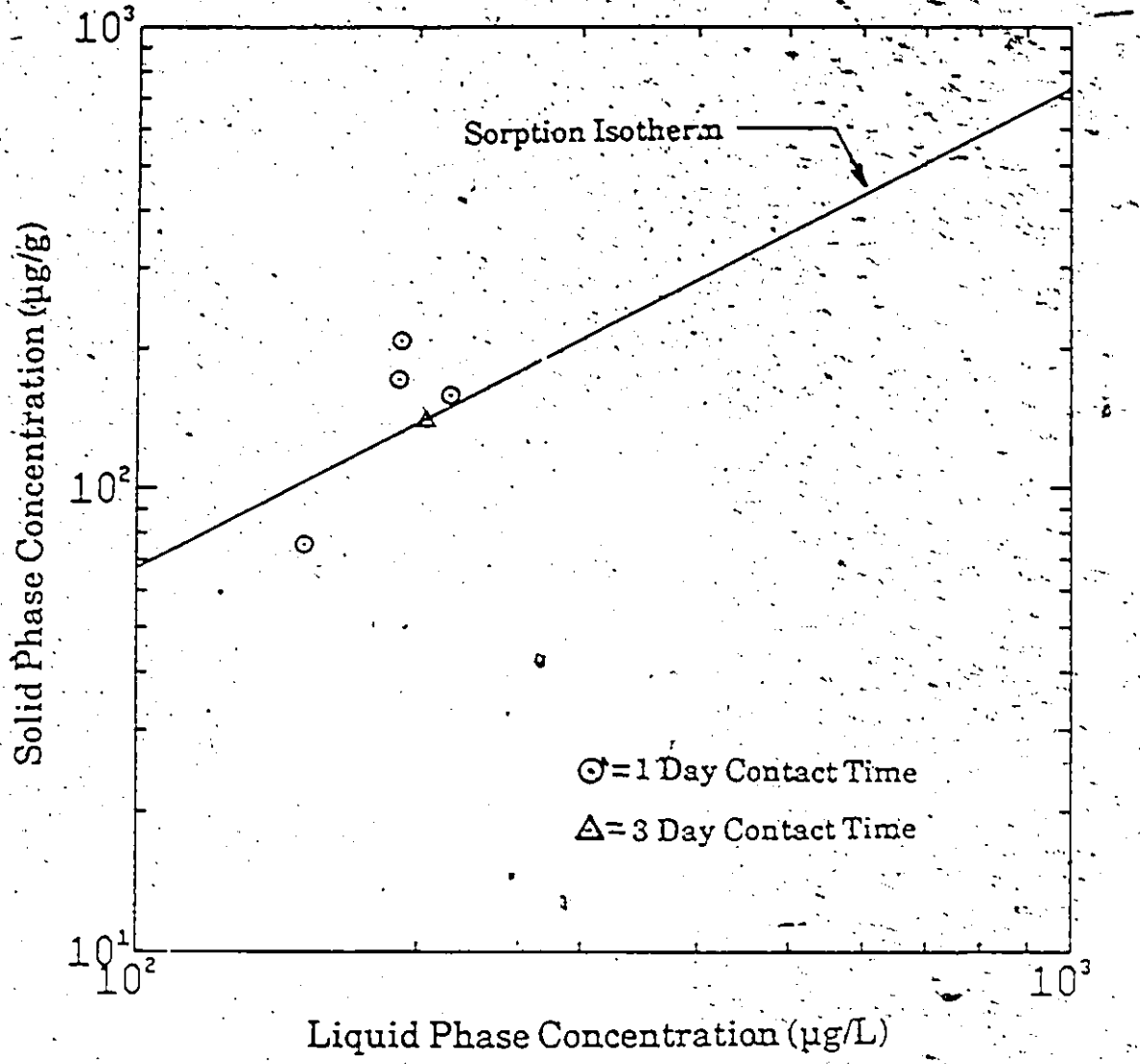


Figure 4.7.6 Desorption of lindane by live activated sludge.

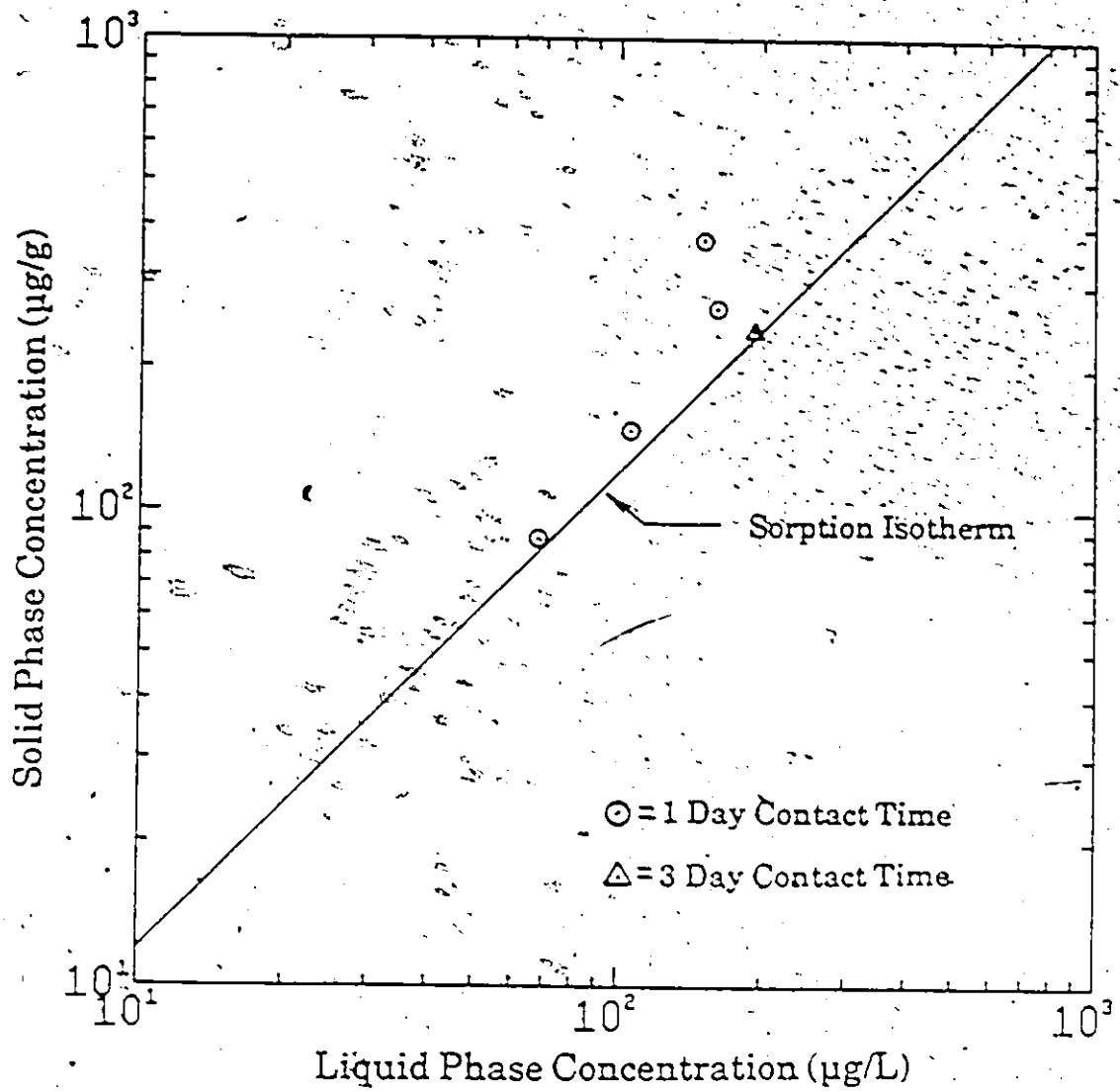


Figure 4.7.7 Desorption of lindane by live *R. arrhizus* at 5°C.

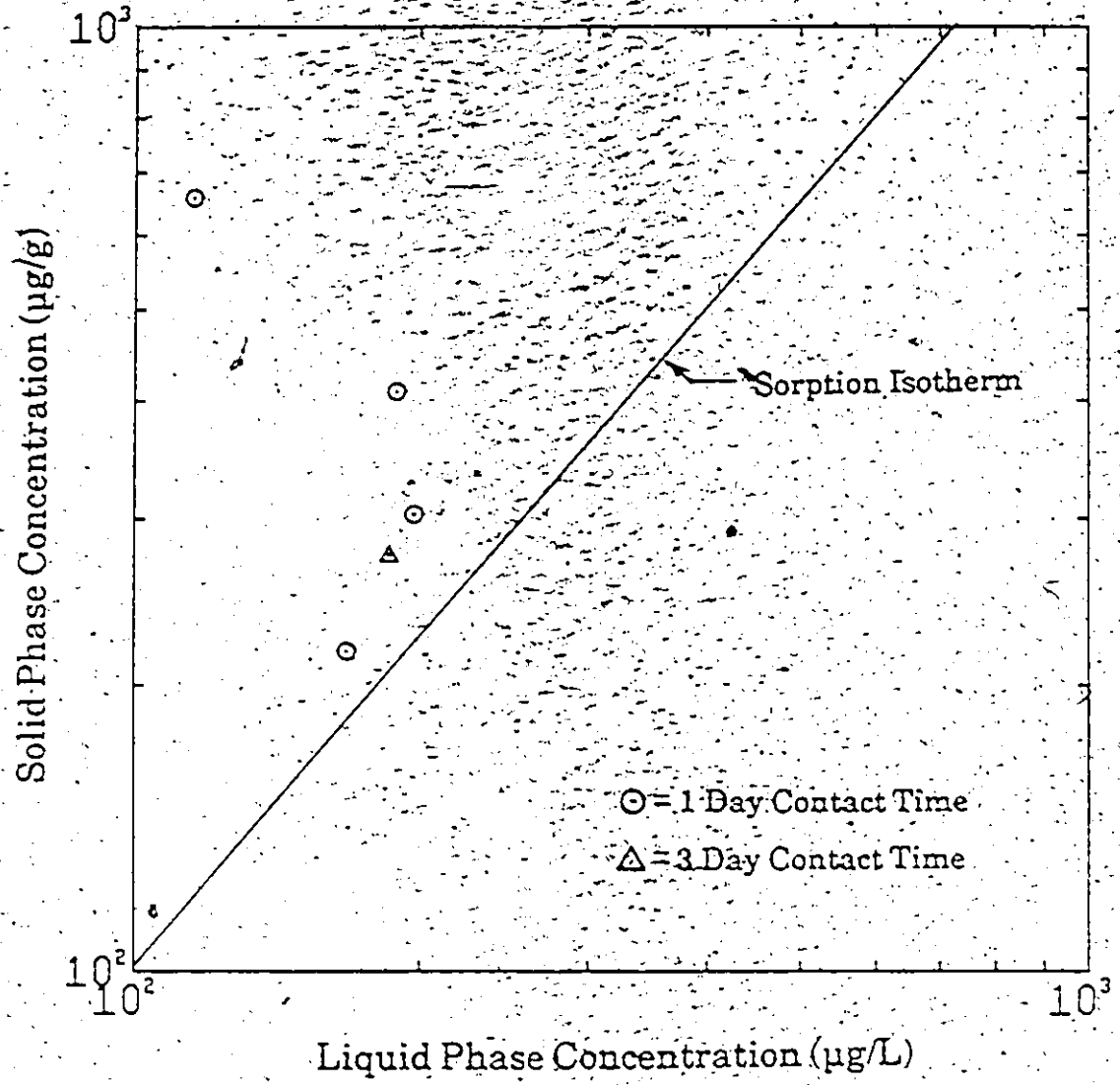


Figure 4.7.8 -- Desorption of lindane by live activated sludge at 5°C.

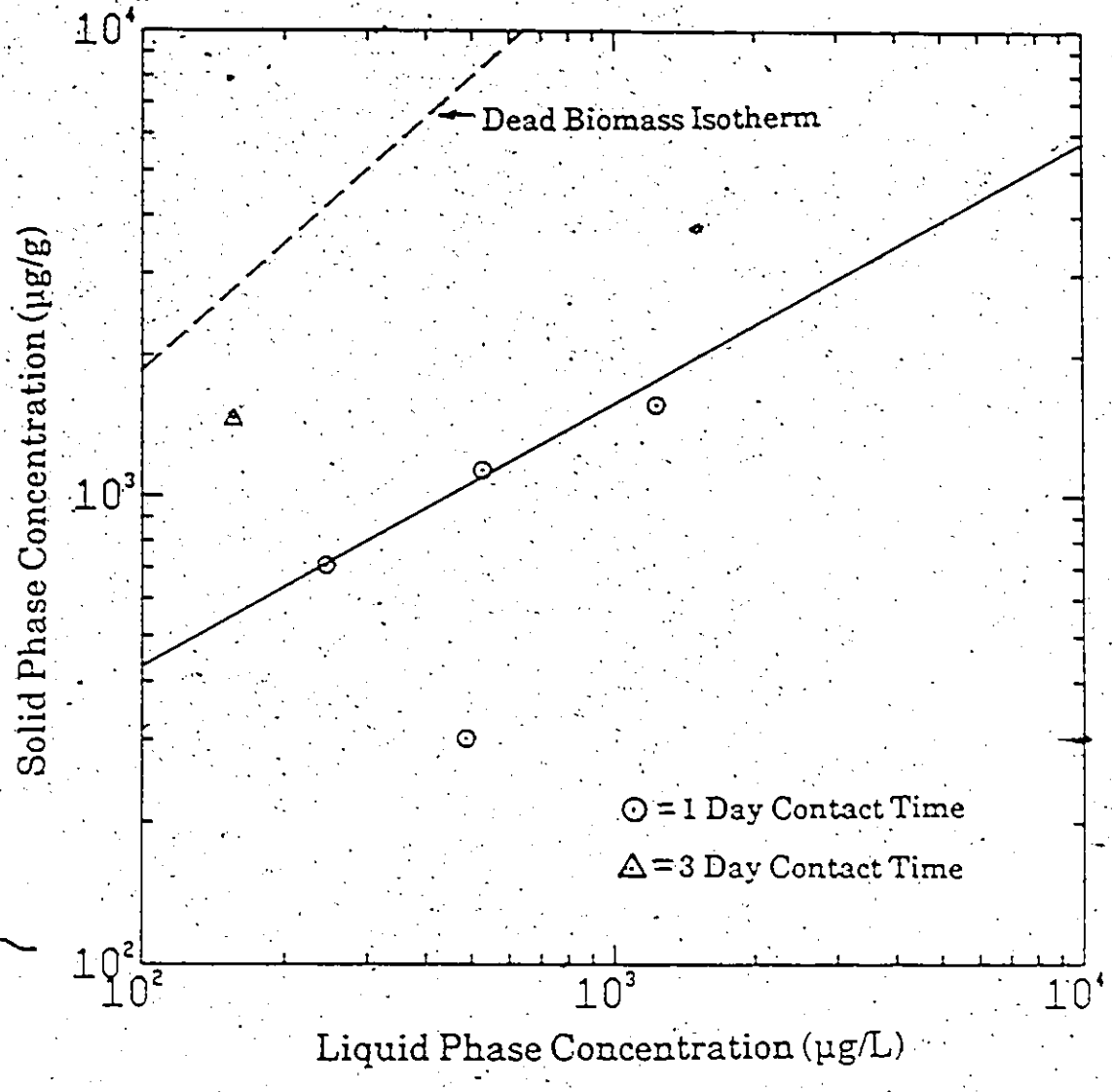


Figure 4.7.9 Biosorption of pentachlorophenol by live *R. arrhizus*.

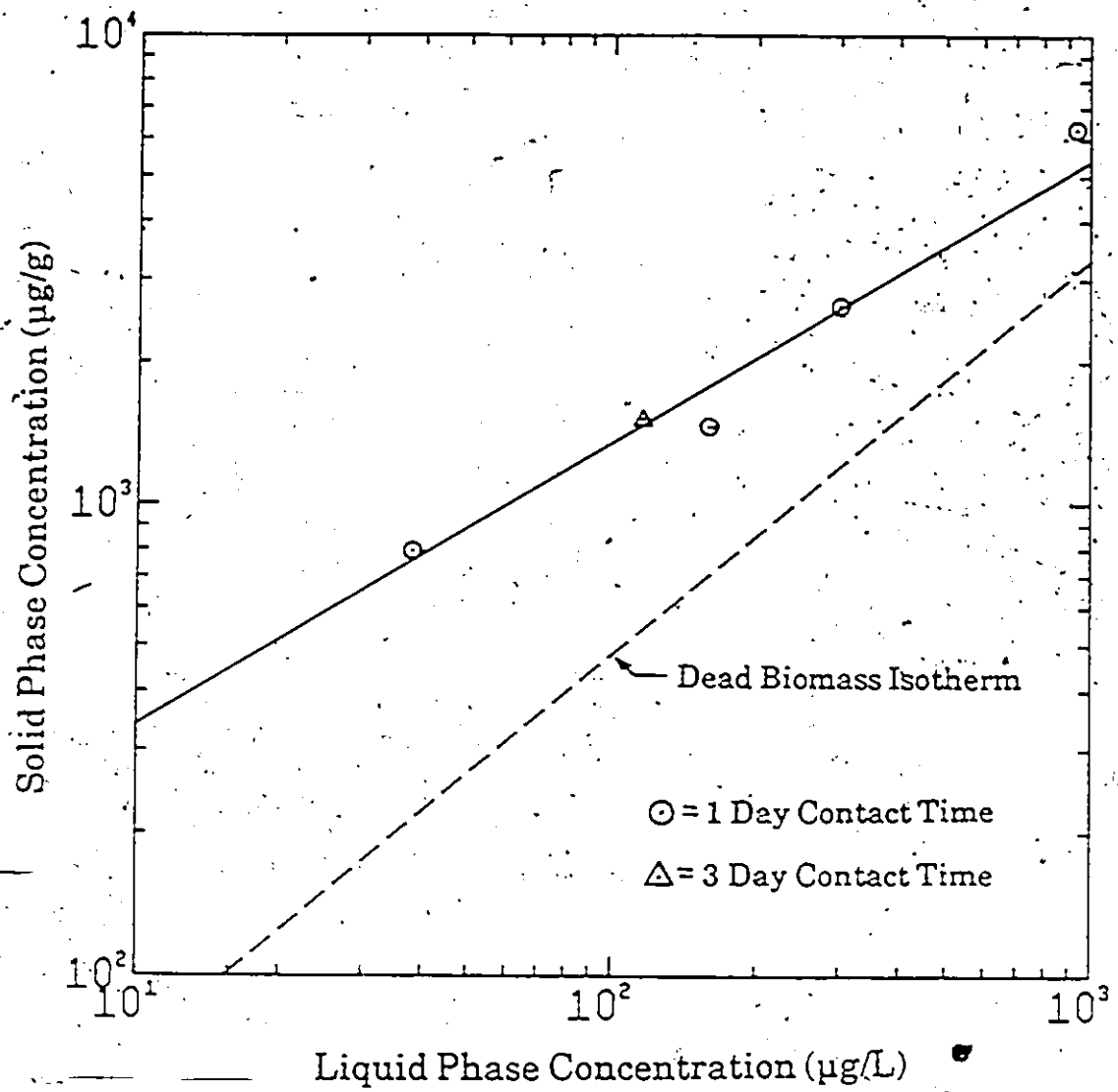


Figure 4.7.10. Biosorption of pentachlorophenol by live activated sludge.

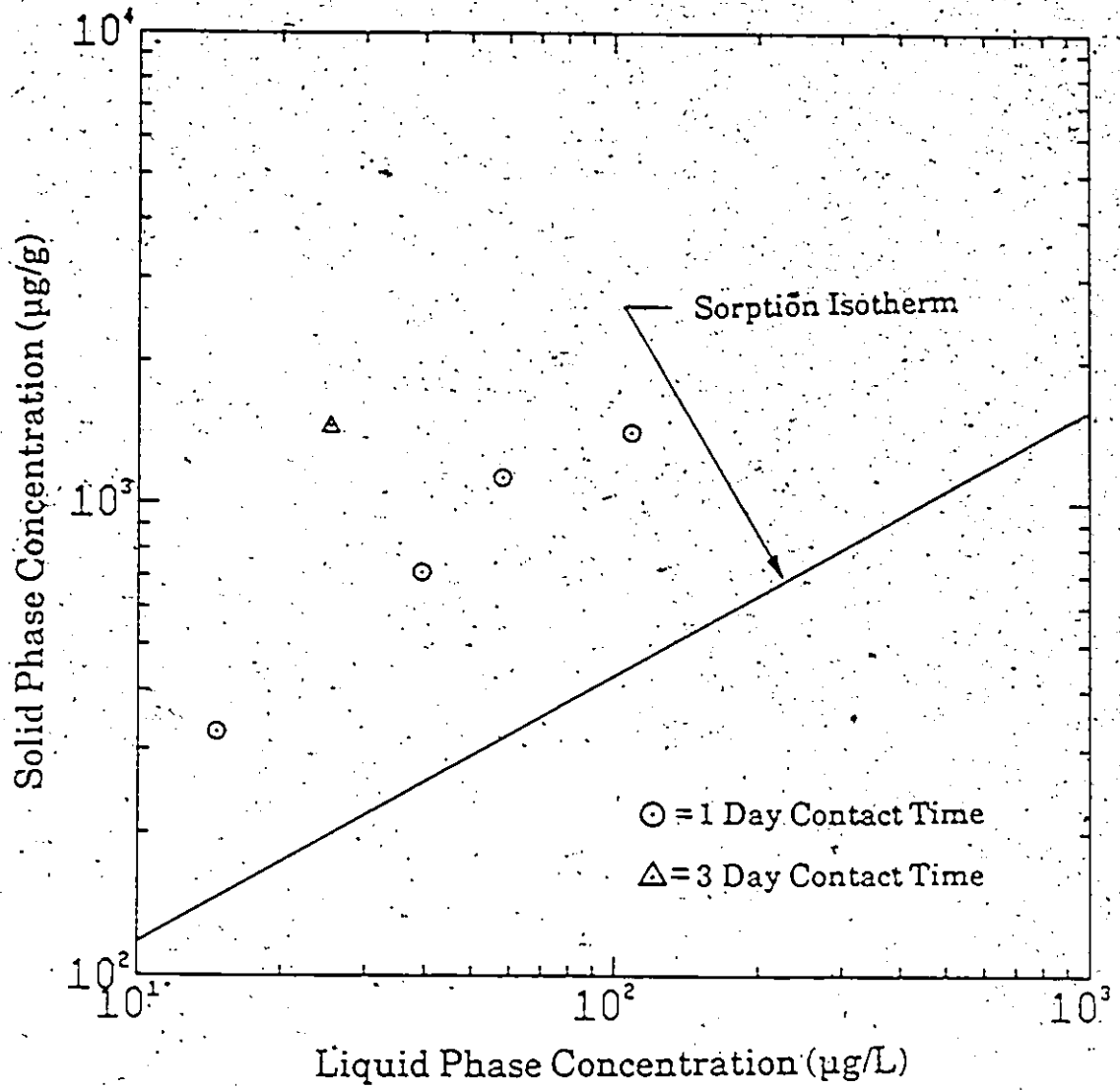


Figure 4.7.11 Desorption of pentachlorophenol by live *R. arrhizus*.

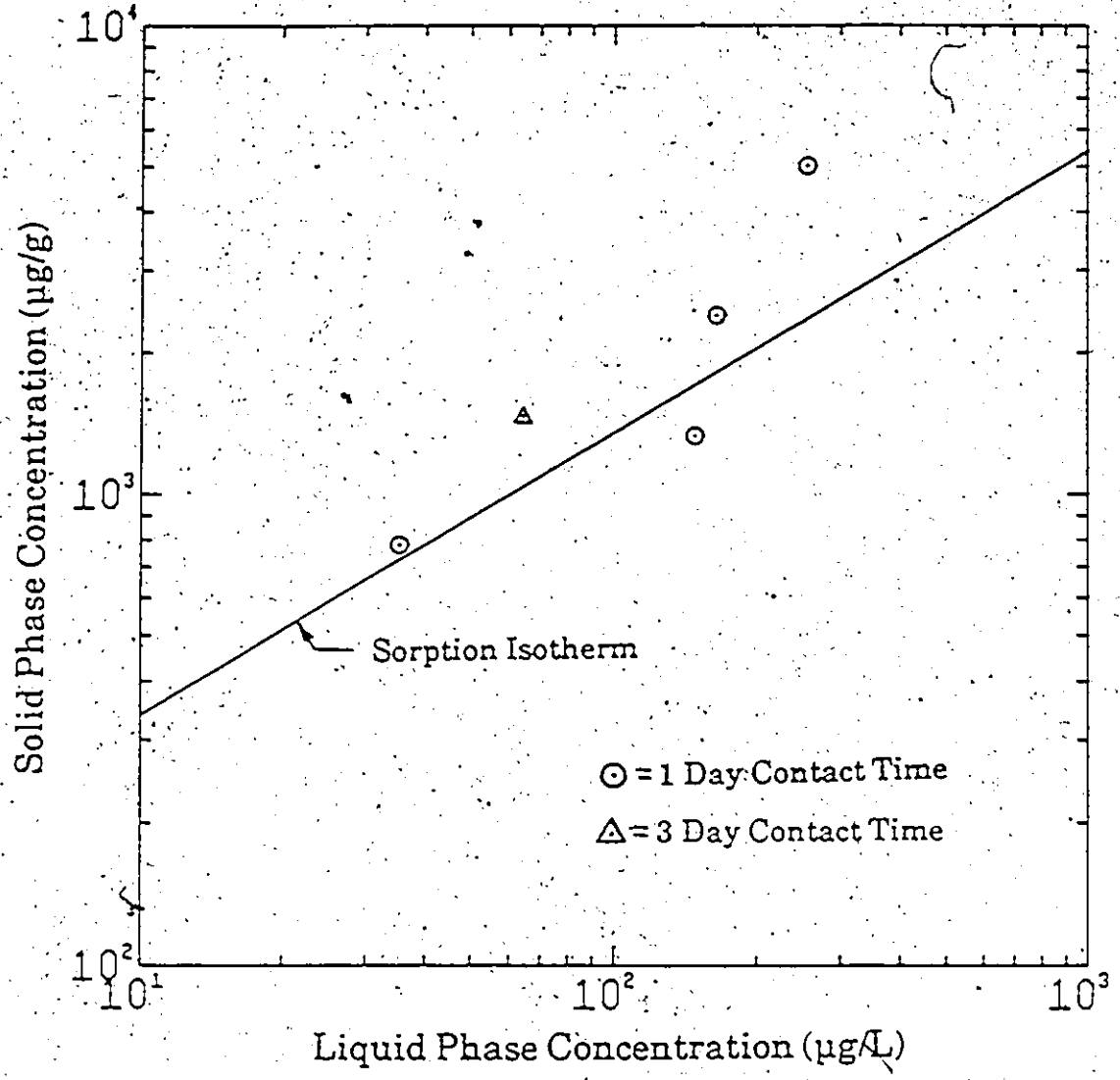


Figure 4.7.12 Desorption of pentachlorophenol by live activated sludge.

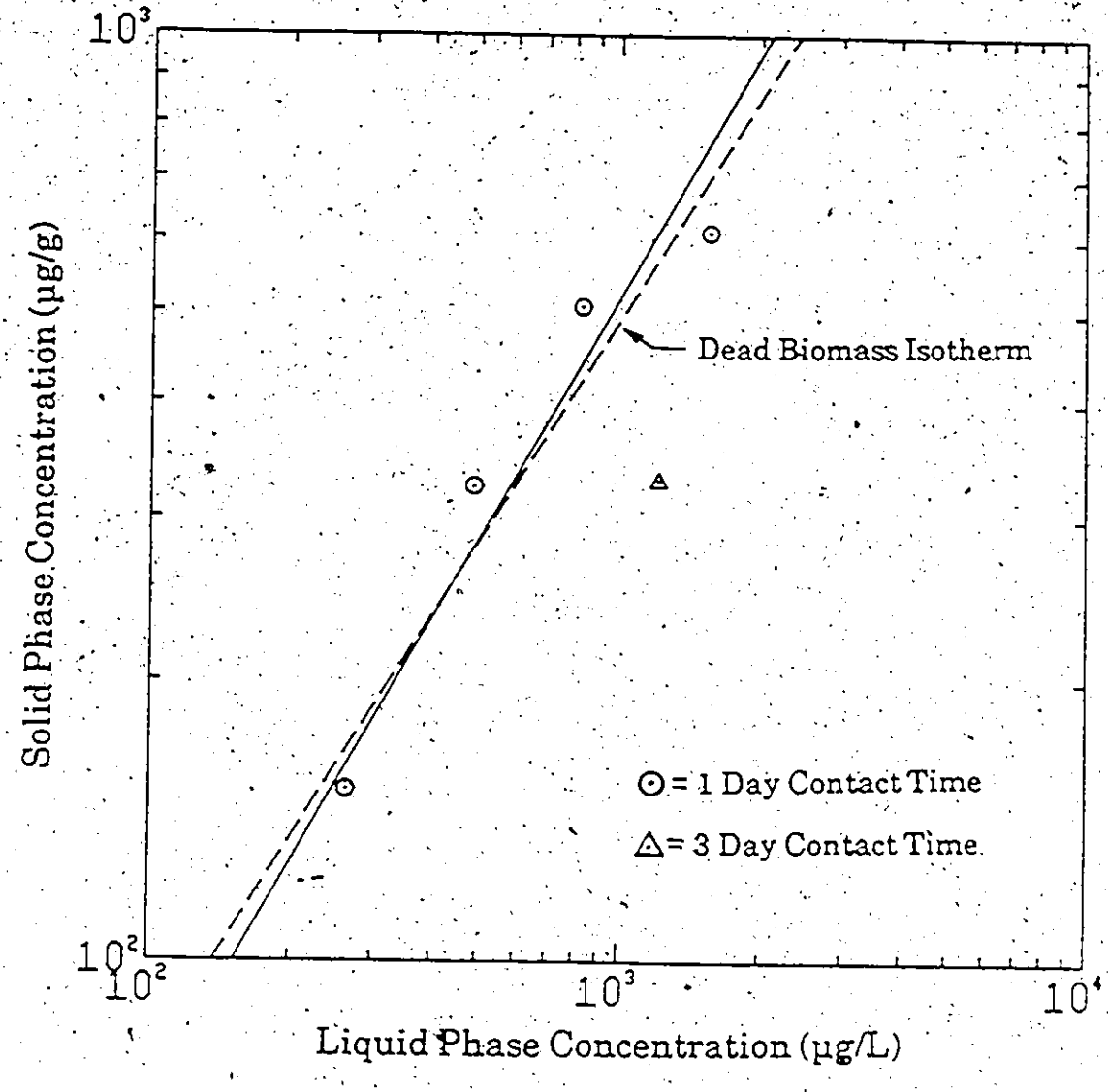


Figure 4.7.13 Biosorption of diazinon by live *R. arrhizus*.

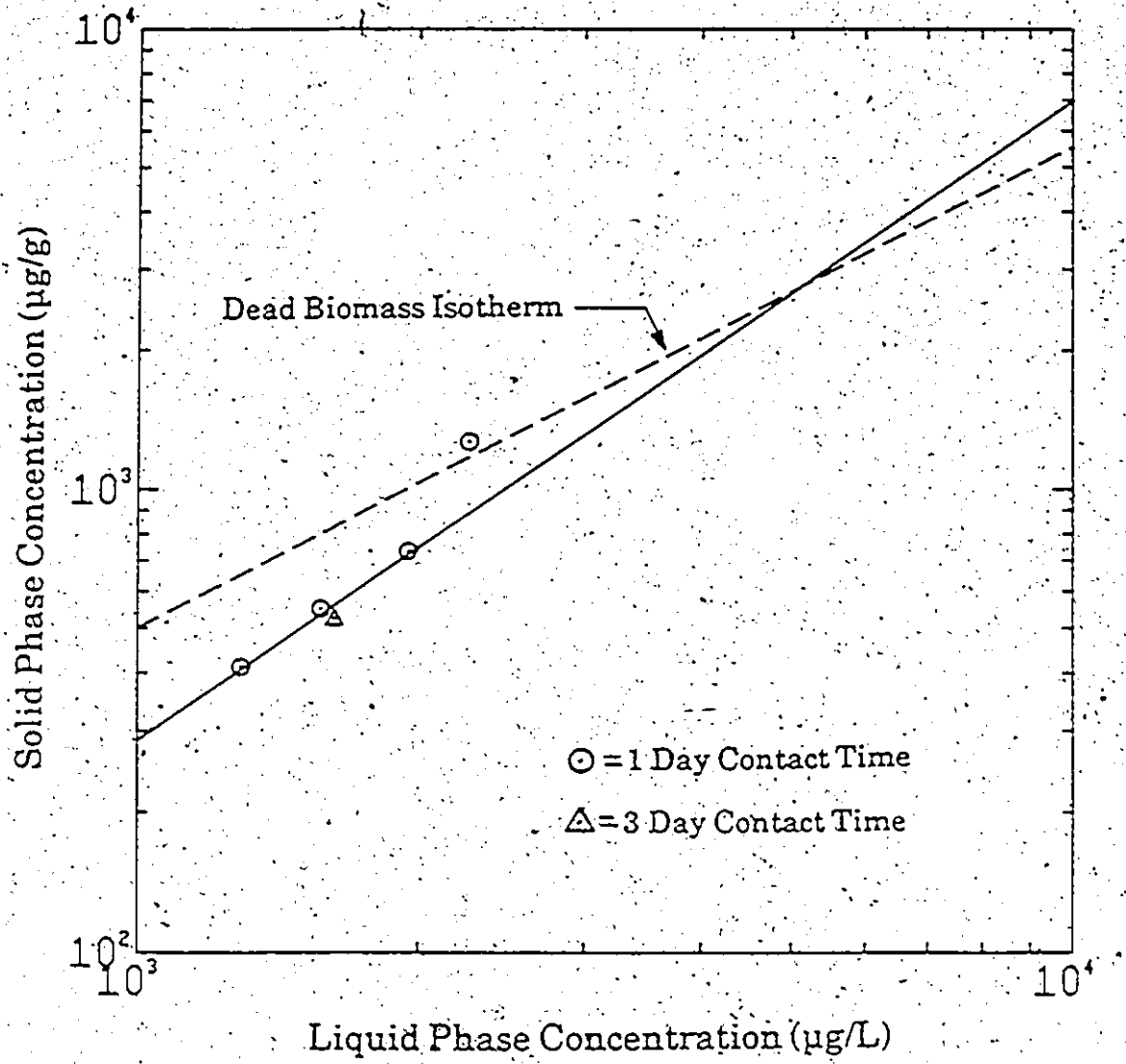


Figure 4.7.14 Biosorption of diazinon by live activated sludge.

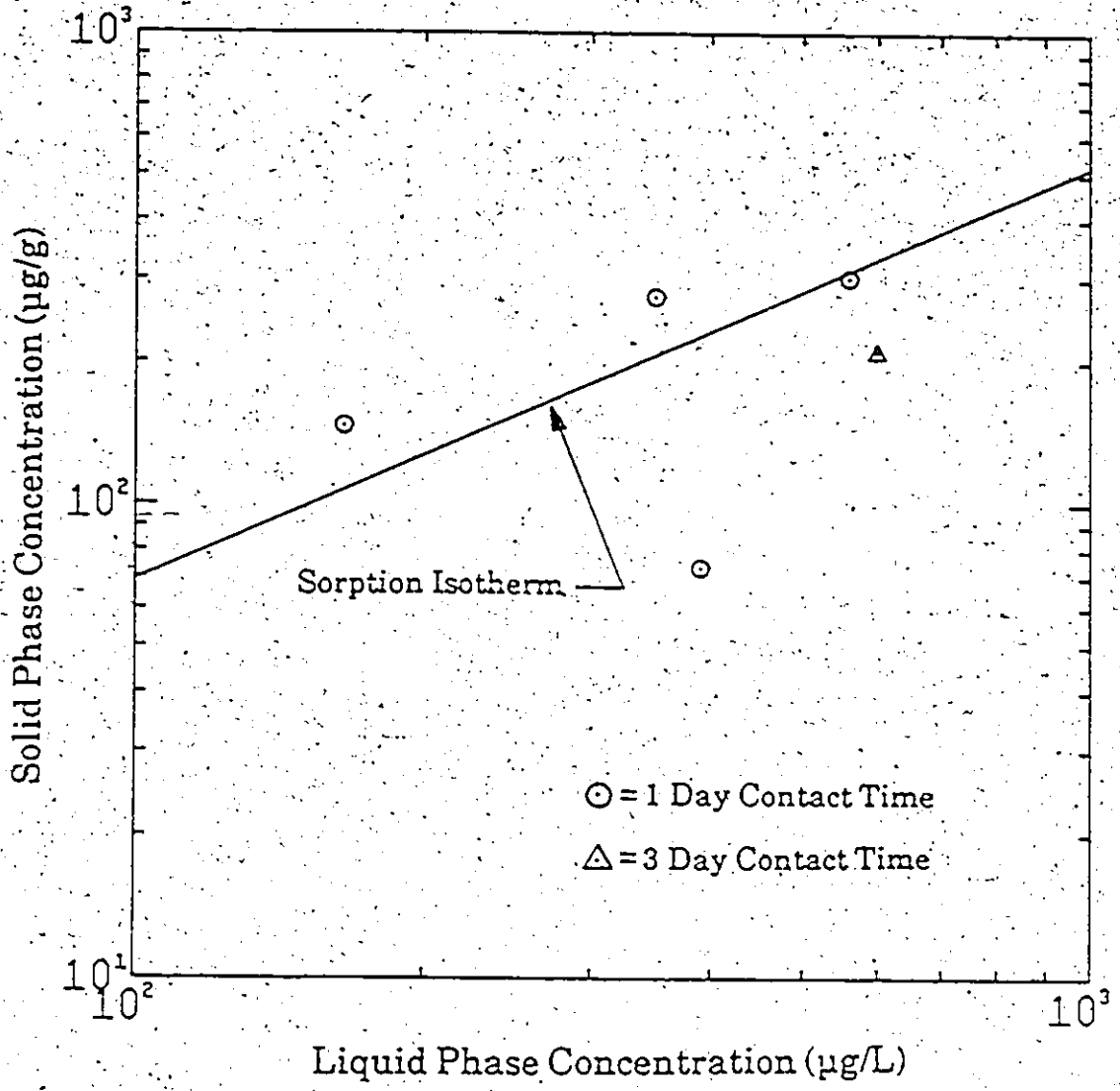


Figure 4.7.15 Desorption of diazinon by live *R. arrhizus*.

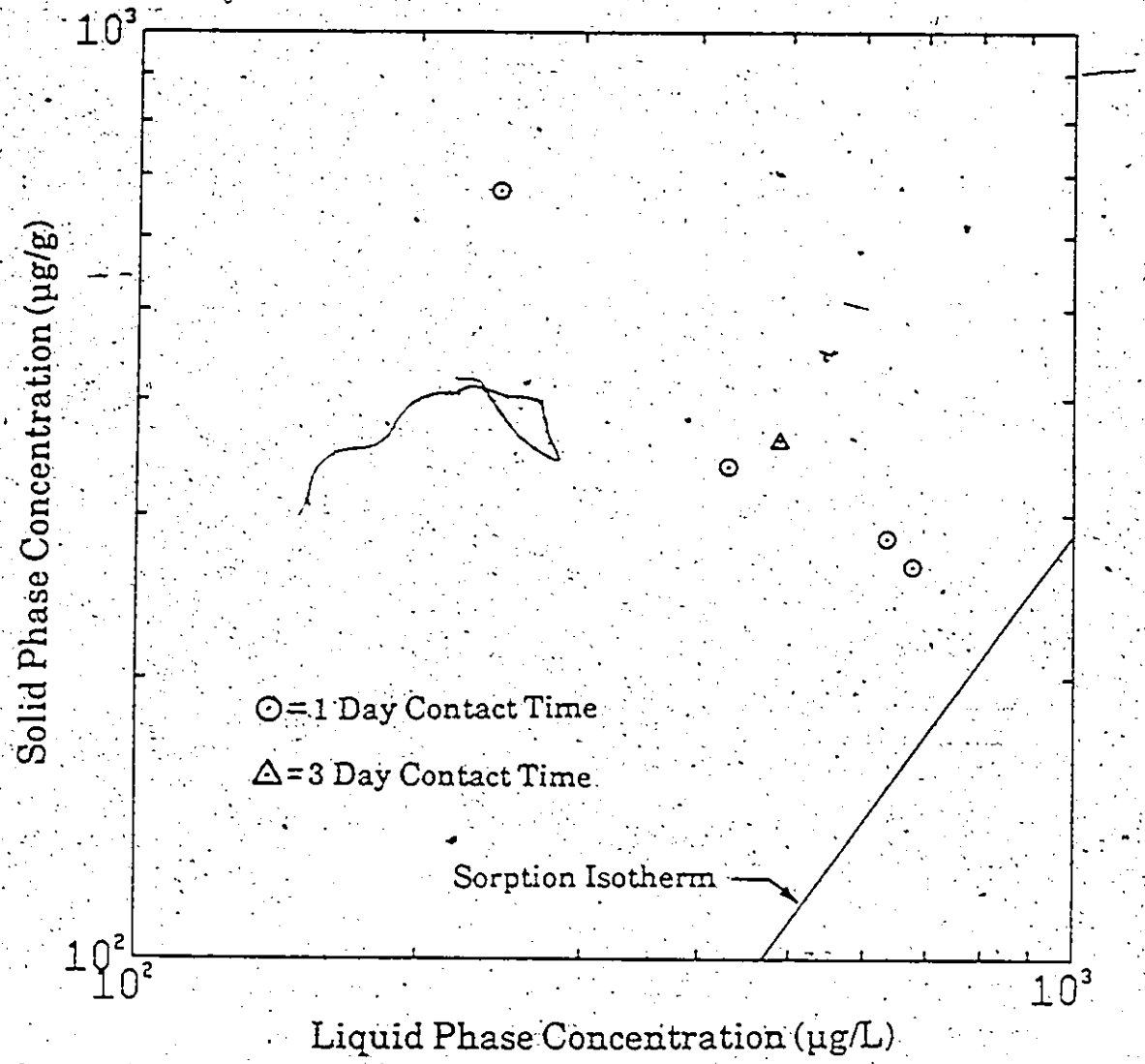


Figure 4.7.16 Desorption of diazinon by live activated sludge.

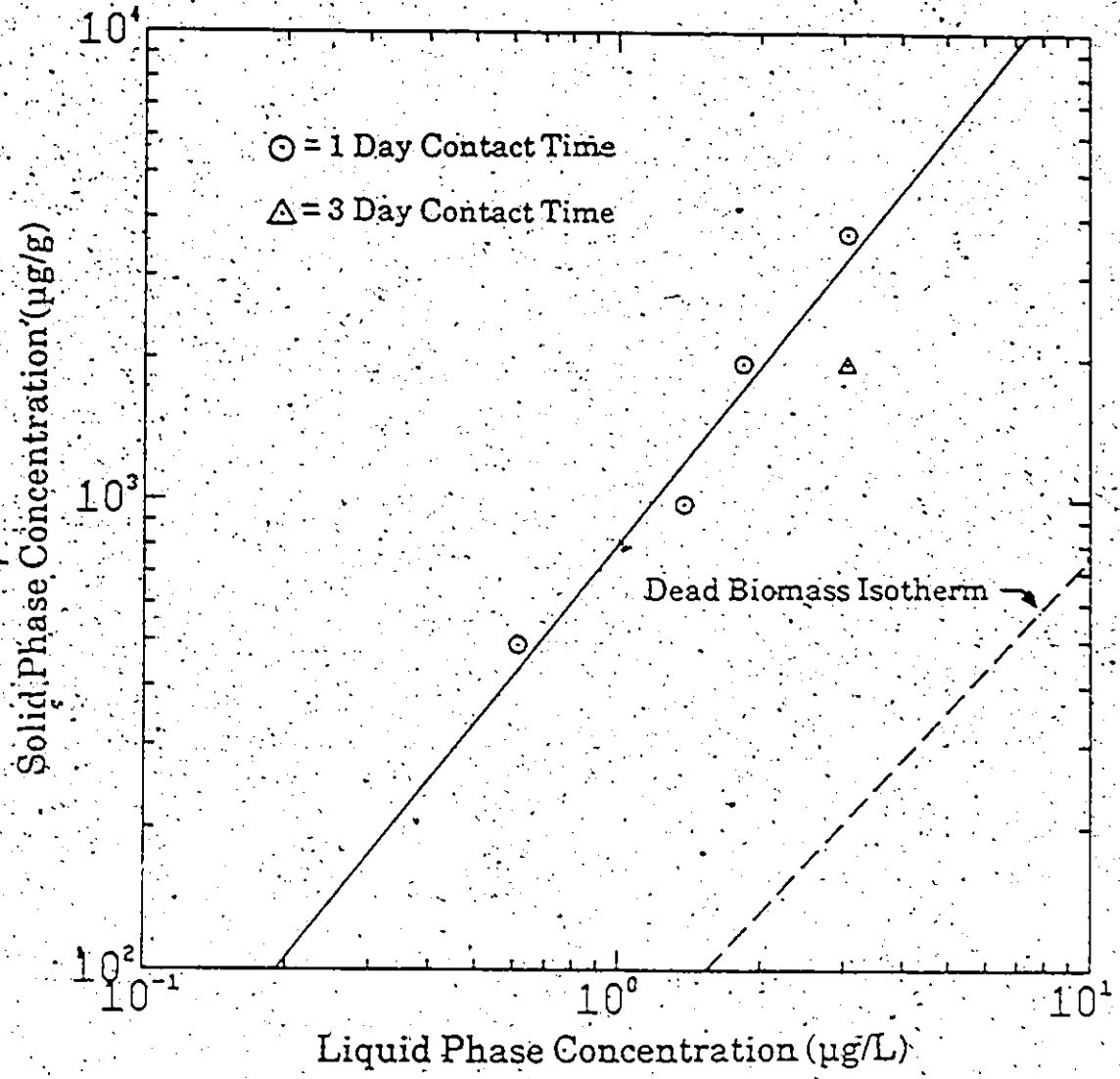


Figure 4.7.17 Biosorption of 2-chlorobiphenyl by live *R. arrhizus*.

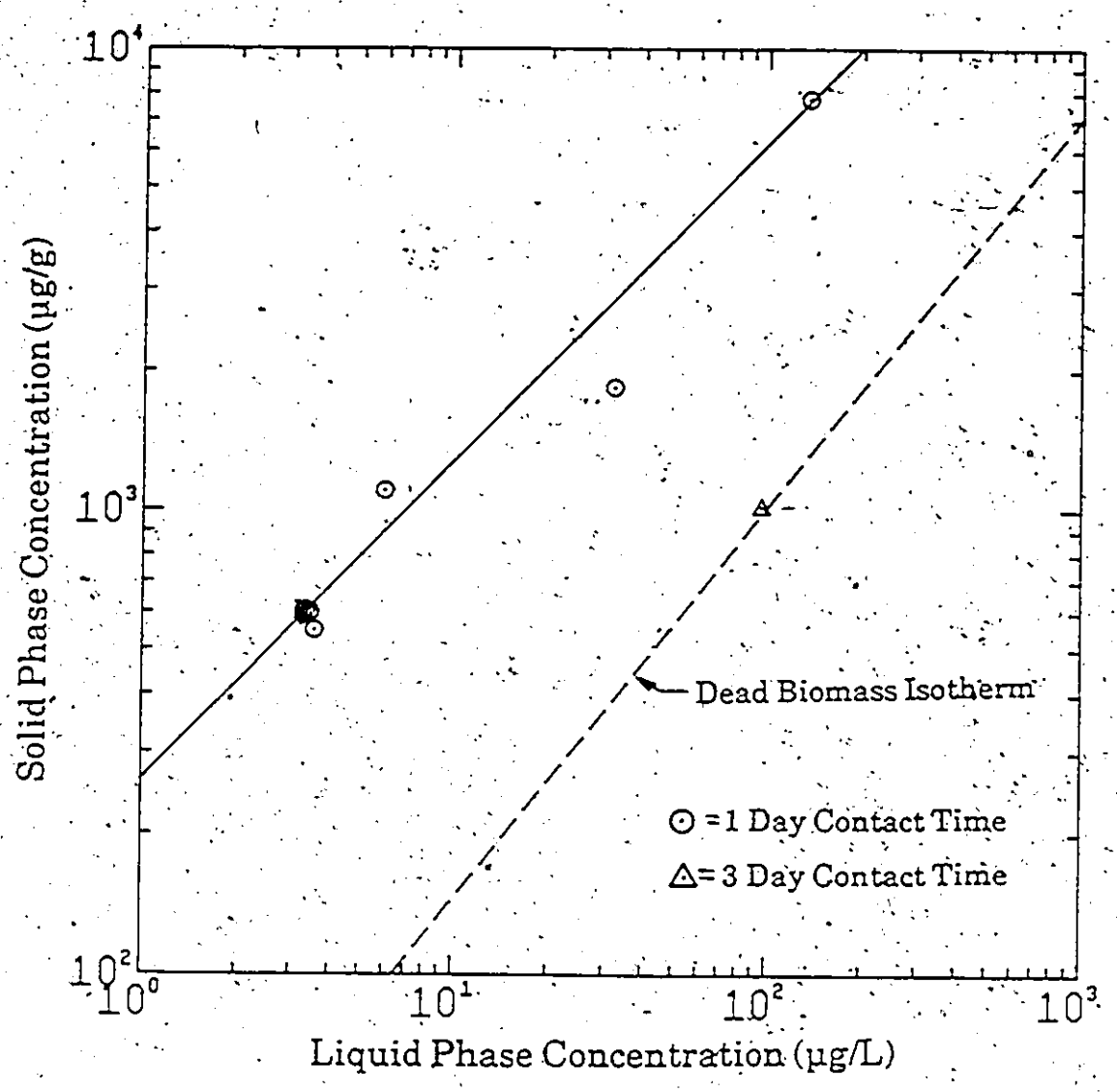


Figure 4.7.18 Biosorption of 2-chlorobiphenyl by live activated sludge.

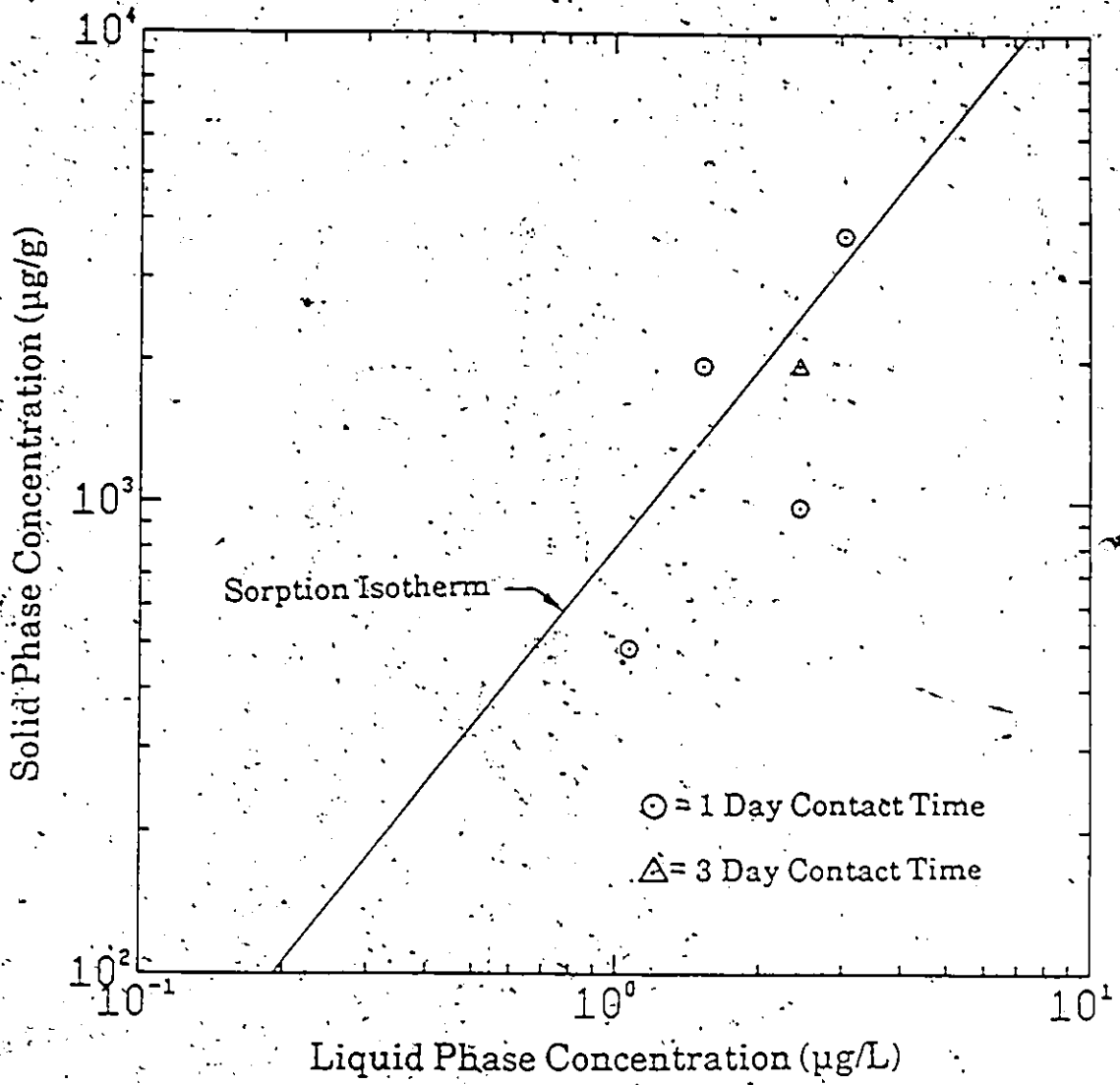


Figure 4.7.19 Desorption of 2-chlorobiphenyl by live *R. arrhizus*.

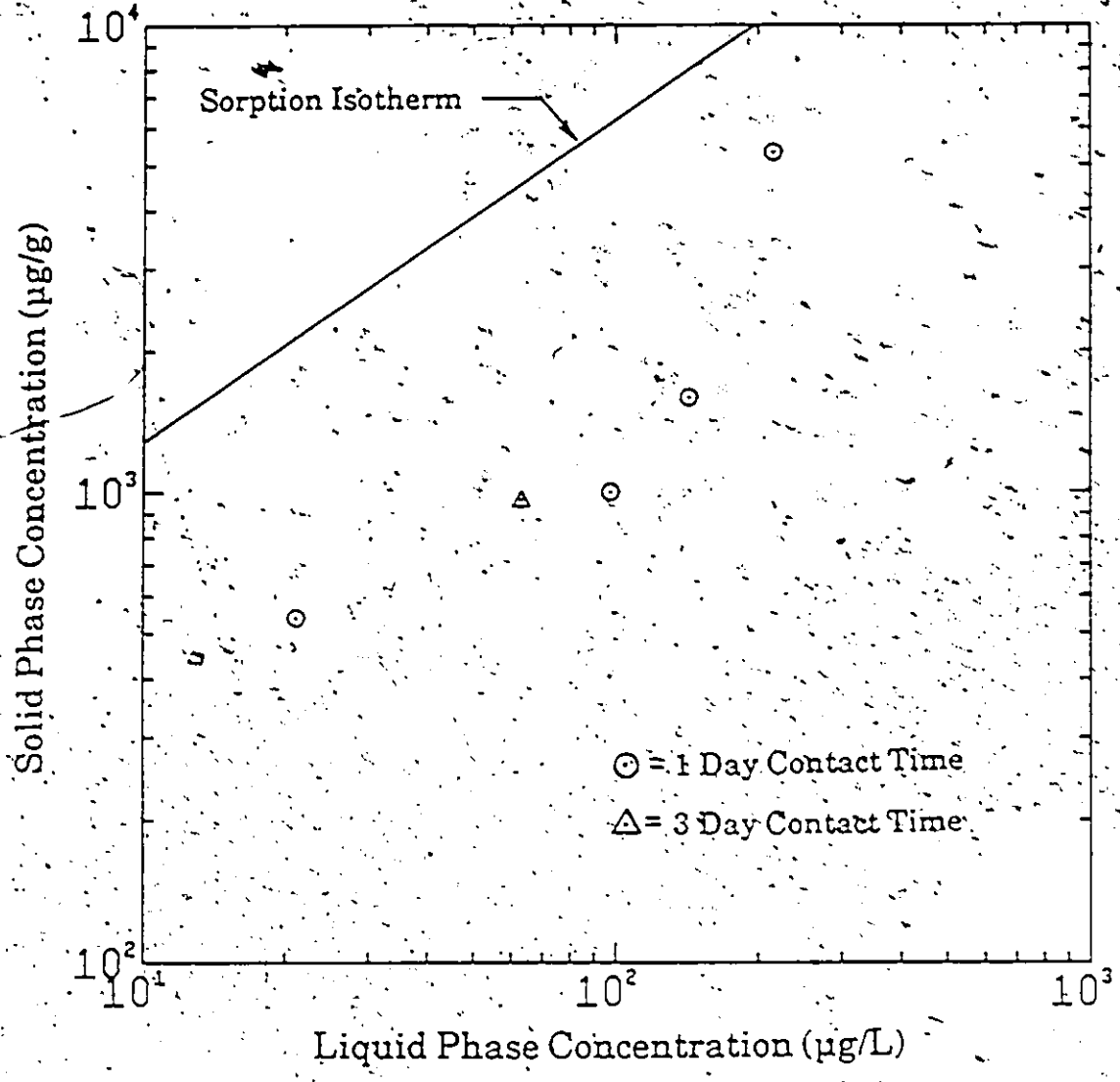


Figure 4.7.20 Desorption of 2-chlorobiphenyl by live activated sludge.

complete a mass balance on the C-14 in each system. If most of the malathion had disappeared from solution and was sorbed on the biomass, then most of the C-14 should be found on the biomass. Table 4.4 gives the malathion removal and the distribution of the C-14 between the solution and the biomass at the end of the contact period.

To determine the extent of quenching of the C-14 counts caused by the presence of chemicals leached from the biomass into the test solutions, various test solutions were spiked with a C-14 labeled citric acid solution. The quantity of C-14 added with each spike was determined by analysing a spiked distilled water solution. It was assumed that the distilled water solution provided no quenching. Since all solutions tested contained a base of distilled water, and differences rather than absolute values were sought, any quenching effects of the distilled water and the container would tend to cancel out. The samples were spiked at two different levels to ascertain whether quenching was concentration dependent. The results of the analyses of the spiked solutions are given in Tables B9.3 and B9.4 in Appendix B9. The percent quenching observed ranged from -3.1 to $+2.8$ indicating negligible quenching effects in the solution samples. Significant quenching did not occur in either concentration range. To determine the quenching effect of filters and biomass, spiked samples containing these components were also analyzed. The data for these tests are given in Table B9.5 in Appendix B9. No quenching effect was observed for a filter with no biomass or for a filter with *R. arrhizus*. Negative quenching appeared to take place with a filter and *R. arrhizus* cell wall preparation. This is probably due to error in the analysis. Quenching of 65% for activated sludge and 16% for activated sludge cell wall preparation was observed. Testing for the quenching effect of biomass and filters is more difficult than for the filtrate solutions. The spiking agent (C-14 citric acid) is highly water soluble and probably is not sorbed significantly on the biomass or filter. The quenching effect of the solid material is likely to be

Table 4.4 Results of C-14 labeled malathion experiment.

Biomass*	A	B	C	D = B + C
R. arrhizus	100	100	1	101
R.arrhizus	100	98	2	100
Activated sludge	93	87	7	94
Activated sludge	95	86	8	94
R. arrhizus cell walls	92	80	10	90
R. arrhizus cell walls	84	73	2	75
Activated sludge cell walls	97	92	3	95
Activated sludge cell walls	94	87	6	93

* Duplicate experiments were run for each type of biomass

A = % of the total malathion originally present in solution which had disappeared from solution after the 7 day contact time

B = % of the total C-14 originally present in solution which remained in solution after the 7 day contact time

C = % of the total C-14 originally present in solution which was sorbed on the biomass after the 7 day contact time

D = B + C = % of the total C-14 originally present in solution which was found in the system after the 7 day contact time (this indicates the extent of closure of the mass balance on the C-14)

greater if the C-14 is sorbed onto the solid than if it is in solution. The extent of desorption of the C-14 from the biomass into the scintillation fluid is also not known. Therefore, the results of the quenching tests for the biomass and filter samples are not considered to be reliable. It is likely, however, that some quenching occurred with the solid samples. This quenching effect could account for the lack of a complete closure of the mass balances on C-14 since all of the C-14 on the biomass would not be detected.

4.9 X-RAY ENERGY DISPERSION ANALYSIS

An attempt was made to use x-ray energy dispersion analysis to determine the location of the biosorbed compounds in or on the microbial cells. Lindane loaded biomass samples were thin sectioned and were analyzed for chlorine, and diazinon loaded samples were analyzed for phosphorus and sulphur under the electron microscope with an EDAX microprobe. No conclusive results were obtained, apparently because the concentrations of the compounds on the biomass were below the detection limit of the instrument. The objective of these analyses was to determine whether the organic compounds were primarily adsorbed onto the cell wall, or if significant quantities entered the cell interior and, if so, how they were distributed in the cell. Unfortunately, with the levels of uptake and the type of compounds used in these experiments no significant observations could be made.

CHAPTER FIVE

DISCUSSION

5.1 SINGLE SOLUTE BIOSORPTION

5.1.1 Biosorption Isotherm Modelling

Since it is probable that the biosorption phenomenon is at least partially due to adsorption on biomass surfaces, adsorption models may be useful in modelling the biosorption process. Some commonly used adsorption models are given in Table 5.1.

Table 5.1 Adsorption Models

Model	Equation	Assumptions
Linear	$q = K_B C$ K_B = constant	Empirical model
Freundlich	$q = K_F C^{1/n}$ K_F, n = constants	Exponential surface energy distribution
Langmuir	$q = QbC/(1 + bC)$ Q, b = constants	Homogeneous surface energy distribution Monolayer surface coverage No interaction among adsorbed molecules
BET	$q = QbC/[(C_s - C)(1 + (B-1)(C/C_s))]$ Q, b = constants C_s = solubility of adsorbate	Homogeneous surface energy distribution No interaction among adsorbed molecules Multilayer surface coverage

Of the commonly used adsorption models, the Freundlich equation appears to provide the best fit to the experimental biosorption data in the present work. Other investigators have modelled the biosorption phenomenon by the Freundlich equation (Baughman and Paris, 1981). Biosorption has also been modelled by a linear relationship between solid phase and liquid phase concentrations where the constant of proportionality is called the bioconcentration factor or the partition coefficient (Baughman and Paris, 1981; Voice and Weber, 1983). The model is of the form:

$$q = K_B C.$$

This linear model can be thought of as a special case of the Freundlich equation where the exponent parameter is equal to one. In cases where only a small fraction of the biosorption capacity of the biomass is being utilized we might expect the uptake to be directly proportional to the equilibrium liquid phase concentration with the result being a linear sorption isotherm. This condition might be expected to occur at low solute concentrations. It can be noted that the Langmuir and BET equations approximate the linear model at low concentrations (Voice and Weber, 1983). Where a large fraction of the available sorption capacity is being utilized, we might expect a nonlinear isotherm, with a declining rate of change of uptake with increasing liquid phase concentration. In that case we would expect an isotherm fitting the Langmuir model, or the Freundlich model with an exponent less than one. Review of the Freundlich parameters given in Table 4.1 shows that many of the exponential parameters are equal to, or nearly equal to, one. From this we could infer that, over the concentration ranges covered, only a small fraction of the total biosorption capacity of the biomass is being utilized. The concentration ranges examined cover the ranges likely to occur in publicly owned wastewater treatment plants and in the cases of lindane, pentachlorophenol and 2-chlorobiphenyl are limited by the relatively low solubilities of the compounds.

Other investigators have also observed linear isotherms for microbial biosorption. Baughman and Paris (1981) noted that in many cases where the Freundlich equation was used to model biosorption, the linear bioconcentration model would have fit the data as well. Weber et al. (1987) and Dobbs et al. (1986) reported that the biosorption of lindane by activated sludge fit a linear sorption model. Voerman and Tammes (1969) used the Freundlich equation to model biosorption of lindane and dieldrin on yeast and reported a value of 1/n equal to one. Steen and Karickhoff (1981) reported linear isotherms for biosorption of polynuclear aromatics by mixed microbial populations. Herbes (1977) reported a 1/n value of one for the Freundlich model of biosorption of anthracene by autoclaved yeast cells. The exponential parameter of the Freundlich equation was reported to have a value of one for biosorption of isomers of hexachlorocyclohexane by bacteria (Sugiura et al., 1975). Ellgehausen et al. (1980) reported 1/n values close to one for a variety of pesticides biosorbed by algae. Paris and Lewis (1976) observed 1/n values near one for the Freundlich models of biosorption of methoxychlor by bacteria, algae, and fungi. An isotherm for biosorption of lindane by algae fit the Freundlich equation but with an exponential parameter greater than one (Hansen, 1979). The value of 1/n for the Freundlich model of sorption of lindane by bacteria adsorbed on magnetite was reported to be 0.8 (MacRae, 1985). Some systems, however, exhibit biosorption isotherms more characteristic of adsorption isotherms covering a range where saturation of the adsorbent is approached. For example, Tsezos and Seto (1986) reported values of 1/n around 0.5 for biosorption of chloroethanes on several different inactive microbial species. Because of the relatively high water solubility of the chloroethanes, the isotherms were carried to higher solute concentration levels where, apparently, the saturation capacity of the sorbents was approached.

The experimental results of the present work, and that of others, suggest that adsorption models, particularly the Freundlich equation are suitable for modelling organic

biosorption systems. It appears that only a small proportion of the ultimate sorption capacity of the biomass is utilized in the low concentration ranges of interest with hazardous organic pollutants, and that over these ranges a linear relationship exists between uptake and liquid phase equilibrium concentration. At higher concentrations there is some evidence that the sorption capacity of the biomass is approached and the increase in loading with an increase in liquid phase concentration diminishes (Tsezos and Seto, 1986).

The fact that biosorption data can be represented by an adsorption model does not necessarily mean that biosorption is the result of a surface adsorption mechanism alone. It is possible that absorption could also follow the Freundlich model, particularly at low concentrations. With the exception of malathion, which will be discussed separately, there do not appear to be any significant differences in the form of the isotherms between the two types of biomass. Since activated sludge consists of a mixed microbial population we might expect that it would have a more nonhomogeneous distribution of surface energies. Considering an adsorption mechanism, the nonhomogeneous energy distribution could be expected to result in nonlinear isotherms even at low concentrations. There does not appear to be any evidence of this from the experimental results, however.

The experimental data for malathion suggest a different biosorptive behaviour. The data are highly scattered and do not seem to fit a single isotherm line (Figures 4.1.9 and 4.1.10). Malathion is the most soluble of the compounds tested and has the lowest octanol/water partition coefficient. It would, therefore, be expected to result in the lowest biosorptive uptake of all the examined compounds. To the contrary, malathion exhibits the highest apparent uptake for activated sludge and the second highest uptake for *R. arrhizus*. This evidence suggests that some mechanism other than, or in addition to, biosorption may be responsible for the observed removal of malathion from solution. Malathion is known to be biodegradable (Paris et al., 1975) and also to hydrolyze in water solution (Muhlmann and

Schrader, 1957). The degradation of malathion has been shown to be catalyzed by sterile soils (Konrad et al., 1969). Malathion is also broken down by various enzymes (Buchel, 1983; Fest and Schmidt, 1982). Because of its unexpectedly high apparent uptake and its suscepability to chemical decomposition, it was suspected that malathion was being removed primarily by a chemical reaction. This hypothesis was further investigated by subsequent experiments and is discussed in Section 5.8.

5.1.2 Dependence of the Biosorption Isotherm on Initial Conditions

If the biosorption process is a true equilibrium process, the biosorption isotherm should be independent of the initial pollutant and biomass concentrations used to experimentally determine the isotherm. In the experimental work the isotherm data were obtained using a number of different initial conditions. In some cases data from two different initial liquid phase concentrations overlapped on the equilibrium isotherm. If the data obtained in this way fit a single isotherm curve this would suggest that the biosorption isotherm was indeed independent of the starting conditions and that it represents true equilibrium. The biosorption data for lindane (Figures 4.1.1 and 4.1.2), pentachlorophenol (Figures 4.1.3 and 4.1.4), and 2-chlorobiphenyl (Figures 4.1.7 and 4.1.8) appear to fit single isotherms regardless of initial conditions, and therefore, appear to represent equilibrium processes. The data for biosorption of diazinon by *R. arrhizus* seems to be in the form of a series of different isotherms, one representing each initial liquid phase concentration (Figure 4.1.5). Taken together, however, all of the data can be represented reasonably well by a single isotherm. Because of the low uptake of diazinon it is difficult to cover a wide concentration range with a single initial concentration and therefore to obtain overlapping data. The apparent effect of initial concentration may be due to the small concentration changes resulting in greater error in the data. However, this phenomenon was not observed with activated sludge, which has

even lower uptake for diazinon than does *R. arrhizus* (Figure 4.1.6). Kinetics data for diazinon confirm that the three day contact time used in the biosorption experiments was sufficient to establish equilibrium (Figures 4.5.5 and 4.5.6). The results for diazinon, therefore, cannot be explained by a failure to reach equilibrium which might account for such behaviour. The experimental data currently available are insufficient to provide a definitive explanation of this observation.

5.1.3 Magnitude of Biosorptive Uptake

The different compounds examined in the present study show a wide variation in biosorptive uptake. Review of Table 4.2 shows that, at a liquid phase concetration of 100 µg/L, for *R. arrhizus* the uptake ranges from 77 µg/g for diazinon to 9250 µg/g for 2-chlorobiphenyl. For activated sludge the range is 45 µg/g for diazinon to 759 µg/g for 2-chlorobiphenyl (excluding malathion). In all cases *R. arrhizus* displayed a higher uptake capacity than activated sludge for the same conditions. The difference in uptake between activated sludge and *R. arrhizus* cannot be accounted for by a difference in specific surface areas of the two types of biomass. The specific surface areas of inactive *R. arrhizus* and activated sludge have been found, using the BET nitrogen adsorption technique, to be 0.52 m^2/g and 1.1 m^2/g, respectively (Tsezos, unpublished). Based solely on surface area we would expect activated sludge to have greater uptake than *R. arrhizus*. Table 5.2 gives a comparison of the uptake per unit surface area for the different compounds and sorbents. Data for activated carbon adsorption of lindane and pentachlorophenol are also given for comparison (Dobbs and Cohen, 1980). The activated carbon has a specific surface area of approximately 1000 m^2/g (Calgon Canada, 1986, personal communication).

Table 5.2 Uptake per unit surface area at C = 100 µg/L.

Compound	Sorbent	Uptake per unit mass (µg/g)	Uptake per unit area (µg/m^2)
Lindane	*R. arrhizus*	254	490
	Activated sludge	156	140
	Activated carbon	80,000	80
Pentachlorophenol	*R. arrhizus*	1860	3600
	Activated sludge	478	430
	Activated carbon	55,000	55
Diazinon	*R. arrhizus*	77	150
	Activated sludge	45	40
2-chlorobiphenyl	*R. arrhizus*	9250	18000
	Activated Sludge	759	690

It is interesting to note that while activated carbon has a much greater uptake per unit mass than the biomass, it has a lower uptake per unit surface area. This could mean that the chemicals have a greater affinity for the biomass surfaces or that absorption as well as adsorption is taking place in the biomass. The latter seems more probable since the hydrophobic carbon surface would be expected to be highly attractive to hydrophobic compounds. This would also account for the lack of a positive correlation between surface area and uptake for the two types of biomass. It is also of interest to estimate the extent of surface coverage of the sorbents at different concentrations, assuming the uptake is totally the result of surface adsorption. To this end the surface area covered by each molecule was estimated by calculating the molecular volumes from the densities and molecular weights and assuming a cubic shape (i.e., area covered by each molecule equals 2/3 power of molecular volume). The uptake corresponding to a complete molecular monolayer coverage of the surface was computed and then the number of molecular layers corresponding to different

levels of uptake were calculated. The results of these calculations are shown in Tables 5.3 and 5.4. It should be noted that, if instead of a cubic shape, closely packed spheres are assumed, the molecular areas would increase by approximately 33% and the corresponding values of uptake at monolayer coverage would be reduced by approximately 25%. The number of molecular layers estimated in Table 5.4 would correspondingly increase by 33%. In view of the rudimentary assumptions made in these calculations these differences are considered to be unimportant. The main purpose of the estimates is to determine whether multilayer surface coverage would be necessary to account for the observed loading if a surface adsorption mechanism is assumed.

Table 5.3 Uptake at complete molecular monolayer coverage.

Compound	Sorbent	Molecular Area (nm^2)	Uptake at Monolayer Coverage (µg/g)
Lindane	*R. arrhizus*	0.41	620
	Activated sludge	"	1300
	Activated carbon	"	1.2×10^6
Pentachlorophenol	*R. arrhizus*	0.37	620
	Activated sludge	"	1300
	Activated carbon	"	1.2×10^6
Diazinon	*R. arrhizus*	0.59	450
	Activated sludge	"	940
2-chlorobiphenyl	*R. arrhizus*	0.42	390
	Activated sludge	"	820

Table 5.4 Number of molecular layers at different concentrations.

Compound	Sorbent	No. of Molecular Layers at Stated Solution Concentration		
		10 µg/L	100 µg/L	1000 µg/L
Lindane	*R. arrhizus*	0.04	0.41	4.3
	Activated sludge	0.01	0.12	1.2
	Activated carbon	0.02	0.07	0.2
Pentachlorophenol	*R. arrhizus*	0.37	3.0	24.0
	Activated sludge	0.05	0.36	2.5
	Activated carbon	0.02	0.05	0.1
Diazinon	*R. arrhizus*	0.03	0.17	1.1
	Activated sludge	0.004	0.05	0.5
2-chlorobiphenyl	*R. arrhizus*	2.0	24.0	290.0
	Activated sludge	0.16	0.93	5.5

If adsorption accounts for the bulk of the biosorptive uptake it appears that, at higher levels of loading, multilayer surface coverage must occur. In fact, for strongly sorbed compounds such as 2-chlorobiphenyl a large number of layers are required. The formation of a large number of layers is suggestive of a condensation or precipitation phenomenon. It may be, however, that adsorption accounts for only a portion of the uptake, and that absorption into the biomass also occurs. The less than complete monolayer coverage calculated for activated carbon adsorption suggests that absorption is taking place in the biomass and could account for the high apparent surface coverage.

The uptake levels reported by other investigators are, in general, lower than those observed in the present investigation. The uptake of lindane by live and dead yeast reported by Voerman and Tammes (1969) is about an order of magnitude lower than that observed in the present experiments. The uptake for the bacterium showing the greatest biosorption of lindane reported by MacRae (1985) is significantly less than that observed for *R. arrhizus* and activated sludge. The highest uptake of lindane by bacteria reported by Grimes and Morrison

(1975) is 10.559 µg/g at a liquid phase concentration of 32 µg/L which is lower than that of *R. arrhizus* by a factor of approximately 7.5 and lower than that of activated sludge by a factor of almost five. The biosorptive uptake of lindane by both live and dead bacteria reported by Sugiura et al. (1975) is about an order of magnitude below that observed in the present work. Hansen (1979) reported uptake of lindane by algae at approximately twice that of *R. arrhizus* at a solution concentration of 1000 µg/L and approximately equal to that of *R. arrhizus* at a solution concentration of 10 µg/L. Sharom et al. (1980) investigated the accumulation of lindane and diazinon by various soils and sediments and reported uptake values lower than in the present study by several to several hundred times. The large differences in reported biosorptive uptake levels may be partially due to experimental techniques in that some investigators determined uptake by extraction of the sorbed compound from the filtered and washed biomass. Washing the biomass could desorb a significant quantity of sorbate as the biosorption process has been shown to be readily reversible (see Section 5.3). The differences may also be due to significant variability in the biosorption capacity of different microorganisms.

5.1.4 Correlation of Biosorption with the Octanol/Water Partition Coefficient

As discussed in Section 2.2.1, water solubility and the octanol/water partition coefficient have been correlated with biosorption by aquatic organisms. The octanol/water partition coefficient is an indicator of lipid solubility of a compound and is, therefore, presumed to be correlated with the partitioning of lipophilic compounds to the lipid phases of organisms. Water solubility is generally inversely related to adsorptive uptake and also inversely related to the lipid solubility so it is also presumed that water solubility would be correlated with biosorptive uptake. Table 5.5 gives the approximate water solubilities and octanol/water partition coefficients, K_{ow}, of the compounds examined.

Table 5.5 Approximate Water Solubilities and Octanol/Water Partition Coefficients

Compound	Water Solubility (mg/L)	Log K_{ow}
Lindane	10[1]	3.72*
Pentachlorophenol	14[2]	4.65*
Diazinon	40[1]	3.14[5]
2-chlorobiphenyl	6[3]	4.87*
Malathion	150[1]	2.89**

* Average of reported values
** Estimated by fragment method

References:

1. McNeely et al. (1979)
2. Verschueren (1977)
3. Hutzinger et al. (1974)
4. Hansch and Leo (1979)
5. Zaroogian et al. (1985)

From the water solubilities we would expect the compounds to be biosorbed in the following order of increasing uptake: malathion, diazinon, pentachlorophenol, lindane, and 2-chlorobiphenyl. As previously discussed, malathion exhibits unusually high apparent uptake and is probably removed primarily by decomposition (see Section 5.8). Of the other four compounds the actual uptake in increasing order is diazinon, lindane, pentachlorophenol, and 2-chlorobiphenyl. Pentachlorophenol is actually significantly more strongly sorbed than lindane, although from water solubility considerations it would be predicted that lindane would be more strongly sorbed. From Table 5.5, however, we can see that the octanol/water partition coefficient correctly predicts that pentachlorophenol would be more strongly sorbed than lindane. In fact the biosorptive uptake in general is reasonably well correlated with the octanol/water partition coefficient as can be seen from Figures 5.1.1 and 5.1.2 which are plots of log q versus log K_{ow} at a liquid phase concentration of 100 µg/L. For *R. arrhizus* the

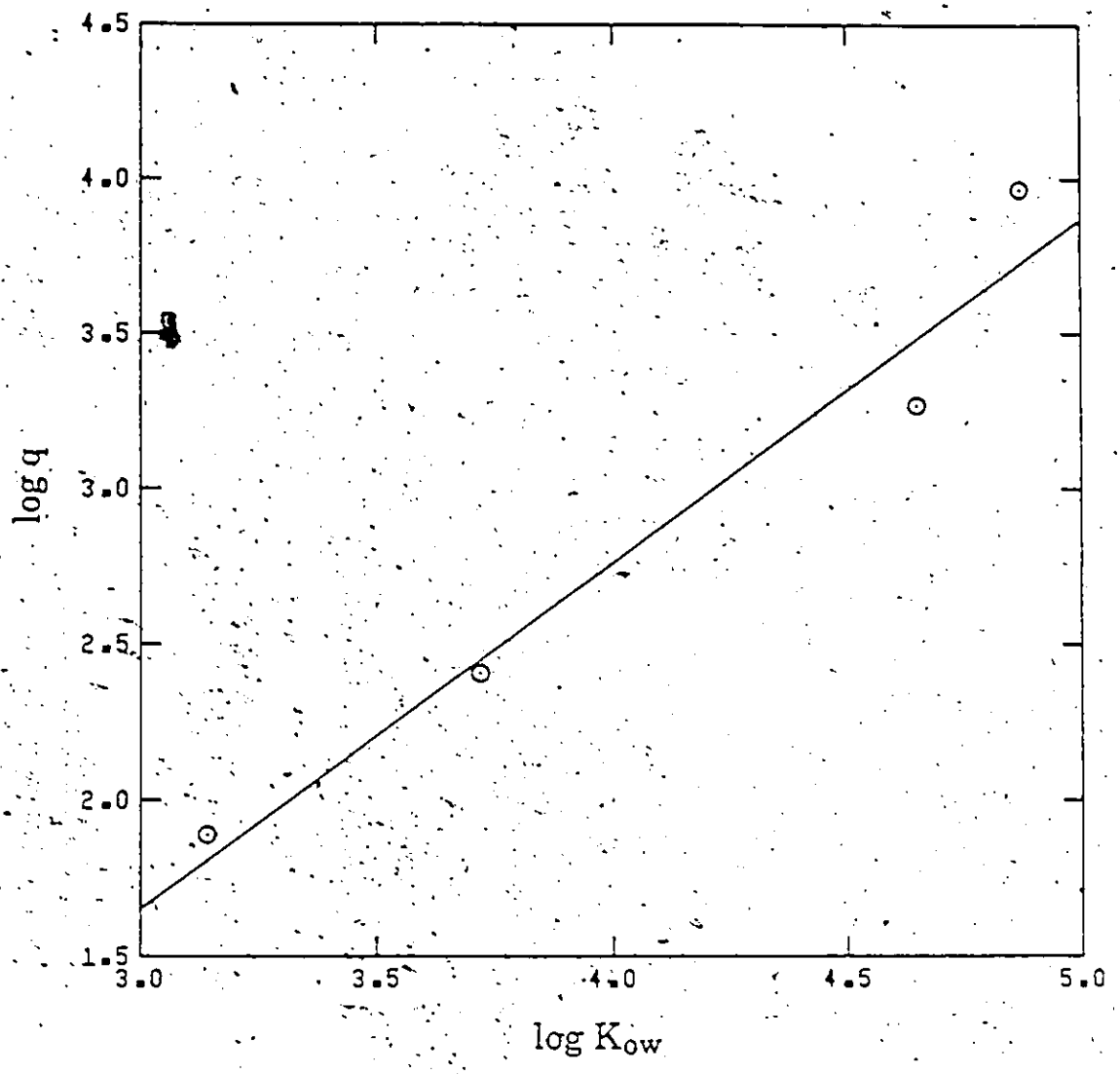


Figure 5.1.1 Biosorptive uptake by *R. arrhizus* at 100 µg/L vs. the octanol/water partition coefficient.

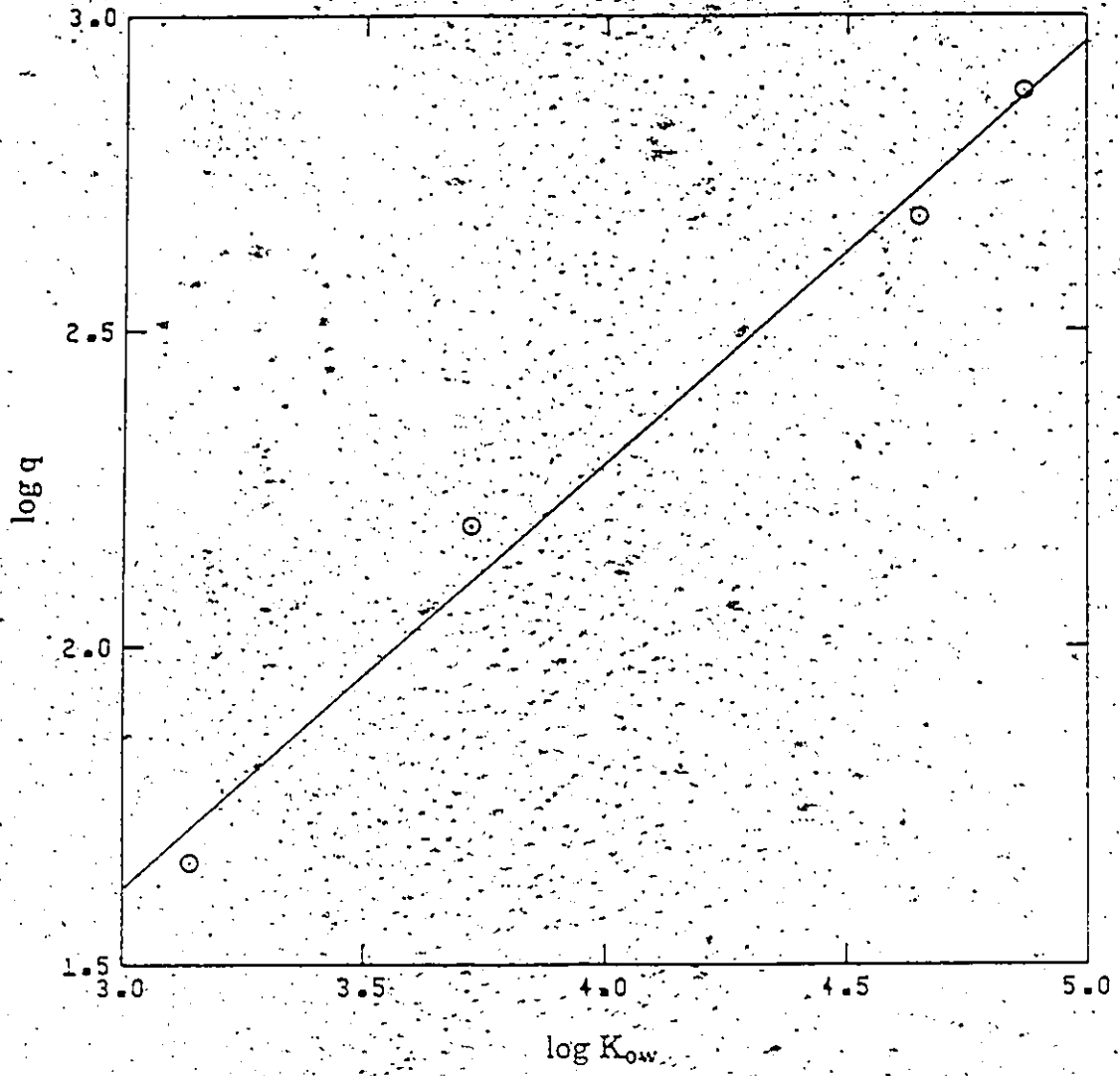


Figure 5.1.2 Biosorptive uptake by activated sludge at 100 µg/L vs. the octanol/water partition coefficient.

equation for the regression line is log q = 1.11 log K - 1.68 with a correlation coefficient of 0.956. For activated sludge the equation is log q = 0.671 log K - 0.396 with a correlation coefficient of 0.985. From a mechanistic viewpoint, the fact that uptake is well correlated with the octanol/water partition coefficient implies that a significant mechanism in biosorption is absorption in the lipid phases of the cells. The lack of correlation of uptake with water solubility tends to reinforce this hypothesis.

5.1.5 Application of the Polanyi Adsorption Potential Theory to Biosorption

The Polanyi adsorption potential theory has been applied to adsorption from solution on activated carbon (Manes and Hofer, 1969; Wohleber and Manes, 1971a, 1971b; Chiou and Manes, 1973; Chiou and Manes, 1974; Schenz and Manes, 1975; Rosene and Manes, 1976). The adsorption potential for a solute is expressed as

$$\varepsilon = RT \ln\left(\frac{C_s}{C}\right)$$

where

ε = adsorption potential of the solute,

R = the gas constant,

T = absolute temperature,

C_s = solubility of the solute in the solvent,

C = equilibrium concentration of the solute in the solvent.

If the volume of solute adsorbed is plotted against the adsorption potential per unit volume the plots obtained for different compounds should converge, with the application of constant scale factors (Manes and Hofer, 1969; Wohleber and Manes, 1971). Figures 5.1.3 and 5.1.4 are plots of volume adsorbed versus adsorption potential per unit volume for all compounds except malathion, for the two types of biomass. Adsorbed volumes were calculated directly from uptake data by dividing loading, in mass units, by the density of the compound. For

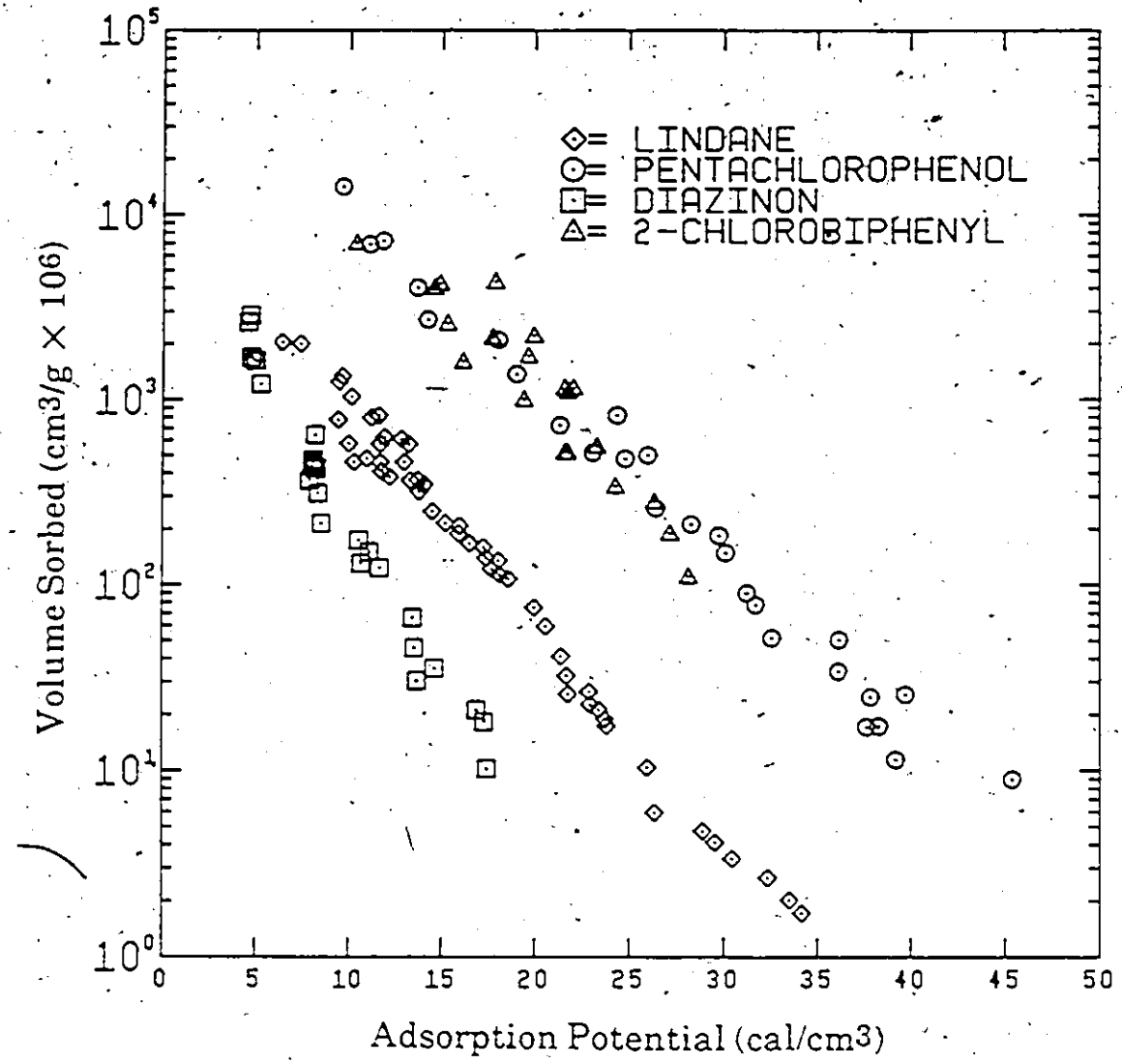


Figure 5.1.3 Volume biosorbed by *R. arrhizus* vs. the Polanyi adsorption potential.

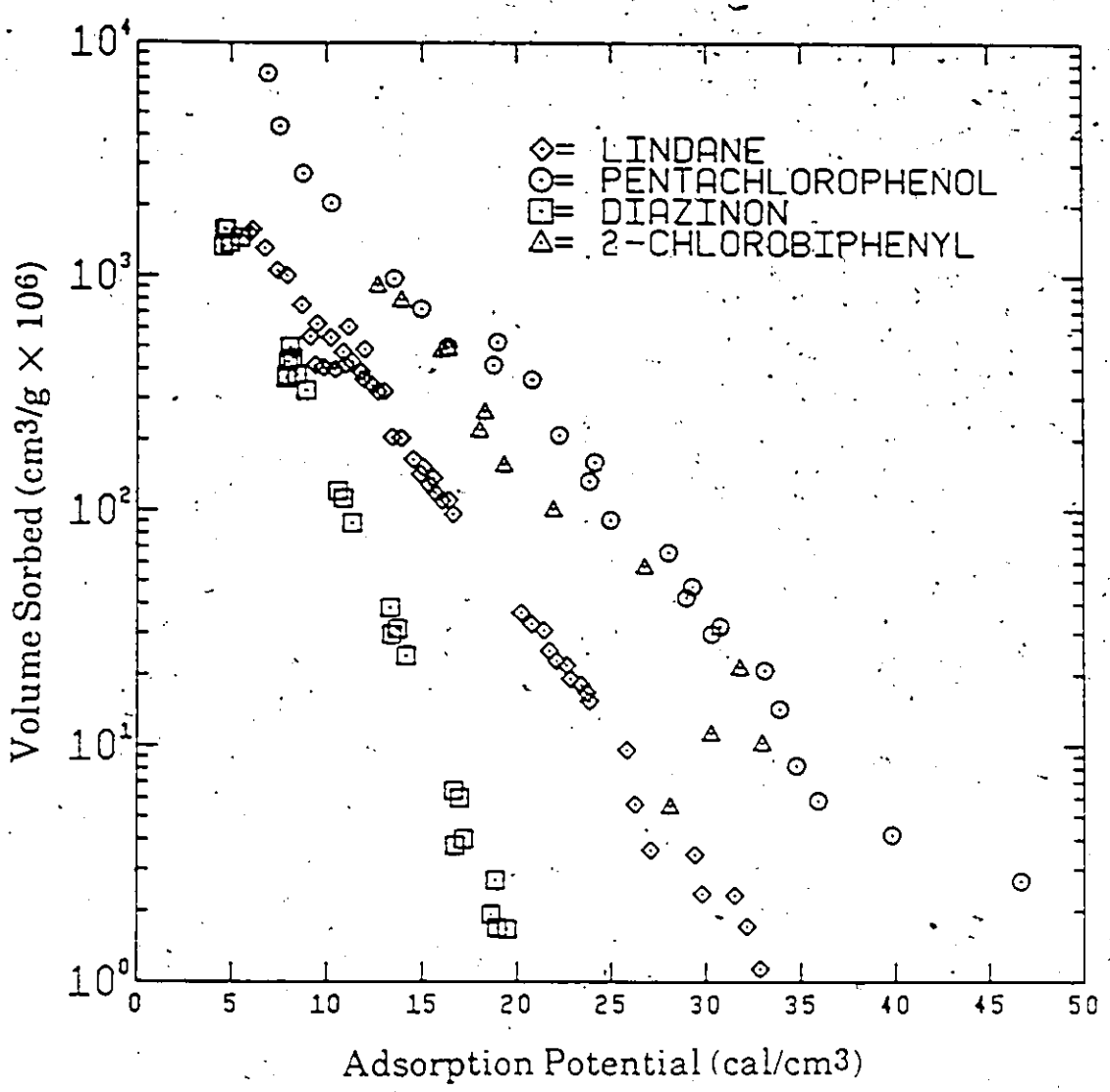


Figure 5.1.4 Volume biosorbed by activated sludge vs. the Polanyi adsorption potential

greater clarity, similar plots were also made by calculating the adsorbed volume from the Freundlich equations for the sorption isotherms rather than from the actual data. In this way the curves plot as straight lines on a semi-log plot and are shown in Figures 5.1.5 and 5.1.6. It can be noted from Figures 5.1.3 and 5.1.4 that the curves for pentachlorophenol and 2-chlorobiphenyl are almost coincident. Coincident curves would be expected of similar compounds such as those in a hydrocarbon series (Wohleber and Manes, 1971). However, pentachlorophenol and 2-chlorobiphenyl are not very similar except for the presence of aromatic rings. For activated sludge the curves for the four compounds could be made to converge to a reasonable degree, by applying an abscissa scaling factor to each curve (Figure 5.1.6). This does not appear to hold for *R. arrhizus* except for the lindane and diazinon curves (Figure 5.1.5). The Polanyi theory appears to apply to a reasonable degree to some biosorption data, suggesting that adsorption may be a significant mechanism in biosorption phenomena. The major benefit of the Polanyi theory lies in its ability to predict adsorption isotherms. Should the proper scaling factors be determined from theory, biosorption isotherms could be predicted without the need for experimental work.

5.2 THERMODYNAMICS OF BIOSORPTION

5.2.1 Heat of Sorpti[redacted]

If we assume that biosorption equilibrium is the result of a dynamic sorption-desorption process involving the solute and solvent in an exchange reaction of the form

$$(1)^{\ell} + m(2)^{s} \rightleftharpoons (1)^{s} + m(2)^{\ell}$$

as given by Everett (1978), where (1) and (2) represent the solute and solvent, and superscripts s and ℓ refer to the sorbed (solid) phase and solution (liquid) phase, respectively, then the equilibrium constant for the above exchange reaction is given by

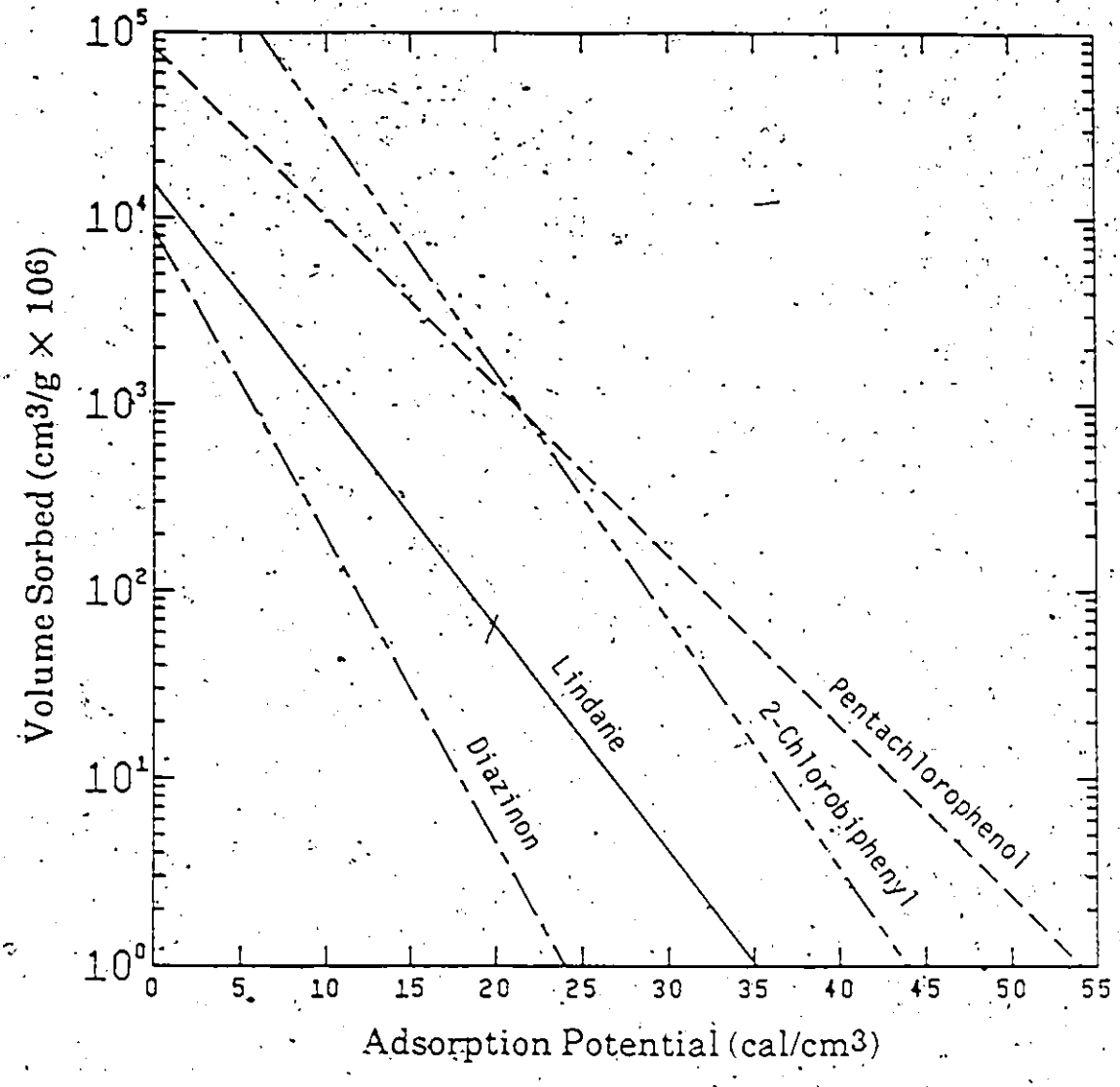


Figure 5.1.5 Volume biosorbed by *R. arrhizus* vs. the Polanyi adsorption potential.

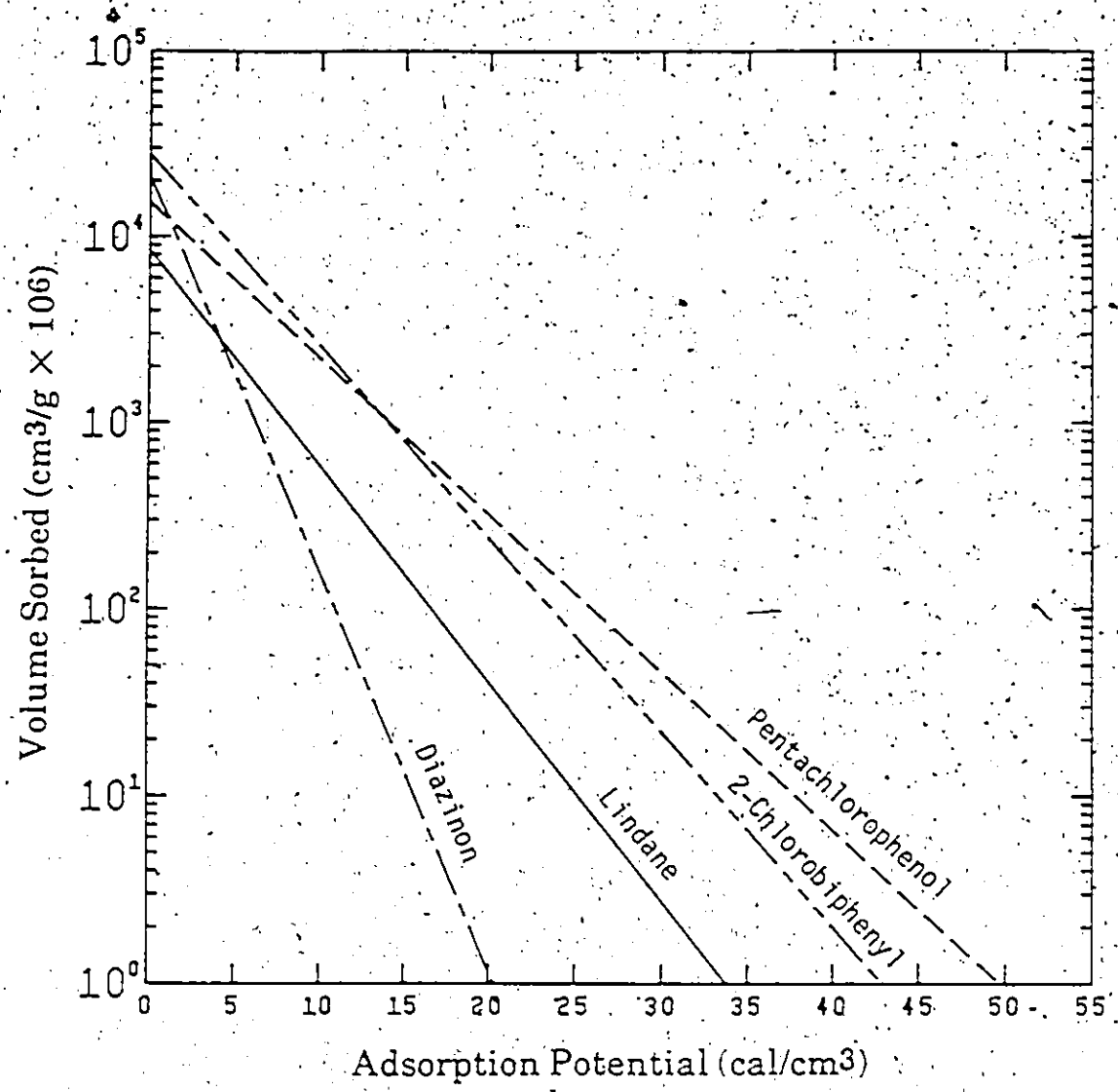


Figure 5.1.6 Volume biosorbed by activated sludge vs. the Polanyi adsorption potential.

$$K = \frac{a_1^s (a_2^\ell)^m}{a_1^\ell (a_2^s)^m} \tag{5.1}$$

where

K = equilibrium constant,

a_1^s = activity of the solute in the solid phase,

a_1^ℓ = activity of the solute in the liquid phase,

a_2^s = activity of the solvent in the solid phase,

a_2^ℓ = activity of the solvent in the liquid phase.

In dilute solution we may assume that activities in the liquid phase can be approximated by mole fractions, therefore

$$a_1^\ell \simeq X_1 \tag{5.2}$$

and

$$a_2^\ell \simeq X_2 \tag{5.3}$$

where

X_1 and X_2 = mole fractions of solute and solvent in liquid phase, respectively.

It can be further assumed, at low solute concentration, that the mole fraction of the solvent remains essentially constant and approximately equal to one, therefore

$$X_2 \simeq 1 \tag{5.4}$$

Considering only conditions at constant loading (i.e. constant solid phase concentration), it can be assumed that the ratio of activities of solute and solvent in the sorbed phase are constant, thus

$$\frac{a_1^s}{(a_2^s)^m} = k_1 = \text{constant} \tag{5.5}$$

Since mole fractions are proportional to concentrations

$$X_1 = k_2 C \tag{5.6}$$

where

k_2 = proportionality constant,

C = equilibrium liquid phase solute concentration.

Substituting equations (5.2) through (5.6) into equation (5.1) we obtain

$$K = \frac{k_1}{k_2 C} \tag{5.7}$$

The enthalpy or heat of sorption is given by the van't Hoff equation

$$\Delta H = \frac{-R\, d\,(\ln K)}{d\,(1/T)} \tag{5.8}$$

where

ΔH = heat of sorption

R = gas constant

T = absolute temperature

Finally, if we assume that ΔH is independent of temperature, we can determine ΔH from liquid phase equilibrium concentrations, at constant loading, at two different temperatures. Integrating equation (5.8), at constant loading, between two different temperatures and substituting for K from equation (5.7), we obtain:

$$\Delta H = \frac{-R \ln (C_1/C_2)}{(1/T_2 - 1/T_1)} \tag{5.9}$$

where

C_1 = equilibrium concentration of solute at $T = T_1$,

C_2 = equilibrium concentration of solute at $T = T_2$,

T_1, T_2 = absolute temperatures.

Using the appropriate Freundlich equations to determine the equilibrium concentrations at different loadings, the heats of sorption shown in Table 5.6 were calculated. The assumption of the temperature independence of ΔH was tested by plotting ln C versus 1/T for lindane at a loading of 500 µg/g for both types of biomass (Figure 5.2.1). If ΔH is independent of temperature these plots should yield straight lines. Linear regression of the data yielded

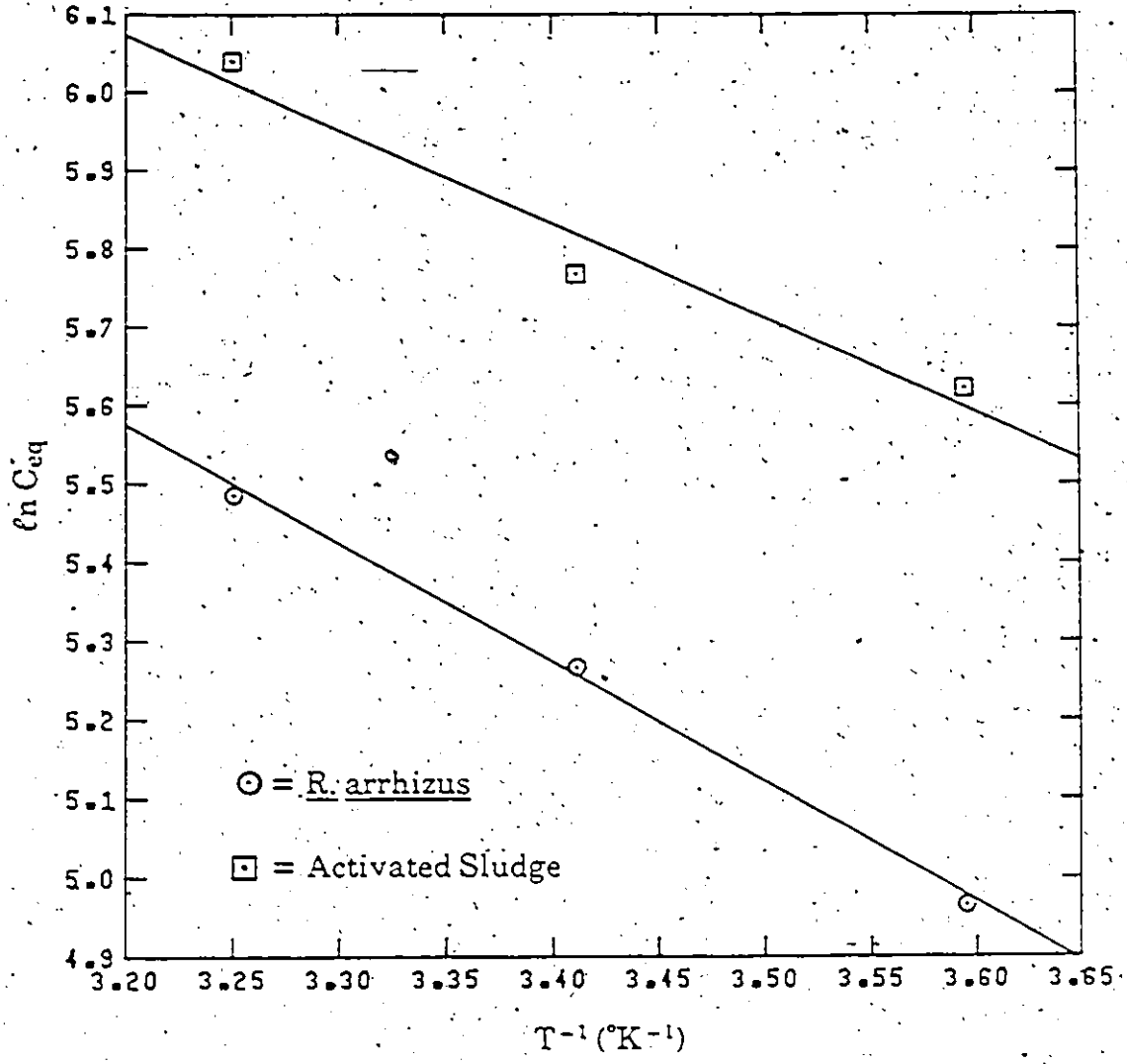


Figure 5.2.1 Equilibrium lindane concentration at 500 µg/g loading vs. reciprocal temperature.

correlation coefficients of 0.99 for *R. arrhizus* and 0.95 for activated sludge, therefore the assumption that ΔH is independent of temperature over the experimental temperature range appears to be reasonable.

Table 5.6 Estimated Heats of Sorption

Compound	Biomass	ΔH at stated loading (kcal/g-mole) 100 µg/g	500 µg/g	1000 µg/g
Lindane	*R. arrhizus*	−3.2	−3.0	−2.9
	Activated sludge	−3.2	−2.4	−2.1
Diazinon	*R. arrhizus*	+7.8	−0.2	−3.6
	Activated sludge	+3.7	+2.0	+1.3
Malathion*	*R. arrhizus*	+39.6	+39.2	+39.1
	Activated sludge	+92.0	+81.0	+76.0

* Data assuming sorption mechanism only

While the estimated heats of sorption may not be accurate because of the assumptions involved in their calculation, they are considered to be of the correct order of magnitude. Crisp (1956) and Kern et al. (1978) compared heats of adsorption for solutions obtained from calorimetric experiments to those calculated from thermodynamic considerations similar to those used in the present work and found reasonable agreement.

The heat of sorption is a measure of the energy released or taken up in forming the bonds between the sorbent and the sorbate and breaking the bonds between the sorbate and the solvent. The magnitude of the heat of sorption is related to the strength of those bonds. In general, chemical bonds are stronger than bonds resulting from physical attractive forces such as van der Waals forces. Thus the magnitude of the energy released or taken up in

forming or breaking those bonds is related to the strength of the bonds. For chemical bonds, or chemisorption, the magnitude of the heat of sorption would be expected to be greater than 10 kcal/g-mole. Physical sorption processes would be expected to have heats of sorption less than 10 kcal/g-mole.

The sign of the heat of sorption indicates the direction of energy transfer. A negative ΔH indicates a release of energy, or an exothermic process, while a positive ΔH indicates a taking up of energy from the surroundings, or an endothermic process. In an exothermic process the temperature of the surrounding solution would increase, and in an endothermic process the temperature of the surounding solution would decrease. Thus heat of sorption could be measured by calorimetric experiments. Adsorption processes have generally been found to be exothermic (Weber, 1972). Absorption could be either exothermic or endothermic. For an adsorption mechanism, the heat of sorption would be expected to be independent of loading if the surface is homogeneous, and the adsorptive bonding energies of all adsorption sites are equal. For non-homogeneous surfaces, where adsorption sites of different energy might exist, the heat of adsorption would be expected to be a function of loading. Further, it might be expected that the higher energy sites would be favoured for adsorption. In that case, a decline in the heat of adsorption with increasing loading would be expected, as the higher energy sites would dominate at lower loading. We would expect biomass surfaces to be non-homogeneous. Since the activated sludge is a mixed culture, whereas the *R. arrhizus* is a pure strain, it might be expected that the activated sludge would have less surface homogeneity than *R. arrhizus*. In that case it would be expected that activated sludge would show a greater change in heat of adsorption with loading than *R. arrhizus*. With absorption, which is a process of dissolution into a homogeneous absorbing medium, change in the heat of sorption with loading would not be expected if the concentration in the absorbing medium remained low so that interactions between sorbate

molecules were not important. The lipid content of *R. arrhizus* has been reported to be in the range of 0.66% to 19.9% (Weete, 1974). Bacteria generally have a lipid content on the order of 10% (Asselineau, 1966). If absorption into the lipid pools of the cells is assumed to account for the total uptake, and the lipids are assumed to make up 10% of the cell mass, then for a range of loading of 100 to 1000 µg/g the sorbate concentration range in the lipids would be 1000 µg/g to 10,000 µg/g. For this relatively low concentration range we could expect that the heat of sorption would be independent of loading. Therefore it is postulated that changes in heats of sorption with loading could primarily be due to adsorption.

The estimated heats of sorption for lindane with both types of biomass are in the range where a physical rather than a chemical mechanism would be expected to dominate. The heats of sorption are negative, indicating an exothermic process as would be expected of adsorption. The heats of sorption for lindane are of the same order of magnitude as found by Herbes (1977) for sorption of anthracene by autoclaved yeast (5.2 kcal/g-mole). Both types of biomass show a decline in the magnitude of the heat of sorption with increased loading, and the decrease is greater for activated sludge than for *R. arrhizus*. It is possible, however, that the observed decline in heats of sorption is an artifact of the model fitting technique. In all cases the exponential parameters of the Freundlich equations are close to one, and the 95% confidence intervals for the exponential parameters overlap. If the isotherms at different temperatures are taken to be linear (1/n = 1.0), then

$$q = K_{F1} C_1 = K_{F2} C_2 \tag{5.10}$$

where

q = loading,

C_1 = equilibrium conclentation at $T = T_1$,

C_2 = equilibrium conclentration at $T = T_2$,

K_{F1} = Freundlich constant at $T = T_1$,

K_{F2} = Freundlich constant at $T = T_2$.

At two fixed temperatures

$$\frac{-R}{(1/T_2 - 1/T_1)} = \text{constant} \tag{5.11}$$

Therefore, from equation 5.9,

$$\Delta H = (\text{constant})\, \ell n\left(\frac{C_1}{C_2}\right) \tag{5.12}$$

From equation 5.10, at constant loading,

$$\frac{C_1}{C_2} = \frac{K_{F2}}{K_{F1}} = \text{constant} \tag{5.13}$$

Substituting equation 5.13 into equation 5.12 we obtain

$$\Delta H = \text{constant} \tag{5.14}$$

From equation (5.14) it can be seen that ΔH will be independent of loading and no decline in heats of sorption with increased loading will be calculated. Therefore, it is not possible to say with a high degree of certainty that the decline in heats of sorption with increased loading do, in fact, occur. The heat of sorption data do suggest that biosorption of lindane involves a physical, rather than a chemical, mechanism, and that the process is exothermic. We cannot conclude from the data whether an adsorption or absorption mechanism is primarily responsible for biosorption.

The magnitude of the heats of sorption for diazinon also suggest that biosorption is a physical process. For *R. arrhizus* the calculated values of heat of sorption appear to indicate that the biosorption process is endothermic at low loading and exothermic at higher loading. This might be possible if the biosorption process involved an endothermic absorption mechanism and an exothermic adsorption mechanism and absorption were dominant at low loading and adsorption were dominant at higher loading. However, it may be that the unusual result is caused by experimental error in the data and the small difference in uptake over the experimental temperature range. From Figure 4.2.5 it can be seen that the data for

the isotherm seem to indicate lower uptake at 5°C than at 20°C in the lower range and higher uptake at 5°C than at 20°C in the higher range. Since there is not a large difference in uptake at the two temperatures, it may be that the apparent change in heat of sorption with loading is an artifact due to experimental error and the model fitting technique. The heats of sorption calculated for biosorption of diazinon by activated sludge also suggest a physical mechanism. The positive values suggest that the process is endothermic in the range of loading over which the heats of sorption were calculated, and the heat of sorption appears to decline with increased loading.

The primary objective of the study of temperature effects and thermodynamics was to determine whether the sorption process involves a physical or chemical mechanism. The magnitude of the heats of sorption for both diazinon and lindane strongly suggest a physical mechanism. The heats of sorption given for malathion are apparent values calculated assuming that malathion was removed only by a biosorption mechanism. The high positive values indicate that the removal of malathion was greater at the higher temperature than at the lower temperature. This is unlikely if an adsorption mechanism is dominant. The large magnitude of the apparent heats of sorption are also unlikely to be caused by a physical absorption phenomenon. These results could be accounted for by a chemical decomposition reaction. In that case, the calculated biosorption uptake does not represent equilibrium conditions but rather indicates the extent to which the chemical reaction has proceeded over the duration of the experiment. If the reaction proceeds more slowly at the lower temperature, as would be expected, the observed removal would appear to be lower than at the higher temperature. In reality the chemical reaction would not have proceeded to as great an extent. These results tend to support the hypothesis that a chemical decomposition of malathion is the primary mechanism responsible for the observed removal of malathion from the solutions. These phenomena are discussed further in Section 5.8.

5.2.2 Free Energy of Biosorption

The free energy change associated with the biosorption process can be estimated from equilibrium sorption data. Two methods of calculating free energy of adsorption have been reported in the literature. The first method (Daniel, 1951; Crisp, 1956; Wright and Powell, 1972; Groszek, 1975; Kern et al., 1978) uses the relationship between the free energy change and the equilibrium constant,

$$\Delta G = -RT \ln K \tag{5.15}$$

where

ΔG = free energy change,

R = gas constant,

T = absolute temperature,

K = equilibrium constant.

The equilibrium constant was previously defined by equation (5.1) as follows:

$$K = \frac{a_1^s (a_2^\ell)^m}{a_1^\ell (a_2^s)^m} \tag{5.1}$$

At low liquid phase equilibrium concentrations, where the solid phase concentrations are also low, we may assume that the activities can be represented by mole fractions. Since the solute concentrations are much less than the solvent concentrations we may take the mole fractions of the solvent in both phases to be equal to one. Equation (5.1) can then be simplified to

$$K = \frac{X_s}{X_\ell} \tag{5.16}$$

where

X_ℓ = equilibrium mole fraction of solute in liquid phase,

X_s = equilibrium mole fraction of solute in solid phase.

Combining equations (5.15) and (5.16) we obtain

$$\Delta G = -RT \ln\left(\frac{X_s}{X_\ell}\right) \tag{5.17}$$

from which we can estimate the free energy change of sorption. At a given concentration the mole fraction of the solute in the liquid phase can be easily calculated. The mole fraction of the solute (sorbate) in the solid phase is given by

$$X_s = \frac{M_1}{M_1 + M_2} \tag{5.18}$$

where

M_1 = number of molecules of solute sorbed per gram of sorbent,

M_2 = number of molecules of solvent sorbed per gram of sorbent.

The number of molecules of solute sorbed per gram of sorbent is given by

$$M_1 = \frac{q\,N_A}{MW_1} \tag{5.19}$$

where

q = solid phase concentration of solute, g/g,

N_A = Avogadro's number, 6.02×10^{23} molecules/mole,

MW_1 = molecular weight of solute, g/mole.

Assuming monolayer surface coverage, the number of molecules of solvent sorbed per gram of sorbent is given by

$$M_2 = \frac{S - M_1 A_1}{A_2} \tag{5.20}$$

where

S = specific surface area of sorbent, m^2/g,

A_1 = area occupied by each solute molecule, m^2,

A_2 = area occupied by each solvent molecule, m^2.

Assuming a cubic shape, the molecular areas can be estimated by

$$A_1 = \left(\frac{MW_1}{\rho_1 N_A}\right)^{2/3} \tag{5.21}$$

and

$$A_2 = \left(\frac{MW_2}{\rho_2 N_A}\right)^{2/3} \tag{5.22}$$

where

MW_1, MW_2 = molecular weights of solute and solvent, g/mole,

ρ_1, ρ_2 = densities of solute and solvent, g/m^3.

Combining equations (5.18) through (5.22), the mole fraction of the solute in the solid phase is found. The free energy change of sorption can then be found from equation (5.17). Table 5.7 gives the free energy changes of sorption estimated using the above equations at an equilibrium liquid phase concentration of 1.0 µg/L. The assumption of closely packed spheres instead of the assumption of a cubic shape in estimating the surface area occupied by each molecule results in less than 2% increase in the calculated values of ΔG. In view of the rudimentary nature of these calculations this change is considered to be unimportant.

The ΔG values are all negative which is expected since the solute is expected to be more concentrated in the solid phase than in the liquid phase. The magnitude of the free energy change is indicative of the extent to which the solute is concentrated in the surface phase.

Table 5.7 Estimated free energies of sorption.

Compound	Biomass	ΔG (kcal/g-mole)
Lindane	*R arrhizus*	−9.5
	Activated sludge	−8.9
Pentachlorophenol	*R. arrhizus*	−11.0
	Activated sludge	−10.0
Diazinon	*R. arrhizus*	−9.4
	Activated sludge	−8.1
2−chlorobiphenyl	*R. arrhizus*	−11.5
	Activated sludge	−10.4
Malathion*	*R. arrhizus*	−9.8
	Activated sludge	−12.3

* Data assuming sorption mechanism only

Another method of determining the free energy change of adsorption has been reported (Daniel, 1951; Bull, 1956; Chattoraj and Birdi, 1984). In this method an expression for the free energy change of the adsorbing surface is derived from the Gibbs adsorption equation, and is given as follows:

$$\Delta G' = -RT \int_0^a N \frac{da}{a} \tag{5.23}$$

where

$\Delta G'$ = total free energy change,

a = activity of adsorbed solute in liquid phase,

N = number of moles of solute adsorbed.

This equation is claimed to be generally applicable and does not depend on knowledge of the thickness of the surface layer as in the previous method (Chattoraj and Birdi, 1984).

To compute the free energy change per unit mass of adsorbent, we can rewrite equation (5.23) in the form

$$\Delta G'' = -RT \int_0^a q \frac{da}{a} \tag{5.24}$$

where

$\Delta G''$ = free energy change of adsorption per unit mass of adsorbent,

q = molar solid phase concentration of solute.

At low solute concentration, we may substitute mole fraction for activity and obtain

$$\Delta G'' = -RT \int_0^x q \frac{dX}{X} \tag{5.25}$$

where

X = mole fraction of solute in liquid phase.

If q is related to X by the Freundlich equation

$$q = K_F X^{1/n} \tag{5.26}$$

then equation (5.25) can be rewritten as

$$\Delta G'' = -RT \int_0^x K_F X^{(1/n-1)} dX \tag{5.27}$$

Integration of equation (5.27) yields

$$\Delta G'' = -nRTK_F X^{1/n} = -nRTq \tag{5.28}$$

To calculate the free energy change per mole of solute adsorbed, we obtain

$$\Delta G = \frac{\Delta G''}{q} = -nRT \tag{5.29}$$

As noted by Bull (1956), this result indicates that at low concentrations where linear isotherms are expected the surface free energy change for any system would equal $-RT$. Table 5.8 gives the values of the surface free energy changes, at 20°C estimated from equation (5.29).

Table 5.8 Estimated surface free energy changes at 20°C.

Compound	Biomass	ΔG (kcal/g-mole)
Lindane	*R. arrhizus*	−0.57
	Activated sludge	−0.58
Pentachlorophenol	*R. arrhizus*	−0.64
	Activated sludge	−0.70
Diazinon	*R. arrhizus*	−0.73
	Activated sludge	−0.56
2− chlorobiphenyl	*R. arrhizus*	−0.54
	Activated sludge	−0.69
Malathion*	*R. arrhizus*	−0.49
	Activated sludge	−1.04

* Data assuming sorption mechanism only

The surface free energy changes given in Table 5.8 are an order of magnitude lower than the free energy changes of sorption given in Table 5.7. Several assumptions are employed in the first method of estimating the free energy changes which are not required in the second method. In both methods the activity of the solute in the liquid phase is assumed to be equal to its mole fraction. For the dilute solutions considered this assumption is probably reasonable (Daniel, 1951). In the first method the activity of the solute in the adsorbed state is also represented by its mole fraction. Although the concentration in the solid phase is low this assumption may not be valid. In addition, it was necessary to assume the thickness of the adsorbed layer, which in this case was taken to be a single molecule thick. The validity of this assumption is also not known (see discussion of molecular layers, Section 5.1.3). If multilayer adsorption is assumed, the estimated solid phase mole fractions will be reduced and thus the estimates of ΔG will be lower and could, in fact, coincide with the estimates made using

the second method. Of course, both methods assume an adsorption mechanism only, which may not be correct.

5.2.3 Entropy Changes Associated with Biosorption

The entropy change associated with the sorption of the solute, ΔS, can be computed from the Gibbs-Helmholtz equation,

$$\Delta S = \frac{\Delta H - \Delta G}{T} \tag{5.30}$$

Entropy changes at 20°C, for lindane and diazinon, at several different loadings, are given in Table 5.9. The entropy changes were computed using equation (5.30) and the free energy changes in Table 5.8.

Table 5.9 Entropy Changes at 20°C.

Compound	Biomass	ΔS at stated loading (cal/g-mole K)		
		100 µg/g	500 µg/L	1000 µg/L
Lindane	*R. arrhizus*	-9.0	-8.3	-8.0
	Activated sludge	-8.8	-6.3	-5.2
Diazinon	*R. arrhizus*	+29.0	+1.9	-9.8
	Activated sludge	+14.5	+8.8	+6.3

The negative values of ΔS represent a decrease in entropy on adsorption which is in accord with a more ordered arrangement of the solute molecules on the sorbent surface than in the liquid phase (Wright and Powell, 1972). Wright and Powell (1972) reported decreases in entropy for adsorption of aromatic hydrocarbons on carbon blacks. However, Wright and Pratt (1974) reported increases in entropy for adsorption of aromatic carboxylic acids on carbon blacks. They conclude that for large molecules the simplified view of the more ordered

arrangement of molecules on the surface may not be appropriate. They also propose that the non-specific interaction energy between the adsorbate and the surface sites coupled with configurational changes in the adsorbate on adsorption could account for the positive entropy changes. In the present work, the change from a positive to a negative entropy change with increased loading for diazinon could be the result of a change in the dominant biosorption mechanism. The more complex molecular structure of diazinon may account for its apparently more complex behaviour which tends to confirm the hypothesis that the biosorption phenomena involves more than a simple adsorption or absorption mechanism.

5.3 DESORPTION

Results of the desorption experiments indicate that the biosorption of lindane, diazinon, and 2-chlorobiphenyl is completely reversible for both types of biomass. In general, the desorption data fall approximately on the sorption isotherms as can be seen in Figures 4.3.1 through 4.3.6. The apparent reversibility is consistent with physical sorption mechanisms which would be expected to be readily reversible. One implication of the reversibility of the biosorption process is that hazardous pollutants sorbed onto biological sludges may be released into the environment if the sludges are disposed of on the land.

Desorption experiments with malathion have produced some interesting results. At a temperature of 5°C, reversibility of the malathion removal process was observed for both types of biomass (Figures 4.3.7 and 4.3.8). At this temperature the process appears to behave as a sorption process. If malathion were removed by a chemical decomposition mechanism we would not expect to recover the malathion by a desorption process. At a temperature of 20°C a different result was obtained. At this temperature desorption was not observed. In fact, additional removal of malathion from solution appeared to take place. When the sorption solutions were decanted and replaced with distilled water it was not possible to completely

decant the liquid so that some of the adsorption solution remained in the flask and was mixed with the fresh distilled water. The initial desorption solution thus contained some residual malathion which was accounted for in the mass balance used to compute apparent loading after desorption. In the desorption experiments at 20°C, rather than desorption being observed, some of the residual malathion remaining from the adsorption step was removed from solution. If a sorption process is assumed, Figures 4.3.9 and 4.3.10 show that rather than desorption, additional uptake appears to take place. These results are consistent with the chemical reaction hypothesis. If the chemical reaction is much slower at 5°C, the removal of malathion by reaction may be insignificant compared to removal by sorption, and thus the observed disappearance of malathion would be due mainly to biosorption. Reversibility of the process would, therefore, be observed, as was the case for the desorption experiments at 5°C. At 20°C, the reaction may be much faster and may be the dominant removal mechanism. In that case reversal of the malathion removal would not be observed since the malathion would have been irreversibly destroyed. This would account for removal of the residual malathion as well. In general, the results of the desorption studies tend to confirm the chemical reaction hypothesis.

5.4 SORPTION BY CELL WALLS

Sorption onto microbial cell walls separated from the *R. arrhizus* and activated sludge biomass was studied to aid in understanding the mechanism of biosorption. The method used to separate the cell walls from the whole cells is described in Section 3.1.3. If the fraction of the total cell mass consisting of cell walls is known, the fraction of the biosorptive uptake attributed to sorption onto the cell walls can be determined from experimental uptake data for cell walls and whole cells. The fraction of the total uptake attributed to sorption onto the cell walls is given by

$$F = \frac{q_w f_w}{q_T} \tag{5.31}$$

where F = fraction of total uptake attributed to sorption by cell walls,

q_w = uptake by cell walls per unit mass of sorbent,

q_T = uptake by whole cells per unit mass of sorbent, with q_w and q_T taken at the same liquid phase concentration,

f_w = fraction of cells consisting of cell walls.

The fraction of cell wall material recovered from separation of the whole cells was measured. The cell walls were found to make up 47.5% of the mass of *R. arrhizus* cells and 67.5% of the mass of activated sludge cells. The cell walls could be expected to make up a larger fraction of the smaller bacterial cells of activated sludge than for the larger fungal cells of *R. arrhizus*. These values are consistent with a cell wall fraction of 44.8% reported by Canton et al. (1977) for algal cells and a cell wall yield of 55.8% reported by Stagg and Feather (1973) for a fungus.

If the dominant mechanism for biosorption were sorption onto the cell walls, the cell walls would be expected to display higher uptake per unit mass than whole cells. For both types of biomass, cell walls showed lower uptake than whole cells, for both lindane and diazinon (Figures 4.4.1 through 4.4.4). Table 5.10 gives the percentages of uptake attributed to sorption onto the cell walls estimated using Equation (5.31) and the cell wall sorption data given in Appendix B4. Uptake values for whole cells at the appropriate liquid phase concentrations were determined from the Freundlich equations.

Table 5.10 Percentage of Total Biosorptive Uptake Attributed to Cell Walls.

Compound	Biomass	C (µg/L)	% Uptake on Cell Walls	
Lindane	*R. arrhizus*	3622	18	
		3260	16	Mean = 16
		2629	14	
Lindane	Activated sludge	3595	46	
		3075	48	Mean = 46%
		2439	46	
Diazinon	*R. arrhizus*	4909	31	
		4818	22	Mean = 25%
		4570	23	
Diazinon	Activated sludge	4838	44	
		4545	48	Mean = 49%
		3954	56	

For both *R. arrhizus* and activated sludge the fraction of the sorbate accumulated by the cell walls is less than that expected if cell walls alone were responsible for biosorption suggesting that other parts of the cell are likely responsible for a substantial portion of the uptake. The fraction of the total uptake attributed to sorption on cell walls is greater for activated sludge than for *R. arrhizus*. Even considering that cell walls make up a greater fraction of the total cell mass for activated sludge, examination of the data in Appendix B4 shows that activated sludge cell walls exhibit stronger uptake than *R. arrhizus*. Microscopic examination of the *R. arrhizus* cell wall preparation showed that the majority of particles consisted of what appeared to be cell fragments. Similar examination of the activated sludge cell wall prepara-

tion did not clearly show the extent to which the cells had been broken. It is therefore possible that the activated sludge cell wall preparation contained some unbroken cells which could partly account for the higher observed uptake. The data do, however, suggest that, assuming the cell wall processing does not alter the biosorptive behaviour of cell walls significantly, the biosorption process involves both sorption by the cell walls and perhaps sorption into the cytoplasmic contents of the cells. For algal cells, Canton et al. (1977) reported that 13% of the uptake of α-HCH occurred in the cell walls and 87% occurred in the cell content of which the majority occurred in the lipid fractions of both the cell walls and cell contents. These results are similar to the results for biosorption of lindane by *R. arrhizus* in the present work.

It is interesting to note that for malathion the apparent uptake by *R. arrhizus* cell walls is approximately the same as for whole cells while the apparent uptake by activated sludge is considerably less than for whole cells. If malathion removal is caused primarily by a chemical reaction effected by the inactive biomass, it appears that the cell wall preparation process does not destroy or remove the active agents in the *R. arrhizus* biomass. The activated sludge biomass is apparently affected by the cell wall preparation process in some way which reduces its capacity to promote the degradation of malathion. If the degradation process is catalyzed by adsorption on the cell walls, perhaps the cell wall preparation process causes changes in the active sites on the activated sludge bacterial cell walls. The data developed in the present study are not sufficient to clearly ascertain the details of the mechanism of malathion removal, although a chemical decomposition is strongly suggested (see Section 5.8).

5.2 KINETICS OF BIOSORPTION

The kinetics of biosorption of lindane by inactive *R. arrhizus* and activated sludge were investigated. Figures 4.5.1 and 4.5.2 show the reduced liquid phase lindane concentra-

tion versus time for the two types of biomass. Experiments were run in each case at two different mixing speeds and the data for the different speeds are shown. Since there appear to be no significant differences due to mixing speed, it can be concluded that the measured sorption rates are intrinsic rates, independent of mass transfer in the bulk solutions. Figures 4.5.3 and 4.5.4 give the solid phase concentration, or uptake, as a function of time for the two types of biomass. Solid phase concentrations were calculated by a mass balance and were corrected for the quantity of lindane removed with each sample taken from the reactor. Biosorption of lindane by *R. arrhizus* appears to be a relatively rapid process, reaching 90% of the equilibrium uptake in less than ten minutes and complete equilibrium within one hour. Biosorption of lindane by activated sludge appears to be somewhat slower and, in fact, did not appear to achieve equilibrium in the 22 hour contact time. For activated sludge, there appears to be an initial relatively rapid uptake followed by a slow approach to equilibrium. This could be the result of a two step sorption mechanism whereby the solute is first adsorbed on the cell surface and then slowly absorbed into the cell interior.

Attempts were made to model the kinetic data with simple first and second order rate models, however the data do not appear to fit these simple models over the entire range. Assuming that the biosorption process involves a two step mechanism consisting of a surface adsorption step followed by absorption of the adsorbed molecules into the cell interior, a rate equation can be postulated to fit this process. The two step process can be represented by the following:

$$A + B \leftrightharpoons S \rightarrow C + B$$

where

A = the sorbate molecules in the liquid phase,

B = the empty adsorption sites,

S = the adsorbed sorbate molecules,

C = the absorbed sorbate molecules.

Assuming simple kinetic mechanisms for each step, the rate equations for the system can be written as follows:

$$r_A = -k_1 AB + k_2 S \tag{5.32}$$

$$r_S = k_1 AB - k_2 S - k_3 S \tag{5.33}$$

$$r_C = k_3 S \tag{5.34}$$

where

r_A, r_S, r_C = rates of formation of A, S, and C, respectively,

k_1, k_2, k_3 = rate constants.

If we assume that the adsorption step is rapid, and that the adsorbed molecules quickly reach a steady state condition, then

$$r_S = 0 = k_1 AB - (k_2 + k_3)S \tag{5.35}$$

and

$$S = \frac{k_1 AB}{k_2 + k_3} \tag{5.36}$$

Substituting equation (5.36) into equation (5.32) yields

$$r_A = -k_1 AB + \frac{k_1 k_2 AB}{k_2 + k_3} \tag{5.37}$$

The number of empty adsorption sites at any time equals the total number of sites, B_0, minus the number of filled sites, S, or

$$B = B_0 - S = B_0 - \frac{k_1 AB}{k_2 + k_3} \tag{5.38}$$

Rearranging equation (5.38) we obtain

$$B = \frac{B_0}{1 + \frac{k_1}{k_2 + k_3} A} \tag{5.39}$$

Substitution of equation (5.39) into equation (5.37) yields

$$r_A = \frac{B_0\left(\frac{k_1 k_2}{k_2 + k_3} - k_1\right) A}{1 + \frac{k_1}{k_2 + k_3} A} \tag{5.40}$$

If we substitute

$$B_0\left(\frac{k_1 k_2}{k_2 + k_3} - k_1\right) = K_1 = \text{constant} \tag{5.41}$$

and

$$\frac{k_1}{k_2 + k_3} = K_2 = \text{constant} \tag{5.42}$$

into equation (5.40) we obtain

$$r_A = \frac{K_1 A}{1 + K_2 A} \tag{5.43}$$

For the biosorption process the concentration of the sorbate in the liquid phase approaches an equilibrium concentration as a limit so the appropriate form of the rate equation is

$$\frac{d(C - C_{eq})}{dt} = \frac{K_1 (C - C_{eq})}{1 + K_2 (C - C_{eq})} \tag{5.44}$$

where

C = the liquid phase concentration of the sorbate at any time,

C_{eq} = the equilibrium liquid phase concentration of the sorbate.

Integrating equation (5.44) we obtain

$$\int_{C_o}^{C} \frac{1 + K_2 (C - C_{eq})}{C - C_{eq}} d(C - C_{eq}) = K_1 \int_o^t dt \tag{5.45}$$

and

$$\ell n\left(\frac{C - C_{eq}}{C_o - C_{eq}}\right) - K_2 (C_o - C) = K_1 t \tag{5.46}$$

where

C_o = the initial liquid phase concentration of the sorbate,

t = time.

Equation (5.46) can be rearranged into a form from which the values of K_1, and K_2 can be obtained by linear regression of the kinetic data.

$$\frac{1}{C_o - C} \ell n\left(\frac{C - C_{eq}}{C_o - C_{eq}}\right) = K_1 \frac{t}{C_o - C} + K_2 \qquad (5.47)$$

This model can be fit to a portion of the kinetic data for both types of biomass, however it does not provide a good fit over the entire time range. For both types of biomass the value of K_2 resulting from linear regression is negative which is inconsistent with the proposed mechanism from which the model was derived. It appears that simple kinetic models are not adequate to describe the biosorption behaviour of lindane.

Since the biosorption data for the diazinon/*R. arrhizus* system exhibited some anomalous behaviour (see Section 5.1.2), further investigation of the kinetics of diazinon biosorption was performed. The purpose of this investigation was to determine whether the three day contact time was sufficient to achieve equilibrium and to observe any evidence of a chemical reaction which might explain the anomalous behaviour. The kinetics experiments were done using individual contact flasks and therefore do not necessarily represent the intrinsic biosorption rate. Review of Figures 4.5.5 and 4.5.6 show that the equilibrium appears to be reached before the three day contact time. However, the data on Figure 4.5.5 show an increase in diazinon concentration after 7 days. It is not within the objectives of the present work to study the kinetics of biosorption in detail. This unusual behaviour is noted, and additional work should be carried out in the future.

Kinetics experiments were also done with malathion. The results of these experiments are discussed in Section 5.8.

5.6. COMPETITIVE BIOSORPTION

The effect of competition between two or more sorbates was investigated by contacting solutions containing several different combinations of solutes with both types of

inactive biomass. For adsorption, if the adsorbates are competing for the same adsorption sites, it would be expected that the uptake of each adsorbate at a given liquid phase concentration would be reduced from the uptake observed in a single solute system. In the case of absorption this effect might also be observed, particularly at high loading where the interactions between the solute molecules in the sorbent may be significant so that the solubilities of the solutes in the absorbing medium would be reduced. Review of Figures 4.6.1 through 4.6.18 indicates that for the combinations of solutes tested in the present work the effects of competition are slight. In many cases it appears that competition has had no effect on biosorptive uptake. Bioconcentration factors, K_B, were determined for each compound as a single solute and in competition with other sorbates. The bioconcentration factors were obtained by fitting the linear sorption model to the data using a least squares routine. The bioconcentration factors are given in Table 5.11 together with the 95% confidence limits for each factor. It is recognized that the two parameter Freundlich equation models the biosorption isotherms better than the one parameter linear model (see Section 4.1). However, the linear model provides the advantage that the relative magnitude of the biosorptive uptake for different systems may be evaluated over the entire concentration range by comparing the bioconcentration factors, K_B. This cannot be done with the Freundlich model if the exponent parameters are different. Thus the linear model provides a convenient method of comparing single solute and multi-solute biosorption isotherms.

From Table 5.11 it can be noted that in most cases the bioconcentration factor confidence limits for the competitive systems overlap those of the corresponding single solute system. In these cases we cannot conclude with confidence that the presence of competing sorbates has affected the biosorptive uptake of the compound in question. In three cases there appears to be a reduction in uptake caused by competition and in one case the uptake appears to increase.

Table 5.11 Bioconcentration factors for competitive biosorption

Compound	Biomass	Competing Compounds	K_B	95% Confidence Limits on K_B	
Lindane	*R. arrhizus*	None	2.53	2.38–2.69	
Lindane	Act. sludge	None	1.56	1.50–1.62	
PCP	*R. arrhizus*	None	22.8	19.3–26.4	
PCP	Act. sludge	None	7.08	5.51–8.64	
Diazinon	*R. arrhizus*	None	0.66	054–0.78	
Diazinon	Act. sludge	None	0.46	0.43–0.49	
Lindane	*R. arrhizus*	PCP	3.05	2.46–3.64	*
Lindane	Act. sludge	PCP	1.44	1.30–1.59	*
PCP	*R. arrhizus*	Lindane	47.8	23.8–71.9	*
PCP	Act. sludge	Lindane	13.4	6.41–20.4	*
Lindane	*R. arrhizus*	Diazinon	2.40	2.27–2.54	*
Lindane	Act. sludge	Diazinon	1.36	1.29–1.44	↓
Diazinon	*R. arrhizus*	Lindane	0.64	0.56–0.73	*
Diazinon	Act. sludge	Lindane	0.45	0.38–0.52	*
Diazinon	*R. arrhizus*	Malathion	0.66	0.60–0.72	*
Diazinon	Act. sludge	Malathion	0.63	0.57–0.70	↑
Lindane	*R. arrhizus*	Diazinon PCP Malathion	1.71	1.50–1.92	↓
Lindane	Act. sludge	Diazinon PCP Malathion	1.42	1.37–1.47	↓
PCP	*R. arrhizus*	Lindane Diazinon Malathion	26.3	23.2–29.4	*
PCP	Act. sludge	Lindane Diazinon Malathion	6.39	4.94–7.84	*
Diazinon	*R. arrhizus*	Lindane PCP Malathion	0.56	0.40–0.72	*
Diazinon	Act. sludge	Lindane PCP Malathion	0.42	0.37–0.48	*

* Confidence limits of the bioconentration factor overlap those of the single solute bioconcentration factor
↑ Biosorptive uptake is greater than uptake in the single solute system
↓ Biosorptive uptake is less than uptake in the single solute system

For the lindane/pentachlorophenol combination, there does not appear to be any reduction in the uptake of either compound due to competition (Table 5.11; Figures 4.6.1 through 4.6.4). This is the case for both *R. arrhizus* and activated sludge. Voerman and Tammes (1969) observed similar results with biosorption of lindane and dieldrin by yeast. The lack of competitive effects may be due to differences in the dominant mechanisms through which each compound is biosorbed. The two compounds may be adsorbed at different sites on the biomass surface, in which case they would not compete for the same adsorption sites so that no competitive effects would be observed. If the adsorption and absorption capacities of the biomass are much greater than the loadings achieved in the experiments, the effects of competition may be very slight and might not be observed. In the case of adsorption the presence of a large number of unoccupied adsorption sites might cause the effects of competition to be insignificant. If adsorption were the dominant mechanism, the range of loading covered in the experiments is beyond the calculated loading needed to achieve a complete adsorbed monolayer (see Table 5.3). This would appear to indicate that all surface sites were utilized, at least at the higher concentrations, and that competitive effects should be observed. The fact that no competitive effects were observed suggests that the dominant mechanism may be absorption and that interactions between the two solutes in the sorbent may be negligible at the experimental concentrations.

For the lindane/diazinon system there also appears to be no reduction in the uptake of either compound by *R. arrhizus* (Table 5.11; Figures 4.6.5 and 4.6.7). For activated sludge, the uptake of lindane appears to be somewhat reduced from that of the single solute system, however, the uptake of diazinon appears to be unaffected (Table 5.11; Figures 4.6.6 and 4.6.8).

Biosorption of diazinon by *R. arrhizus* does not appear to be affected by competition with malathion (Table 5.11; Figure 4.6.9). Comparison of bioconcentration factors indicates

an increase in uptake of diazinon by activated sludge in the presence of malathion (Table 5.11). Review of Figure 4.6.10, however, indicates, in general, an increase in uptake at the lower concentrations and a decrease in uptake at the higher concentrations. If malathion is not strongly sorbed and is removed primarily by a chemical reaction mechanism, it would not be expected to have a strong competitive effect on the sorption of diazinon. This seems to be the case with *R. arrhizus*, however in the case of activated sludge it is unclear whether the presence of malathion actually affects the uptake of diazinon. The apparent uptake of malathion appears to be reduced by the presence of diazinon (Figures 4.6.11 and 4.6.12). If the reaction is catalyzed by the biomass surface, possibly the adsorption of diazinon interferes with the reaction process and causes a reduction in the reaction rate and thus a reduction in the apparent uptake. If the reaction is catalyzed by an enzyme, the diazinon may interfere with the reaction process in some way.

Systems of four compounds were also tested. Reduction in uptake of lindane by both types of biomass appeared to result from competition with pentachlorophenol, diazinon, and malathion (Table 5.11; Figures 4.6.13 and 4.6.14). The uptake of pentachlorophenol and diazinon, however, appear to be unaffected by the presence of the other compounds (Table 5.11; Figures 4.6.15 through 4.6.18).

In general, the competitive biosorption data suggest that there is little or no effect on biosorptive uptake of the compounds tested caused by competition from other compounds, at least over the concentration ranges of the data. The concentration ranges of the experimental data cover the upper end of the range of concentrations expected to occur in public wastewater treatment plants. Therefore, the data suggest that the effect of competition on biosorption of hazardous organic compounds in biological treatment plants may be minimal. Therefore it may be possible to model the behaviour of individual compounds from single solute biosorption isotherm data without regard for competitive effects.

5.7 BIOSORPTION AND DESORPTION BY LIVE BIOMASS

Isotherms for biosorption of lindane, pentachlorophenol, diazinon, and 2-chlorobiphenyl by live *R. arrhizus* and live activated sludge were developed. The reversibility of the sorption process was also studied by desorbing the compounds from the live biomass. The parameters for the Freundlich equations fit to the biosorption data for live biomass are given in Table 5.12.

Table 5.12 Freundlich Equation Parameters for Bisorption by Live Biomass.

Compound	Biomass	K_F	1/n
Lindane, 20°C	*R. arrhizus*	1.37	0.93
	Activated Sludge	0.56	1.04
Lindane, 5°C	*R. arrhizus*	1.17	1.01
	Activated Sludge	0.49	1.16
Pentachlorophenol	*R. arrhizus*	32.13	0.56
	Activated Sludge	85.06	0.60
Diazinon	*R. arrhizus*	1.22	0.88
	Activated Sludge	0.02	1.39
2-chlorobiphenyl	*R. arrhizus*	798.50	1.26
	Activated Sludge	261.50	0.69

At 20°C and at 5°C the uptake of lindane by both types of live biomass is less then the uptake observed with dead biomass (Figures 4.7.1 through 4.7.4). Other investigators observed that the accumulation of organic pollutants by dead biomass was greater than or

equal to the accumulation by live microorganisms. If at least a portion of the accumulation is due to absorption into the cell, the greater accumulation by dead cells may be caused by the greater permeability of the cell membrane in dead cells (Davson and Danielli, 1952). The live cells may be protected, to some extent, from transport of pollutants into the cell interior. The autoclaving and drying processes may also affect the cell surface in such a way that surface adsorption is enhanced in the dead cells. From Table 5.12 it can be noted that the biosorption isotherms for lindane are approximately linear as was observed with dead biomass. From the isotherms at the two different temperatures, the heats of sorption can be estimated as described in Section 5.2. At a loading of 100 μg/g the heat of sorption for live *R. arrhizus* is −2.2 kcal/mol and the heat of sorption for live activated sludge is −4.3 kcal/mol. Heats of sorption of this magnitude are indicative of a physical rather than a chemical mechanism, as found for biosorption by dead biomass. The negative heats of sorption indicate an exothermic process as is the case with dead biomass.

In each experiment a one day contact time was allowed for sorption and desorption except for one experimental point in which a three day contact was allowed for sorption and desorption. The one day contact time was used, rather than the three day period used for dead biomass, to reduce the potential for biodegradation. The three day contact time was used to observe the effect of additional contact time and to help determine whether any biodegradation did occur. From Figures 4.7.1 through [redacted] can be seen that in all cases the uptake after the three day period is less than for the one day contact time. This suggests that biodegradation has not occurred since we would expect the apparent uptake to increase with time if biodegradation were taking place. The decrease in uptake with time could be caused by reduction of the mass of microbial cells in the system due to autooxidation and lysis of cells. Lindane sorbed onto the cells after one day could subsequently be released to the solution with the destruction of cells, resulting in a higher solution concentration and lower apparent

loading. In the *R. arrhizus* experiments the quantity of biomass remaining after sorption and desorption was measured by collecting the biomass on a preweighed filter, drying and reweighing the filter and biomass. After compensating for the quantity of biomass decanted prior to desorption it was found that the mass of *R. arrhizus* decreased an average of approximately 17% over the combined six day contact period. This result suggests that some decomposition of the biomass does occur during the biosorption and desorption experiments. If the calculated uptake of the 3 day contact data for the lindane/*R. arrhizus* system is corrected for the loss of biomass the data fit more closely to the isotherm lines suggesting that the biomass quantity has indeed decreased. Desorption data indicate that biosorption of lindane by live *R. arrhizus* at 20°C is reversible (Figure 4.7.5). The reversibility of the process is further evidence that the removal of lindane results from sorption and not biodegradation. For activated sludge at 20°C (Figure 4.7.6) and *R. arrhizus* at 5°C (Figure 4.7.7) the desorption data points fall generally along the sorption isotherm lines indicating that the processes for these systems are reversible. The data points above the isotherm lines for the higher concentrations, however, indicate some irreversibility. The apparent irreversibility could be due to a slow desorption rate, resulting in failure to reach equilibrium in the one day contact period. Since biosorption of lindane by *R. arrhizus* appears to be reversible at 20°C it seems unlikely that it would not be reversible at the lower temperature. The desorption rate, however, may be slowed sufficiently at 5°C so that equilibrium is not achieved in one day. The fact that the three day desorption experiment appears to show reversibility tends to confirm this hypothesis. For activated sludge at 5°C (Figure 4.7.8) all of the desorption data points are above the sorption isotherm line indicating a lack of complete reversibility. Again this could result from failure to reach equilibrium at the lower temperature due to a decrease in the desorption rate. Taken together, the experimental evidence suggests that biosorption

of lindane by *R. arrhizus* and activated sludge is reversible, although the desorption rate may be slower than the sorption rate and may be appreciably affected by temperature.

The uptake of pentachlorophenol by live *R. arrhizus* was found to be less than that observed for dead biomass (Figure 4.7.9). In the case of activated sludge, however, the uptake is greater for live biomass than for dead biomass (Figure 4.7.10). For *R. arrhizus* the three day contact time resulted in apparently greater uptake than for a one day contact period. This could have resulted from biodegradation of pentachlorophenol or from a slow biosorption rate so that equilibrium is not attained in the one day contact period. The greater uptake of pentachlorophenol by live activated sludge than by dead sludge disallows the possibility of a generalization concerning the relative uptake of live and dead biomass. One case was reported in the literature in which dead cells sorbed slightly less than live cells (Werner and Morschel, 1978). It is possible that biodegradation accounts for the higher uptake of live sludge, however, the desorption data (Figure 4.7.12) show evidence of reversibility of the process which would not be expected if biodegradation had occurred. In the case of *R. arrhizus* the desorption data appear to indicate that the removal process is at least partly irreversible (Figure 4.7.11). This could be the result of biodegradation or a slow desorption rate. Biodegradation of pentachlorophenol has been reported (Moos et al., 1983; Tabak et al., 1981), although Petrasek et al. (1983) found no evidence of biodegradation of pentachlorophenol in an activated sludge pilot plant.

Diazinon was found to be sorbed by live *R. arrhizus* to approximately the same level as by dead biomass (Figure 4.7.13). Uptake by live activated sludge was observed to be somewhat lower than by dead sludge (Figure 4.7.14). Diazinon appears to be recovered by desorption in the case of *R. arrhizus* (Figure 4.7.15), but not in the case of activated sludge (Figure 4.7.16). The data from the three day contact experiments (Figures 4.7.13 and 4.7.14) do not show an increase in apparent uptake with time, and thus tend to indicate that

biodegradation did not occur. Paris et al. (1975) observed no biodegradation of diazinon by various bacterial and fungal populations. The apparent lack of reversibility of sorption of diazinon by activated sludge may be due to slow desorption kinetics.

The uptake of 2-chlorobiphenyl by live *R. arrhizus* is greater than the uptake by dead biomass (Figure 4.7.17). This result is also observed for uptake of 2-chlorobiphenyl by live activated sludge (Figure 4.7.18). In both cases the three day uptake appears to be less than the one day uptake, suggesting that the sorbate may be released as a result of decomposition of the biomass over the extended time period. Desorption data for both types of biomass indicate that the sorption processes are reversible (Figures 4.7.19 and 4.7.20). The observed reversibility suggests that biodegradation did not take place, and thus the higher uptake with live biomass is unlikely to be caused by biodegradation. For activated sludge the loading after desorption is significantly less than would be predicted by the sorption isotherm (Figure 4.7.20). This may be caused, among others, by destruction of the microbial cells, possibly as a result of contact with the 2-chlorobiphenyl. The greater uptake of 2-chlorobiphenyl by live biomass than by dead biomass suggests perhaps an active transport mechanism for this compound. Norris and Kelly (1979), however, observed that metal uptake by active metabolic transport was limited in the absence of a food source. In the present work the biosorption experiments were carried out in the absence of a microbial food source so that active transport may not be a prominent mechanism. Perhaps the lower uptake of 2-chlorobiphenyl by dead biomass is due to changes in cellular membranes or in the case of bacteria in the extracellular polysaccharide matrix. Friedman and Dugan (1967) showed that the extracellular matrix of zoogloeal bacteria contributed to the uptake of metals.

The results of the present work suggest that a generalization cannot be made concerning the difference in biosorptive uptake by live and dead biomass. The difference in uptake appears to depend on the specific sorbate.

It is of interest to examine the use of biosorption isotherms to predict the fate of hazardous organic compounds in biological treatment systems. Petrasek et al. (1983) investigated the fate of a number of toxic organic compounds injected into the influent of an activated sludge pilot plant. The following operating parameters were reported for the process:

Mean influent flow:	0.088 L/s
Mixed liquor solids concentration:	1900 mg/L
Waste sludge solids concentration:	6400 mg/L
Solids retention time:	7 days
Aeration basin volume:	2549 L*

* From Petrasek et al. (1980)

From the above information the waste sludge flow can be estimated from equation 5.48.

$$Q_w = \frac{VX}{\theta X_w} \tag{5.48}$$

where

Q_w = waste sludge flow

V = aeration basin volume

X = mixed liquor solids concentration

X_w = waste sludge solids concentration

θ = solids retention time

The waste sludge flow calculated using equation (5.48) and the reported parameters is 108 L/d. For a linear isotherm, assuming that the biosorption system is in equilibrium at the effluent concentration of the compound in question, at steady state the mass balance equation for the compound is given by equation 5.49 (see Figure 5.7.1).

$$Q_o C_o = (Q_o - Q_w)C + Q_w X_w K_B C + Q_w C \tag{5.49}$$

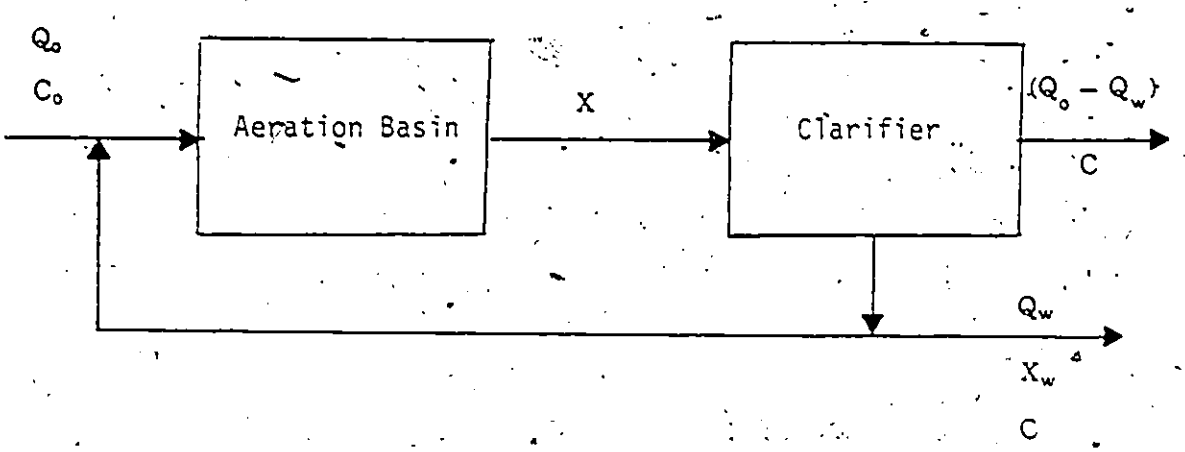


Figure 5.7.1 Schematic flow diagram of activated sludge system.

where

Q_o = influent flow

C_o = influent concentration of compound

C = effluent concentration of compound

K_B = bioconcentration factor

If K_B is known, the effluent concentration of the compound can be predicted by rearranging equation 5.49 to solve for C.

$$C = \frac{Q_o C_o}{Q_o + Q_w X_w K_B} \qquad (5.50)$$

The total concentration in the waste sludge effluent, C_W, (including the portion sorbed onto the sludge and the portion in solution) is given by equation 5.51.

$$C_W = X_W K_B C + C \qquad (5.51)$$

The Freundlich constant K_F can be substituted for K_B for lindane since the isotherm for live activated sludge is nearly linear. From Table 5.11 the K_F value is 0.56. Using the activated sludge influent concentration of 41.8 µg/L for lindane reported by Petrasek et al. (1983) the predicted effluent concentration from equation 5.50 is 41.6 µg/L. From equation 5.51 the predicted lindane concentration in the waste sludge effluent is 192 µg/L. The actual mean concentration of lindane in the waste sludge effluent was reported to be 173 µg/L which is reasonably consistent with the predicted value. The effluent concentration, however, was reported to be 25.8 µg/L. If this value is used to predict the waste sludge effluent concentration, a value of 119 µg/L is predicted. In their mass balance the authors were unable to account for approximately 25% of the lindane. They postulated that the loss was due to biodegradation since the loss by volatilization was expected to be minimal. This may account for the the fact that the actual effluent concentration is less than the predicted concentration. Weber et al. (1987) used equilibrium biosorption data to predict removal of lindane in an

activated sludge reactor and reported a predicted removal of 12% and an actual removal of 7%. They observed no biodegradation of the lindane. It appears that the biosorption data can be used to predict the concentration in the waste sludge effluent to the correct order of magnitude. This method predicts that the removal efficiency of lindane due to biosorption in a single stage, complete mix activated sludge plant would be slight. The concentration of lindane in the sludge, however, may be great enough to pose an environmental hazard upon disposal of the sludge.

5.8 MECHANISM OF MALATHION REMOVAL

The unexpectedly high apparent uptake of malathion by the inactive biomass initially suggested that malathion was being removed by some means other than biosorption. Based on its relatively high water solubility and low octanol/water partition coefficient malathion was expected to exhibit less biosorptive uptake than the other compounds. However, the apparent uptake of malathion by activated sludge was observed to be greater than that of all the other compounds. The uptake of malathion by *R. arrhizus* was found to be greater than that of all compounds except 2-chlorobiphenyl. The removal of malathion from solution was suspected to be the result of a chemical reaction in which malathion is decomposed. Additional experimental work was undertaken to test this hypothesis.

Biosorption experiments with malathion were carried out at a temperature of 5°C (see Section 5.2). At this temperature the apparent uptake of malathion was considerably less than that observed at a temperature of 20°C (Figures 4.2.5 and 4.2.6). The apparent heats of sorption calculated from the apparent isotherms at the two different temperatures are characteristic of a chemical rather than a physical process (see Section 5.2). If the mechanism of malathion removal primarily involved physical sorption, the uptake would not be expected to be greatly affected by moderate temperature changes. The observed temperature effect can

be accounted for by the chemical reaction hypothesis. At a reduced temperature the chemical reaction rate would be expected to be reduced. The extent to which the reaction would proceed in a given time would thus be reduced and would account for the lower apparent uptake.

If removal of malathion occurs by chemical decomposition, the decomposed malathion would not be recovered by desorption. Desorption experiments carried out at 20°C show that the malathion is not recovered by desorption (Figures 4.3.9 and 4.3.10). The irreversibility of the process tends to confirm the chemical reaction hypothesis. At a temperature of 5°C however, the malathion appears to be recovered by desorption (Figures 4.3.7 and 4.3.8). Since the process appears to be reversible at this temperature it seems likely that sorption is the primary mechanism observed at the lower temperature over the contact times used in the experiments. A reduced reaction rate would account for this observation. If the reaction rate at the lower temperature is very slow, the quantity of malathion decomposed will be small and may be negligible compared with the sorbed quantity. Thus reversibility is observed at the lower temperature but not at the higher temperature where the reaction rate is greater.

Kinetics of malathion removal by live and dead biomass were investigated. Kinetics experiments were done at 20°C with two different concentrations each of inactive *R. arrhizus* and activated sludge and at 5°C with one concentration of each biomass. A control test at 20°C with no biomass was also performed to evaluate the rate of hydrolysis of malathion. Experiments with live biomass were also performed at 20°C. With live activated sludge the malathion had been removed to below the detection limit before the first sample time of 24 hours and therefore no rate curve was plotted for this experiment. Data for the inactive biomass and for live *R. arrhizus* are shown in Figures 4.5.7 through 4.5.9. For both types of dead biomass at 20°C malathion appears to be removed by a zero order rate process with the rate dependent on the biomass concentration (Figures 4.5.7 and 4.5.8). At the higher

biomass concentrations the malathion was eventually removed to below the detection limit of approximately 1 µg/L. The complete removal of malathion is further evidence that the primary mechanism is not sorption and tends to confirm the chemical reaction hypothesis. At 5°C the rate of malathion removal is much slower than at 20°C (Figures 4.5.7 and 4.5.8). There appears to be an initial more rapid disappearance of malathion followed by a slower removal. The initial disappearance of malathion is probably due mainly to sorption, while the subsequent slow removal is probably the result of a chemical reaction. This process also appears to follow a zero order rate as observed at the higher temperature. Disappearance of malathion is also observed at 20°C with no biomass present, at a much lower rate than in the presence of the dead biomass. This result was expected since malathion is known to hydrolyze in water solution (Muhlmann and Schrader, 1957; Freed et al., 1979). Apparently the inactive biomass in some way acts as a catalyst for the decomposition of malathion. Perhaps the reaction is catalyzed by adsorption on the biomass surface. A similar mechanism was proposed by Konrad et al. (1969) who observed rapid decomposition of malathion in the presence of sterile soil. Although malathion is known to decompose under the influence of various enzymes (Buchel, 1983; Fest and Schmidt, 1982), it is not expected that enzymes would be present after processing the dead biomass.

The rate of removal of malathion by live biomass is greater than that of dead biomass (Figure 4.5.9). There appears to be an initial rapid removal, probably due to sorption, followed by a lag phase and then by a rapid disappearance of the malathion. The rate of removal by live activated sludge is greater than that of *R. arrhizus* in that the malathion was observed to completely disappear within 24 hours. Paris et al. (1975) found that malathion was degraded by both bacteria and fungi when the malathion provided the sole source of carbon. They reported the major metabolite to be the β-monoacid of malathion.

To further aid in understanding the removal of malathion by inactive biomass carbon-14 labeled malathion solution was contacted with dead *R. arrhizus* and activated sludge. Since no decomposition products were detected in the solutions from which malathion had been removed, it was suspected that the products of decomposition were highly water soluble and thus were not extracted by the organic solvent in detectable concentrations. If this were the case the decomposition products would not be expected to be sorbed significantly by the biomass and should remain mainly in solution. If C-14 labeled malathion were decomposed by the biomass, we would then expect to find the majority of the C-14 remaining in the solution. Review of Table 4.3 shows that this is indeed the case. In the case of *R. arrhizus*, where the malathion completely disappeared from the solution, virtually all of the C-14 was found in the solution. This result tends to confirm the hypothesis of a chemical reaction in which malathion is broken down into water soluble byproducts. In the other experiments, where the labeled malathion was contacted with dead sludge and cell walls, the malathion was not completely removed, and more C-14 was found on the biomass. In all cases except one, 90% or more of the initially added C-14 was accounted for. The higher concentrations of C-14 on the biomass where the malathion was not totally removed are probably due to biosorption of part of the remaining malathion. One of the solutions in which the malathion had been completely removed was extracted with hexane and the hexane was analyzed for C-14. Less than one percent of the C-14 was found in the hexane, indicating that the decomposition products are highly water soluble. In general the labeled malathion experiments add further evidence to support the chemical reaction hypothesis. It is of interest to note that malathion is apparently also decomposed by contact with the cell walls. These results seem to suggest that the reaction may be catalyzed by adsorption on the cell walls. At the present time the evidence is not sufficient to support a more detailed mechanistic understanding of the process, although the overall evidence strongly supports the general chemical reaction hypothesis.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

For the hazardous organic compounds and biomass types examined the following conclusions are drawn:

1. Biosorption is an equilibrium process, and biosorptive equilibrium is independent of initial sorbent and sorbate concentrations.
2. The Freundlich equation is suitable for modelling biosorption isotherms.
3. *R. arrhizus* displays greater biosorptive uptake capacity than does activated sludge.
4. Biosorptive uptake of organics is positively correlated with the octanol/water partition coefficient.
5. Sorbed organic compounds are readily desorbed, and the desorption isotherms appear to be essentially the same as the sorption isotherms.
6. Based on the thermodynamic study of the process, biosorption appears to be a physical rather than a chemical process.
7. The biosorption phenomenon appears to involve both adsorption of the sorbate on the biomass surface and absorption of the sorbate into the cells.
8. Biosorptive uptake generally appears not to be strongly affected by competition from other sorbing compounds.
9. The kinetics of biosorption of lindane are characterized by a rapid initial uptake followed by a slower accumulation process.

10. Live and dead biomass do not generally exhibit the same biosorptive uptake, and the differences appear to depend on the specific sorbate.

11. Biosorption equilibrium isotherm data are useful in estimating the order of magnitude of removal of hazardous organics in biological treatment plants.

12. Decomposition of malathion is brought about by contact with dead biomass and microbial cell walls, as well as by live biomass via a mechanism that appears to be of chemical and not of physical nature.

6.2 RECOMMENDATIONS FOR FUTURE RESEARCH

The following are recommendations for future research to expand understanding of the phenomenon of biosorption:

1. Investigation of biosorption of additional organic pollutants and additional types of biomass.

2. Study of the effects of wastewater characteristics and treatment plant operating variables on the biosorption process.

3. Investigation of the fate of sorbed pollutants during sludge digestion and other sludge processing operations.

4. Examination of the behaviour of sorbed pollutants after land disposal of waste sludges.

5. Expansion of biosorption kinetics studies, including desorption kinetics, and development of kinetic models for biosorption and desorption.

6. Development of models to predict the fate of hazardous organic pollutants in biological wastewater treatment plants.

7. Development of models to predict the behaviour of sorbed pollutants in landfilled sludges.

8. Study of the mechanism of biosorption by analysis of single cells using x-ray energy dispersion analysis or other suitable techniques.
9. Investigation of the mechanism of malathion removal by dead biomass.
10. Determination of the degradation products of the malathion removal process.
11. Investigation of the effect of dead biomass on compounds similar to malathion.
12. A detailed examination of the biosorption kinetics of diazinon.

REFERENCES

Asselineau, J., *The Bacterial Lipids*, Holden-Day, San Francisco (1966).

Baughman, G.L. and Paris, D.F., "Microbial Bioconcentration of Organic Pollutants from Aquatic Systems - A Critical Review", CRC Crit. Rev. Microbiol., 8, 205 (1981).

Bishop, D.F., "The Role of Municipal Wastewater Treatment in Control of Toxics", Presented at the NATO/CCMS Meeting, Bari, Italy (1982).

Blackburn, J.W., Troxler, W.L., and Sayler, G.S., "Prediction of the Fates of Organic Chemicals in a Biological Treatment Process - An Overview", Environ. Prog., 3, 163 (1984).

Bridle, T.R., "The Impact of Hazardous Organics on Sludge Management and Disposal", Presented at the PCAO/MOE Seminar Hazardous Substances in Wastewaters, Toronto, Ontario (1982).

Buchel, K.H. (ed.), *Chemistry of Pesticides*, John Wiley and Sons, New York (1983).

Buckingham, J. (ed.), *Dictionary of Organic Compounds*, Chapman and Hall, New York (1982).

Bull, H.B., "Adsorption of Bovine Serum Albumin on Glass", Biochim. Biophys. Acta., 19, 464 (1956).

Burns and Roe Industrial Services Corp., "Fate of Priority Toxic Pollutants in Publicly Owned Treatment Works", Volume I, Report No. EPA-440/1-82-303-VOL-1, U.S. Environmental Protection Agency (1982).

Calgon Canada, personal communication (1986).

Canton, J.H., Greve, P.A., Sloof, W. and van Esch, G.J., "Toxicity, Accumulation and Elimination Studies of α-Hexachlorocyclohexane (α-HCH) with Freshwater Organisms of Different Trophic Levels", Water Res., 9, 1163 (1975).

Canton, J.H., van Esch, G.J., Greve, P.A., and van Hellemond, A.B.A.M., "Accumulation and Elimination of α-Hexachlorocyclohexane (α-HCH) by the Marine Algae *Chlamydomonas* and *Dunaliella*", Water Res., 11, 111 (1977).

Casserly, D.M., Davis, E.M., Downs, T.D., and Guthrie, R.K., "Sorption of Organics by *Selenastrum capricornutum*", Water Res., 17, 1591 (1983).

Chacko, C.I. and Lockwood, J.L., "Accumulation of DDT and Dieldrin by Microorganisms", Can. J. Microbiol., 13, 1123 (1967).

Chattoraj, D.K. and Birdi, K.S., *Adsorption and the Gibbs Surface Excess*, Plenum Press, New York (1984).

Chiou, C.T. and Manes, M., "Application of the Polanyi Adsorption Potential Theory to Adsorption from Solution on Activated Carbon. IV. Steric Factors, as Illustrated by the Adsorption of Planar and Octahedral Metal Acetylacetonates", J. Phys. Chem., 77, 809 (1973).

Chiou, C.T. and Manes, M., "Application of the Polanyi Adsorption Potential Theory to Adsorption from Solution on Activated Carbon. V. Adsorption from Water of Some Solids and Their Melts, and a Comparison of Bulk and Adsorbate Melting Points", J. Phys. Chem., 78, 622 (1974).

Clayton, J.R., Pavlou, S.P. and Breitner, N.F., "Polychlorinated Biphenyls in Coastal Marine Zooplankton: Bioaccumulation by Equilibrium Partitioning", Environm. Sci. Technol., 11, 676 (1977).

Clevenger, T.E., Hemphill, D.D., Roberts, K. and Mullins W.A., "Chemical Composition and Possible Mutagenicity of Municipal Sludges", J. WPCF, 55, 1470 (1983).

Crisp, D.J., "The Adsorption of Alcohols and Phenols from Nonpolar Solvents on to Alumina", J. Colloid Sci., 11, 356 (1956).

Daniel, S.G., "The Adsorption on Metal Surfaces of Long Chain Polar Compounds from Hydrocarbon Solutions", Trans. Faraday Soc., 47, 1345 (1951).

Davson, H. and Danielli, J.F., *The Permeability of Natural Membranes*, University Press, Cambridge (1952).

Dobbs, R.A. and Cohen, J.M., "Carbon Adsorption Isotherms for Toxic Organics", Report No. EPA-60018-80-123, U.S. Environmental Protection Agency (1980).

Dobbs, R.A., Jelus, M. and Cheng, K., "Partitioning of Toxic Organic Compounds on Municipal Wastewater Treatment Plant Solids", Report No. EPA/600/D-86/137, U.S. Environmental Protection Agency (1986).

Ellgehausen, H., Guth, J.A. and Esser, H.O., "Factors Determining the Bioaccumulation Potential of Pesticides in the Individual Compartments of Aquatic Food Chains", Ecotoxicol. Environm. Safety, 4, 134 (1980).

Ernst, W., "Determination of the Bioconcentration Potential of Marine Organisms - A Steady State Approach", Chemosphere, 6, 731 (1977).

Everett, D.H., "Thermodynamics of Adsorption from Non-aqueous Solutions", Progr. Colloid and Polymer Sci., 65, 103 (1978).

Fest, C. and Schmidt, K., *The Chemistry of Organophosphorus Pesticides*, Springer-Verlag, New York (1982).

Freed, V.H., Chiou, C.T. and Schmedding, D.W., "Degradation of Selected Organophosphate Pesticides in Water and Soil", J. Agric. Food Chem., 27, 706 (1979).

Friedmann, B.A. and Dugan, P.R., "Concentration and Accumulation of Metallic Ions by the Bacterium, Zoogloea", Dev. Ind. Microbiol., 9, 381 (1967).

Grimes, D.J. and Morrison, S.M., "Bacterial Bioconcentration of Chlorinated Hydrocarbon Insecticides from Aqueous Systems", Microbial Ecology, 2, 43 (1975).

Groszek, A.J., "Adsorption of Polycyclic Aromatic Hydrocarbons onto Graphite", Faraday Discuss. Chem. Soc., 59, 109 (1975).

Hansch, C. and Leo, A., *Substituent Constants for Correlation Analysis in Chemistry and Biology*, John Wiley and Sons, New York (1979).

Hansen, P., "Experiments on the Accumulation of Lindane (γ-BHC) by the Primary Producers *Chlorella spec.* and *Chlorella pyrenoidosa*", Arch. Environm. Contam. Toxicol., 8, 721 (9179).

Hawley, G.G. (ed.), *The Condensed Chemical Dictionary*, Van Nostrand Reinhold Co., New York (1971).

Herbes, S.E., "Partitioning of Polycyclic Aromatic Hydrocarbons Between Dissolved and Particulate Phases in Natural Waters", Water Res., 11, 493 (1977).

Holwerda, B.C., "The In Vitro Degradation of [^{14}C]-Malathion and [^{14}C]-Maloxon in Resistant and Susceptible Strains of *Drosophila melanogaster*", M.Sc. Thesis, Mc Master University, Hamilton, Ontario (1983).

Hutzinger, O., Safe, S. and Zitko, V., *The Chemistry of PCB's*, CRC Press, Boca Raton (1974).

Jackson, J. and Weller, P., *Chemical Nightmare*, Between the Lines, Toronto (1982).

Johnson, B.T. and Kennedy, J.O., "Biomagnification of p,p'-DDT and Methoxychlor by Bacteria", Appl. Microbiol., 26, 66 (1973).

Kenaga, E.E., "Guidelines for Environmental Study of Pesticides: Determination of Bioconcentration Potential", Residue Rev., 44, 73 (1972).

Kern, H.E., Piechocki, A., Brauer, U. and Findnegg, G.H., "Adsorption from Solution of Chain Molecules onto Graphite: Evidence for Lateral Interactions in Ordered Monolayers", Progr. Colloid Polymer Sci., 65, 118 (1978).

Ko, W.H. and Lockwood, J.L., "Accumulation and Concentration of Chlorinated Hydrocarbon Pesticides by Microorganisms in Soil", Can. J. Microbiol., 14, 1075 (1968).

Konrad, J.G., Chesters, G. and Armstrong, D.E., "Soil Degradation of Malathion, a Phosphorodithioate Insecticide", Soil Sci. Soc. Amer. Proc., 33, 259 (1969).

Lal, R. and Saxena, D.M., "Accumulation, Metabolism, and Effects of Organochlorine Insecticides on Microorganisms", Microbiol. Rev., 46, 95 (1982).

Lederman, T.C. and Rhee, G., "Bioconcentration of a Hexachlorobiphenyl in Great Lakes Planktonic Algae", Can. J. Fish. Aquat. Sci., 39, 380 (1982).

Lieb, W.R. and Stein, W.D., "Biological Membranes Behave as Non-porous Polymeric Sheets with Respect to the Diffusion of Non-electrolytes", Nature, 224, 240 (1969).

Lue-Hing, C., Lordi, D.T. and Kelada, N., "Fate of Priority Pollutants in a Large Metropolitan Treatment Facility", Report No. 80-28, The Metropolitan Sanitary District of Greater Chicago (1980).

Mac-Rae, I.C., "Removal of Pesticides in Water by Microbial Cells Adsorbed to Magnetite", Water Res., 19, 825 (1985).

Mahadevan, P.R. and Tatum, E.L., "Relationship of the Major Constituents of the *Neurospora crassa* Cell Wall to Wild-Type and Colonial Morphology", J. Bacteriol., 90, 1073 (1965).

Manes, M. and Hofer, L.J.E., "Application of the Polanyi Adsorption Potential Theory to Adsorption from Solution on Activated Carbon", J. Phys. Chem., 73, 584 (1969).

McNeely, R.N., Neimanis, V.P. and Dwyer, L., "Water Quality Sourcebook", Environment Canada (1979).

Meleer, H. and Bedford, W.K., "The Fate of 4,6-Dinitro-O-Cresol in Municipal Activated Sludge Systems", Presented at the International Conference on Innovative Biological Treatment of Toxic Wastewaters, Arlington, Virginia (1986).

Moos, L.P., Kirsch, E.J., Wukasch, R.F. and Grady, C.P.L., "Pentachlorophenol Degradation - I", Water Res., 17, 1575 (1983).

Muhlmann, V.R. and Schrader, G., "Hydrolyse der Insektiziden Phosphorsaureester", Z. Naturforschg., 12b, 196 (1957).

Norris, P.R. and Kelly, D.P., "Accumulation of Metals by Bacteria and Yeast", Dev. Ind. Microbiol., 20, 299 (1979).

Paris, D.F. and Lewis, D.L., "Accumulation of Methoxychlor by Microorganisms Isolated from Aqueous Systems", Bull. Environm. Cotam. Toxicol., 15, 24 (1976).

Paris, D.F., Lewis, D.L., Barnett, J.T. and Baughman, G.L., "Microbial Degradation and Accumulation of Pesticides in Aquatic Systems", Report No. EPA-660/3-75-007, U.S. Environmental Protection Agency (1975).

Paris, D.F., Lewis, D.L. and Barnett, J.T., "Bioconcentration of Toxaphene by Microorganisms", Bull. Environm. Contam. Toxicol., 17, 564 (1977).

Petrasek, A.C., Austern, B.M., Pressley, T.A., Winslow, L.A. and Wise, R.H., "Behaviour of Selected Organic Priority Pollutants in Wastewater Collection and Treatment Systems", Presented at the WPCF 53rd Annual Conference, Las Vegas, Nevada (1980).

Petrasek, A.C., Kugelman, I.J., Austern, B.M., Pressley, T.A., Winslow, L.A. and Wise, R.H., "Fate of Toxic Organic Compounds in Wastewater Treatment Plants", J. WPCF, 55, 1286 (1983).

Reinert, R.E., "Accumulation of Dieldrin in an Alga (*Scenedesmus obliquus*, *Daphnia magna*, and the Guppy *Poecilia reticulata*)", J. Fish. Res. Bd. Canada, 29, 1413 (1972).

Rosene, M.R. and Manes, M., "Application of the Polanyi Adsorption Potential Theory to Adsorption from Solution on Activated Carbon. VII. Competitive Adsorption of Solids from Water Solution", J. Phys. Chem., 80, 953 (1976).

Schenz, T.W. and Manes, M., "Application of the Polanyi Adsorption Potential Theory to Adsorption from Solution on Activated Carbon. VI. Adsorption of Some Binary Organic Liquid Mixtures", J. Phys. Chem., 79, 604 (1975).

Sharom, M.S., Miles, J.R.W., Harris, C.R. and McEwen, F.L., "Behaviour of 12 Insecticides in Soil and Aqueous Suspensions of Soil and Sediment", Water Res., 14, 1095 (1980).

Sodergren, A., "Uptake and Accumulation of C-14-DDT by *Chlorella sp.* (*Chlorophyceae*)", Oikos, 19, 126 (1968).

Southworth, G.R., Beauchamp, J.J. and Schmieder, P.K., "Bioaccumulation Potential of Polycyclic Aromatic Hydrocarbons in *Daphnia pulex*", Water Res., , 973 (1978).

Stagg, C.M. and Feather, M.S., "The Characterization of a Chitin-associated D-Glucan from the Cells Walls of *Aspergillus Niger*", Biochim. Biophys. Acta, 320, 64 (1973).

Steen, W.C. and Karickhoff, S.W., "Biosorption of Hydrophobic Organic Pollutants by Mixed Microbial Populations", Chemosphere, 10, 27 (1981).

Sugiura, K., Sato, S. and Goto, M., "The Adsorption-Diffusion Mechanism of BHC-Residues", Chemosphere, 4, 189 (1975).

Tabak, H.H., Quave, S.A., Mashni, C.I. and Barth, E.F., "Biodegradability Studies with Organic Priority Pollutant Compounds", J. WPCF, 53, 1503 (1981).

Tsezos, M., "Determination of Specific Surface Area of Biomass by BET Method", Unpublished.

Tsezos, M., "Biosorption of Uranium and Thorium", Ph.D. Thesis, McGill University, Montreal (1980).

Tsezos, M., "The Role of Chitin in Uranium Adsorption by *R. arrhizus*", Biotechnol. Bioeng., 25, 2025 (1983).

Tsezos, M., "Recovery of Uranium from Biological Adsorbents - Desorption Equilibrium", Biotechnol. Bioeng., 26, 973 (1984).

Tsezos, M. and Keller, D.M., "Adsorption of Radium-226 by Biological Origin Adsorbents", Biotechnol. Bioeng., 25, 201 (1983).

Tsezos, M. and Seto, W., "The Adsorption of Chloroethanes by Microbial Biomass", Water Res., 20, 851 (1986).

Tsezos, M. and Volesky, B., " Biosorption of Uranium and Thorium", Biotechnol. Bioeng., 23, 583 (1981).

Tsezos, M. and Volesky, B., "The Mechanism of Uranium Biosorption by *Rhizopus arrhizus*", Biotechnol. Bioeng., 24, 385 (1982a).

Tsezos, M. and Volesky, B., "The Mechanism of Thorium Biosorption by *Rhizopus arrhizus*", Biotechnol. Bioeng., 24, 955 (1982b).

Verschueren, K., *Handbook of Environmental Data on Organic Chemicals*, Van Nostrand Reinhold Co., New York, (1977).

Voerman, S. and Tammes, P.M.L., "Adsorption and Desorption of Lindane and Dieldrin by Yeast", Bull. Environm. Contam. Toxicol., 4, 271 (1969).

Voice, T.C. and Weber, W.J., "Sorption of Hydrocarbon Compounds by Sediments, Soils and Suspended Solids – I", Water Res., 17, 1433 (1983).

Weast, R.C. and Astle, M.T., (eds.), *CRC Handbook of Data on Organic Compounds*, CRC Press, Boca Raton (1985).

Weber, W.J., *Physicochemical Processes for Water Quality Control*, Wiley-Interscience, New York (1972).

Weber, W.J., Jones, B.E. and Katz, L.E., "Fate of Toxic Organic Compounds in Activated Sludge and Integrated PAC Systems", Water. Sci. Tech., 19, 471 (1987).

Wedemeyer, G., "Uptake of 2,4-Dichlorophenoxyacetic Acid by *Pseudomonas fluorescens*", Appl. Microbiol., 14, 486 (1966).

Weete, J.D., *Fungal Lipid Biochemistry*, Plenum Press, New York (1974).

Werner, D. and Morschel, E., "Preferential Elimination of Dieldrin by Some Diatoms Compared to *Chlamydomonas* and *Scenedesmus* Species", Bull. Environm. Contam. Toxicol., 20, 313 (1978).

Wohleber, D.A. and Manes, M., "Application of the Polanyi Adsorption Potential Theory to Adsorption from Solution on Activated Carbon. II. Adsorption of Partially Miscible Organic Liquids from Water Solution", J. Phys. Chem., 75, 61 (1971a).

Wohleber, D.A. and Manes, M., "Application of the Polanyi Adsorption Potential Theory to Adsorption from Solution on Activated Carbon. III. Adsorption of Miscible Organic Liquids from Water Solution", J. Phys. Chem., 75, 3720 (1971b).

Wright, E.H.M. and Powell, A.V., "Solid-Solution Interface Equilibria for Aromatic Molecules Adsorbed from Non-Aromatic Media", J. Chem. Soc. Faraday Trans., I68, 1908 (1972).

Wright, E.H.M. and Pratt, N.C., "Solid/Solution Interface Equilibria for Aromatic Molecules Adsorbed from Non-Aromatic Media", J. Chem. Soc. Faraday Trans., I70, 1461 (1974).

Zaroogian, G., Heltshe, J.F. and Johnson, M., "Estimation of Toxicity to Marine Species with Structure-Activity Models Developed to Estimate Toxicity to Freshwater Fish", Aquat. Toxicol., 6, 251 (1985).

APPENDIX A

ANALYTICAL PROCEDURES FOR ORGANIC COMPOUNDS

The following procedures were used for analysis of water solutions of organic chemicals:

1. A predetermined weight of the solution to be analyzed was added to a septum vial which was tared on a digital balance. Solution weight was recorded to the nearest microgram.

2. A predetermined volume of Fisher pesticide grade hexane or iso-octane was added to the vial with a volumetric pipette. The vial was sealed with a Teflon lined septum held by a crimped on aluminum cap.

3. The vial was placed on a wrist action shaker and agitated at maximum speed for 30 min. The vial was stored in an inverted position until ready for analysis. This was done to insure that the seal was covered by the water phase to prevent loss of the organic solvent and also to allow separation of any emulsified material.

4. Solvent solution standards for lindane, diazinon, and malathion were prepared by dissolving a known quantity of a prepared standrd into iso-octane and then diluting with iso-octane to a known volume in a volumetric flask. Prepared standards were purchased from Chromatographic Specialties Ltd. and were added with Hamilton gas-tight microliter syringes. Since prepared standard solutions for pentachlorophenol and 2-chlorobiphenyl could not be found, the standards were prepared by weighing a known quantity of the pure chemical on an analytical balance and adding it to iso-octane and diluting the solution to a known volume in a volumetric flask.

5. Water solution standards were prepared by dissolving quantities of the pure organic compounds in distilled water. The organic compounds were of 99+% purity and were purchased from Crescent Chemical Co. The concentrations of the primary water solution standards were determined by extracting the water solutions with iso-octane, as described above, with a high solvent to water ratio (e.g. 100:1). The extracts were then analyzed by gas chromatography, as described below, in comparison with the solvent standards. Additional standards were then prepared by making known dilutions of the primary standards.

6. Concentrations of organic compounds were determined by gas chromatographic analysis of the solvent extracts. Water solution standards covering the concentration range of the samples were analyzed along with the unknown samples. The standards were extracted in the same way as the unknowns so that it was not necessary to know the extraction efficiency of the samples. Concentration of each unknown was found by linear interpolation between adjacent lower and higher standards and was based on peak areas computed by a digital integrator.

7. The following are details of the gas chromatograph operation:

   a. Gas chromatograph: Hewlett-Packard Model 5830A

   b. Detector: Ni-63 electron capture detector

   c. Carrier gas: 95% argon, 5% methane

   d. Chromatographic columns: 0.61m long, 2.0mm I.D., glass column packed with CSP-633 for pentachlorophenol analysis: 0.61m long, 2.0mm I.D., glass column packed with 1.5% OV-17 and 1.95% OV-210 on Chromosorb W, HP 100/120 mesh for all other analyses; columns were purchased from Chromatographic Specialties Ltd.

e. Carrier gas flow rate: 60 ml/min. for CSP-633 column; 30 ml/min. for OV-17, OV-210 column

f. Detector temperature: 300°C

g. Injection port temperature: 225°C

h. Oven temperature: 175°C for CSP-633 column; 200°C for OV-17, OV-210 column except 160°C for 2-chlorobiphenyl analysis

i. Injection volume: 1-5 µL

APPENDIX B1

BIOSORPTION DATA AT 20°C

Table B1.1 Biosorption data for lindane on *R. arrhizus*

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
1807.0	0.171	2458.0	3807.0
1387.0	0.287	2458.0	3731.7
808.0	0.134	1002.0	1447.8
797.0	0.506	1973.0	2324.1
764.0	0.678	2458.0	2498.5
695.0	0.285	1002.0	1077.2
668.0	0.172	1000.0	1930.2
643.0	0.419	1002.0	856.8
537.0	0.521	1002.0	892.5
504.0	0.334	1000.0	1485.0
454.0	0.997	1973.0	1523.4
443.0	0.333	799.0	1069.4
442.0	0.659	1002.0	849.8
434.0	0.747	1002.0	760.4
417.0	0.501	1000.0	1163.7
387.0	0.867	1002.0	709.3
327.0	0.340	714.0	1138.2
314.0	1.943	1973.0	853.8
294.0	0.662	1000.0	1066.5
288.0	1.049	1002.0	680.6
258.0	0.665	714.0	685.7
253.0	0.134	332.0	589.6
235.0	1.191	1002.0	644.0
209.0	0.265	332.0	464.2

Table B1.1 Biosorption data for lindane on *R. arrhizus* (continued)

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
173.0	0.397	332.0	400.5
143.0	0.537	332.0	352.0
141.0	0.496	333.0	387.1
122.0	0.675	332.0	311.1
100.0	0.781	332.0	297.1
96.0	0.912	332.0	258.8
89.3	1.072	332.0	226.4
81.0	1.007	333.0	250.2
79.8	1.198	332.0	210.5
70.5	1.310	332.0	199.6
48.9	2.034	333.0	139.7
41.4	0.130	55.8	110.8
33.3	0.294	55.8	76.5
30.8	0.415	55.8	60.2
30.2	0.533	55.8	48.0
22.4	0.677	55.8	49.3
22.1	0.799	55.8	42.2
19.5	0.921	55.8	39.4
17.9	1.079	55.8	35.1
17.5	1.186	55.8	32.3
9.9	0.333	16.3	19.3
8.9	0.672	16.3	11.1
4.5	1.336	16.3	8.8
3.8	0.166	5.1	7.7
3.0	0.332	5.1	6.3
1.8	0.659	5.1	5.0
1.3	0.995	5.1	3.8
1.1	1.236	5.1	3.2

Table B1.2 Biosorption data for lindane on activated sludge

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2043.0	0.691	4010.0	2846.6
1963.0	0.168	2458.0	2946.4
1643.0	0.333	2458.0	2447.4
1382.0	1.336	4010.0	1967.1
1210.0	0.667	2458.0	1871.1
984.0	2.167	4010.0	1396.4
873.0	0.126	1002.0	1023.8
815.0	0.241	1002.0	775.9
794.0	0.177	1000.0	1163.8
726.0	0.364	1002.0	758.2
658.0	0.337	1000.0	1014.8
621.0	0.513	1002.0	742.7
555.0	0.504	1000.0	882.9
542.0	0.330	799.0	778.1
514.0	0.177	714.0	1129.9
488.0	0.641	1002.0	801.9
439.0	0.769	1000.0	729.5
430.0	0.788	1002.0	725.9
416.0	0.869	1002.0	674.3
410.0	0.335	714.0	907.5
371.0	0.989	1002.0	638.0
341.0	1.106	1002.0	597.6
312.0	0.672	714.0	598.2
279.0	0.139	332.0	381.3
247.0	1.338	752.0	377.4
209.0	0.401	332.0	306.7
189.0	0.539	332.0	265.3
181.0	2.007	752.0	284.5
167.0	0.690	332.0	239.1
157.0	0.681	332.0	257.0

Table B1.2 Biosorption data for lindane on activated sludge (continued)

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
153.0	0.808	332.0	221.5
139.0	0.941	332.0	205.1
126.0	0.998	332.0	206.4
119.0	1.185	332.0	179.7
46.1	0.142	55.8	68.3
39.7	0.263	55.8	61.2
33.3	0.391	55.8	57.5
30.8	0.530	55.8	47.2
27.7	0.658	55.8	42.7
24.0	0.776	55.8	41.0
22.7	0.924	55.8	35.8
19.6	1.066	55.8	34.0
18.1	1.202	55.8	31.4
17.4	1.330	55.8	28.9
10.3	0.335	16.3	17.9
9.2	0.673	16.3	10.5
7.4	1.320	16.3	6.7
3.6	0.336	5.1	4.4
2.3	0.645	5.1	4.3
1.9	0.983	5.1	3.2
1.6	1.641	5.1	2.1

Table B1.3 Biosorption data for pentachlorophenol on *R. arrhizus*

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
1509.0	0.117	4778.0	27940.2
1082.0	0.272	4778.0	13588.2
914.0	0.272	4778.0	14205.9
594.0	0.529	4778.0	7909.3
522.0	0.801	4778.0	5313.4
218.0	0.159	872.0	4113.2
174.0	0.259	872.0	2695.0
102.0	0.540	872.0	1425.9
67.8	0.792	872.0	1015.4
50.6	0.158	306.0	1616.6
45.8	0.276	306.0	942.9
34.7	0.276	306.0	983.1
31.4	0.537	306.0	511.4
20.5	0.171	91.7	416.6
14.6	0.801	306.0	364.0
13.4	0.268	91.7	292.2
10.3	0.103	28.6	177.0
9.3	0.539	91.7	152.9
7.6	0.830	91.7	101.4
3.3	0.061	7.4	67.1
3.3	0.255	28.6	99.1
2.3	0.150	7.4	33.8
2.2	0.538	28.6	49.0
2.0	0.779	28.6	34.1
1.6	0.256	7.4	22.5
1.3	0.538	28.6	50.4
0.4	0.397	7.4	17.7

Table B1.4 Biosorption data for pentachlorophenol on activated sludge

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2830.0	0.134	4778.0	14537.3
2463.0	0.269	4778.0	8605.9
2452.0	0.269	4778.0	8646.8
1851.0	0.543	4778.0	5390.4
1314.0	0.857	4778.0	4042.0
615.0	0.134	872.0	1917.9
443.0	0.301	872.0	1425.2
321.0	0.561	872.0	982.2
183.0	0.839	872.0	821.2
174.0	0.127	306.0	1039.4
115.0	0.268	306.0	712.7
115.0	0.268	306.0	712.7
82.0	0.541	306.0	414.0
56.8	0.133	91.7	262.8
53.2	0.796	306.0	317.8
44.0	0.265	91.7	180.1
21.7	0.534	91.7	131.1
17.5	0.132	28.6	84.1
16.3	0.805	91.7	93.7
12.9	0.267	28.6	58.9
11.6	0.267	28.6	63.5
6.7	0.528	28.6	41.3
5.6	0.809	28.6	28.3
4.6	0.173	7.4	16.3
3.5	0.336	7.4	11.6
1.4	0.724	7.4	8.2
0.3	1.347	7.4	5.3

Table B1.5 Biosorption data for diazinon on *R. arrhizus*

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
4621.0	0.070	4827.0	2926.1
4386.0	0.139	4827.0	3172.7
4303.0	0.278	4827.0	1884.9
3881.0	0.524	4827.0	1805.3
3398.0	1.058	4827.0	1350.7
1001.0	0.672	1275.0	407.7
946.0	0.672	1275.0	489.6
931.0	0.673	1275.0	511.1
920.0	0.673	1275.0	527.5
875.0	0.173	999.0	716.8
860.0	0.294	999.0	472.8
813.0	0.534	999.0	348.3
748.0	1.050	999.0	239.0
288.0	0.149	317.0	194.6
274.0	0.296	317.0	145.3
223.0	0.560	317.0	167.9
172.0	1.057	317.0	137.2
74.5	0.137	84.6	73.7
71.3	0.262	84.6	50.8
66.7	0.529	84.6	33.8
42.8	1.060	84.6	39.4
14.8	0.178	19.0	23.6
12.2	0.335	19.0	20.3
11.4	0.669	19.0	11.4

Table B1.6 Biosorption data for diazinon on activated sludge

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
4703.0	0.083	4827.0	1485.0
4601.0	0.128	4827.0	1765.6
4370.0	0.261	4827.0	1751.0
3998.0	0.538	4827.0	1540.9
3108.0	1.058	4827.0	1624.8
1003.0	0.668	1275.0	407.2
993.0	0.671	1275.0	420.3
955.0	0.668	1275.0	479.0
953.0	0.671	1275.0	479.9
913.0	0.155	999.0	554.8
857.0	0.287	999.0	494.8
772.0	0.538	999.0	421.9
612.0	1.072	999.0	361.0
284.0	0.245	317.0	134.7
250.0	0.533	317.0	125.7
201.0	1.177	317.0	98.6
78.9	0.133	84.6	42.9
76.2	0.255	84.6	32.9
66.3	0.527	84.6	34.7
53.9	1.146	84.6	26.8
16.6	0.334	19.0	7.2
16.1	0.690	19.0	4.2
14.4	0.689	19.0	6.7
13.0	1.344	19.0	4.5
6.6	0.172	7.0	2.2
6.0	0.333	7.0	3.0
5.7	0.677	7.0	1.9
4.4	1.360	7.0	1.9

Table B1.7 Biosorption data for 2-chlorobiphenyl on *R. arrhizus*

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
321.0	0.070	883.0	8040.1
98.0	0.171	883.0	4590.6
90.2	0.039	277.0	4839.4
80.3	0.067	277.0	2944.6
73.2	0.504	920.0	1679.8
63.5	0.160	356.0	1828.1
41.6	0.237	920.0	3712.6
40.5	0.341	883.0	2470.7
38.9	0.171	883.0	4936.3
29.0	1.001	920.0	890.5
25.5	0.290	356.0	1139.7
23.8	0.129	277.0	1962.8
21.9	0.341	883.0	2525.2
13.8	0.662	883.0	1313.0
13.8	0.579	356.0	591.0
13.3	0.579	356.0	591.9
13.1	0.210	277.0	1256.7
12.1	0.662	883.0	1315.6
8.5	0.421	277.0	637.7
6.5	0.698	277.0	387.6
3.6	1.099	356.0	320.6
2.9	0.329	74.0	216.1
2.2	0.570	74.0	126.0

Table B1.8 Biosorption data for 2-chlorobiphenyl on activated sludge

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
267.0	0.242	920.0	2698.3
168.0	0.180	356.0	1044.4
134.0	0.516	920.0	1523.3
119.0	0.263	356.0	901.1
65.6	0.527	356.0	551.0
59.9	1.000	920.0	860.1
59.3	0.527	356.0	563.1
37.5	0.145	74.0	251.2
34.7	1.067	356.0	301.1
26.0	0.268	74.0	178.9
12.5	0.531	74.0	115.8
3.2	1.066	74.0	66.4
2.2	1.319	10.6	6.4
1.2	0.726	10.6	12.9
0.8	0.399	10.6	24.6
0.6	0.853	10.6	11.8

Table B1.9 Apparent biosorption data for malathion on *R. arrhizus*

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2151.0	0.168	5150.0	17851.2
1898.0	0.334	5150.0	9736.5
1342.0	0.686	5150.0	5551.0
1241.0	0.686	5150.0	5698.3
703.0	1.393	5150.0	3192.4
274.0	0.167	456.0	1089.8
244.0	0.167	1040.0	4766.5
193.0	0.336	456.0	782.7
190.0	0.679	456.0	391.8
186.0	0.679	456.0	397.6
180.0	1.375	456.0	200.7
143.0	0.349	1040.0	2570.2
118.0	0.670	1040.0	1376.1
67.4	1.332	1040.0	730.2
54.3	0.168	95.8	247.0
52.6	0.337	95.8	128.2
44.3	1.328	145.0	75.8
43.3	0.172	145.0	591.3
40.0	0.669	95.8	83.4
37.1	0.662	145.0	163.0
31.2	0.662	145.0	171.9
29.9	0.331	145.0	347.7
25.6	1.385	95.8	50.7
4.2	0.164	13.1	54.3
4.2	0.329	13.1	27.1
3.3	0.687	13.1	14.3
3.2	0.692	13.1	14.3
3.1	1.347	13.1	7.4

Table B1.10 Apparent biosorption data for malathion on activated sludge

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
1912.0	0.138	4820.0	21072.5
1535.0	0.138	4820.0	23804.3
921.0	0.263	4820.0	14825.1
826.0	0.263	4820.0	15186.3
706.0	0.170	5150.0	26141.2
508.0	0.396	4820.0	10888.9
488.0	0.396	4820.0	10939.4
363.0	0.541	4820.0	8238.4
319.0	0.541	4820.0	8319.8
127.0	0.326	5150.0	15408.0
124.0	0.796	4820.0	5901.7
112.0	0.223	1040.0	4161.4
86.1	0.795	4820.0	5950.9
78.7	0.161	456.0	2343.6
56.3	0.383	1040.0	2568.3
12.5	0.330	456.0	1343.9
11.9	0.172	95.8	487.8
11.0	0.727	5150.0	7068.7
5.7	0.727	5150.0	7076.1
3.1	0.667	456.0	679.0
3.1	0.337	95.8	275.1
2.7	0.686	1040.0	1512.1
2.5	0.667	456.0	680.0
1.5	0.673	95.8	140.1
0.6	1.338	456.0	340.4

APPENDIX B2

BIOSORPTION DATA AT 34.5°C AND 5°C

Table B2.1 Biosorption data for lindane on *R.arrhizus* at 34.5°C

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3215.0	0.129	4208.0	7697.7
2025.0	0.396	4208.0	5512.6
2013.0	0.396	4208.0	5542.9
1551.0	0.663	4208.0	4008.8
729.3	0.133	900.2	1285.0
568.0	0.416	900.2	798.6
554.4	0.416	900.2	831.2
411.3	0.668	900.2	731.8
152.1	0.128	186.0	264.8
117.7	0.400	186.0	170.8
114.9	0.400	186.0	177.8
89.3	0.665	186.0	145.5
51.5	0.135	65.0	100.6
37.3	0.398	65.0	69.7
33.8	0.398	65.0	78.5
23.5	0.699	65.0	59.4

Table B2.2 Biosorption data for lindane on *R. arrhizus* at 5°C

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2749.0	0.128	4194.0	11289.1
1720.0	0.398	4194.0	6216.1
1691.0	0.398	4194.0	6288.9
1363.0	0.667	4194.0	4245.0
618.9	0.129	959.2	2638.0
393.1	0.398	959.2	1422.4
392.9	0.398	959.2	1422.9
306.5	0.671	959.2	973.3
202.4	0.128	304.6	798.4
122.2	0.395	304.6	461.8
115.0	0.395	304.6	480.0
95.3	0.664	304.6	315.2
46.0	0.131	76.2	230.5
32.8	0.418	76.2	103.8
31.8	0.418	76.2	106.1
25.1	0.674	76.2	75.8

Table B2.3 Biosorption data for lindane on activated sludge at 34.5°C

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3572.0	0.129	4208.0	4930.2
2420.0	0.460	4208.0	3887.0
2355.0	0.460	4208.0	4028.3
2022.0	0.795	4208.0	2748.3
797.4	0.127	900.2	809.4
614.6	0.460	900.2	620.9
614.6	0.460	900.2	620.9
499.9	0.796	900.2	502.9
165.5	0.133	186.0	154.1
131.6	0.469	186.0	116.0
130.8	0.469	186.0	117.7
102.6	0.801	186.0	104.1
57.3	0.129	65.0	60.3
43.0	0.461	65.0	47.8
42.7	0.461	65.0	48.4
34.3	0.793	65.0	38.8

Table B2.4 Biosorption data for lindane on activated sludge at 5°C

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3418.0	0.125	4194.0	6208.0
2437.0	0.410	4194.0	4285.4
2350.0	0.410	4194.0	4497.6
1831.0	0.695	4194.0	3402.0
769.1	0.129	959.2	1473.6
551.0	0.400	959.2	1020.5
541.7	0.400	959.2	1043.8
426.1	0.663	959.2	804.1
249.8	0.143	304.6	383.2
182.2	0.410	304.6	298.5
180.8	0.410	304.6	302.0
146.7	0.673	304.6	234.8
59.0	0.134	76.2	128.0
54.0	0.389	76.2	57.0
52.8	0.389	76.2	60.0
26.3	0.798	76.2	62.5

Table B2.5 Biosorption data for diazinon on *R. arrhizus* at 5°C

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3387.0	0.285	3897.0	1791.4
2825.0	0.572	3897.0	1874.8
2230.0	1.144	3897.0	1457.2
566.3	0.294	624.4	198.0
526.8	0.572	624.4	170.8
426.2	1.093	624.4	181.3

Table B2.6 Biosorption data for diazinon on activated sludge at 5°C

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3339.0	0.285	3897.0	1958.6
3081.0	0.579	3897.0	1408.6
2576.0	1.113	3897.0	1186.9
555.8	0.307	624.4	223.3
508.6	0.590	624.4	196.4
446.5	1.161	624.4	153.2

Table B2.7 Apparent biosorption data for malathion on *R. arrhizus* at 5°C

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3181.0	0.284	3405.0	788.7
3086.0	0.483	3405.0	661.0
2728.0	1.146	3405.0	590.8
702.3	0.287	746.0	152.5
702.2	0.565	746.0	77.5
628.2	1.122	746.0	105.0

Table B2.8 Apparent biosorption data for malathion on activated sludge at 5°C

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3175.0	0.289	3405.0	797.2
3011.0	0.607	3405.0	648.8
2854.0	1.138	3405.0	484.2
704.6	0.299	746.0	138.6
658.0	0.581	746.0	151.4
588.3	1.148	746.0	137.4

APPENDIX B3

DESORPTION DATA

Table B3.1 Desorption data for lindane on *R. arrhizus*

C_{eq} (µg/L)	q (µg/g)
548.7	1254.0
455.4	1036.0
329.0	682.2
95.9	197.3
74.0	171.6
52.2	111.5

Table B3.2 Desorption data for lindane on activated sludge

C_{eq} (µg/L)	q (µg/g)
1100.0	1543.0
1014.0	1295.0
780.7	1090.0
172.5	266.1
45.0	221.5
86.3	150.7

Table B3.3 Desorption data for diazinon on *R. arrhizus* at 5°C

C_{eq} (μg/L)	q (μg/g)
1334.0	705.3
1246.0	784.7
1150.0	348.7
246.7	51.3
219.2	−11.0
190.6	−25.4

Table B3.4 Desorption data for diazinon on activated sludge at 5°C

C_{eq} (μg/L)	q (μg/g)
1265.0	595.1
1238.0	517.9
1037.0	948.9
216.4	60.9
201.1	53.0
196.6	8.2

Table B3.5 Apparent desorption data for malathion on *R. arrhizus*

C_{eq} (µg/L)	q (µg/g)
248.9	5433.0
102.0	3065.0
34.8	1923.0

Table B3.6 Apparent desorption data for malathion on activated sludge

C_{eq} (µg/L)	q (µg/g)
526.0	4007.0
236.1	3353.0
0.0	2405.0

Table B3.7 Apparent desorption data for malathion on *R. arrhizus* at 5°C

C_{eq} (µg/L)	q (µg/g)
1077.0	187.9
956.5	164.6
873.4	304.3
239.8	25.3
222.7	−20.8
205.7	−6.5

Table B3.8 Apparent desorption data for malathion on activated sludge at 5°C

C_{eq} (µg/L)	q (µg/g)
1013.0	172.2
879.7	185.8
207.8	58.2
206.1	77.0
194.5	31.8

Table B3.9 Desorption data for 2-chlorobiphenyl on *R. arrhizus*

C_{eq} (µg/L)	q (µg/g)
121.0	3073.0
63.5	1543.0
7.3	883.3

Table B3.10 Desorption data for 2-chlorobiphenyl on activated sludge

C_{eq} (µg/L)	q (µg/g)
233.1	1732.0
162.0	1208.0
98.0	764.3

APPENDIX B4

BIOSORPTION DATA FOR CELL WALLS

Table B4.1 Biosorption data for lindane on *R. arrhizus* cell walls

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3622.0	0.152	4195.0	3762.3
3260.0	0.316	4195.0	2956.1
2629.0	0.731	4195.0	2143.4

Table B4.2 Biosorption data for lindane on activated sludge cell walls

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3595.0	0.156	4195.0	3838.8
3075.0	0.324	4195.0	3456.8
2439.0	0.674	4195.0	2605.0

Table B4.3 Biosorption data for diazinon on *R. arrhizus* cell walls

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
4909.0	0.178	5114.0	1151.0
4818.0	0.368	5114.0	804.1
4570.0	0.672	5114.0	810.0

Table B4.4 Biosorption data for diazinon on activated sludge cell walls

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
4838.0	0.162	5114.0	1698.5
4545.0	0.330	5114.0	1724.2
3954.0	0.670	5114.0	1730.1

Table B4.5 Apparent biosorption data for malathion on *R. arrhizus* cell walls

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
1564.0	0.662	4407.0	4295.2
1360.0	0.333	6714.0	16054.0
1145.0	0.655	6714.0	8495.8
1048.0	0.176	6714.0	32138.4
296.0	0.333	4407.0	12363.9
270.0	0.162	4407.0	25505.5

Table B4.6 Apparent biosorption data for malathion on activated sludge cell walls

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
4069.0	0.171	4407.0	1976.6
3776.0	0.327	4407.0	1931.4
3377.0	0.892	4407.0	1155.1

APPENDIX B5

KINETIC DATA

Table B5.1 Kinetic data for lindane biosorption on *R. arrhizus* at 510 rpm

Time (min)	C (µg/L)	q (µg/g)*
0.0	1276.0	0.0
1.9	911.3	692.0
3.0	820.3	862.8
4.0	773.1	950.7
5.0	743.1	1005.9
7.5	715.5	1056.3
10.0	691.5	1099.8
15.0	672.1	1134.8
20.0	649.6	1175.2
30.0	622.0	1224.3
40.0	626.4	1216.5
50.0	617.7	1231.8
60.0	601.4	1260.2
80.0	604.0	1255.7
100.0	608.4	1248.1
120.0	609.9	1245.5
150.0	607.1	1250.3
181.7	610.0	1245.4
240.0	604.0	1255.5

* corrected to account for sample removal

Table B5.2 Kinetic data for lindane biosorption on *R. arrhizus* at 700 rpm.

Time (min)	C (µg/L)	q (µg/g)*
0.0	1187.0	0.0
2.5	876.6	567.7
5.0	706.4	877.7
7.0	655.1	970.7
12.5	619.6	1034.7
22.0	573.2	1118.0
32.0	551.3	1157.1
47.0	550.4	1158.7
62.0	552.2	1155.5
82.0	552.7	1154.6

* corrected to account for sample removal

Table B5.3 Kinetic data for lindane biosorption on activated sludge at 510 rpm

Time (min)	C (µg/L)	q (µg/g)*
0.0	975.1	0.0
3.0	970.6	6.5
4.1	937.4	56.1
5.0	917.9	85.1
7.5	885.3	133.4
10.0	859.7	171.1
15.0	824.9	222.1
20.0	795.3	265.2
30.0	755.4	323.1
45.0	717.0	378.6
60.0	698.2	405.6
90.0	675.7	437.8
120.0	657.8	463.3
180.0	640.8	487.4
240.0	638.5	490.6
360.0	618.4	518.8
450.0	618.2	519.1
680.0	605.8	536.3
1325.8	524.0	649.8

* corrected to account for sample removal

Table B5.4 Kinetic data for lindane biosorption on activated sludge at 680 rpm

Time (min)	C (µg/L)	q (µg/g)*
0.0	928.0	0.0
5.0	842.8	129.2
10.0	797.5	197.3
15.0	752.6	264.4
20.0	716.5	318.2
30.0	697.9	345.8
50.0	662.9	397.5
85.0	626.6	450.9
120.0	594.3	498.2
180.0	602.1	486.9
240.0	574.3	527.1

* corrected to account for sample removal

Table B5.5 Kinetic data for diazinon biosorption on *R. arrhizus*

Biomass concentration = 0.527 g/L

Time (d)	C (µg/L)	q (µg/g)*
0.00	6046.0	0.0
0.04	4678.0	2595.8
0.24	4464.0	3001.9
0.99	4243.0	3421.3
2.99	4137.0	3622.4
7.02	4443.0	3041.7
10.02	4604.0	2736.2

Table B5.6 Kinetic data for diazinon biosorption on activated sludge

Biomass concentration = 0.539 g/L

Time (d)	C (μg/L)	q (μg/g)*
0.00	6046.0	0.0
0.04	5414.0	1172.5
0.25	5811.0	436.0
0.99	5188.0	1591.8
3.00	5276.0	1428.6
6.98	5099.0	1757.0
9.98	5223.0	1526.9

Table B5.7 Kinetic data for malathion removal by *R. arrhizus*

Biomass concentration = 0.850 g/L

Time (d)	C (μg/L)
0.00	4357.0
2.73	2494.0
5.81	306.6

Table B5.8 Kinetic data for malathion removal by *R. arrhizus*

Biomass concentration = 0.418 g/L

Time (d)	C (μg/L)
0.00	4357.0
2.73	3246.0
5.81	594.8
10.81	12.0

Table B5.9 Kinetic data for malathion removal by *R. arrhizus* at 5°C

Biomass concentration = 0.578g/L

Time (d)	C (µg/L)
0.00	14831.0
1.06	13124.0
3.00	13001.0
4.98	12477.0
8.13	12209.0
17.00	11501.0

Table B5.10 Kinetic data for malathion removal by activated sludge

Biomass concentration = 0.839 g/L

Time (d)	C (µg/L)
0.00	4357.0
2.73	2448.0
5.81	133.8

Table B5.11 Kinetic data for malathion removal by activated sludge

Biomass concentration = 0.413g/L

Time (d)	C (µg/L)
0.00	4357.0
2.73	3032.0
5.81	863.4
10.81	35.0

Table B5.12 Kinetic data for malathion removal by activated sludge at 5°C

Biomass concentration = 0.585 g/L

Time (d)	C (µg/L)
0.00	14831.0
1.06	12376.0
3.00	12091.0
4.99	11334.0
8.13	13620.0
17.01	10734.0

Table B5.13 Kinetic data for malathion removal with no biomass

Biomass concentration = 0.000g/L

Time (d)	C (µg/L)
0.00	4357.0
2.73	4285.0
5.81	4250.0
10.81	4114.0

Table B5.14 Kinetic data for malathion removal by live *R. arrhizus* at 20°C

Biomass concentration = 2.155g/L

Time (h)	C (µg/L)
0.00	1886.0
1.83	1507.0
3.88	1442.0
6.77	1419.0
21.92	1135.0
29.52	817.3
47.83	123.3

APPENDIX B6

COMPETITIVE BIOSORPTION DATA

Table B6.1 Biosorption data for lindane on *R. arrhizus* in competition with pentachlorophenol

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2203.0	0.040	2421.0	5463.7
1947.0	0.065	2248.0	4616.6
1900.0	0.099	2421.0	5252.0
1799.0	0.135	2421.0	4607.4
1753.0	0.135	2421.0	4948.1
1728.0	0.171	2421.0	4052.6
1455.0	0.260	2421.0	3715.4
1177.0	0.231	2248.0	4636.4
1130.0	0.410	2248.0	2726.8
1071.0	0.524	2421.0	2576.3
1052.0	0.410	2248.0	2917.1
831.4	0.807	2248.0	1755.0
690.7	1.088	2421.0	1590.3
167.6	0.066	188.4	315.6
145.7	0.197	188.4	216.8
109.9	0.421	188.4	186.5
35.2	0.806	188.4	190.1

Table B6.2 Biosorption data for lindane on activated sludge in competition with pentachlorophenol

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2311.0	0.032	2421.0	3481.0
2228.0	0.067	2421.0	2893.6
2060.0	0.134	2421.0	2894.0
2033.0	0.134	2421.0	2895.5
1962.0	0.090	2248.0	3184.9
1898.0	0.204	2421.0	2563.7
1511.0	0.400	2421.0	2275.0
1424.0	0.278	2248.0	2964.0
1414.0	0.278	2248.0	3000.0
1234.0	0.532	2248.0	1906.0
1162.0	0.799	2421.0	1576.3
924.2	1.071	2248.0	1236.0
805.2	1.332	2421.0	1213.1
177.7	0.096	188.4	111.8
155.4	0.282	188.4	117.0
117.6	0.532	188.4	133.1
31.6	1.064	188.4	147.4

Table B6.3 Biosorption data for pentachlorophenol on *R. arrhizus* in competition with lindane

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
953.5	0.040	1636.0	17105.3
899.5	0.065	1729.0	12722.4
653.4	0.099	1636.0	9905.2
487.8	0.135	1636.0	8505.2
461.8	0.171	1636.0	6866.7
388.1	0.135	1636.0	9243.7
364.3	0.231	1729.0	5907.8
312.9	0.260	1636.0	5088.8
293.4	0.410	1729.0	3501.5
221.6	0.410	1729.0	3676.6
177.7	0.524	1636.0	2783.0
156.7	1.088	1636.0	1359.7
122.7	0.807	1729.0	1990.0
71.7	0.066	158.7	1319.7
42.1	0.197	158.7	591.9
14.7	0.421	158.7	342.0
1.7	0.806	158.7	194.8

Table B6.4 Biosorption data for pentachlorophenol on activated sludge in competition with lindane

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
1455.0	0.032	1636.0	5727.8
1315.0	0.067	1636.0	4812.6
1241.0	0.090	1729.0	5434.3
1116.0	0.134	1636.0	3880.6
1103.0	0.134	1636.0	3977.6
956.4	0.204	1636.0	3331.4
916.2	0.278	1729.0	2923.7
837.8	0.278	1729.0	3205.8
791.9	0.400	1636.0	2110.3
711.2	0.532	1729.0	1913.2
448.7	0.799	1636.0	1486.5
340.3	1.071	1729.0	1296.6
284.1	1.332	1636.0	1014.9
97.8	0.096	158.7	636.3
78.8	0.282	158.7	283.4
53.7	0.532	158.7	197.4
4.6	1.064	158.7	144.8

Table B6.5 Biosorption data for lindane on *R. arrhizus* in competition with diazinon

Ceq (µg/L)	B (g/L)	Co (µg/L)	q (µg/g)
1491.0	0.124	1884.0	3169.4
1058.0	0.271	1884.0	3048.0
863.1	0.415	1884.0	2460.0
835.0	0.415	1884.0	2527.7
800.1	0.566	1884.0	1915.0
725.3	0.677	1884.0	1712.8
629.4	0.823	1884.0	1524.4
516.0	1.097	1884.0	1247.0
414.3	1.483	1884.0	991.0
347.8	0.060	404.8	951.6
297.3	0.150	404.8	716.7
237.0	0.267	404.8	628.5
178.8	0.548	404.8	412.4
176.0	0.548	404.8	417.5
159.0	0.805	404.8	305.5
112.7	1.067	404.8	273.8
101.3	1.465	404.8	207.2

Table B6.6 Biosorption data for lindane on activated sludge in competition with diazinon

Ceq (µg/L)	B (g/L)	Co (µg/L)	q (µg/g)
1729.0	0.098	1884.0	1584.9
1438.0	0.265	1884.0	1683.0
1281.0	0.401	1884.0	1503.7
1277.0	0.401	1884.0	1513.7
1161.0	0.544	1884.0	1329.0
1055.0	0.648	1884.0	1279.3
945.4	0.807	1884.0	1163.1
744.0	1.162	1884.0	981.1
645.7	1.473	1884.0	840.7
381.2	0.058	404.8	404.1
348.3	0.130	404.8	434.6
304.3	0.265	404.8	379.2
238.2	0.524	404.8	317.9
236.2	0.524	404.8	321.8
187.6	0.810	404.8	268.1
149.7	1.069	404.8	238.6
120.9	1.471	404.8	193.0

Table B6.7 Biosorption data for diazinon on *R. arrhizus* in competition with lindane

Ceq (µg/L)	B (g/L)	Co (µg/L)	q (µg/g)
1716.0	0.124	1878.0	1306.5
1499.0	0.415	1878.0	913.3
1490.0	0.271	1878.0	1431.7
1441.0	0.415	1878.0	1053.0
1422.0	0.566	1878.0	805.7
1353.0	0.677	1878.0	776.1
1189.0	0.823	1878.0	837.2
1140.0	1.097	1878.0	672.7
995.5	1.483	1878.0	595.1
370.0	0.060	395.0	417.4
347.1	0.805	395.0	59.5
341.3	0.150	395.0	358.0
315.1	0.267	395.0	299.3
275.9	0.548	395.0	217.3
271.9	0.548	395.0	224.6
235.6	1.067	395.0	149.4
191.4	1.465	395.0	139.0

Table B6.8 Biosorption data for diazinon on activated sludge in competition with lindane

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
1822.0	0.098	1878.0	572.6
1719.0	0.265	1878.0	600.0
1680.0	0.401	1878.0	493.8
1665.0	0.401	1878.0	531.2
1636.0	0.544	1878.0	444.9
1560.0	0.648	1878.0	490.7
1536.0	0.807	1878.0	423.8
1353.0	1.162	1878.0	451.8
1243.0	1.473	1878.0	431.1
385.8	0.058	395.0	157.5
362.3	0.130	395.0	251.5
336.1	0.265	395.0	222.3
308.6	0.524	395.0	164.9
302.1	0.524	395.0	177.3
256.2	0.810	395.0	171.4
254.8	1.069	395.0	131.2
214.5	1.471	395.0	122.7

Table B6.9 Biosorption data for diazinon on *R. arrhizus* in competition with malathion

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2126.0	0.060	2156.0	495.9
1921.0	0.169	2156.0	1390.5
1801.0	0.289	2156.0	1228.4
1672.0	0.453	2156.0	1068.4
1632.0	0.453	2156.0	1156.7
1597.0	0.599	2156.0	933.2
1516.0	0.821	2156.0	779.5
1385.0	1.097	2156.0	702.8
1183.0	1.565	2156.0	621.7
466.4	0.068	473.3	101.8
432.7	0.135	473.3	300.7
393.8	0.263	473.3	302.3
368.3	0.405	473.3	259.3
340.6	0.536	473.3	247.6
337.5	0.405	473.3	335.3
303.6	0.794	473.3	213.7
260.4	1.108	473.3	192.1
232.2	1.343	473.3	179.5

Table B6.10 Biosorption data for diazinon on activated sludge in competition with malathion

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2174.0	0.131	2156.0	−137.4
2133.0	0.039	2156.0	594.3
2133.0	0.068	2156.0	339.2
2022.0	0.271	2156.0	494.5
1854.0	0.398	2156.0	758.8
1852.0	0.398	2156.0	763.8
1725.0	0.524	2156.0	822.5
1535.0	0.698	2156.0	890.1
1496.0	0.798	2156.0	826.9
462.6	0.067	473.3	160.9
417.9	0.150	473.3	369.3
409.5	0.276	473.3	231.2
368.1	0.402	473.3	261.7
352.6	0.402	473.3	300.2
333.4	0.535	473.3	261.5
305.1	0.798	473.3	210.7
263.5	1.066	473.3	196.8
257.1	1.316	473.3	164.3

Table B6.11 Apparent biosorption data for malathion on *R. arrhizus* in competition with diazinon

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2107.0	0.060	2959.0	14082.6
1615.0	0.169	2959.0	7952.7
1410.0	0.289	2959.0	5359.9
1292.0	0.599	2959.0	2783.0
1267.0	0.453	2959.0	3735.1
1261.0	0.821	2959.0	2068.2
1238.0	0.453	2959.0	3799.1
1204.0	1.097	2959.0	1599.8
769.3	1.565	2959.0	1399.2
475.5	0.068	600.3	1840.7
406.2	0.135	600.3	1437.8
332.3	0.263	600.3	1019.0
298.8	0.405	600.3	744.4
297.1	0.405	600.3	748.6
273.7	0.536	600.3	609.3
246.8	0.794	600.3	445.1
208.1	1.108	600.3	354.0
61.3	1.343	600.3	401.3

Table B6.12 Apparent biosorption data for malathion on activated sludge in competition with diazinon

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2752.0	0.039	2959.0	5348.8
2348.0	0.068	2959.0	9011.8
1695.0	0.131	2959.0	9648.9
956.4	0.271	2959.0	7389.7
661.0	0.398	2959.0	5773.9
646.2	0.398	2959.0	5811.1
612.5	0.524	2959.0	4478.1
508.6	0.698	2959.0	3512.1
444.8	0.798	2959.0	3149.8
561.8	0.067	600.3	578.9
432.7	0.150	600.3	1117.3
268.7	0.276	600.3	1201.4
202.5	0.402	600.3	989.6
198.1	0.402	600.3	1000.5
132.1	0.535	600.3	875.1
82.1	0.798	600.3	649.2
57.8	1.066	600.3	508.9
57.0	1.316	600.3	412.8

Table B6.13 Biosorption data for lindane on *R. arrhizus* in competition with diazinon, malathion, and pentachlorophenol

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
856.8	0.133	1166.0	2324.8
729.5	0.263	1166.0	1659.7
654.1	0.396	1166.0	1292.7
634.6	0.533	1166.0	997.0
625.2	0.533	1166.0	1014.6
579.6	0.796	1166.0	736.7
574.7	0.659	1166.0	897.3
436.1	1.069	1166.0	682.8
323.9	1.338	1166.0	629.4

Table B6.14 Biosorption data for lindane on activated sludge in competition with diazinon, malathion, and pentachlorophenol

Ce_q (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
986.2	0.131	1166.0	1372.5
841.0	0.268	1166.0	1212.7
747.0	0.400	1166.0	1047.5
694.7	0.530	1166.0	889.2
665.9	0.530	1166.0	943.6
618.8	0.667	1166.0	820.4
549.8	0.796	1166.0	774.1
453.4	1.065	1166.0	669.1
384.8	1.366	1166.0	571.9

Table B6.15 Biosorption data for pentachlorophenol on *R. arrhizus* in competition with diazinon, lindane, and malathion

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
425.6	0.133	1539.0	8371.4
208.3	0.263	1539.0	5059.7
164.4	0.396	1539.0	3471.2
139.2	0.533	1539.0	2626.3
89.4	0.533	1539.0	2719.7
75.2	0.659	1539.0	2221.3
70.9	0.796	1539.0	1844.3
51.6	1.069	1539.0	1391.4
36.4	1.338	1539.0	1123.0

Table B6.16 Biosorption data for pentachlorophenol on activated sludge in competition with diazinon, lindane, and malathion

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
759.8	0.268	1539.0	2907.5
735.6	0.131	1539.0	6132.8
511.6	0.400	1539.0	2568.5
484.6	0.530	1539.0	1989.4
443.5	0.530	1539.0	2067.0
367.7	0.667	1539.0	1756.1
239.8	0.796	1539.0	1632.2
230.6	1.065	1539.0	1228.5
107.9	1.366	1539.0	1047.7

Table B6.17 Biosorption data for diazinon on *R. arrhizus* in competition with lindane, malathion, and pentachlorophenol

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
742.7	0.133	794.0	385.7
625.3	0.263	794.0	641.4
620.8	0.396	794.0	437.4
685.4	0.533	794.0	203.8
649.7	0.533	794.0	270.7
629.3	0.659	794.0	249.9
658.5	0.796	794.0	170.2
545.9	1.069	794.0	232.1
377.6	1.338	794.0	311.2

Table B6.18 Biosorption data for diazinon on activated sludge in competition with lindane, malathion, and pentachlorophenol

C_{eq} (μg/L)	B (g/L)	C_o (μg/L)	q (μg/g)
750.9	0.131	794.0	329.0
711.2	0.268	794.0	309.0
690.3	0.530	794.4	196.4
664.4	0.400	794.0	324.0
662.4	0.530	794.0	248.3
636.1	0.667	794.0	236.7
609.4	0.796	794.0	231.9
565.9	1.065	794.0	214.2
465.3	1.366	794.0	240.6

APPENDIX B7

BIOSORPTION DATA FOR LIVE BIOMASS

Table B7.1 Biosorption data for lindane on live *R. arrhizus*, 5°C.

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
574.9	0.455	885.7	683.1
297.8	1.784	885.7	329.5 *
257.6	1.784	885.7	352.1
152.0	4.315	885.7	170.0
75.4	8.699	885.7	93.2

* 3 day contact time

Table B7.2 Biosorption data for lindane on live activated sludge, 5°C

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
687.2	0.117	822.4	1155.6
488.2	0.505	822.4	661.8
371.1	1.078	822.4	418.6 *
366.1	1.012	822.4	450.9
237.9	2.044	822.4	286.0

* 3 day contact time

Table B7.3 Biosorption data for lindane on live *R. arrhizus*

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
609.6	0.432	835.1	522.0
326.9	1.899	835.1	267.6 *
292.3	1.897	835.1	286.1
147.8	4.828	835.1	142.4
82.6	9.188	835.1	81.9

* 3 day contact time

Table B7.4 Biosorption data for lindane on live activated sludge

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
748.7	0.101	792.9	437.6
548.5	0.491	792.9	497.8
500.3	1.072	792.9	272.9 *
438.4	1.111	792.9	319.1
323.1	1.973	792.9	238.1

* 3 day contact time

Table B7.5 Biosorption data for pentachlorophenol on live *R. arrhizus*

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
1230.0	0.355	1787.0	1569.0
526.2	1.112	1787.0	1133.8
486.1	4.308	1787.0	302.0
245.6	2.179	1787.0	707.4
155.6	1.116	1787.0	1461.8 *

* 3 day contact time

Table B7.6 Biosorption data for pentachlorophenol on live activated sludge

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
935.6	0.121	1700.0	6317.4
299.8	0.530	1700.0	2641.9
158.5	1.054	1700.0	1462.5
115.4	1.045	1700.0	1516.4 *
38.1	2.095	1700.0	793.3

* 3 day contact time

Table B7.7 Biosorption data for diazinon on live *R. arrhizus*

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
1553.0	0.600	1920.0	611.7
1211.0	2.151	1920.0	329.6 *
827.8	2.153	1920.0	507.3
490.0	4.406	1920.0	324.6
264.4	10.839	1920.0	152.7

* 3 day contact time

Table B7.8 Biosorption data for diazinon on live activated sludge

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
2262.0	0.120	2414.0	1266.7
1947.0	0.636	2414.0	734.3
1623.0	1.510	2414.0	523.8 *
1569.0	1.534	2414.0	550.8
1288.0	2.729	2414.0	412.6

* 3 day contact time

Table B7.9 Biosorption data for 2-chlorobiphenyl on live *R. arrhizus*

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
3.1	0.226	842.0	3712.1
3.1	0.430	842.0	1951.0 *
1.8	0.430	842.0	1953.9
1.4	0.860	842.0	977.5
0.6	1.720	842.0	489.2

* 3 day contact time

Table B7.10 Biosorption data for 2-chlorobiphenyl on live activated sludge

C_{eq} (µg/L)	B (g/L)	C_o (µg/L)	q (µg/g)
134.3	0.123	1090.0	7769.9
96.3	0.977	1090.0	1016.9 *
32.2	0.574	1090.0	1842.9
6.0	0.987	1090.0	1098.9
3.6	1.979	1090.0	549.0

* 3 day contact time

APPENDIX B8

DESORPTION DATA FOR LIVE BIOMASS

Table B8.1 Desorption data for lindane on live *R. arrhizus*

C_{eq} (µg/L)	q (µg/g)
248.1	182.0 *
221.3	193.8
207.1	118.4
156.6	116.0
77.4	75.4

* 3 day contact time

Table BS.2 Desorption data for lindane on live activated sludge

C_{eq} (µg/L)	q (µg/g)
216.5	158.7
203.9	140.4 *
191.8	208.0
190.7	171.5
151.6	75.5

* 3 day contact time

Table B8.3 Desorption data for lindane on live *R. arrhizus* at 5°C

C_{eq} (µg/L)	q (µg/g)
192.7	241.4 *
161.3	269.0
150.7	374.6
105.8	148.1
68.0	87.4

* 3 day contact time

Table B8.4 Desorption data for lindane on live activated sludge at 5°C

C_{eq} (µg/L)	q (µg/g)
197.2	303.3
189.0	409.3
185.6	274.9 *
167.6	217.3
115.3	657.8

* 3 day contact time

Table B8.5 Desorption data for pentachlorophenol on live *R. arrhizus*

C_{eq} (µg/L)	q (µg/g)
108.4	1413.0
57.9	1131.0
39.3	712.1
25.3	1453.0 *
14.6	327.0

* 3 day contact time

Table B8.6 Desorption data for pentachlorophenol on live activated sludge

C_{eq} (µg/L)	q (µg/g)
254.5	5016.0
164.5	2408.0
147.9	1330.0
64.3	1464.0 *
35.5	779.0

* 3 day contact time

Table B8.7 Desorption data for diazinon on live *R. arrhizus*

C_{eq} (µg/L)	q (µg/g)
598.2	210.4 *
559.3	302.5
389.9	73.4
350.0	275.3
164.8	146.2

* 3 day contact time

Table B8.8 Desorption data for diazinon on live activated sludge

C_{eq} (µg/L)	q (µg/g)
675.8	264.2
633.7	283.2
485.9	360.6 *
428.7	338.2
243.8	672.2

3 day contact time

Table B8.9 Desorption data for 2-chlorobiphenyl on live *R. arrhizus*

C_{eq} (µg/L)	q (µg/g)
3.1	3698.0
2.5	1945.0 *
2.5	974.8
1.5	1951.0
1.1	488.6

* 3 day contact time

Table B8.10 Desorption data for 2-chlorobiphenyl on live activated sludge

C_{eq} (µg/L)	q (µg/g)
215.1	5312.0
143.0	1589.0
97.5	998.8
63.0	961.5 *
21.2	538.5

* 3 day contact time

APPENDIX B9

DATA FOR CARBON – 14 MALATHION EXPERIMENT

Table B9.1 Analytical data for C – 14 malathion solutions at end of contact period

Biomass	Biomass Conc. (g/L)	Malathion (µg/L)	C – 14 (dpm)	% of Initial C – 14 Remaining in Solution
R. arrhizus	0.953	0.0	32585	100
R. arrhizus	0.953	0.0	31699	98
Act. Sludge	0.976	31.41	28356	87
Act. Sludge	0.976	22.76	27882	86
R. arrhizus cell walls	0.361	34.16	26098	80
R. arrhizus cell walls	0.361	72.40	23677	73
Act. sludge cell walls	0.645	11.66	29752	92
Act. sludge cell walls	0.645	28.48	28228	87
None	None	441.10	32469	*
R. arrhizus	0.953	–	99	**
Act. sludge	0.958	–	51	**
R. arrhizus cell walls	0.361	–	46	**
Act. sludge cell walls	0.645	–	51	**
None	None	–	75	**

* stock solution (initial solution concentrations)

** contacted with distilled water

Table B9.2 Analytical data for biomass and filters

Biomass	C–14 (dpm)	% of Initial C–14 Found in Biomass	% C–14 Recovered (Mass Balance)
R. arrhizus	141013	1	101
R. arrhizus	163466	2	100
Act. sludge	593237	7	94
Act. sludge	719817	8	94
R. arrhizus cell walls	862109	10	90
R. arrhizus cell walls	205467	2	75
Act. sludge cell walls	303359	3	95
Act. sludge cell walls	492878	6	93

Table B9.3 Analytical data for C – 14 spiked filtrate samples

Sample	C – 14 without spike	C – 14 with spike	Difference	% Quench
Dist. water	75	36806	36731	–
R. arrhizus	32530	69102	36572	0.4
Act. sludge	13330	50819	37489	–2.1
R. arrhizus cell walls	25832	62884	37052	–0.9
Act. sludge cell walls	29667	65381	35714	2.8
Filtered stock soln.	20492	57552	37060	–0.9
Stock soln.	30873	68754	37881	–3.1
R. arrhizus	99	36686	36587	0.4 *
Act. sludge	51	36935	36884	–0.4 *
R. arrhizus cell walls	46	36775	36729	0.0 *
Act. sludge cell walls	51	36866	36815	–0.2 *

* filtrate samples from biomass contacted with distilled water.

Table B9.4 Analytical data for C – 14 spiked filtrate samples

Sample	C – 14 without spike	C – 14 with spike	Difference	% Quench
Dist. water	75	74591	74516	–
R. arrhizus	31577	104130	72553	2.7
Act. sludge	28116	101401	73285	1.8
R. arrhizus cell walls	23924	97278	73354	1.7
Act. sludge cell walls	27764	101866	74102	0.7
Filtered stock soln.	21043	94688	73645	1.3

Table B9.5 Analytical data for spiked filters and biomass

Sample	C – 14 without spike	C – 14 with spike	Difference	% Quench
Dist. water	75	143066	142991	–
Filter + *R. arrhizus*	141013	284415	143402	– 0 3
Filter + act. sludge	593237	642831	49594	65 0
Filter + *R. a.* cell walls	862109	1021804	159695	– 11 7
Filter + a. s. cell walls	303359	423323	119964	16.1
Filter only	392095	535683	143588	– 0.4