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# Reactor design for hazardous waste treatment using a white rot fungus

Pak, Daewon, D.Eng.Sc.

New Jersey Institute of Technology, 1988



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# REACTOR DESIGN FOR HAZARDOUS WASTE TREATMENT USING A WHITE ROT FUNGUS

by

Daewon Pak

Dissertation submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Engineering Science

1988

APPROVAL SHEET

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## ABSTRACT

Title of Thesis : Reactor Design for Hazardous Waste Treatment Using a White Rot Fungus

Daewon Pak, Doctor of Engineering Science, 1988

Thesis directed by : Dr. Gordon A. Lewandowski
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Dr. Piero M. Armenante
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Various nutrient media and reactor configurations have been explored in order to grow the white rot fungus Phanerochaete chrysosporium, induce its active enzyme, develop kinetic data for the degradation of 2-chlorophenol, and use chemical engineering analysis to design an efficient reactor.

Preliminary experiments indicated that the biodegradation rate was improved by two to three orders of magnitude when the fungus was immobilized. As a result, emphasis shifted to two types of reactor design: a packed-bed reactor employing a silica-based porous support, and a fluidized-bed reactor employing alginate beads. Both were very effective in degrading 2-chlorophenol at inlet concentrations up to 520 ppm. Apparent Michaelis-Menten kinetic rate constants were developed for both reactors. To our knowledge, these are the first such constants to be published for this fungus.

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Finally, he express his sincere appreciation to his family, especially his uncle and aunt, for their help and encouragement which made this thesis possible.

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# I. INTRODUCTION

Until recently, the inability of microorganisms to produce enzymes capable of dehalogenating organic compounds has been a primary reason for the recalcitrance of chlorocarbons in biological treatment systems. The reason for this inability is the near absence of such compounds in the natural environment, and the consequent lack of exposure of naturally occurring microorganisms to the carbon-chlorine bond. A white rot fungus has been found in the environment which is readily capable of disrupting that bond, and holds tremendous promise as a biological treatment tool.

The white rot fungus Phanerochaete chrysosporium belongs to a family of wood-rotting fungi that are found all over the Northern Hemisphere. They decompose wood by breaking down lignin, a complex aromatic polymer that is otherwise very resistant to decay. Advantages of using this fungus are the following :

1. It grows rapidly and produces abundant conidia which simplifies handling;
2. Its optimum temperature for growth is 39 - 40$^{o}$C, which, together with its optimum pH of 4.5, means that bacterial contamination is minimized or avoided altogether.
3. It has a highly active ligninolytic enzyme system of broad specificity.

It is therefore the purpose of this research to demonstrate a new technology, employing a white rot fungus, to biodegrade otherwise recalcitrant compounds such as chlorinated phenol, and to apply chemical engineering practice to design bioreactors for this special microorganism.

## II. LITERATURE REVIEW

Phanerochaete chrysosporium, a white-rot wood-decaying fungus, has recently been shown to produce extracellular enzymes that not only degrade lignin, but a broad spectrum of chlorinated hydrocarbons as well.

These enzymes were first isolated from shallow stationary cultures grown in 125 ml Erlenmyer flasks, which completely oxidized lignin to carbon dioxide (1,2). Culture parameters influencing this enzyme system have been studied using standing cultures.

The ligninolytic activity of this fungus can be induced by nitrogen starvation. The onset of enzyme activity, however, could be delayed by addition of ammonia to nitrogen starved cultures (3,4,5,6). Although the source of nitrogen had little influence on this enzyme system, its concentration was found to be critical. The optimum concentration of nitrogen was 2 to 4 mM.

The oxygen concentration was found to be an important rate-determining factor (1,7). The rate and extent of conversion of lignin to carbon dioxide was two to threefold greater under 100 % oxygen than 21 % oxygen (air).

The medium pH was also an important factor. Phanerochaete chrysosporium metabolized lignin optimally when grown at approximately pH 4.5 (8). An extensive survey of possible buffers showed 2,2-dimethylsuccinate to be superior

to o-phthalate (9).

Limitation of carbohydrate or sulfur was also reported to trigger ligninolytic activity of the fungus (10), and the balance of trace elements (such as Mg, Cu, Mn, etc.) was also considered to be important for ligninolytic activity.

Appearance of the secondary metabolite veratryl alcohol paralleled the induction of ligninolytic activity. Veratryl alcohol was reported to be synthesized ex novo from glucose. The relationship between veratryl alcohol and lignin degradation was not clear. A recent study by Faison, et al. (11) concluded that veratryl alcohol caused an increase in ligninase activity by increasing the amount of certain ligninase proteins.

Harvey, et al. (12) reported that the role of veratryl alcohol in lignin degradation was as a mediator with the enzyme. Veratryl alcohol was oxidized to a radical cation which was not rapidly degraded. Therefore it could act as a one-electron oxidant and, in the process of electron transfer, was regenerated. It was suggested that the lignin degrading enzyme functioned not as an oxygenase but as a peroxidase and that the oxidation reaction was brought about by initial single electron transfer between the aromatic ring and an active site in the enzyme.

Leisola et al. (13) isolated two oxidation products of veratryl alcohol from ligninolytic cultures of Phanerochaete chrysosporium. IR spectra and H-NMR spectra of the purified

compounds showed the absence of an aromatic ring. However whether the ring cleavage product is formed by the action of the major ligninase protein or whether a so far non-identified protein is responsible for this reaction was not known.

Haemmerli et al. (14) investigated the oxidation product of veratryl alcohol by the lignin peroxidase. Five products were identified : veratraldehyde, two quinones and two aromatic ring cleavage lactones.

Kirk, et al. (15) suggested two methods for increasing the production of ligninase by the fungus. The first method involves addition of veratryl alcohol (0.4 mM) and excess trace metals to nitrogen-starved cultures. Addition of veratryl alcohol resulted in a two-fold increase in activity. Increasing trace elements seven-fold without veratryl alcohol also resulted in higher ligninase activity. The addition of veratryl alcohol with increased trace elements resulted in more than an additive increase in ligninase activity (nearly five-fold). Increasing the veratryl alcohol to 0.8 mM or the trace elements to ten times did not cause further increases. It was observed that Cu or Mn caused an increase in activity equal to that observed with the complete trace element solution.

Studies on the effect of agitation have been conducted (16,17,18,19). At first, it was found that agitation of the cultures, although resulting in the beneficial formation of

mycelial pellets, suppressed ligninase activity (16,17). However, Reid, et al. (18) showed that cultures agitated on a gyratory shaker degraded lignin to carbon dioxide about as effectively as static cultures. A recent study by Kirk, et al. (19) reported the beneficial effect of detergent addition on the development of ligninase activity in agitated submerged cultures. These cultures were grown in a chemically defined medium containing 2.2 mM ammonium tartrate, 1 % glucose, and 0.4 mM veratryl alcohol. 10 mM dimethyl succinate (pH 4.3) was used as buffer. Results showed that addition of Tween 80, Tween 20, or 3-[(3-colamidopropyl)dimethylammonio]1-propanesulfonate to the cultures permitted development of ligninase activity comparable to that routinly obtained in stationary cultures. He suggested that two of the effective detergents, Tween 20 and Tween 80, probably supplied fatty acids to the cultures (lauric and oleic acids respectively).

Leisola et al. (20) showed that the time needed for the onset of enzyme activity and the final activity level was dependent on the average diameter of the mycelial pellets. The highest activity (180 U/ml) was obtained with the pellets which had an average diameter of about 1 - 2 mm. With somewhat smaller or longer pellets only 60 U/ml were produced. They also observed that the highest activity was obtained with C-limited agitated pellets when veratryl alcohol was used. However it was questionable whether

veratryl alcohol itself was the inducing compound.

Although the metabolic pathways for lignin degradation have been studied extensively (21,22,23,24), those for chlorinated organics are just beginning to be investigated.

Leatham, et al. (25) found that a mutant of Phanerochaete chrysosporium could degrade phenolic compounds and cleave aromatic rings. Thirty-six phenolic compounds were tested by this mutant. 16 compounds were degraded by more than 50 % and 28 compounds were degraded by at least 20 % in 3 days. He concluded that this fungus was among the most versatile and nonspecific degraders of aromatic molecules yet examined.

Sanglard, et al. (26) showed that the fungus was able to degrade benzopyrene which is representative of polycyclic aromatic hydrocarbons. It was concluded that the ligninase is fairly non-specific and non-stereoselective, as one would expect from an enzyme that is able to attack a basically random polymer like lignin.

Eaton, et al. (27) tested Phanerochaete chrysosporium and several other white rot fungi for PCB (polychlorinated biphenyls) degradation. Phanerochaete chrysosporium mineralized the most PCB and was chosen for his detailed study. It was suggested that PCB degradation also begins at the onset of secondary metabolism, triggered by nitrogen limitation.

Arjmand and Sandermann (28) determined that P.

chrysosporium was capable of degrading chloro and dichloroanilines.

Bleach plant effluents were decolorized with the fungus (29,30). About 60 % color reduction was reported to be achieved in 2-4 days with fungal decolorization, along with a 40% reduction in BOD and COD. It was found that color reduction by Phanerochaete chrysosporium BKM F-1767 was much better than that shown by the strains ME-446 and HHB-6251. Phlebia brevispora Nakas consistently reduced color slightly faster than did Phanerochaete chrysosporium BKM F-1767. However, growth of Phlebia brevispora is somewhat less vigorous than that of Phanerochaete chrysosporium. Both of them produced abundant conidia which made inoculation and the handling of stock cultures easier. The fungal biomass could be recycled for at least 60 days. The fungal decolorization was found to require a co-substrate such as glucose or cellulose.

Huynh, et al. (31) used Phanerochaete chrysosporium to degrade chlorinated organics in wastewater treatment systems. Most of the chlorinated phenols and other low-molecular weight components and their chlorinated derivatives were removed. Veratryl alcohol was the major product. It was concluded that the degradation mechanism involved methylation, oxidation, and reduction.

Bumpus, et al. (32,33) reported the ability of the fungus to degrade such normally recalcitrant organohalides as

DDT [1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane], polychlorinated biphenyls, polychlorinated dibenzo(p)dioxins, and lindane [hexachlorocyclohexane]. The fungus was also able to completely degrade alkanes that were chlorinated on every carbon atom. The pathway for DDT degradation in Phanerochaete chrysosporium is clearly different from the major pathway proposed for microbial degradation of DDT. It was concluded that biotreatment systems inoculated with this organism under nitrogen-limiting conditions might provide an effective and economical means for the biological detoxification and disposal of hazardous chemical waste.

The importance of extracellular hydrogen peroxide in lignin degradation has become increasingly apparent. Kersten and Kirk (34) reported that under ligninolytic conditions, P. chrysosporium produced extracellular hydrogen peroxide as well as the corresponding peroxidases. A number of potential substrates such as simple aldehydes, hydroxycarbonyl, and dicarbonyl compounds were tested. The highest activity of extracellular $H_2O_2$-producing oxidases was observed at pH 6, with methylglyoxal and glyoxal as secondary metabolites. No $H_2O_2$-producing oxidase activity was observed with the sugars glucose, xylose, galactose, and cellubiose.

Asada et al. (35) suggested that one of the possible physiological roles of NADH-peroxidases was to supply $H_2O_2$ to lignin-peroxidase by oxidizing NADH. Mn stimulated this reaction 3.4-fold.

Greene and Gould (36) suggested that intracellular fatty acyl-coenzyme A oxidase may be an important source of extracellular $H_2O_2$. They observed increased $H_2O_2$ production and $O_2$ consumption in the presence of stearoyl-coenzyme A with mycelia permeabilized with the detergent Triton X-100.

Possible involvment of intracellular enzymes in $H_2O_2$ production has also been studied. Kelley and Reddy (37) reported the isolation of intracellular glucose-1-oxidase, and suggested that this enzyme is the primary source of peroxide in ligninolytic cultures. However, it has not been demonstrated that the action of these intracellular $H_2O_2$-producing glucose oxidases actually result in extracellular $H_2O_2$.

Leisola, et al. (38) showed that in the culture fluid of P. chrysosporium, 21 extracellular hemoproteins can be found which all have peroxidative activity. Fifteen of these enzymes oxidized veratryl alcohol (lignin peroxidase) in the presence of $H_2O_2$. Six enzymes were Mn-dependant peroxidases, which reached their maximum activity earlier than the lignin peroxidases in the cultures. It was suggested that the many extracellular peroxidases of P. chrysosporium can simply be divided into two basic groups: Mn-dependant peroxidase and Lignin peroxidases. However it was not clear why the fungus produces two types of extracellular peroxidases. The fact that they appear and reach their maximal activity at different times indicates that they may have different

functions in lignin degradation.

Glenn, et al. (39) reported that the $H_2O_2$-requiring enzyme is responsible for the generation of ethylene from 2-keto-4-thiomethyl butyric acid which is lignin model compound.

Leisola, et al. (40) reported that the extracellular $H_2O_2$-dependent ligninase activity of P. chrysosporium was produced in agitated culture conditions when veratryl alcohol or veratraldehyde were added to the cultures. Too high an agitation speed, however, led to complete inhibition of the ligninolytic activity and reduction of veratraldehyde. They analyzed the medium during secondary ligninolytic metabolism of cultures using glucose as the primary carbon source. The major soluble organics accumulating in the culture filtrate were veratryl alcohol (60%), veratraldehyde (5-10 %) and two unknown metabolites. It was shown that these two unknown compounds were two isomers of a ring cleavage product from veratryl alcohol. IR spectra and H-NMR spectra of isomers showed the absence of aromatic rings.

Huynh, et al. (41) observed that the fungus produced an extracellular aromatic methyl ester esterase and a separate aromatic methoxyl demethylase. It was reported that both esterase and demethylase were components of the ligninolytic enzyme complex.

Umezawa, et al. (42) reported that demethylation was not essential for the enzymatic aromatic ring cleavage of the

methoxylated aromatic substrates

Various wild-types strains have been isolated and mutants with altered properties have been developed. Kirk and Tien (43) compared selective strains of the fungus on the basis of their total ligninolytic activity and production of lignin-depolymerizing enzymes. The seven strains included three wild-type isolates (ME-446, K-3, and BKM-F-1767), three cellulase-negative (cel) mutants (3113, 13132-176, and 85118-22, all derived from K-3) and one mutant strain (SC26 derived from BKM-F-1767). The highest activity was seen in strain SC26 and BKM-F-1767. It was confirmed that the extracellular $H_2O_2$, which is required by ligninase, is rate-limiting.

It is also interesting to note that there are a few bacteria (e.g. Streptomyces viridosporus) capable of producing ligninolytic enzymes (44).

Buswell et al. (45) obtained a very high levels of the lignin-degrading enzyme from P. chrysosporium INA-12 under conditions where nitrogen is non-limiting. Although nitrogen limitation has been a prerequisite for the onset of significant lignin degradation, P. chrysosporium INA-12 produced higher level of ligninase activity than other strains when glycerol served as an alternative carbon sources to glucose. It may be related to the relatively poor growth rate of P. chrysosporium INA-12 on glycerol compared to glucose.

In order to overcome the sensitivity of the lignin peroxidase production to agitation, the utilization of immobilized P. chrysosporium spores both in agarose and agar gel beads, and different reactor configuations, have been studied. Linko et al. (46) has shown that agarose biocatalyst produced higher lignin peroxidase activities in a shorter time than the agar biocatalyst. The enzyme activity of lignin peroxidase in a medium with glucose (0.5 g/l) was 30 % higher than without medium.

Kirk and Tien (47) used bench-scale rotating biological contactors (RBC) to increase the production of ligninase from P. chrysosporium. When veratryl alcohol and excess trace metals were added, the fungus adhered to the disc poorly and produced negligible levels of ligninase activity. The problem of adherence to the disc was solved by using a mutant strain, SC26, derived from the wild-type strain, BKM-F-1767. SC26 produced levels of ligninase activity comparable to or higher than those observed with BKM in flask cultures.

Solid substrates for growth of P. chrysosporium offer the crucial benefit of a low shear environment, which has a dramatic effect on ligninase activity. Mudgett et al. (48) investigated the effects of the gas environment and culture conditions in the conversion of a natural wood lignin by attached growth of P. chrysosporium. It was suggested that oxygen and carbon dioxide pressures in solid-state

fermentations influenced significantly the regulation of microbial metabolism, favoring biomass or product formation, depending on the gas phase composition.

# III. EXPERIMENTAL PROCEDURES

## Organism and Inoculum

Phanerochaete chrysosporium BKM-1767 (ATCC 24725) was obtained both from American Type Culture Collection, and from the Department of Wood and Paper Science at North Carolina State University. As recommended by the providers, the ATCC culture was maintained on potato dextrose agar, while the NC State culture was maintained on yeast malt extract agar. The schematic description of experiment for both culture are shown in Fig 1. Results using either culture were the same.

## Culture Media

All media were prepared with distilled, deionized water.

**Potato Dextrose Agar (PDA)**

| | |
|---|---|
| Diced Potatoes | 300 g |
| Glucose | 20 g |
| Agar | 15 g |
| Water | 1 liter |

The diced potatoes were boiled in about 500 ml of water until throughly cooked and were filtered through cheese cloth. The volume was brought up to one liter with water. Agar was dissolved in the filtrate by heating, and glucose was added prior to sterilization.

**Yeast Malt Extract Agar**

| | |
|---|---|
| Yeast extract | 0.3 g |
| Malt extract | 0.3 g |
| Peptone | 0.5 g |
| Agar | 2.0 g |
| Glucose | 1.0 g |
| Water | 100.0 ml |

All nutrients were dissolved in warm water before autoclaving. After autoclaving, the medium was cooled to about 50 $^{o}$C, followed by addition of 0.7 ml of 1 N HCl to adjust the pH to 4.5.

**Growth and Enzyme Induction Medium**

| | Growth Medium | Induction Medium |
|---|---|---|
| $KH_2PO_4$ | 2.0 g | 2.0 g |
| $MgSO_4$ | 0.5 g | 0.5 g |
| $CaCl_2$ | 0.1 g | 0.1 g |
| $NH_4Cl$ | 0.12 g | 0.012 g |
| Glucose | 10.0 g | 2.0 g |
| Thiamine | 0.001 g | 0.001 g |
| Water | 1.0 liter | 1.0 liter |

The final pH of the media was 4.3 to 4.4.

## Standing Cultures

Standing cultures were grown in 125 ml flasks containing 20 ml of growth media and 0.067 g/l of veratryl alcohol.

Conidia from agar plates were used as inoculum. The culture was kept in an incubator at 39 $^{o}$C and flushed with saturated filtered air every two days. The pH was maintained at 4.4 to 4.5 by using a $K_2HPO_4$ buffer. 2-Chlorophenol was added to the standing culture on the seventh day.

## Shaker Cultures

Shaker cultures were grown in 250 ml flasks containing 50 ml of growth media along with 0.067 g/l veratryl alcohol and 0.05 g/l of Tween 80. Conidia from agar plates were used as inuculum. Once again, the pH was kept constant at 4.4 to 4.5 using a potassium phosphate buffer. The flasks were placed in a constant temperature (39 $^{o}$C) shaker (New Brunswick Scientific Model G-24), operating at 80 rpm with a 1.5" radius of agitation. Because of the (relatively) mild agitation, the fungus grew in the form of pellets, about 1/4" to 1" in size. The flasks were closed with cotton plugs to allow sufficient air transfer while preventing contamination. 2-Chlorophenol was added to the culture on the seventh day.

## Batch Fermentation

A schematic diagram of the experimental arrangement is shown in Figure 2.

Two types of batch fermentors were used: Multigen (working volume: 1.5 liter), and Microferm MF114 (working volume: 10 liters) both manufactured by New Brunswick

Scientific.

Mycelial inocula were used throughout the investigation. Inocula for the batch fermentors were prepared by homogenizing a 5-day old culture grown in 250 ml shaker flasks containing 100 ml of growth media. One ml of homogenized culture was used for the Multigen, and 10 ml for the MF114 fermentor.

Fermentation was carried out at 39 $^{o}$C at an agitation speed of 400 rpm for both types of fermentor. In the larger fermentors, the pH was automatically controlled to within +/- 0.1 pH units using a 10N NaOH solution metered by a New Brunswick pH Controller. In the smaller fermentors, a potassium phosphate buffer maintained the pH in the range 4.4 to 4.5.

The aeration rate was maintained at 0.2 liters/min for every liter of operating volume (i.e. 0.3 liters/min for the smaller fermentors, and 2 liters/min for the larger fermentors).

The fungus was allowed to grow in a batch mode utilizing only the nutrients initially present in the medium. After about 5 days, the nitrogen was completely depleted. After 7 days, 2-chlorophenol was introduced, and its concentration was gradually increased. Occasionally, nitrogen and glucose were also added to the fermentor after ligninase induction in order to test their effect on 2-chlorophenol degradation.

## Packed-Bed Reactor

A schematic of the packed-bed arrangement is shown in Figure 3.

A 500 ml glass cylinder (2" I.D. x 10" length), sealed at both ends by rubber plug, was used as a packed bed reactor. Originally, 1/4" ceramic saddles were used as the packing material. The temperature of the system was maintained constant by external circulation of water at 39 $^{o}$C. Conidia from agar plates were used as inoculum and the fungus allowed to grow on the surface of the packing material. The aeration rate was 250 ml/min.

After seven days, a feed stream containing 350 ppm of 2-chlorophenol in induction media was introduced from the bottom at the rate of 0.25 ml/min.

A second set of experiments was run utilizing silica-based porous spheres and silica-based porous cylinders (Manville Celite Catalyst Carrier, R-635) in place of the ceramic packing. The chemical and physical properties of the carrier (cylinders) are listed in Tables 1 and 2. A fungal culture grown in a shaker flask for 7 days (90 mg of dry biomass) was homogenized in a blender and used as inoculum. The fungus was allowed to grow on for 5 days the surface of the packing material. 2-chlorophenol was introduced at concentration up to 500 ppm and with flow rate from 0.1 to 1 ml/min. The working volume of reactor was 400

ml. The void fractions of reactor with sphere and cylinder-shaped carrier were 30 % and 38 % , respectively. The aeration rate was 500 ml/min.

A 3300 ml glass cylinder (4" I.D. x 16" length) was used to determine if there was an unaccounted scale-up factor (such as wall effect) on the performance of the packed-bed reactor. Reactor inoculation and operation were conducted as before. The working volume and void volume were 1800 ml and 800 ml, respectively. The aeration rate was 2300 ml/min.

A third set of experiments was run utilizing balsa wood chips cut to size (5 mm x 5 mm x 5 mm), which could provide a carbon source (cellulose) to this fungus. A fungal culture grown in a shaker flask for 7 days was homogenized in a blender and used as inoculum. The fungus was allowed to grow on the surface of wood chipsfor 5 days. The working volume and void volume were 250 ml and 120 ml, respectively. The aeration rate was 300 ml/min. The induction solution had only 10 % of the glucose growth requirement (1 g/l), and was introduced from the bottom at the rate of 0.4 ml/min.

**Immobilized Cell Reactor**

Sodium alginate (2 g) was dissolved in a 100 ml saline solution and sterilized. The sodium alginate gel was then added to a fungal culture grown in a shaker flask for 7 days, and the mixture was homogenized in a blender. The fungus-alginate suspension was then extruded ,at room temperature,

by means of a syringe pump (Sage Instrument, Model 341A) moving at a uniform speed of 6 ml/min and equipped with a 18 gauge syringe needle into a 0.2 M calcium chloride solution at room temperature. The droplets formed into 3.5 to 4 mm beads upon contact with the calcium chloride solution.

All the 2000 beads so produced were then placed in an upflow reactor of the same size as used before for the packed column experiments.

The next day, a feed stream containing 520 ppm of 2-chlorophenol in induction media was introduced from the bottom at the rate of 0.1 to 1 ml/min. Enough air (approximately 300 cc/min) was introduced to keep the beads fluidized and maintain the dissolved oxygen concentration above 2.0 mg/l.

## IV. ANALYTICAL METHODS

### Dry Mycellium Weight

The samples taken from the batch fermentor were centrifuged at 4000 rpm and the supernatant drained. The residue was then washed several times with distilled water and dried overnight at 70 $^{o}$C to determine the dry mycellium weight.

### Nitrogen Assay

Since $NH_4Cl$ was used as the nitrogen source, an Orion Model 95-12 ammonia electrode was used for assay of nitrogen during fermentation. An Orion Model 701-A pH meter was used to measure the mV response of the electrode to the nitrogen concentration in each sample. The ammonia electrode was calibrated by placing it in 50 ml of 10 ppm nitrogen standard solution, together with 0.5 ml of pH adjusting ISA solution (Orion research). The reading of 10 ppm $N_2$ was arbitrarily set to zero (enough time was allowed for the electrode response to stabilize). This calibration procedure was carried out for a series of standards. The resulting calibration curve [electrode potential in mV vs. log(ppm $N_2$)] was linear in the range 0.1 to 1000 ppm.

### Glucose assay

The o-toluidine method was used to follow the depletion

of glucose during fermentation. At high enough temperature (100 $^{o}$C) O-toluidine reacts with glucose in the presence of an acid to form a color complex, the intensity of which is proportional to the glucose concentration.

## Substrate Analysis

Gas chromatographs (Varian 3700) were used to determine the concentration of 2-chlorophenol in reactor samples. 15 ml of sample were taken from the reactor and centrifuged for 4 minutes at 4000 rpm. 1 ul of sample were injected onto a 6 ft x 1/8 inch stainless steel column containing 10 % SP2100 on 100/200 Supelcoport. The oven temprature was 140 $^{o}$C, The injection and detector temperatures were 270 $^{o}$C and 300 $^{o}$C, respectively. Air (flow rate 400 cc/min) and hydrogen (flow rate 30 cc/min) were used in the Flame Ionization Detector, and nitrogen (flowrate 40 cc/min) was the carrier gas. An electronic integrator (Hewlett-Packard 3390A) was used to determine the peak area.

## Dissolved Oxygen

During fermentation, the dissolved oxygen concentration was determined by a sterilizable DO electrode (New Brunswick Scientific, Model M1016-0310), coupled with a dissolved oxygen analyzer (New Brunswick Scientific Model DO-50) and a strip-chart recorder.

## Assay of 2-Chlorophenol in the Off-Gas

As a result of volatilization, the off-gas coming from the batch fermentors contained some 2-chlorophenol. In order to determine the stripping rate, the following tests were carried out. First, P. chrysosporium was grown in a 14 liter batch fermentor containing 10 liters of media for 6 days. The entire reactor was then autoclaved at 121 $^{o}$C for two hours to kill the fungus. 500 ppm of 2-chlorophenol were added to the liquid phase, and the air was turned on at a flow rate of 2 liters/min. The off-gas was passed through three flasks connected in series, each containing 2 liters of water, in order to absorb the 2-chlorophenol. Samples from each flask, and from the reactor, were taken every 12 hours and analyzed by GC. Also every 12 hours, the water in the absorption flasks was changed to ensure complete removal of 2-chlorophenol from the off-gas. Finally, a material balance was performed on the system in order to calculate the stripping rate constant.

## V. RESULTS AND DISCUSSION

### Standing and Shaker Cultures

These cultures were mainly used for:

a. screening purposes, i.e. for determining the effects of a number of operating variables and nutrients on the biological degradation rates of 2-chlorophenol;

b. producing enough biomass to be used as inoculum for larger scale experiments.

In the standing cultures, the fungus grew in the form of one large agglomerate which tended to cover the bottom of the entire flask. In the shaker cultures, up to several smaller pellets of the fungus were formed, depending on the agitation speed.

Table 3 reports some of the results obtained as a result of the screening test program. When exposed to 2-chlorophenol after 5 days, the fungus showed a significant degradation activity, as reported in Figures 4 and 5. Both standing and shaker cultures showed the same pattern for decreasing 2-chlorophenol. Tween 80 did not appear to influence the biodegradaton rate for these types of culture. On the other hand, veratryl alcohol produced an increase of the biodegradation activity, as shown in Figure 6.

### Batch Fermentation Cultures

The bulk of these experiments were conducted in the 14-

liter fermentors. The 1.5-liter fermentors were mainly used for intermediate determination of scale-up effects on the operating variables. In one particular instance, a culture grown in the smaller fermentor exibited very high degradation rates (up to 700 ppm/day) and showed extremely good tolerance to high concentrations of 2-chlorophenol (up to 1000 ppm). Unfortunately we were not able to duplicate these results.

The growth in the 14-liter fermentor was qualitatively very similar to that observed in the smaller fermentors. The rates of nitrogen and glucose consumption were higher during growth, as shown in Figure 7. After the fifth day, the nitrogen source was usually completely depleted and the glucose consumption rate decreased accordingly (see Table 4).

Table 5 and Figure 8 show the relationship between glucose and nitrogen consumption, and the corresponding change in dry mycelial weight. It is not clear why the biomass concentration continued to increase after nitrogen depletion. This may be a result of utilization of intracellular nitrogen, or may represent accumulation of polysaccharides within the cells (see also Table 5).

In order to compare the overall efficiency of different reactor configurations, a first-order kinetic model was initially used to describe the fungal detoxification activity against 2-chlorophenol. In a batch reactor, the mass balance for 2-chlorophenol can be written as :

$$V \frac{dC}{dt} = - V k n C = - V k_c C$$

Hence:

$$k_c = (1/t) \ln (Ci/Co)$$

where:

Ci = initial concentration of 2-chlorophenol (mg/l)

Co = final concentration of 2-chlorophenol (mg/l)

$k_c$ = overall kinetic constant ($hr^{-1}$)

k = kinetic constant ( mg of $biomass^{-1}$ $hr^{-1}$)

n = biomass concentration (mg/l)

V = volume of reactor (ml)

Figure 9 presents the rate of biodegradation of 2-chlorophenol in the 14 liter batch fermentor when veratryl alcohol and Tween 80 were used in the induction medium. These data were fit to a first-order rate model. Similar results were obtained without Tween 80 (Figure 10). By comparing the data contained in Tables 6 and 7 we concluded that, contrary to literature results, Tween 80 does not seem to be effective in improving the rate of biodegradation in agitated cultures.

Changing other parameters, such as the glucose and nitrogen concentration, failed to improve the biodegradation rate of 2-chlorophenol, while elimination of veratryl alcohol decreased the biodegradation rate. Addition of veratryl alcohol extracellulary could enhance the enzyme activity in

batch culture.

Figure 11 shows the stripping data for 2-chlorophenol from 14 liter batch fermentor. The stripping process follows a first-order expression, with a rate constant of 5.5 x $10^{-3}$ $hr^{-1}$. This value can also be predicted from thermodynamic considerations by assuming a 30% saturation of the air leaving the reactor.

The overall removal rate for 2-chlorophenol in a 14 liter batch fermentor, from Figures 8 and 9, also appears to follow a first-order expression, with a rate constant of 13 x $10^{-3}$ $hr^{-1}$ . Since the overall rate constant (for both biodegradation and stripping) was 13 x $10^{-3}$, while the rate constant for stripping alone was 5.5 x $10^{-3}$, it appears that the rate of biodegradation in a suspended growth reactor was 7.5 x $10^{-3}$ $hr^{-1}$. This result is in qualitative agreement with other investigators in this field who claim that the fungus is not very active in suspended growth reactors, but rather needs to be attached to a surface.

## Packed-Bed Reactor

Immobilization appeared to be crucial to effective induction of the appropriate degradative enzyme system for P. chrysosporium. Additional advantages of cell immobilization are the following:

- It provides high cell concentrations and therefore, higher reaction rates per unit volume of reactor;

- o It eliminates the need for cell separation and recycle;
- o It protects the cells against high shear stress;
- o It protects the cells against high concentrations of toxic chemicals due to the effect of mass transfer resistance within the immobilizing medium.

Therefore, to induce the appropriate degradative enzyme system, the utilization of immobilized Phanerochaete chrysosporium, was studied in different bioreactor designs.

The first system to be studied was a packed-bed reactor with a ceramic saddle packing. Table 8 contains some results for the mineralization of 2-chlorophenol in the ceramic-saddle, packed-bed reactor. After inoculating the reactor, fungus began to grow up from the bottom. The induction media contained 0.44 mM ammonium chloride as a nitrogen source. After one month, the reactor was full of biomass, and 2-chlorophenol was being biodegraded.

In these preliminary experiments, the same first-order kinetic model described in the batch fermentation section was also used to initially describe the fungal detoxification activity against 2-chlorophenol. The model assumed that the reactor operated in a plug flow mode. These degradation rate constants were used to compare the relative performance of the different types of bioreactors and packing materials. From a mass balance for the 2-chlorophenol it is:

$$F\ C - F\ (\ C + dC\ ) = k_c\ C\ dV$$

Therefore, from a mass balance for the reactor at steady state it is:

$$F\ dC = -\ k_c\ C\ dV$$

and hence:

$$k_c = (F/V)\ \ln\ (Ci/Co)$$

where:

$k_c$ = overall kinetic constant ( $hr^{-1}$ )

Ci = concentration of 2-chlorophenol in feed (mg/l)

Co = conc of 2-chlorophenol in outgoing stream (mg/l)

F = liquid flow rate ( ml/min )

V = reactor working volume ( ml )

These preliminary first-order degradation rate constants are reported in Table 14. The packed-bed reactor shows a marked improvement over the batch fermentor by almost an order of magnitude, when the first-order rate constants were compared.

The use of the Manville packing represented an additional improvement in operation. With the ceramic saddles, the fungus clumped together unevenly, particularly at the top of the column. However, with the Manville support there appeared to be relatively uniform growth and excellent 2-chlorophenol removal. Electron scanning micrographs showed the porous interior of the Manville spherical carrier (Figures 12 and 13). The fungus had grown through the pore system (20 um) and into the center of biocatalyst.

The saturation concentration of dissolved oxygen in the induction medium at 39 $^{o}C$ was 5.9 ppm. This number is

reasonable when compared with glucose solution at different concentration (Table 16). After inoculation, the concentration of dissolved oxygen decreased to 4.1 ppm after two days but then increased again back to 5.3 ppm on the following day. When the induction medium containing 2-chlorophenol was introduced from the bottom, the concentration of dissolved oxygen decreased to 4.7 ppm, and thereafter remained constant. The oxygen concentration remained constant at this level in spite of any imposed variation of the liquid flow rate. This can be attributed to the high volumetric flow rate of air as compared to that of the liquid feed stream. As a result, the relative velocity of the air bubbles with respect to the liquid can be taken to be a constant despite the liquid flow rate variations. In addition, the metabolic consumption of oxygen per unit liquid volume can be considered to be approximately constant and dependent on the primary metabolite consumption rate only. In absence of nitrogen this rate is also a constant and equal to the maintenance metabolism rate. Consequently, by making a mass balance for the reactor, we can see that any small variation of the liquid flow rate (as compared to the air flow rate) should not affect the oxygen concentration in the outgoing liquid stream.

In order to better determine the fungal degradation rate in this reactor configuration, the steady-state concentration of 2-chlorophenol in the outlet stream of packed-bed reactor

with the Manville carrier (cylinder shape) was studied at 8 different feed flow rates. The enzyme concentration in the reactor was assumed to be constant at each feed flow rate, which implies that the enzyme produced by fungus using the nitrogen source in the inlet stream made up for the loss of enzyme in the outlet stream. The results are presented in Table 10 and Figure 14. These results were interpreted using a more appropriate model for this enzymatically catalyzed reaction. The Michaelis-Menten equation was chosen for this purpose:

$$\frac{dS}{dt} = \frac{Vm\ S}{Km + S}$$

where:

S = substrate concentration (mg/l)

Vm = maximum velocity

Km = Michaelis-Menten constant

From a linear regression of experimental data, the values for Vm and Km were calculated to be 119 ppm/hr and 131 ppm, respectively (Figure 15).

Since 2-chlorophenol has been known to be toxic to microorganisms, the substrate inhibition model was also used to predict the kinetic parameters:

$$\frac{ds}{dt} = \frac{Vm\ S}{K + S + S^2/K_I}$$

where:

S : substrate concentration (mg/l)

Vm : maximum velocity

$K_I$ : inhibition constant

From the linear regression of experimental data, the values for Vm, K, and $K_I$ are 11.7 ppm/hr, 7.7 ppm, and -121, respectively. One of the kinetic constants, however, is negative. This makes the constant physically meaningless. The negative inhibition constant ,however, implies that 2-chlorophenol up to 500 ppm does not inhibit the enzyme activity.

The steady state outflow concentrations of 2-chlorophenol for this 4" diameter packed-bed reactor with cylinder-shaped carrier are listed in Table 11 and Figure 16. The regressed values of Vm and Km in the Michaelis-Menten equation are 57 ppm/hr and 132.6 ppm, respectively (Figure 17). When these values were compared with those for 2" diameter reactor, the almost same values for km were obtained because the identical enzyme was produced in both reactors. The difference of the value of Vm could be attributed to different biomass concentration. The inhibition model was also tested. The corresponding values for Vm, K, and $K_I$ are 8.1 ppm/hr, 3.2 ppm, and -348.8, respectively. The negative kinetic constant is physically meaningless. Hence, the model was discarded.

In the long term study of 2-chlorophenol degradation,

the decrease in the activity of 2-chlorophenol degradation was not noticed for two months but breakage of silica-based catalyst (about 5 %) was observed.

The use of wood chips as a packing material can provide a co-substrate instead of glucose with this fungus. Therefore, we also experimentally determined the performance of such a reactor. The degradation rates of the packed-bed reactor with Balsa wood chips are reported in Table 12. The Balsa wood chips showed a 20 % increase in first-order rate constant when compared to the Manville carrier (cylinder shape). The wood chip can serve as both a carrier and a co-substrate. This type of packing need to be studied in the future.

## Immobilized Cell Reactor

This reactor contained the fungus entrapped in alginate beads. In order to improve bead stability, 2 % alginate beads were used instead of 1 % alginate. 2 % alginate beads had maintained their shape for more than 3 weeks.

Due to the mixing effect caused by aeration, the immobilized beads were moving from top to bottom throughout the column reactor. However, by growing inside the beads, the fungus was protected against high shear rates. After introducing a feed stream, the immobilized beads changed color from ivory to yellow-brown as the flow rate increased.

As the 2-chlorophenol concentration increased, the beads got darker. Immobilized beads exposed to higher concentrations of 2-chlorophenol kept their shape longer than those exposed to low concentrations. After the experiments were completed, the total bead weight was less than the weight taken before the experiment. It was suspected that the fungus could degrade the alginate.

The performance of the fluidized bed reactor with immobilized beads at 10 different feed flow rates is shown in Table 13 and Fig 18. Once again, the Michaelis-Menten equation was used to fit the data and get the kinetic parameters. From the linear regression of experimental data, the values for the Vm and Km are 52.6 ppm/hr and 16 respectively (Figure 19). This is the same type of kinetic expression comparing with packed-bed reactor. The difference of the values of Vm could be attributed to different biomass concentration. The values of km, however, were expected to be the same as that for packed-bed reactor. The different mass transfer resistances in the packed-bed reactor versus the immobilized cell reactor was suspected to affect km.

The shell mass balance method and Fick's law were used to describe the diffusion of 2-chlorophenol on the inside of an alginate bead, assuming that the substrate disappeared according to Michaelis-Menten equation. The resulting second-order nonlinear differential equations is the following:

$$\frac{d^2C_A}{dr^2} + \frac{2}{r}\frac{dC_A}{dr} = \frac{Vm}{D}\frac{C_A}{km + C_A}$$

The boundary conditions are as follow:

$$\text{at } r = 0 \qquad \frac{dC_A}{dr} = 0$$

$$\text{at } r = R \qquad C_A = C_{As}$$

These equations were solved using the method of orthogonal collocation with five interior points and also by using a 4th order Runge-Kutta routine. The method of orthogonal collocation is explained in Appendix A. The program for orthogonal collocation and Runge-Kutta is listed in Appendix B.

In order to utilize the model, the diffusion coefficient for 2-chlorophenol was estimated using Wilke-Chang equation:

$$D_{AB} = 7.48 \times 10^{-8} \frac{(a\ M_B)\ T}{n_B\ V}$$

where:

$D_{AB}$ = diffusion coefficient of solute A at very low oncentrations in solvent B, $cm^2/s$

$M_B$ = molecular weight of solvent B

T = absolute temperature, K

$n_B$ = viscosity of solvent B, cp

$V_A$ = molal volume of solute A at its boiling temperature, $cm^3/g\text{-mole}$

a = association factor of solvent B, dimensionless (2.6 for water)

The result represents an estimate for diffusion through water at infinite dilution. It was assumed that this would be representative of 2-chlorophenol diffusion in alginate. Table 15 shows the comparision between experimental and estimated diffusion coeffients at infinite dilution. The estimated diffusion coefficient for 2-chlorophenol at 39 $^oC$ is $8.74 \times 10^{-6}$ $cm^2/sec$.

2-chlorophenol concentration gradients in an alginate bead are shown in Figures 20 and 21. The same result was obtained from both methods of orthogonal collocation and Runge-Kutta. The small size bead has a high effectiveness factor, which implies that diffusional effects are not as important as the "inherent" reaction rate.

# VI. CONCLUSIONS

1. Phanerochaete chrysosporium can significantly degrade 2-chlorophenol at concentrations up to at least 800 ppm.

2. Phanerochaete chrysosporium is not particularly active in a suspended growth reactor, but needs to be attached to a surface.

3. In continuous flow systems, certain compounds (such as Tween 80 and veratryl alcohol) were found to be less important than some investigators have claimed.

4. Wood chips can serve as both carrier and co-substrate in a packed-bed reactor.

5. A packed-bed reactor employing silica-based porous support, and a fluidized bed reactor employing alginate beads, were designed and operated to degrade 2-chlorophenol at feed concentrations up to 520 ppm.

6. The Michaelis-Menten enzyme kinetic equation can represent 2-chlorophenol degradation data from both types of reactor in continuous mode. The substrate inhibition kinetic model was unable to describe 2-chlorophenol degradation data since regression of data

yielded negative values for the inhibition constant.

7. For the packed-bed reactor, the rate constants obtained with a 2" column were consistant with those obtained with a 4" column.

8. For the fluidized-bed reactor, the diffusional effects in the alginate beads do not appear to be as important as the inherent reaction rate.

## VII. FUTURE WORK

- The glucose concentration should be reduced and the use of other co-substrates ( such as wood chips ) investigated.
- Additional changes to the reactors should be made to enhance their performance ( e.g. better air distribution, packing material design, etc.)
- The effect of temperature change from the optimum (39 $^{o}C$) on reactor performance should be investigated.
- Other recalcitrant compounds of interest to industry should be tested along with a real waste.

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## Table 1

## Chemical Properties of Silica-based Porous Cylinders

| Chemical | Wt % |
|---|---|
| $SiO_2$ | 82.0 |
| $Al_2O_3$ | 7.0 |
| CaO | 3.0 |
| MgO | 1.0 |
| $Fe_2O_3$ | 2.0 |
| $Na_2O$ | 3.0 |
| $K_2O$ | 1.0 |
| $P_2O_5$ | 0.5 |
| $TiO_2$ | 0.2 |
| LOI | 1.2 |
| pH of slurry | 8 - 8.5 |

## Table 2

### Typical Physical Properties of Silica-based Porous Cylinders

| | |
|---|---|
| Form | Cylinder (1/4" diameter, 1/4"-1/2" height) |
| Mean Pore Diameter, | 18 |
| Surface area, B.E.T., m/g | 1 - 2 |
| Total Pore Volume, cc/g | 1.08 |
| Volume Fraction 1 - 10 um , cc/g | 0.08 (12.5%) |
| Volume Fraction 10 - 20 um , cc/g | 0.22 (36.0%) |
| Volume Fraction 10 - 30 um , cc/g | 0.24 (39.0%) |
| Volume Fraction 10 - 50 um , cc/g | 0.51 (84.0%) |
| Water Absorption, % by weight, pellet method | 60 % |
| Hardness - Monsanto Hardness Test, Kg | 8 |
| Bed Density (compacted) lbs/ft | 32 |

## Table 3

## Trial Runs

| Basal Medium Plus..... | Cuture Condition | Initial 2-CP Conc (ppm) | Time to Degrde to < 1 ppm |
|---|---|---|---|
| -- | standing | 16 | 2 days |
| -- | shaken | 16 | 3 days |
| saw dust | standing | 20 | 2 days |
| saw dust | shaken | 20 | 2 days |
| after 100 ppm phenol acclimation | shaken | 20 | 2 days |
| Tween 80 | standing | 16 | 2 days |
| Tween 80 | shaken | 16 | 3 days |
| veratry alcohol | standing | 30 | 2 days |
| veratry alcohol + Tween 80 | standing | 30 | 3 days |
| veratry alcohol + Tween 80 | shaken | 20 | 3 hours |
| Immobilized Inside | batch/aerated | 20 | 2 days |
| or Outside Alginate Beads | batch/aerated | 20 | 2 days |

## Table 4

### Change of Primary Nutrient Concentrations with Time in a Batch Fermentor

| Culture Age (days) | Glucose Conc (g/l) | Nitrogen Conc (ppm) |
|---|---|---|
| 1 | 10 | 30 |
| 2 | 9.1 | 13 |
| 3 | 8.9 | 10.5 |
| 4 | 8.6 | 5.5 |
| 5 | 7.5 | 0 |
| 6 | 5.6 | - |
| 7 | 4.8 | - |
| 8 | 4.3 | - |
| 9 | 4.1 | - |
| 10 | 3.5 | - |
| 11 | 3.2 | - |
| 12 | 3.2 | - |

## Table 5

### Relationship Between Culture Growth and Depletion of Glucose and Nitrogen in a Batch Fermentor

| Culture Age (days) | Glucose Conc (g/l) | Nitrogen Conc (ppm) | Biomass Conc (mg/l) |
|---|---|---|---|
| 1 | 5 | 33 | 0 |
| 2 | 4 | 17 | 224 |
| 3 | 2.7 | 0 | 760 |
| 4 | 1.6 | - | 792 |
| 5 | 1.2 | - | 875 |
| 6 | 0.6 | - | 1032 |
| 7 | 0.2 | - | 1201 |
| 8 | 0 | - | 1309 |
| 9 | - | - | 1220 |

## Table 6

### Biodegradation of 2-Chlorophenol in the Presence of Veratryl Alcohol and Tween 80 as Inducers

| Time hour | Concentration in reactor ppm | Stripping with air ppm | Biodegradation ppm |
|---|---|---|---|
| 0 | 838 | 0 | 0 |
| 16 | 503 | 88 | 246 |
| 24 | 433 | 114 | 290 |
| 48 | 346 | 146 | 344 |
| 64 | 283 | 194 | 358 |
| 80 | 238 | 210 | 388 |
| 96 | 193 | 228 | 415 |
| 112 | 155 | 233 | 456 |
| 120 | 138 | 242 | 488 |
| 144 | 87 | 256 | 493 |
| 164 | 73 | 269 | 495 |
| 188 | 61 | 279 | 496 |

## Table 7

### Biodegradation of 2-Chlorophenol in a Batch Fermentor with Veratryl Alcohol Alone as Inducer

| Time hour | Concentration in reactor ppm | Stripping with air ppm | Biodegradation ppm |
|---|---|---|---|
| 0 | 193.0 | 0 | 0 |
| 20 | 172.0 | 21.2 | 28.0 |
| 43 | 111.4 | 32.7 | 48.9 |
| 52 | 95.7 | 37.3 | 60.0 |
| 76 | 65.2 | 48.6 | 79.2 |
| 96 | 48.2 | 59.1 | 85.7 |

Table 8

Performance of the Packed-Bed Reactor with Ceramic Saddles

| Culture Age (days) | Flow Rate (ml/min) | 2-CP Conc in Induction Medium (ppm) | 2-CP Conc in Outflow (ppm) |
|---|---|---|---|
| 11 | 2 | 312 | 0 |
| 12 | | | 25.6 |
| 13 | | | 26.8 |
| 14 | | 354 | 20.3 |
| 15 | | | 20.4 |
| 16 | | | 20.8 |
| 17 | | | 20.1 |

Table 9

Performance of the Packed-Bed Reactor with Silica-Based Spheres

| Flow Rate (ml/min) | 2-CP Conc in Induction Medium (ppm) | 2-CP Conc in Outflow (ppm) |
|---|---|---|
| 0.35 | 500 | 55 |
| 0.50 | | 103 |
| 0.70 | | 136 |

## Table 10

### Performance of 500 ml Packed Bed Reactor with Silica-Based Cylinders

| Flow Rate (ml/min) | 2-CP Conc in Induction Medium (ppm) | 2-CP Conc in Outflow (ppm) |
|---|---|---|
| 0.2 | 460 | 0 |
| 0.3 | | 23 |
| 0.4 | | 37 |
| 0.5 | | 39 |
| 0.6 | | 47 |
| 0.7 | | 114 |
| 0.8 | | 160 |
| 0.9 | | 166 |

## Table 11

### Performance of 3.3 Liter Packed-Bed Reactor with Silica-Based Cylinders

| Flow Rate (ml/min) | 2-CP Conc in Induction Medium (ppm) | 2-CP Conc in Outflow (ppm) |
|---|---|---|
| 0.5 | 500 | 0 |
| 1 | | 104 |
| 2 | | 160 |
| 2.5 | | 182 |
| 3 | | 204 |
| 4 | | 233 |
| 5 | | 292 |
| 6 | | 323 |
| 10 | | 375 |
| 12 | | 397 |
| 14 | | 410 |

## Table 12

### Performance of Packed-Bed Reactor with Balsa Wood Chips

| Flow Rate (ml/min) | 2-CP Conc in Induction Medium (ppm) | 2-CP Conc in Outflow (ppm) |
|---|---|---|
| 0.4 | 300 | 43 |

## Table 13

### Performance of Fluidized Bed Reactor with Immobilized Alginate Beads

| Flow Rate (ml/min ) | 2-CP Conc in Induction Medium (ppm) | 2-CP Conc in Outflow (ppm) |
|---|---|---|
| 0.1 | 520 | 0 |
| 0.2 | | 0 |
| 0.3 | | 18 |
| 0.4 | | 20 |
| 0.5 | | 69 |
| 0.6 | | 78 |
| 0.7 | | 130 |
| 0.8 | | 146 |
| 0.9 | | 176 |
| 1.0 | | 215 |

## Table 14

**Comparison of the Preliminary First-order Kinetic Rate Constants for Removal of 2-chlorophenol by *P. chrysosporium* in Different Reactor Configurations**

| | |
|---|---|
| o 14 Liter Batch Fermentor | $Kc = 1.3 \times 10^{-2}\ hr^{-1}$ |
| o Air Stripping Mass Transfer Coefficient in Batch Reactor | $K_L a = 5.5 \times 10^{-3}\ hr^{-1}$ |
| o Packed-Bed Reactor (Continuous Flow) (with Ceramic Saddles) | $Kc = 0.11\ hr^{-1}$ |
| o Packed-Bed Reactor (Continuous Flow) (with Silica-based Sphere) | $Kc = 0.123\ hr^{-1}$ |
| o Packed-Bed Reactor (Continuous Flow) (with Silica-based Cylinder) | $Kc = 0.155\ hr^{-1}$ |
| o Packed-Bed Reactor (Continuous Flow) (with Balsa Wood Chips) | $Kc = 0.186\ hr^{-1}$ |

## Table 15

### Comparison between Experimental and Estimated Diffusion Coefficients

| Substrate | Temp °C | Diffusion Coefficient (x $10^{-5}$) ($cm^2/s$) | | |
|---|---|---|---|---|
| | | in Water | 2% Ca-alginate | Wilke-Chang |
| Glucose | 30 | 0.68 | 0.68 | 0.68 |
| L-Tryptophan | 30 | 0.67 | 0.67 | 0.62 |
| Ethanol | 30 | 1.1 | 1 | 1.2 |
| Methanol | 15 | 1.26 | | 1.4 |

**Table 16**

**Oxygen Solubilities mg $O_2$/Liter**

| Solution | Temperature ( °C ) | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 20 | 25 | 30 | 37 | 45 |
| Distilled Water | 11.3 | 9.0 | 8.5 | 7.5 | 6.9 | 6.0 |
| Glucose, 0.7 M | | 8.0 | | | | |
| Glucose, 1.5 M | | 6.7 | | | | |
| Sucrose, 0.4 M | | 8.9 | | | | |
| Sucrose, 0.9 M | | 7.3 | | | | |
| Sodium Chloride, 0.1 N | | | | 7.3 | | |
| Sodium Chloride, 1.0 N | | | 6.0 | | | |
| Potasium Chloride, 1.0 N | | | | 5.6 | | |

FIGURE 1

**SCHEMATIC DESCRIPTION OF EXPERIMENTAL CULTURE FOR PHANEROCHAETE CHRYSOSPORIUM**

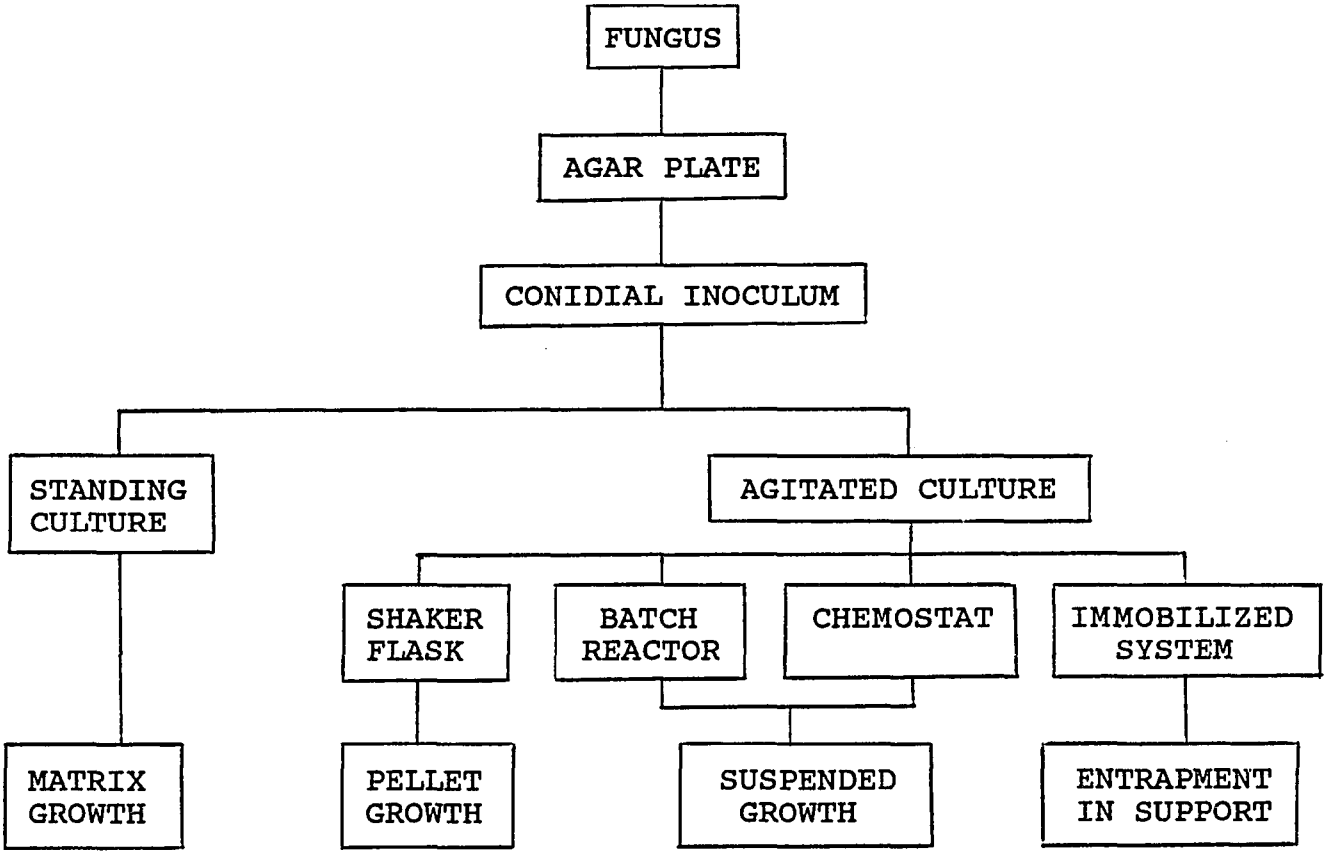


FIGURE 2

SCHEMATIC DIAGRAM OF EXPERIMENTAL SETUP FOR BATCH CULTURE

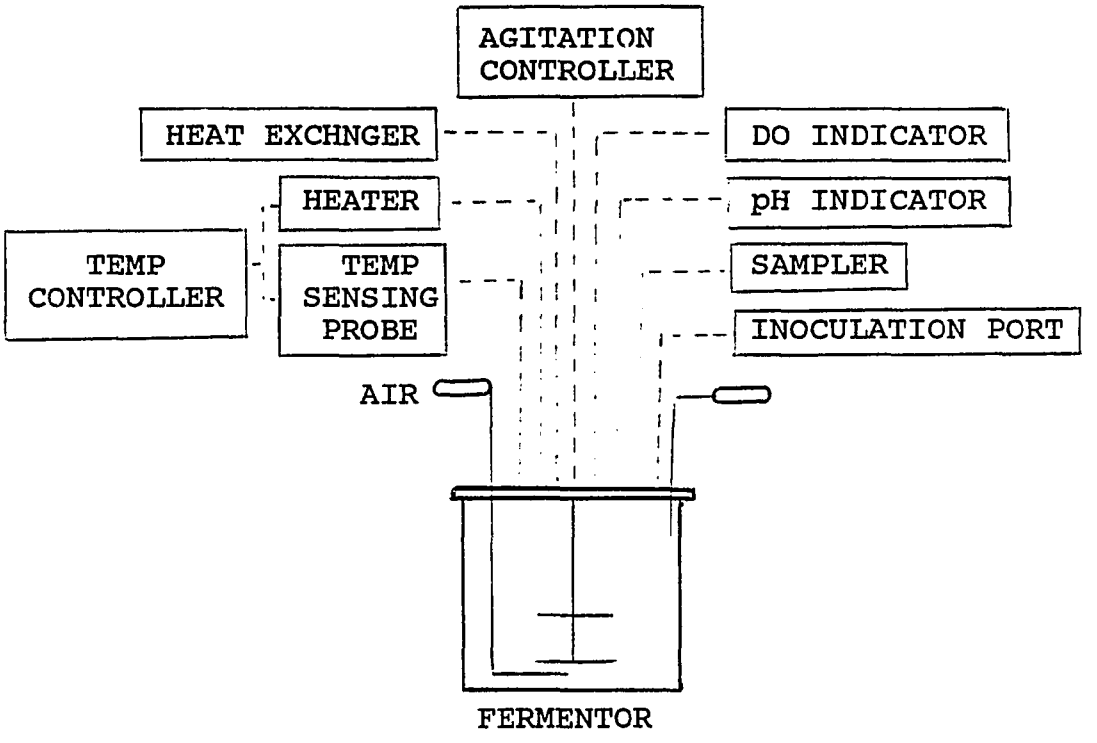


FIGURE 3

SCHEMATIC DIAGRAM OF EXPERIMENTAL SET-UP FOR IMMOBILIZED SYSTEM

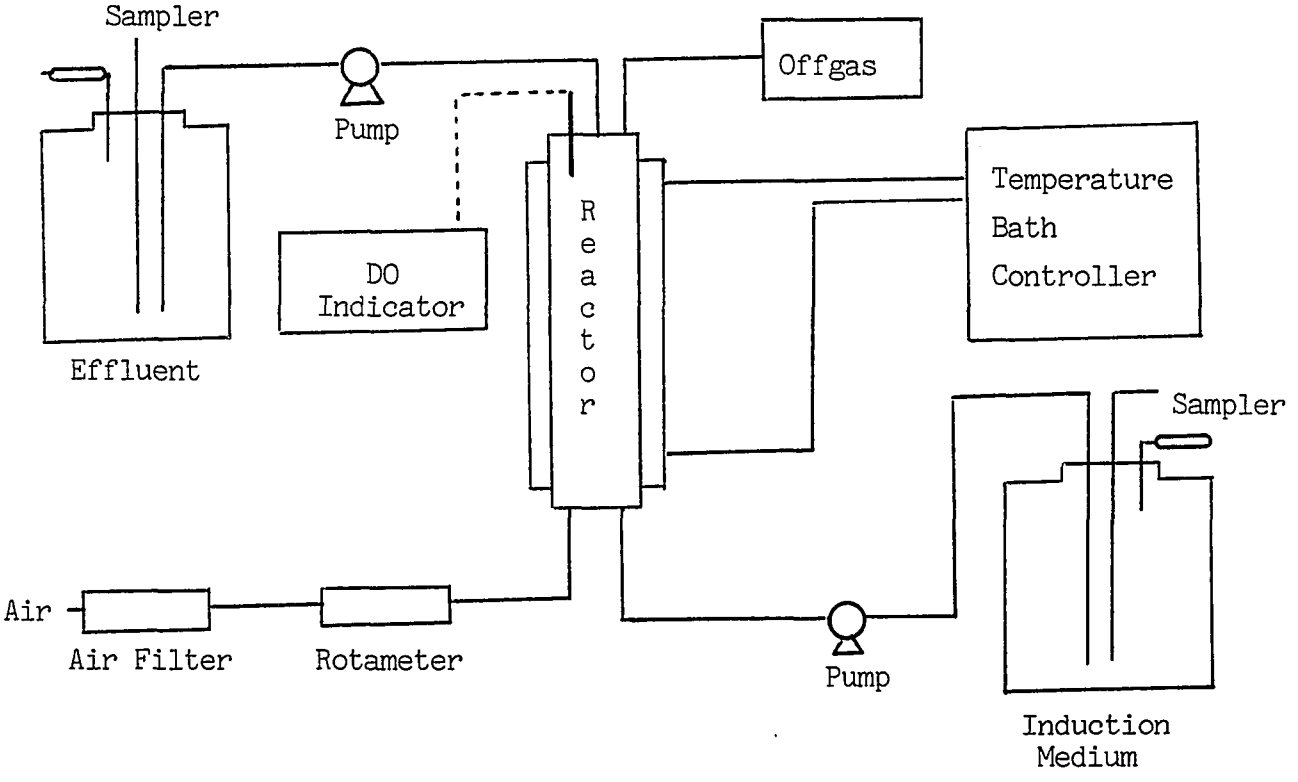


FIGURE 4

BIODEGRADATION OF 2-CHLOROPHENOL IN STANDING CULTURE

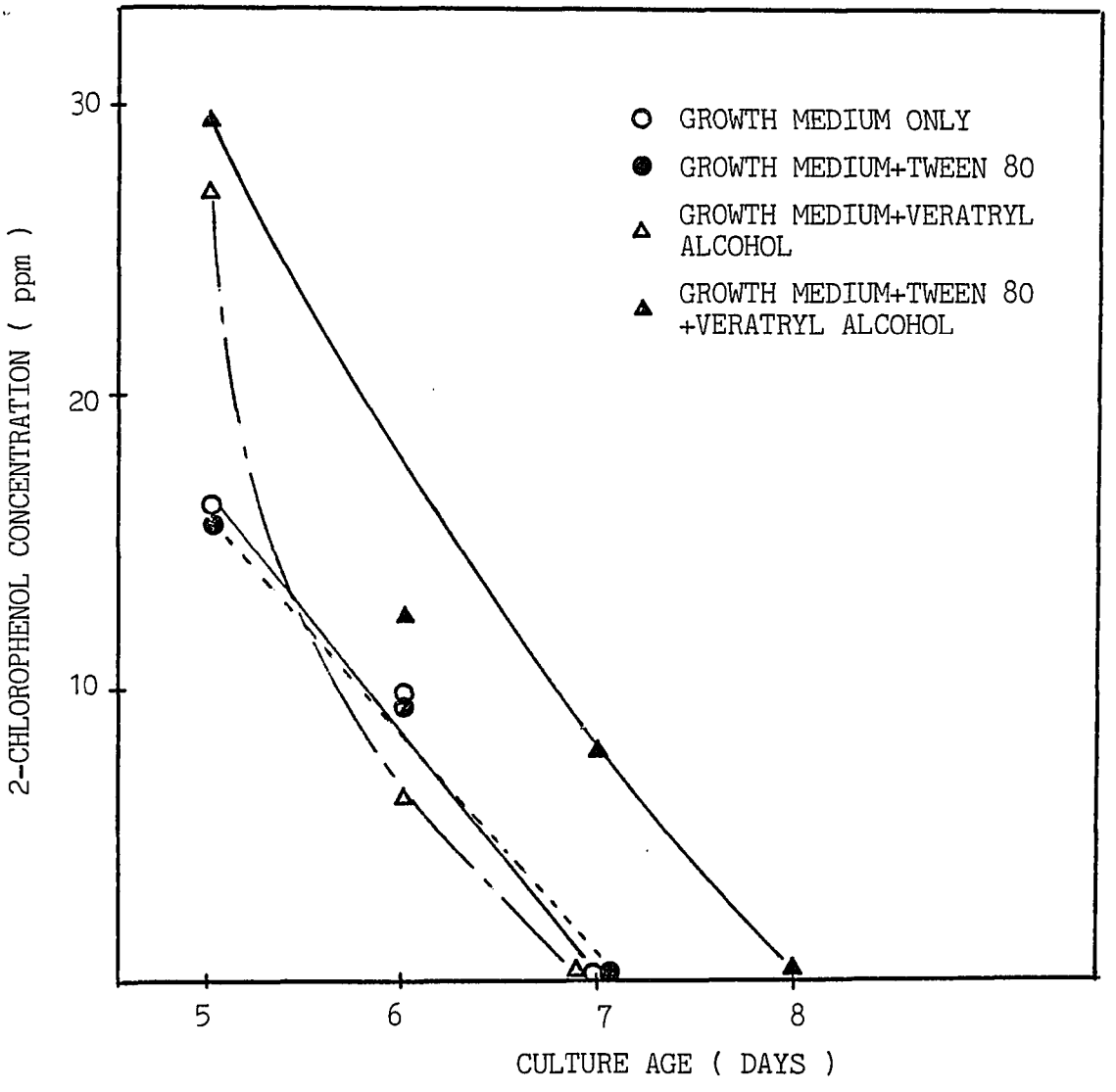


FIGURE 5

BIODEGRADATION OF 2-CHLOROPHENOL IN SHAKER FLASK

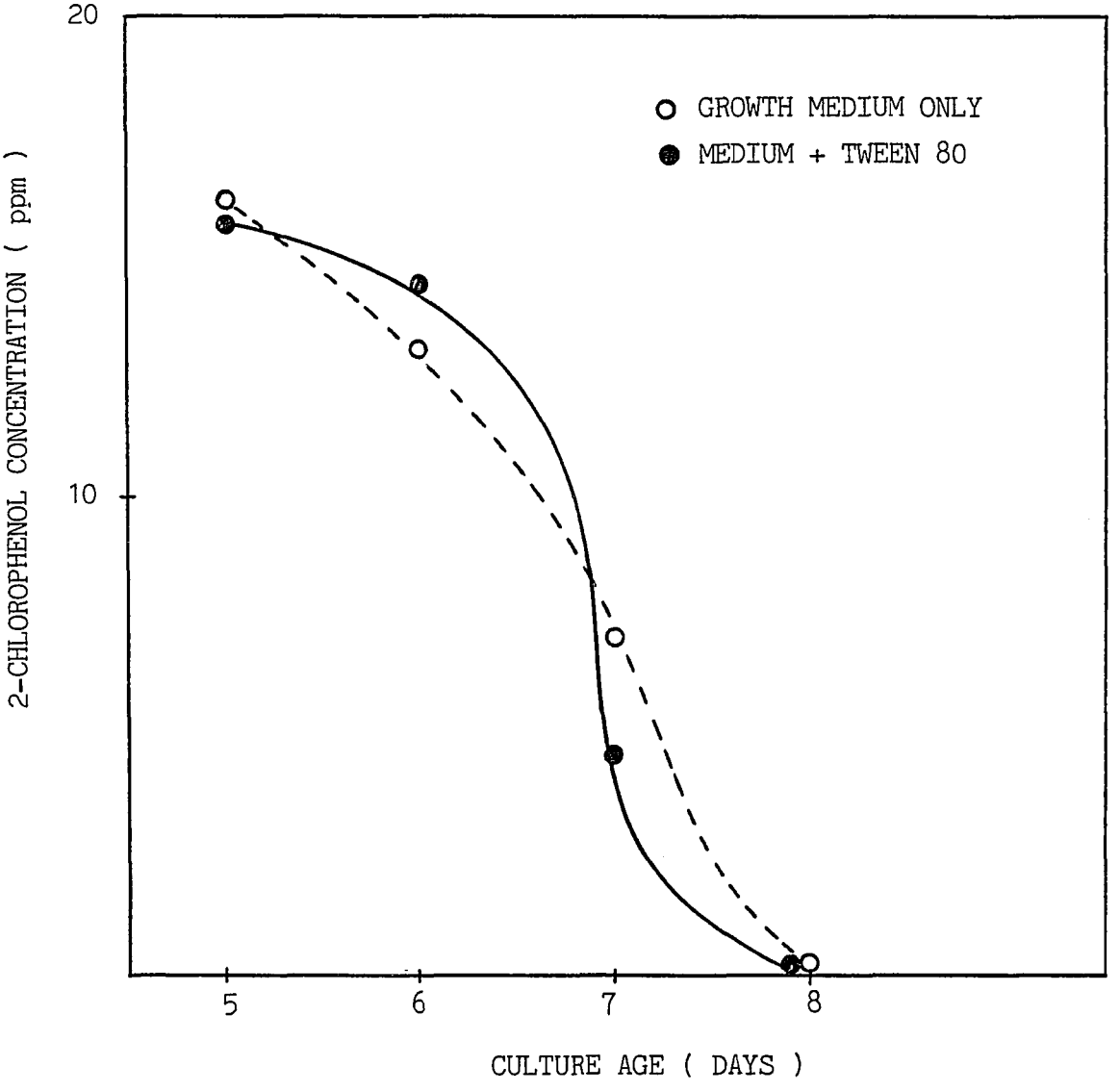


FIGURE 6

BIODEGRADATION OF 2-CHLOROPHENOL IN SHAKER FLASK

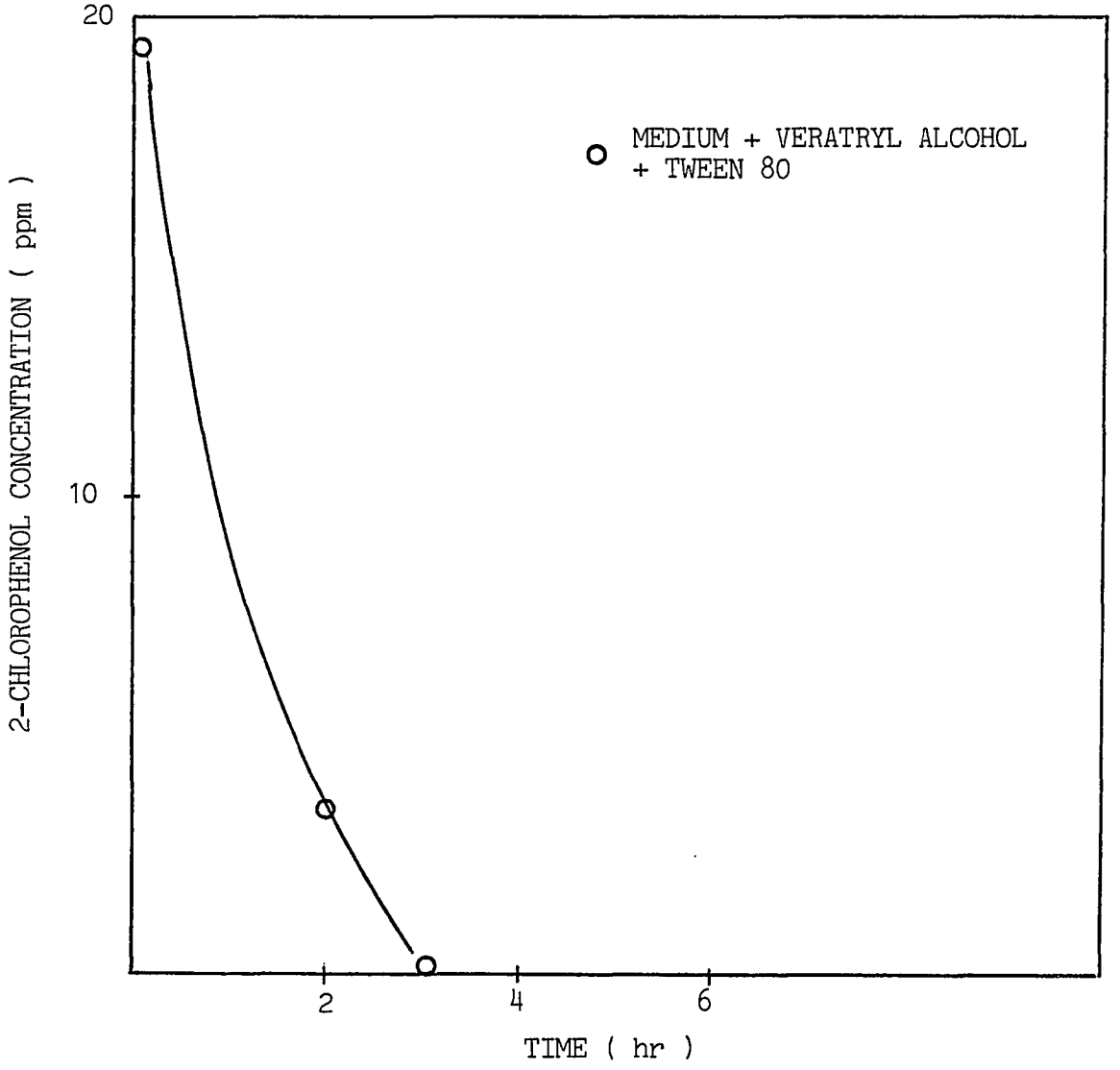


FIGURE 7

CULTURE OF PHANEROCHAETE CHRYSOSPORIUM IN BATCH FERMENTOR

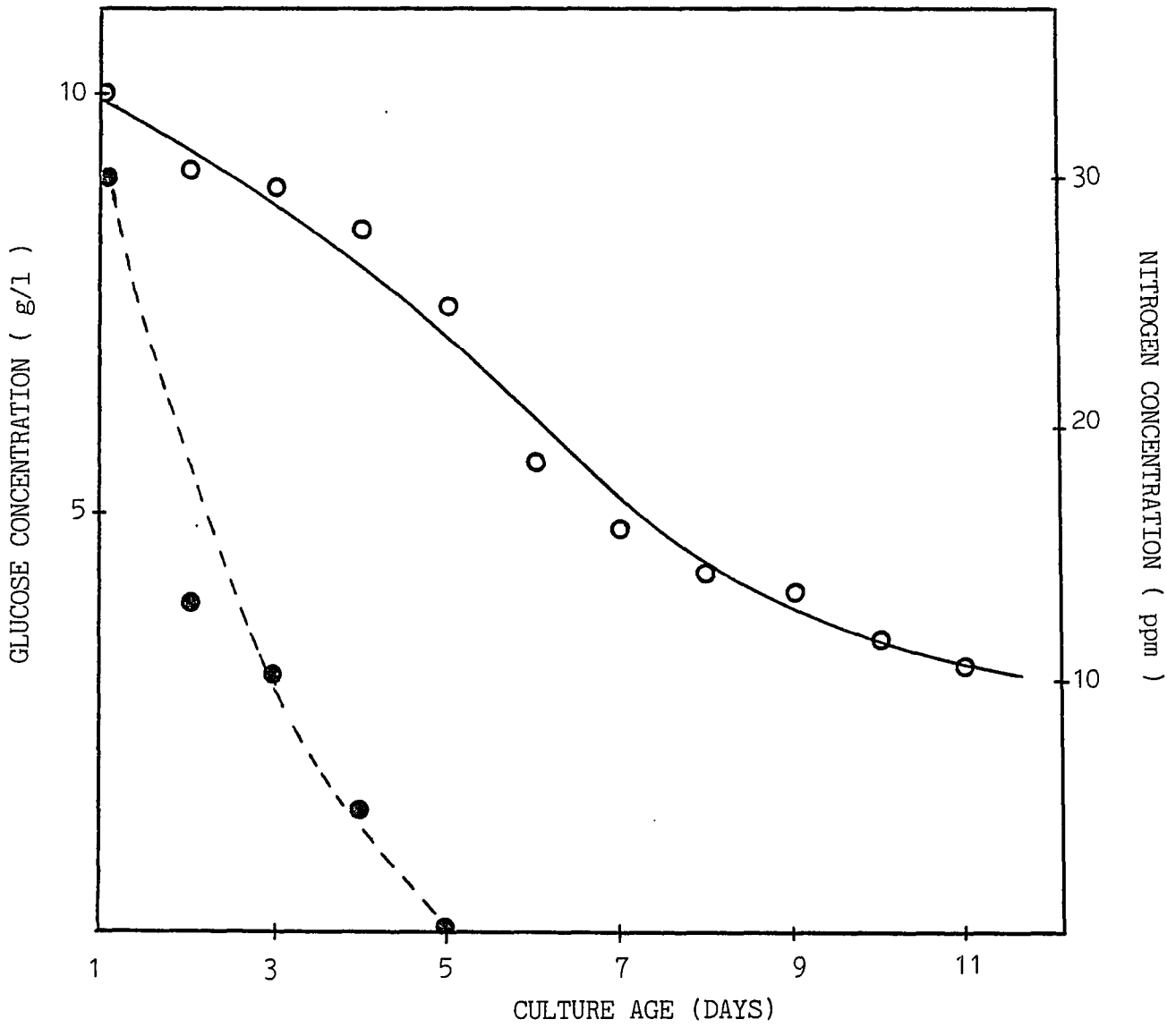


FIGURE 8

RELATIONSHIP BETWEEN CULTURE GROWTH AND DEPLETION OF GLUCOSE AND NITROGEN

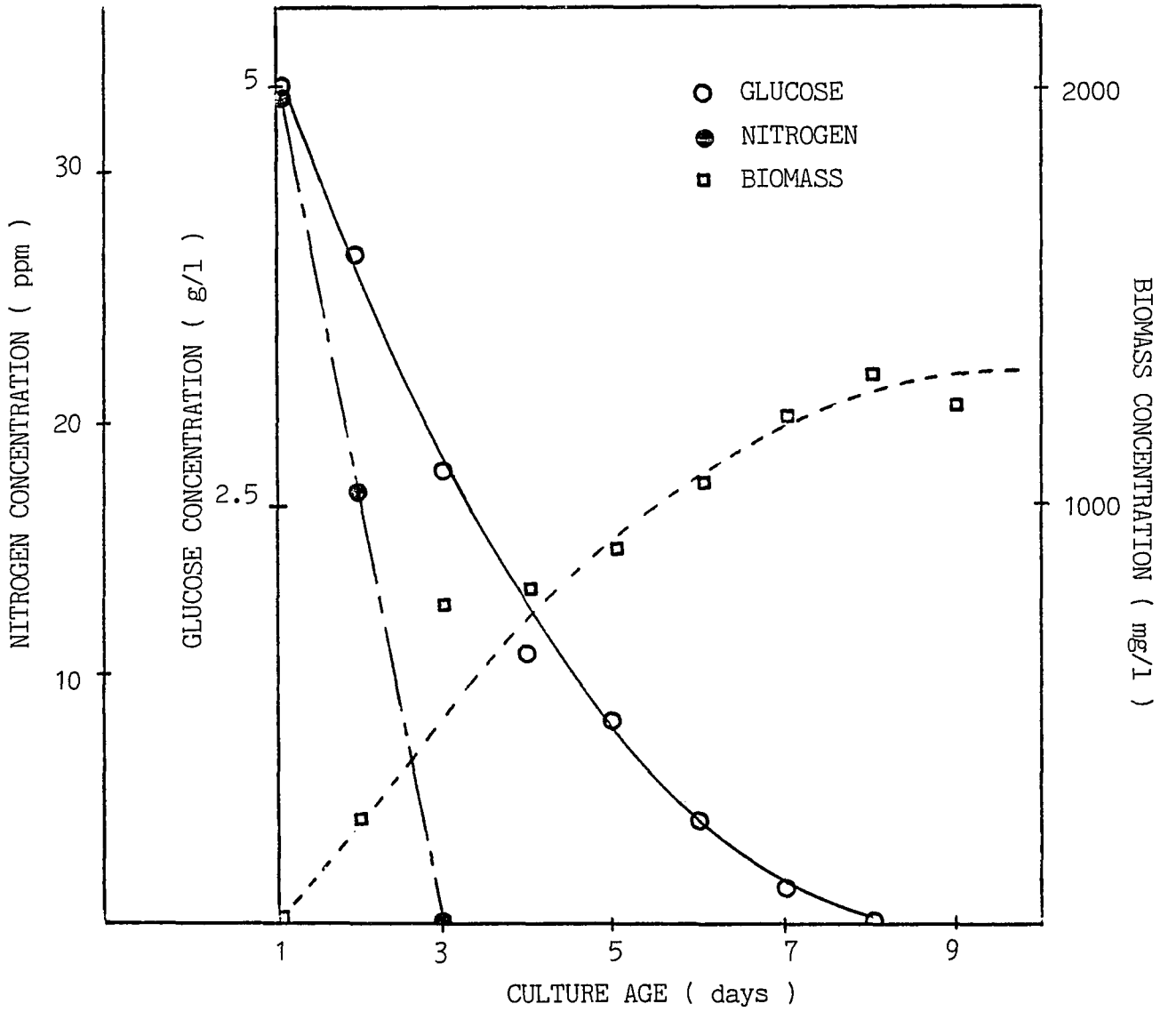


FIGURE 9

DEGRADATION OF 2-CHLOROPHENOL IN THE PRESENCE OF VERATRYL ALCOHOL AND TWEEN 80 AS INDUCER

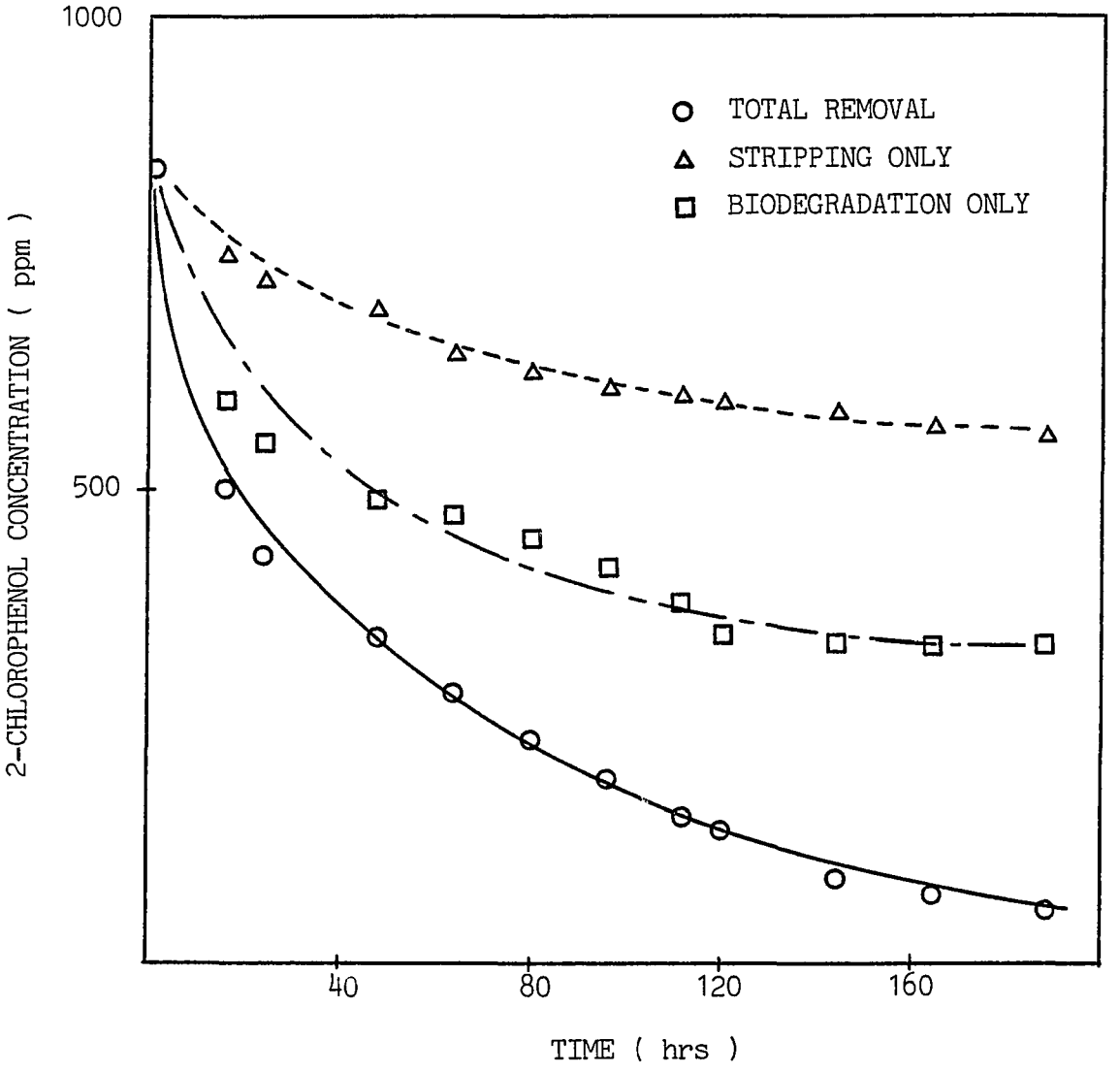


FIGURE 10

DEGRADATION OF 2-CHLOROPHENOL IN THE PRESENCE OF VERATRYL ALCOHOL AS INDUCER

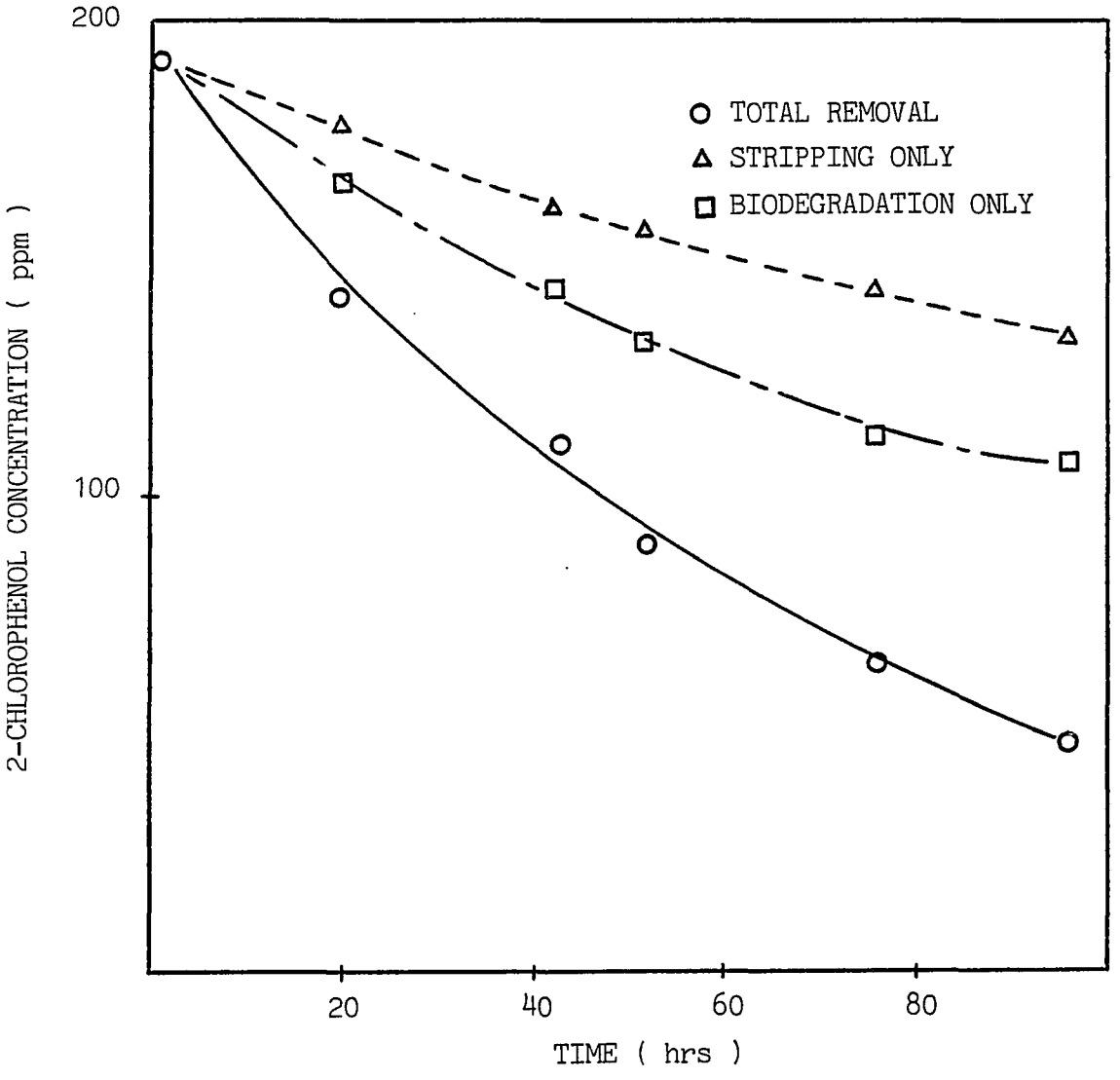


FIGURE 11

STRIPPING OF 2-CHLOROPHENOL IN BATCH FERMENTOR

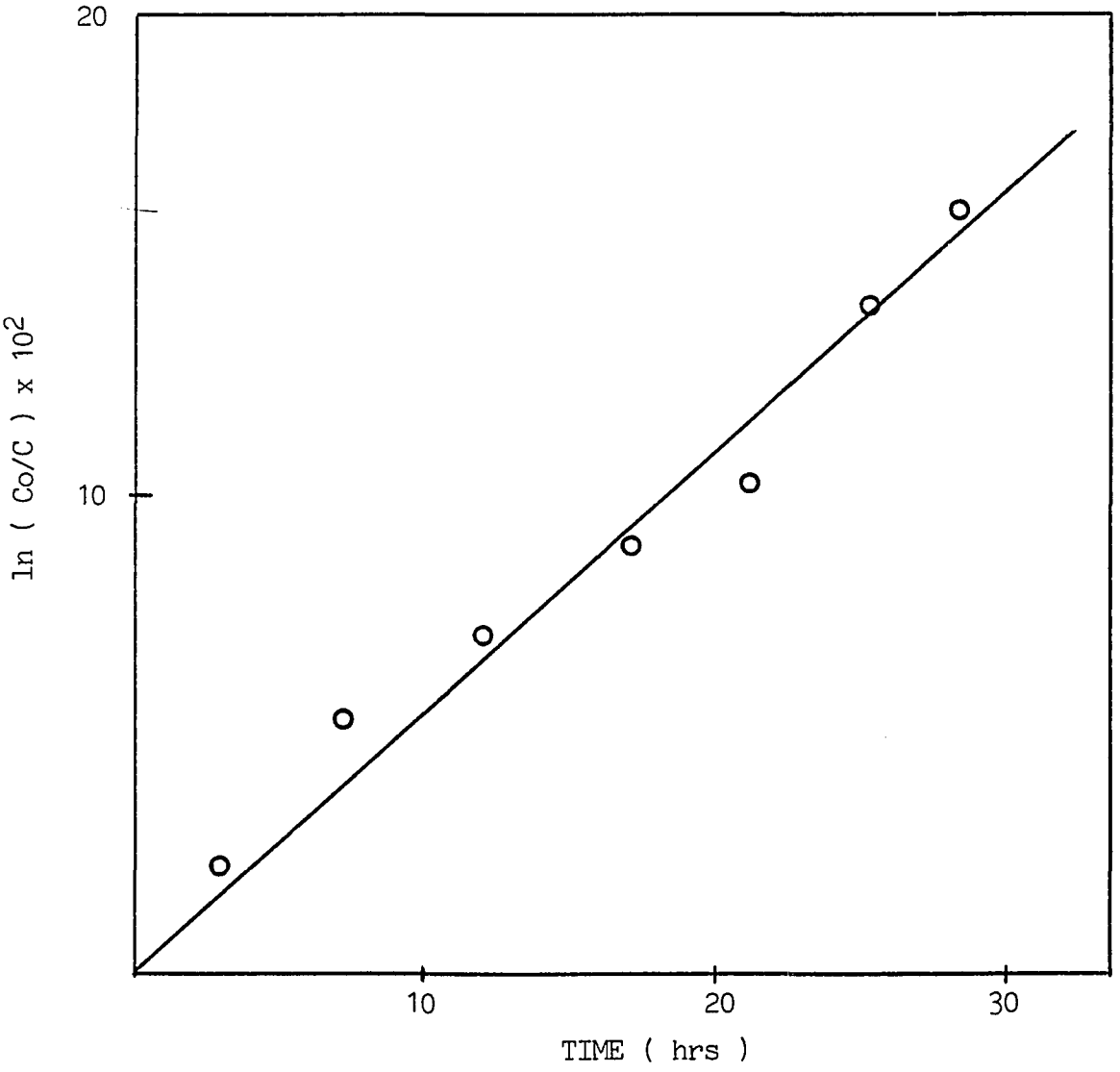


FIGURE 12

Electron Scanning Micrographs of Cross Sections of Silica-Based Sphere A: Cross Section of Silica-Based Sphere (35x). B: Fungus inside Sphere (1000x)

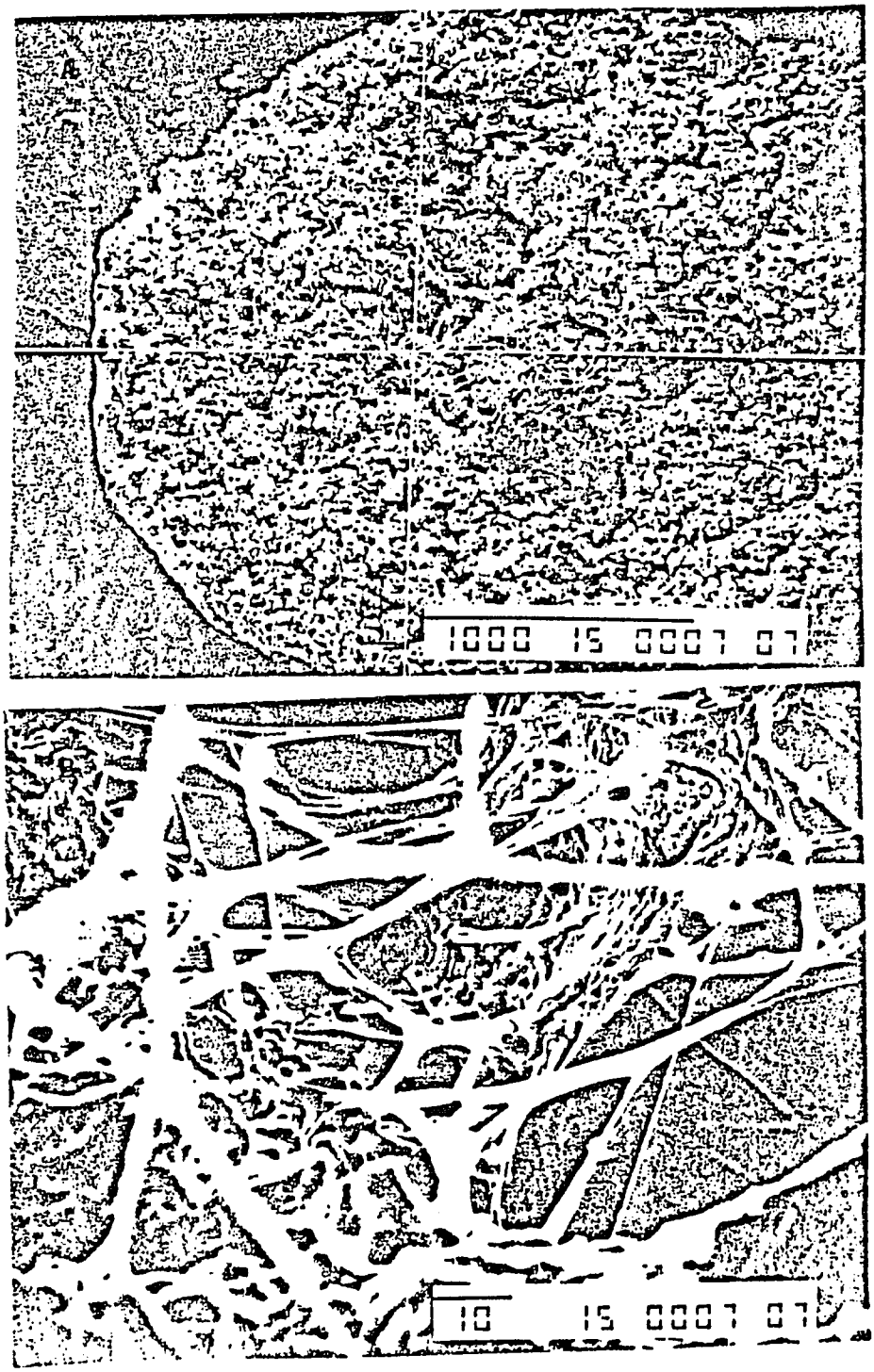


FIGURE 13

Electron Scanning Micrographs of Cross Sections of Silica-Based Sphere A: Cross Section of Silica-Based Sphere (35x). B: Fungus inside Sphere (2000x)

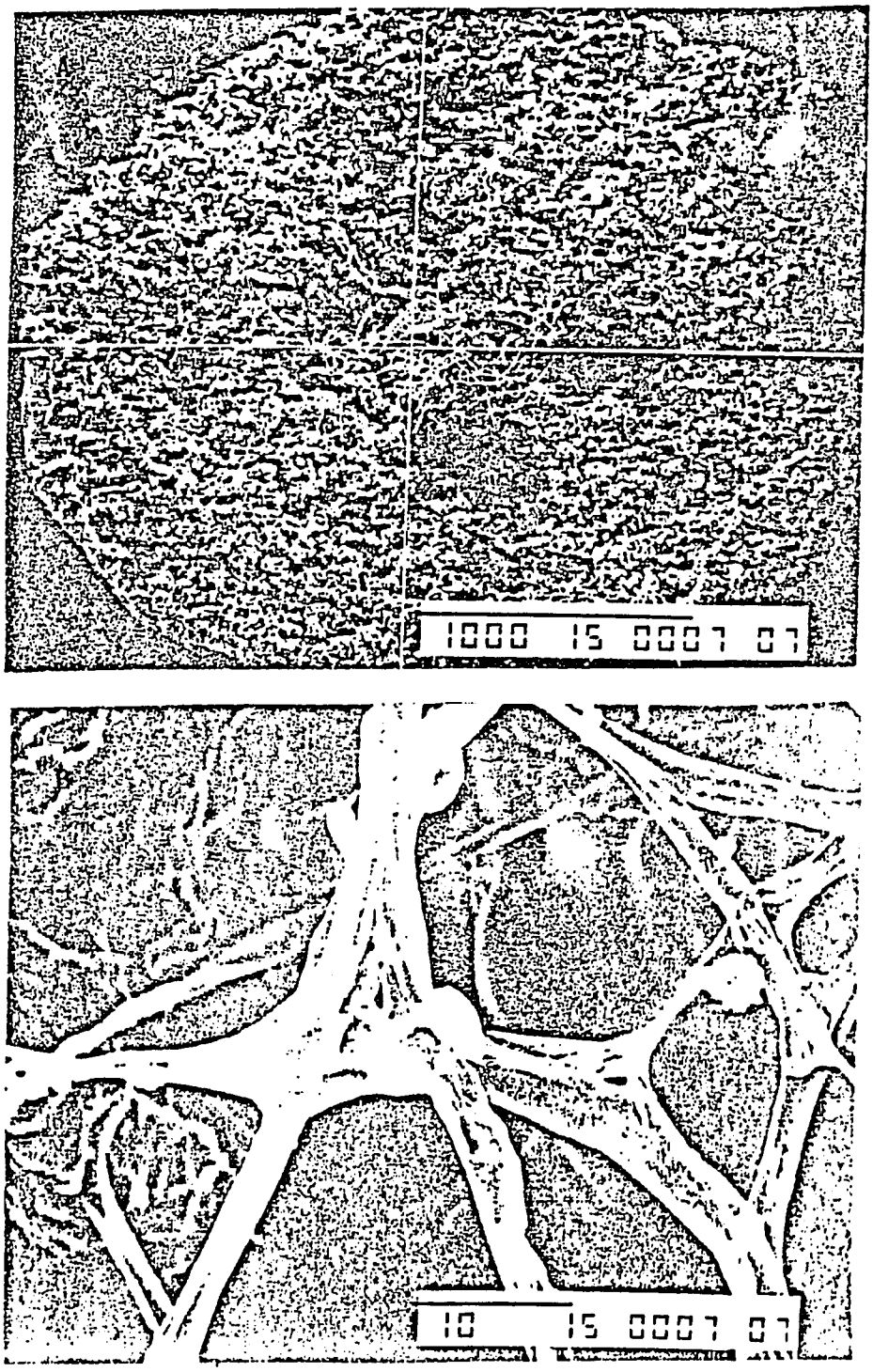


FIGURE 14

PERFORMANCE OF PACKED-BED REACTOR (2" DIAMETER) WITH SILICA BASED CYLINDER SHAPED CARRIER

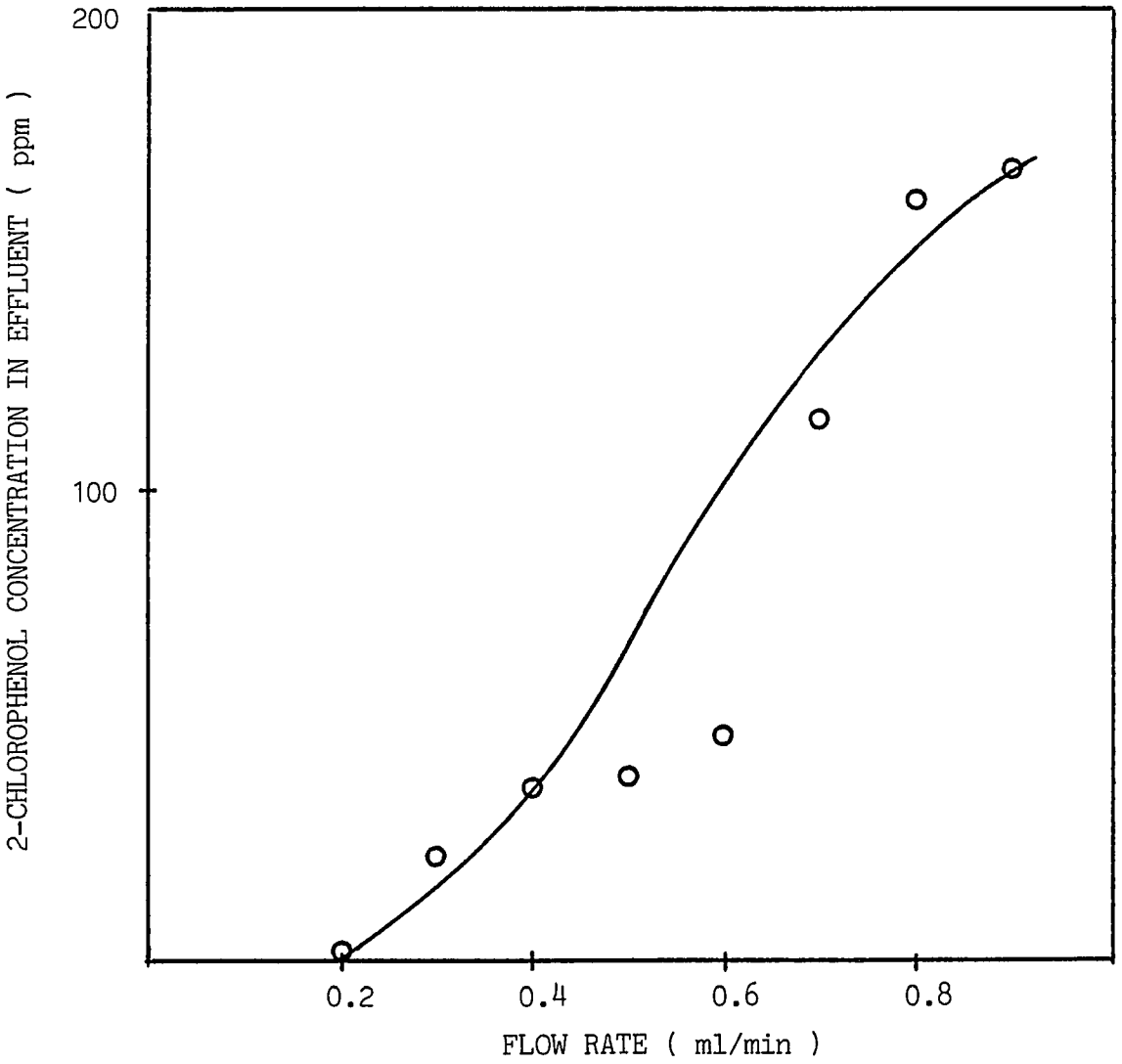


FIGURE 15

COMPARISON BETWEEN MICHAELIS-MENTEN EQUATION AND EXPERIMENTAL DATA FROM PACKED-BED REACTOR ( 2" DIAMETER )

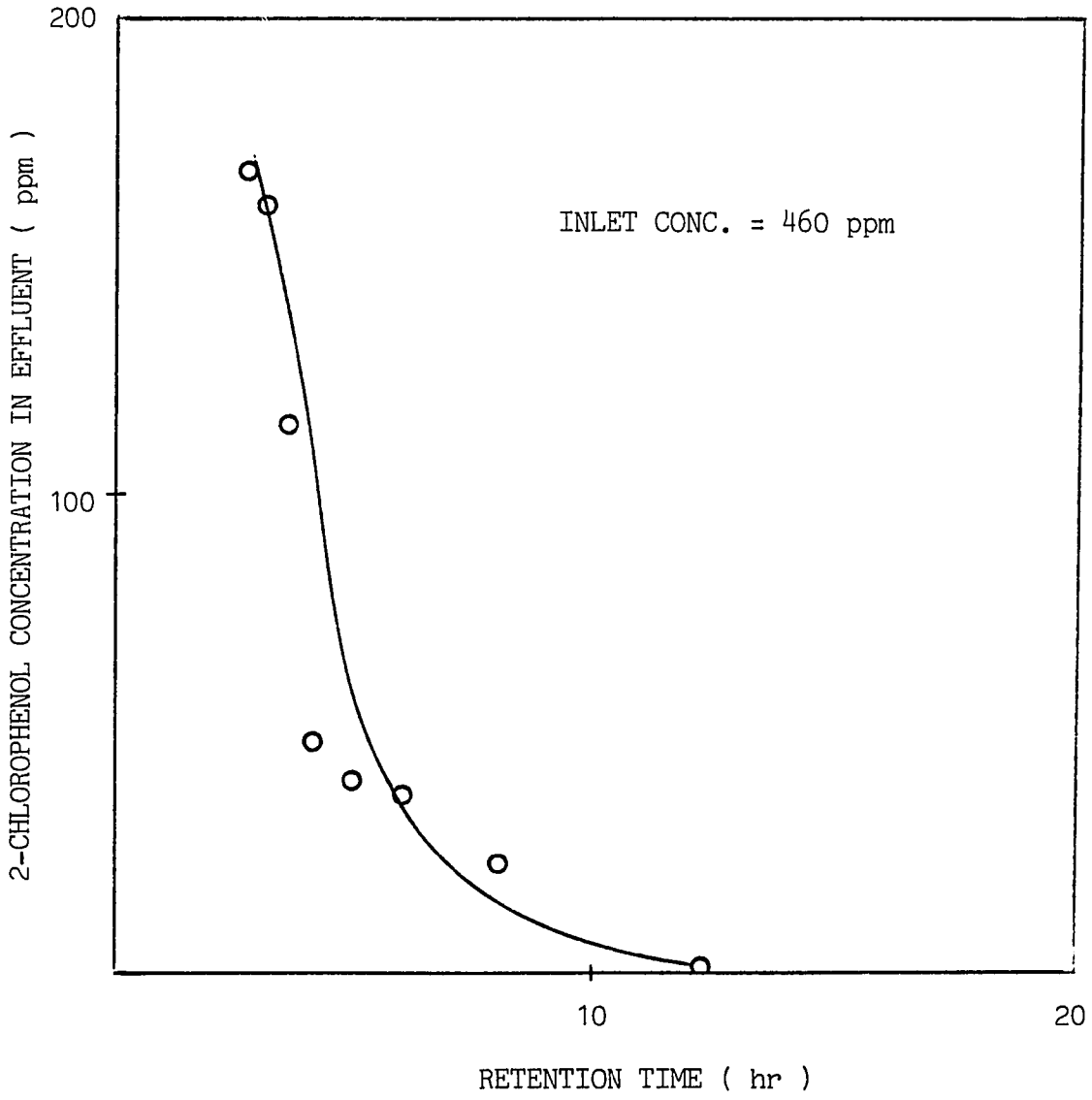


FIGURE 16

PERFORMANCE OF PACKED-BED REACTOR (4" DIAMETER) WITH SILICA BASED CYLINDER SHAPED CARRIER

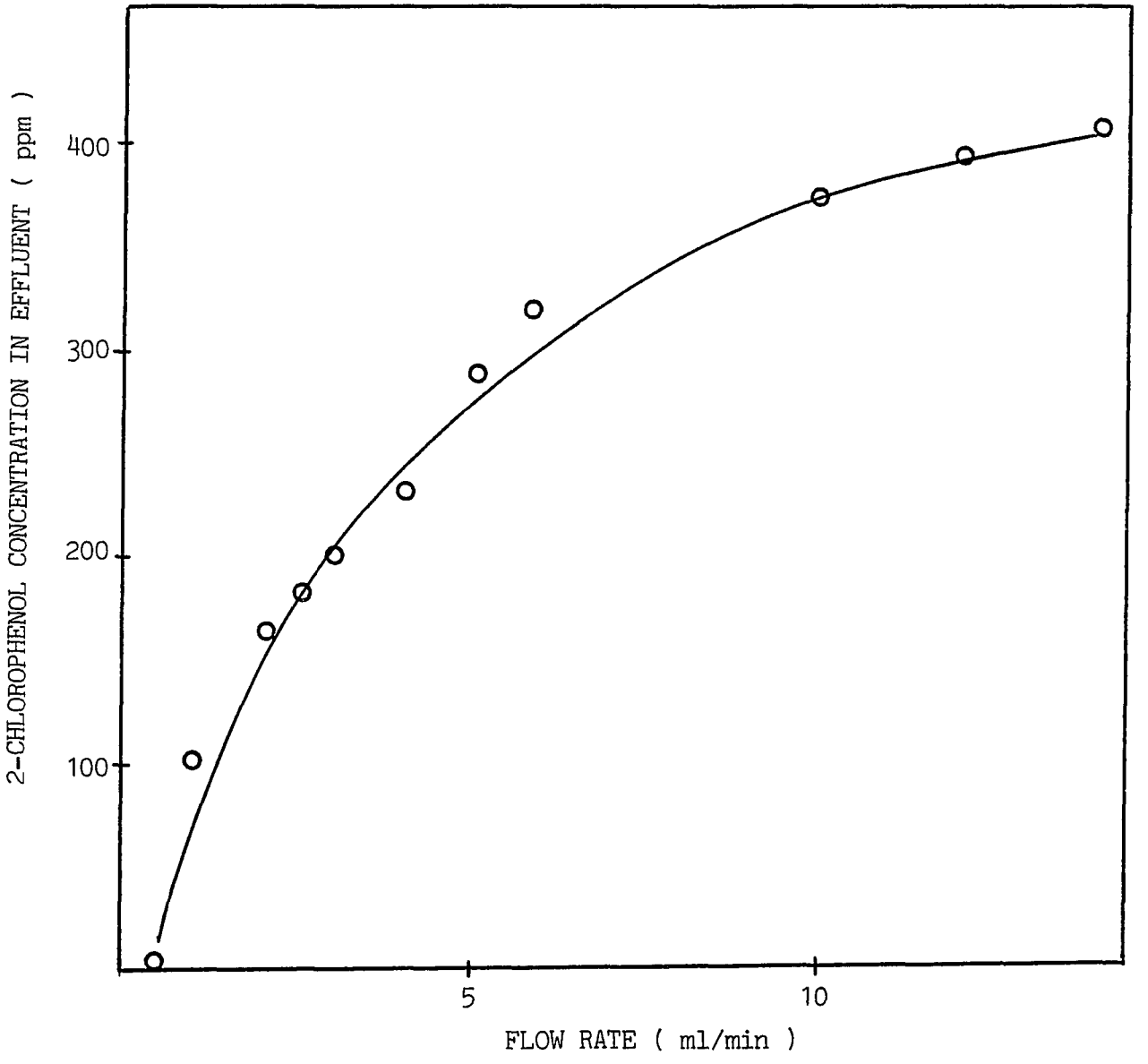


FIGURE 17

COMPARISON BETWEEN MICHAELIS-MENTEN EQUATION AND EXPERIMENT
DATA FROM PACKED-BED REACTOR ( 4" DIAMETER )

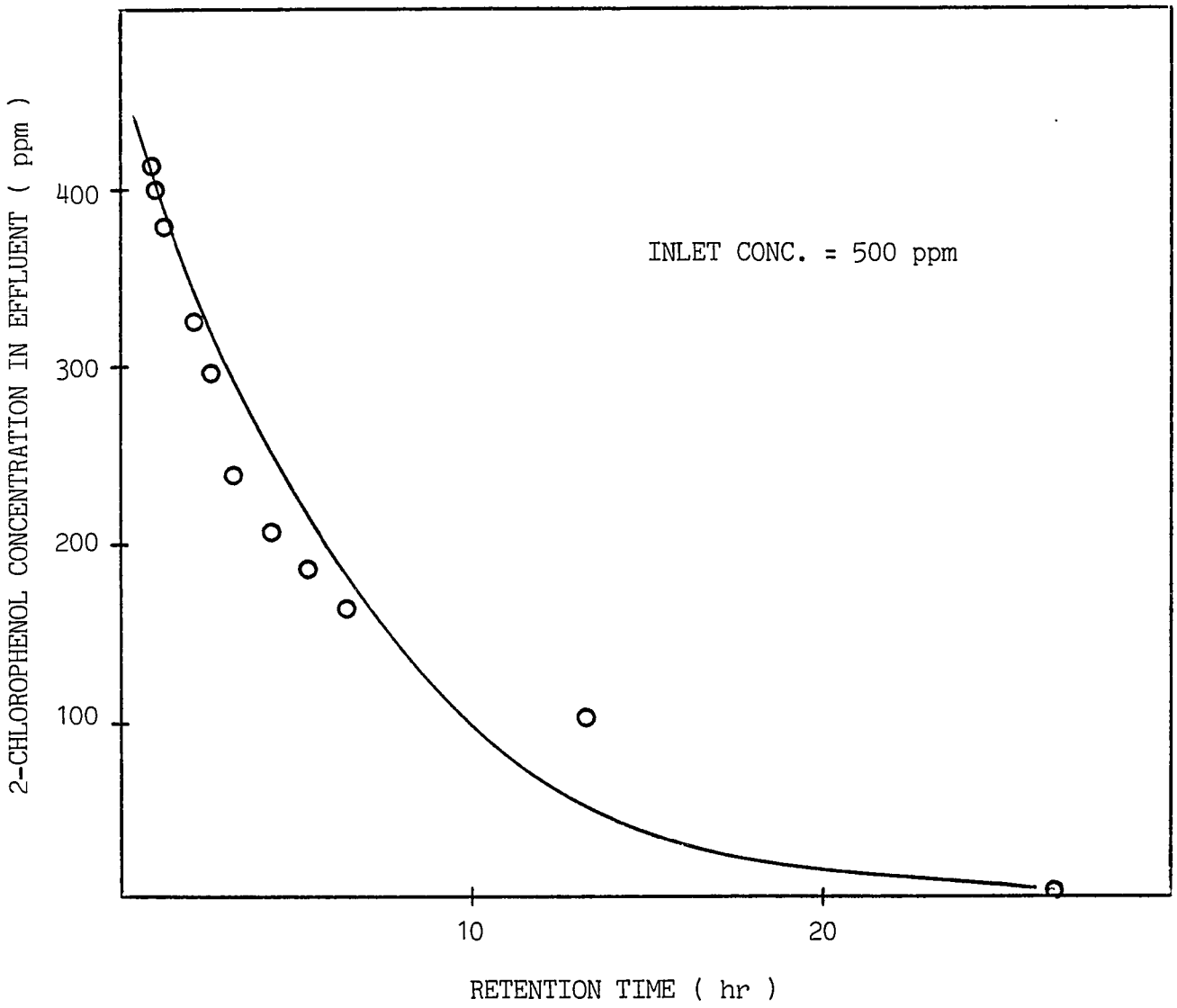


FIGURE 18

PERFORMANCE OF FLUIDIZED-BED REACTOR WITH IMMOBILIZED ALGINATE BEADS

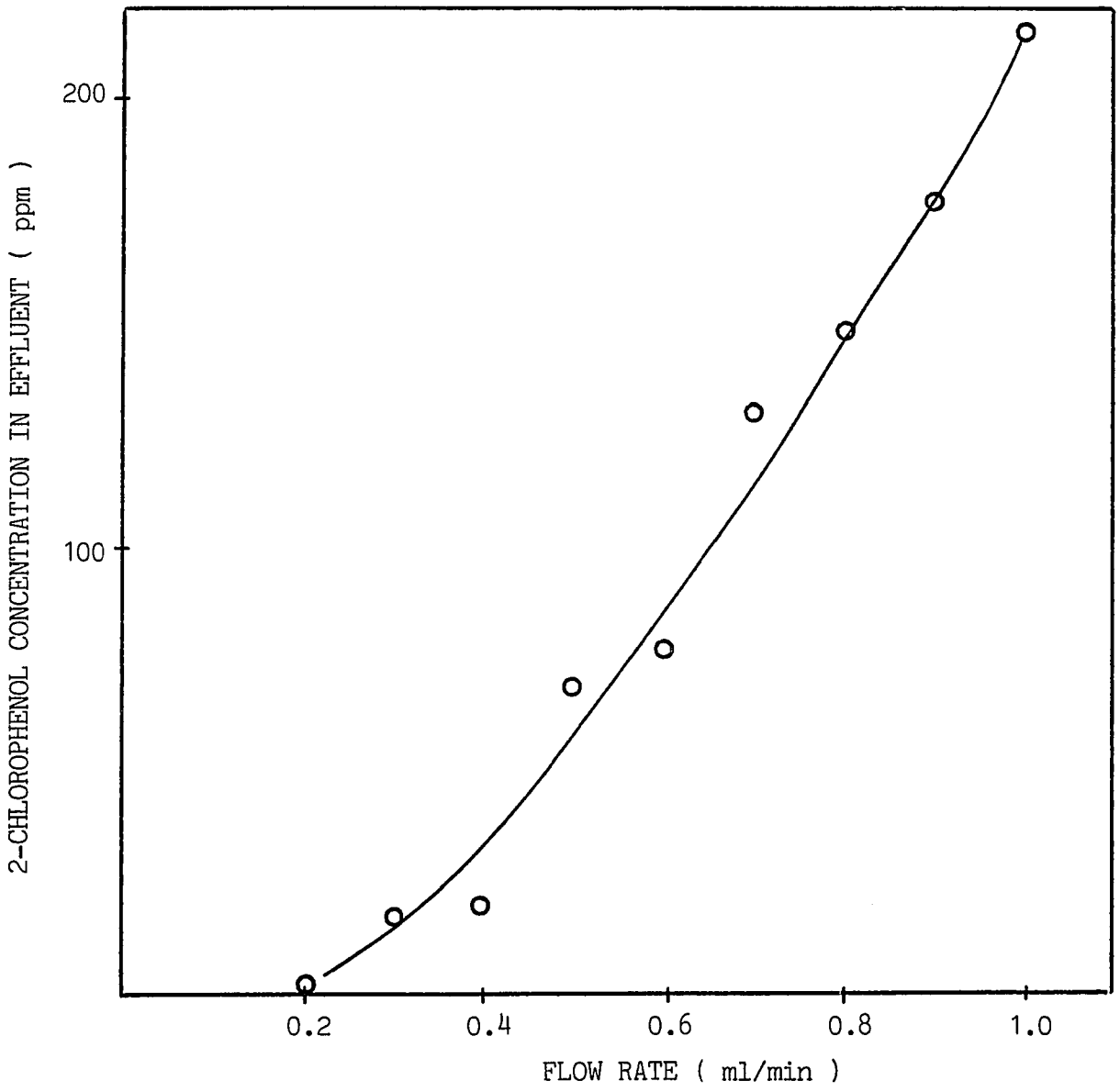


FIGURE 19

COMPARISON BETWEEN MICHAELIS-MENTEN EQUATION AND EXPERIMENT DATA FROM FLUIDIZED-BED REACTOR

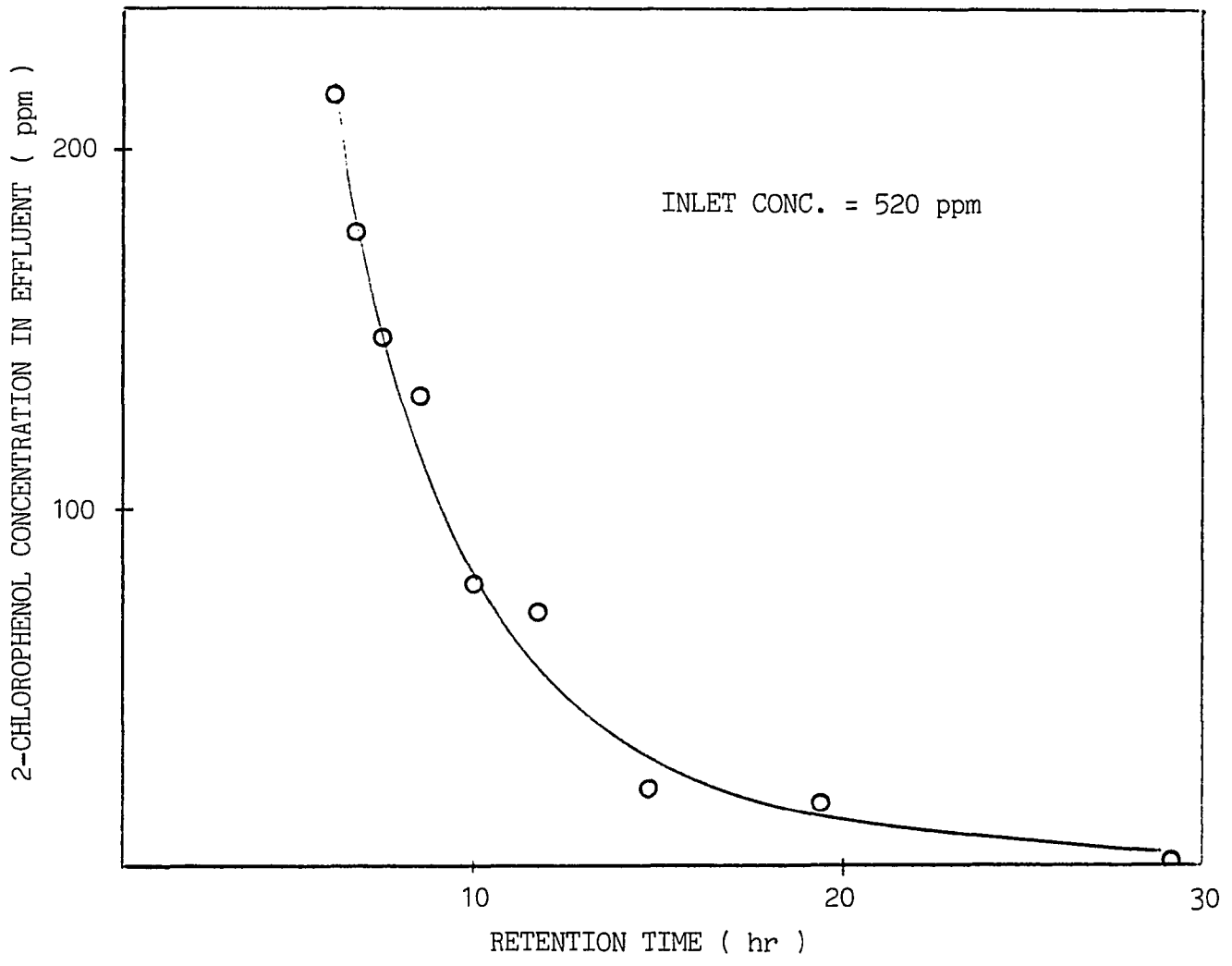


FIGURE 20

CONCENTRATION GRADIENT IN IMMOBILIZED ALGINATE BEAD

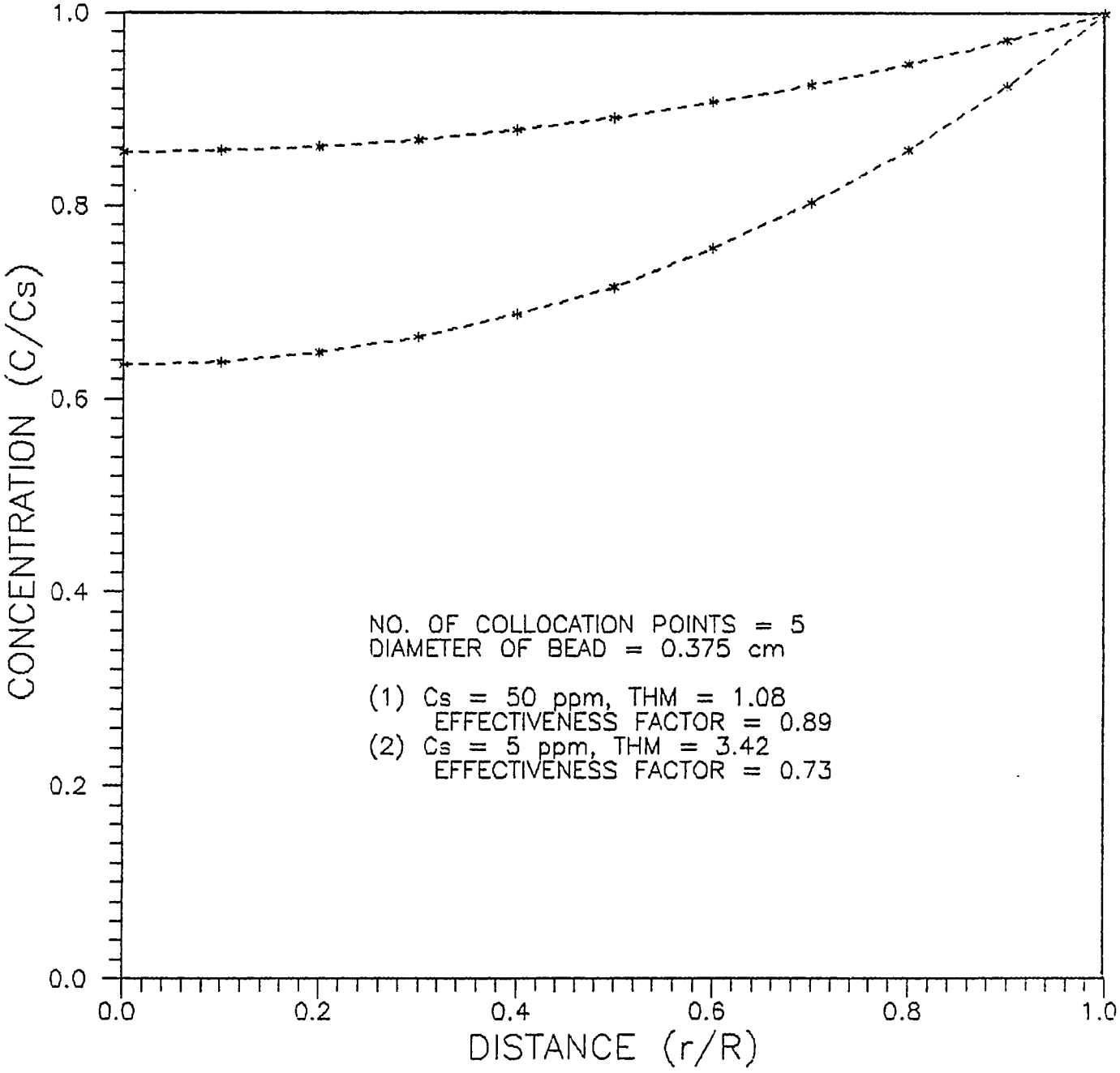
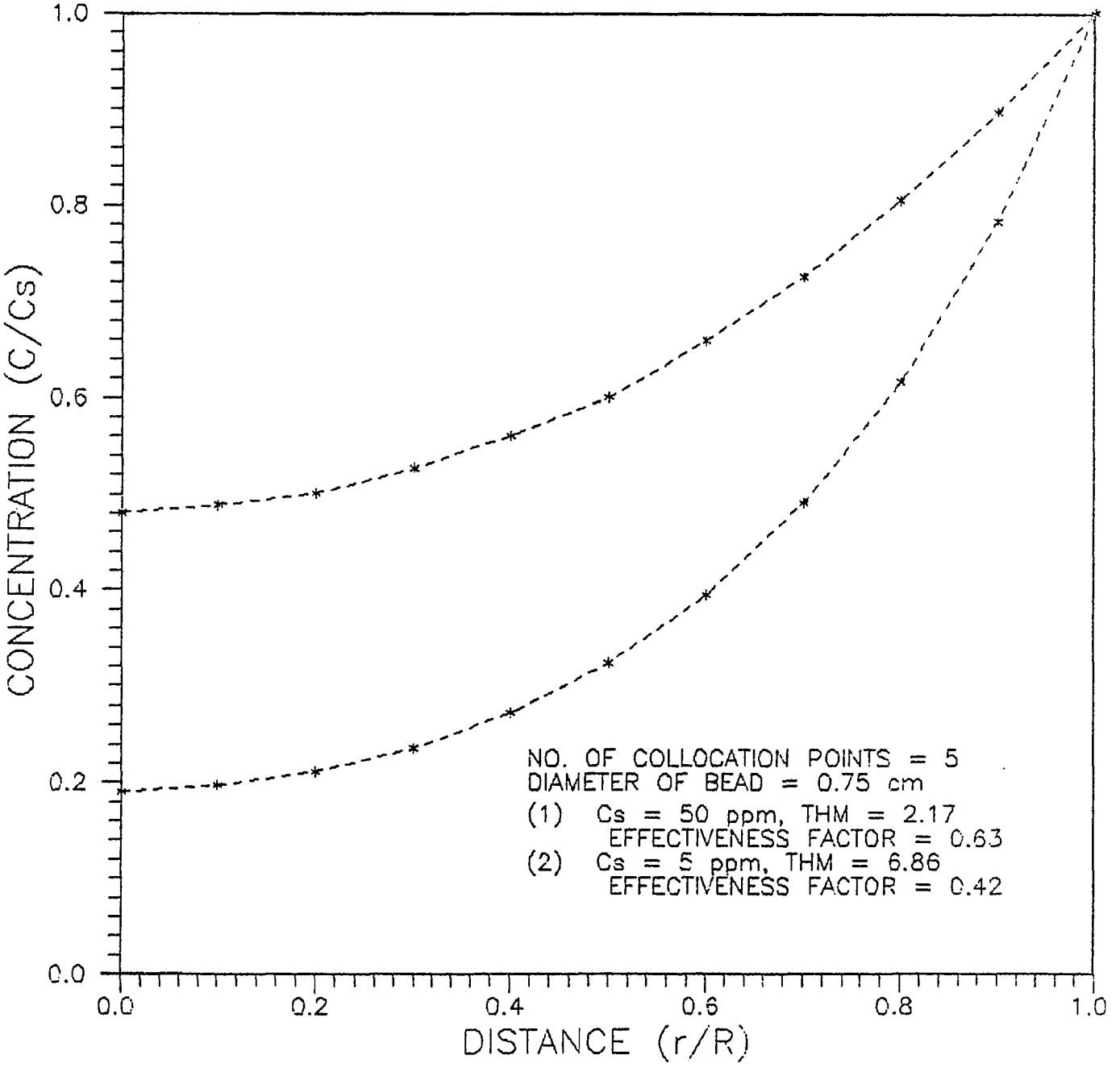


FIGURE 21

CONCENTRATION GRADIENT IN IMMOBILIZED ALGINATE BEAD



# APPENDIX A

## USE OF ORHTOGONAL COLLOCATION TO SOLVE NONLINEAR DIFFERENTIAL EQUATIONS

## Diffusion and Enzyme Catalytic Reaction Inside an Alginate Bead

The shell mass-balance method and Fick's law are used to describe the diffusion and reaction processes inside an alginate bead. An alginate bead of Radius R is submerged in a liquid stream containing substrate A. In the neighborhood of the surface of an alginate bead, the concentration of substrate A is $C_{As}$ moles per unit volume. Substrate A disappears according to Michaelis-Menten kinetic equation. The resulting mass balance is:

$$\frac{d^2C_A}{dr^2} + \frac{2}{r}\frac{dC_A}{dr} = \frac{Vm}{D}\frac{C_A}{km + C_A} \qquad (1)$$

The boundary conditions are as follows:

at $r = 0$ $\qquad \dfrac{dC_A}{dr} = 0$

at $r = R$ $\qquad C_A = C_{As}$

Equation (1) can be expressed in the dimensionless form by the introduction of the following parameters:

$$y = \frac{C_A}{C_{As}} \qquad x = \frac{r}{R}$$

$$\Phi^2 = \frac{(Vm)(R^2)}{D\ C_{As}} \qquad G = \frac{km}{C_{As}}$$

Equation (1) then becomes

$$\frac{d^2y}{dx^2} + \frac{2}{x}\frac{dy}{dx} = \Phi^2\frac{y}{G + y} \qquad (2)$$

with the following boundary conditions:

$$\text{at} \quad x = 1 \qquad\qquad y = 1$$

$$\text{at} \quad x = 0 \qquad\qquad \frac{dy}{dx} = 0$$

Equation (2) can be rewritten in terms of $u = x^2$ for $0 \leq x \leq 1$ giving:

$$u \frac{d^2y}{du^2} + \frac{3}{2} \frac{dy}{du} = \frac{\Phi^2}{4} \frac{y}{6 + y} \qquad (3)$$

**Power Series Representation of $y_N$**

An approximate solution to equation (3) is obtained in the following form:

$$y_N = 1 + ( 1 - u ) \sum_{i=1}^{N} a_i \, u^{i-1} \qquad (4)$$

The collocation method consists in substituting $y_N$ into the differential equation and and imposing the residual to be zero at N interior points $u_i$. The resulting N equations can be solved for **a**. Since $y_N$ in equation (4) is polynomial of degree N in u may be reformulated into an Nth-degree Lagrangian interpolation polynomial:

$$y_N = \sum_{i=1}^{N+1} l_i(u) \; y(u_i) \qquad (5)$$

where $y_{N+1} = y_{u=1} = 1$

$$l_i = \frac{P_{N+1}(x)}{(x - x_i)\, P^{(1)}_{N+1}(x_i)}$$

$P_{N+1}(x) = (x - x_1)\ldots\ldots (x - x_{N+1})$ is a polynomial of degree N+1 with leading coefficient 1. Equation (5) contains N unknown ordinates $y(u_i)$, i = 1.....N instead of the N unknown coefficients **a**.

N interior collocation points $u_1, u_2, u_3, \ldots\ldots u_N$ are equal to zeros of Jacobi polynomial and yield the node polynomial

$$P_{N+1}(u) = \sum_{j=1}^{n+1} (u - u_j) \qquad (6)$$

in which $u_{N+1} = 1$

The residual

$$R_N = u \frac{d^2 y_N}{du^2} + \frac{3}{2} \frac{dy_N}{du} - \frac{\Phi^2}{4} \frac{y_N}{G + y_N}$$

is evaluated and equated to zero at the N interior $u_j$.

$$R_N(u_j) = u_j \left(\frac{d^2 y_N}{du^2}\right)_{u=u_j} + \frac{3}{2} \left(\frac{dy_N}{du}\right)_{u=u_j} - \frac{\Phi^2}{4} \left(\frac{y_N}{G+y_N}\right)_{u=u_j}$$

$$= u_j \left(\sum_{i=1}^{n+1} B_{ji}\, y_i\right) + \frac{3}{2} \sum_{i=1}^{n+1} A_{ji}\, y_i - \frac{\Phi^2}{4} \left(\frac{y_j}{G+y_j}\right)$$

$$= 0 \qquad j = 1, 2, \ldots\ldots, N \qquad (7)$$

$$A_{ji} = \frac{1}{x_j - x_i} \frac{P^{(1)}_{N+1}(x_j)}{P^{(1)}_{N+1}(x_i)} = l_i^{(1)}(x_j)$$

$$B_{ji} = \frac{1}{x_j - x_i} \left( \frac{P^{(2)}_{N+1}(x_j)}{P^{(1)}_{N+1}(x_i)} - 2\, l^{(1)}_i(x_j) \right)$$

$$= 2\, l^{(1)}_i(x_j) \left( l^{(1)}_j(x_j) - \frac{1}{x_j - x_i} \right)$$

Equation (7) can be rearranged to

$$u_j \sum_{i=1}^{n} B_{ji}\, y_i + \frac{3}{2} \sum_{i=1}^{n} A_{ji}\, y_i - \frac{\Phi^2}{4} \left( \frac{y_j}{G + y_j} \right)$$

$$= - u_j\, B_{j,N+1} - \frac{3}{2}\, A_{j,N+1} \qquad j = 1, 2, \ldots, N \qquad (8)$$

The unknown $y_i$ can be obtained from equation (8).

## Jacobi Polynomials

Jacobi polynomials can be written in the following form:

$$P^{(\alpha,\beta)}_N(x) = \sum_{i=0}^{N} (-1)^{N-1}\, \gamma_i\, x^i \qquad (9)$$

$\gamma_0$ is taken to be 1 and the remaining N coefficients can be found either directly from the orthogonal property

$$\int_0^1 x^{\beta} (1 - x)^{\alpha} P_j(x)\, P_N(x)\, dx = 0 \qquad j = 0, 1, \ldots, N-1 \qquad (10)$$

or from any of a number of other relations that are all in one way or the other derived from the fundamental relation (10). A more convenient form of (10) is

$$\int_0^1 x^{\beta} (1 - x)^{\alpha} x^{j} P_N(x)\, dx = 0 \qquad j = 1, 2, \ldots, N-1 \qquad (11)$$

The following set of linear equations for $\gamma_i$ is derived from the integrals (11).

$$\mathbf{M}\,\gamma = 0$$

$$M_{ji} = \frac{\Gamma(\beta + 1 + i + j)\,\Gamma(\alpha + 1)}{\Gamma(\alpha + \beta + 2 + i + j)} (-1)^{N-1}$$

$$i = 0, 1, \ldots\ldots, N \qquad j = 0, 1, \ldots\ldots, N-1$$

since

$$\int_0^1 x^{m} (1 - x)^{n}\, dx = \frac{\Gamma(m + 1)\,\Gamma(\alpha + 1)}{\Gamma(m + n + 2)}$$

## Notation

| | |
|---|---|
| D | Diffusion coefficient of substrate |
| Vm | Maximum velocity in Michaelis-Menten equation |
| Km | Michaelis-Menten constant |
| $\Phi$ | Thiele modulus |
| R | Radius of catalysis |
| $C_{As}$ | Concentration of A at the surface of catalysis |

# APPENDIX B

## COMPUTER PROGRAM

1. ORTHOGOANL COLLOCATION METHOD
2. RUNGEKUTTA METHOD
3. LINEAR REGRESSION

```
C
C       ************************************************
C       *                                              *
C       *        PROGRAM    ORTHOGONAL COLLOCATION     *
C       *                                              *
C       ************************************************
C
C       THIS PROGRAM IS TO SOLVE THE SECOND ORDER NONLINEAR
C       DIFFERENTIAL EQUATION USING ORTHOGONAL COLLOCATION
C       METHOD
C
        PROGRAM SOLVE
        IMPLICIT REAL*8 (A-H,O-Z)
        REAL K2
        DIMENSION DIF1(10),DIF2(10),DIF3(10),ROOT(10),V1(10)
        DIMENSION XINTP(10),Y(10),BMAT(10,10),B(10,10),V2(10)
6        FORMAT(1X,8F10.4)
200      WRITE(*,7)
7        FORMAT(' INPUT N,IS,THM '/)
        READ(*,*) N,IS,THM
        WRITE(*,8)
8        FORMAT(' INPUT K2 AND CS '/)
        READ(*,*) K2,CS
        IF (N .EQ. 0) GOTO 100
        S=IS
        ALFA=1.
        BETA=(S-1)/2
        CALL JCOBI(10,N,0,1,ALFA,BETA,DIF1,DIF2,DIF3,ROOT)
        NT=N+1
        WRITE(*,6) (ROOT(I),I=1,NT)
C
C       SET UP LAPLACIAN MATRIX
C
        DO 10 I=1,NT
        CALL DFOPR(10,N,0,1,I,1,DIF1,DIF2,DIF3,ROOT,V1)
        CALL DFOPR(10,N,0,1,I,2,DIF1,DIF2,DIF3,ROOT,V2)
        DO 11 J=1,NT
11       BMAT(I,J)=4*(ROOT(I)*V2(J)+(S+1)/2*V1(J))
10      continue
C
C       INITIAL ESTIMATION OF SOLUTION VECTOR
C
        DO 20 I=1,NT
20       Y(I)=1.
        ITNO=0
        G=K2/CS
C
C       SET UP JACOBIAN AND SOLVE ALGEBRAIC EQUATION
C
30      DO 25 I=1,N
        B(I,NT)=BMAT(I,NT)*Y(NT)-THM**2*Y(I)/(G+Y(I))
        DO 26 J=1,N
```

```
          B(I,J)=BMAT(I,J)
          B(I,NT)=B(I,NT)+BMAT(I,J)*Y(J)
26        CONTINUE
          B(I,I)=B(I,I)-G/(G+Y(I))**2*THM**2
25        CONTINUE
          CALL  GAUSL(10,10,N,1,B)
          RES=0.
          DO 28 I=1,N
          Y(I)=Y(I)-B(I,NT)
28         RES=RES+B(I,NT)**2
          ITNO=ITNO+1
          IF (ITNO .GT. 100) GOTO 36
          IF (RES .GT. 1.D-16) GOTO 30
36        WRITE (*,15) ITNO, RES
15         FORMAT(1X,'ITNO=',I4,' RES=',D10.1)
          WRITE(*,6) (Y(I),I=1,NT)
C
C         EVALUATE EFFECTIVENESS FACTOR BY INTEGRATION
C
         CALL RADAU(10,N,0,1,1,0.D0,BETA,ROOT,DIF1,V1)
         ETA=V1(NT)
         DO 45 I=1,N
45        ETA=ETA+V1(I)*Y(I)**2
C
C         FIND SOLUTION AT X=0 TO 1
C
         DO 40 I=1,11
         X=(I-1)/10.D0
         CALL INTRP(10,NT,X*X,ROOT,DIF1,XINTP)
         YV=0.
         DO 41 J=1,NT
41        YV=YV+XINTP(J)*Y(J)
40       WRITE(*,17)  X,YV
17        FORMAT(1X,F7.3,F19.9)
         WRITE(*,50)  ETA
50        FORMAT(1X,'EFFECTIVENESS FACTOR=',F15.9)
         GOTO 200
100      STOP
         END
```

```
C
C       THIS PROGRAM CALCULATES THE ZEROS OF JACOBI
C       POLYNOMIALS AND THE THREE FIRST DERIVATIVES OF THE
C       NODE POLYNOMIAL
C
      SUBROUTINE JCOBI(ND,N,N0,N1,AL,BE,DIF1,DIF2,DIF3,ROOT)
      IMPLICIT REAL*8 (A-H,O-Z)
      DIMENSION DIF1(ND),DIF2(ND),DIF3(ND),ROOT(ND)
      AB=AL+BE
      AD=BE-AL
      AP=BE*AL
      DIF1(1)=(AD/(AB+2)+1)/2
      DIF2(1)=0.
      IF (N.LT.2) GOTO 15
      DO 10 I=2,N
      Z1=I-1
      Z=AB+2*Z1
      DIF1(I)=(AB*AD/Z/(Z+2)+1)/2
      IF (I .NE. 2) GOTO 11
      DIF2(I)=(AB+AP+Z1)/Z/Z/(Z+1)
      GOTO 10
11    Z=Z*Z
      Y=Z1*(AB+Z1)
      Y=Y*(AP+Y)
      DIF2(I)=Y/Z/(Z-1)
10    CONTINUE
C
C       ROOT DETERMINATION BY NEWTON METHOD WITH SUPPRESSION
C       OF PREVIOUSLY DETERMINED ROOTS
C
15     X=0.
      DO 20 I=1,N
25     XD=0.
      XN=1.
      XD1=0.
      XN1=0.
      DO 30 J=1,N
      XP=(DIF1(J)-X)*XN-DIF2(J)*XD
      XP1=(DIF1(J)-X)*XN1-DIF2(J)*XD1-XN
      XD=XN
      XD1=XN1
      XN=XP
30     XN1=XP1
      ZC=1.
      Z=XN/XN1
      IF (I .EQ. 1) GOTO 21
      DO 22 J=2,I
22     ZC=ZC-Z/(X-ROOT(J-1))
21     Z=Z/ZC
      X=X-Z
      IF (DABS(Z) .GT. 1.D-09) GOTO 25
      ROOT(I)=X
```

```
      X=X+.0001
20    CONTINUE


C
C     ADD EVENTUAL INTERPOLATION POINTS AT X=0 OR X=1
C
      NT=N+N0+N1
      IF  (N0 .EQ. 0) GOTO 35
      DO 31 I=1,N
      J=N+1-I
31     ROOT(J+1)=ROOT(J)
      ROOT(1)=0
35     IF (N1 .EQ. 1) ROOT(NT)=1.
C
C     EVALUATE DERIVATIVES OF POLYNOMIAL
C
      DO 40 I=1,NT
      X=ROOT(I)
      DIF1(I)=1.
      DIF2(I)=0.
      DIF3(I)=0.
      DO  40 J=1,NT
      IF (J.EQ.I) GOTO 40
      Y=X-ROOT(J)
      DIF3(I)=Y*DIF3(I) +3*DIF2(I)
      DIF2(I)=Y*DIF2(I) +2*DIF1(I)
      DIF1(I)=Y*DIF1(I)
40    CONTINUE
      RETURN
      END
```

```
C
C       THIS PROGRAM EVALUATES DISCRETIZATION MATRICES AND
C       GAUSSIAN QUADRATURE WEIGHTS, NORMALIZED TO SUM 1
C         ID = 1 :    DISCRETIZATION MATRIX FOR Y(1)
C         ID = 2 :    DISCRETIZATION MATRIX FOR Y(2)
C         ID = 3 :    GAUSSIAN QUADRATURE WEIGHTS
C
        SUBROUTINE DFOPR(ND,N,N0,N1,I,ID,DIF1,DIF2,DIF3,ROOT,VECT)
      IMPLICIT REAL*8 (A-H,O-Z)
      DIMENSION DIF1(ND),DIF2(ND),DIF3(ND),ROOT(ND),VECT(ND)

      NT=N+N0+N1
      IF (ID .EQ. 3) GOTO 10
      DO 20 J=1,NT
      IF (J .NE. I) GOTO 21
      IF (ID .NE. 1) GOTO 5
      VECT(I)=DIF2(I)/DIF1(I)/2
      GOTO 20
5     VECT(I)=DIF3(I)/DIF1(I)/3
      GOTO 20
21    Y=ROOT(I)-ROOT(J)
      VECT(J)=DIF1(I)/DIF1(J)/Y
      IF (ID .EQ. 2) VECT(J)=VECT(J)*(DIF2(I)/DIF1(I)-2/Y)
20    CONTINUE
      GOTO 50
10    Y=0.
      DO 25 J=1,NT
      X=ROOT(J)
      AX=X*(1-X)
      IF  (N0 .EQ. 0) AX=AX/X/X
      IF (N1 .EQ. 0) AX=AX/(1-X)/(1-X)
      VECT(J)=AX/DIF1(J)**2
25    Y=Y+VECT(J)
      DO 60 J=1,NT
60    VECT(J)=VECT(J)/Y
50    RETURN
      END
```

```
C
C       THIS PROGRAM SOLVES A*X = B BY GAUSSIAN ELIMINATION
C       WITH PARTIAL PIVOTING.
C
        SUBROUTINE GAUSL(ND,NCOL,N,NS,A)
        IMPLICIT REAL*8 (A-H,O-Z)
        DIMENSION A(ND,NCOL)

        N1=N+1
        NT=N+NS
        IF (N .EQ. 1) GOTO 50

        DO 10 I=2,N
        IP=I-1
        I1=IP
        X=DABS(A(I1,I1))
        DO  11 J=1,N
        IF (DABS(A(J,I1)) .LT. X) GOTO 11
        X=DABS(A(J,I1))
        IP=J
11      CONTINUE
        IF (IP .EQ. I1) GOTO 13

        DO 12 J=I1,NT
        X=A(I1,J)
        A(I1,J)=A(IP,J)
12       A(IP,J)=X
13      DO 10 J=I,N
        X=A(J,I1)/A(I1,I1)
        DO 10 K=I,NT
10       A(J,K)=A(J,K) - X*A(I1,K)

50      DO 20 IP=1,N
        I=N1-IP
        DO  20 K=N1,NT
        A(I,K) = A(I,K)/A(I,I)
        IF (I .EQ. 1) GOTO 20
        I1=I-1
        DO 25 J=1,I1
25       A(J,K)=A(J,K) - A(I,K)*A(J,I)
20      CONTINUE
        RETURN
        END
```

```
C
C       RADAU AND LOBATTO QUADRATURE WEIGHTS
C          ID = 1 : RADAU QUADRATURE WITH X = 1
C          ID = 2 : RADAU QUADRATURE WITH X = 0
C          ID = 3 : LOBATTO QUADRATURE WITH BOTH END POINTS
C
        SUBROUTINE RADAU(ND,N,N0,N1,ID,AL,BE,ROOT,DIF1,VECT)
        IMPLICIT REAL*8 (A-H,O-Z)
        DIMENSION ROOT(ND),DIF1(ND),VECT(ND)
        S=0.
        NT=N+N0+N1
        DO 40 I=1,NT
        X=ROOT(I)
        IF (ID-2) 10,20,30
10      AX=X
        IF (N0 .EQ. 0) AX=1/AX
        GOTO 40
20      AX=1-X
        IF (N1 .EQ. 0) AX=1/AX
        GOTO 40
30       AX=1.
40       VECT(I)=AX/DIF1(I)**2
        IF (ID .NE. 2) VECT(NT)=VECT(NT)/(1+AL)
        IF (ID .GT. 1) VECT(1)=VECT(1)/(1+BE)
        DO 50 I=1,NT
50       S=S+VECT(I)
        DO 60 I=1,NT
60       VECT(I)=VECT(I)/S
        RETURN
        END
```

```
C
C       THIS PROGRAM EVALUATES LAGRANGIAN INTERPOLATION
C       COEFFICIENTS
C
        SUBROUTINE INTRP(ND,NT,X,ROOT,DIF1,XINTP)
        IMPLICIT REAL*8 (A-H,O-Z)
        DIMENSION ROOT(ND),DIF1(ND),XINTP(ND)
        POL=1.
        DO  5 I=1,NT
        Y=X-ROOT(I)
        XINTP(I)=0.
        IF (Y. EQ. 0.D0) XINTP(I)=1.
5       POL=POL*Y
        IF (POL .EQ. 0.D0) GOTO 10
        DO 6 I=1,NT
6        XINTP(I)=POL/DIF1(I)/(X-ROOT(I))
10      RETURN
        END
```

```
C
C    ********************************************************
C    *                                                      *
C    *        PROGRAM        RUNGEKUTTA                     *
C    *                                                      *
C    ********************************************************
C     THIS PROGRAM SOLVES THE SECOND ORDER NONLINEAR
C     DIFFERENTIAL EQUATION BY RUNGEKUTTA METHOD
C
        IMPLICIT REAL*8 (A-H,O-Z)
        REAL K2, K11, K12, K21, K22, K31, K32
        REAL K41, K42
        WRITE(*,2)
2       FORMAT(' THM, YO, K2, CS  '/)
        READ(*,*) THM, YO, K2, CS
        H=1./1000
        G=K2/CS
        U1=YO
        U2=0
        DO 11 I=1,10
          DO 12 J=1,100
            XO=((I-1)*100+J-1)*H
            IF (I .EQ. 1 .AND. J .EQ. 1) THEN
              K12=THM**2*(U1/(G+U1))*H
            ELSE
              K12=H*F2(XO,U1,U2,THM,G)
            END IF
            K11=H*U2
            XO=XO+H/2
            U1=U1+K11/2
            U2=U2+K12/2
            K21=H*U2
            K22=H*F2(XO,U1,U2,THM,G)
            U1=U1-K11/2+K21/2
            U2=U2-K12/2+K22/2
            K31=H*U2
            K32=H*F2(XO,U1,U2,THM,G)
            XO=XO+H/2
            U1=U1-K21/2+K31
            U2=U2-K22/2+K32
            K41=H*U2
            K42=H*F2(XO,U1,U2,THM,G)
            U1=U1-K31+(K11+2*K21+2*K31+K41)/6
            U2=U2-K32+(K12+2*K22+2*K32+K42)/6
12        CONTINUE
          WRITE(*,5) U1
5          FORMAT(1X,F19.9)
11      CONTINUE
        STOP
        END
        FUNCTION   F2  (X,U1,U2,THM,G)
        IMPLICIT REAL*8 (A-H,O-Z)
```

```
      F2=-2./X*U2+THM**2*(U1/(G+U1))
      RETURN
    END
```

```
C     ***************************************************
C     *                                                 *
C     *         PROGRAM        REGRESS                  *
C     *                                                 *
C     ***************************************************
C
C     THIS PROGRAM IS TO FIND A KINETIC CONSTANT FOR
C     THE MONOD EQUATION (PACKED-BED REACTOR)
C
C     DATA INPUT   :     NO   =  NO. OF POINTS
C                        TAU  =  RETENTION TIME
C                        SO   =  SUBSTRATE CONC IN EFFLUENT
C
      DIMENSION    A(10,10), X(10), COPY(10)
      DIMENSION    TAU(20), SO(20)
      N=3
      READ (3,*)  NO
      DO 10 I=1 , NO
        READ (3,*)  TAU(I), SO(I)
10    CONTINUE
      SUM1=0
      SUM2=0
      SUM3=0
      SUM4=0
      SUM5=0
      SUM6=0
      SUM7=0
      SUM8=0
      DO 11 J=1 , NO
        SUM1=SUM1+ALOG(SO(J))
        SUM2=SUM2+SO(J)
        SUM3=SUM3+TAU(J)
        SUM4=SUM4+(ALOG(SO(J)))**2
        SUM5=SUM5+ALOG(SO(J))*SO(J)
        SUM6=SUM6+SO(J)*TAU(J)
        SUM7=SUM7+SO(J)**2
        SUM8=SUM8+ALOG(SO(J))*TAU(J)
11    CONTINUE
      A(1,1)=SUM4
      A(1,2)=SUM5
      A(1,3)=SUM1
      A(1,4)=SUM8
      A(2,1)=SUM5
      A(2,2)=SUM7
      A(2,3)=SUM2
      A(2,4)=SUM6
      A(3,1)=SUM1
      A(3,2)=SUM2
      A(3,3)=NO
      A(3,4)=SUM3
      CALL  GAUSS (N, A, X)
      CALL  AAR (NO, TAU, X, SO, R)
```

```
      DO  6  J=1 , 3
      WRITE(4,*)  X(J)
6     CONTINUE
      DO  7  K=1 , 3
      WRITE(4,*)  (A(K,L), L=1 , 4)
7     CONTINUE
      WRITE(4,14)    R
14    FORMAT(/,'CORRELATION COEFFICIENT =',F7.5)
      STOP
      END
```

```
C
C     THIS PROGRAM IS TO FIND A KINETIC CONSTANT FOR
C     SUBSTRATE INHIBITION MODEL (PACKED-BED REACTOR)
C
      DIMENSION    A(10,10), X(10), COPY(10)
      DIMENSION    TAU(20), SO(20)
      N=4
      READ (1,*)  NO
      DO 10 I=1 , NO
        READ (1,*)  TAU(I), SO(I)
10    CONTINUE
      SUM1=0
      SUM2=0
      SUM3=0
      SUM4=0
      SUM5=0
      SUM6=0
      SUM7=0
      SUM8=0
      SUM9=0
      SUM10=0
      SUM11=0
      SUM12=0
      DO 11 J=1 , NO
        SUM1=SUM1+ALOG(SO(J))
        SUM2=SUM2+(ALOG(SO(J)))**2
        SUM3=SUM3+SO(J)
        SUM4=SUM4+SO(J)**2
        SUM5=SUM5+SO(J)**3
        SUM6=SUM6+SO(J)**4
        SUM7=SUM7+TAU(J)*ALOG(SO(J))
        SUM8=SUM8+TAU(J)*SO(J)
        SUM9=SUM9+TAU(J)*SO(J)**2
        SUM10=SUM10+TAU(J)
        SUM11=SUM11+SO(J)*ALOG(SO(J))
        SUM12=SUM12+SO(J)**2*ALOG(SO(J))
11    CONTINUE
      A(1,1)=SUM2
      A(1,2)=SUM11
      A(1,3)=SUM12
      A(1,4)=SUM1
      A(1,5)=SUM7
      A(2,1)=SUM11
      A(2,2)=SUM4
      A(2,3)=SUM5
      A(2,4)=SUM3
      A(2,5)=SUM8
      A(3,1)=SUM12
      A(3,2)=SUM5
      A(3,3)=SUM6
      A(3,4)=SUM4
      A(3,5)=SUM9
```

```
      A(4,1)=SUM1
      A(4,2)=SUM3
      A(4,3)=SUM4
      A(4,4)=NO
      A(4,5)=SUM10
      CALL  GAUSS (N, A, X)
      CALL  AAR (NO, TAU, X, SO, R)
      DO  6  J=1 , N
      WRITE(2,*)  X(J)
6     CONTINUE
      DO  7  K=1 , N
      WRITE(2,*)  (A(K,L), L=1 , N+1)
7     CONTINUE
      WRITE(2,12)     R
12    FORMAT(/,' CORRELATION COEFFICIENT =',F7.5)
      STOP
      END
```

```
C
C     THIS PROGRAM IS TO FIND A KINETIC CONSTANT FOR
C     MONOD MODEL (FLUIDIZED BED REACTOR)
C
      DIMENSION    A(10,10), X(10), COPY(10)
      DIMENSION    TAU(20), SO(20)
      N=3
      READ (1,*)  NO
      DO 10 I=1 , NO
        READ (1,*)  TAU(I), SO(I)
10    CONTINUE
      SUM1=0
      SUM2=0
      SUM3=0
      SUM4=0
      SUM5=0
      SUM6=0
      SUM7=0
      DO 11 J=1 , NO
        SUM1=SUM1+1./SO(J)
        SUM2=SUM2+SO(J)
        SUM3=SUM3+TAU(J)
        SUM4=SUM4+(1./SO(J))**2
        SUM5=SUM5+TAU(J)/SO(J)
        SUM6=SUM6+SO(J)*TAU(J)
        SUM7=SUM7+SO(J)**2
11    CONTINUE
      A(1,1)=SUM4
      A(1,2)=NO
      A(1,3)=SUM1
      A(1,4)=SUM5
      A(2,1)=NO
      A(2,2)=SUM7
      A(2,3)=SUM2
      A(2,4)=SUM6
      A(3,1)=SUM1
      A(3,2)=SUM2
      A(3,3)=NO
      A(3,4)=SUM3
      CALL  GAUSS (N, A, X)
      CALL  AAR (NO, TAU, X, SO, R)
      DO  6  J=1 , 3
      WRITE(2,*)  X(J)
6     CONTINUE
      DO  7  K=1 , 3
      WRITE(2,*)  (A(K,L), L=1 , 4)
7     CONTINUE
      WRITE(2,12)     R
12    FORMAT(/,' CORRELATION  COEFFICIENT =',F7.5)
      STOP
      END
```

```
C
C     THIS PROGRAM IS TO FIND A KINETIC CONSTANT FOR
C     SUBSTRATE INHIBITION MODEL (FLUIDIZED BED REACTOR)
C
      DIMENSION    A(10,10), X(10), COPY(10)
      DIMENSION    TAU(20), SO(20)
      N=4
      READ (1,*)  NO
      DO 10 I=1 , NO
        READ (1,*)  TAU(I), SO(I)
10    CONTINUE
      SUM1=0
      SUM2=0
      SUM3=0
      SUM4=0
      SUM5=0
      SUM6=0
      SUM7=0
      SUM8=0
      SUM9=0
      SUM10=0
      DO 11 J=1 , NO
        SUM1=SUM1+1./SO(J)
        SUM2=SUM2+1/SO(J)**2
        SUM3=SUM3+SO(J)
        SUM4=SUM4+SO(J)**2
        SUM5=SUM5+SO(J)**3
        SUM6=SUM6+SO(J)**4
        SUM7=SUM7+TAU(J)/SO(J)
        SUM8=SUM8+TAU(J)*SO(J)
        SUM9=SUM9+TAU(J)*SO(J)**2
        SUM10=SUM10+TAU(J)
11    CONTINUE
      A(1,1)=SUM2
      A(1,2)=NO
      A(1,3)=SUM3
      A(1,4)=SUM1
      A(1,5)=SUM7
      A(2,1)=NO
      A(2,2)=SUM4
      A(2,3)=SUM5
      A(2,4)=SUM3
      A(2,5)=SUM8
      A(3,1)=SUM3
      A(3,2)=SUM5
      A(3,3)=SUM6
      A(3,4)=SUM4
      A(3,5)=SUM9
      A(4,1)=SUM1
      A(4,2)=SUM3
      A(4,3)=SUM4
      A(4,4)=NO
```

```
      A(4,5)=SUM10
      CALL  GAUSS (N, A, X)
      CALL  AAR (NO, TAU, X, SO, R)
      DO  6  J=1 , N
      WRITE(2,*)  X(J)
6     CONTINUE
      DO  7  K=1 , N
      WRITE(2,*)  (A(K,L), L=1 , N+1)
7     CONTINUE
      WRITE(2,12)     R
12    FORMAT(/,' CORRELATION COEFFICIENT =',F7.5)
      STOP
      END
```

```
C
C     GAUSSIAN ELIMINATION WITH MAXIMAL COLUMN PIVOTING
C     ALGORITHM
C
      SUBROUTINE  GAUSS  (N, A, X)
      DIMENSION  A(10,10),  X(10),  COPY(10)
      DO  10  I=1 , N-1
        AMAX=ABS(A(I,I))
        DO  20  J=I , N
          DUM=ABS(A(J,I))
          IF (DUM-AMAX)  20, 20, 19
19        AMAX=DUM
          DO  30  K=I , N+1
            COPY(K)=A(I,K)
            A(I,K)=A(J,K)
            A(J,K)=COPY(K)
30        CONTINUE
20      CONTINUE
        DO  40  J=I+1 , N
          R=A(J,I)/A(I,I)
          DO  50  K=I , N+1
            A(J,K)=A(J,K)-R*A(I,K)
50        CONTINUE
40      CONTINUE
10    CONTINUE
      X(N)=A(N,N+1)/A(N,N)
      DO  60  I=1 , N
        J=N-I
        SUM=0
        DO  70  K=J+1 , N
          DUM=A(J,K)*X(K)
          SUM=SUM+DUM
70      CONTINUE
        X(J)=(A(J,N+1)-SUM)/A(J,J)
60    CONTINUE
      RETURN
      END
```

```
C
C       THIS PROGRAM IS TO CACULATE THE ABSOLUTE AVERAGE
C       RESIDUAL BETWEEN EXPERIMENTAL AND PRIDICTED VALUES
C
        SUBROUTINE AAR (NO, TAU, X, SO, R)
        DIMENSION    TAU(20),  X(10),  SO(20)
        SUM1=0
        SUM2=0
        DO 21 I=1,NO
          TE=X(1)/SO(I)+X(2)*SO(I)+X(3)*SO(I)**2+X(4)
          SUM1=SUM1+(TAU(I)-TE)**2
          SUM2=SUM2+TAU(I)
21      CONTINUE
        TA=SUM2/NO
        DUM=SUM1/(NO-1)
        R=SQRT(DUM)/TA
        RETURN
        END
```