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ABSTRACT
MINERALIZATION OF 2-CHLOROPHENOL USING A HOLLOW FIBER ULTRAFILTRATION SYSTEM IN COMBINATION WITH A REACTOR CONTAINING AN IMMOBILIZED WHITE ROT FUNGUS

by
Sheau-Ming Tung

Phanerochaete chrysosporium, a white rot fungus was immobilized on a packed-bed reactor, and was used to mineralize an aqueous stream contaminated with 50 ppm of 2-chlorophenol. Hollow fiber ultrafiltration membranes (for 5,000 daltons and 10,000 daltons molecular weight cut-off) were utilized to retain the extracellular enzyme resulting from the immobilized fungus in a recirculation loop outside of the reactor.

It was found here that the concentration of proteins (roughly proportional to the extracellular enzyme releasd by the fungus) does not correlate with the rate of degradation of 2-chlorophenol in the system. The protein was concentrated 1.5 fold in the recycle loop by the ultrafiltration unit, but the biodegradation rate did not show any significant improvement.

At least four kinds of protein whose molecular weights were around 13,000 , 16,000, 23,000 and 40,000 daltons were observed in the concentrated enzyme solution using SDS polyacrylamide gel electrophoresis.

Extracellular enzyme solution containing a high amount of fungal biomass appeared to have a positive effect on the degradation rate. Mineralization of 2-chlorophenol cannot be obtained without fungal biomass.

MINERALIZATION OF 2-CHLOROPHENOL USING A HOLLOW FIBER ULTRAFILTRATION SYSTEM IN COMBINATION WITH A REACTOR CONTAINING AN IMMOBILIZED WHITE ROT FUNGUS

by
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MINERALIZATION OF 2-CHLOROPHENOL USING A HOLLOW FIBER ULTRAFILTRATION SYSTEM IN COMBINATION WITH REACTOR CONTAINING AN IMMOBILIZED WHITE ROT FUNGUS

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This thesis is dedicated to
my dear family

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CHAPTER 1

INTRODUCTION

The use of microorganisms for the biological treatment of pollutants is often a cost effective alternative and results in environmentally acceptable end products such as water, CO_2 and other inorganic salts with low or no toxicity. However, microorganisms need to be found which can treat specific pollutants and at a practical reaction rate.

Chlorinated phenols are commonly found pollutants due to their wide utilization in industry and agriculture. There are three major contamination sources [1]. First, chlorinated phenols and their derivatives have been used widely as insecticides, fungicides and herbicides, which has caused serious local contamination of soil [2] and groundwater [3-4]. The second major source of chlorinated phenols is bleached pulp [5], and the third contamination source arises from combustion processes [6].

The toxicity of chlorinated phenols tends to increase with their degree of chlorination, and because few microorganisms can decompose them, the more highly chlorinated phenols tend to accumulate in the environment.

Phanerochaete Chrysosporium, commonly referred to as white rot fungus, is one of very few microoganisms that can degrade chlorocarbons and polycyclic aromatics [7-13]. This fungus grows on wood by excreting an extracellular oxidative enzyme system capable of metabolizing lignin, which is a highly complex, nonrepeating heteropolymer that binds the cellulose in wood. The cellulose is the principal carbon source for this organism. Due to the irregularity of lignin polymers, the ligninase system is not specific, and therefore possesses the capability of degrading a variety of toxic pollutants with carbon skeletons similar to that lignin. Otherwise recalcitrant organohalides such as lindane (1,2,3,4,5,6-hexachloro-cyclohhexane) [10], DDT

[1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane] [10], polychlorinated dibenzo-p-dioxin [10], polychlorinated biphenyl [7] Benzopyren [13] and pentachlorophenol (PCP) [9] can be mineralized by *Phanerochaete chrysosporium*. Sodroski et al. [15] suggested that the ability of *Phanerochaete chrysosporium* to metabolize synthetic organohalides is dependent on the extracellular lignin-degrading enzyme system.

Lewandowski, et al [16] found that immobilized *Phanerochaete chrysosporium* can degrade 2-chlorophenol at a rate 40 times faster than a suspended growth system. Immobilized fungus was applied to several types of reactor configurations and the results obtained suggested that a packed-bed reactor with porous silica beads as the support material resulted in an effective system. They also successfully obtained the Michaelis-Menten kinetic constants for packed-bed and well-mixed reactors.

Leisola et al [17] and Kirk [18] both concentrated the extracellular enzyme solution 10-20 fold before purification by ultrafiltration using an Amicon PM 10 and YM 10 respectively (10,000 dalton cut-off both) membrane filter in their research. This procedure was used to concentrate the extracellular enzyme solution to increase the overall degradation rate of chlorinated phenol.

As membrane technology improved and as industry demanded more selective separation, the applications of ultrafiltration have increased [19]. These include treatment of industrial effluents and process water; concentrating, purifying, and separating macromolecular solutions in the chemical, food and drug industries; sterilizing, clarifying and purifying biological solutions and beverages; purifying ultra pure water; and pretreating sea water in resverse osmosis (RO) processes [20-25].

There are four primary ultrafiltration (UF) configurations [21]: tubular, spiral-wound flat-sheet and hollow fiber designed for different requirements. The tubular form is generally used in low-volume, high-value applications. The spiral-wound has greater membrane surface area than the tubular, and can economically treat large

volumes. The hollow fiber UF has more flexible operating conditions and less fouling problems than the other two, and is widely used in lab experiments as well as in manufacturing.

My research work was focused on the mineralization of 2-chlorophenol in a packed-bed reactor with immobilized fungus, utilizing an external hollow fiber ultrafiltration recirculation system to retain the extracellular enzyme solution.

CHAPTER 2

MATERIALS AND APPARATUS

2.1 Microorganism, Media and Material

2.1.1 Microorganism

Phanerochaete Chrysosporium BKM-1767 (ATCC 24725), commonly referred to as white rot fungus, was obtained from the American Type Culture Collection. The fungus was maintained at 4°C on Bacto nutrient agar plate. 23 g of Bacto nutrient agar powder (Difco Lab., Detroit Michigan) (consisting of Bacto beef extract, 3 grams, Bacto peptone, 5 grams and Bacto agar, 15 grams) were suspended in 1 liter distilled or deionized water, and heated to boiling to dissolve the components completely. This agar solution was sterilized in an autoclave for 15 minutes at 15 pounds pressure (121° C). Disposable petri dishes were used to contain the autoclaved agar solution. The Bacto nutrient agar plates were formed after cooling down, and were spread with fungal inoculum. Incubation was carried out at 37°C for 24 hours. The conservation temperature was 4°C.

2.1.2 Growth and Induction Media

Two kinds of media were used in the experiment. The growth medium was used to grow the fungus in suspension. The induction medium was used to stimulate the production of the active enzyme system by the fungus. The compositions of the media are listed in Table 1. The pH of the prepared media was around 4.0~4.5.

Table 1. Composition of Growth Medium and Induction Medium

Chemical Species	Growth Medium	Induction Medium
KH_2PO_4	2.0 g	2.0 g
$MgSO_4$	0.5 g	0.5 g
$CaSO_4$	0.1 g	0.02 g
$NaNO_3$	0.2 g	0.02 g
Glucose	10.0 g	0.8 g
Thiamine Hydrochloride	1.0 mg	1.0 mg
Mineral Salt Solution*	5.0 ml	5.0 ml
Deionized Water	1.0 liter	1.0 liter

*Composition of Mineral Salt Solution: $MgSO_4 \cdot 7H_2O$, 3 g ; $MnSO_4 \cdot H_2O$, 0.5 g ; NaCl, 1 g ; $FeSO_4 \cdot 7H_2O$, 100 mg ; $CoSO_4$, 100 mg ; $CaCl_2$, 82 mg ; $ZnSO_4$, 100 mg ; $CuSO_4 \cdot 5H_2O$, 10 mg ; H_3BO_3, 10 mg ; $NaMoO_4$, 10 mg ; $AlK(SO_4)$, 10 mg ; Deionized Water 1 liter.

2.1.3 2-Chlorophenol

2-chlorophenol (99% purity, Aldrich Chemical Co., Inc., Milwaukee, WI) was used to prepare 2-chlorophenol solutions of different concentration by mixing it with induction medium as the feed of the packed-bed reactor system.

2.1.4 Polyethylene (PET)

Polyethylene terephthalate (PET) flakes obtained from the recycling plant at Rutgers University, New Brunswick, were used as random immobilization support material for the fungus in the packed bed reactor. The shape of PET flakes was irregular. The width varied from 0.5 cm to 2.0 cm. The crude edge and crumpled surface were helpful for fungus to attach.

2.2 Experimental Apparatus

2.2.1 Fermenter

A 8" i.d.×16" high glass batch fermenter (Figure 1) (New Brunswick Scientific Co. Inc, N.J. Model No: MG 14) with built-in aeration, temperature setting and stirring systems was used to cultivate the fungus. The maximun liquid volume which the fermenter can process is 15 liters.

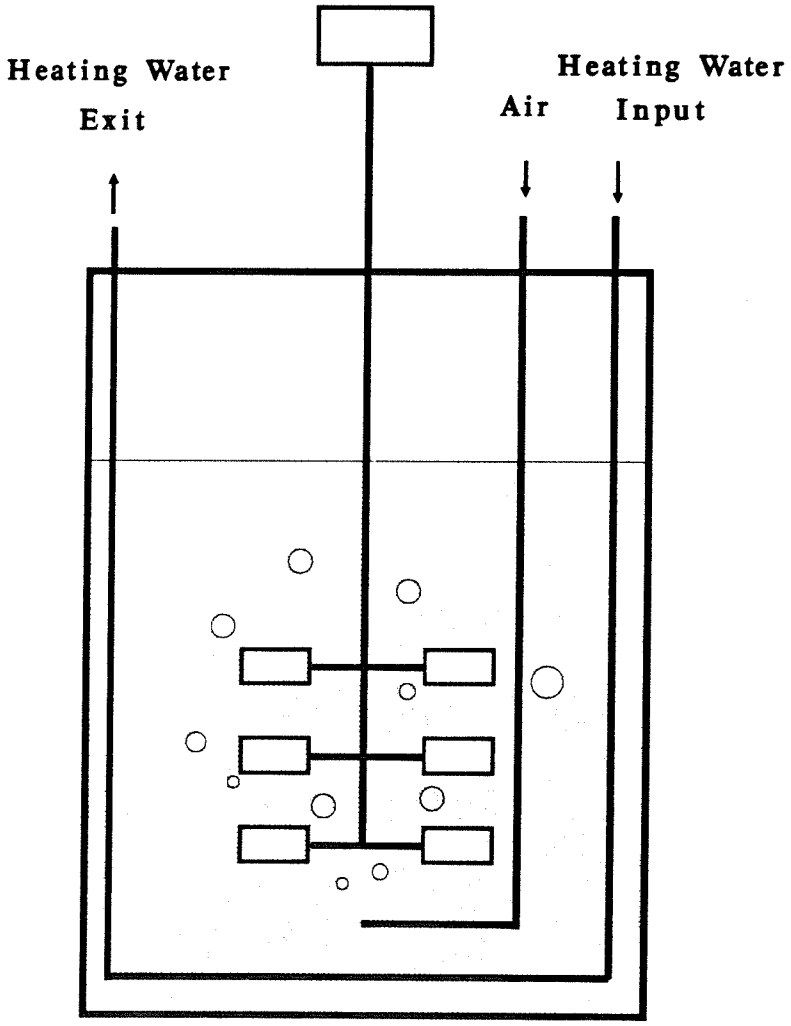


Figure 1. Diagram of the Fermenter

2.2.2 Packed-Bed Reactor

A 2" i.d.× 20" long jacketed vessel made of glass was used as the packed-bed reactor (Figure 2). The temperature of the vessel was controlled by circulating hot water in the jacket. The air and liquid were pumped in from the bottom. A circulation pump was available to recycle the solution from top to bottom to make the concentration of solution homogeneous.

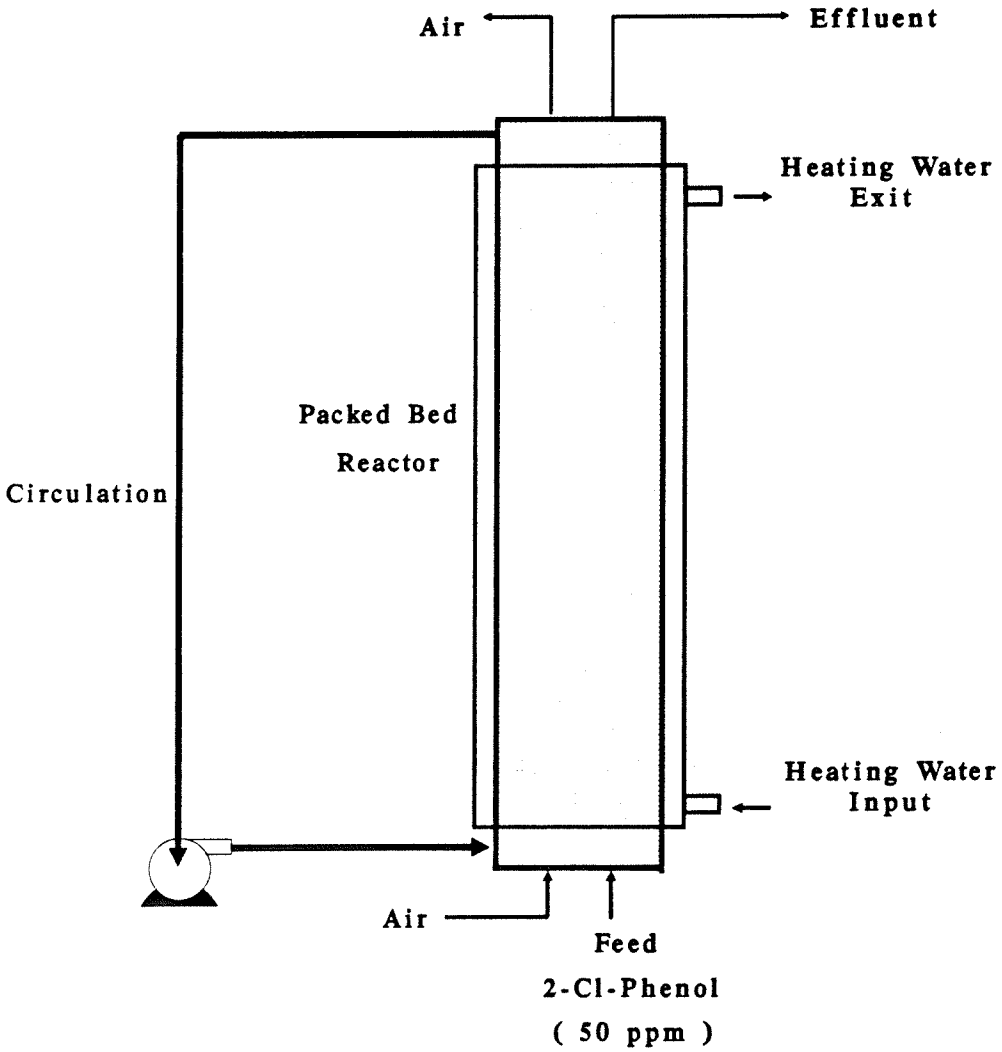


Figure 2. Diagram of the Packed-Bed Reactor

2.2.3 Hollow Fiber Ultrafiltration System

The hollow fiber ultrafiltration (abbreviated as UF) system (kindly donated by Supelco, Inc. PA.) consisted of three major parts: a hollow fiber UF cartridge, a peristaltic pump and a collection tank (Figure 3).

The cartridges consisted of bundles of hollow fiber membranes sealed in transparent cylinders. The hollow fiber membrane was made of acrylic copolymer and had a 0.1-μm skin on the inside that was supported by a spongelike outer substructure. The fibers were formed into bundles and epoxy adhesive was applied to both ends. The bundles were placed in a low-pressure vessel of polysulfone that had a length of 43 inches and a diameter of 3 inches. The feedwater entered the device at one end and flowed through the fiber bores. Matter rejected by the membrane was concentrated and left the fiber bores at the other end. The solvent and low-molecular-weight solutes permeated the membrane, flowed through the support substructure, and were removed through the product port [26].

The hollow fiber ultrafiltration cartridge (Supelco, Inc. PA., model no: HF1.0-43·PM10 and model no.: HF1.0-43·PM5) connected with a variable speed 1/4 HP Masterflex peristaltic pump (Cole-Parmer Instrument Co. Inc, IL , Model No: 7549-30) constituted the ultrafiltration system (according to the Model HF-LAB-1 System of Romicon, Inc. MA) (Figure 3). The recirculation rate was adjusted by setting the linear front panel speed control of peristaltic pump from 100 to 650 rpm. The permeate flow rate was adjusted by pinching two permeate hoses with hosecock clamps. The pressure of the cartridge was detected by two sanitary pressure gauges installed at the entrance and exit of the cartridge. The molecular weight cut-offs (MWCO) of the hollow fiber membranes, were 10,000 Daltons for model no. HF1.0-43·PM10 and 5000 Daltons for the HF1.0-43·PM5. The new cartridges had to be conditioned before use by following the conditioning procedure of the operating

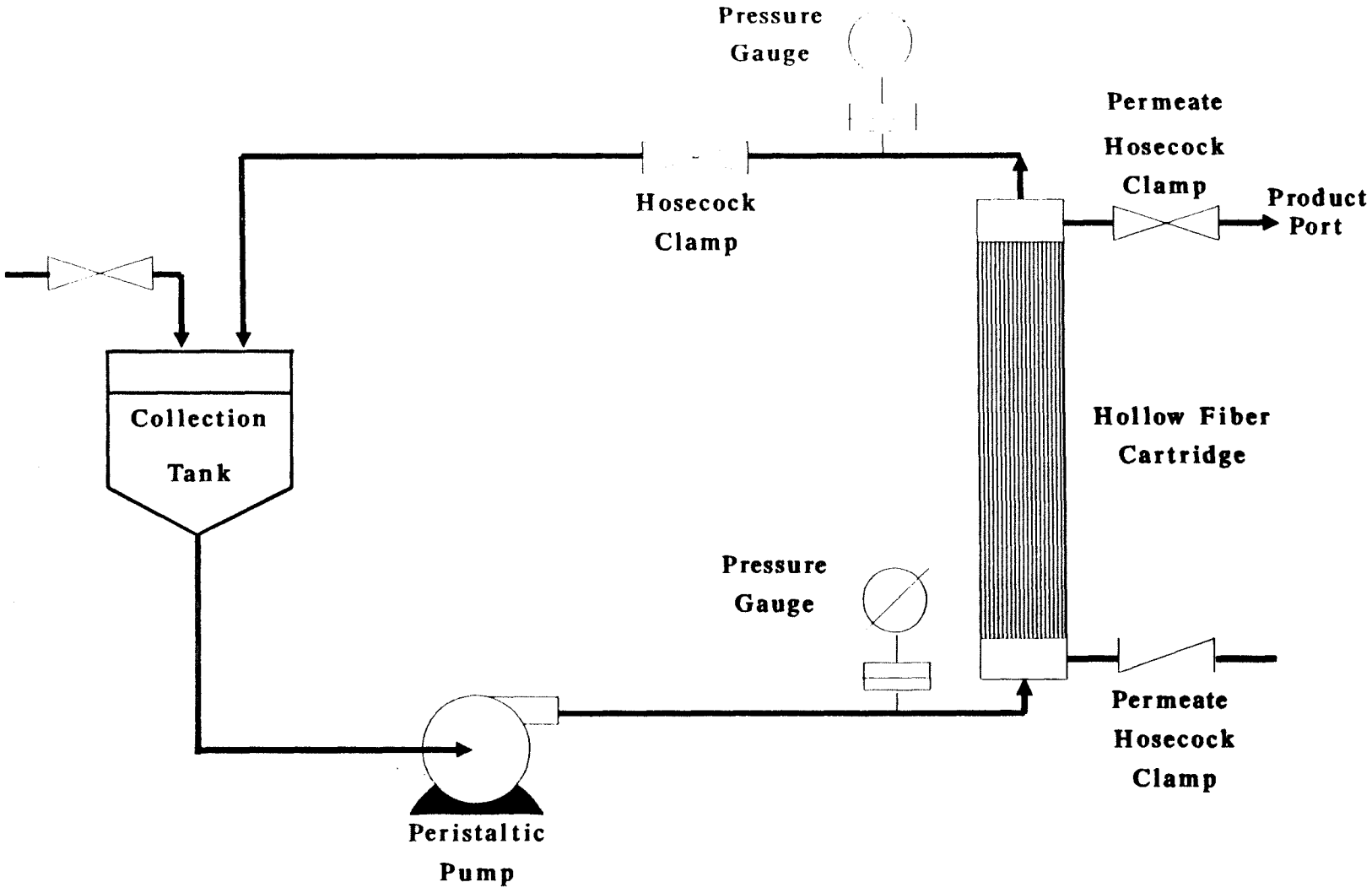


Fig 3. The Hollow Fiber Ultrafiltration System

instructions offered by Romicon. The specifications of the cartridges are listed in Table 2.

Table 2. Specifications of Hollow Fiber Ultrafiltration Membrane Cartridges

Model No.	HF1.0-43·PM10	HF1.0-43·PM5
MW Cut-off	10,000	5000
Water Flux	0.26 - 0.34	0.39-0.51 L/Min
Materials of Construction:		
Cartridge Shell	Polysulfone	
Potting Material	Epoxy	
Fibers	Polysulfone	
Max. Inlet Pressure	40 psig	
Max. Transmembrane Pressure	25 psig	
Fiber Internal Diameter	1.1 mm (0.043 in)	
Cartridge Diameter	2.5 cm (1.0 in)	
Surface Area	0.09 m^2 (1 ft^2)	
Length	45.7 cm (18 in)	
Water Recirculation Rates(25°C)	2 - 16 L/Min	

CHAPTER 3

EXPERIMENTAL PROCEDURE

3.1 Experiments in Packed Bed Reactor

3.1.1 Biomass Growth in Fermenter

The fungus, maintained on the nutrient agar plates, was transferred to a 10 liter growth medium in the well aerated batch fermenter as described in the previous chapter, with continuous stirring at 100 rpm and 32°C. The concentrations of glucose and nitrogen were measured daily. After four days, the amount of consumed nutrient was calculated from deducting the initial concentration from the current concentration and then new additions of glucose and sodium nitrate were made to the fermenter, to compensate for the consumed nutrient, to achieve initial concentrations. After six days, a thick fungal slurry was formed.

3.1.2 Immobilization in Packed-Bed Reactor

About 1 liter of fungal slurry was taken out and mixed with PET flakes, which were sterilized in the autoclave at 121°C for 15 minutes in advance, in a 2 liter flask by vigorous shaking. The mixture was poured into the packed bed reactor which has not been sterilized but only rinsed with bleach. Aeration rate for the bed was maintained at 20 ml/min, and temperature was kept at 32°C by circulating water in the jacket. Then the fungus was allowed to settle on the PET flakes for 24 hours. After 24 hours fresh growth medium was continuously introduced into the reactor from the bottom at a flow rate 2 ml/min. The rate of circulation (about 30 ml/min) which was accomplished by pumping out the solution from the upper vessel into the bottom, was

much higher than the feed rate (3.5 ml/min) so that the packed bed reactor behaved like a well mixed reactor (CSTR). The fungus was grown in the packed bed reactor for 4~5 days. Then the filamentous fungus embraced the PET flakes to form a well developed network in the packed bed reactor. The void volume was about 600 ml. This was measured before transferring the fungus in the bed by filling up the reactor (which was packed with PET flakes) with water. The void volume was the amount of water which was added to the vessel.

3.1.3 Degradation of 2-Chlorophenol in the Continuous Packed-Bed Reactor with the Ultrafiltration System

A 50 ppm 2-chlorophenol solution (prepared by mixing 0.0403 ml of pure 2-chlorophenol with one liter of induction medium), was continuously introduced to the packed-bed reactor containing the fungus at a flow rate of 3.5 ml/min. The retention time was about 3 hrs. The effluent (Stream #1) containing extracellular enzyme and residual 2-chlorophenol was collected in a collection tank that was connected to the hollow fiber ultrafiltration system. The hollow fiber ultrafiltration system was operated in a continuous mode (Figures 4.a and 4.b) to retain the extracellular enzyme within the system. The effluent stored in the collected tank was circulated into the UF unit (Stream #2) at 1500 ml/min flow rate by the peristaltic pump. The concentrate (Stream #4) was recycled to the collected tank. In order to compare the effect of the UF unit, the permeate (Stream #3) was operated in the two different modes, Mode A and Mode B, in sequence.

Mode A

In Mode A (Figure 4.a), the UF unit was a component of the process but any separation effect was eliminated by recycling the permeate (Stream #3) back to the UF loop (Stream #4). The final product (Stream #5) was pumped out at the same flow

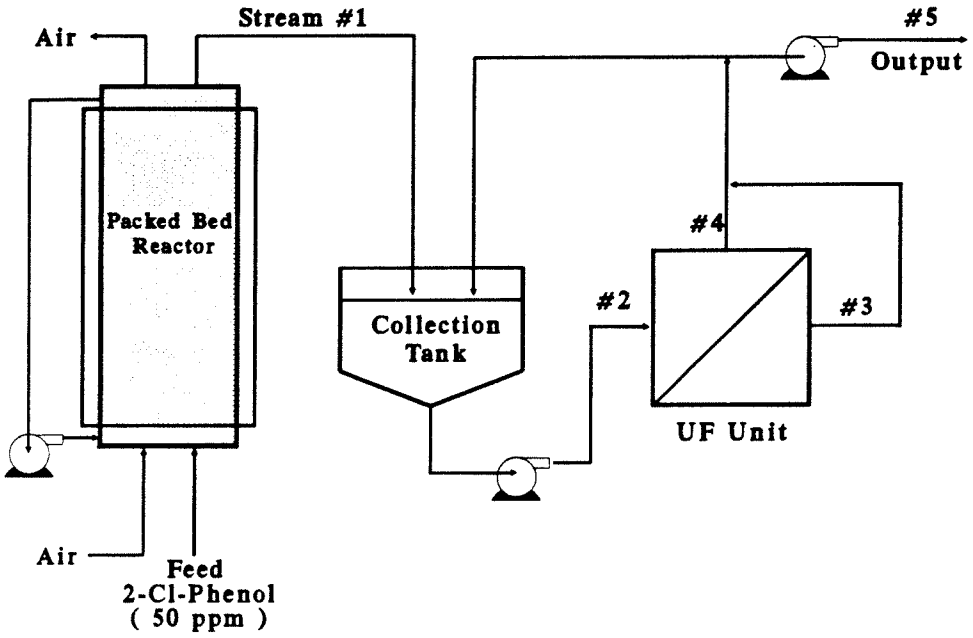


Figure 4.a Diagram of Mode A Process
The permeate (Stream 3) flows back to the loop (Stream 4), and the final output of system is pumped out from Stream 5.

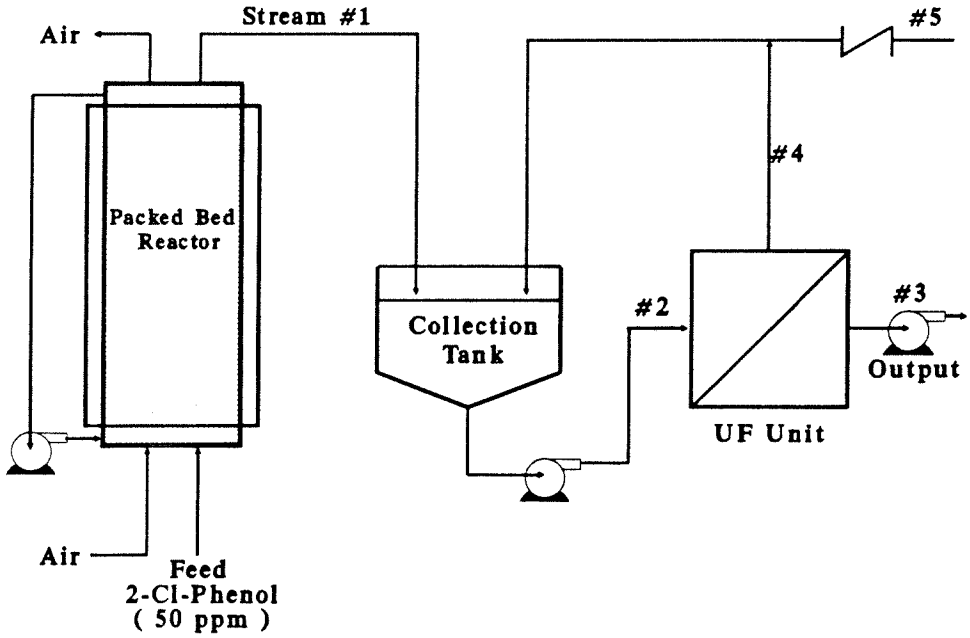


Figure 4.b Diagram of Mode B Process
The permeate (Stream 3) becomes the system output, and the flow rate of Stream 5 is zero.

rate as the feed of the packed bed reactor, to satisfy the overall mass balance. The pH and concentrations of 2-chlorophenol, chloride, and protein in every stream (from #1 to #5) were measured every day by the analytical methods described in the next chapter. After reaching steady state, Mode A was switched to Mode B.

Mode B

In Mode B (Figure 4.b), the flow rate of Stream #5 was zero and the permeate became the final product by exiting the system. The pH and concentrations of 2-chlorophenol, chloride, and protein in every stream (from #1 to #4) were measured every day.

Mode A (Figure 4.a) was used to run control experiments. This mode had the same retention time of the UF system, the same volatilization rate of 2-chlorophenol and the same operating conditions of Mode B, but the UF unit was not used to separate the components of the effluent.

There were three sets of different operating conditions in the packed-bed reactor experiments :

System I: 10000 mw cut-off hollow fiber membrane and 3.5 ml/min feed rate.

System II: 10000 mw cut-off hollow fiber membrane and 2.0 ml/min feed rate.

System III: 5000 mw cut-off hollow fiber membrane and 2.0 ml/min feed rate.

The procedures described above were repeated for each system.

3.2 Experiments in Shaker Bottles

In order to investigate the effect of air and biomass concentration on the mineralization of 2-chlorophenol, shaker bottle experiments were designed as shown in Figure 5. The effluent from the packed-bed reactor (containing the induced enzyme system and residual 2-chlorophenol) was poured into two 20 ml bottles which were

sterilized in autoclave at 121°C for 15 minutes in advance. The first bottle (A) was full without any air bubble while the second (B) was 20% full. Teflon caps sealed the bottles tightly to avoid any volatilization. Two other sterilized bottles (C and D) were prepared following a similar procedure. However these bottles were also added with a high amount of fungal biomass which was taken from the packed-bed tower. Another two sterilized bottles (E and F) were prepared by filling the fresh 40 ppm 2-chlorophenol in full and 20% full respectively without any microorganism. These two were control sets to present the volatilization of 2-chlorophenol in the bottles. These six bottles were shaken at 150 rpm at room temperature for six days. 1 ml solution was sampled from every bottles respectively after 1, 2, 3, and 6 days to measured the concentration of 2-chlorophenol by HPLC.

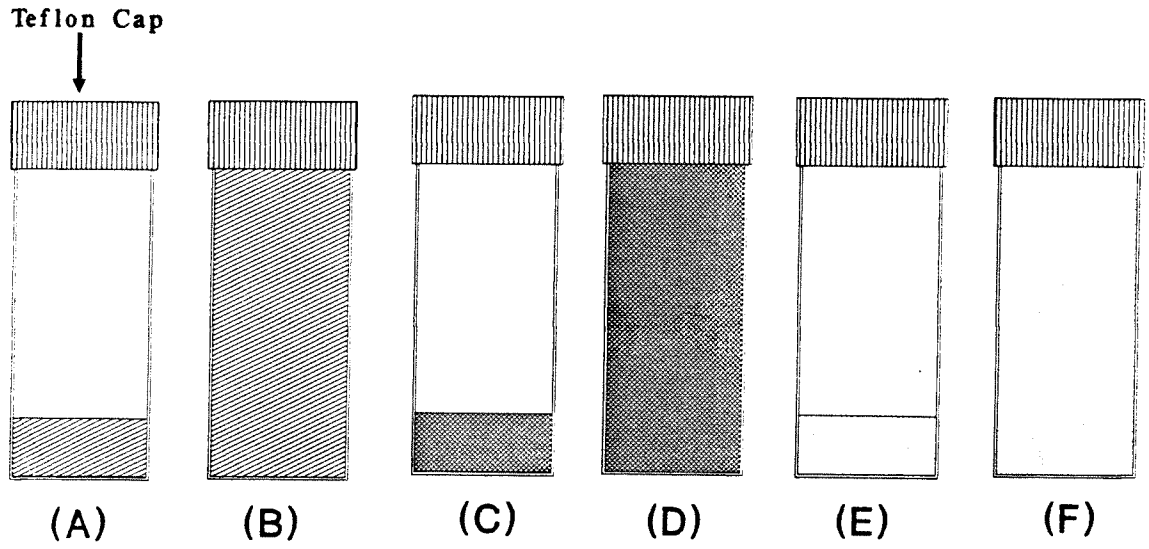


Figure 5 Strategy Used in Shaker Bottle Experiments
The solutions in bottle (A),(B),(C) and (D) was the effluent of packed-bed reactor
(A) with air, adding no fungal biomass,
(B) without air, adding no fungal biomass,
(C) with air, adding fungal biomass,
(D) without air, adding fungal biomass,
(E) and (F), control sets.

CHAPTER 4

ANALYTICAL METHODS

Fixed nitrogen (as nitrate ion), dissolved oxygen and pH were measured using specific electrodes inserted through the reactor lid or in samples from the reactor. Glucose was assayed using the ortho toluidine method [26].

4.1 2-Chlorophenol Assay

HPLC (Waters model 600E with 484 tunable detector and 715 ultra WISP autosampler) provided with a C18 bonded phase column (Altech Associates Inc. Deerfield, IL) was used to measure the concentration of 2-CP. The flow rate of the mobile phase, which was a mixture of methanol with 1% acetic acid and pure water with 1% acetic acid at 60:40 ratio, was 1 ml/min. The absorbance was detected at 280 nM. The samples taken from the system were centrifuged at 13000 rpm in an ultracentrifuge (International Equipment Company ; Needham, MA; model No: IEC Centra-M) for 10 minutes. Then 25 µl of the supernatant liquid were injected for analysis via an autosampler. Precalibration was done by injecting a series of different known concentrations of 2-CP. Nelson software was used for converting detected area to concentration in ppm directly.

4.2 Protein Concentration Assay

BCA Protein Assay reagent (Pierce Chemical Company, Rockford, IL, Series No.: 23225X) was used to measure the concentration of protein. The samples taken from the streams were centrifuged at 13000 rpm for 15 minutes in the ultracentrifuge. Then 0.15 ml of supernatant liquid was mixed with 3 ml reagent and incubated at 37°C for

30 minutes. Absorbance was then measured spectrophotometrically at 562 nM. Bovine serum albumin (BSA) obtained from Pierce Chemicals was used as the calibration standard.

4.3 Polyacrylamide Gel Electrophoresis Analysis

In order to observe the effect of protein separation by UF unit, the enzyme systems in the permeate (Stream #3) and the concentrate (Stream #4) were analyzed using denaturing (SDS) discontinuous gel electrophoresis, Laemmli gel method (abrieved as SDS-PAGE). The enzyme solutions sampled from the permeate (Stream #3) and the concentrate (Stream #4) were concentrated 3-4 fold in a vacuum freezing dryer. 7%~ 14% gradient acrylamide separating gel under denaturing condition (in the presence of 0.1% SDS), pH 8.8, was used to separate proteins in the concentrated enzyme solution based on molecular size, as they moved through the gel matrix toward the anode. The stacking gel was 3% acrylamide, buffered with tris-HCl, pH 6.8. The gel was run overnight at 7 mAmp. The SDS-PAGE molecular standard (obtained from Bio-Rad Inc.) appeared on the gel in a series of bands which represent the different known molecular weights. The molecular weight of a unknown sample can be estimated by comparing the position of the band on the gel with the bands of standard.

After electrophoretic separation, the gels were stained with the Coomassie Blue staining method. The proteins were precipitated in a fixing solution containing 50% methanol and 10% acetic acid, and then detected by using the Coomassie Blue. The 7% acetic acid solution was used to immerse the gels overnight to destain the blue color on the gels where there was no protein.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Results for Experiments in Packed-Bed Reactor

In order to easily compare the differences between Mode A and Mode B, in all the tables and figures the day zero indicates the time when the process was switched from Mode A to Mode B (but only after steady state had be achieved when the process was in Mode A). The previous days were labeled as negative numbers (-1, -2,...etc.). The following days were labeled as positive numbers (1, 2,.. etc.). Stream #1 was the input of the UF system. Stream #2 was the circulation stream of the UF system. The final output was Stream #5 before day zero, and changed to Stream #3 after day zero.

For System I, (using 10,000 mw cut-off hollow fiber membrane and 3.5 ml/min feed rate), the degradation of 2-chlorophenol in every stream is presented in Figure 6 and Table 3. Figure 7 and Table 4 present the percentage ratio of residual 2-chlorophenol concentration relative to inlet concentration in each stream. When the steady state was reached in Mode A and the system was switched to Mode B (at day zero) about 20% of the incoming 2-chlorophenol was reduced from 52 ppm (inlet stream) to 42 ppm (Stream #1) (Figure 6), as a result of biodegradation in the packed-bed reactor (having a retention time of 3 hours). Then the effluent from the reactor was circulated in the ultrafiltration system. The concentration of 2-chlorophenol in the final outlet (Stream #5) went down to 30 ppm (57.3% residual) due to the extra 4.75 hour retention time in the ultrafiltration system. The protein concentration in the circulation (Stream #2) was 0.0509 g/l (Figure 10).

After switching to Mode B, the concentration of 2-chlorophenol in the final output (Stream #3) was supposed to go down gradually because the extracellular enzyme systems were expected to concentrate in the circulation loop because of the ultrafiltration unit. The results showed that in the next three days, the protein of circulation (Stream #2) increased from 0.0509 gm/l to 0.0691 gm/l, at the rate 0.0061 gm/l/day (Figure 10), but the residual 2-chlorophenol of final output (Figure 8) was about 60% of the inlet concentration (30 ppm) without any significant decline.

For System II (Figure 11-15 and Table 7-10), the operating conditions were the same as System I except for the feed rate which was reduced from 3.5 ml/min to 2 ml/min. After switching to Mode B, the protein concentration in the circulation (Stream #2) increased at an average rate of 0.0041 gm/l/day, from 0.0412 gm/l to 0.0618 gm/l in five days (Figure 15). The percentage of residual 2-chlorophenol in the final output (Figure 13) went down to 2.1 ppm, i.e., from 49% of the input concentration (25.50 ppm) to 45.7% (22.40 ppm) in the first day, and then fluctuated in the range 47.5%~53.3% (23.5 ppm to 28.4 ppm).

For System III (Figure 16-20 and Table 11-14) the hollow fiber ultrafiltration cartridge was changed to the smaller pore size, i.e., 5,000 molecule weight cut-off. The protein concentration in the circulation stream (Figure 20) sharply increased at a rate of 0.0121 gm/l/day ,2~3 fold faster than the previous two system, from 0.0631 gm/l to 0.0995 gm/l in first three days, and then varied between 0.0922 gm/l and 0.0958 gm/l in the next three days. Even though such a dramatic change occurred, the residual 2-chlorophenol only decreased by 5% (2 ppm) from 59.7% (31.12 ppm) to 54.4% (28.46 ppm) (Figure 18), and then remained between 60% (30 ppm) and 54% (28 ppm) of the input concentration.

The protein concentration still could be detected in the output (Figures 10, 15 and 20), even in System III for which the molecular weight cut-off of the membrane

(5,000 daltons) was smaller than the molecular weight of extracellular enzyme system of fungus (13,000~40,000 daltons).

5.2 Results of Electrophoresis Analysis

Figure 24 shows the results of the SDS polyacrylamide gel. The samples in Figure 24(a) were obtained using the 10,000 mw cut-off membrane. The permeate was labeled as "P" and concentrate as "C". There were no bands showing up in the permeate (Stream #3), but at least 4 bands appearing in the concentrate (Stream #4). The molecular weight of these four bands are about 13,000 , 16,000, 23,000 and 40,000 daltons, respectively. In Figure 24(b), the samples obtained using 5,000 MWCO membrane are shown. There were no vivid bands but two darker color lines stained on the gel for the concentrate. There was no bands for the permeate (#3).

The permeate could not be found on the gel because the concentration of protein in the permeate was too low to be stained, even after a 2-3 fold concentration was done using the vacuum freezing dryer.

5.3 Results for Experiments in Shaker Bottles

The results of biodegradation in the shaker bottle experiments are presented in Figure 25 and Table 15. The dimensionless data for 2-chlorophenol showed in Figure 26 and Table 16 were obtained by dividing the concentration at any given time by the initial concentration. The 2-chlorophenol in the Bottles A,B and D varied in the range ±4% (±1 ppm) and were regarded as constant. That meant there was no degradation in these three bottles. The 2-chlorophenol in Bottle C, which contained a high amount of fungus biomass and air (four fifth of the bottle), was degraded by 16%, from 42.12

ppm to 35.40 ppm, in six days. The biodegradation rate was 1.12 ppm/day. These results show that mineralization of 2-chlorophenol can not be processed without fungal biomass and air. This can explain why the protein concentration increased in the circulation, but the degradation of 2-chlorophenol did not change significantly.

Table 3. Concentration (in ppm) of 2-Chlorophenol in Different Streams for System I

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5	Stream Inlet
-3	40.15	31.08	30.32	31.54	30.63	53.64
-1	41.69	34.69	32.80	34.78	32.87	53.75
0	42.21	32.41	30.66	32.40	30.09	52.51
1	40.63	33.69	31.83	34.08		54.02
3	38.74	32.28	30.33	32.19		49.01
4	39.03	29.81	27.65	29.79		50.33

Table 4. **Percentage of Residual 2-Chlorophenol in Different Streams for System I**

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5
-3	74.85%	57.94%	56.53%	58.80%	57.10%
-1	77.56%	64.55%	61.02%	64.71%	61.16%
0	80.38%	61.72%	58.39%	61.70%	57.30%
1	75.21%	62.36%	58.92%	63.08%	
3	79.04%	65.87%	61.88%	65.68%	
4	77.54%	59.23%	54.94%	59.20%	

Table 5. Concentration (in g/L) of Protein in Different Streams for System I

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5	Stream Inlet
-3	0.0618	0.0582	0.0485	0.0533	0.0558	0.0801
-1	0.0485	0.0461	0.0412	0.0485	0.0485	0.0363
0	0.0558	0.0509	0.0473	0.0546	0.0461	0.0509
1	0.0558	0.0533	0.0473	0.0509		0.0448
3	0.0655	0.0691	0.0570	0.0716		0.0631
4	0.0436	0.0485	0.0388	0.0497		0.0558

Table 6. pH Values in Different Streams for System I

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5	Stream Inlet
-3	3.34	3.47	3.47	3.51	3.5	4.33
-1	3.5	3.65	3.65	3.66	3.66	3.68
0	3.46	3.78	3.76	3.78	3.79	3.61
1	3.49	3.82	3.82	3.83		3.58
3	3.64	4.16	4.15	4.15		4.17
4	3.68	4.81	4.75	4.81		3.74

Table 7. Concentration (in ppm) of 2-Chlorophenol in Different Streams for System II

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5	Stream Inlet
-7	34.20	28.10	27.70	27.30	24.30	54.00
-6	34.50	27.10	27.60	26.90	24.40	51.50
-5	38.20	29.60	29.90	29.00	26.50	53.30
-3	32.30	25.00	24.90	24.80	22.80	53.40
-2	34.50	22.90	23.00	22.80	21.10	54.80
-1	35.50	26.60	27.00	26.80	24.20	53.90
0	35.30	28.30	29.30	28.30	25.50	52.00
2	33.70	25.00	22.40	24.70		49.00
3	34.80	26.40	24.20	26.50		47.13
4	35.10	26.70	23.56	26.50		49.60
5	34.01	29.15	26.80	28.22		50.29
8	36.98	31.66	28.44	32.39		54.00

Table 8. Percentage of Residual 2-Chlorophenol in Different Streams for System II

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5
-7	63.3%	52.0%	51.3%	50.6%	45.0%
-6	67.0%	52.6%	53.6%	52.2%	47.4%
-5	71.7%	55.5%	56.1%	54.4%	49.7%
-3	60.5%	46.8%	46.6%	46.4%	42.7%
-2	63.0%	41.8%	42.0%	41.6%	38.5%
-1	65.9%	49.4%	50.1%	49.7%	44.9%
0	67.9%	54.4%	56.3%	54.4%	49.0%
2	68.8%	51.0%	45.7%	50.4%	
3	73.8%	56.0%	51.3%	56.2%	
4	70.8%	53.8%	47.5%	53.4%	
5	67.6%	58.0%	53.3%	56.1%	
8	68.5%	58.6%	52.7%	60.0%	

Table 9. Concentration (in g/L) of Protein in Different Streams in System II

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5	Stream Inlet
-7	0.0521	0.0509	0.0497	0.0521	0.0546	0.0570
-6	0.0509	0.0351	0.0339	0.0363	0.0351	0.0424
-3	0.0631	0.0388	0.0363	0.0400	0.0388	0.0533
-2	0.0776	0.0485	0.0436	0.0570	0.0509	0.0485
-1	0.0679	0.0643	0.0606	0.0667	0.0679	0.0570
0	0.0509	0.0412	0.0303	0.0339	0.0412	
2	0.0801	0.0533	0.0448	0.0533		0.0618
3	0.0570	0.0521	0.0388	0.0497		0.0473
4	0.0582	0.0570	0.0339	0.0521		0.0716
5	0.0594	0.0618	0.0412	0.0570		0.0582

Table 10. pH Values in Different Streams for System II

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5	Stream Inlet
-7	4.23	5.02	4.84	4.84	4.84	3.58
-6	4.05	4.75	4.77	4.78	4.76	3.38
-2	4.71	4.88	4.89	4.89	4.85	3.75
-1	4.14	4.71	4.68	4.75	4.78	3.94
0	4.36	4.93	4.94	4.92		4.93
2	4.34	4.81	4.79	4.83		3.85
3	4.00	4.86	4.84	4.84		4.54
4	3.94	4.80	4.80	4.84		3.55
5	4.07	4.83	4.84	4.94		3.80

Table 11. Concentration (in ppm) of 2-Chlorophenol in Different Streams for System III

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5	Stream Inlet
-7	41.5	16.88	15.99	16.79	15.48	50.58
-6	42.1	23.47	21.34	22.77	20.89	48.86
-5	45.23	26.67	25.06	26.27	23.46	52.76
-3	46.55	30.21	28.29	30.06	27.85	53
-2	47.5	33.51	30.8	34.56	32.88	57.18
-1	44.18	33.15	31.14	32.81	25.71	54
0	44.5	33.53	32.16	33.24	31.12	52.15
1	45.76	33.38	29.8	33.03		52.68
2	46.18	32.69	28.46	32.68		52.3
3	45.74	32.8	28.74	32.71		51.78
4	45.66	35.07	29	31.92		48.18
5	47.19	35.64	28.18	34.78		49.92
6	48.64	37.36	29.8	36.05		55.83

Table 12. Percentage of Residual 2-Chlorophenol in Different Streams for System III

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5
-7	82.0%	33.4%	31.6%	33.2%	30.6%
-6	86.2%	48.0%	43.7%	46.6%	42.8%
-5	85.7%	50.5%	47.5%	49.8%	44.5%
-3	87.8%	57.0%	53.4%	56.7%	52.5%
-2	83.1%	58.6%	53.9%	60.4%	57.5%
-1	81.8%	61.4%	57.7%	60.8%	47.6%
0	85.3%	64.3%	61.7%	63.7%	59.7%
1	86.9%	63.4%	56.6%	62.7%	
2	88.3%	62.5%	54.4%	62.5%	
3	88.3%	63.3%	55.5%	63.2%	
4	94.8%	72.8%	60.2%	66.3%	
5	94.5%	71.4%	56.5%	69.7%	
	87.1%	66.9%	53.4%	64.6%	

Table 13. Concentration (in g/L) of Protein in Different Streams for System III

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5	Stream Inlet
-7	0.0412	0.0388	0.0376	0.0363	0.0679	0.0618
-6	0.0448	0.0448	0.0473	0.0436	0.0436	0.0631
-2	0.0752	0.0740	0.0691	0.0667	0.0752	0.0764
-1	0.0728	0.0703	0.1214	0.0691	0.0679	0.0910
0	0.0703	0.0631	0.0606	0.0631	0.0643	0.0594
1	0.0825	0.0995	0.0667	0.1371		0.0570
2	0.0910	0.0801	0.0679	0.1110		0.0958
3	0.1007	0.0995	0.0801	0.0983		0.0898
4	0.0837	0.0922	0.0764	0.0910		0.2780
5	0.0813	0.0958	0.0776	0.0946		0.1335
6	0.0873	0.0958	0.0776	0.0886		0.0825

Table 14. pH Values in Different Streams for System III

Day	Stream #1	Stream #2	Stream #3	Stream #4	Stream #5	Stream Inlet
-7	3.7	3.81	3.79	3.8	3.82	3.51
-2	3.75	4.19	3.98	4.15	4.2	3.39
-1	3.78	4.34	4.19	4.37	4.5	3.53
0	3.86	4.39	4.34	4.39	4.4	3.54
1	4.15	4.33	4.3	4.33		3.42
2	3.96	4.35	4.31	4.36		3.54
3	3.96	4.47	4.42	4.46		3.67
4	4.53	4.89	4.75	4.87		3.53
5	4.4	4.98	4.92	5		3.59
6	3.89	4.75	4.84	4.84		4.08

Table 15. Concentration of 2-Chlorophenol in the Shaker Bottles

Bottle \ Day	0	1	2	3	6
Bottle A	35.48	34.62	35.68	34.88	35.34
Bottle B	35.48	35.06	36.39	36.97	36.89
Bottle C	42.12	41.37	39.41	37.40	35.40
Bottle D	42.12	42.98	42.51	42.45	42.40
Bottle E	40.53	40.67	40.88	40.94	41.91
Bottle F	40.53	42.07	41.50	41.04	41.33

Table 16. Percentage of Residual 2-Chlorophenol in the Shaker Bottles

Bottle \ Day	0	1	2	3	6
Bottle A	100%	98%	101%	98%	100%
Bottle B	100%	99%	103%	104%	104%
Bottle C	100%	98%	94%	89%	84%
Bottle D	100%	102%	101%	101%	101%
Bottle E	100%	100%	101%	101%	103%
Bottle F	100%	104%	102%	101%	102%

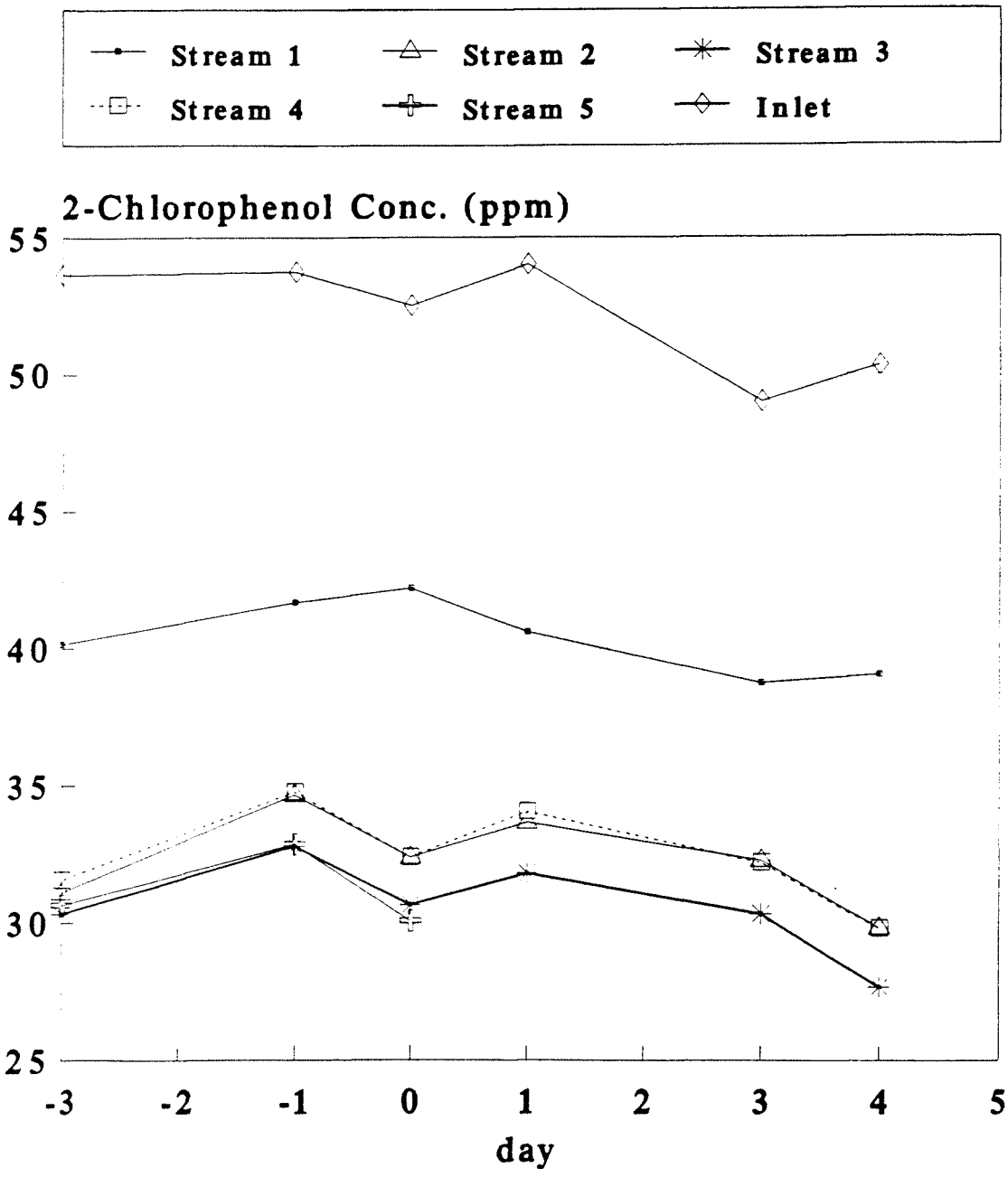


Figure 6. Degradation of 2-Chlorophenol in Different Streams for System I

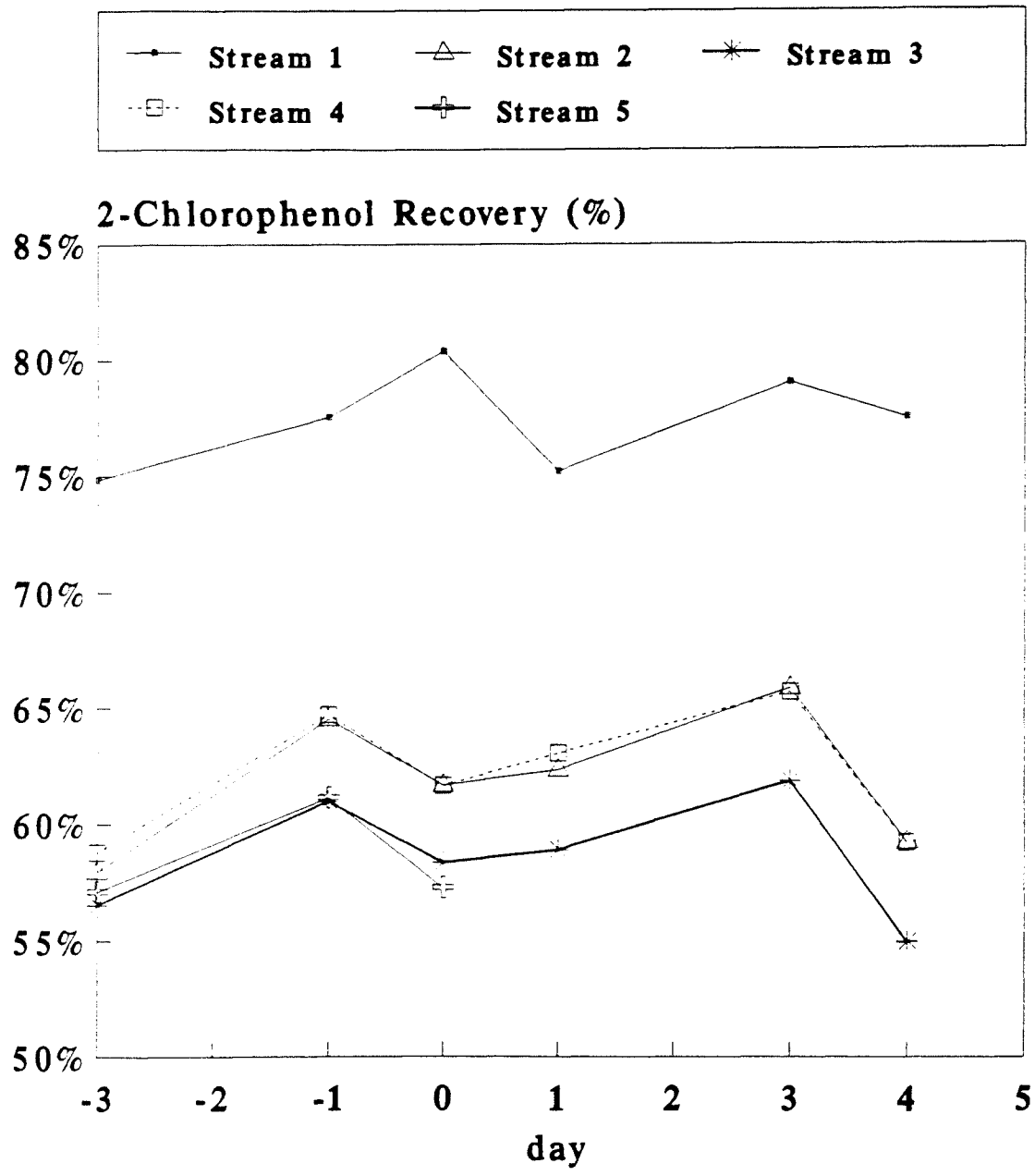


Figure 7. Percentage of Residual 2-Chlorophenol Recovered in Different Streams for System I

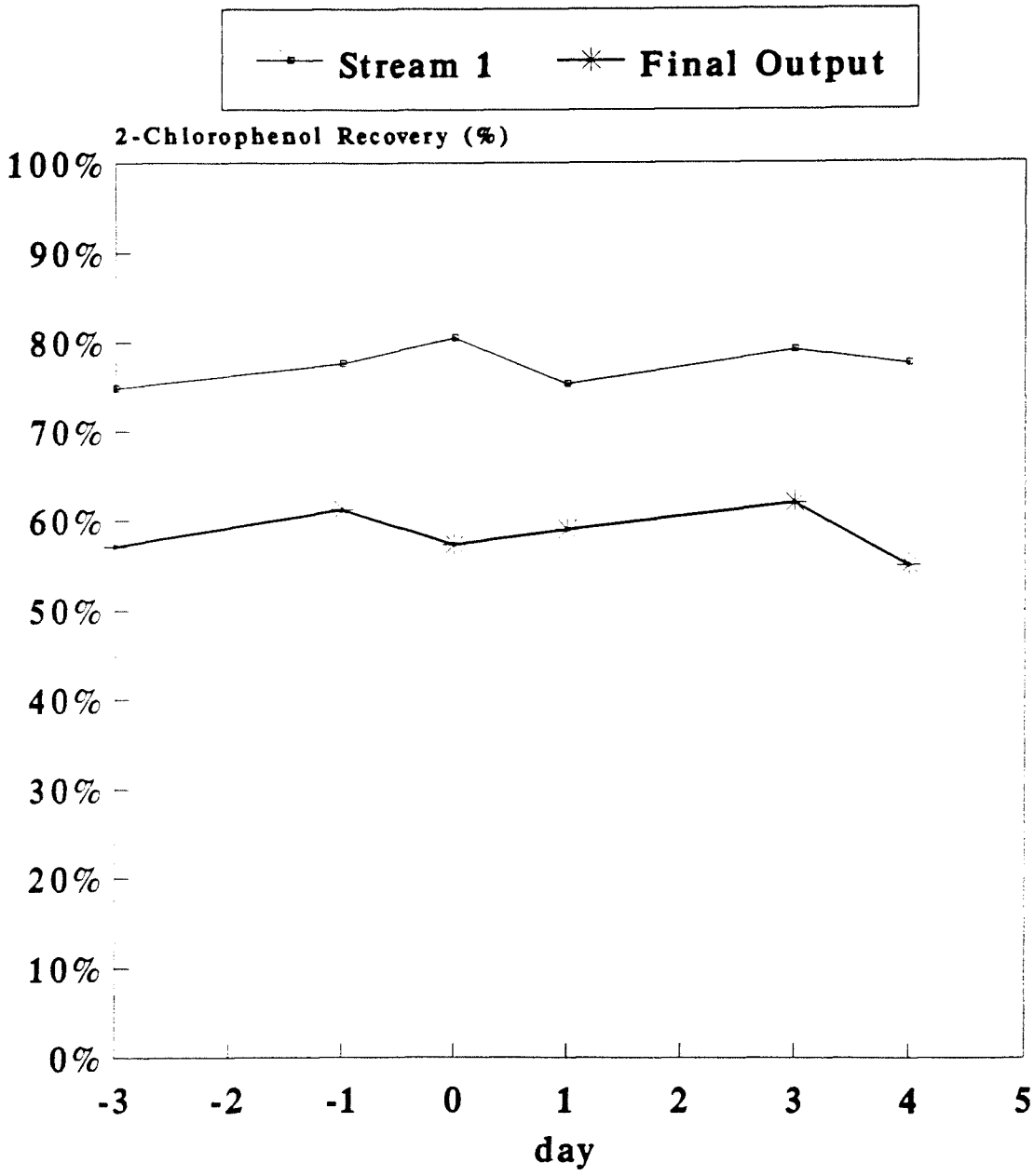


Figure 8. Percentage of Residual 2-Chlorophenol Recovered in Stream #1 and Final Output in System I

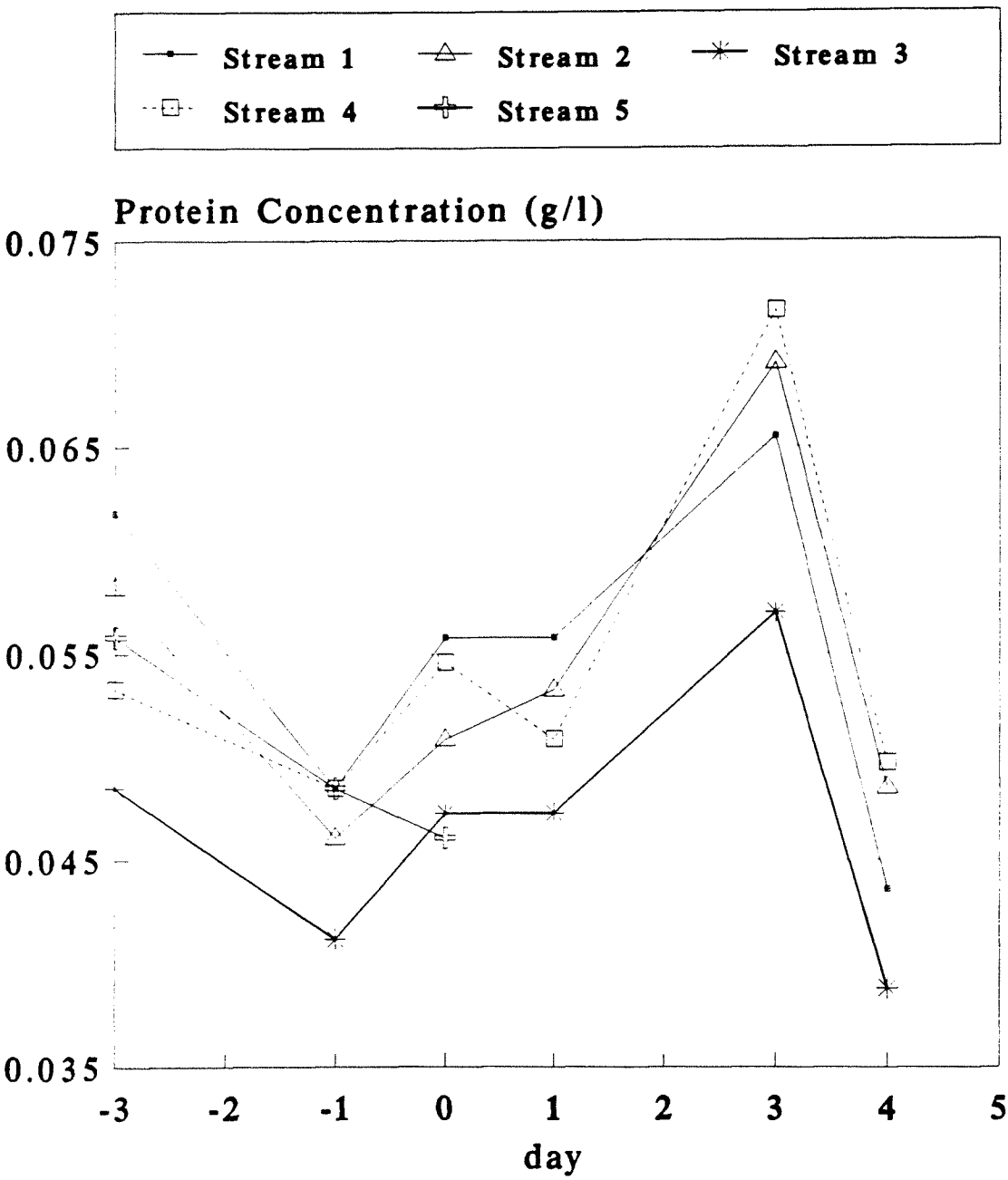


Figure 9. Protein Concentration in Different Streams for System I

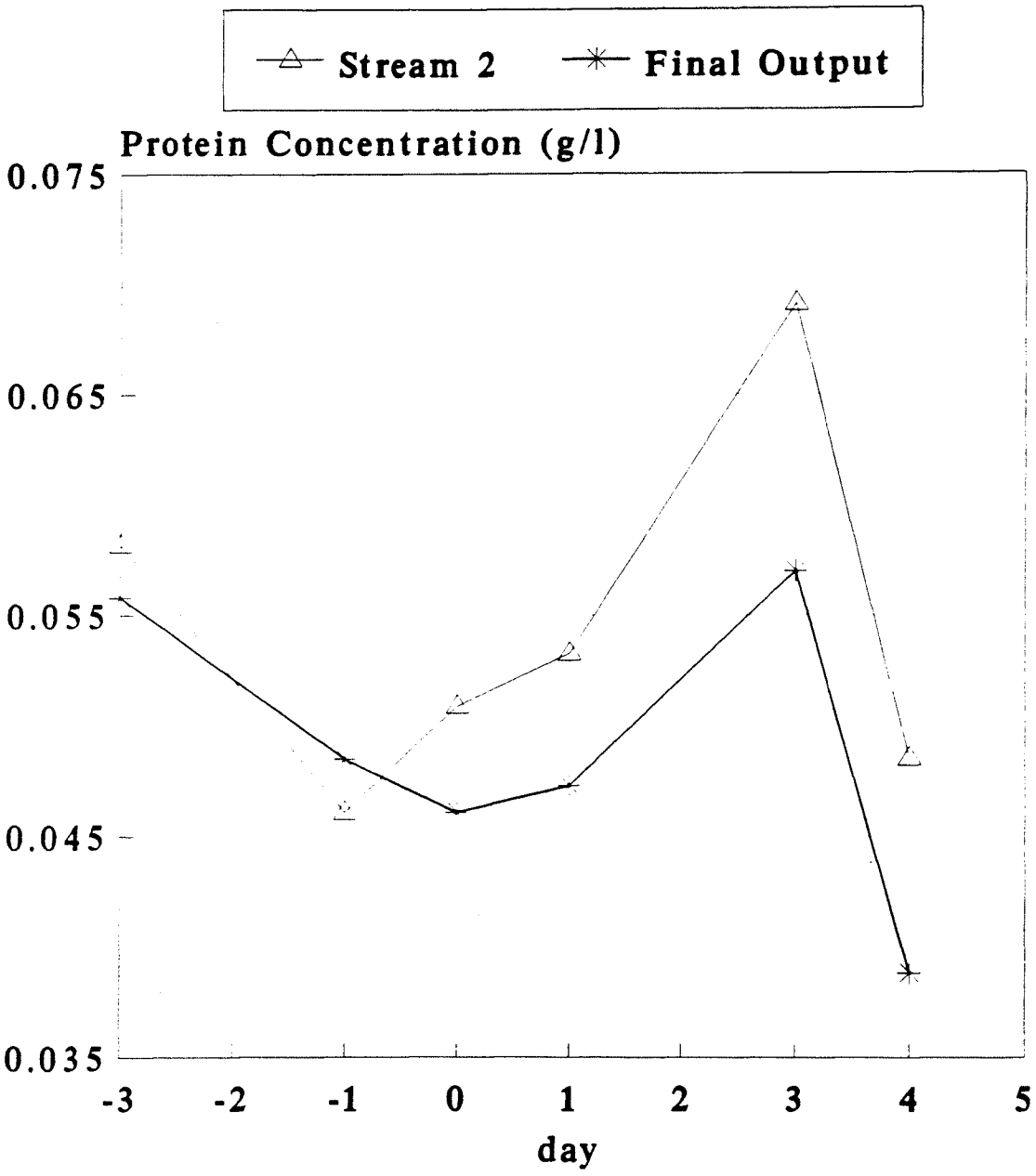


Figure 10. Protein Concentration in Circulation and Final Output Streams for System I

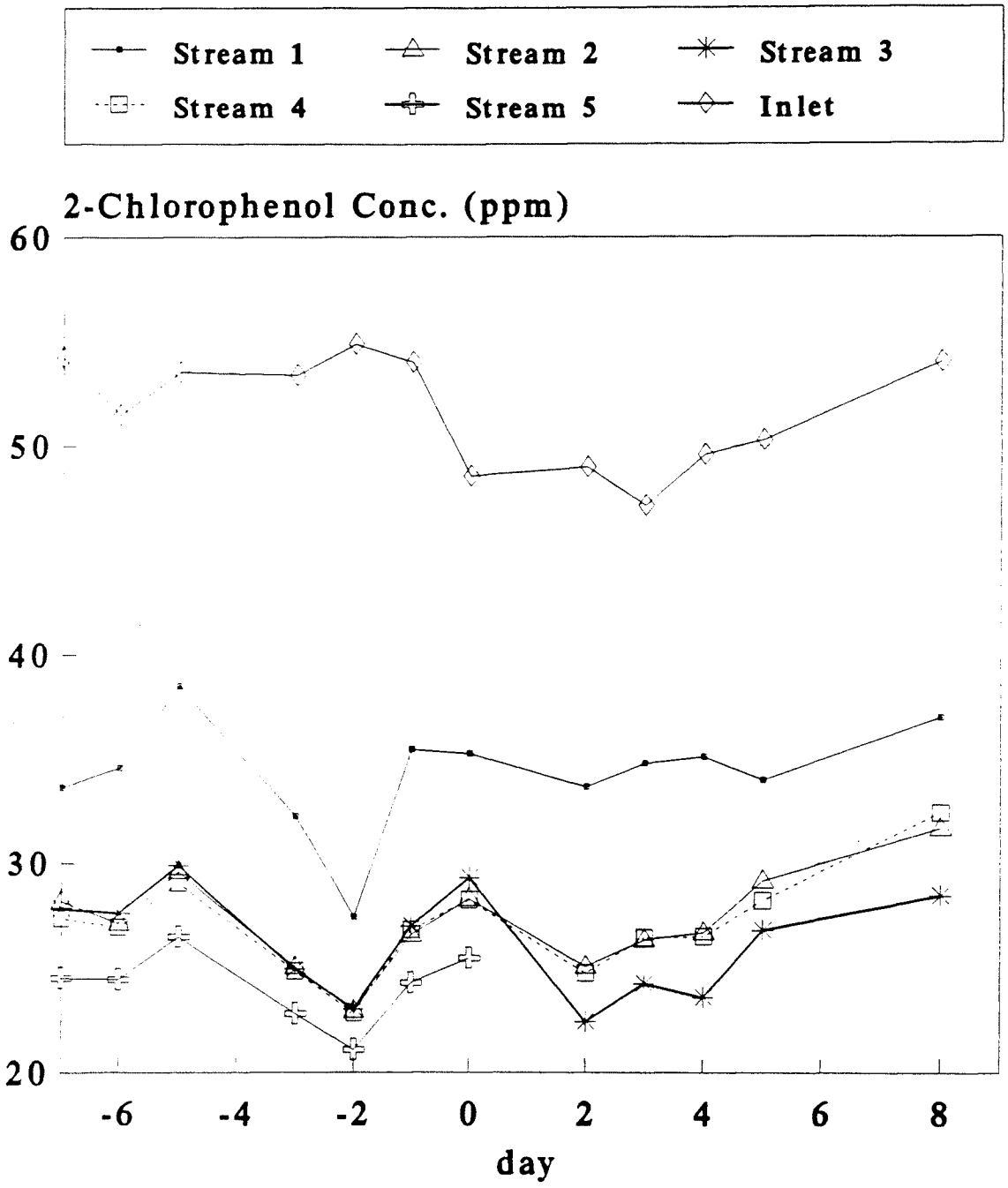


Figure 11. Degradation of 2-Chlorophenol in Different Streams for System II

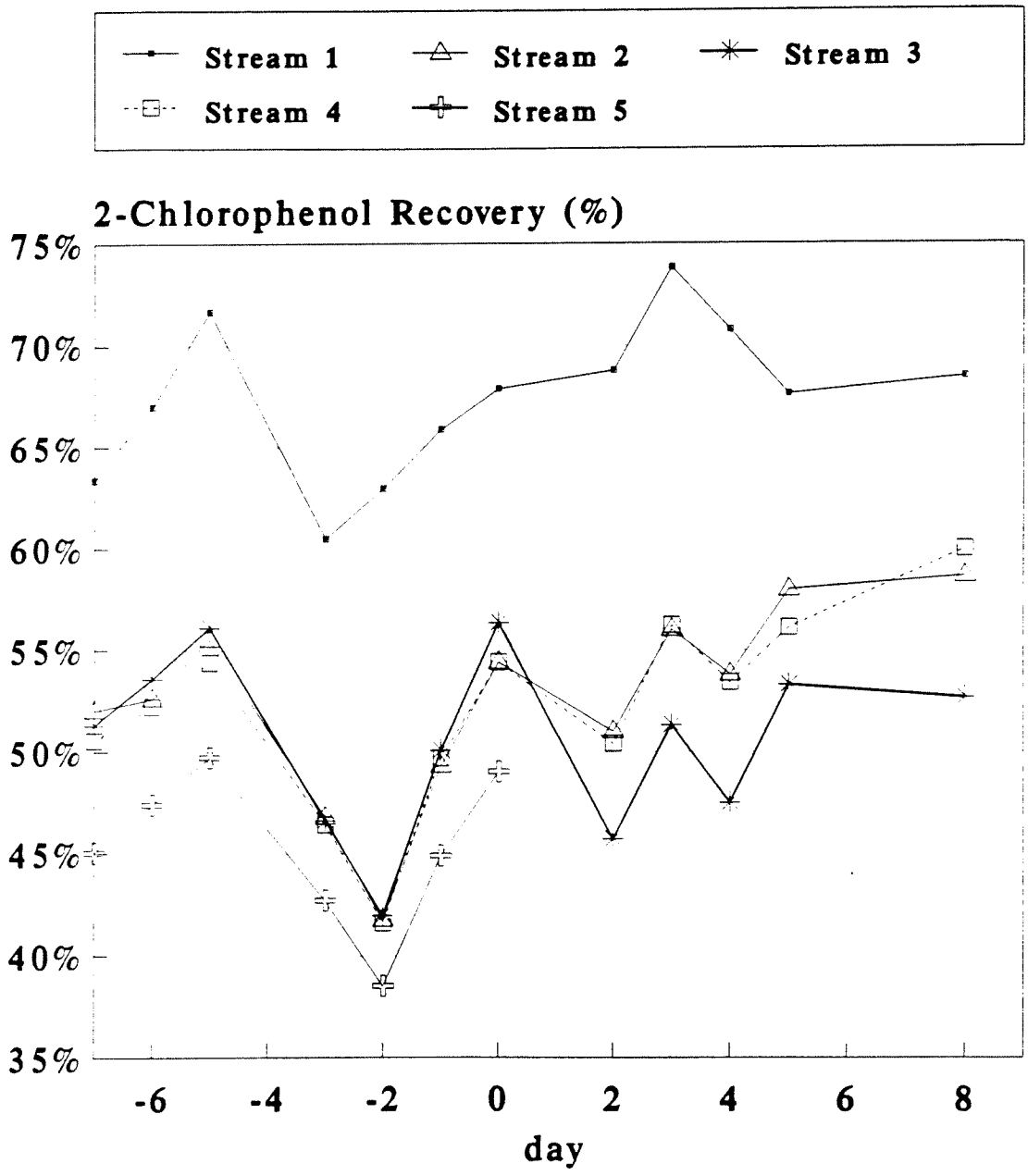


Figure 12. Percentage of Residual 2-Chlorophenol Recovered in Different Streams for System II

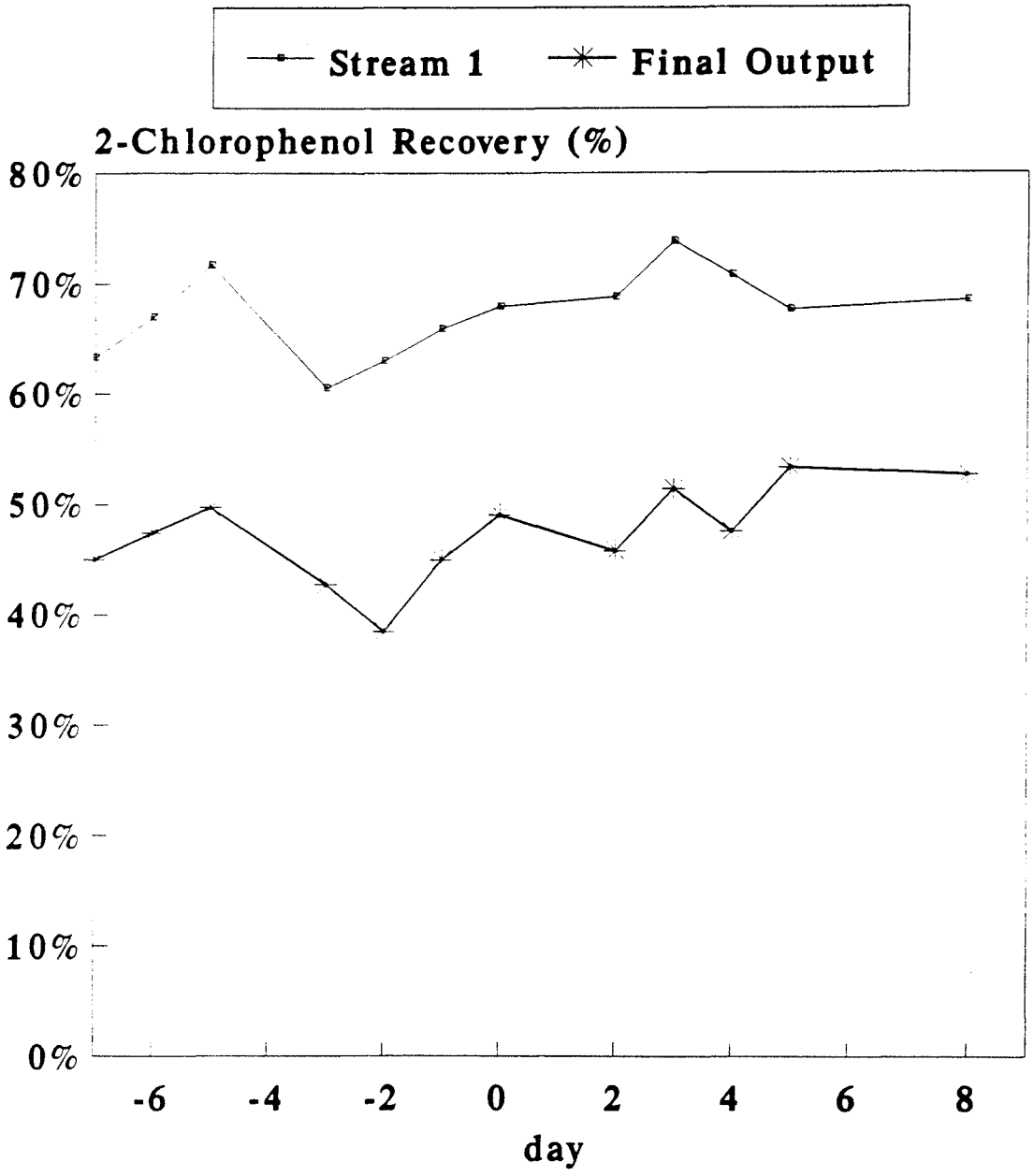


Figure 13. Percentage of Residual 2-Chlorophenol Recovered in Stream 1 and Final Output for System II

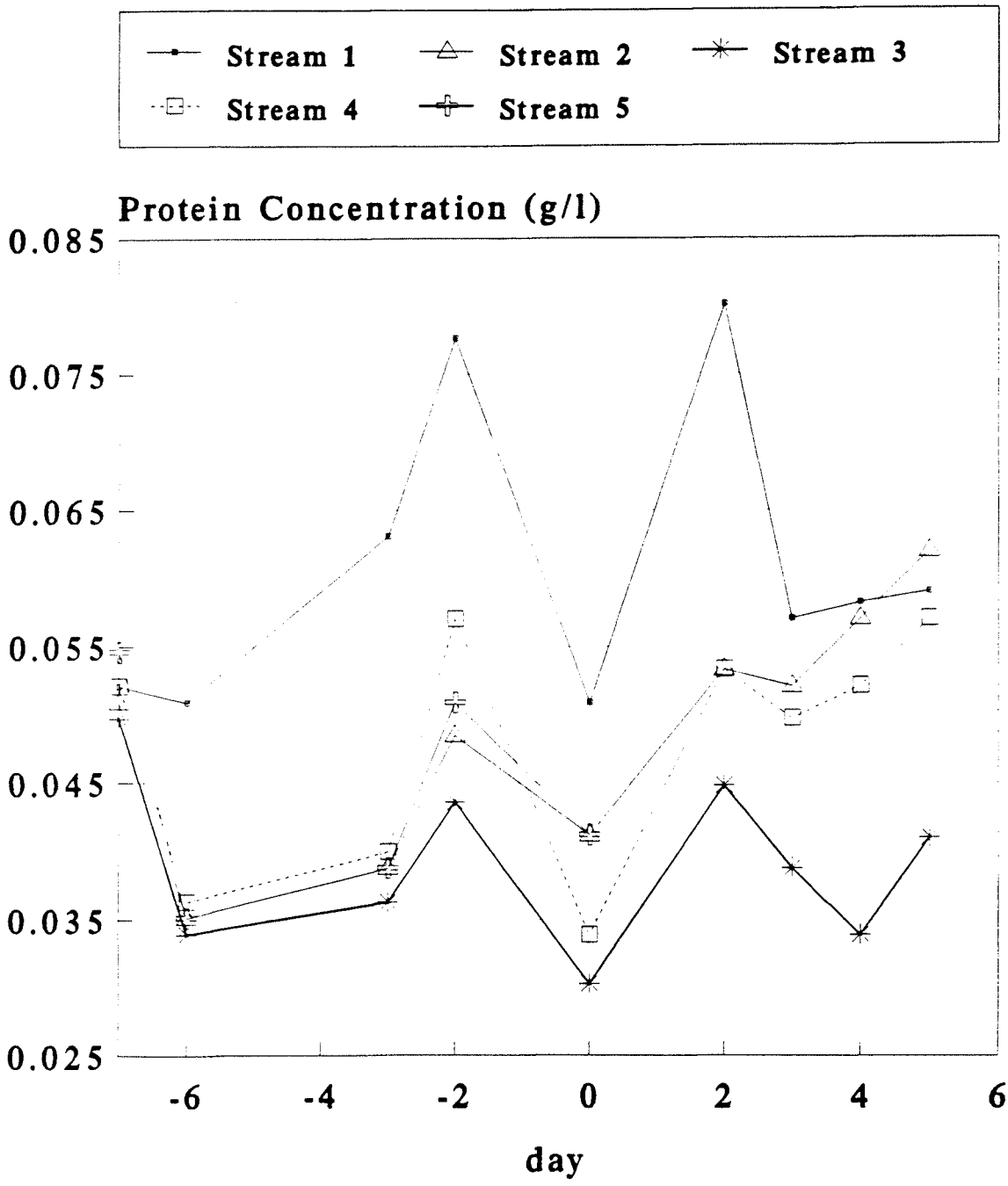


Figure 14. Protein Concentration in Different Streams for System II

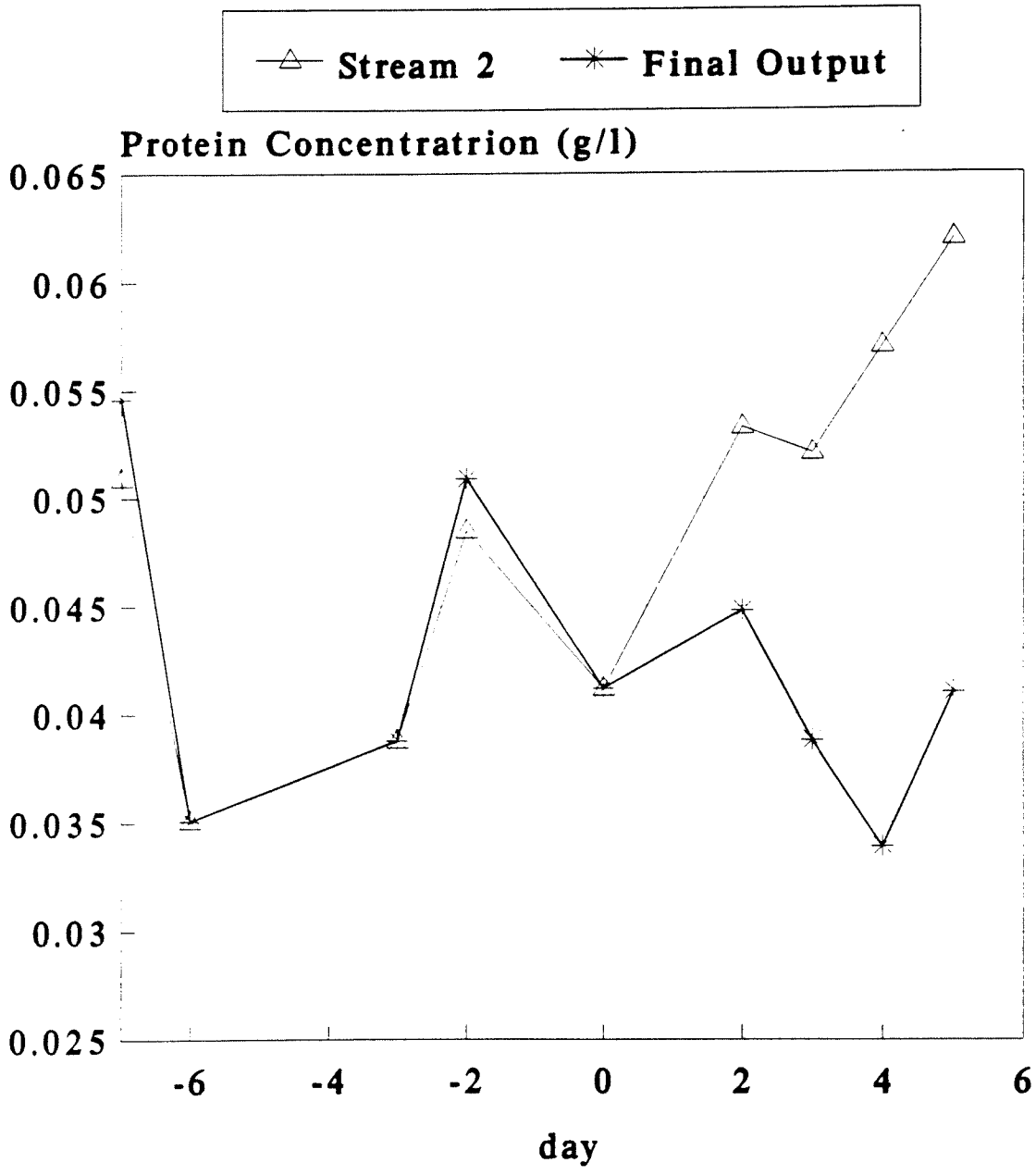


Figure 15. Protein Concentration in Circulation and Final Output Streams for System II

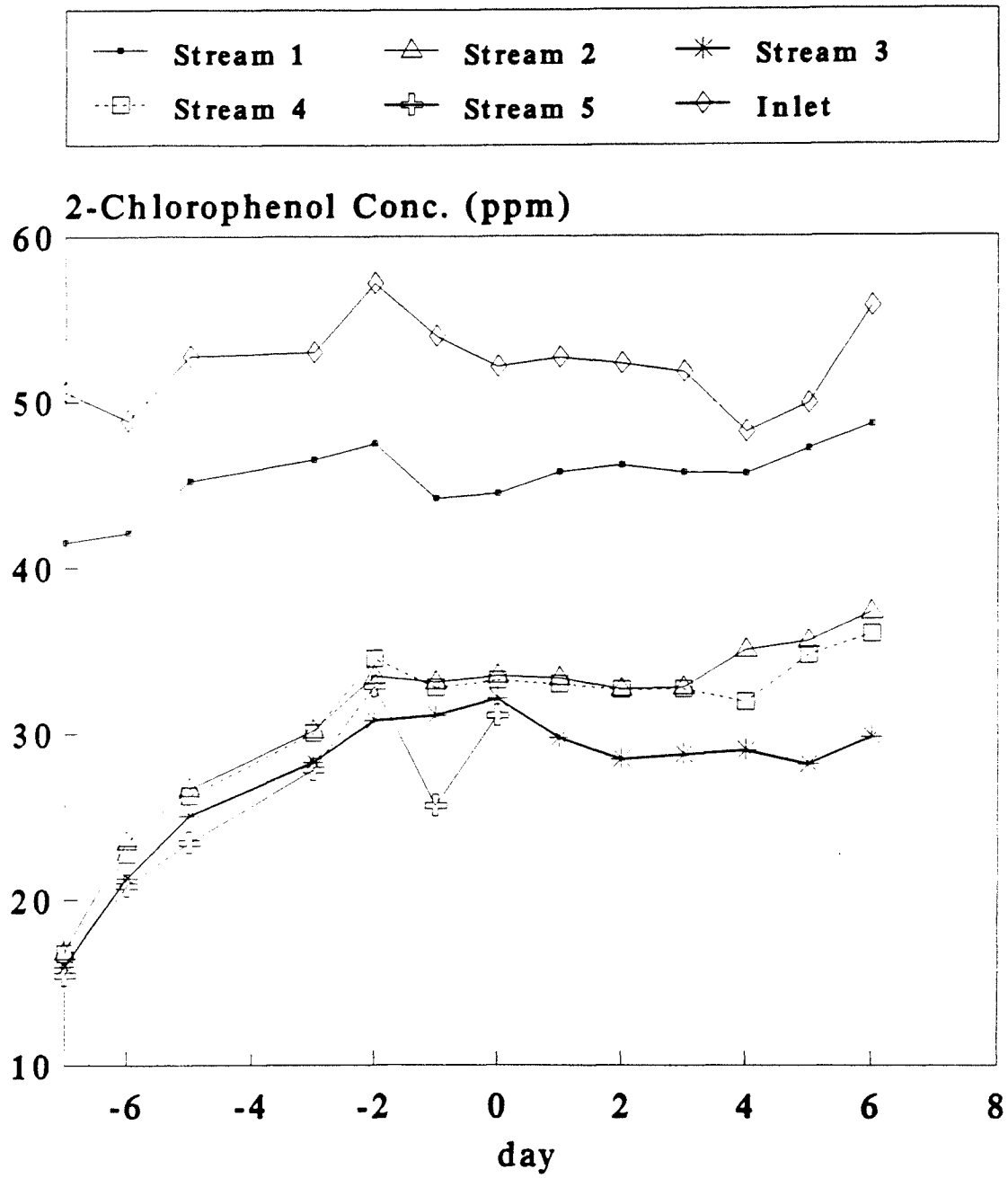


Figure 16. Degradation of 2-Chlorophenol in Different Streams for System III

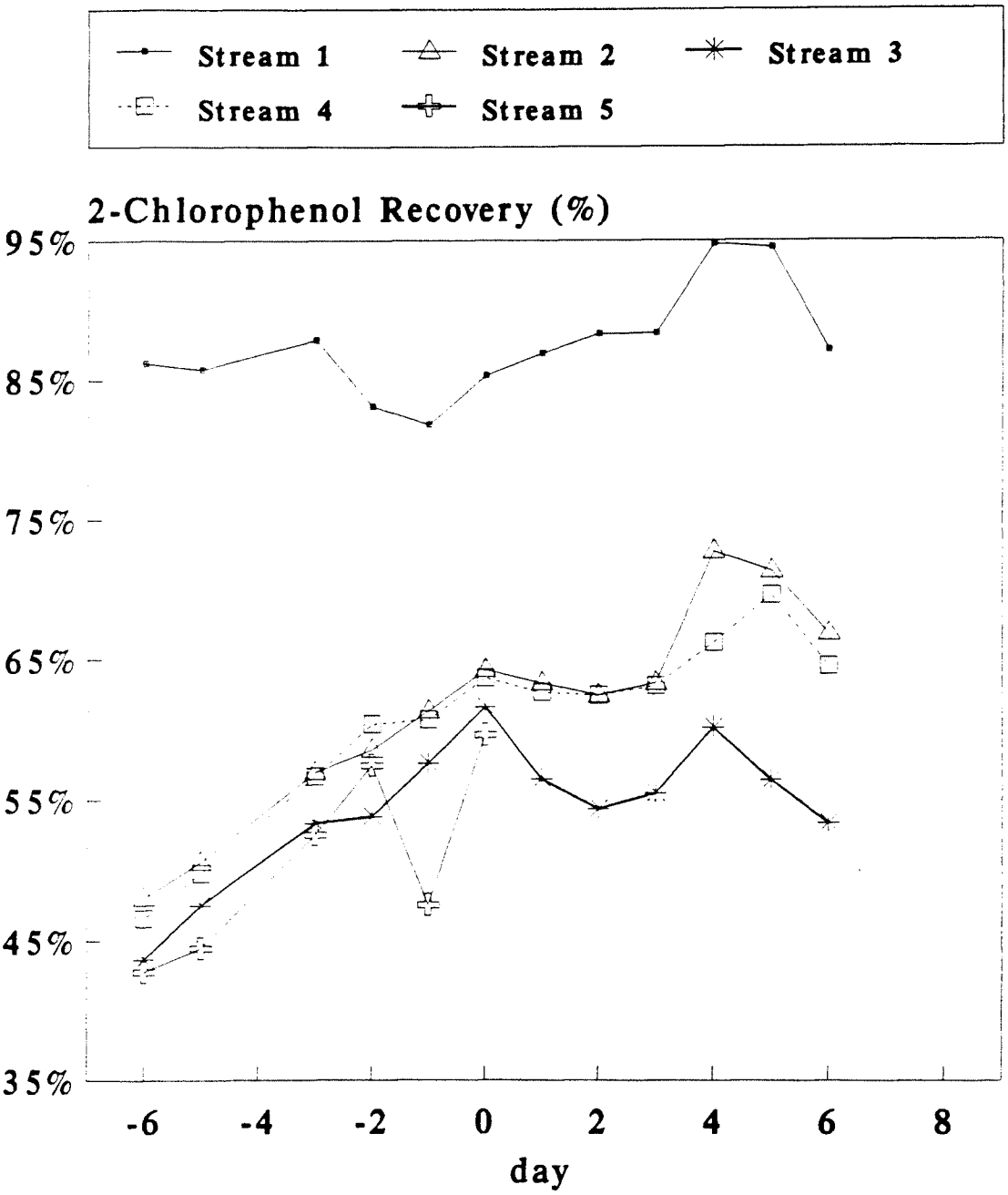


Figure 17. Percentage of Residual 2-Chlorophenol Recovered for System III

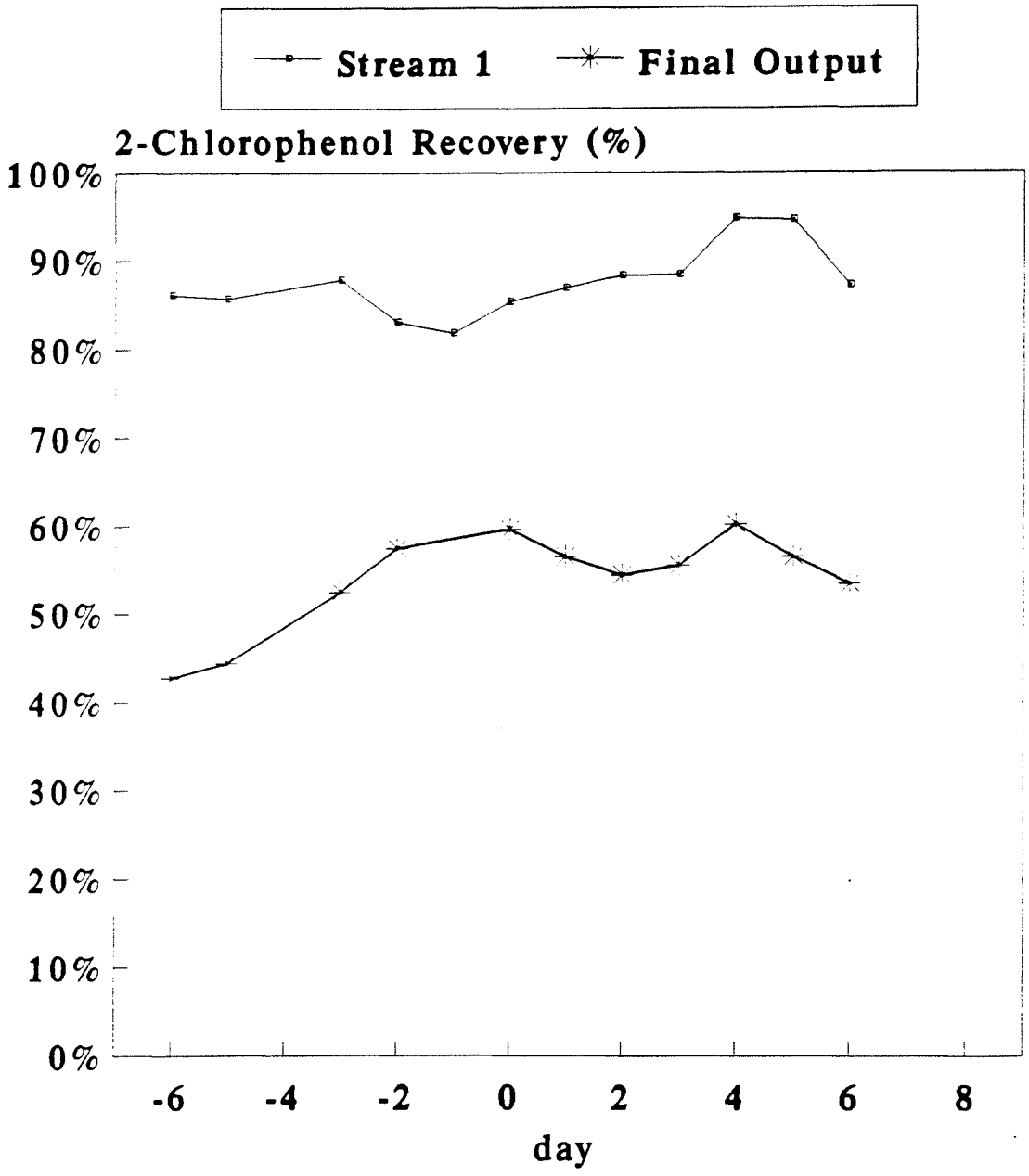


Figure 18. Percentage of Residual 2-Chlorophenol Recovered in Stream 1 and Final Output for System III

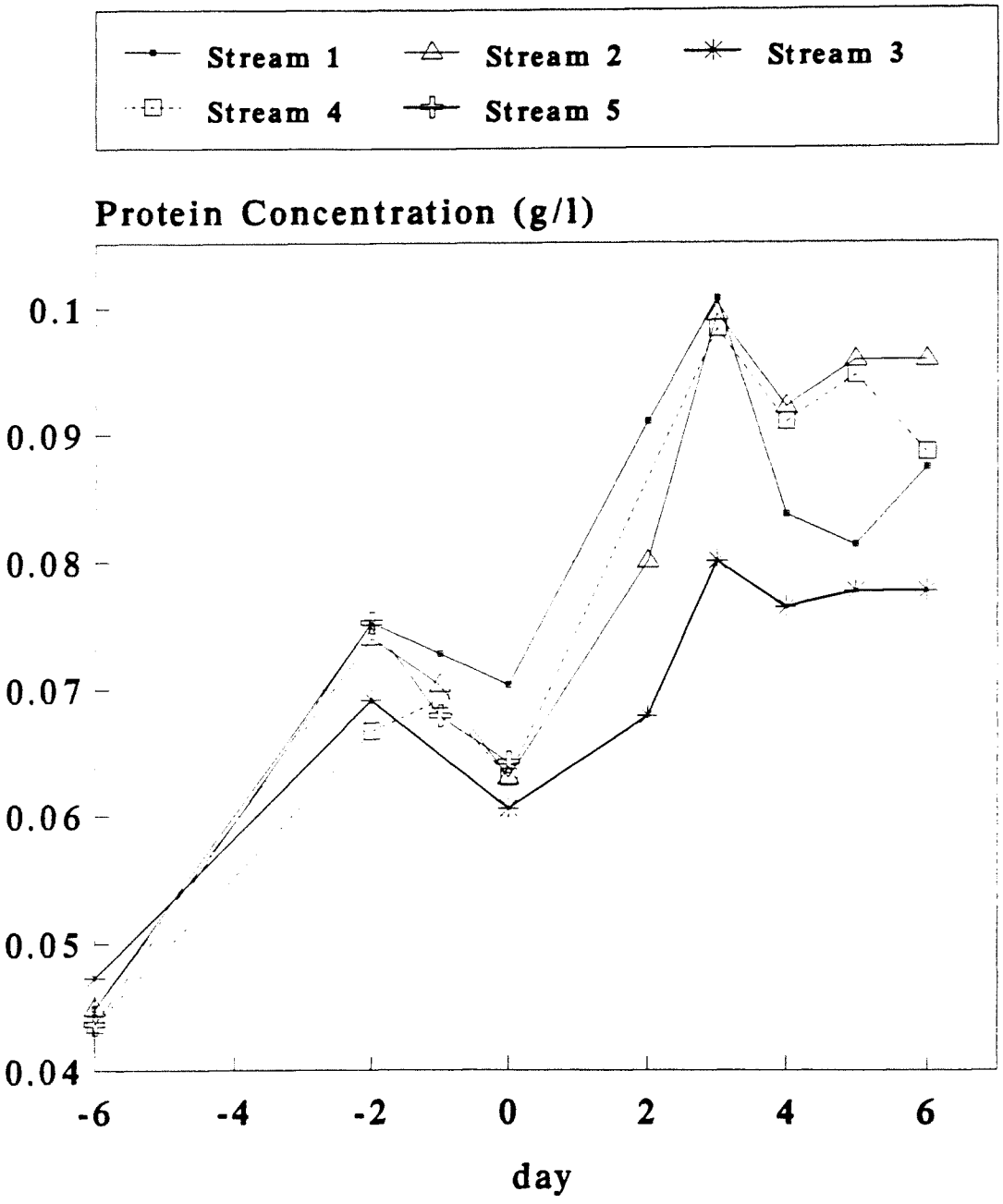


Figure 19. Protein Concentration in Different Streams for System III

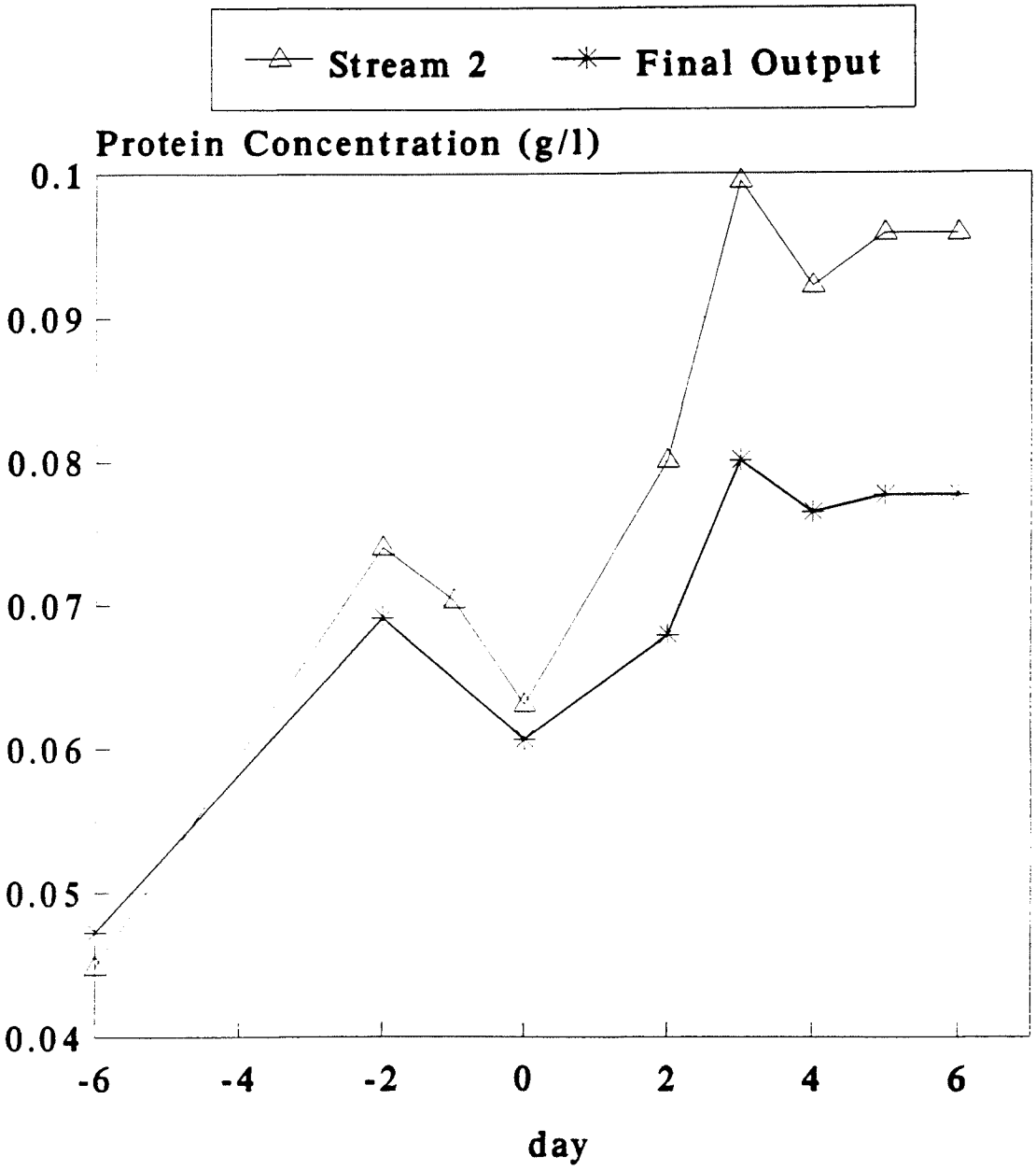


Figure 20. Protein Concentration in Circulation and Final Output Streams for System III

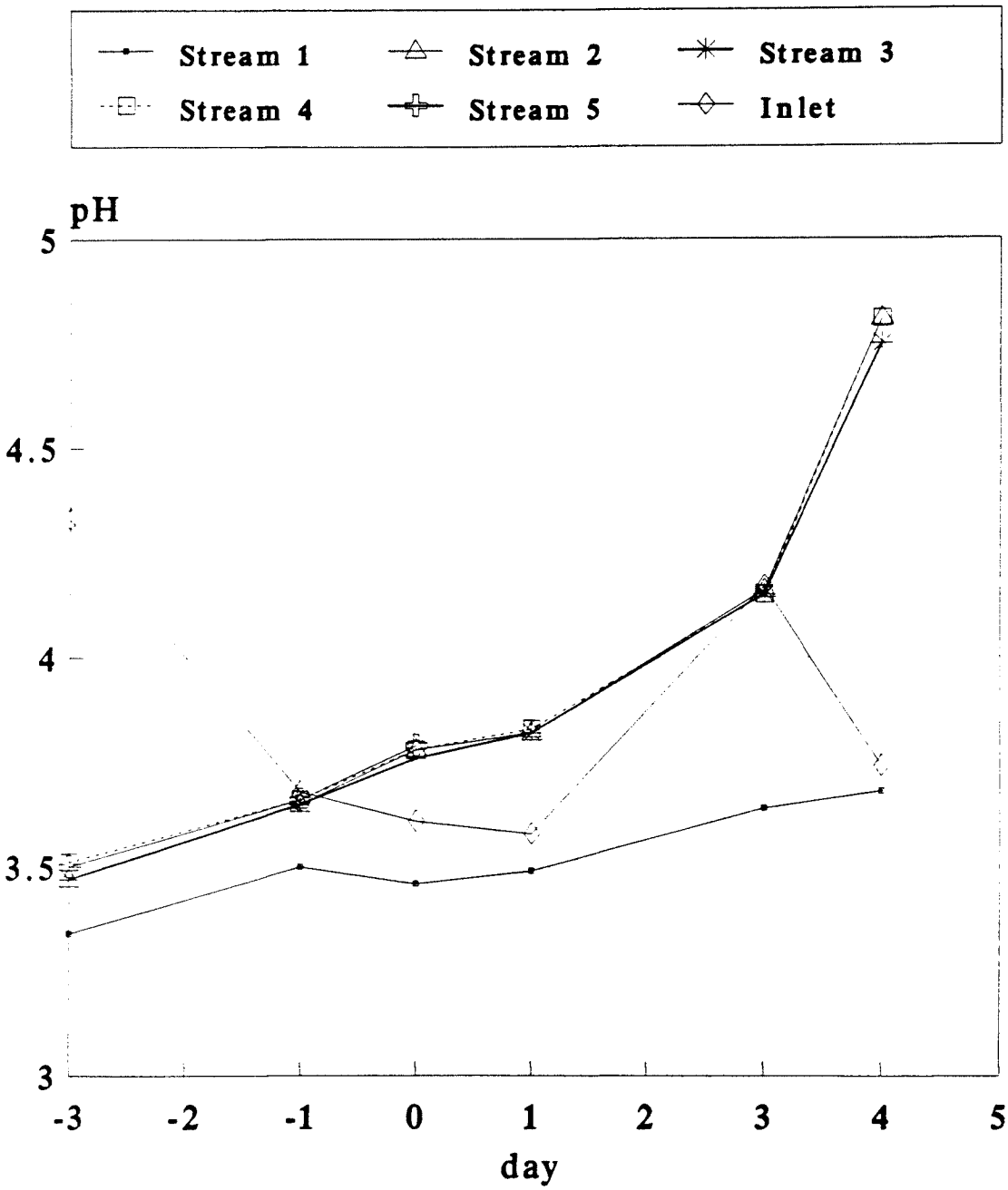


Figure 21. pH Values in Different Streams for System I

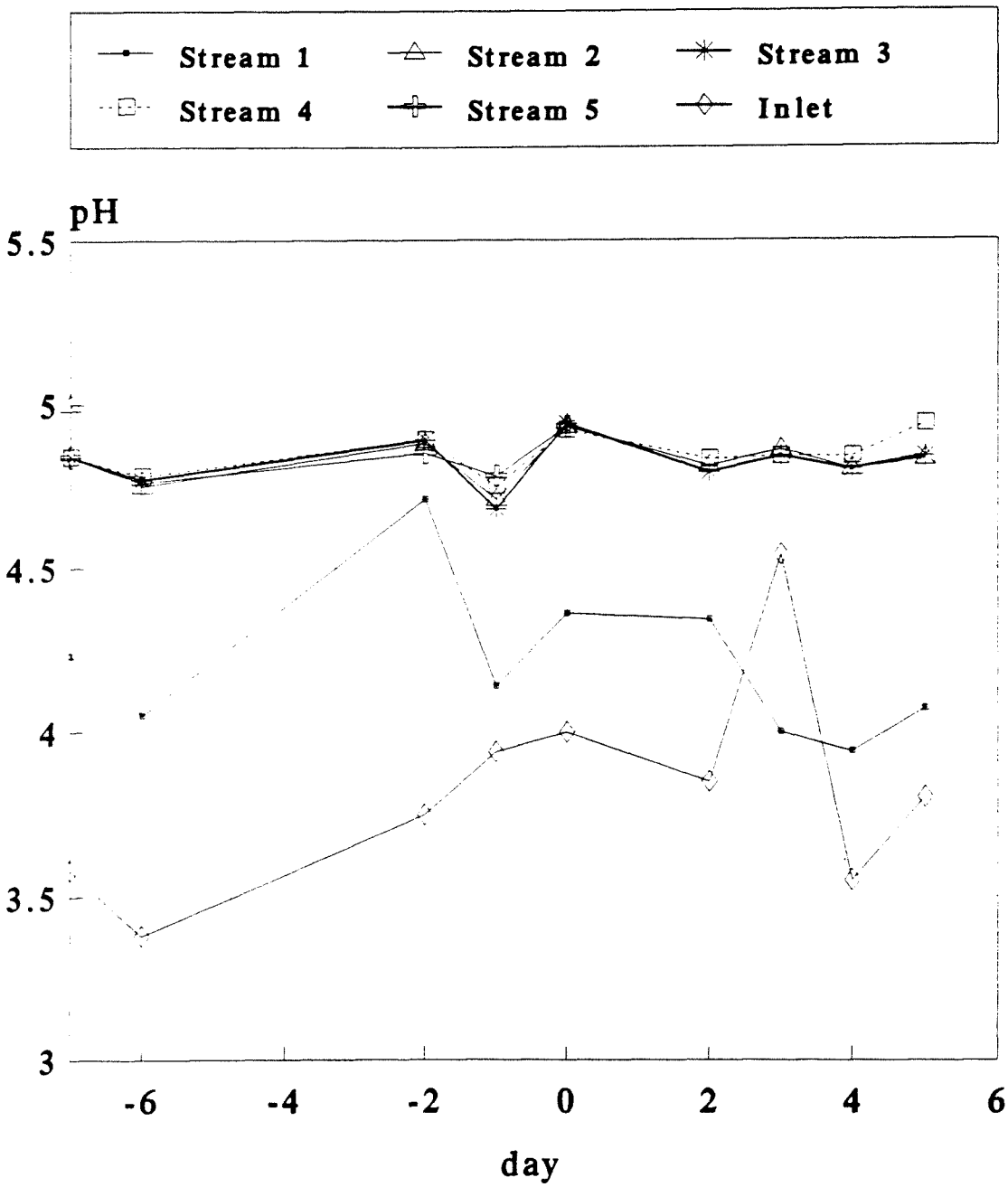


Figure 22. pH Values in Different Streams for System II

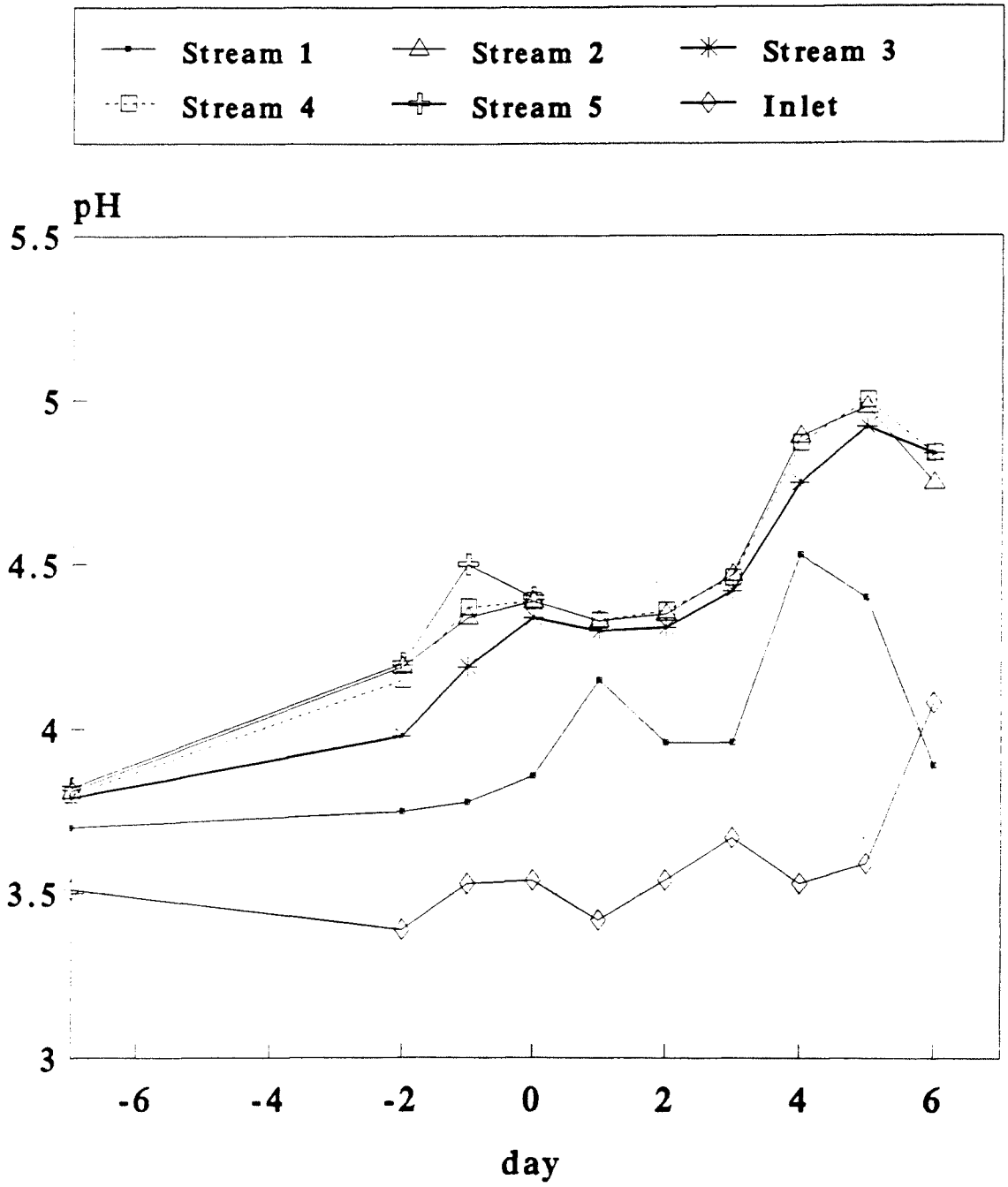
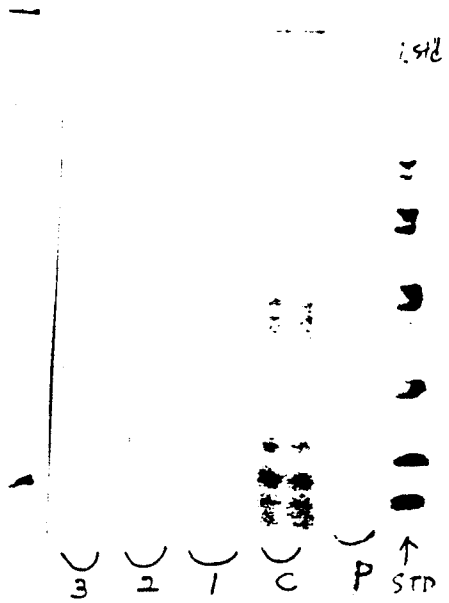
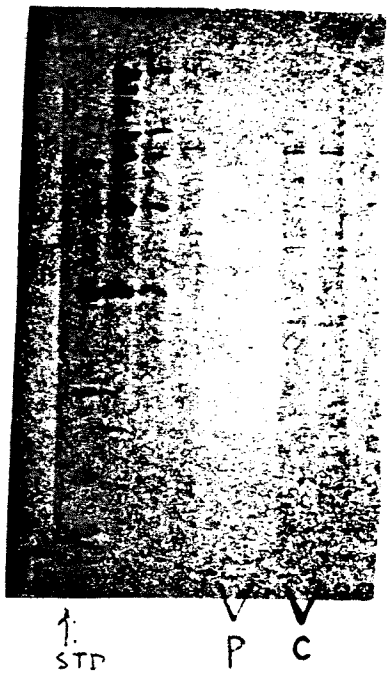


Figure 23. pH Values in Different Streams for System III



(a) Samples were processed with 10,000 MWCO membrane.



(b) Samples were processed by 5,000 MWCO membrane.

Figure 24. Polyacrylamide Gel Electrophoresis

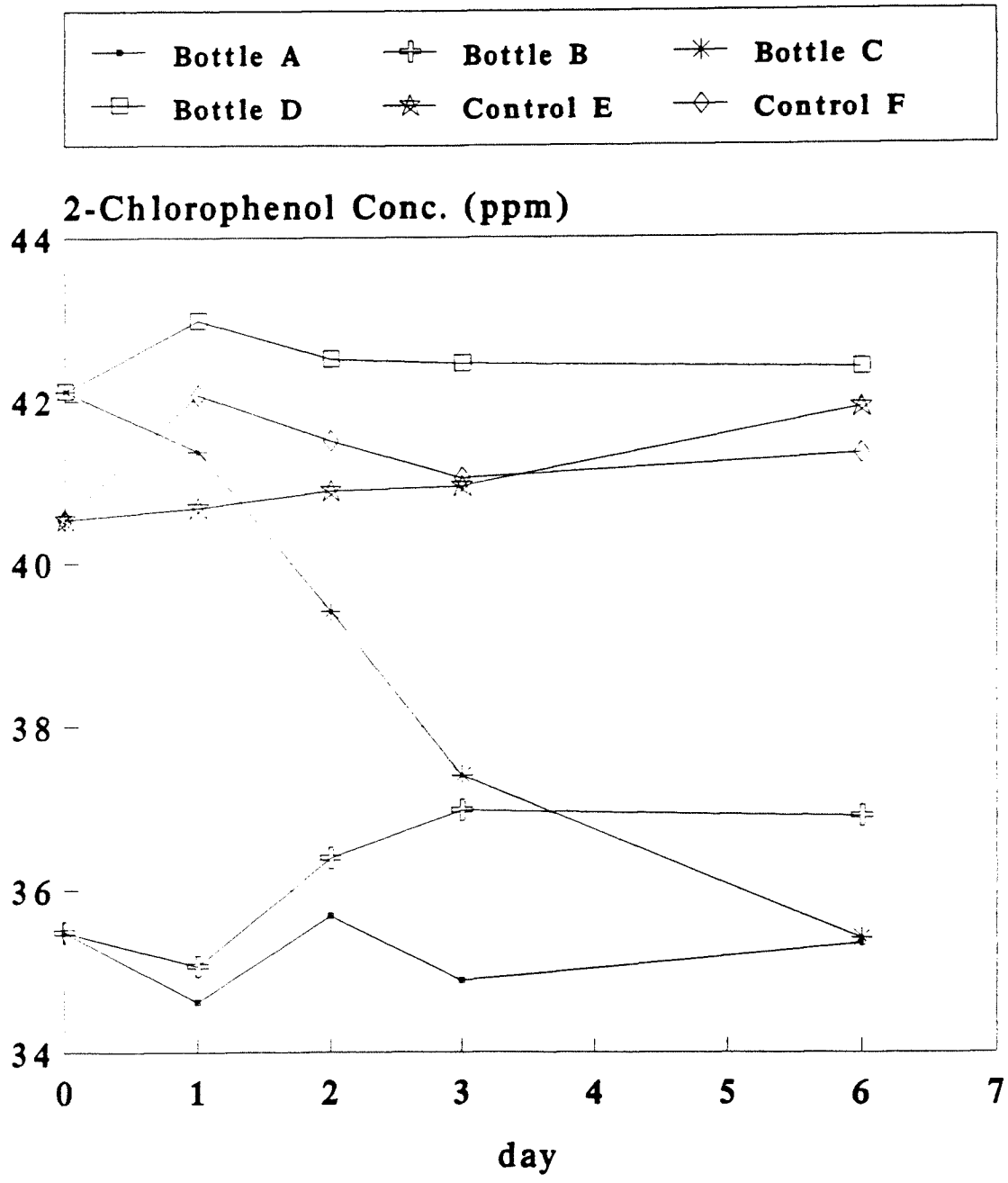


Figure 25. Degradation of 2-Chlorophenol in the Shaker Bottle Experiments

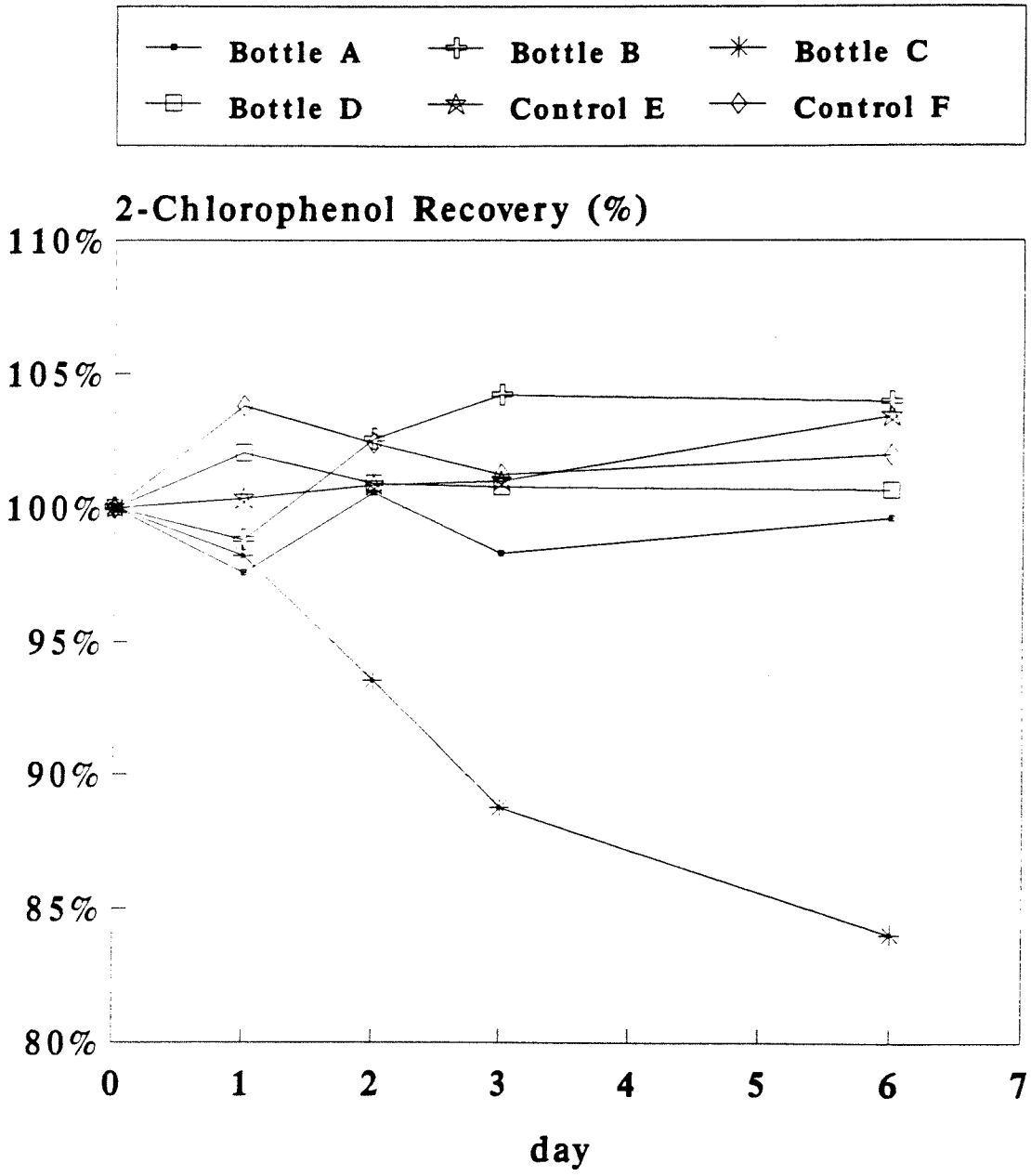


Figure 26. Percentage of Residual 2-Chlorophenol Recovered in Shaker Bottles

CHAPTER 6
CONCLUSIONS

1. From the SDS electrophoresis gel showing the fungus appear to have excreted at least four kinds of enzyme to mineralize 2-chlorophenol. The molecule weights were around 1,3000 , 1,6000, 23,000 and 40,000 daltons.
2. The hollow fiber ultrafiltration system can partially concentrate the proteins in the system. A part of proteins excreted by the fungus can still pass through 5,000 mwco hollow fiber membrane, probably because of the linear structure of the proteins.
3. The concentration of proteins does not correlate with the rate of degradation of 2-chlorophenol in this system. In this work the protein was concentrated 1.5 fold in the recycle loop by the ultrafiltration unit but the biodegradation rate did not show any significant improvement. Therefore, it appears that the high protein concentration is not a sufficient but a necessary condition for degrading 2-chlorophenol.
4. In the shaker bottle experiments, extracellular enzyme solution containing a high amount of fungal biomass and air appeared to play a positive role on degradation rate. Hence, mineralization of 2-chlorophenol cannot be processed without a significant amount of fungal biomass and air.

CHAPTER 7

FUTURE WORK

The mechanisms of mineralization of chlorinated phenol by the fungus are still unclear. The roles of extracellular enzymes, biomass and co-enzyme in the mineralization and the relations between them can only be answered by further research.

The results obtained from this project show that the degradation rate of 2-chlorophenol by the white rot fungus can not be increased merely by increasing the extracellular enzyme concentration. The presence of biomass seem to be necessary.

Based on this hypothesis, if the flow process was changed to that shown in Figure 27, a part of concentrated enzyme solution would flow back to the packed-bed reactor containing an abundant biomass. In this case the degradation rate of 2-chlorophenol may increase because of the combination of abundant biomass and concentrated extracellular enzyme solution. Additional work will be necessary to clarify this point.

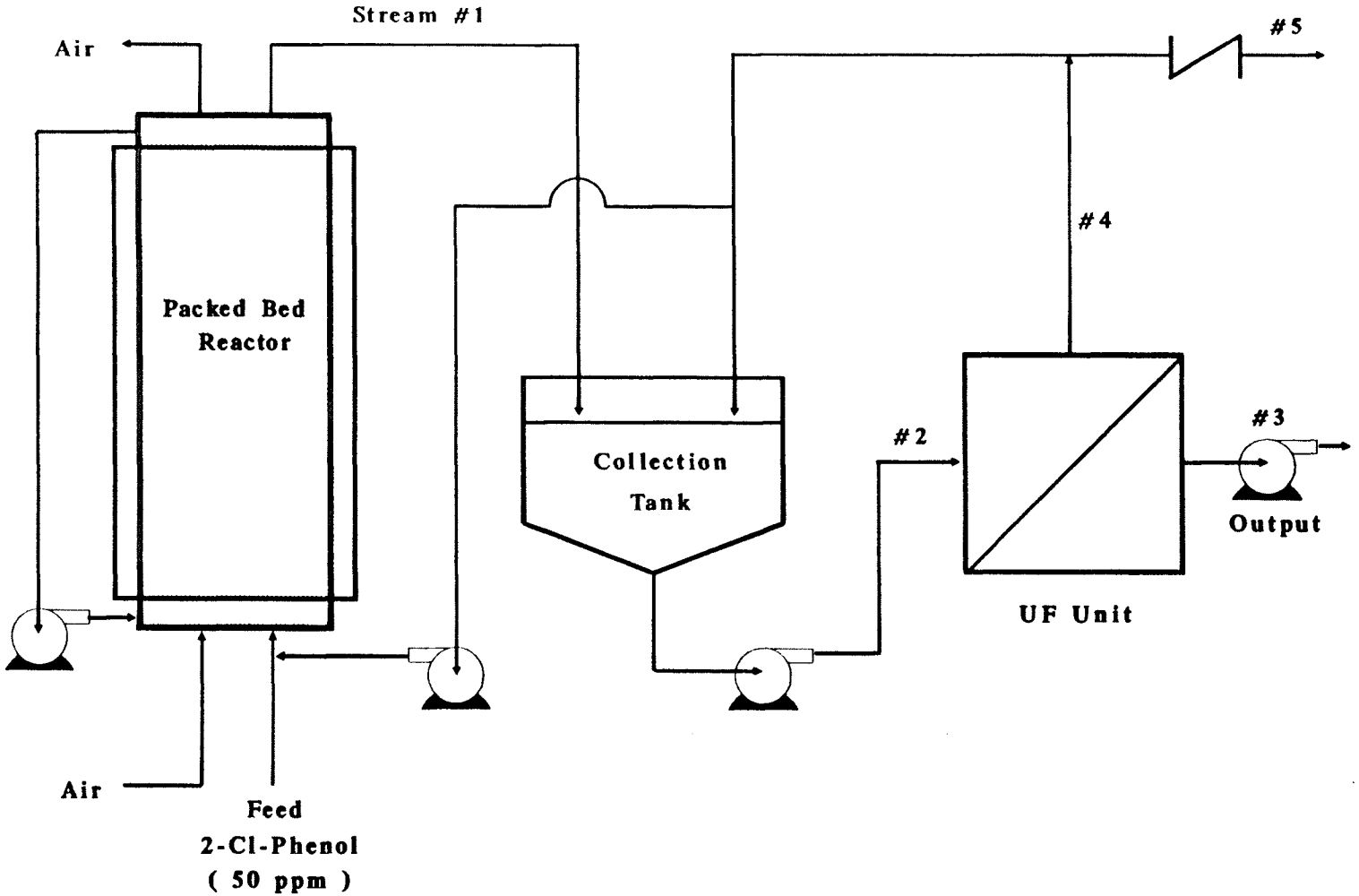


Figure 27. Diagram of Proposed Process for Future Work

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