TREATMENT STUDIES ON A XENOBIOTIC CONTAINING INDUSTRIAL EFFLUENT

by

IAN PAUL CLARK

A thesis submitted to the
Faculty of Engineering
of the
University of Birmingham
for the Degree of
Doctor of Philosophy (Engineering)

Biochemical Engineering Section
School of Chemical Engineering
University of Birmingham
P.O. Box 363
Birmingham
B15 2TT
England

April 1990

SYNOPSIS

The aim of this project was to assess treatment processes for an effluent generated from the manufacture of a mixture of dinitroethylbenzene and trinitroethylbenzene, known as K10.

No directly related literature was available, although there were many references to the closely related TNT manufacture and effluent treatment. Hence this was used to obtain possible solutions to difficulties arising in treatment of such an effluent.

Mixed microbial cultures were adapted, within laboratory scale activated sludge plants, to treat either a mixed phenolic waste or K10 wash water effluent supplemented with mixed phenolic waste (as a carbon supplement). Plant performance was assessed using standard wastewater treatment techniques such as COD, TOC and phenol analyses together with high performance liquid chromatography. These tests demonstrated that some of the K10 wash water effluent components were biodegraded or transformed, although the coloured components were poorly biodegraded (comparable with results obtained for TNT wastewater treatment).

Studies on the rate of oxygen uptake by activated sludge in response to exposure to various concentrations of K10 wash water effluent showed, surprisingly, that unadapted organisms had a higher uptake rate than adapted organisms. It was suggested that the K10 wash water effluent was active in "uncoupling" oxidative phosphorylation, a suggestion further supported by the demonstration that K10 waste dissipated electrochemical membrane potential. As a point of interest, the analogous TNT red water was also found to dissipate electrochemical membrane potential.

The use of activated carbon to adsorb the wastewater components was successful. However, since the carbon would not be regenerated but instead discarded, as with the analogous TNT red water treatment, this process would be environmentally as well as economically unsound. It was thus not recommended for treatment of K10 wash water effluent.

Ozone gas was found to react quite readily with the K10 wash water effluent components. The orange coloration of the wastewater was removed completely as was the absorbance in the UV range of 240 to 300nm thus suggesting that the effluent was no longer aromatic. Over 95% of the COD value and over 60% of the carbon content of the wastewater could be removed by ozonolysis. The COD and TOC values correlated with absorbance at 250nm and thus absorbance measurements could be used to estimate these values during the treatment process. The compounds remaining after treatment were considered to be organic acids which were readily metabolised by microorganisms. Ozonolysis of the analogous TNT red water was not as successful since the ozone contactor design did not allow sufficient ozone contact time for this effluent. The reaction rate of TNT red water components with the ozone gas was much slower resulting in large quantities of ozone in the contactor off-gas. The COD and absorbance values decreased linearly with ozone utilised (as with K10 wash water effluent).

To conclude: K10 wash water effluent is a highly potent metabolic inhibitor: however, at low concentrations the non-coloured components can be treated by adapted biological systems. Of the treatment processes investigated, only ozone or activated carbon treatments were successful in removing the coloured components. Ozonolysis is environmentally sounder since it destroys the aromaticity of the wastewater and the remaining compounds can be successfully metabolised by microorganisms.

DEDICATION

To my Mother

ACKNOWLEDGMENTS

The author wishes to express many thanks to those people who have been very helpful during the course of this work. In particular to Dr. D. A. J. Wase from Birmingham University for his guidance, supervision and enthusiasm, and also to Dr. M. Phillips from the Royal Ordnance Explosives Division at Waltham Abbey for his supervision, useful ideas and financial support.

Thanks are also extended to the following:

The technical staff - Joe Farmer, Hazel Jennings, Elaine Mitchell and Graham Titmus for their guidance and assistance.

Gill Wheeler for the production of photographs in this thesis.

The glass blowers - Stan Clabon and Vince Merral for their ever-ready and efficient service.

The departmental workshop and electronics staff for construction of apparatus and maintainance of equipment.

Finally, to my fellow workers for helpful suggestions and ideas.

CONTENTS

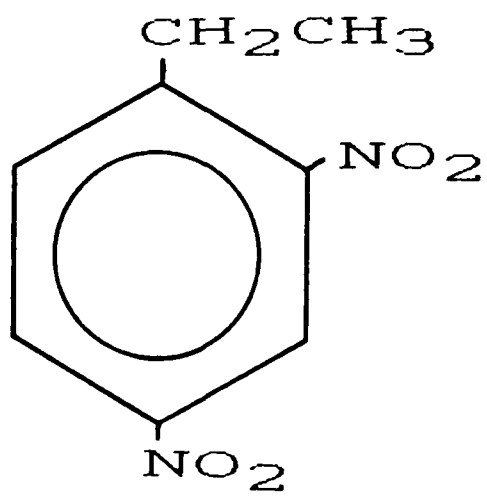
CHAPTER 1

INTRODUCTION

1.1 THE TASK

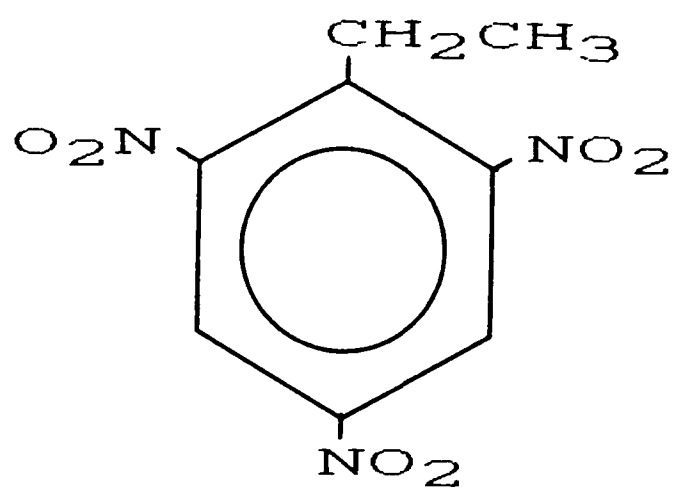
A pilot plant based at Waltham Abbey in Essex used to manufacture a mixture of dinitro- and trinitro-ethylbenzene (DNEB and TNEB, Figure 1), on a batch-wise basis, but has now been decommissioned. A new plant is under construction at Bridgwater in Somerset which will also operate on a batch-wise basis. The aim of this project is to assess the treatability of the effluent generated, preferably by biological methods, so as to render it harmless to the environment. Because of its unique pollutant content, the treatment of such an effluent requires new techniques or modification of old ones (Smith et al., 1982).

Essentially there is no relevant information pertaining to the disposal of DNEB/TNEB manufacturing effluent in the literature. Thus it was necessary to look at an analogous system such as the waste generated from the manufacture of trinitrotoluene (TNT). The chemical structure of TNT (Figure 2), a solid, is very similar to that of DNEB/TNEB (Figure 1), a liquid. However, although they are related, there is no direct evidence that effluents from their manufacture will have similar properties. In contrast to the dearth of information for DNEB/TNEB, the literature related to TNT waste is voluminous, and therefore only a selection of papers will



DNEB

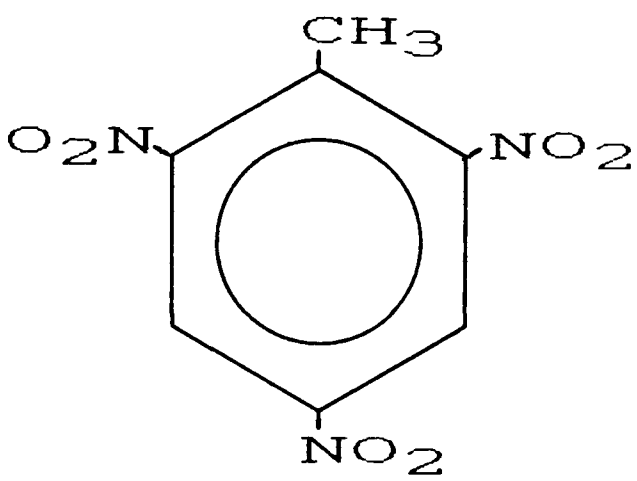
Molecular Formula $C_8H_8N_2O_4$



TNEB

Molecular Formula $C_8H_7N_3O_6$

Figure 1. Structure of Dinitroethylbenzene (DNEB) and Trinitroethylbenzene (TNEB)



Molecular Formula $C_8H_5N_3O_6$

Figure 2. Structure of Trinitrotoluene (TNT)

be chosen to help illustrate, clarify and emphasise problems involved in treating such an effluent.

1.2 WHY TREAT A WASTE?

The removal of organic material from wastewater is one of the most important tasks of the waste engineer. There are many authors such as Callely et al. (1977); Eckenfelder (1980); Forster (1985); Koziorowski and Kucharski (1972) and Nemerow (1971) who all stress the problems, basic as they are, of discharging organic compounds into receiving waters.

Problems arise because organic compounds are usually oxidised rapidly by microorganisms in the receiving streams, resulting in reduced dissolved oxygen levels and the accompanying ill effects of deoxygenated water. In severe cases this can mean a stinking, lifeless body of water. Other problems can accompany certain wastes such as direct toxicity to environmental life forms. Discharge of these toxic wastes to domestic sewers may cause difficulties in the watercourse receiving the treated effluent, and in extreme cases can even kill the bacteria present at the local sewage treatment plant. Mere discharge of such an effluent stream, untreated, to a watercourse is obviously potentially highly damaging to the environment, although there is always the problem of precisely estimating the effect on environmental life forms, since waste streams are often mixed and invariably complex. Therefore treatment of such wastes must be of paramount importance if a pollution-free environment is to be maintained. Micro-organisms are sensitive to environmental conditions such as temperature; pH (acidity and alkalinity); oxygen tension;

degree of mixing; the presence of toxic substances and also the character and quality of their foodstuffs in general. Therefore due care must always be taken to avoid such extremes as far as is possible (e.g. by dilution, pH adjustment, etc.) in order to permit the inhabitants of the sewage works to continue to operate efficiently, even in the presence of the toxic material to be treated.

1.3 DNEB AND TNEB (K10) WASH WATER EFFLUENT

1.3.1 Why are DNEB and TNEB Manufactured?

DNEB/TNEB as a mixture, known as K10, is manufactured for use as a plasticiser, not an explosive. It does nevertheless possess explosive properties and was therefore considered for inclusion in the hazard classification UN Class 1.1, (an explosive (pyrotechnic) substance). However, many of the tests and criteria used for the classification of explosive materials indicated that K10 was too insensitive for inclusion in this category. K10 was also found very difficult to ignite and had a high flashpoint temperature. As K10 could not be classified as a flammable liquid it was finally classified in UN Class 6.1, (a poisonous (toxic) substance). However, this refers to K10 which is a mixture of both DNEB and TNEB. Pure DNEB would be expected to behave in the same way as the whole K10 liquid and also as dinitrotoluene and dinitrobenzene which are also classified in UN Class 6.1. TNEB is, of course, not normally separated from K10, and it is therefore not entirely clear how it would be classified. However, it is reasonable to expect it to behave as TNT and thus be classified in UN Class 1.1.

1.3.2 The Chemistry of K10 Manufacture

K10 is produced by the treatment of ethylbenzene with sulphuric and nitric acids in three separate stages. This process is discussed below and the reaction process is shown in Figure 3.

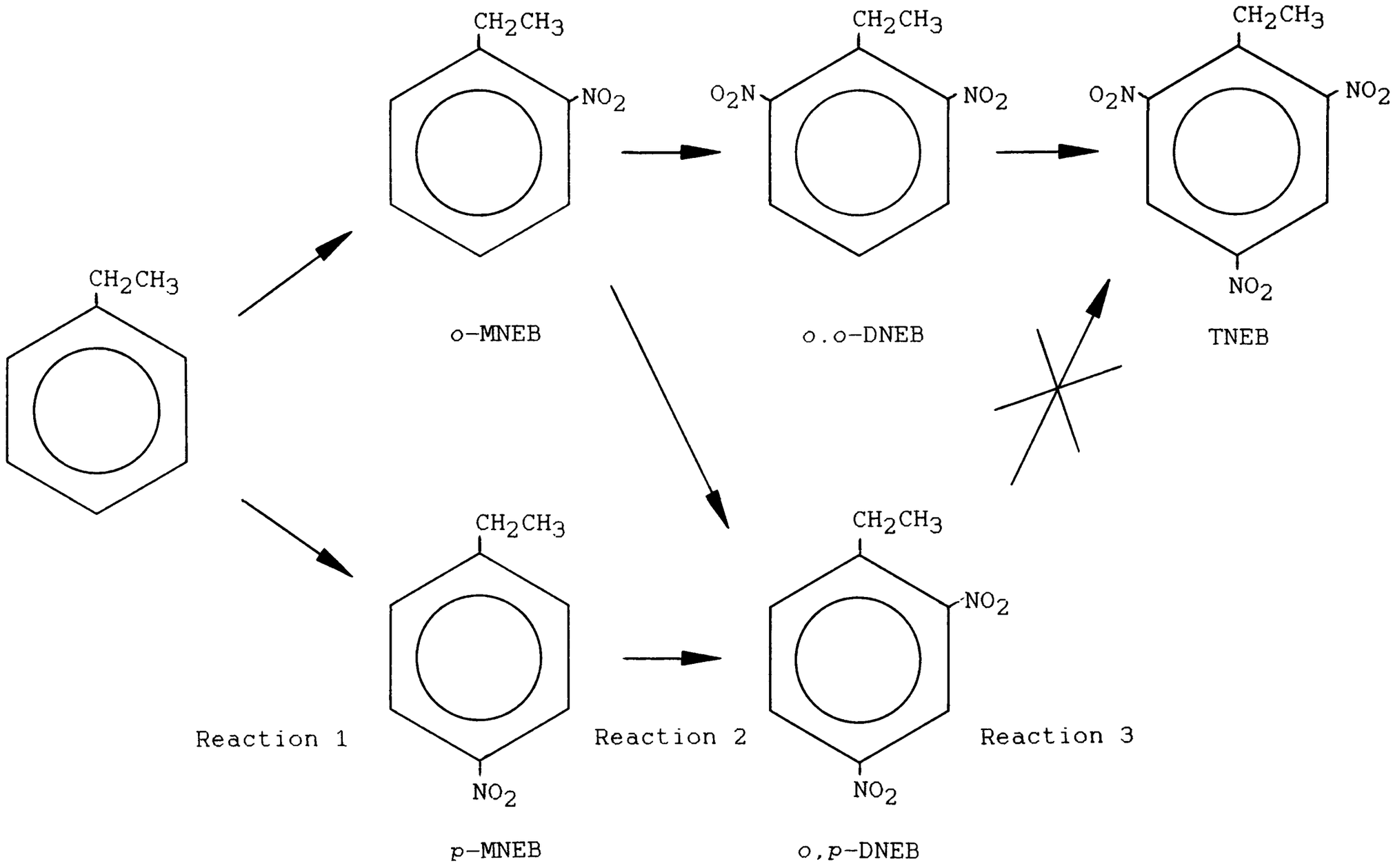


Figure 3. Manufacture of Dinitroethylbenzene and Trinitroethylbenzene

1.3.2.1 Reaction 1. Production of Mononitroethylbenzene (MNEB)

Ethylbenzene is stirred with concentrated sulphuric acid and about 50% nitric acid at a temperature not exceeding 30^{o}C for two hours. The MNEB is then separated from the reaction mixture. The substitution position is either ortho or para at a ratio of 2:1 respectively whilst little or no meta substitution occurs. It should be noted that all substitution positions mentioned here are relative to the ethyl group position.

1.3.2.2 Reaction 2. Production of Dinitroethylbenzene (DNEB)

The MNEB mixture is stirred with both concentrated sulphuric and concentrated nitric acids at 70^{o}C for two hours. The DNEB is separated from the reaction mixture and then washed in distilled water, followed by sodium carbonate solution and finally urea solution. After this treatment all of the para-MNEB is substituted in the ortho position thus forming ortho,para-DNEB. The ortho-MNEB is substituted either at the ortho or at the para position with equal frequencies thus forming ortho,ortho-DNEB and ortho,para-DNEB at a ratio of 1:1. Thus ortho,para-DNEB predominates over ortho,ortho-DNEB by a ratio of 2:1 respectively.

1.3.2.3 Reaction 3. Production of DNEB/TNEB mixture

The DNEB mixture is stirred with both concentrated sulphuric and concentrated nitric acids at 85^{o}C for four hours. The DNEB/TNEB is separated from the reaction mixture and then washed in distilled water, followed by sodium carbonate solution and finally urea solution.

The ortho,para-DNEB is not very reactive and does not take part in this reaction to any great extent. However the ortho,ortho-DNEB is very reactive and is readily substituted in the para position forming TNEB. Thus the final reaction mixture contains 2,4 DNEB and 2,4,6 TNEB at a ratio of 2:1 respectively. This reaction mixture is known as K10.

1.3.3 General Chemical Properties of K10 and Wash Water Effluent.

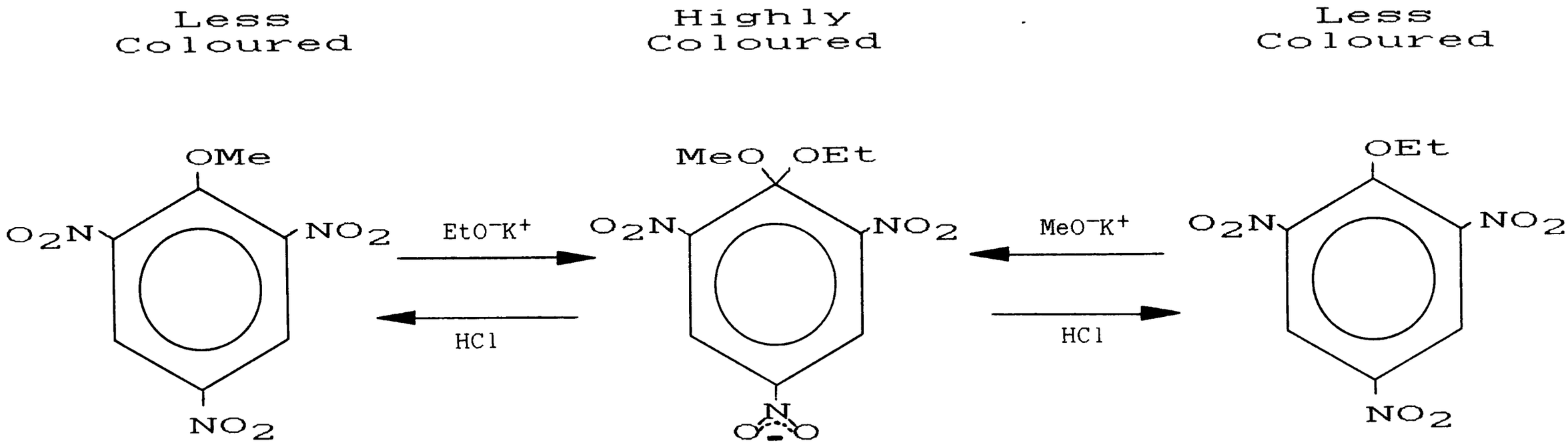
This manufacturing process generates two types of waste, the first being spent acids which are currently dumped and the other the wash water effluent. The possibility of cleaning and detoxifying this wash water effluent is the basis of this project. The wash water effluent can be expected to contain urea and sodium carbonate, both at concentrations of less than 1%, and an assortment of aromatic compounds. No relevant published information is available as to the nature of this effluent. Consequently environmental toxicity and chemical behaviour can be estimated only from comparisons with similar

effluents, allied to some deduction and guess-work. Thus, it was expected to contain Meisenheimer complexes (Figure 4) which are responsible for an absorbance peak at about 350nm due to their orange colouration. The waste also absorbed light strongly in the short ultra-violet region (190 to 260nm) and its components can be absorbed onto activated charcoal, the loading of which, with TNT wastewater, is pH dependent (Nay et al., 1972). During the course of this work, the effluent was found to have a total organic carbon content of around 4000mg/L and a chemical oxygen demand of 8000mg/L and must therefore be considered as a fairly strong effluent.

1.3.4 General Physical Properties of K10 and Wash Water Effluent.

For most investigations, the physical properties of the materials under investigation are known. Although, in this case, they had to be determined by preliminary experimentation, it seems appropriate to list them at this point.

K10 is a yellow liquid with a flash point of 86^{o}C and an ignition temperature of 244^{o}C, whereas wash water effluent has an orange colouration and no reported ignition or flash point temperatures. The density of K10 and that of three separate batches of wash water effluent were measured using a 50cm^{3} specific gravity (or density) bottle. The K10 sample received had a density of 1.367g/cm^{3} (Royal Ordnance density value = 1.34g/cm^{3}) whilst that of the effluent ranged from 1.013g/cm^{3}



Meisenheimer Salt

Figure 4. Structure of the Meisenheimer Salt

to 1.015g/cm^3.

Solubilities of K10 and the wash water effluent, the latter of which is mainly water, were observed for a range of solvents as shown in Table 1. Equal volumes of solvent and sample were added and shaken vigorously for five minutes. It should be noted that if solvent and sample were not miscible then, as a rule, colour was not visibly detectable in the solvent layer. Ethyl acetate proved the only exception to this rule when in contact with the wash water effluent.

1.3.5 Analysis of K10

The composition of K10 can be determined by High Performance Liquid Chromatography (HPLC). Good separation is achieved using a C_{18} reverse phase column with a mobile phase comprising methanol, water and tetrahydrofuran at a ratio of 2:2:1 respectively. Figure 5 shows a typical separation of K10 (Supplied by Royal Ordnance, Waltham Abbey, Essex and reproduced by the author). The peak areas for DNEB and TNEB were 2.60×10^7 and 1.25×10^7 respectively and indicate a 2:1 ratio assuming that both compounds absorbed light equally at 260nm. The largest contamination peak, at 260nm, had an area of 7.25×10^5 which is less than 3% of the DNEB peak at this wavelength. This analysis confirms that DNEB and TNEB are clearly the predominant components of K10.

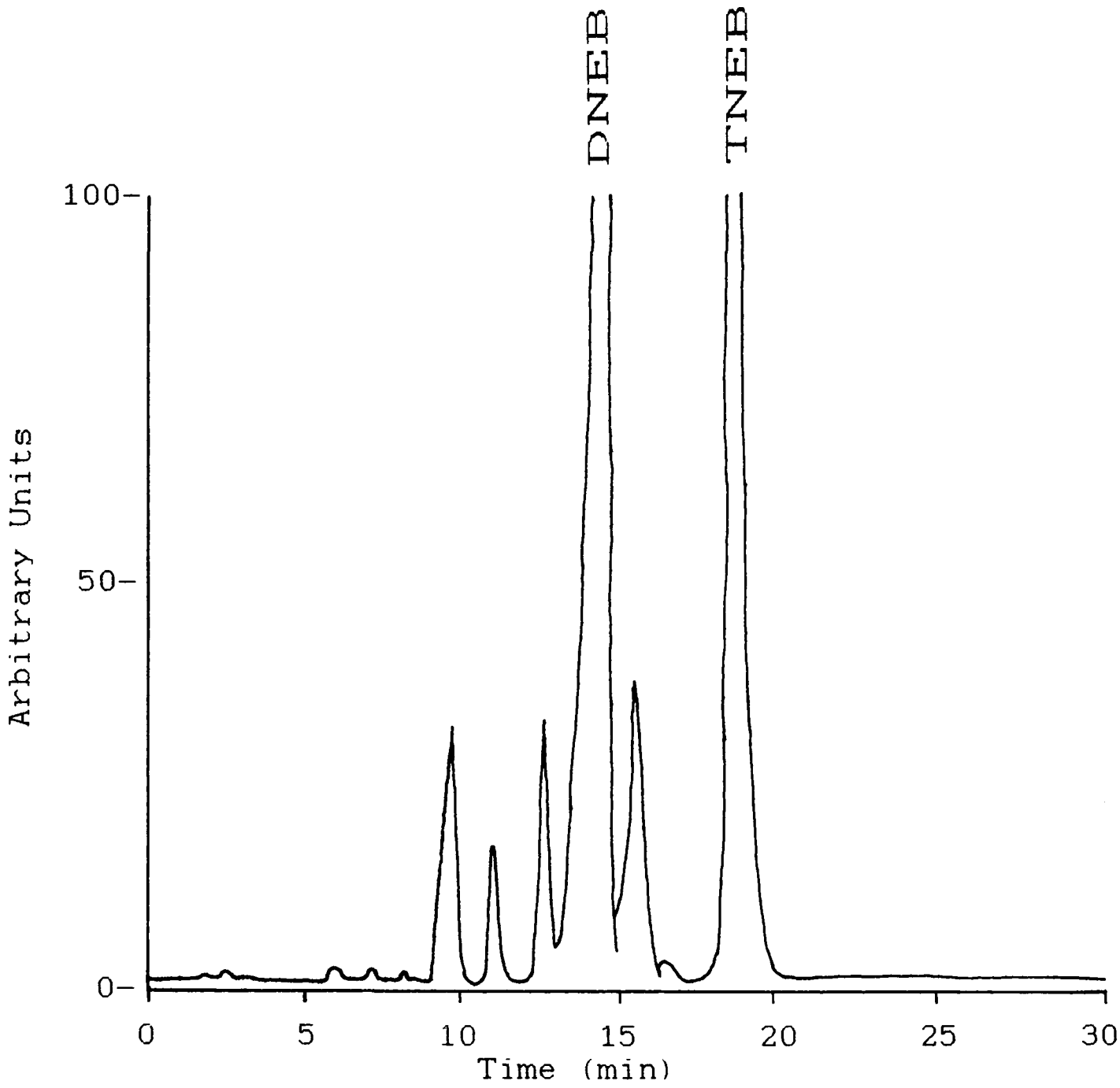
Solvent	Solvent Polarity Index	K10	K10 Effluent
Water	9.0	-	+
Methanol	6.6	+	+
Acetonitrile	6.2	+	+
Acetone	5.4	+	+
Chloroform	4.4	+	-
Ethyl acetate	4.3	+	- *
Tetrahydrofuran	4.2	+	+
Propan-2-ol	4.2	-	+
1,2 Dichloroethane	3.5	+	-
Dichloromethane	3.4	+	-
Diethylether	2.8	+	-
Heptane	0.1	-	-
Hexane	0.1	-	-
2,2,4 Trimethyl-pentane	-0.4	-	-

Key: + = Solvent and sample readily miscible

\- = Solvent and sample immiscible

* = Solvent and sample immiscible, however the solvent layer aquired an orange hue

Table 1. Solubilities of K10 and the K10 wash water effluent in a range of solvents.



Conditions of Analysis

Run Time:	30 minutes
Injection Volume:	20µl
Sample Rate:	2 per second
Sample Concentration:	33.4mg in 50ml solvent
Solvent Flow Rate:	1ml/min
Solvent Composition:	Water : MeOH : THF at 2:2:1
Detector Wavelength:	260nm. 0.1 AU FSD
Stationary Phase:	Zorbax ODS (C_{18}), 250mm x 4.6mm ID

Figure 5. HPLC Analysis of K10

1.4 POSSIBLE TREATMENT PROCESSES FOR CHEMICAL WASTES.

Treatment processes for chemical wastes can be divided into two main categories, namely chemical or biological treatments. A list of established treatments which may be applicable to an aqueous, possibly hazardous waste stream, such as K10 wash water effluent, which contains a moderate concentration of organic compounds is given below:

Physical-Chemical Treatments:

Carbon Adsorption

Resin Adsorption

Freeze-crystallisation

Liquid-liquid Extraction

Oxidation

Ozonolysis (with or without UV light)

Reduction (catalytic)

Biological Treatments:

Aerobic Activated Sludge

Aerated Lagoons

Percolating Filters

Anaerobic Digestion

Enzyme Treatment

It may be possible to obtain complete treatment of a waste using only one of these processes but it is often the case that a combination of several processes is required. These treatments will be discussed individually: whilst costs of such treatment processes are not exhaustively covered, it be remembered that costs, both capital and operating, must be considered when choosing a waste treatment process.

1.4.1 Activated Carbon

Adsorption has been used for many years in the reduction of tastes, odours and colours in the treatment of water and wastewater. Adsorption is a process by which molecules in solution, the adsorbate, attach to a solid surface, the adsorbent, through van der Waals forces. This adsorption continues until equilibrium is attained. Carbon adsorption is a well established technique for the removal of organic compounds and has a great potential for removing either non-biodegradable or toxic organics from wastewaters (Besselierre and Schwartz, 1976; Burns and Shell, 1974; Calleley et al., 1977; Cooper and Hager, 1966; Eckenfelder, 1980; Holiday and Hardin, 1981; Kul'skii and Shabolina, 1967; Saleh et al., 1982).

Several factors must be established before an adsorption process is employed, namely:

i) Selection of adsorbent type

ii) Adsorbent regeneration

iii) Operating conditions (flow rate, contact time, bed depth and grain size).

Selection of the correct adsorbent is very important and adsorbents can be classified into active and less active forms, but classification depends on the molecules to be adsorbed. For example, a carbon with very fine pores and a high activity is not suitable for large molecules, whilst a carbon with larger pores and a lower activity might prove to be a more effective adsorbent. Mistakes of this nature are often made in industry where wastes can be very varied. The best policy in selecting an adsorbent is by trial and error using a wide range of adsorbents with the waste to be treated.

Adsorbent regeneration is the second consideration, and in particular whether regeneration is economically viable. Regeneration of the carbon can be achieved using a variety of techniques including steam stripping, solvent extraction and heat treatment. However, steam treatment is not commonly used as the adsorbate must have a boiling point well below that of the steam and its concentration in the condensate must be much higher than in the wastewater. Organic solvent extraction is sometimes employed although the solvent must readily dissolve the adsorbed phase and also be miscible with water. Heat

treatment is the most commonly used process, especially when recovery of the adsorbed phase is of no importance. This process involves drying the carbon and heating (650 to 1000^{o}C) with limited amounts of water, flue gas and oxygen. Much of the current interest in activated carbon is centred about the problems involved in the economically efficient use and reuse of activated carbon (Hemphill et al., 1977). For instance thermal regeneration results in carbon losses of between five to ten percent and is often coupled with a reduction in the carbon loading capacity. The latter is usually due to a pore size increase and also by blocking due to deposition of residual materials. Moreover thermal regeneration is expensive and if the carbon is not regenerated it presents a further disposal problem.

The final considerations are those of the operating conditions. Activated carbon is said to be able to adsorb up to thirty percent of its weight in mixed organic materials with an efficiency exceeding seventy percent and with a contact time of less than forty minutes. However, it must be remembered that many factors will affect the efficiency of such a process. These were discussed by Eckenfelder (1980) and summarised as follows:

Rate of adsorption is a function of:

i) Diffusion of the adsorbate to the adsorbent surface through a liquid film resistance

ii) Diffusion of the adsorbate into the pores of the adsorbent and adsorption at the interior surface sites

In most applications, these first two mechanisms are controlling, but the rate of adsorption also increases with:

i) An increase in adsorbate concentration

ii) A decrease in adsorbent particle size

iii) A smaller adsorbate molecule and an increase in surface area of adsorbent.

Adsorption is proportional to the time of contact, and for granular carbon the adsorbate must penetrate the channels in the carbon which requires a longer time period before equilibrium is attained.

Adsorption capacity may increase with:

i) An increase in adsorbate concentration

ii) An increase in surface area of the adsorbent

iii) An increase in molecular weight of the adsorbate

iv) A decrease in pH of the solution that changes the organic molecules into a less soluble form.

Molecular structure of the molecule to be adsorbed is also an important factor and is shown in Table 2. It can be seen that nitro groups often enhance adsorbability and that increased molecule size can result in more solute carbon chemical bonds and hence better adsorption.

1) Increased solubility of the solute in the liquid carrier decreases its adsorbability.
2) Branched chain molecules are usually more adsorbable than straight chains.
3) Substituent groups affect adsorbabiliy

Substituent Group	Nature of Influence
Hydroxyl	-Generally reduces adsorbability; extent of which depends on structure of host molecule.
Amino	-Effect similar to hydroxyl but somewhat greater.
Carbonyl	-Effect varies with host molecule
Double Bonds	-As carbonyl
Halogens	-As carbonyl
Sulphonic	-Usually decreases adsorbability
Nitro	-Often increases adsorbability

4) Undissociated molecules (weakly ionised) are in general preferentially adsorbed.
5) Unless screening of carbon pores intervene, large molecules are more sorbable than small molecules of similar chemical nature.
6) Molecules with low polarity are more sorbable than highly polar molecules.

Table 2. Influence of Molecular Structure and other Factors on Adsorption (Eckenfelder, 1980)

Some work has been carried out on both nitroaromatic solutions and effluents. Eckenfelder (1980) discusses the removal of 96% of nitrobenzene (1g/l) from solution with a doseage of powdered carbon (5g/l). In light of this, one might expect activated carbon to be a suitable process for treatment of nitroaromatic effluents. Indeed, many researchers have investigated the use of activated carbon for treatment of munitions manufacturing wastes (including TNT wastes). Whilst such treatment is technically feasible the experimental data obtained for carbon suitability are not promising (Schulte et al., 1973). Whilst nitroaromatics and colour can be adsorbed by carbon (a process which is more efficient if the waste is acidic) there is a serious deficiency in the activated carbon system. This is the inability to successfully regenerate the exhausted carbon either by thermal or chemical methods (Fochtman and Huff, 1975 and Patterson et al., 1976). As a consequence the carbon must be disposed of, by incineration, after a single use.

1.4.2 Resin Adsorption

Resin adsorption, like carbon adsorption, is a useful process for the extraction of organic solutes from aqueous waste streams. However, carbons can be thermally regenerated resulting in adsorbate molecule destruction whereas resins are always chemically regenerated. This regeneration is with an organic solvent, or a caustic one, with the reuse of organic solvents after distillation.

Resin adsorption is thus similar to carbon although the former is preferred if the adsorbate molecule is to be recovered. Unfortunately these synthetic resins are much more expensive than carbon, and thermal regeneration operating costs are higher than solvent regeneration. The last consideration is that resin adsorption serves only to concentrate the waste and does not destroy it, unlike thermal treatment. As a result further consideration needs to be given to disposal.

Patterson et al. (1976) discuss problems of polymeric resins used in the military explosives industry. They state that whilst resins are easily regenerated, the loading capacity is not as high as for carbon adsorption. TNT itself is reported to adsorb, elute and be suitable for reprocessing but the coloured bodies are poorly adsorbed. Thus polymeric resins are essentially unsuitable for treatment of TNT red water effluent.

1.4.3 Freeze-crystallisation

This process involves the formation of "pure" ice crystals from a solution, and concentration of dissolved solutes in the residual solution. These crystals are then separated from the concentrated solution which must then either be treated further or disposed of.

Thus freeze crystallisation is another process which is concerned with effluent concentration and, due to the fairly high energy requirement, is not generally used on a commercial basis for wastewater treatment.

1.4.4 Liquid-liquid Extraction

Liquid-liquid extraction involves the removal of wastewater constituents from the aqueous phase to an organic phase. This process is dependent on several factors. Firstly, the components of the waste must be readily soluble in the extracting solvent, whilst the extracting solvent must have as low a solubility in water as is possible. The final consideration must be in the separation of the extracting solvent and solute. The difference in their boiling points is most often used for their separation. This process, as with freeze-crystallisation, serves only to concentrate the waste components and thus does not solve the waste disposal problem.

1.4.5 Oxidation

Chemical oxidation may be employed to oxidise pollutants. Common oxidants are chlorine, air, potassium permanganate and ozone (discussed separately, Section 1.4.6).

Chlorine is generally used to oxidise cyanides to cyanates which can be oxidised further to carbon dioxide and nitrogen gases. Chemical oxidation has also been applied to

dilute aqueous waste streams containing phenols and also for odour reduction of wastes in the papermaking industries. Unfortunately, some of these processes introduce new components into the waste stream which might themselves need further treatment. Further problems arise when hazardous materials, often from industrial wastes, are only partially removed due to inefficient or incomplete oxidation. Thus it must be stressed that chemical oxidation requires careful evaluation due to the addition of chemicals into the waste stream which might increase the pollutant load and require stringent safety measures for their use. Although these processes can, and often do, solve the waste problem, high operating costs, a factor dependent on the waste stream itself, often restrict their use on industrial waste streams.

1.4.6 Ozone

1.4.6.1 Properties and Toxicity

Ozone is a highly reactive allotrope of ordinary atmospheric oxygen in which the molecule is composed of three, rather than two atoms (Figure 6). Ozone gas is about one and a half times heavier than air; it boils at -112^{o}C (1 atm); is thirteen times more soluble than oxygen in water (over temperature range 0 to 30^{o}C) and has a distinctly blue colouration. This blue colouration is not however, apparent in "ozonised air" which is produced when part of the air's oxygen is converted to ozone, producing ozonised air, a colourless gas (Anon, 1983 and Rice et al., 1981).

Only a few substances are resistant to the very strong oxidising effect of ozone. They include glass, stainless steel, PTFE and viton and hyphalon rubbers. Since ozone is such a strong oxidising agent it is also a very toxic gas and the breathing of ozone has serious implications which include:

structural changes in lung tissue

enzymatic changes in blood

reduced resistance to respiratory bacterial infection

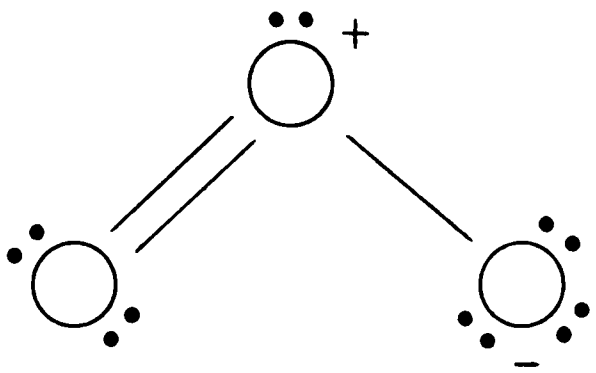
reduced pulmonary function

disruption of cellular biochemistry

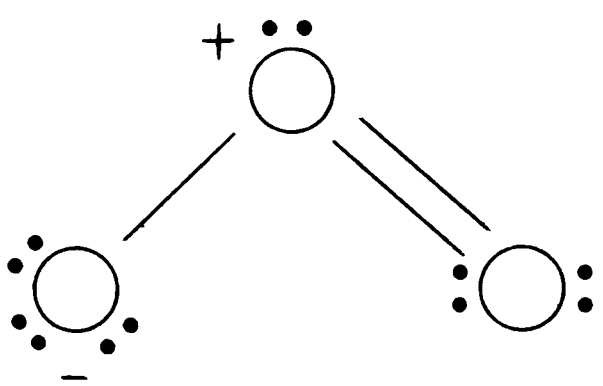
fragilisation of cell membranes

increased oxidation of unsaturated fatty acids

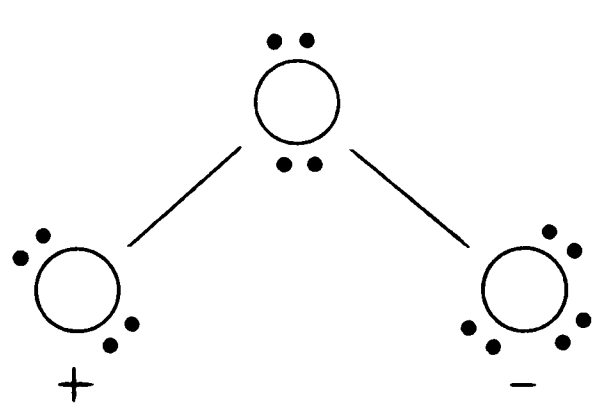
i)



i i)



i i i)



i v)

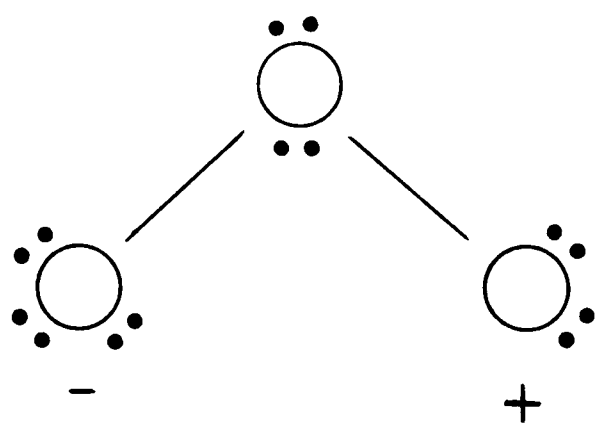


Figure 6. The four Canonical Forms of the Ozone Resonance Hybrid (Bailey, 1978)

However there is no evidence of carcinogenic or mutagenic effects or effects on reproduction (Leh, 1973 and Rice et al., 1981). Fortunately the risk of exposure to harmful or toxic levels is relatively small, since ozone has a characteristic penetrating odour which is readily detectable at concentrations as low as 0.01 to 0.05 ppm (Rice et al., 1981) and the recommended limit for ozone exposure is 0.1 ppm ($0.2mg/m^3$) calculated as an 8-hour time weighted average concentration (Anon, 1983).

1.4.6.2. Production of Ozone

Due to the reactive nature of ozone and its relatively short half-life (12 hours in dry air, as measured by the US Environmental Pollution Agency) it cannot be shipped or stored and is thus generated on site immediately prior to use. Even so, it can be piped considerable distances from the generator to the contactor with no fear of significant decomposition to diatomic oxygen.

Ozone is produced when high-voltage electricity is imposed across a discharge gap in the presence of a dry, oxygen containing gas. Ozone has been used in France for water disinfection since 1906. This plant could generate ozone using $73kWh/kgO_3$. Today, ozone generation at an Indianapolis plant requires only $6.31kWh/kgO_3$ when utilising pure oxygen or 12.6 kWh/kgO_3 when using air (Rice et al., 1981). This is clearly a marked improvement in efficiency, in terms of energy

costs, compared with the 1906 figure. Even so the potential for improvement remains high, since the basic method of production is grossly inefficient. Currently the situation is that only about 10% of the energy supplied is used to make ozone. The remainder is lost as light, sound and primarily heat. Unless this heat is removed the ozone yield will suffer due to the sensitivity of ozone decomposition to temperature.

Yield is also a function of the oxygen and water content of the feed gas; moreover, a given ozone generator can produce over twice the quantity of ozone and consume less than half the power when fed with 100% oxygen as opposed to air (Hardisty and Rosen, 1977). In oxygen feed ozone production systems the effluent gas stream from the ozone contactor, which now should contain only the residual oxygen is often recycled. However, in this case problems may arise in processes which involve the complete oxidation of organics as carbon dioxide is invariably produced. This will dilute the high purity oxygen and so reduce the ozonator efficiency. Once-use air systems are obviously unaffected by gaseous reaction products such as carbon dioxide and are thus favourable for processes involving complete oxidation, such as the one under consideration in this thesis.

1.4.6.3 Mechanisms of Ozone Attack

The oxidation potential of ozone in alkaline solution is very considerable. Indeed it is ranked second only to fluorine, among the readily available water treatment chemicals, and is capable of oxidising a great many organic and inorganic compounds. However, it is not selective in this function.

Ozone can react in two ways. The first is directly as molecular ozone in which the ozone molecule attacks a chemical bond which is specifically reactive to ozone, such as a double bonded carbon atom. The other method of ozone attack is for the molecule to decompose to radicals before reacting in this form. These two distinctly different methods of ozone attack need careful consideration since the partial oxidation products will be quite different for the same substrate. This would also be reflected in the amount of organic carbon removal. These two methods of attack are discussed further:-

The first method discussed involves a direct attack. There are two general methods by which ozone could directly attack an unsaturated bond. The first mechanism involves a one-step simultaneous attack of both reactive centres of the ozone molecule on both reactive centres of the unsaturated molecule. The other possibility is a two-step attack of the reactive centres of the ozone molecule on the unsaturated system. However many additional questions remain. If, for

example, the first method were to operate, would it involve the terminal oxygen atoms or adjacent atoms? Or if the second method were to apply, would the first step for this method imply an electrophilic or nucleophilic attack? Would the first step involve a central or terminal atom? If the attack involved a terminal atom would the attack then be completed in the second step by the middle or the other terminal atom?

It is generally considered that the reaction which occurs is a two step process in which a terminal oxygen atom makes the initial electrophilic attack, after which either the central or other terminal atom could complete the attack (Figure 7). Reaction (i) was favoured by some workers during the 1950's since this molecule should be relatively stable. However, this was the best argument against it, as it was never isolated. The structure formed in Reaction (ii) should be very unstable and would be expected to break down very rapidly (Bailey et al., 1959). The oxidation of phenol is through a direct attack mechanism and has been shown to produce over fifteen different intermediate compounds (Figure 8). It is also worth noting that complete oxidation of phenol to carbon dioxide and water would not necessarily be required in a large scale process since many of the intermediate compounds are readily utilised by microorganisms.

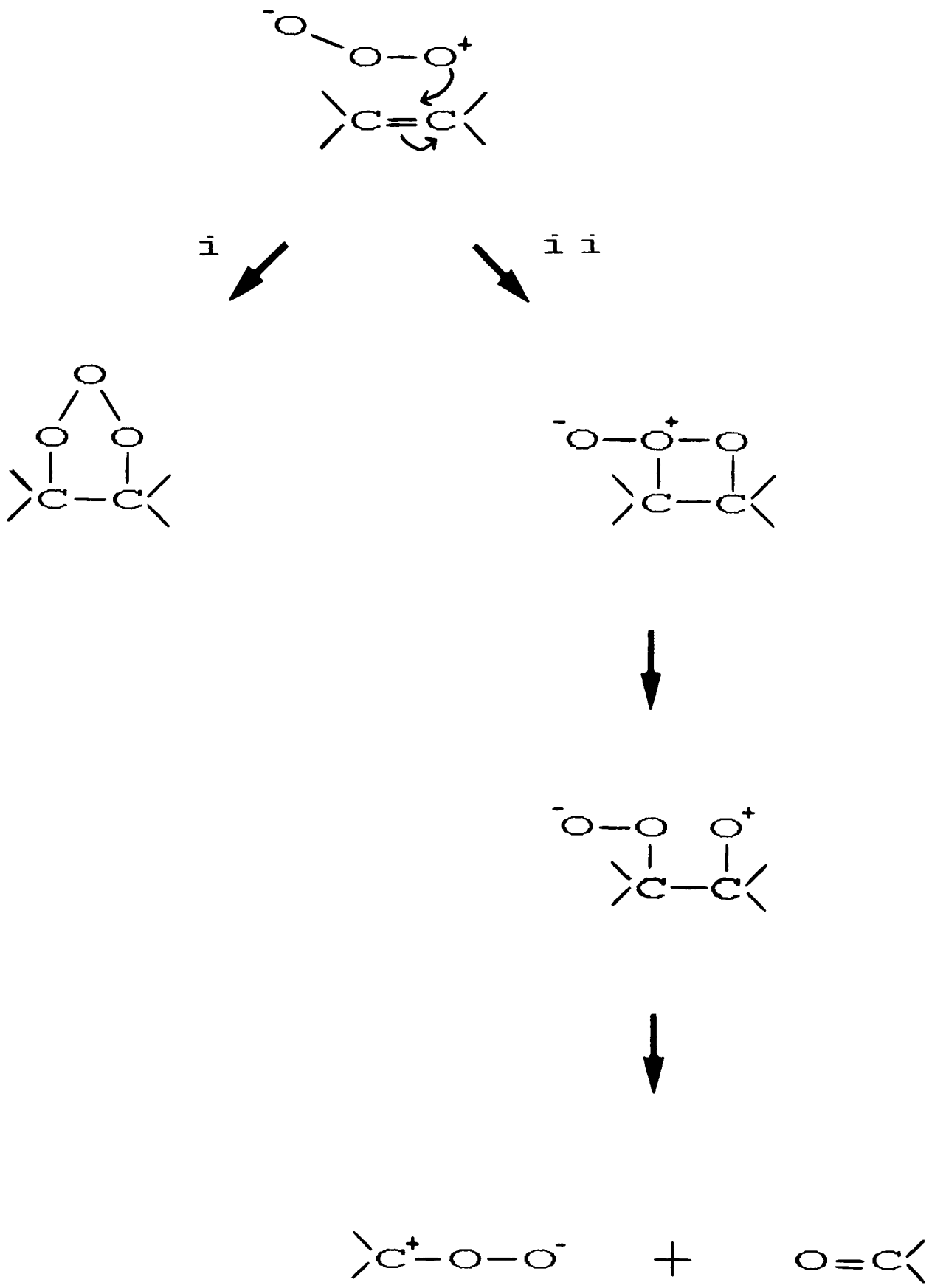


Figure 7. The attack of an Unsaturated Bond by Ozone (Bailey et al., 1959)

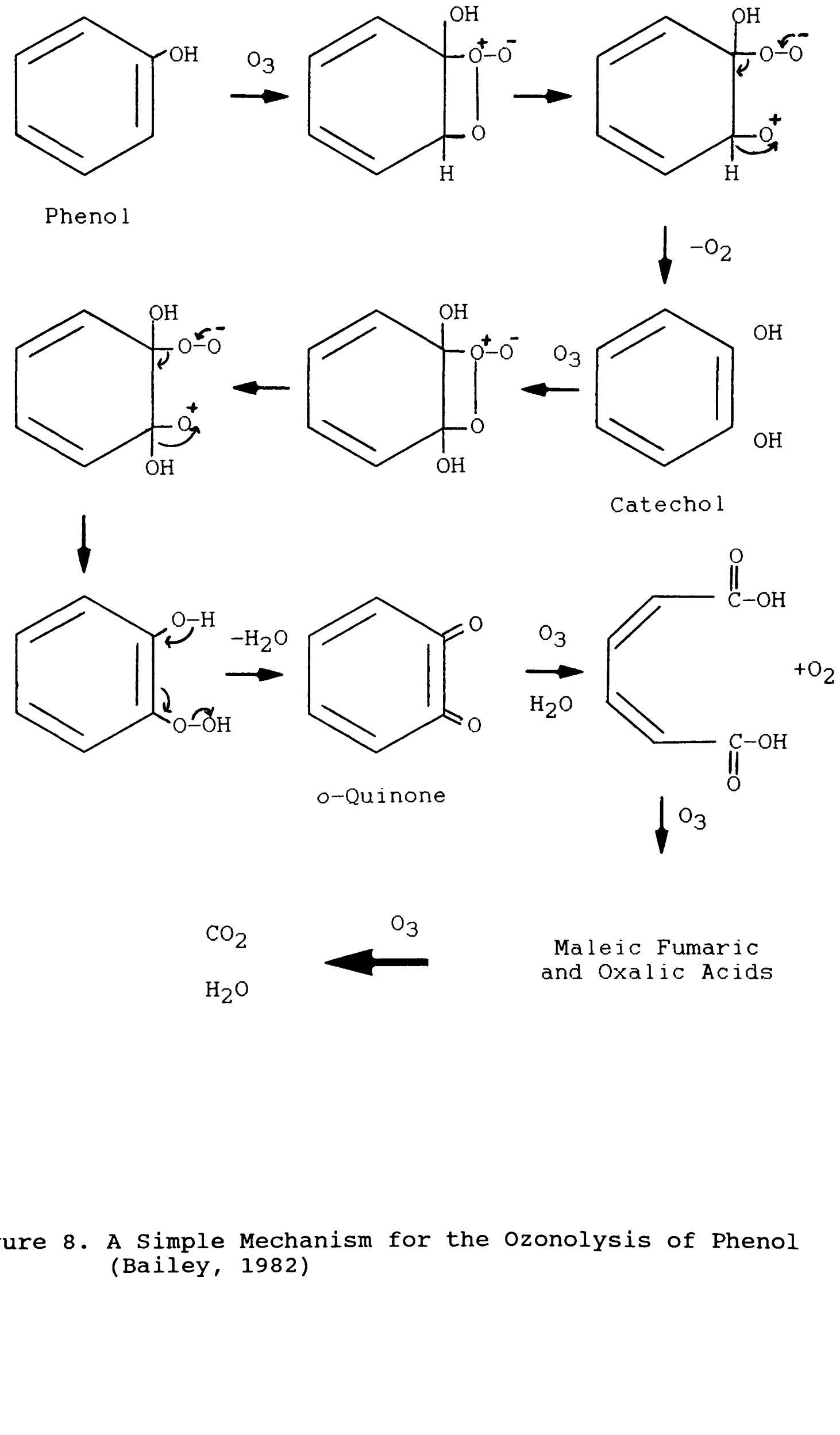


Figure 8. A Simple Mechanism for the Ozonolysis of Phenol (Bailey, 1982)

The second method to be discussed could involve hydroxyl radical attack. Ozone can decompose in water to form hydroxyl radicals. These radicals are one of the most powerful and effective oxidants that occur in aqueous solution. The auto-decomposition of ozone to radicals is catalysed by the hydroxide ion (OH^-) and thus a high pH, about 9, is required for radical formation. However 'scavengers' such as bicarbonate ions (HCO_3^-), carbonate ions (CO_3^{2-}) and aliphatic alcohols are well known for their abilities to quench formation of radicals (Sierka, 1985).

In this connection it is of incidental interest that several workers have shown radical attack to be important in the ozonation of chlorinated nitrobenzenes. Thus Duguet et al. (1988) removed o-chloronitrobenzene from ground-water by combining ozone treatment with addition of hydrogen peroxide. This was claimed to increase radical production and they managed to reduce o-chloronitrobenzene from 1.8mg/l to 0.02mg/l with an ozone dose of 8mg/l and a hydrogen peroxide addition of 3mg/l. Furthermore, Guittonneau et al. (1988) investigated the use of ozone coupled with ultra-violet light over a range of pH values and demonstrated that the predominant mechanism for the oxidation of p-chloronitrobenzene to be the free radical mechanism. Decisive proof that this was the case was obtained by the addition of the radical-quenching reagent 0.1 mol/l bicarbonate, which effectively quenched the reaction.

1.4.6.4 Ozone Contactors

Adsorption of ozone in contaminated water is a gas-liquid process comprising ozone mass transfer, generally accompanied by irreversible chemical reactions. There are a number of problems in evaluating the capacities and heights of process equipment from laboratory trials, and these appear not always to have been appreciated even by those who have undertaken the tests. For instance, many compounds, such as phenol, present a great reactivity towards ozone and this makes calculations of parameters such as kinetic rate constants and mass transfer coefficients difficult to calculate (Sotelo, 1988). On the other hand, not all compounds react rapidly, and many researchers have simply used small, bubble contactors which were unable to utilise 100% of the applied ozone to oxidise their more refractory materials (Caprio et al., 1984; Fochtman and Huff, 1975; Kuo and Sierka, 1983; Shelby et al., 1984 and Sierka, 1985)

Large bubble contactors (over 5m in height) would have overcome this problem if the ozone reaction rate had been high. However, really refractory compounds tend to react relatively slowly and are 'reaction rate limited'. Such a system would then require a longer ozone contact period in order to utilise significant quantities of the applied ozone. Thus the type of ozone application determines the requirements placed on the ozone contactor.

An alternative to the bubble contactors mentioned earlier is the spinning disc contactor (Anderson et al., 1984 and Saw et al., 1985). Barberis and Howarth (1988) give mass transfer coefficients for both bubble columns and rotating contactors as follows:-

		$K_L \times 10^4 (ms^{-1})$
Bubble Contactor	6.2mm Bubble Diameter	1.4
	2.6mm Bubble Diameter	2.1
Spinning Disc	Flat	4.1
(0.38m)	Perforated	6.0-11.5

Saw et al. (1985) suggest that the enhanced mass transfer of thin films lies in the fact that the internal resistance to mass transfer is minimised by the presence of a wave motion characteristic of these films and that thin films present a very large surface area per unit volume of throughput. Thus, spinning disc reactors should be conducive to promotion of 'direct reaction' of molecular ozone, or 'free radical' type reactions (Anderson et al., 1984). It seems obvious to the author that spinning disc contactors are only of use when the ozonation of wastewater is limited by mass transfer of ozone. If the mass transfer of ozone is 'reaction-rate' limited then increased mass transfer potential will be of little value.

1.4.6.5 Ozonation Analysis Considerations

The use of ozone as a water treatment process generally results in the following effects: a reduction in TOC due to the oxidation of carbon compounds to carbon dioxide; a reduction in colour due to the disruption of conjugated double bonds; and also to a reduction in the COD value. These tests are often utilised for the monitoring of ozonolysis treatment.

1.4.6.6 Colour reduction using Ozone

Coloured organic compounds, by nature, should be particularly amenable to oxidation by ozone. The reason is that organic colour is generally caused by the excess electrons present at unsaturated bonds and at resonant bonds in cyclic compounds. Cleaving such double bonds with ozone often leads to the production of ketones, aldehydes or acids in the case of carbon-carbon bonds. However, the products formed also depend on the other substituent groups.

The disappearance of colour does not necessarily mean that all of the colour-causing compound has been converted to carbon dioxide and water, but simply that the conjugated unsaturated groups have been destroyed (Nebel et al., 1973). As a practical example of the problems in the field, Eckenfelder (1980) discusses the use of ozone to remove colour from dye production wastewater (Figure 9). Whilst there was a decrease in the total organic carbon (TOC) value due to

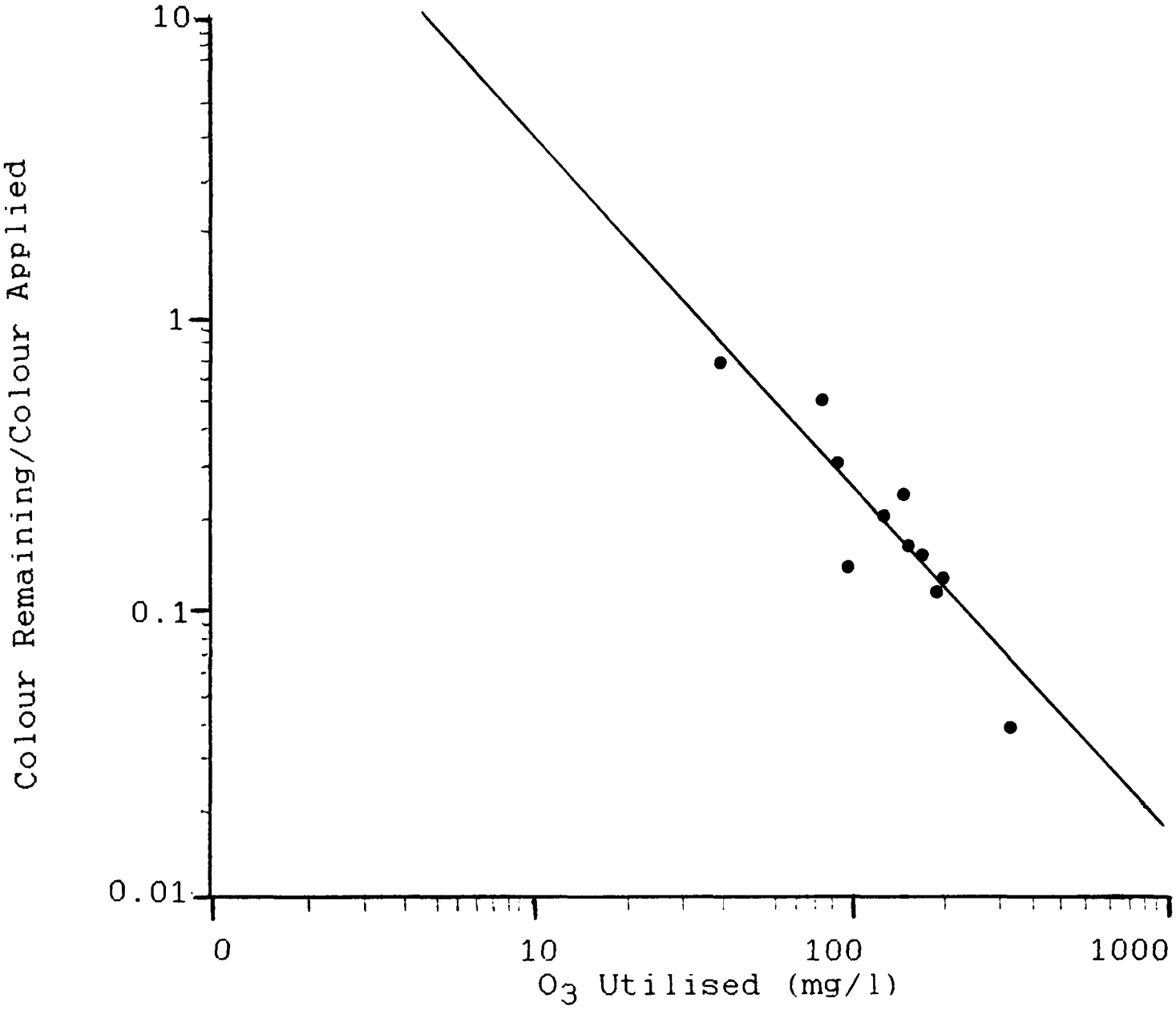


Figure 9. Colour Removal from a Secondary Dye Waste Effluent by Ozonolysis (Eckenfelder, 1980)

production of carbon dioxide, there was an increase in the biological oxygen demand (BOD).

This effect compares well with the results of Anderson et al. (1984), who also found that during the ozonation of coloured effluents the BOD increased. This is because the ozone oxidised some of the non-biodegradable material leaving it in a more biologically amenable form. Continued ozonation would oxidise these compounds further, and a decrease in BOD would occur. Thus the effect of ozonation on BOD is not readily predictable. In this connection Metzer and Miyamoto (1975) have reported the same phenomenon in the COD test, attributed to ozone making compounds more accessible to oxidation by the dichromate used in this test.

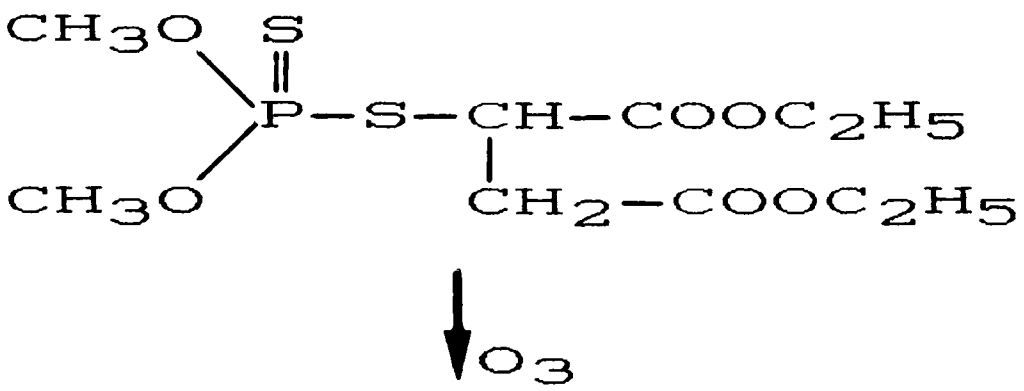
The use of ozone for high level COD reduction is not always economically practical and De Renzo (1978) recommends that ozone is only used for complete oxidation of wastes containing up to one percent oxidisable material, or as a preliminary treatment for more concentrated wastes which are not amenable to other techniques.

1.4.6.7 Ozone and Pesticides

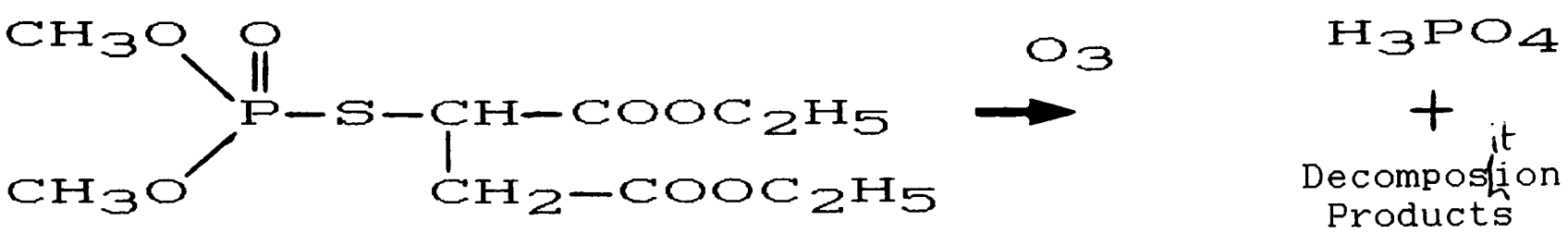
Ozone is often integrated into groundwater purification processes. Ozone is known to reduce taste and colour, and also to 'remove' (oxidise) phenols, detergents and pesticides. However, pesticides differ widely in their reactivity towards ozone. Phosalone, for example, is reported to be oxidised to destruction with only small amounts of ozone (Rice et al., 1981). On the other hand, lindane, DDT and PCB's are only slightly reactive with ozone under normal conditions. Ozonation of malathion and parathion (Figure 10) presents a different problem. During ozonation intermediate compounds are produced (their corresponding oxons) which are more toxic than the starting thions. Thus sufficient ozone must be applied to ensure complete oxidation of these intermediates. The ozonation of heptachlor is yet another example of a problem pesticide. This reacts with ozone to form heptachlorepoxide (Figure 11). This product is also toxic and in addition to this it is stable to further ozonation. It is thus incumbent to identify the specific pesticides within a treatment system and ascertain the most effective method of removal.

In the same way, in general, it is important to have as much information as possible about the waste to be oxidised in order that the most effective treatment regime can be devised.

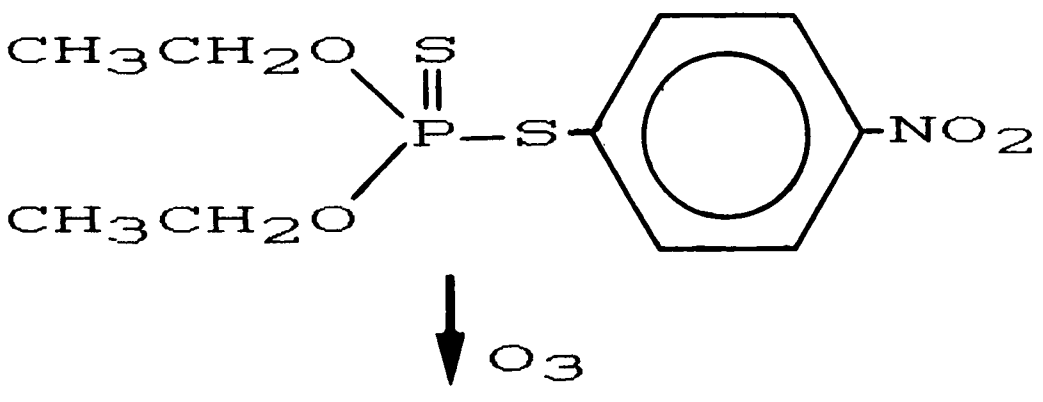
Malathion



Malaoxon



Parathion



Paraoxon

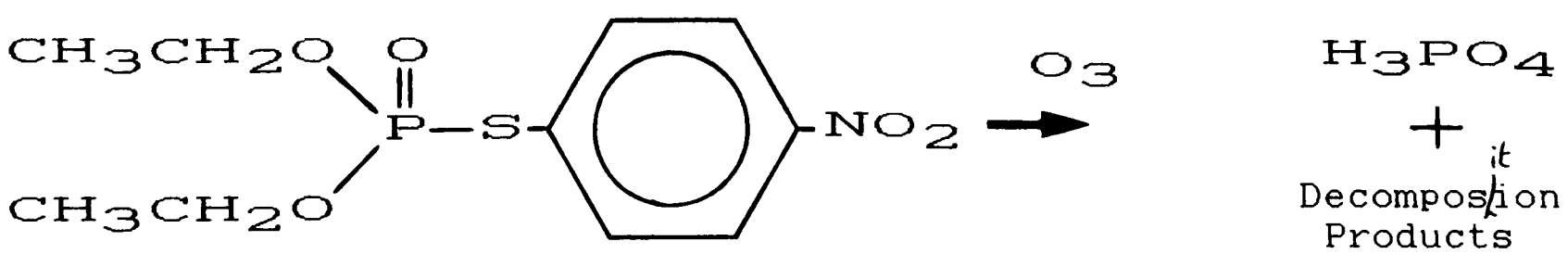
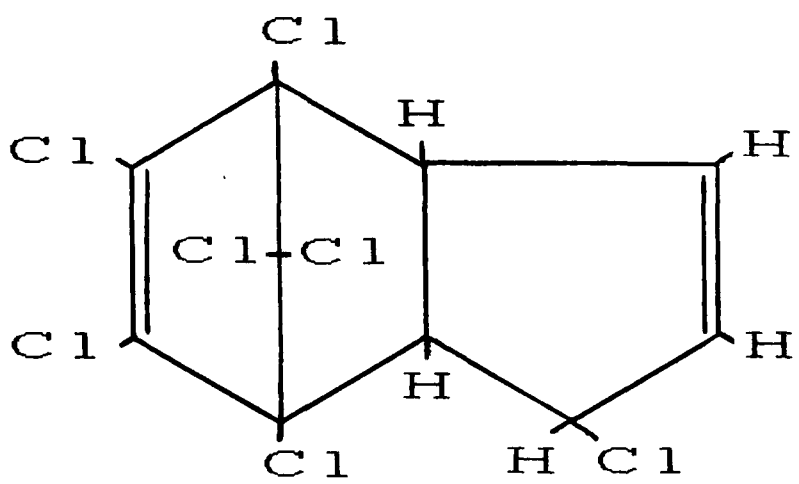


Figure 10. Ozonation of Malathion and Parathion
(Rice et al., 1981)

Heptachlor



O_3

Heptachlorepoxide

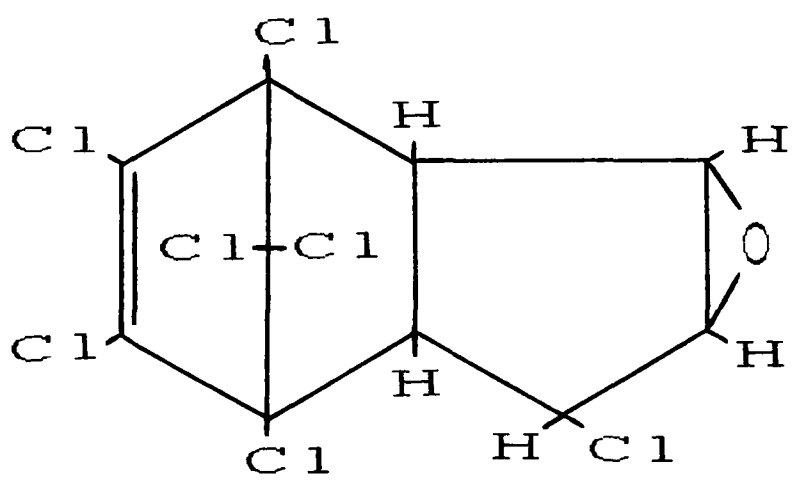


Figure 11. Ozonation of Heptachlor (Rice et al., 1981)

1.4.6.8 Ozone with Nitroaromatics

During the manufacture of TNT a primary waste, termed 'red water' originates when trinitrated oil is washed with a solution of sodium sulphite. This absorbs the unsymmetrical isomers of TNT and the resulting solution is very difficult to dispose of in an economical or environmentally sound manner. Further wastewater called 'pink water' is generated in shell loading plants when work surfaces and loaded munitions are washed down with water to remove TNT. This water is not as highly coloured as red water but it contains explosive material which could precipitate and settle out in the receiving waters.

Much research has been carried out on these wastewaters including ozonation. Brabets and Marks (1974) claimed to have successfully oxidised the TNT found in their synthetic pink water. However, whilst coloured compounds were readily destroyed they found that part of the colour reappeared if the pH was raised. Anderson et al. (1984) discuss this further and claim that a higher dose of ozone brings about complete destruction of these colour forming compounds and so prevents reversion of colour. Brabets and Marks also found carbon dioxide in the exit gas from their treatment system thus indicating that the benzene ring was being attacked and completely destroyed. An increase in biodegradability was noted.

The major drawback in this process was poor utilisation

of ozone. UV light was investigated for ozone oxidation enhancement. For UV light to be useful it must be absorbed by the ozone. Figure 12 shows the absorbance spectrum of ozone. This peaks at about 254nm and thus a low pressure UV lamp is required. When ozone is exposed to UV light it becomes 'excited' and decomposes to an oxygen atom and diatomic oxygen which, along with intact ozone, attack the organic species. UV energy can also activate some organic species, thus rendering them more susceptible to oxidation.

Fochtman and Huff (1975) found that the effect of UV light dramatically increased the rate of TOC and colour removal during the ozonation of pink water. However mass transfer in the system was poor due to the small contactor size (4 litres) and the high air/ozone flow rate (over 1.5 l/min). When they reduced the air/ozone flow rate the utilisation of ozone improved by over four fold. This is a good example of an unsuitable size of test reactor, and inadequate test design, as mentioned in section 1.4.6.4. Nevertheless, UV light does appear to assist systems by promoting formation of hydroxyl radicals.

Sierka (1985) looked at the effect of both temperature and pH on an ozone and ozone/ultrasound treatment process for a synthetic wastewater comprising TNT and RDX (cyclotrimethylene-trinitramine)(Figure 13) in the weight ratio of 70%:30% respectively. The rate of both TOC and explosive removal from the sample was found to increase with

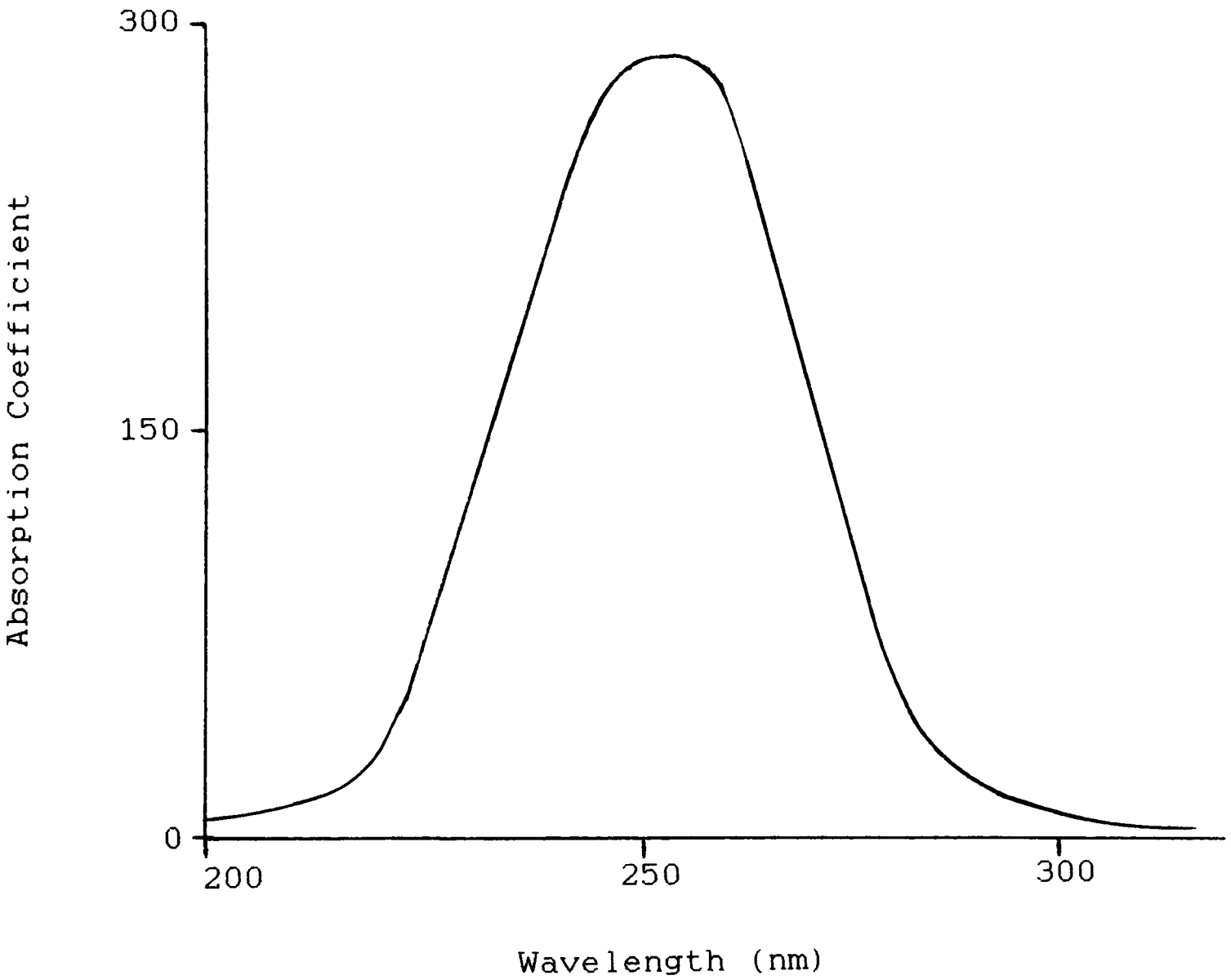


Figure 12. Absorption of Ultraviolet Light by Ozone (Fochtman and Huff, 1975)

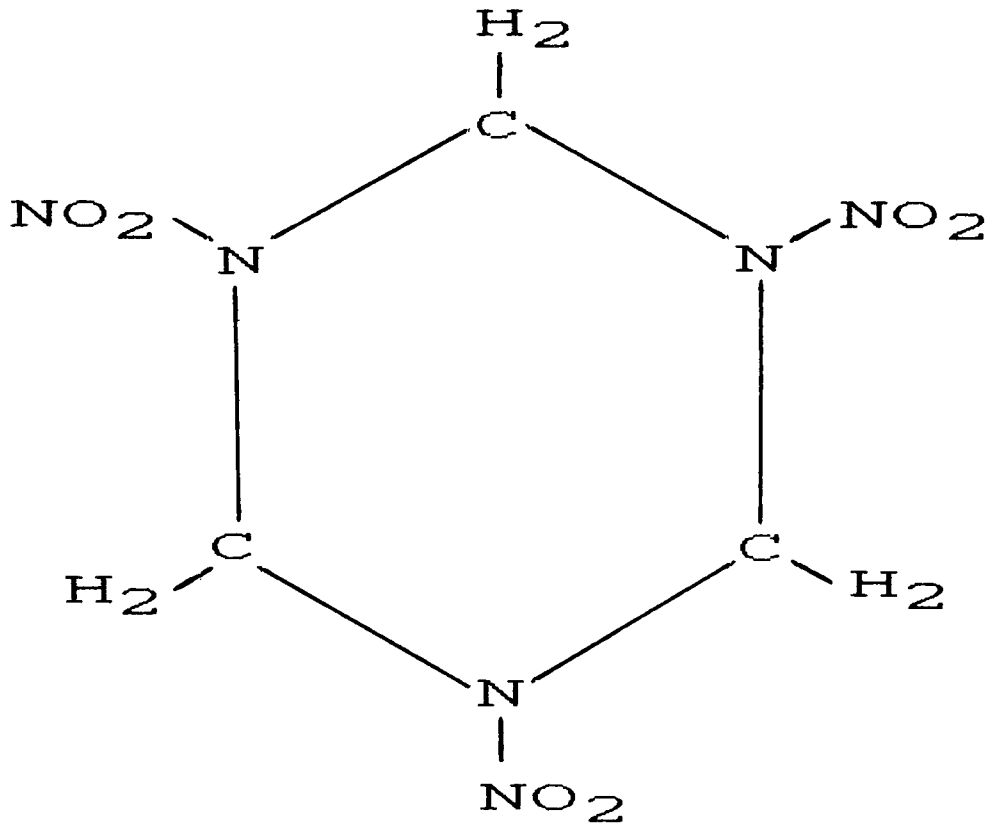


Figure 13. Chemical Structure of RDX
(Cyclotrimethylene-trinitramine)

increasing pH and with increasing temperature. The use of ultrasound in ozone oxidation was based on the argument that both ozone mass transfer and oxidation reaction rate would be enhanced by its presence in the reactor. The optimum frequency for this effect was unknown so a range of frequencies was examined at a fixed power rating. The results showed maximum destruction of explosives and TOC removal at a frequency of about 850 kHz and also that at that frequency, increasing power rates increased the removal further, although it was possible to attribute this to the increase in temperature during insonation. It was also noted that a combination of high pH and temperature was not beneficial, perhaps because radical formation was extinguished. In conclusion, the ozone/ultrasound process does not seem viable.

Whilst many authors have concentrated on promoting effects for ozonation, little attention has been given to the chemical development of the reaction process. Only simple determinations such as COD, TOC, BOD, colour and odour have been applied. However, Caprio et al. (1984) have investigated the effect of ozonation on aqueous nitrobenzene: care has been taken with the recognition and quantitative estimation of the process intermediates, with the aim of providing to a proper chemical description. These estimations were of nitrobenzene, nitric acid, glyoxal, glyoxylic acid, oxalic acid, formic acid and carbon dioxide (in the gas leaving the reactor). The initial ozone reactions were found to involve the oxidation of the nitro-substituent group with the elimination of nitric

acid and the formation of maleic acid and maleinaldehyde as unsaturated intermediates (Figure 14). This first oxidation stage merges into the appearance of concentration maxima for those species which are still reactive towards ozone. These intermediates are oxidised to form carbon dioxide, oxalic acid, glyoxylic acid, formic acid and glyoxal as the final reaction products. All of these remaining organic species appear to be integrated directly into the Glyoxylate Cycle (or TCA Cycle) and thus could be oxidised by microorganisms, with the exception of glyoxal. No evidence could be found for the metabolism of glyoxal by the author of this thesis. However, on further ozonolysis glyoxal will yield equimolar amounts of glyoxylic and formic acids (Caprio et al., 1984) and could then enter the glyoxylate cycle.

Unfortunately, it is a little difficult to draw parallels between nitrobenzene and the present system. The nearest work is that of Rice et al. (1981) who also suggest that organic nitriles, nitrites, nitroso compounds, hydroxylamines and the like will be oxidised, first to the corresponding nitro groups. These will decompose upon continued ozonation liberating nitrate ions (NO_3^-) and then on to smaller carbonaceous compounds. However, the amount of detail available within this report was regrettably very small compared with the detailed work of Caprio et al. (1984).

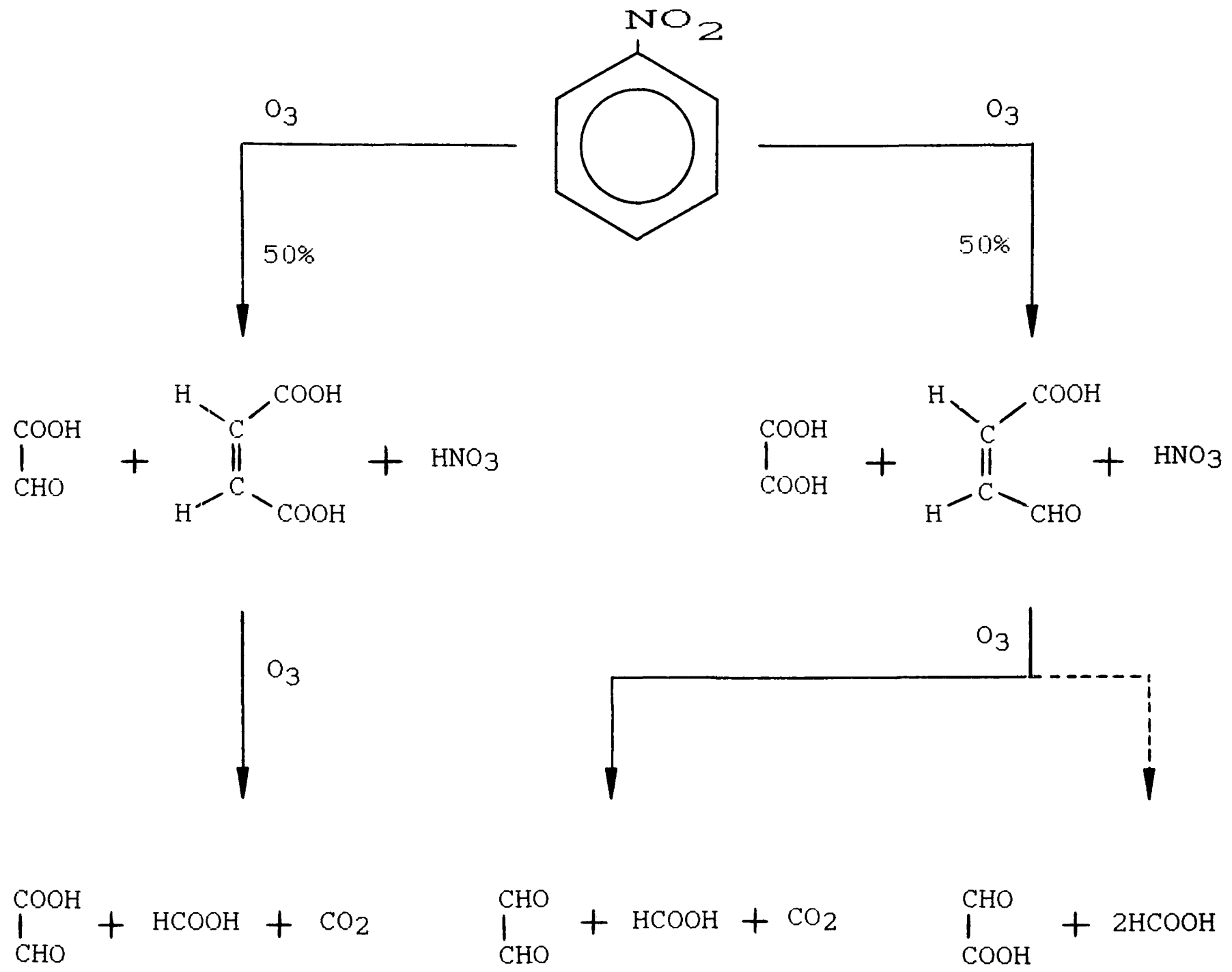


Figure 14. Proposed Mechanisms of Nitrobenzene Ozonation (Caprio et al., 1984)

1.4.6.9 Cost of Ozone Systems

The capital cost of an ozone system is very high. For example, a small laboratory scale generator with compressor and ozone ambient monitor is £20,000. Such a system can only generate 18g of ozone per hour which for wastewater treatment purposes is not very high. Figure 15 shows the relationship of capital cost per gram of ozone production potential verses the generating capacity of the plant. It is quite clear from this graph that the relative cost of a larger system is much lower in terms of capital cost per unit of generating capacity. Moreover, these larger generators can utilise low pressure air drying systems which are more energy efficient. For example, a 3Kg/h low pressure ozone stream would only require 60kW compared to 105kW for the high pressure one (Appendix 1).

1.4.6.10 Summary and Conclusions

Ozone generation and application is becoming a fairly established technology with numerous applications. The key advantage offered by ozone is its strong oxidising capability and its clean 'add nothing' feature. With the future prospects of water re-use and the effectiveness of ozone at removing refractive materials that are toxic, carcinogenic, or colour-, taste- and odour-producing to innocuous products means that industry and water treatment companies will have to take the capital investment seriously and will undoubtedly be swayed in favour of the ozone process.

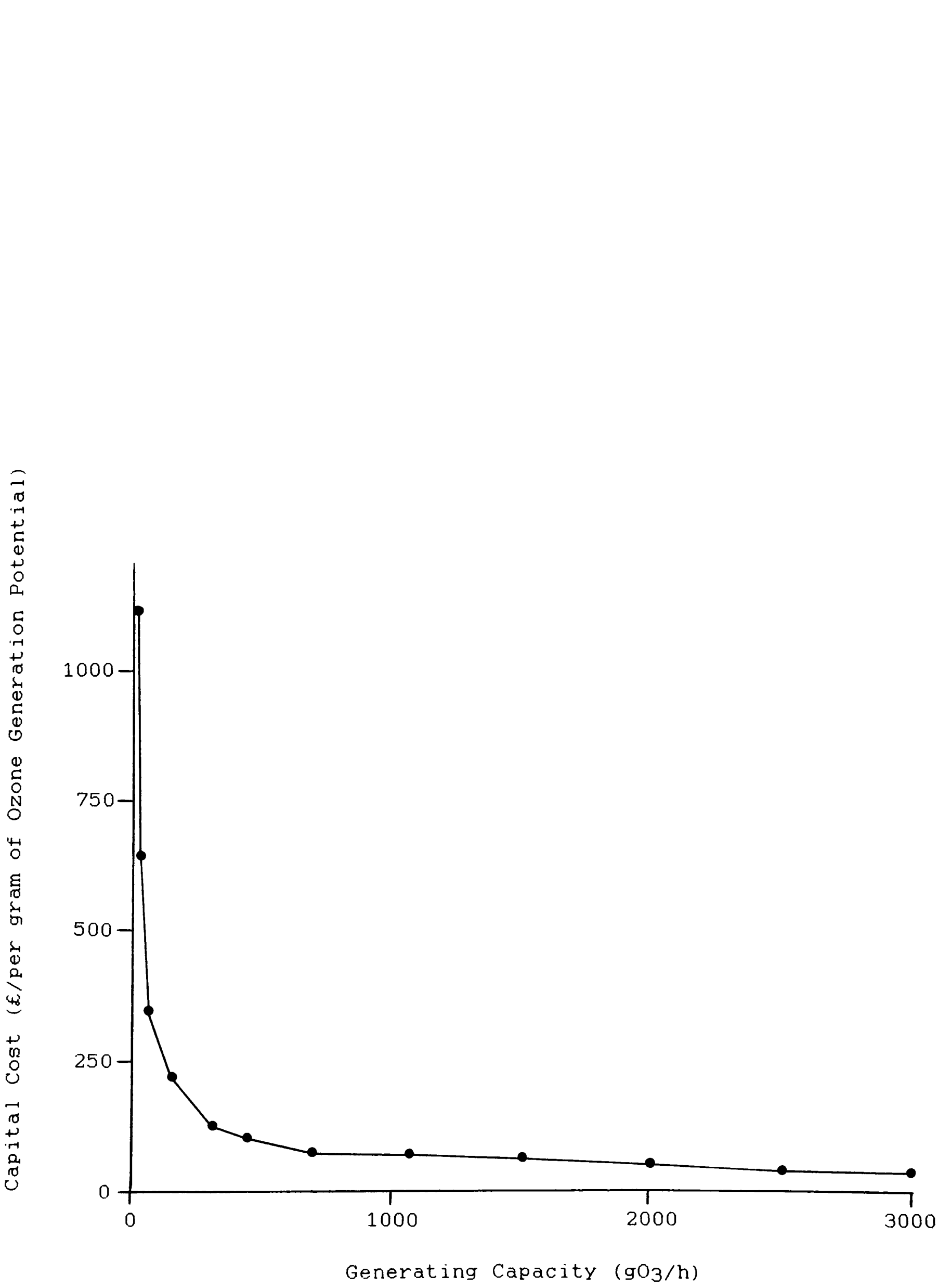


Figure 15. Relationship of Generating Potential with Capital Cost of Ozone Generating Equipment

1.4.7 Reduction - Catalytic

This process involves reducing the components of the waste, as opposed to oxidising them, and is to be considered only as a pre-treatment to other treatment processes.

For example this process may be useful where biological systems are to be utilised. Biological attack on the aromatic nucleus is electrophilic in character (Mason, 1957); thus, substitution in an aromatic molecule with electron repulsing constituents, such as methyl groups, would be expected to increase its biodegradability. Conversely, electron with-drawing groups, such as halogens or nitro groups, would increase the resistance of the nucleus to attack.

K10 wash water effluent is expected to contain many nitro aromatic compounds, and would therefore be expected to be highly resistant to microbial degradation. On this basis, a reductive pre-treatment might replace these nitro groups with amino groups which are, in general, slightly electron repulsive and might increase the susceptibility of the aromatic nucleus to attack.

Bacteria themselves are capable of reducing nitro groups to the corresponding amine but intermediate compounds, such as nitroso substituted aromatics, can produce azoxy compounds (McCormick et al., 1976; Kaplan and Kaplan, 1982a) which themselves are not susceptible to biological attack. A

chemical pre-treatment, such as reduction, is far more controllable and production of azoxy compounds (Figure 16, Reaction vi) can be avoided. March (1985) proposes many mechanisms and reagents for transformation of organic compounds (Figure 16, Reactions i,ii,iii,v and vi) and Spanggord et al. (1982) describe the diazotisation and decomposition of 4-amino-3,5,dinitrotoluene to 3,5,dinitrotoluene (Figure 16, Reaction iv).

Unfortunately, the most convenient method of hydrogenation involves the use of zinc (or tin) with hydrochloric acid, for the reduction of nitro groups to amines (Figure 16, Reaction i) and this, per se, introduces new components into the waste which may themselves need to be removed or treated. It is this factor which makes catalytic hydrogenation a more favourable process.

1.4.7.1 Catalytic Hydrogenation

The aromatic nitrogen group is easily reduced to the corresponding amine by catalytic hydrogenation with high efficiency. Trinitrotoluene has been reduced easily to triaminotoluene at normal temperature and pressure (Figure 17, Reaction A) using palladium on barium sulphate as a catalyst. Phenol has been reduced in the presence of sodium (as sodium carbonate) at moderate temperature and pressure to cyclohexanone, a non-aromatic ketone (Figure 17, Reaction B). These reactions are discussed by Rylander (1967).

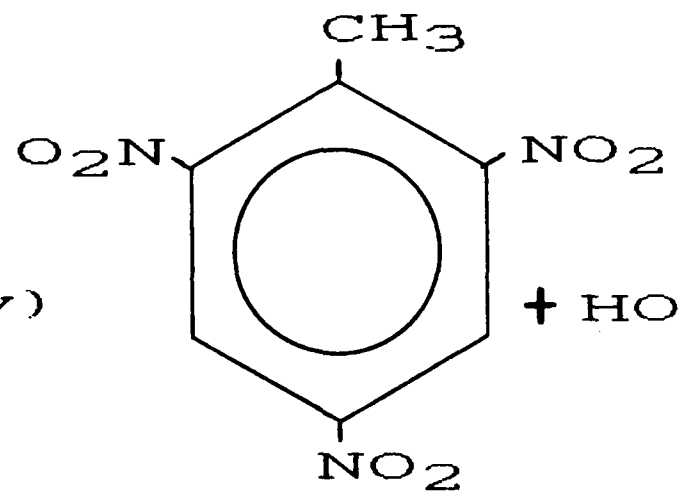
i) $ArNO_2$ —(Zinc/HCl; or Catalytic Hydrogenation)→ $ArNH_2$

ii) $ArNH_2$ —(HONO)→ ArN_2^+

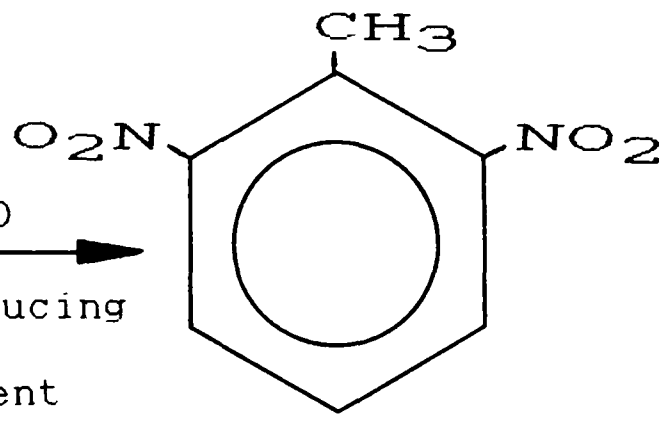
iii) ArN_2^+ —(H_3PO_2)→ ArH

Diazotisation

iv)



+ HONO —(H_2O; Reducing Agent)→



v) $ArNO_2$ —(Zinc/Water; Neutral pH)→ $ArNHOH$

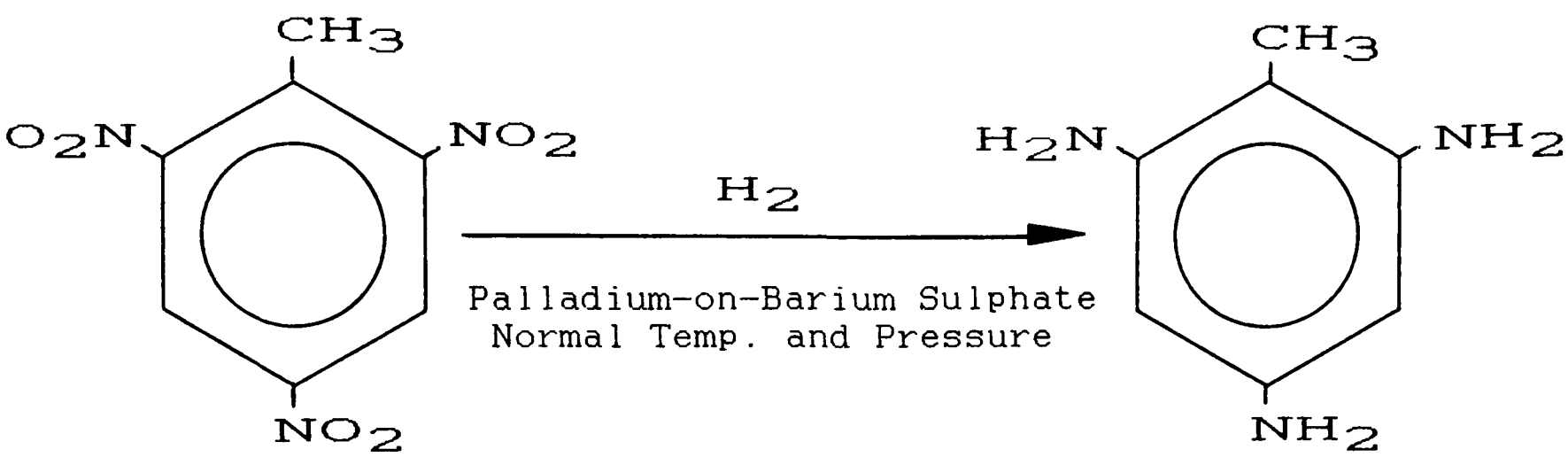
Nitroso Compound

vi) $2ArNO_2$ —(Weak Reducing Agent; (Including Glucose))→ $Ar-N^+(O^-)=N-Ar$

Azoxy Compound

Figure 16. Some Reactions of Aromatic Nitro Groups

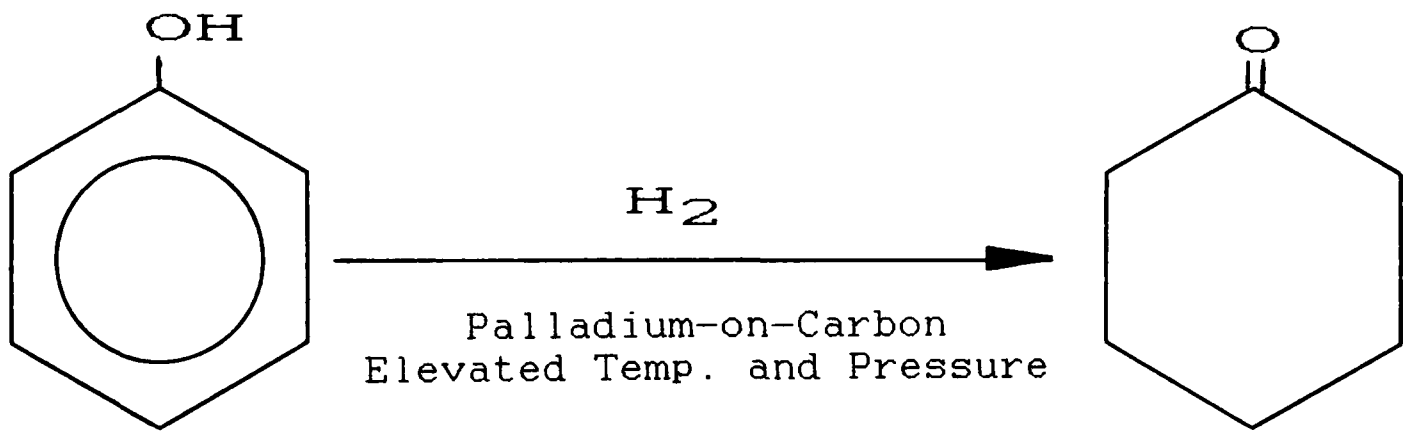
A



Trinitrotoluene

Triaminotoluene

B



Phenol

Cyclohexanol

Figure 17. Catalytic Hydrogenation of trinitrotoluene and Phenol

When using catalytic hydrogenation several factors need consideration. These are:

i) Type of catalyst metal
ii) If supported, the type of catalyst support
iii) Catalyst stability
iv) Catalyst poisoning
v) Catalyst reuse

The first of these considerations is to decide on a catalyst metal. All six of the elements in the platinum metals group (ruthenium, rhodium, palladium, osmium, iridium and platinum) make excellent hydrogenation catalysts. Palladium and platinum are probably the most widely used and both can be utilised either unsupported as a finely divided metal or supported on other substances such as carbon, alumina, zinc and asbestos to name but a few.

Supported catalysts are more efficient but their performance is dependent on the support material. This is due to the physical properties of the support, such as the surface area, the average pore size and the pore size distribution. The products of hydrogenation can also vary with the type of support (Augustine, 1963). Another factor influencing the choice of support is catalyst poisoning as illustrated with the hydrogenation of nitrobenzene using palladium-on-alumina in cyclohexane. For this situation, the rate of hydrogenation was dramatically reduced when the reaction was only 30%

complete, this reduction being ascribed to catalyst poisoning/inhibition. However, with palladium-on-carbon the reaction proceeded unabated, almost until completion (Rylander, (1967).

When catalysts are not in use they do not generally lose catalytic activity if contained in an air tight vessel, but in use the catalysts do lose activity. There is no way of predicting the rate at which catalytic activity will decrease and careful monitoring of reactions for many repeated hydrogenations is required to assess this. Although regeneration of the catalyst is possible, it is not uncommon for the catalytic nature to be changed and different compounds to be formed during hydrogenation (Farmer and Galley, 1933).

A report by Pitter (1985) confirmed that electron repulsing groups in general increase the susceptibility of the benzene nucleus to biological attack. However, when Pitter examined a range of aminotoluenes, aminophenols and amino sulphonic and benzoic acids for biological susceptibility, he discovered that the amino constituent increased resistance to biological attack, and thus was an exception to the rule. This indicates that hydrogenation of the K10 wash water effluent will not improve susceptibility of the aromatic nucleus to biological degradation.

1.4.8 Aerobic Activated Sludge

The activated sludge process is a biological system in which microorganisms oxidise the organic components of the wastewater. The process is, in effect, an artificial intensification of the process of self-purification which occurs in streams.

Activated sludge plants are fed continuously (as opposed to batch-wise) with the wastewater, which is then mixed with flocculated biological growths, mainly bacteria and protozoa, and aerated. Bacteria, usually Gram negative, comprise carbon oxidisers and nitrogen oxidisers; aerobes and facultative anaerobes; floc-formers and non floc-formers (Forster, 1985) and are responsible for the oxidation of organic components of the wastewater. The protozoa, such as cilliates, are also very important since they feed on free-swimming bacteria which, if left unchecked, would otherwise cause a turbid final effluent.

Wastewater which has continuously entered the plant is thus contacted with microorganisms for a suitable time (the retention time) during which waste components are adsorbed into the flocs and oxidised. This mixture of microorganisms and wastewater then passes into a clarifier where the flocs settle out as a sludge leaving a clarified final effluent. The majority of this sludge is returned to the aeration vessel and mixed with the fresh incoming wastewater and the remainder is removed (wasted) and disposed of by such processes as

anaerobic digestion, incineration or return to land.

The wastes fed to treatment plants vary from those of purely domestic origin through to effluents from the manufacture of complex and (sometimes) unpleasant chemicals, and the case with which these are treated differs accordingly.

The efficiency of a treatment plant in terms of Biochemical Oxygen Demand removal (Section 2.4.1) can often be very high, particularly if the principal component of the waste is domestic effluent. However, this is not necessarily the case for all organic compounds in solution. The Chemical Oxygen Demand estimation (Section 2.4.2) is capable of oxidising compounds which the microorganisms find difficult, if not impossible, to metabolise. To get high chemical oxygen demand removal efficiency the organisms must be capable of oxidising most of the components of the wastewater. This requires specialised communities of microorganisms and the adaptation of these microorganisms is the limiting factor of such biological treatment process. Without these microorganisms, biological treatment processes are not a viable proposition for treatment of many of those less-pleasant wastewaters.

1.4.9 Aerated Lagoons

Lagooning in oxidation ponds is a common method for the oxidation of organic material. The process can be considered as a design extension of the stabilisation pond. The main difference is that aerated lagoons are mechanically aerated tanks and thus are not dependent merely on algae and natural diffusion for the supply of oxygen. The use of aerators leads to the formation of flocculated biological growths as found in activated sludge plants and hence the process is much the same but without a sludge recycle. The absence of this recycle means that organisms are not able to acclimatise to the same extent as in activated sludge plants. Moreover, the removal efficiencies are generally not as high, and the process is less stable under varied influent loading (De Renzo, 1978). Thus, overall, the aerated lagoon is not as attractive a proposition as the activated sludge process for industrial wastewater treatment.

1.4.10 Percolating Filters

A percolating filter is a packed bed, usually of stone or plastic but sometimes wood. Wastewater is distributed over the top surface of the bed by spray, or static, or moving distributors. The wastewater then percolates down the bed against an upward flow of air. A slimy film which consists of microorganisms embedded in extracellular polymer, develops on the surfaces of the packing material, and the organisms

oxidise the organic components of the wastewater. Although this is the basis of all such filters, this system can often also be subject to various problems. For instance, excessive accumulation of solids on the surface can block the voids between the packing and the filter system can "pond". This occurs during winter months when grazing organisms, such as nematode worms, are suppressed, due to the cold, resulting in increased film growth. However, this film is removed during warmer weather and results in the "spring sloughing" problem whereby the humus generated overloads removal tanks giving rise to a low quality final effluent.

In general a percolating filter system is less efficient than an activated sludge system; mainly due to the short contact period of the wastewater with the microorganisms. The process does, however, allow the long term adaptation of organisms, but so, of course, does the activated sludge system. However as this process is essentially plug flow, it is not generally suitable for industrial wastewaters even though energy requirements are much lower than for the activated sludge process (De Renzo, 1978).

1.4.11 Anaerobic Digestion

Anaerobic digestion is a biological treatment process for the degradation of organic compounds in an oxygen free environment. The organisms present in such a system can be classified into two interdependent groups: the hydrolytic

species, which degrade large molecules into their component monomers, and the post-hydrolytic species which degrade these monomers and other compounds present in the waste to methane and hydrogen sulphide (Forster, 1985). The main problem, in operating such a system, is the maintenance of these organisms so that they operate in a balanced and controlled way.

There are two predominant types of anaerobic digester. The first operates rather like the activated sludge process (Section 1.4.8) except that it is dealing with slurries rather than dilute liquids. The other type is much more like a percolating filter (Section 1.4.10). Generally these devices operate with an upward flow of wastewater (Eckenfelder, 1980) which passes through a packed or fluidised bed or a blanket (which is really a bed in which the organisms are self supporting). Both processes are not, of course, aerated. Anaerobic digestion is less effective at removing complex organics, such as those expected in K10 wash water effluent, than aerobic processes; indeed, biodegradation of many xenobiotics is almost impossible except by means of aerobic attack. Nitro-aromatics are within this group of compounds, and consequently Kobayashi and Rittmann (1982) suggest the use of aerobic organisms for the degradation of such materials.

1.4.12 Enzyme Treatment

Enzymes are highly selective chemical catalysts produced by living organisms and act on specific substrate molecules. For example, urease is an enzyme which catalyses the degradation of urea to carbon dioxide and ammonia.

However, enzymes are generally unstable protein molecules and expensive to produce. Thus enzyme treatment of complex industrial wastes is not a practical proposition since enzyme action cannot be accommodated to match the varying compositions and toxicities of waste streams. It is thus not surprising that there are no full scale applications of enzyme treatment processes for wastewater treatment known to the author.

1.5 BIOLOGICAL AND CHEMICAL METHODS COMPARED

Various processes have been discussed (Section 1.4) which could well be suitable as a treatment, or combination of treatments, for K10 wash water effluent. Considerations both of the efficiency and of the costs of these techniques are necessary to permit rational selection of a preferred option.

In general, biological treatments are the most cost-effective processes for the treatment of aqueous waste streams containing organics, providing suitable organisms are available. In this connection, Gale (1947) proposed his microbial infallibility principle in which he stated that "microorganisms capable of oxidising any compound that can be theoretically oxidised will exist wherever the compounds are found". However, it must be remembered that these theoretical organisms must first be found, selected, adapted and produced in quantity. If this is not readily feasible, then a biological system is not an appropriate device, and physical-chemical methods should be adopted. Of these, activated carbon or ozone are probably the most promising as single treatment processes for K10 wash water effluent, although adsorbent regeneration can be costly in the case of carbon treatment. A chemical pre-treatment such as hydrogenation, or limited ozonolysis, may render the waste more amenable to biological systems.

In general biological systems have over-riding advantages for treatment of wastes containing low organic pollutant concentrations due to the high affinity of microorganisms for substrate molecules. These compounds are then, in general, mineralised to innocuous compounds such as water and carbon dioxide. Some molecules are exceptions to this general rule, and are not mineralised but merely transformed into new compounds which might possess more serious pollutant properties than the parent molecule. This occurs with both nitrobenzenes (Section 1.6.6.1) and aminobenzenes (Section 1.6.6.2), and this is of particular relevance to the present study, which involves nitrobenzenes.

Tabak et al. (1981) have described as "priority" pollutants those compounds which fall into the following classes of organic compounds: phenols, phthalate esters, naphthalenes, monocyclic aromatics, polycyclic aromatics, polychlorinated biphenyls, halogenated ethers, nitrogenous organics, halogenated aliphatics and organochlorine insecticides. Again, nitrogenous organics feature in Tabak's priority listing. However, his research group have shown that appropriate microbial metabolism is an important factor for the treatment of some of these "priority" pollutants (Tabak et al., 1981). Clearly, then, wastes of the types under consideration in this work are considered particularly polluting, and it has been suggested that appropriate biological treatment might be a useful option. However, industrial wastes consist of complex mixtures of pollutants,

rather than a solution of one defined material, and this confuses the issue. Since no specific treatment can be applied to all situations each industrial waste needs to be reviewed individually, often by trial and error, although the more promising treatments can be quickly identified if the approximate composition of the waste is known.

In the case of K10 manufacture a biological system would be the most convenient for the wash water effluent. Of the available biological systems, the aerated activated sludge system is the most attractive since it is the most compact, and the recirculation of sludge allows for the development of acclimatised microorganisms. The factors affecting the adaptation of microorganisms and the metabolism of aromatic compounds are very complex and it seems appropriate to discuss these further.

1.6 BIODEGRADATION

Biodegradation has been defined as the microbial transformation of chemical organic compounds to, ultimately, inorganic compounds. The biodegradation of aromatic compounds has received particular attention (Alexander, 1981; Alexander, 1985; Bull, 1980; Painter, 1974; Slater and Bull, 1982; Spain and Van Veld, 1983). The varying fates of these chemical compounds when exposed to biological systems fall into three major categories.

i) The compound will remain totally unchanged (recalcitrant)

ii) The compound will be transformed or partially degraded

iii) The compound will be completely mineralised to carbon dioxide, water, sulphate, etc.

1.6.1 Partial Degradation

Whilst the latter of the possibilities listed in the previous section is the ultimate aim of any wastewater engineer proposing to use biological systems, problems are posed by partially degraded compounds. Thus difficulties often arise due to the nature of the new compounds which are formed. For instance, this occurs with the transformation of 3-chlorocatechol which forms acyl halide, a highly toxic compound, whilst 4-chlorocatechol is transformed to a recalcitrant molecule (Figure 18, Slater and Bull, 1982).

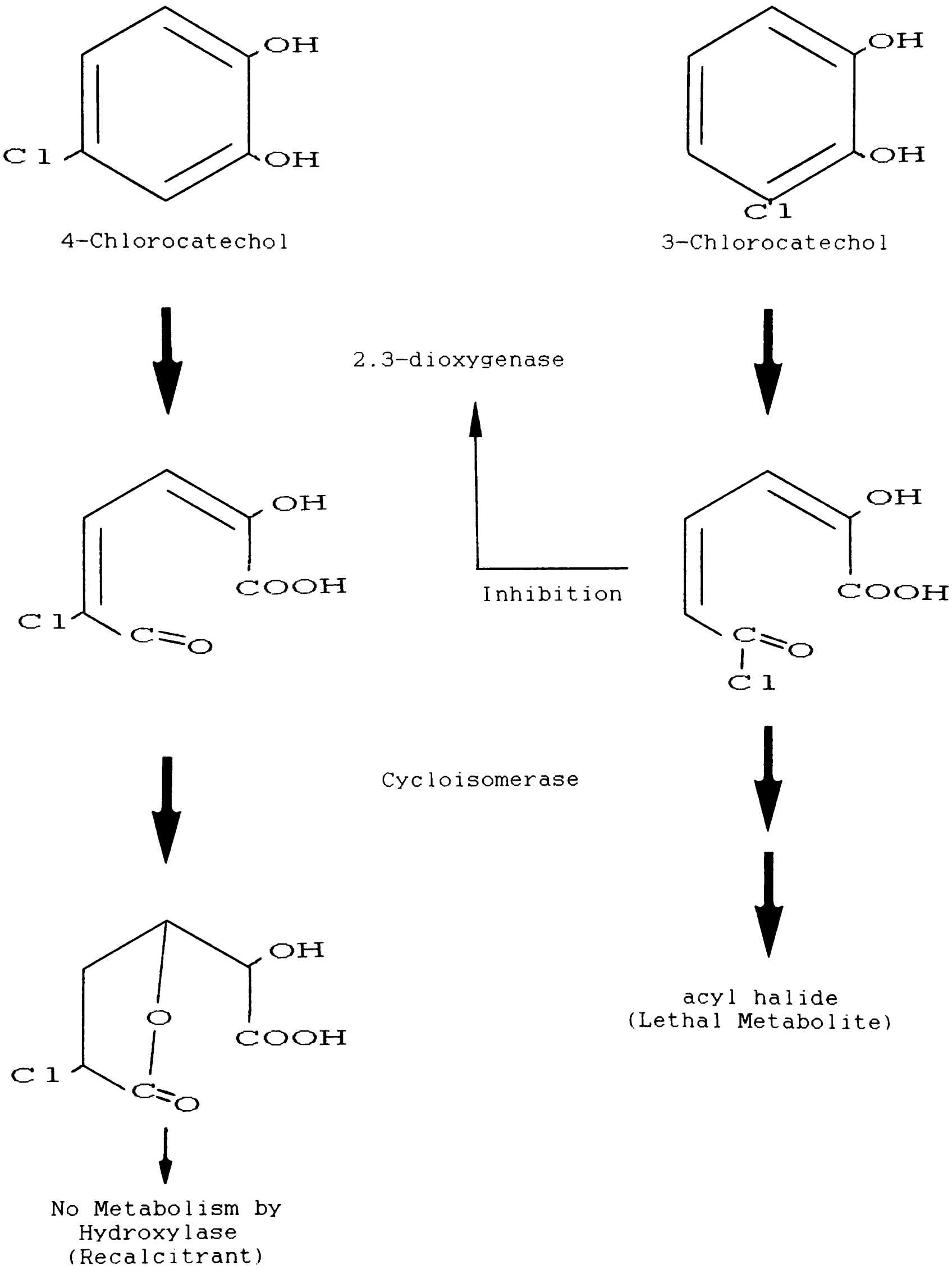


Figure 18. Metabolism of two Chlorocatechols
(Slater and Bull, 1982)

Similar problems have also been reported regarding the metabolism of nitro and amino benzenes with the production of nitroso compounds (Liu et al., 1984; McCormick et al., 1976; McCormick et al., 1978).

1.6.2 Recalcitrance

Through the process of evolution, virtually all naturally occurring organic compounds can be completely mineralised, usually by microorganisms, without ill-effects to the environment and so continuing the cycle of elements. However, man has been synthesising large quantities of new compounds over a time period which is short in evolutionary terms. The introduction of these compounds, often referred to as xenobiotics, into the environment has not always resulted in complete mineralisation. Such compounds are termed recalcitrant meaning that they are not readily biodegradable. This idea was suggested by Alexander (1965) with reference to the fate of pesticides, who emphasised the need for systematic research into their biological degradation.

A combination of microbiology, biochemistry and bioengineering over the last decade has shown many compounds, initially thought recalcitrant, to be biologically degradable. This was demonstrated by Knackmuss (1981) whereby organisms assimilating naphthalene as a carbon source were given small amounts of naphthalyl-2-sulphonic acid, a recalcitrant compound which is related to naphthalene. The naphthalene was

gradually replaced with naphthalyl-2-sulphonic acid, thus applying a selective pressure on the organisms to utilise this new substrate. Once organisms were considered to have adapted to the naphthalyl-2-sulphonic acid, its concentration was increased further and the naphthalene removed forming a new strain of the organism. Findings such as this help to confirm the hypothesis that all organic compounds can be biodegraded if the correct organism can be isolated, the right enzymes induced and if favourable environmental conditions prevail. However, a selection of recalcitrant compounds, even if related one to another, will not necessarily behave similarly since many factors are involved. These factors have been reviewed extensively by Faber (1979) and are summarised in Table 3 (Sayler et al., 1984).

1.6.3 Co-metabolism

Organisms usually metabolise organic compounds in order to derive energy and carbon for growth. However, in some instances, the oxidation of a non-metabolisable compound occurs when organisms are assimilating some other compound. The oxidised form of this recalcitrant molecule might then be oxidised further by some other organism and result in mineralisation of the compound. This is termed co-metabolism, or co-oxidation, and has been reviewed by Horvath (1972). As an example, co-metabolism occurs with metabolism of cyclohexane where one microbial species in a consortium, metabolising paraffin, oxidises the cyclohexane to

Structural Properties	Consequences
1) Molecular Weight or Size	-Limited Active Transport
2) Polymeric Nature	-Extracellular Metabolism Required
3) Aromaticity	-O_2 requiring enzymes
4) Halogen Substitution	-Lack of dehalogenating Enzymes
5) Solubility	-Competative Partitioning
6) Toxicity	-Enzyme inhibition, cell damage
7) Xenobiotic Origin	-Evolution of new degradative pathways
Environmental Factors	
1) Dissolved Oxygen	-O_2 sensitive and O_2 requiring enzymes
2) Temperature	-Mesophilic temperature optimum
3) pH	-Narrow pH optimum
4) Dissolved Carbon	-Organic/pollutant complexes Concentration dependent growth
5) Particulates, Surfaces	-Competition for substrate
6) Light	-Photochemical enhancement
7) Nutrient and Trace Elements	-Limitation on growth and enzyme synthesis
Biological Factors	
1) Enzyme Ubiquity	-Low frequency of degradative species
2) Enzyme specificity	-Analogous substrates not metabolised -Re-acclimation
3) Plasmid Encoded Enzymes	-Low frequency of degradative species -Instability
4) Enzyme Regulation	-Repression of catabolic enzyme synthesis -Required acclimation or induction
5) Competition	-Extinction or low density populations
6) Habitat Selection	-Lack of establishment of degradative populations
7) Population Regulation	-Low population density of degradative organisms

Table 3. Factors Influencing Biodegradability of Organic Pollutants (Sayler et al., 1984)

cyclohexanol with no benefit to itself. Another species, which cannot metabolise paraffin or cyclohexane, then metabolises the cyclohexanol degrading it completely.

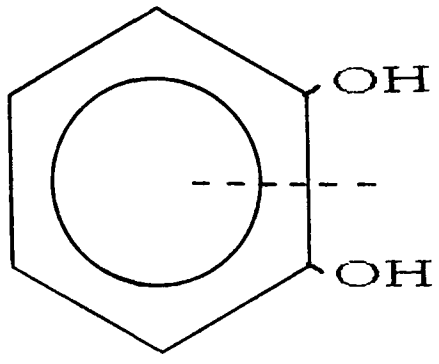
1.6.4 General Metabolism of Aromatic Compounds

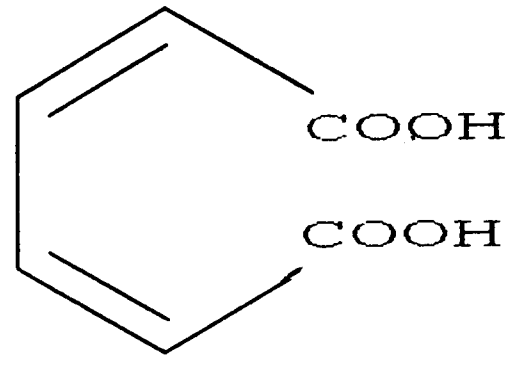
During the late 1950's the opening of the benzene nucleus and the use of its carbon as the sole source of energy by microorganisms was considered an exceptional biochemical activity. However, the degradation of aromatic compounds is an essential step in Nature's carbon cycle and is performed by a wide variety of bacteria and several types of fungi and yeasts. The ability of animals to degrade aromatic compounds is extremely restricted, being mainly confined to the amino acids phenylalanine, tyrosine and tryptophan (Dagley, 1983). Today it is more widely appreciated that aromatic compounds supply, as it were, the daily bread of some of the microbial world and there are, of course, vast quantities of lignin, an aromatic based polymer of plants, which is more abundant than protein in the environment.

The benzene nucleus is relatively inert due to its stable resonance structure. However, microorganisms are exceptional in their ability to input (or advance) energy in reactions which lower the energy barrier of the nucleus, so allowing ring fission. This energy investment is repaid by the subsequent energy-releasing reactions from the metabolism of the aliphatic acids produced. Before this ring fission can

occur the benzene nucleus must carry two hydroxyl groups situated either on adjacent carbons, as in catechol, or placed across the ring as in gentisic acid. The enzymes which carry out this fission of the nucleus are called oxygenases and they utilise oxygen gas (dioxygen) (Evans, 1963). Fission can be realised either via the ortho-cleavage pathway (intradiol-cleavage) or via the meta-cleavage pathway (extradiol-cleavage) (Figure 19), although the ortho pathway is used almost exclusively for the metabolism of catechol itself and the meta pathways are more tolerant of substituted catechols. These particular degradative pathways are discussed in detail by Sayler (1984).

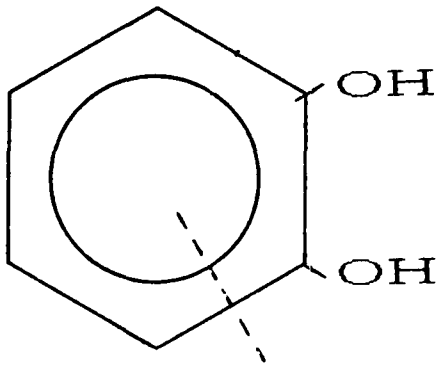
When an aromatic compound carries only one hydroxyl group a second must be introduced by a hydroxylase. If it carries none at all, then two must be added. This process is directed by cytochrome P-450 in mammals, but in bacteria the enzymes involved are flavoproteins. These monooxygenases utilise oxygen and NADPH to achieve this hydroxylation (Figures 20 and 21) and they exhibit narrow substrate specificity. The use of NADH that could otherwise be used to generate ATP must be regarded as an energy investment. In contrast, the hydroxylation, or ring-fission, reactions themselves neither consume or generate energy. These reactions and those that follow simply involve breaking up the original carbon chain to form, eventually, metabolites for the tricarboxylic acid (TCA) cycle. Figure 22 shows the main pathways for the degradation of some dihydric compounds, the products of which can enter the TCA cycle and repay the energy investment.

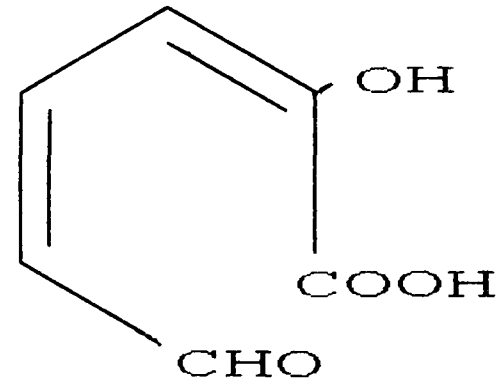




ortho-Cleavage

cis.cis-Muconic Acid

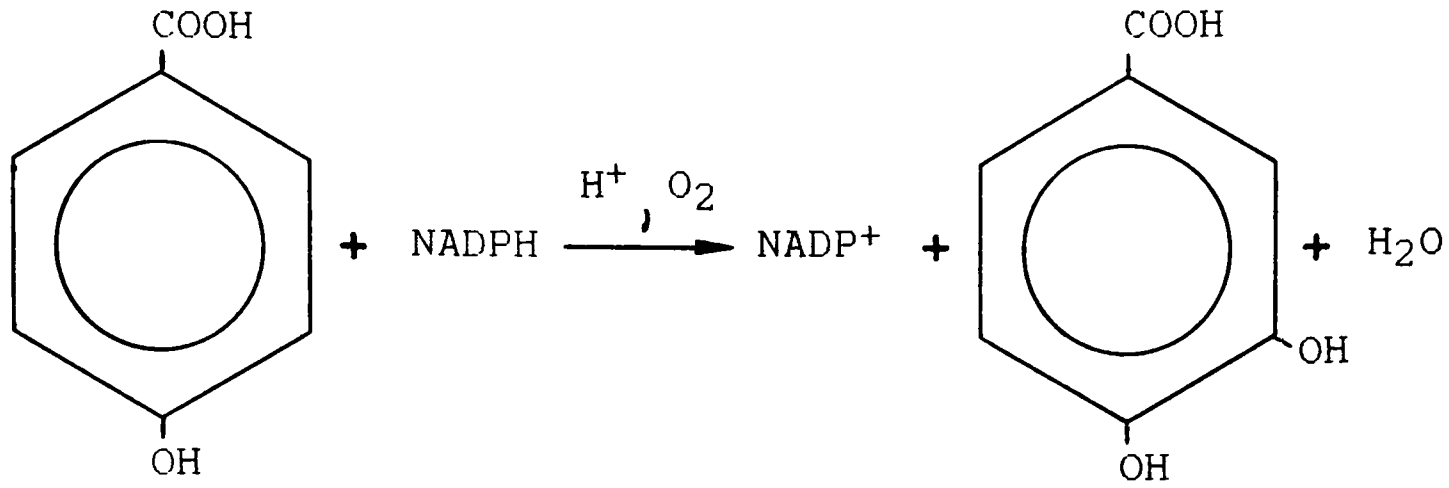




meta-Cleavage

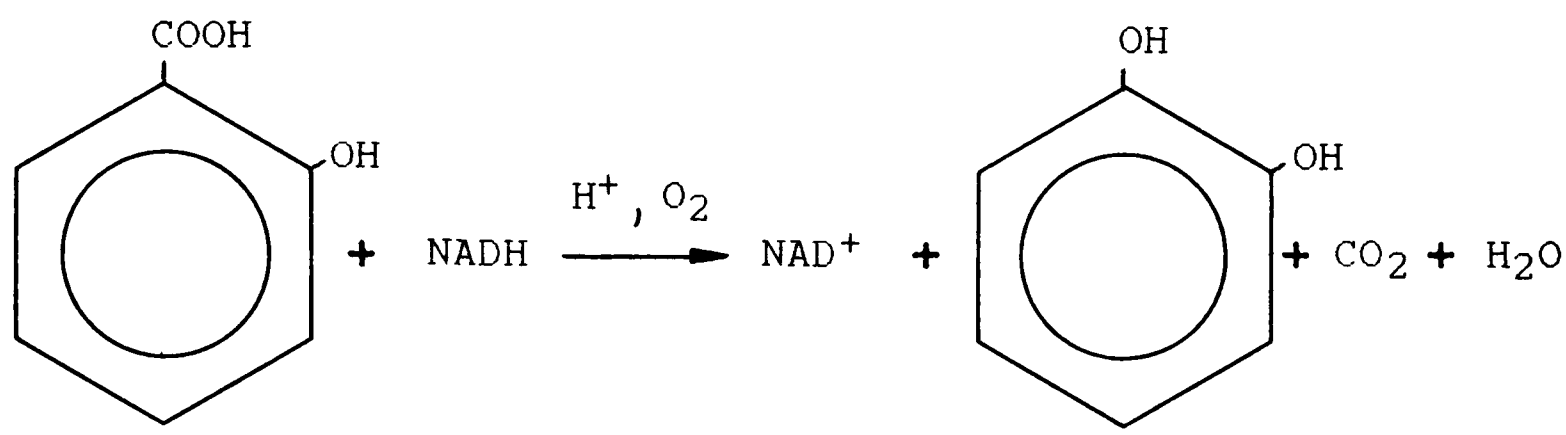
2-Hydroxymuconic
semi aldehyde

Figure 19. Ortho and Meta Cleavage Positions of Catechol (Faber, 1979)



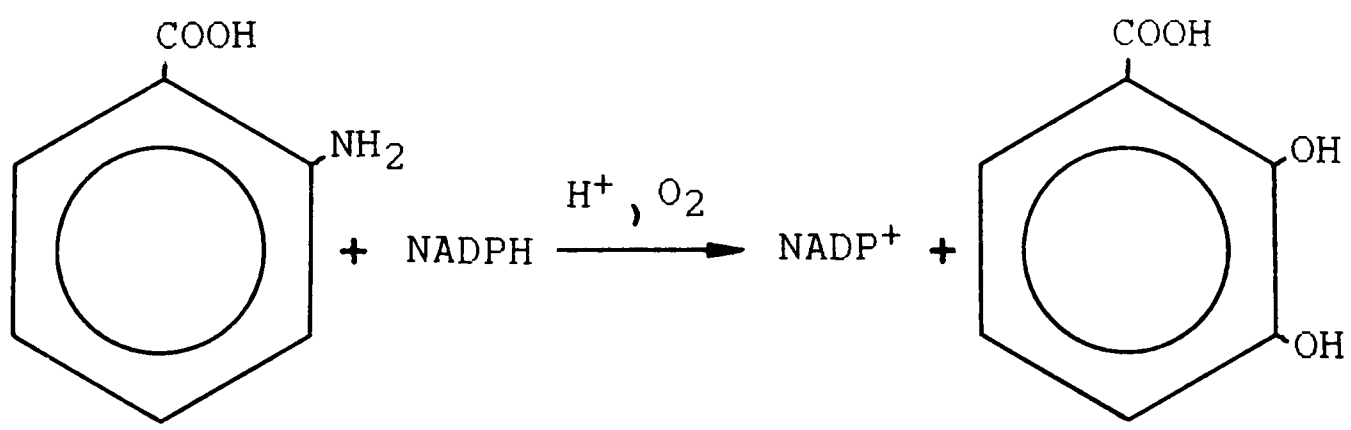
p-Hydroxybenzoic Acid

2,3-Hydroxybenzoic Acid



Salicylic Acid

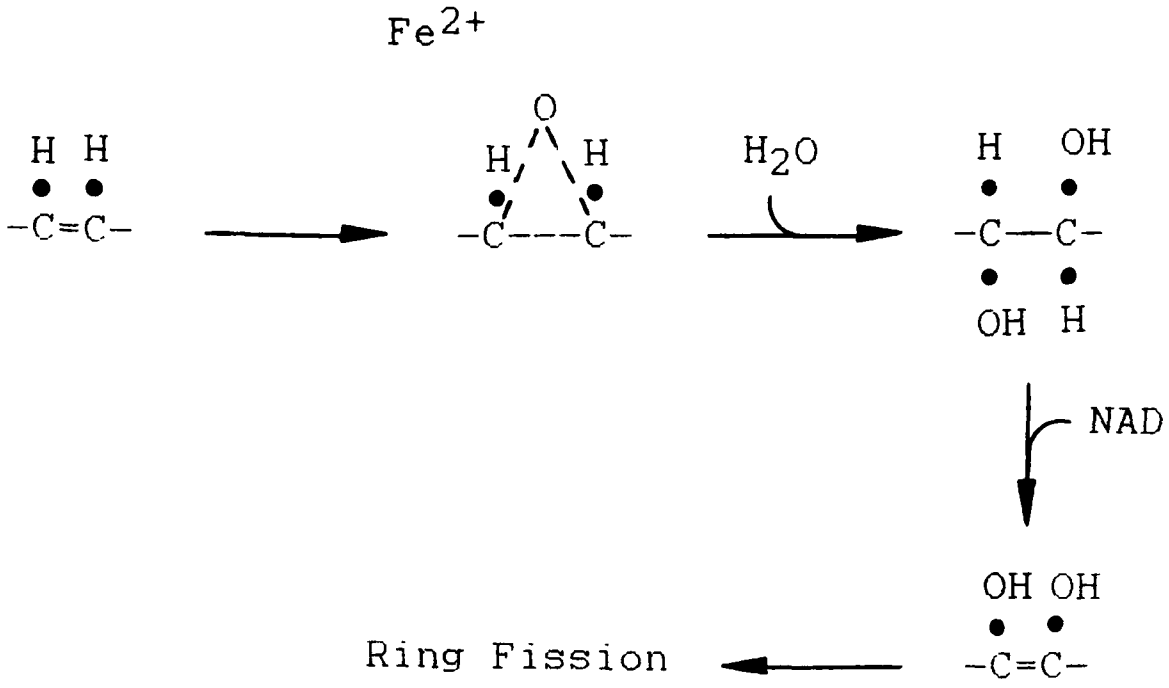
Catechol



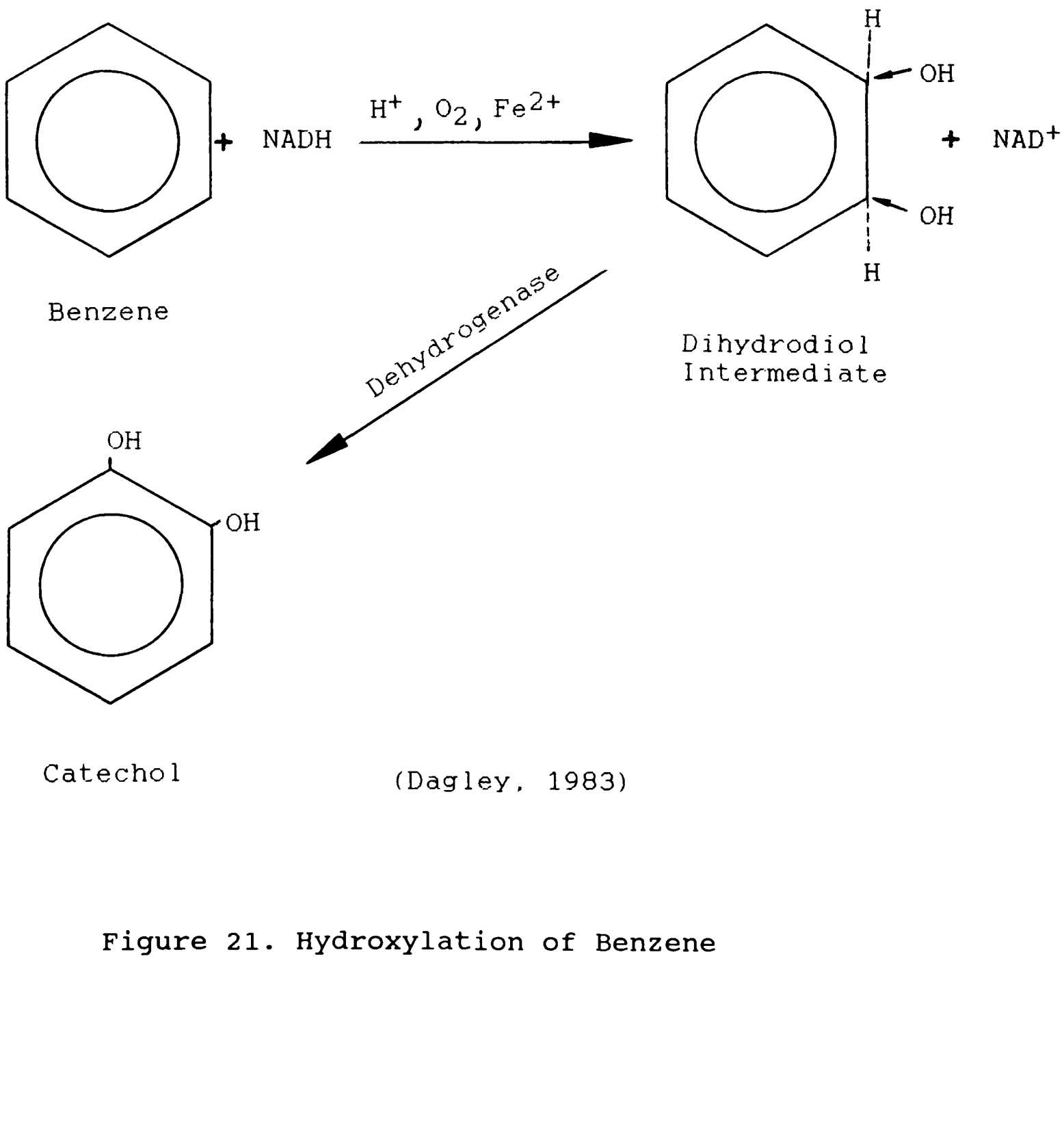
Anthranilic Acid

Protocatechuic Acid

Figure 20. Examples of some Energy requiring Hydroxylations by Flavoproteins (Dagley, 1983)



(Evans, 1963)



(Dagley, 1983)

Figure 21. Hydroxylation of Benzene

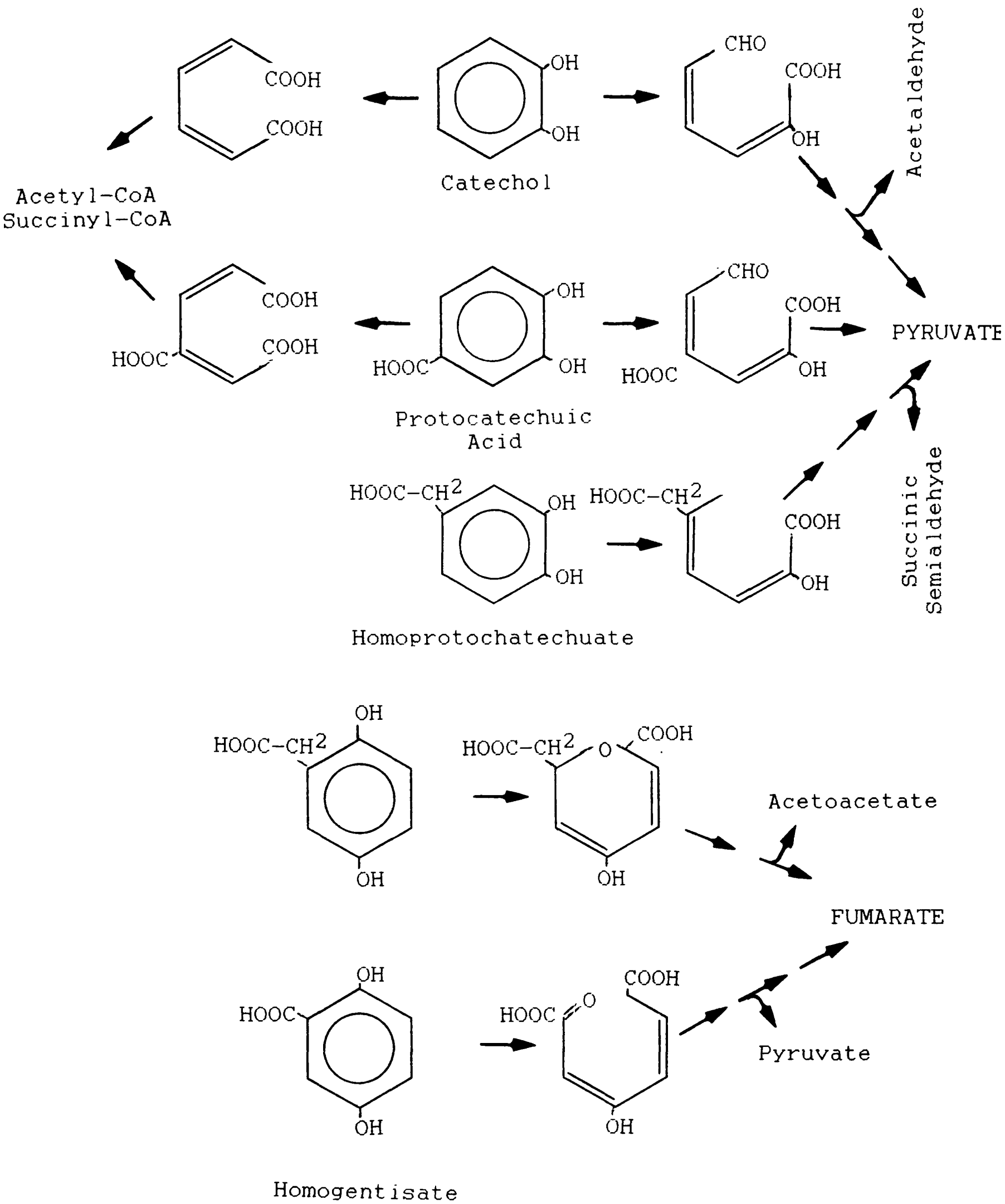


Figure 22. Degradation of Dihydric Phenols (Dagley, 1983)

1.6.5 Anaerobic Metabolism of Aromatic Compounds

Under anaerobic conditions bacteria have been found to metabolise aromatic compounds. Degradation can be divided into three distinct categories: photometabolism, nitrate reduction and methanogenic fermentation, as reviewed by Evans (1977). Cleavage of the aromatic nucleus is preceded by reduction of the ring (Dutton and Evans, 1968) after which inorganic electron-acceptors, such as nitrate, sulphate and carbon dioxide which are reduced to ammonia, sulphide and methane respectively, are used for further metabolism.

In comparison with aerobic degradation of aromatics, the literature concerning anaerobic degradation is sparse, although this process is clearly ecologically significant. The majority of authors describe the degradation of nitro-aromatic compounds only by aerobic means. Kobayashi and Rittmann (1982) give examples of aerobic microorganisms that can attack a variety of aromatic compounds including benzene, toluene, nitrotoluenes, cresol and phenol. Some of these anthropogenic compounds may have similarities with those expected to be present in K10 wash water effluent. It is evident from the literature that anaerobic degradation of such compounds is strictly limited by the restricted range of compounds attacked, and the general difficulty of the anaerobic systems involved; in fairness, there are a few odd compounds incapable of aerobic dissimilation which can be broken down under anaerobic conditions (Hallas and Alexander, 1983; Kobayashi

and Rittmann, 1982). Overall, however, on this basis, aerobic metabolism, as opposed to anaerobic metabolism, was considered a more viable treatment process for this effluent.

1.6.6 Compounds of Immediate Interest

Extensive studies have been carried out on the biological degradation of aromatic compounds although no literature pertaining to K10 manufacture or effluent treatment has been located by the author. However, since the chemical structure of TNEB is very similar to TNT, it would seem a reasonable proposition to study the treatment of TNT wastewater. Due to the voluminous nature of this literature only a general overview of metabolism and transformations will be given.

1.6.6.1 Nitrobenzenes

Environmental contamination from the manufacture of pesticides, dyes and explosives is of great concern since many of these compounds are toxic and mutagenic. For example, in large scale TNT manufacturing and handling operations, workers were reported to have liver damage and anaemia before the hazards had been fully evaluated (Sax, 1983). TNT has since been found to be mutagenic in the Ames test and constitutes a serious environmental hazard. TNT and related compounds have been isolated in soil and waterways after leaching from disposal sites (Pereira et al., 1979) and the degradation of TNT has been widely investigated (Carpenter et al.,1978;

Channon et al., 1944; Hallas and Alexander, 1983; Kaplan and Kaplan, 1982a, 1982b, 1984; Liu et al., 1984; McCormick et al., 1978; Mitchell and Dennis, 1982; Nay et al., 1974; Spanggord et al., 1982; Won et al., 1974, 1976; Wyman et al., 1979).

The general consensus is that TNT and related compounds are difficult, if not impossible, to mineralise completely and that functional groups undergo biological transformations (Carpenter et al., 1978; Hallas and Alexander, 1983; McCormick et al., 1978). These transformations are thought to occur under both aerobic and anaerobic conditions although Liu et al. (1984) reported that 2,4-dinitrotoluene will be transformed only under anaerobic conditions. However, McCormick et al. (1976) have shown the nitro group of TNT to be reduced under both aerobic and anaerobic conditions according to the following equations under mesophilic conditions:-

$$\underset{\text{Nitro}}{Ar\text{-}NO_2} \xrightarrow{H_2} \underset{\text{Nitroso}}{Ar\text{-}NO} + H_2O$$

$$\underset{\text{Nitroso}}{Ar\text{-}NO} \xrightarrow{H_2} \underset{\text{Hydroxylamino}}{Ar\text{-}NHOH}$$

$$\underset{\text{Hydroxylamino}}{Ar\text{-}NHOH} \xrightarrow{H_2} \underset{\text{Amino}}{Ar\text{-}NH_2} + H_2O$$

The pathways for the reduction of the nitro groups of TNT to amino groups or transformation products are shown in Figures 23 and 24 for mesophilic (McCormick et al., 1976) and thermophilic (Kaplan and Kaplan, 1982a) transformations respectively. The transformations of TNT by thermophiles are basically identical to mesophilic processes, and so the two systems were considered indifferent in terms of enzymatic action (Kaplan and Kaplan, 1982a). Partial reduction of the nitro groups to the hydroxylamino intermediate may lead to the non-enzymatic (chemical) formation of the corresponding azoxy compounds. Certain amino surfactants at alkaline pH's are reported to precipitate TNT as water insoluble complexes (Kaplan and Kaplan, 1982b) which were more mutagenic than TNT (itself a frame shift mutagen (Won et al., 1976)). However, Won reports the metabolites of TNT to be non-toxic and non-mutagenic.

The problems associated with TNT wastewater include all of the mutagenic/toxic problems of the parent TNT, whilst in addition, it is environmentally obvious and damaging because of its persistent bright red colour. The problems of biological treatment have therefore incited intense investigation into other possible waste treatments such as carbon adsorption. This is reported to have a loading capacity which is dependent not only on pH but also the amount of colour development of the waste (Nay et al., 1972). Nay also reports that photochemical, as well as pH dependent, colour development occurs with TNT waste and that an increased colour

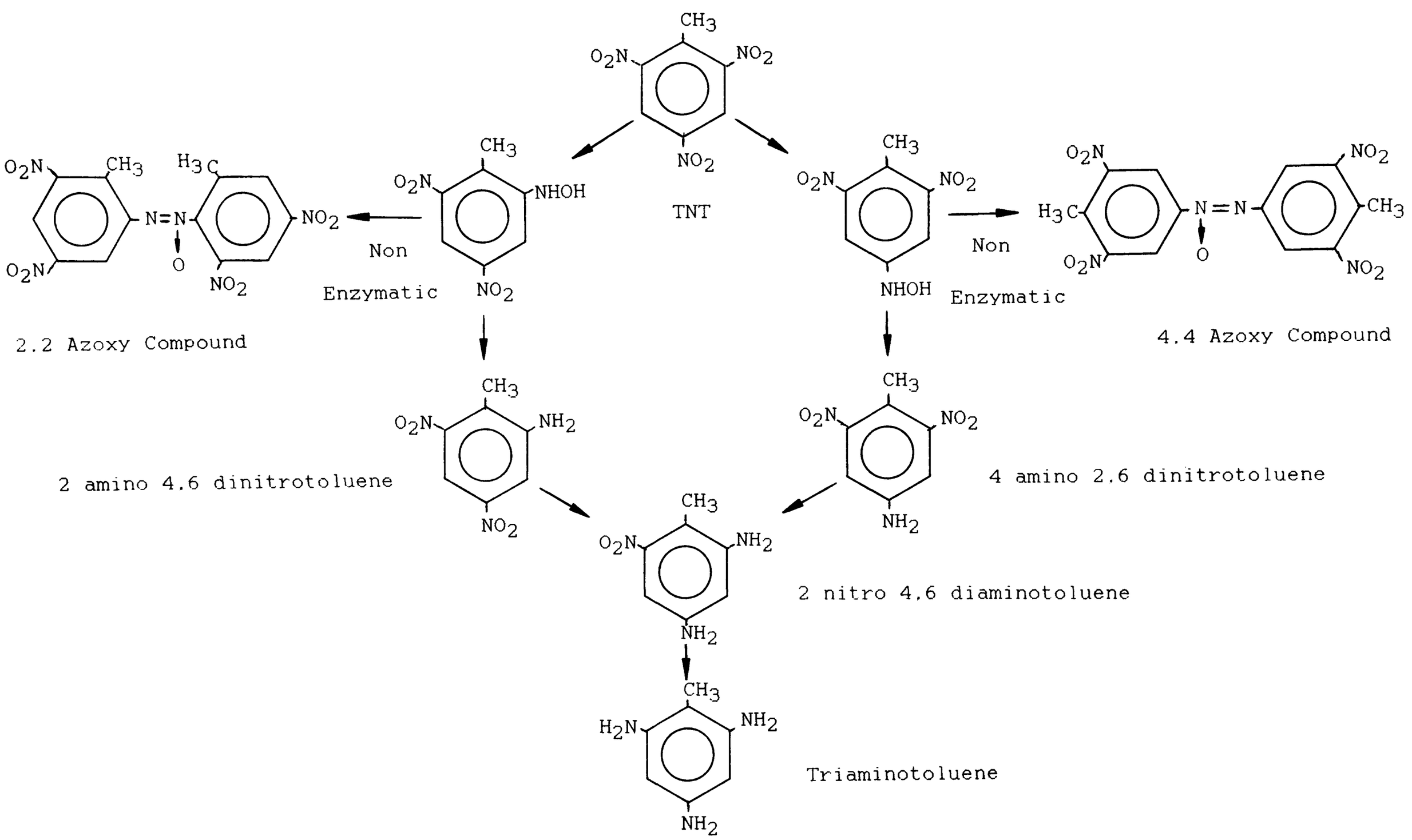


Figure 23. Proposed Mesophilic Pathway for the Transformation of TNT (McCormick et al., 1976)

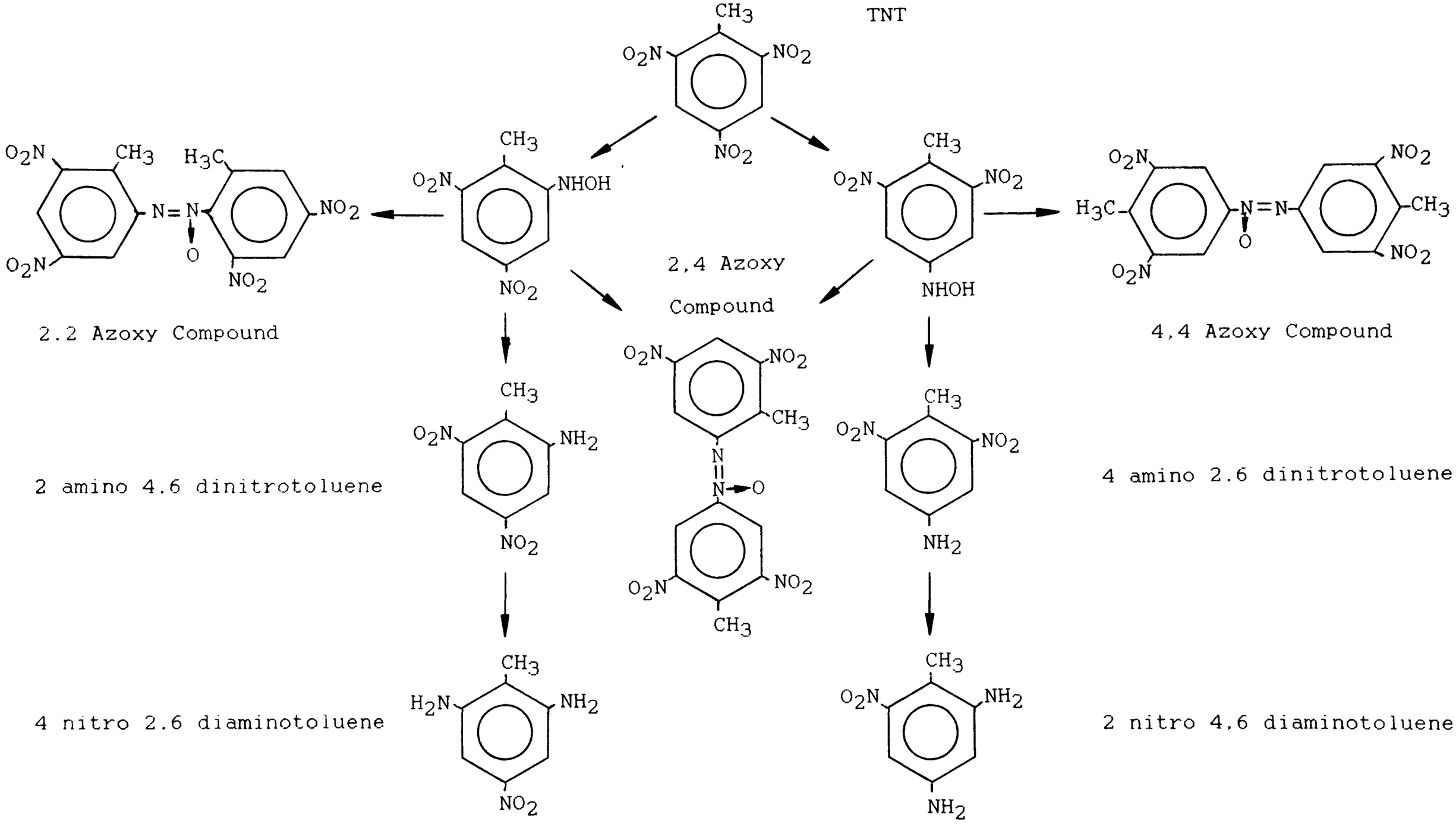


Figure 24. Proposed Thermophilic Pathway for the Transformation of TNT (Kaplan and Kaplan, 1982a)

increases the relative recalcitrance of the waste.

Other treatments such as chemical methods and amino surfactant precipitation (Kaplan and Kaplan, 1982b) have proved to be relatively unsuccessful and so the problem of TNT waste disposal still remains. Further work is required on the use of mixed microbial systems and there is a need to identify intermediates and end products in the environment with the subsequent problems of mutagenicity and toxicity of these compounds.

1.6.6.2 Aminobenzenes

Amino aromatic compounds are produced in large quantities by chemical industries which manufacture substances such as pesticides, dyes and pharmaceuticals. Many of these compounds can cause severe environmental problems if they are not mineralised but biologically transformed instead (Fewson, 1981). Such transformations include acylation (Kaufman et al., 1973), nitro product formation and polymerisation to persistent macromolecules (Bartha and Pramer, 1970). However, the oxidation of aromatic amines does not occur under anoxic conditions (Braun and Gibson, 1984). Transformations can be complicated further by autoxidation, photooxidation and chemical binding reactions. If conventional sewage treatment plants are exposed to high levels of aminobenzenes the effluent does not clarify and exhibits a brown/black colouration due to high levels of polymerised products. This

results in a high carbon content.

The degradation of nitrogen-containing compounds was studied in detail by Symons et al. (1961) using activated sludge and a comparison of substituted sodium benzoates was made (Table 4). The amino substituted compounds were metabolised more readily than the nitro substituted compounds. However, 3,5-dinitro sodium benzoate and 2,4,6-trinitro sodium benzoate were not biodegraded by activated sludge and, unfortunately, the corresponding amino compounds were not included in this study. Since then many microbial cultures have been found which oxidise a wide range of aminobenzenes including anilines and chloroanilines (Bartha and Pramer, 1970; Brown and Laboureur, 1983; Fewson, 1981; Kaufman et al., 1973; Latorre et al., 1984; Lyons et al., 1984). Aniline and chloroanilines are subject to attack by dioxygenases which results in deamination to the corresponding catechols (Figure 25). Figure 26 shows the possible fate of aniline in the environment including the metabolic pathway for complete mineralisation.

It is clear that the continual advances in the elucidation of metabolic pathways and improved mixed biological strains, from better adaption and selection techniques, are increasing the importance of biological degradation for the treatment of many xenobiotic compounds. Modern knowledge of the biodegradative aromatic plasmids and transformation have ensured that maximum value is obtained

Compound	Time to be Degraded (Days)
ortho amino sodium benzoate	2.8
para amino sodium benzoate	5.2
meta amino sodium benzoate	16.5
ortho nitro sodium benzoate	5.2
para nitro sodium benzoate	5.5
meta nitro sodium benzoate	46.0

Table 4. Relative Rates of Degradation of Two Substituted Sodium Benzoates (Symons et al., 1961).

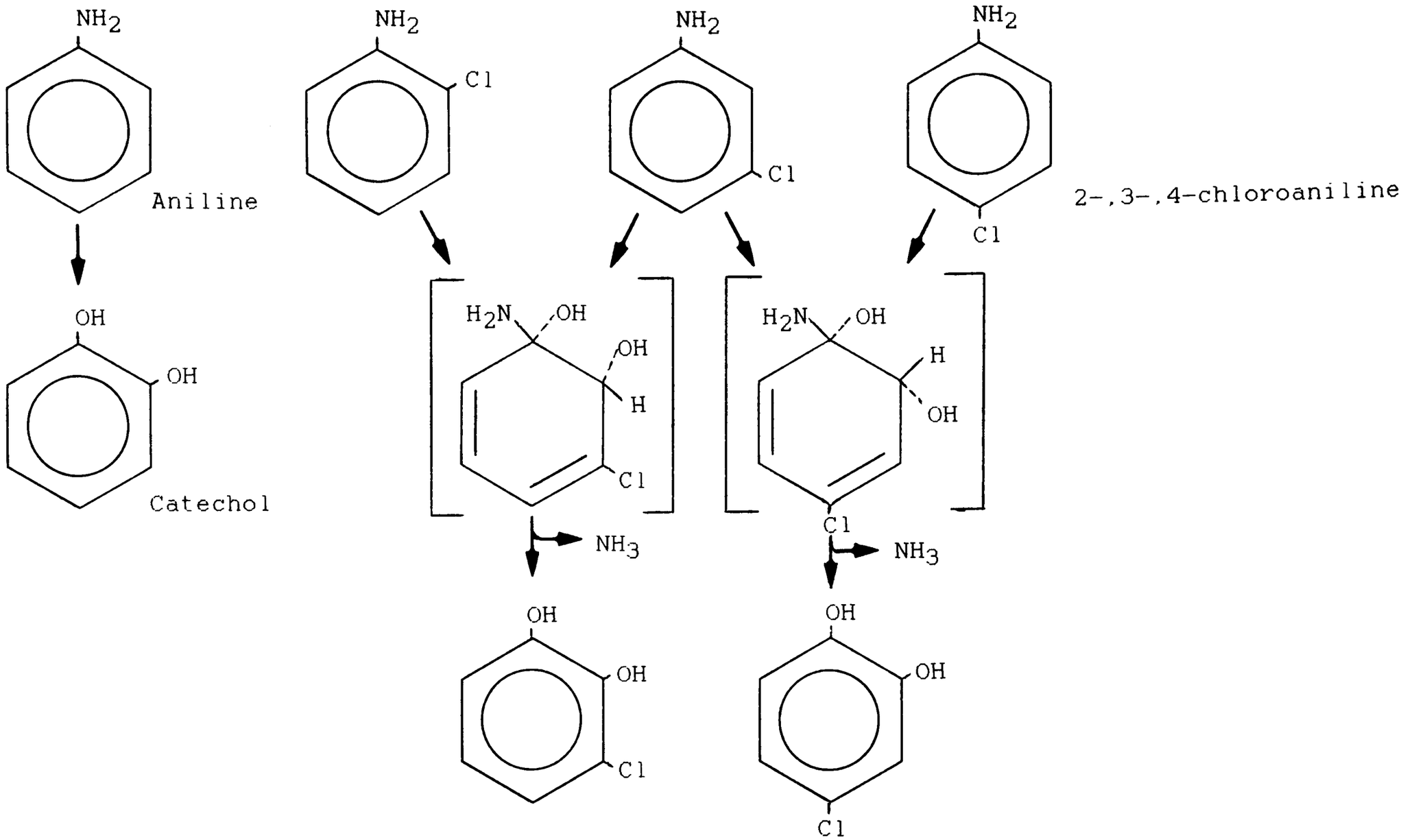


Figure 25. Initial Reactions of Aniline and Chloroaniline Degradation (Latorre et al., 1984)

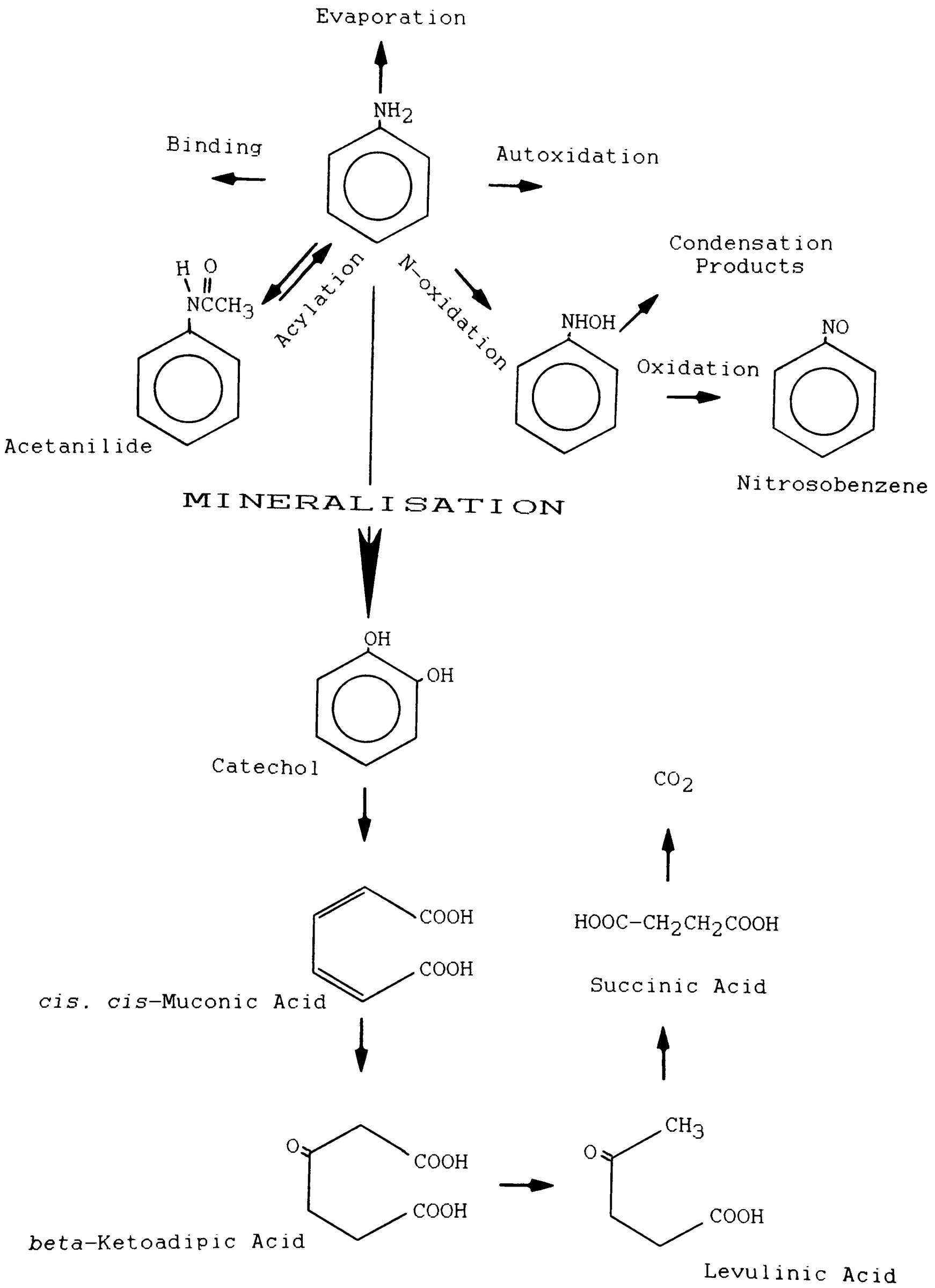


Figure 26. Suggested Mechanisms of Aniline Elimination from Pond Water by Sewage Sludge (Lyons et al., 1984)

from these findings. Sadly, there is still a very considerable way to go before satisfactory biological disposal of TNT wastewater becomes a reality.

1.6.7 Effect of Chemical Structure on Biodegradability

During the 1950s, it was discovered that newly introduced pesticides and detergents were accumulating in soil and food chains. These compounds at best lock up carbon and perhaps nitrogen and sulphur so that they are no longer available for elemental recycle. At worst, if toxic, they can accumulate until they reach harmful levels. This incited much attention to the concept of biodegradability and the effects of chemical structure. Such influencing factors include molecular size, insolubility and the nature, position and number of substituent groups (Painter, 1974).

The position and nature of substituents on the benzene nucleus is very important in terms of effect on biodegradability. Ortho and para substituted compounds have been reported as generally more amenable to biological attack than their meta substituted counterpart (Symons et al., 1961) (Table 4, Section 1.6.5.2). Pitter (1985) attributed biodegradability to the electronic effects of substituents and has indicated that the initial attack on the aromatic nucleus is of electrophilic character.

By collating the data from several sources (Campbell, 1966; Kobayashi and Rittmann, 1982; Painter, 1974; Pitter, 1985; Symons et al., 1961) a general view of the correlation of chemical structure with biodegradability can be drawn.

i) The type of substituent

COO^- > OH, CH_3 > Cl, SO_3^- > NH_2, NO_2

ii) The position of the substituent - this is dependent to some extent on the substituent group.

Ortho > para >>> meta

iii) The number of substituents

Phenol > nitrophenol > dinitrophenol > trinitrophenol

1.7 CONCLUSIONS

In conclusion, K10 wash water effluent is likely to be a complex mixture of both organic and inorganic compounds and be toxic and/or inhibitory to biological systems as raw, undiluted effluent. It is envisaged that a continuous, mixed, aerobic microbial system might be most suitable as a treatment process. A supplementary physical-chemical process may also be required, such as activated charcoal, for the removal of biologically refractory and dead-end products which might be formed. However, if biological systems are unsuccessful, a waste pre-treatment may be necessary to enhance biodegradability. If this too is unsuccessful then a treatment process based on physical-chemical processes alone may be required for the treatment of the wash water effluent.

CHAPTER 2

MATERIALS, METHODS AND EQUIPMENT

2.1 AROMATIC WASTES

2.1.1 K10 Wash Water Effluent

This was derived from the manufacture of DNEB and TNEB and was expected to contain a large number of nitroaromatic compounds, urea and possibly sodium carbonate as previously discussed (Section 1.3.3). The waste was found to have a mean chemical oxygen demand (COD) value of 8000mg/L and a mean total organic carbon (TOC) value of 4000mg/L.

2.1.2 Mixed Phenolic Waste

This waste was obtained from a large industrial site (Synthetic Chemicals Ltd., Four Ashes, Wolverhampton) and contained a large number of mixed phenolic compounds ranging from simple phenols to catechols, resorcinols, cresols and more highly polymerised derivatives. This waste was supplied in a concentrated form (26000mg/L phenol) and had a mean COD value of 70000mg/L and a mean TOC value of 21000mg/L.

2.2 GROWTH MEDIA

2.2.1 Simple Salt Medium

This was used for preliminary shake flask experiments and comprised:

In 1 litre of distilled water:

Potassium dihydrogen orthophosphate	1.36 g
Ammonium sulphate	0.20 g
Magnesium sulphate	0.02 g
Ferric chloride	0.0002 g
Carbon source (varied)	2.00 g

pH adjusted to 7 using potassium hydroxide

Carbon sources used: fructose
galactose
glucose
maltose
starch
sucrose

K10 wash water effluent (replaced appropriate volume of distilled water) at concentrations of 0.1, 1.0 and 10% (v/v).

2.2.2 Mixed Phenolic Medium

Mixed phenolic waste was diluted to 2% (v/v) with tap water. At a later stage of the experimental work this was supplemented with 44mg/L of tri-ammonium orthophosphate for each 1000mg/L of COD present. This gave a ratio 1000:14:10 of COD:N:P.

2.2.3 Synthetic Phenolic Medium

In 1 litre of tap water:

Potassium dihydrogen orthophosphate	0.04 g
Dipotassium hydrogen orthophosphate	0.16 g
Ammonium sulphate	0.20 g
Magnesium sulphate	0.02 g
Ferric chloride	0.0002 g
Phenol	0.5 g
COD of medium	1250 mg/l

2.2.4 K10 Medium (12.5%)

1 litre of K10 wash water effluent (the COD of which is 8000mg/l) was diluted with seven parts of water giving a final COD of 1000mg/L and relative concentration of 12.5% (v/v). At a later stage in the experimental work this stock medium was supplemented with tri-ammonium orthophosphate as for medium 2.2.2, i.e. 44mg/l since the COD is now 1000mg/l.

2.2.4.1 1% K10 and Mixed Phenolic Medium

Medium 2.2.2 was supplemented with 1% (v/v) of K10 wash water effluent.

2.2.4.2 2.5% K10 and Mixed Phenolic Medium

One part of medium 2.2.4 was added to four parts of medium 2.2.2.

2.2.4.3 5% K10 and Mixed Phenolic Medium

Two parts of medium 2.2.4 were added to three parts of medium 2.2.2.

2.2.5 Synthetic K10 Media

These media were essentially the same as for media 2.2.4.1 to 2.2.4.3 except that mixed phenolic medium (Section 2.2.2) was replaced with synthetic phenolic medium (Section 2.2.3).

2.2.5.1 Static Phenol Media

Medium 2.2.3 was supplemented with either 2.5, 5.0, 7.5 or 10% v/v of K10 wash water effluent, replacing the equivalent volume of water. Since the phenol concentration remains unchanged, the COD value (Section 2.4.2) of these media increased with increasing concentration of K10 wash water effluent.

2.2.5.2 Decreasing Phenol Media

Medium 2.2.3 was supplemented with either 2.5, 5.0, 7.5 or 10% v/v of K10 wash water effluent, replacing the equivalent volume of water. The concentration of phenol was reduced with increasing K10 wash water effluent concentration so as to maintain a static COD value for the range of media.

The concentrations of phenol used were 500, 415, 330 and 250 for the respective K10 wash water effluent concentrations. It should be noted that the concentrations of nutritional supplements were not reduced.

2.3 INOCULA

Several mixed microbial cultures were used as sources of inoculum. Two were domestic sewage obtained from separate local sewage treatment works and another was activated sludge obtained from a phenolic waste treatment plant.

2.4 ANALYTICAL METHODS FOR ASSESSMENT OF TREATMENT PLANT PERFORMANCE

2.4.1 Biochemical Oxygen Demand

This test is a biological procedure which attempts to simulate the natural process of oxidation of organic matter in rivers and streams (Wheatstone, 1977). However, difficulties arise with the test since microorganisms are required which can readily oxidise the sample components and as many wastes are inhibitory and/or toxic the test is not always reliable. Montgomery (1967) goes as far to say "The 5-day dilution BOD test is not ideally suited for any of the purposes for which it is used". Moreover, the test is labour intensive, unreliable, inconsistent and the results possess no theoretical significance. It was therefore not used for analysis in this project. Instead the more reliable Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC) analyses were used.

2.4.2 Chemical Oxygen Demand (COD)

COD is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. The dichromate open reflux method with titrimetric determination was adopted for this assay as described in detail in "Chemical Oxygen Demand" (Anon, 1977 and 1986). This method is preferred over procedures using other oxidants because of the strong

oxidising ability of the dichromate and its applicability to a wide range of substrates. Oxidation is quoted at 95 to 100% of the theoretical value (Anon, 1985). However the presence of other oxidising or reducing agents can give unreliable values and chloride ions have to be precipitated, usually by mercuric sulphate, otherwise the silver salt catalyst used is precipitated as silver chloride. *

An aluminium, temperature controlled, dry block digester which could hold sixteen individual sample tubes and condensers was constructed within the Departmental Workshop (Plate 1). Several assays were run using standard solutions of known strength in order to check the accuracy of the equipment.

This assay does not differentiate between organic compounds that are not biodegradable and those which are susceptible to attack, and for this reason it probably gives an over-estimate of the amount of material that can be readily oxidised by biological means.

---

* The analysis method used in this project is described in the 1977 edition of the "Chemical Oxygen Demand" (Anon, 1977). Although, consideration was given to the method described in the 1986 revised edition (in which mercury sulphate is replaced by a combination of silver nitrate and chromium III potassium sulphate which is more environmentally friendly) it did not prove possible to make this change.

Plate 1. COD apparatus

2.4.3 Total Organic Carbon (TOC)

A Beckman (Model 915) total organic carbon analyser was used for this assay. The apparatus consisted of two separate catalyst lined combustion tubes into which the samples were injected. One tube was used for the estimation of inorganic carbon at 150^{o}C whilst the other was used for the estimation of total carbon at a higher temperature of 900^{o}C in which all of the carbon in the injected sample (30µl) was oxidised to carbon dioxide. This was measured using a non-dispersive infra red analyser and the results recorded on a Beckman chart recorder. The difference between the total carbon estimation and that for inorganic carbon indicated the organic carbon content of the sample.

Calibration curves were prepared for the inorganic and the total carbon values using the following stock solutions.

Inorganic carbon (1000mg/L)

In 1 litre of distilled water:

Anhydrous sodium carbonate	4.404 g
Anhydrous sodium bicarbonate	3.497 g

Total Carbon (4000mg/L)

In 1 litre distilled water:

Potassium hydrogen phthalate	8.505 g

The calibration curves (Figures 27 and 28) were checked each time an assay was performed, using the same sample volume and an instrument gain setting of 5.5.

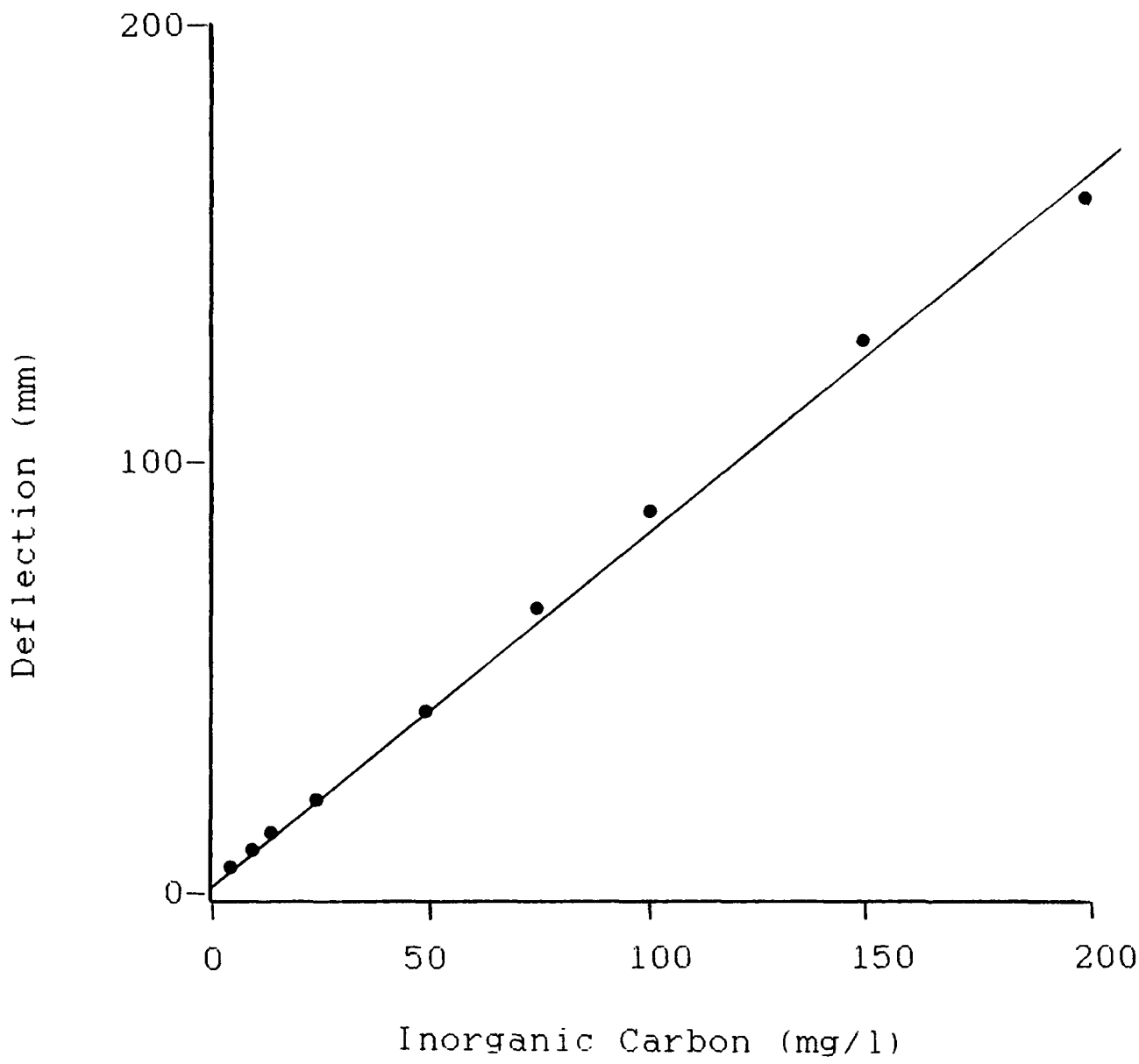


Figure 27. Calibration Curve for Inorganic Carbon

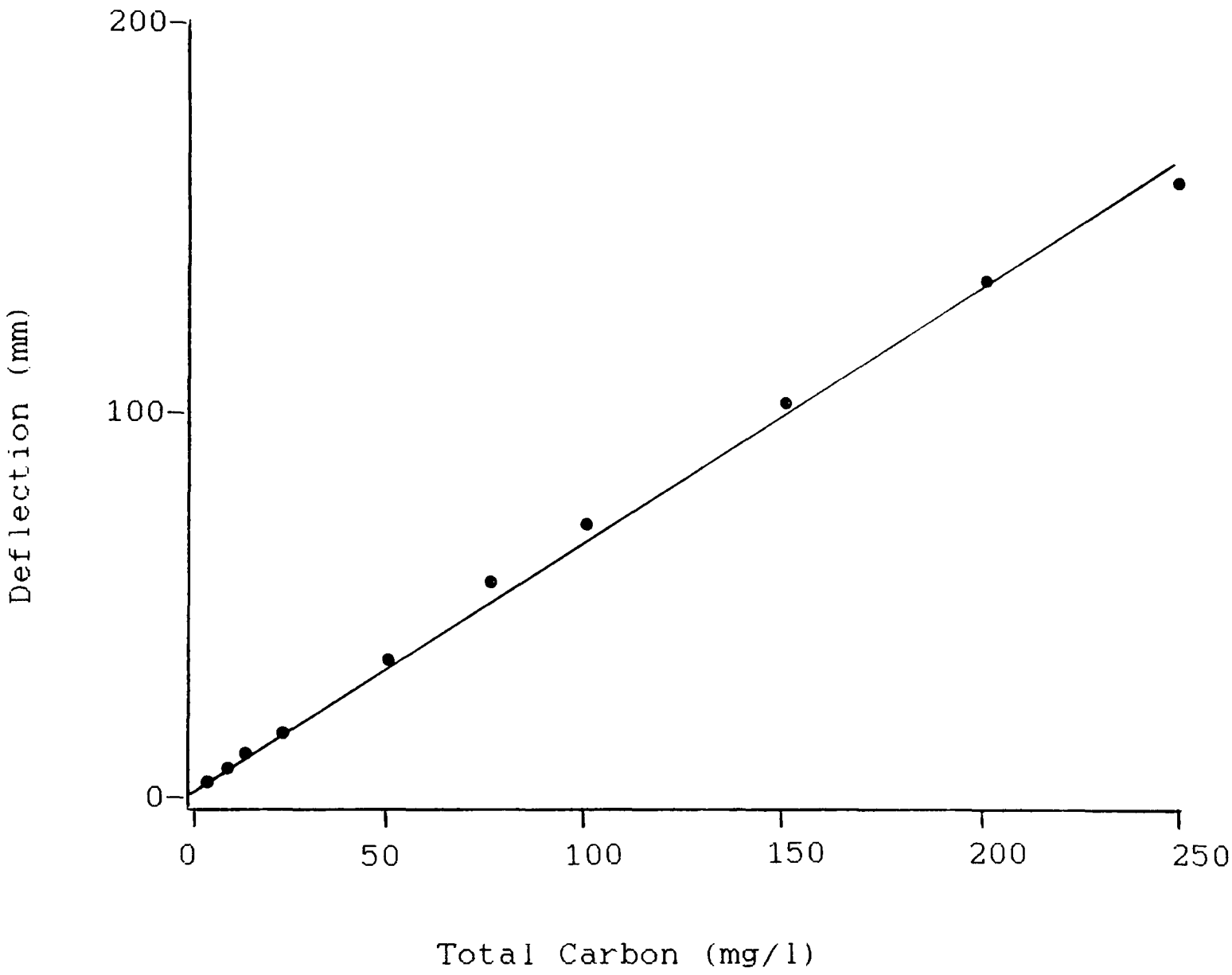


Figure 28. Calibration Curve for Total Carbon

Studies to compare organic carbon values with the corresponding BOD or COD showed that TOC compared much better with COD values (Jones and Jennelle, 1972). It is important to note that TOC concentrations are expressed as carbon, whereas the oxygen demand tests are expressed in terms of oxygen consumed during oxidation.

2.4.4 Total Suspended Solids

Total suspended solids were determined by filtering 50cm^3 of sample through a 9.0cm pre-weighed and dried glass fibre paper (Whatman GF/C). The paper and solids were then dried in an oven at 110^{o}C and cooled in a desiccator. The weight gain gave the amount of total suspended solids present (Anon, 1985).

2.4.5 Phenol Analysis

Phenols were estimated quantitatively by the 4-aminophenazone method (Anon, 1985 and Shaw, 1951). This method depends on the reaction of phenolic compounds with 4-aminophenazone at pH 7.9 in the presence of potassium ferricyanide to form a coloured antipyrine dye. The dye was kept in aqueous solution and the absorbance measured at 500nm. This method can be used for the determination of most phenols including ortho- and meta-substituted and some para-substituted phenols. A standard calibration curve was prepared using known concentrations of phenol (Figure 29).

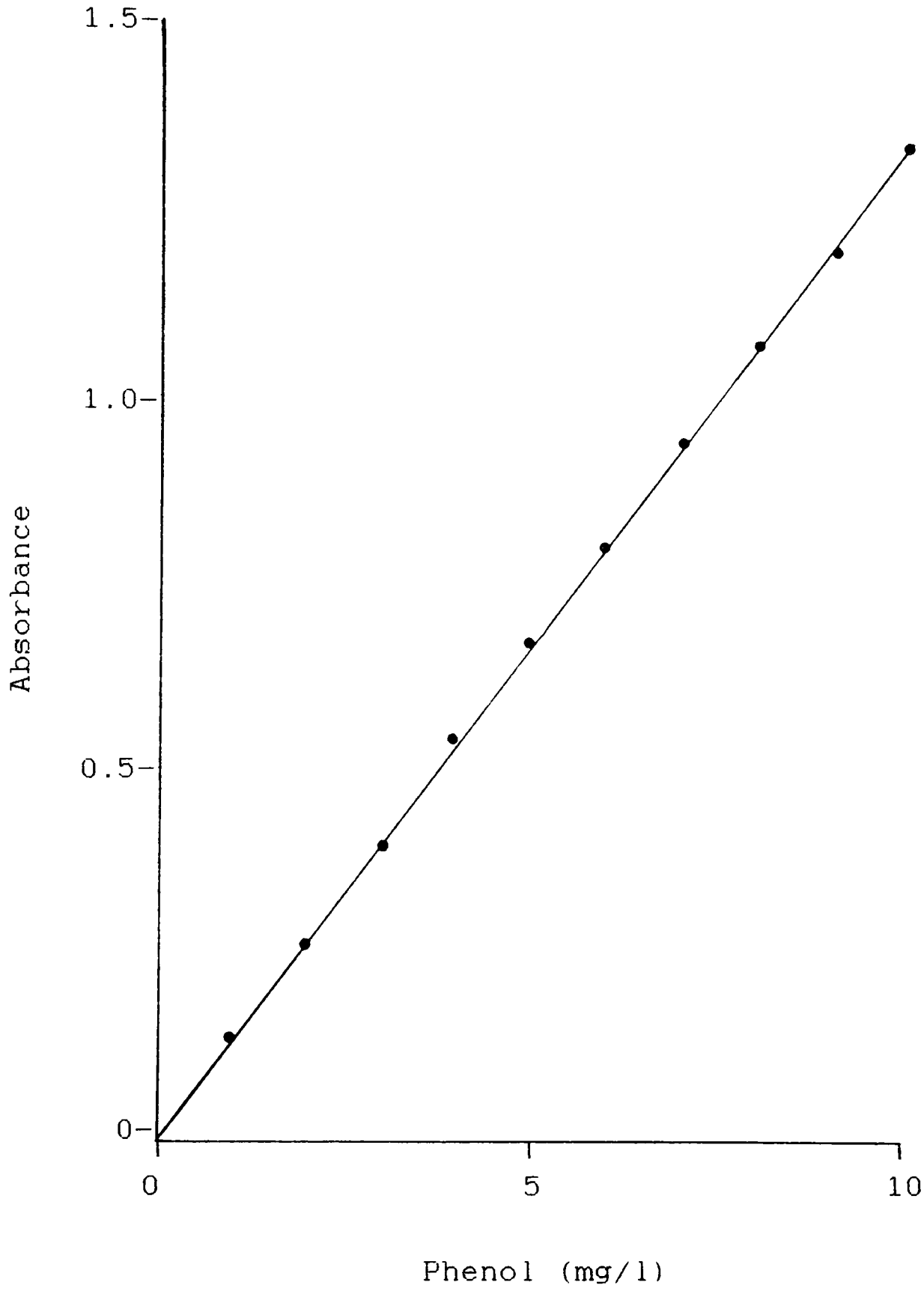


Figure 29. Calibration Curve for Phenol

2.4.6 Microscopic Examination

Microscopic examinations were carried out in order to check the quality of the sludge. Photographs were occasionally taken using an Olympus microscope model CH-2 fitted with a Nikon F3 camera, in order to assist comparisons of the sludge and provide a record of the mixed population.

2.5 UV WAVELENGTH SCANS

The feed and effluent to the activated sludge plants were regularly examined using ultra violet wavelength scans. This was done using a Cecil CE588 microcomputer scanning spectrophotometer (Cecil Instruments Ltd., Cambridge) which was linked to a CE580 chart recorder. The samples were diluted with an equal volume of water so that they gave an absorbance value between 0 to 2 units. They were placed in a quartz cuvette and scanned from 230 to 320nm with a full scale absorbance of 2.0 units and from 320 to 550nm with a full scale absorbance of 1.0 units.

2.6 GAS LIQUID CHROMATOGRAPHY

Although a Pye Series gas chromatography system was made available it was not used. This is because high temperatures are used in such analyses and K10 wash water effluent was observed to change from a translucent orange to a black opaque liquid when under reflux. A chemical change had occurred, and therefore gas chromatography did not seem a viable proposition since it operates at temperatures well in excess of boiling water.

2.7 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

A Cecil CE2010 system with a CE588 LC microcomputer scanning monitor and a CE1220 variable wavelength monitor, connected in series, were used. The output from the monitors was initially fed to potentiometric chart recorders but at a later stage a two channel CI3000 computing integrator (LDC/Milton Roy, Stone, England) was installed. Plate 2 shows the initial system utilised for HPLC analysis.

Columns were obtained from various sources and were generally 25cm long, 4.6mm internal diameter. A variety of packing materials were utilised, each protected by a suitable guard column and pre-column solvent filter. Combinations of solvents including methanol, water, tetrahydrofuran and acetonitrile were degassed immediately prior to use.

At the end of this research project a new HPLC pumping and integration system was made available. This gradient elution system comprised two Gilson 302 pumps with 5ml heads, a Gilson 803C manometric module and a Gilson 811B dynamic mixer. The system was controlled using an IBM PS/2 computer with Gilson 712 HPLC controller software.

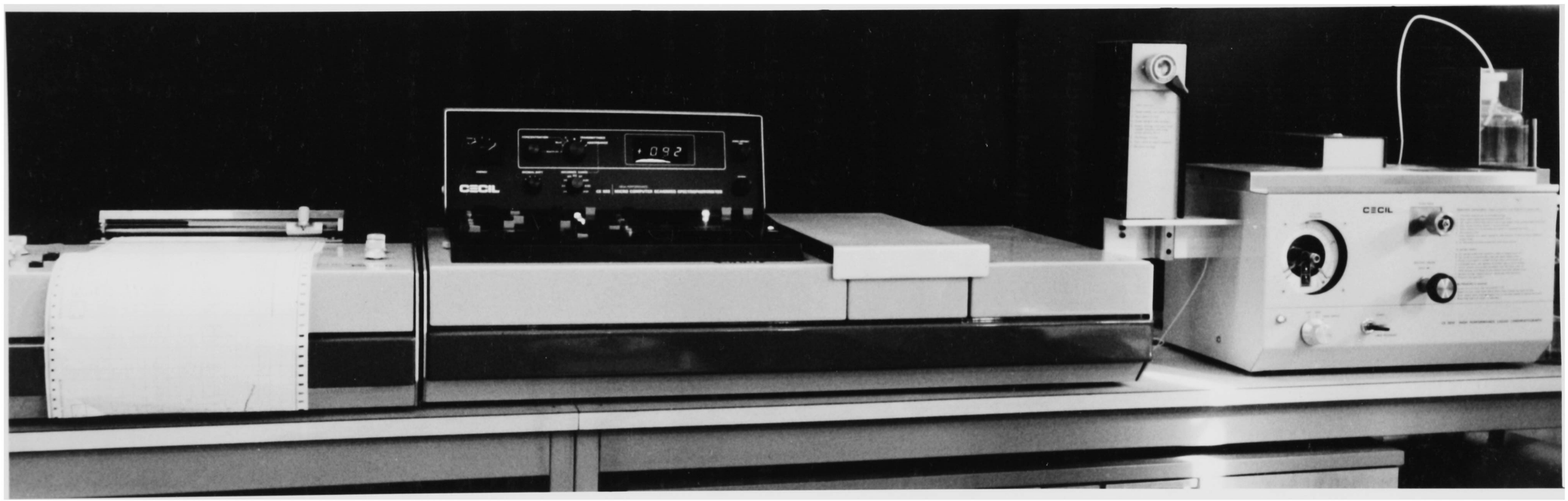


Plate 2. HPLC equipment

2.8 MEASUREMENT OF OXYGEN UPTAKE BY ACTIVATED SLUDGE

A respirometer was constructed (Figure 30). This consisted of a 120cm^3 closed cell containing a thermometer and a lead/silver type (Galvanic) oxygen probe (constructed by the departmental workshop) which was used to measure the decrease in oxygen concentration as a function of time. Since the equipment operated at room temperature (21^{o}C) and a large sample volume was used, there was no requirement for a temperature controlled water jacket for the respiration chamber. Temperatures never varied by more than 0.25^{o}C within an assay or by more than 0.5^{o}C between assays.

The system was calibrated by first aerating one litre of tap water for about an hour by which time it was assumed to be fully saturated with oxygen (taken as 100% which at 21^{o}C is 8.27mgO_2/L). The water was then deaerated using oxygen-free nitrogen to give the 0% oxygen reading.

The sludge used in this assay was washed five times with BOD water (Anon, 1985) and then sparged with air for 15 hours prior to use so as to reach the endogenous respiration rate. Respiration rates were measured by filling the respiration vessel with 125ml of solution containing a known quantity of sludge (in terms of MLSS) and test solution. The rubber bung was pressed into position and the excess liquid extruded up the vent tube which was subsequently sealed in order to produce a totally air free environment.

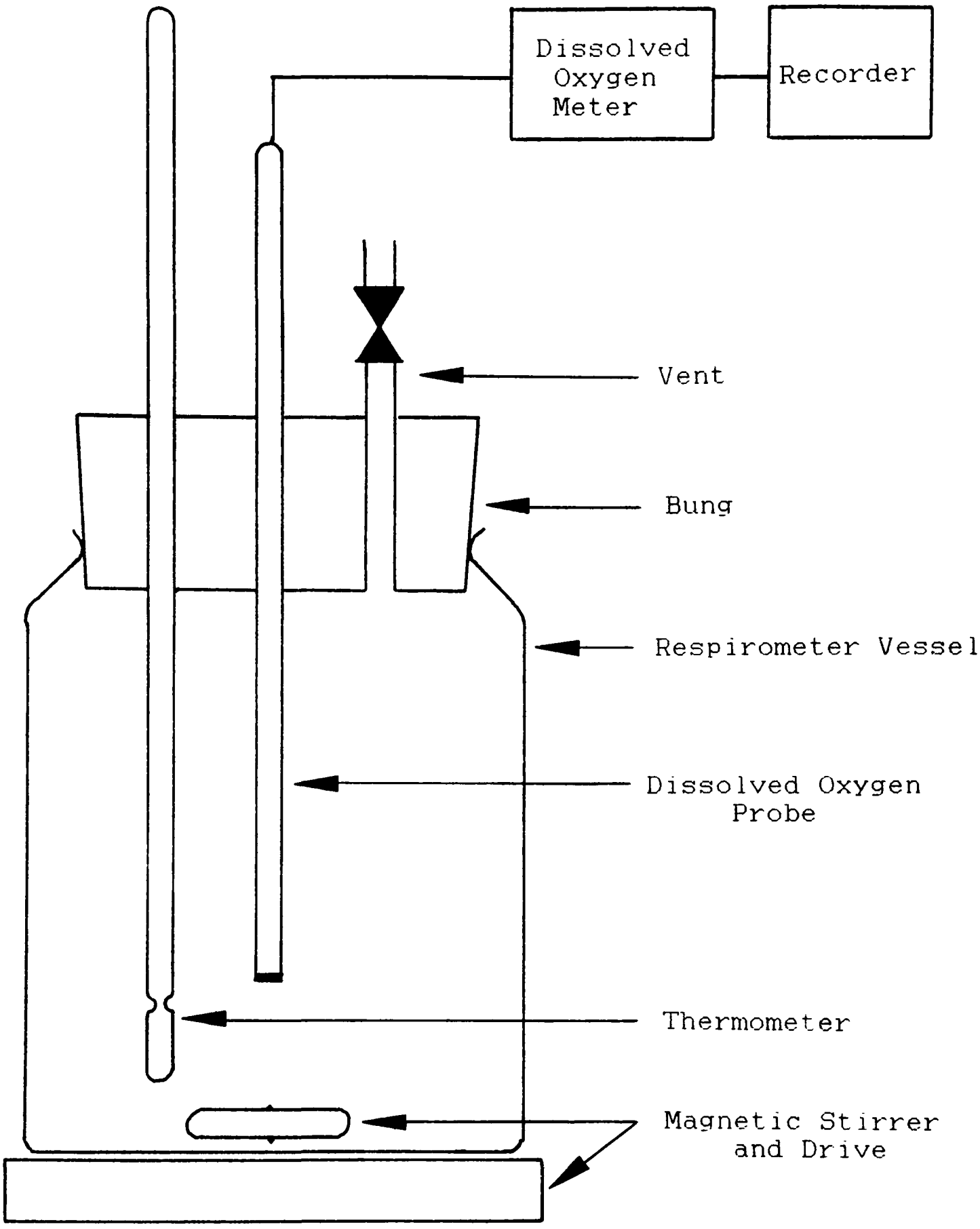


Figure 30. Respirometer used for Oxygen Uptake Studies

Since all assays used an equivalent amount of sludge in terms of MLSS, there was no requirement for incorporation of this value in calculations. Thus oxygen uptake rate was expressed as mgO_2/L/unit time. The use of equal quantities of sludge is an important point to note since the toxicity of a waste and its effect is dependent on the amount of sludge present. Thus if more sludge is incorporated in the respirometer, then the net amount of toxin encountered by an individual organism will be reduced. As a result comparisons between assays will be difficult, if not impossible.

2.9 DETECTION OF OXIDATIVE PHOSPHORYLATION UNCOUPLING

In order for a microorganism to produce adenosine triphosphate (ATP) an electrochemical membrane potential is required. If this potential is short circuited then the organism will most certainly die since ATP is an essential energy carrier of living organisms.

Strain N22 of Rhodobacter capsulatus, a photosynthetic bacterium, possesses an unusual pigment which exhibits an absorbance wavelength shift, towards the red, in response to a membrane potential. Chromatophore membrane vesicles of this strain were prepared by the Biochemistry Department, University of Birmingham. The preparation involved French-pressing a culture of organisms which was followed by differential centrifugation in order to concentrate the vesicles. These vesicles are essentially inside out, i.e. the inner cell membrane is now on the outside of the vesicle (Figure 31).

A flash of light will now cause the transport of electrons across the membrane. However, ATP synthase will immediately consume this potential and must be inactivated by addition of venturicidin. The cytochrome complex bc_1 also allows this reverse transport of electrons (Figure 32) but is inactivated using myxothiazol. The only part of the system remaining operative is that which transports electrons across the membrane from the inside of the cell to the outside (which

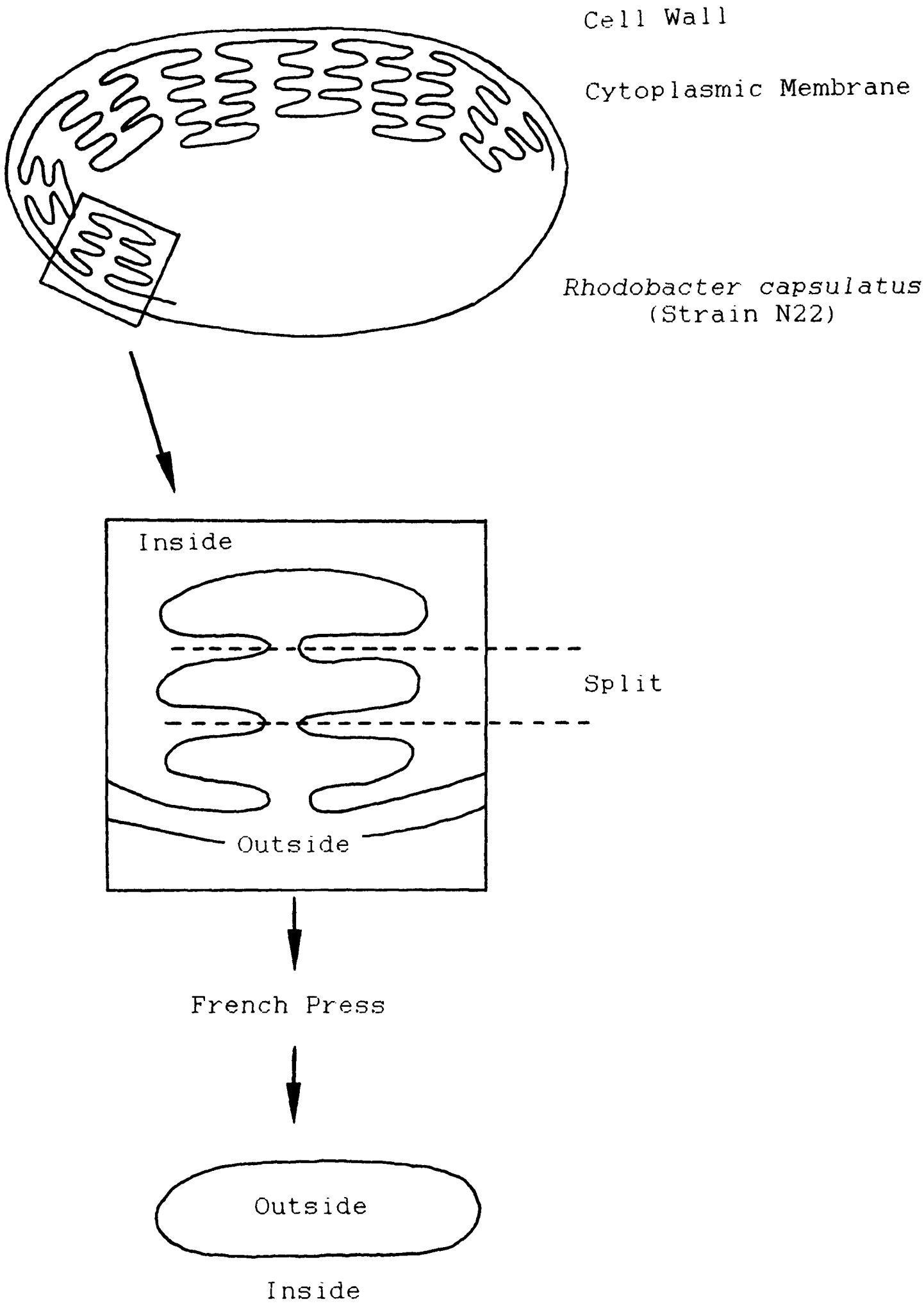
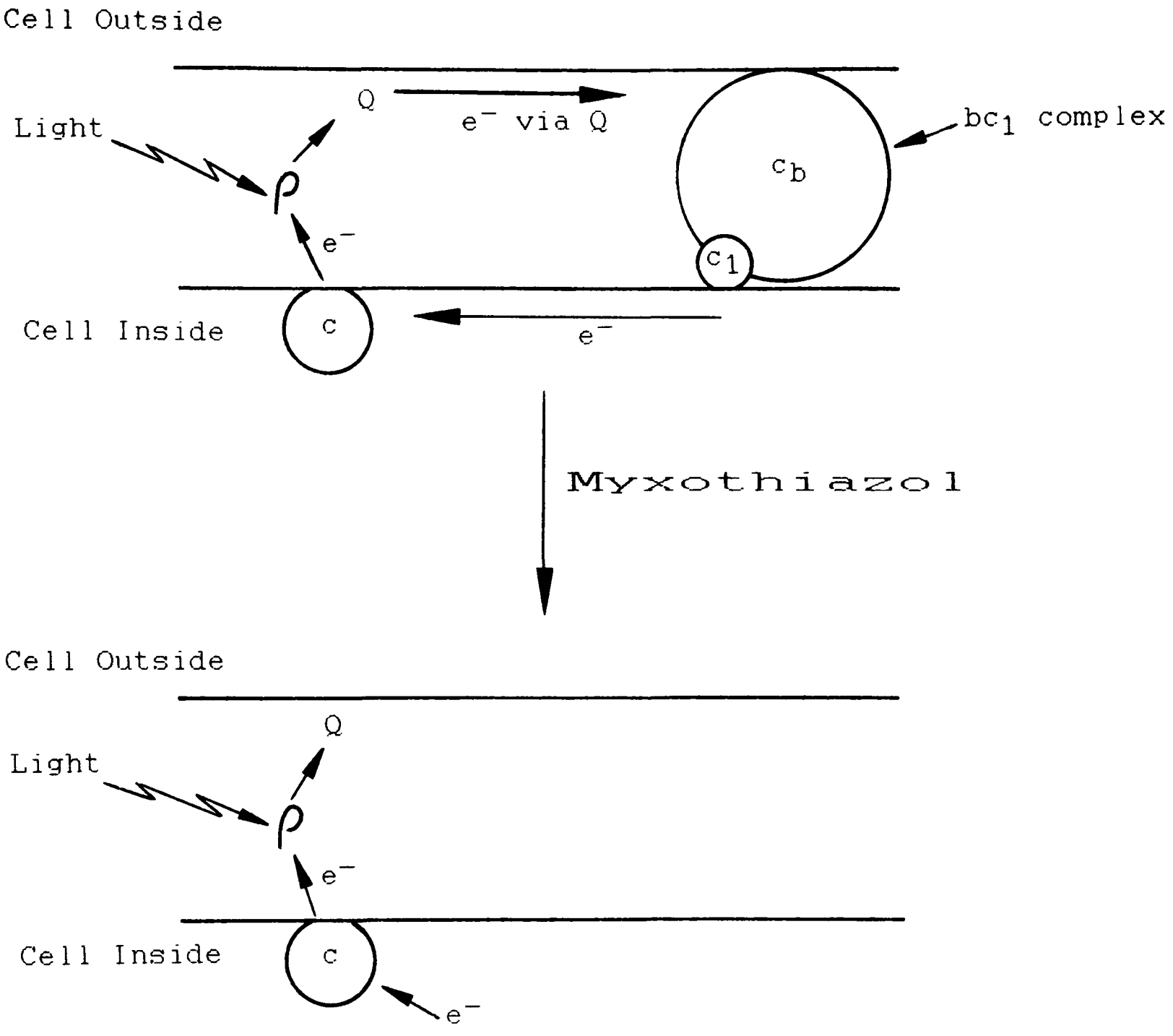


Figure 31. Formation of Vesicles

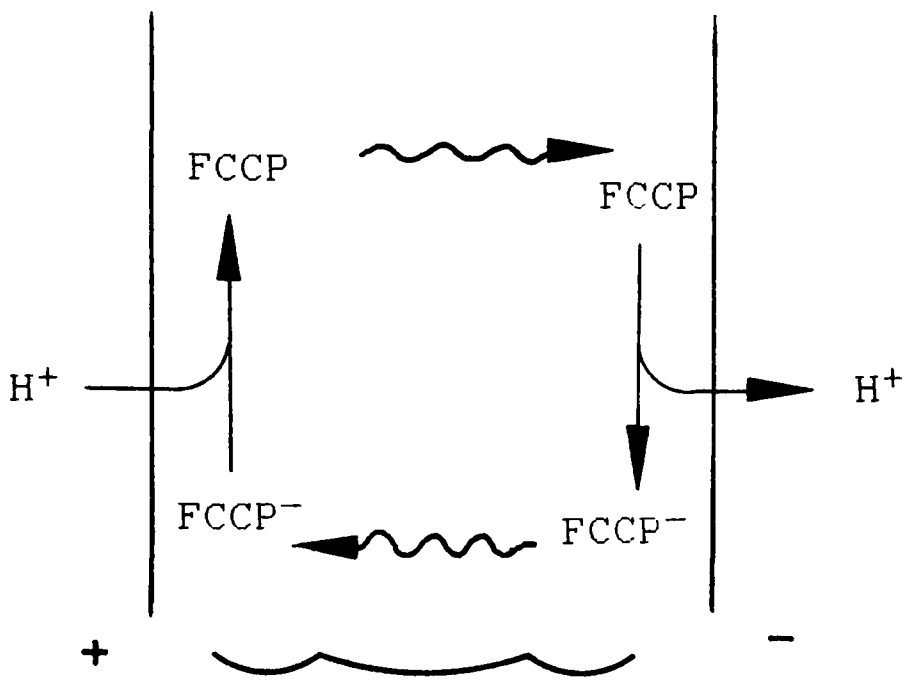


c - cytochrome
Q - quinone
c_1 - cytochrome c_1
c_b - cytochrome c_b

Figure 32. Membrane Structure - Photosynthetic Electron Transport (Cyclic)

is now the inside of the vesicle). A flash of light now causes a membrane potential to be generated which takes a couple of minutes to dissipate completely. Any compound which can dissipate this membrane potential more rapidly, by increasing the membrane conductance, will obviously uncouple ATP production. Carbonyl cyanide-p-trifluromethoxyphenol hydrazone is a very potent uncoupling agent (Figure 33) even at very low concentrations.

The sample was placed into a modified spectrophotometer and a line diagram (Figure 34) shows the equipment used. The sampling duration was 2 seconds with the flash of light occurring about 0.2 seconds into the the sampling period. The results were plotted on a chart recorder with a scale of 0.1s/cm and a full scale deflection of 0.02 absorbance units (5% transmittance).



FCCP - Carbonyl cyanide-*p*-triflurомethoxyphenol hydrazone

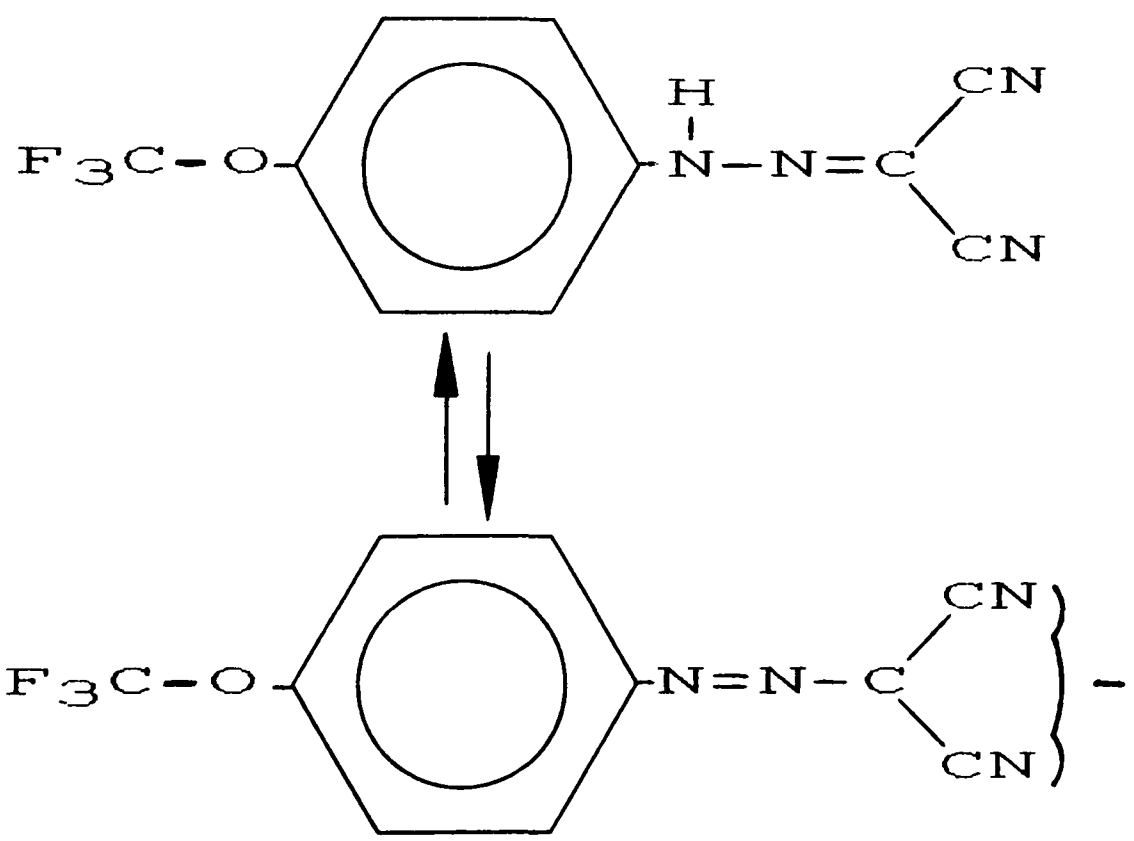
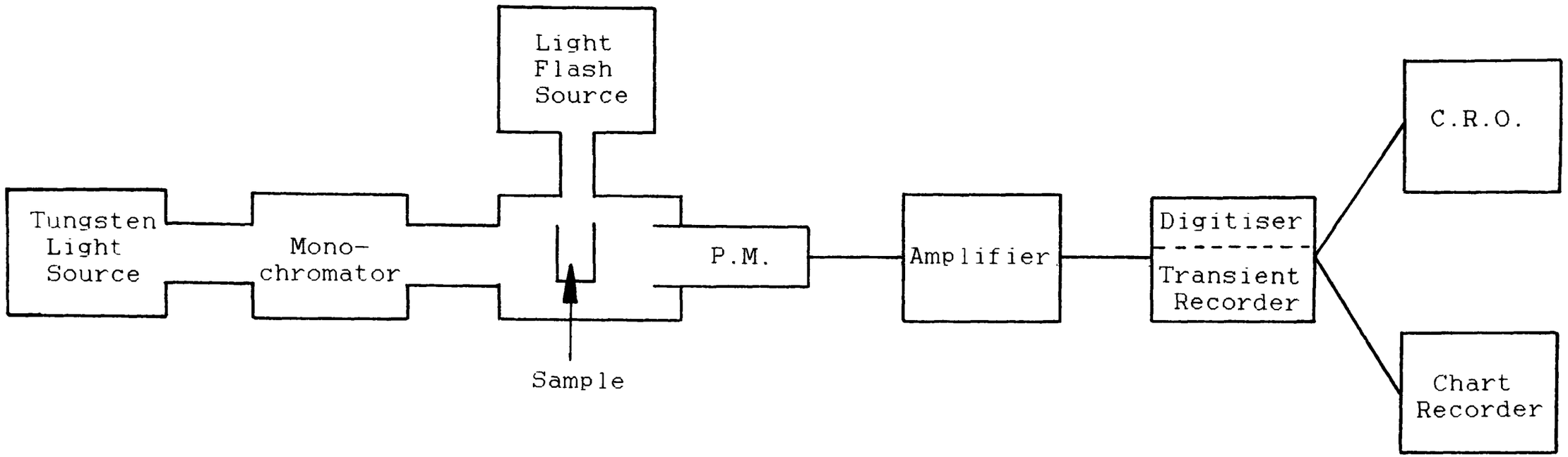


Figure 33. Dissipation of Membrane Potential by an Uncoupling Agent (Nicholls, 1982)



P.M. - Photon Multiplier Tube
C.R.O. - Cathode Ray Oscilliscope

Figure 34. Schematic Diagram of the Apparatus used to Detect Uncoupling Activity

2.10 ACTIVATED CARBON

A carbon contacting column was constructed from pyrex glass and is shown in Figure 35. The column was packed with 20g of powdered activated carbon (BDH Decolourizing Powder) and the Quickfit joint sealed with sealastic silicone sealant (Dow Corning). The column was connected to a Cecil CE2010 HPLC pump which was used to apply K10 wash water effluent to the column at a constant flow rate and pressure. The treated effluent then passed through two wavelength monitors set at 260 and 350nm. The first detected unsaturated bonds whilst the second monitored orange colouration. The use of wavelength monitors was described by Schulte (1973) for following the progress of TNT-activated carbon processes. The breakthrough of colour from the column was stated almost always to precede the breakthrough of nitro aromatic compounds (however Schulte does not identify these coloured compounds). Nevertheless, colour determinations are a simple and effective method for monitoring coloured aromatic effluents from either analytical or plant-scale carbon filters and are more simple than wet chemical methods for nitro aromatic compounds.

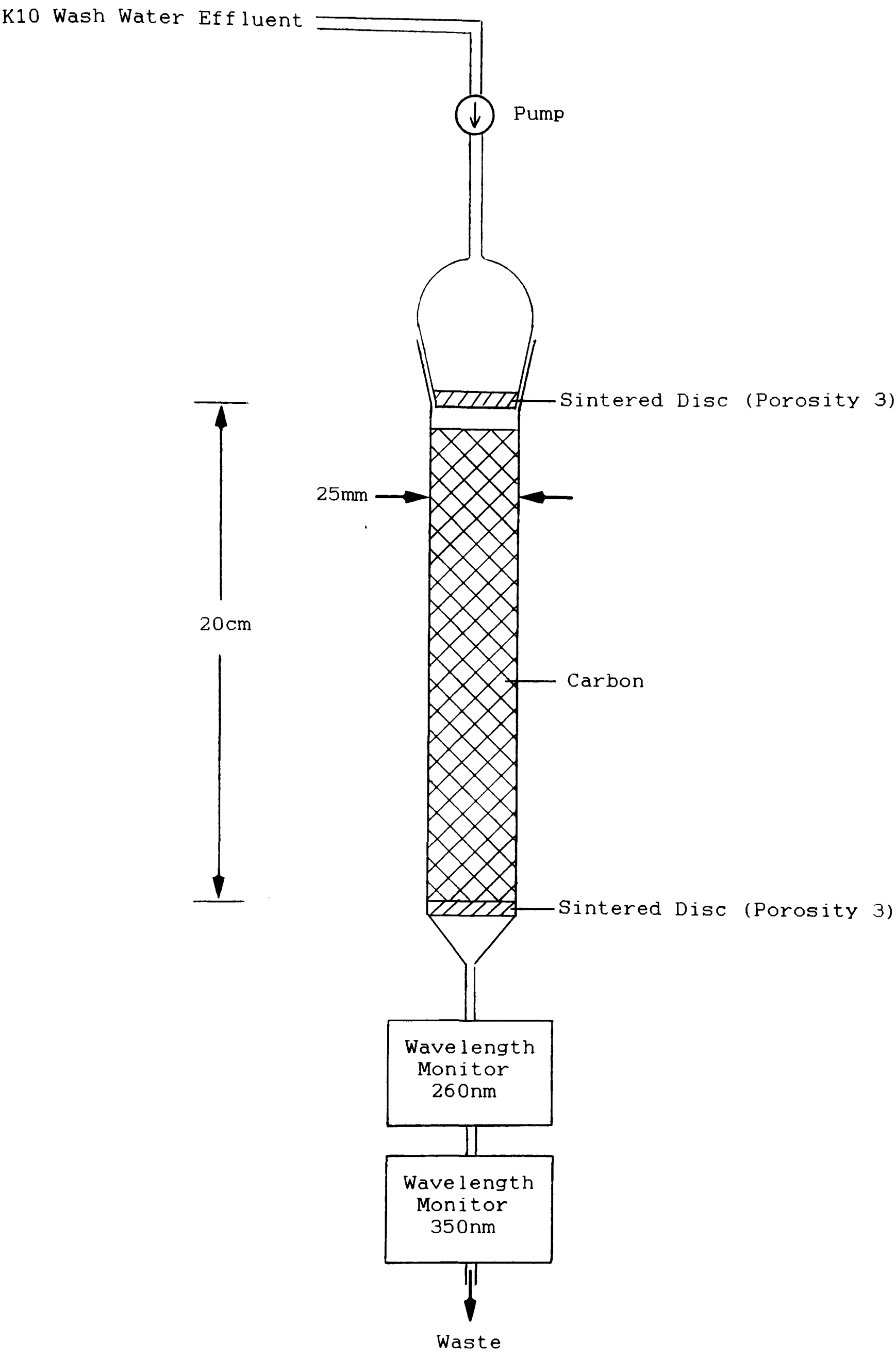


Figure 35. Activated Carbon Contacting System

2.11 OZONOLYSIS

Ozone was generated from the part of oxygen in air using a Labo ozone generator which was on loan from Ozotech Ltd., 115 Station Road, Burgess Hill, Sussex.

2.11.1 Ozone Generator Operating Principle

Figure 36 is a schematic diagram of the ozone generating equipment used.

Air was drawn from the atmosphere by an oil-free tank-mounted air compressor to a pressure of approximately 100psig. and then fed to the ozone generator. The air was dried to a dew point better than -50^{o}C by an air dryer unit. This consisted of two dryer cells mounted in parallel. These were filled with finely granulated activated alumina which removed moisture from the air. Since the drying process was continuous each cell was alternately used whilst the other reactivated. The cycle time for this was two minutes, i.e. each cell dried the air for one minute and was then reactivated for one minute.

After drying, the air was expanded such that it could overcome all the pressure losses in the ozone production cell and the application system (0.75bar gauge for this application). The air then passed through the annular space between the high tension dielectric tube and the earthed

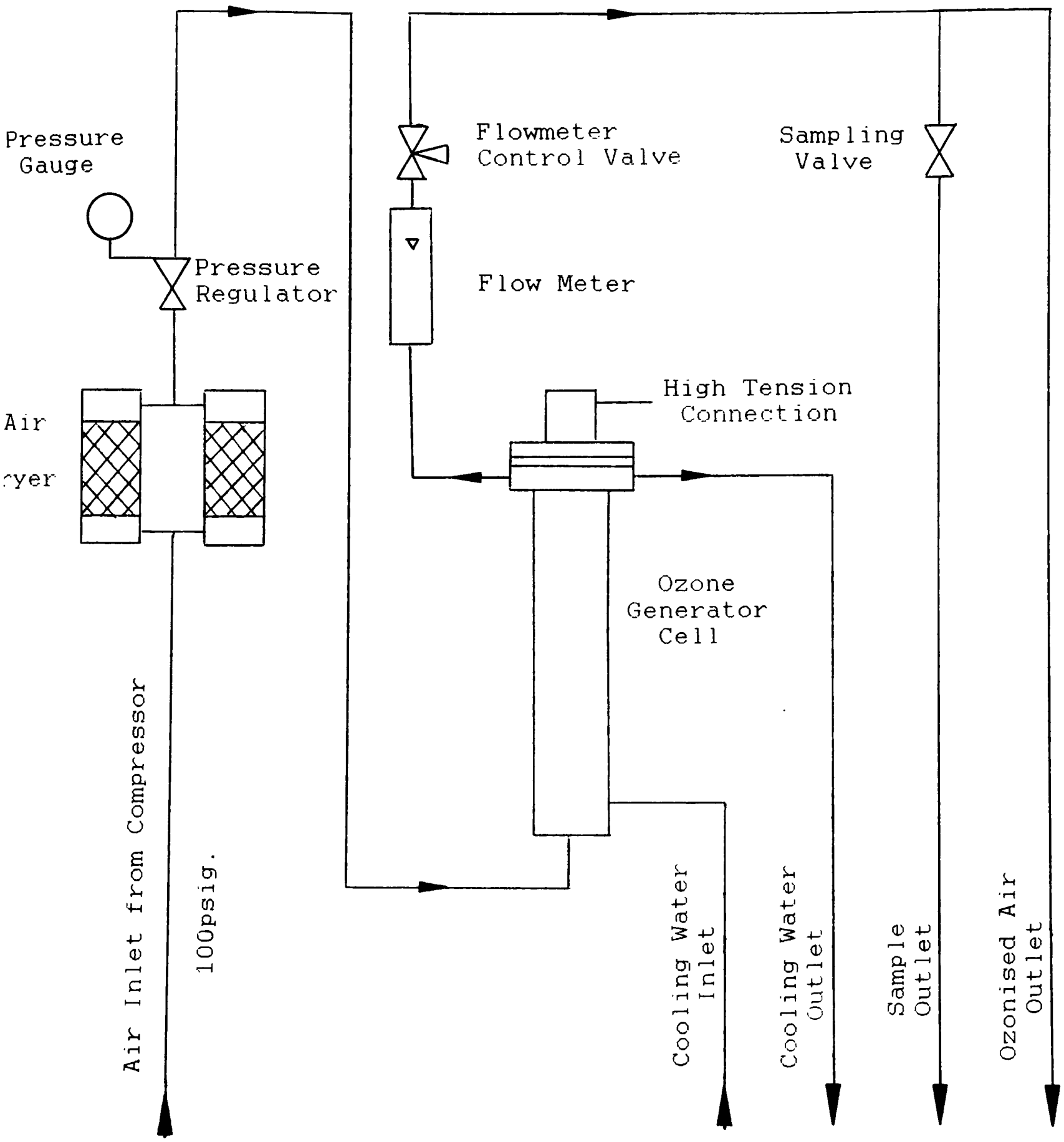


Figure 36. Ozone Generator Equipment: System Flow Scheme

stainless steel jacket where it was subjected to a high voltage silent electric discharge. A proportion of the oxygen molecules in the air were converted to ozone. The ozonised air then passed through a flow rate meter with a combined flow regulating valve which controlled the amount of ozonised air going to the contactor vessel.

An important point to note is that high tension silent electric discharge causes molecular agitation and thus heating. Since ozone undergoes a self-destruction process at elevated temperatures a water cooling jacket surrounded the discharge chamber.

The amount of oxygen converted to ozone in this system was influenced by two parameters: the amount of air flow and the amount of electrical power applied to the electric discharge chamber. Figure 37 shows the relationship between these two parameters.

2.11.2 Ozone Contactor

A contactor was required that would give good utilisation of the ozone gas applied. To achieve this, a contactor was constructed which was five metres tall with a volume of 36 litres. The materials used needed to be resistant to the strong oxidising effect of ozone. Thus glass was used for the contactor vessel and stainless steel for the reactor base plate. Viton rubber gaskets were used at all glass-glass and

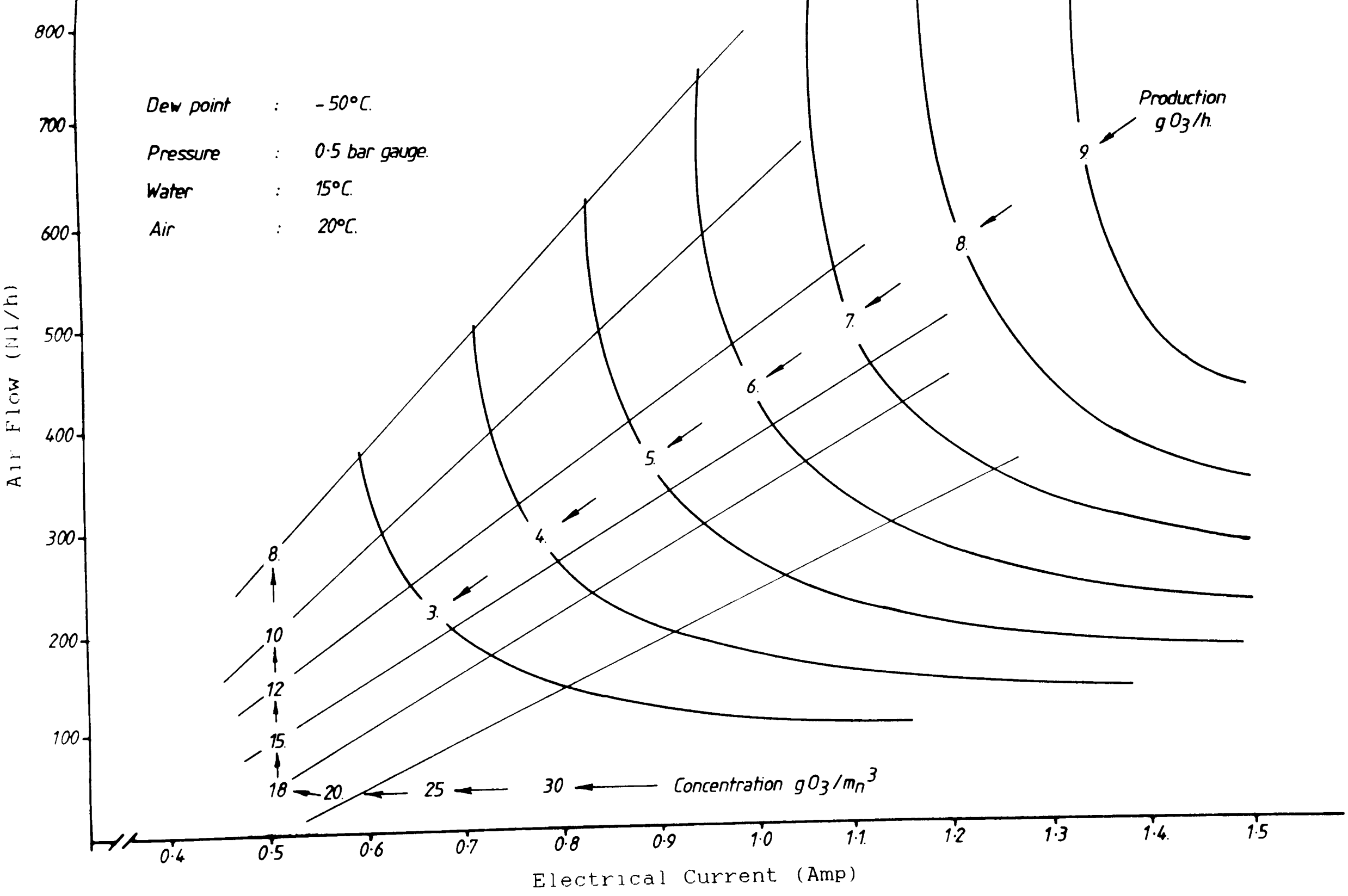


Figure 37. Effect of Power and Air Flow Rate on Ozone Yield

glass-stainless steel interfaces whilst PTFE tubing (1/4 inch O.D) was used to transport the ozonised air from the generator to the contactor. This equipment is shown in Plate 3 and schematically in Figure 38.

The ozonised air entered the column through a stainless steel base plate and was dispersed into small bubbles (<5mm) using a three inch diameter stainless steel sintered disc. The rising bubbles circulated the effluent in the contactor (basically an elongated air lift fermenter) which had a liquid circulation time of less than sixty seconds. The reacted ozonised air left the reactor via the air detrainer and was then washed, to remove traces of effluent, by two water filled aspirators before entering the ozone destructor (an electrically heated chamber).

2.11.3 Ozone Assay

An ozone assay was used to determine the production of ozone from the ozone generator and to determine the ozone in the contactor exit gas stream.

This assay is based on an iodimetric chemical titration. The reaction of ozone with potassium iodide takes place in a neutral buffered aqueous solution according to the equation:-

$$O_3 + 2KI + H_2O \longrightarrow I_2 + 2KOH + O_2$$

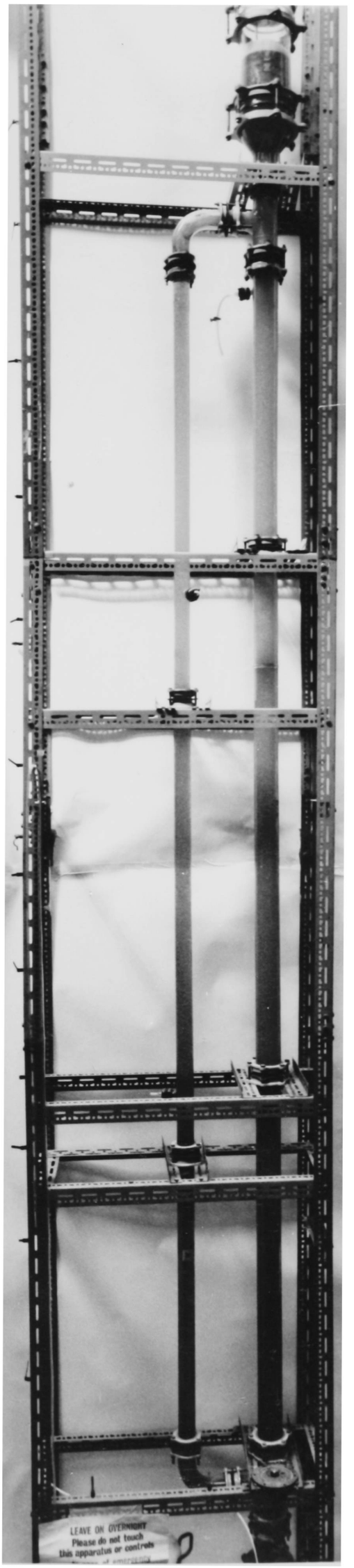
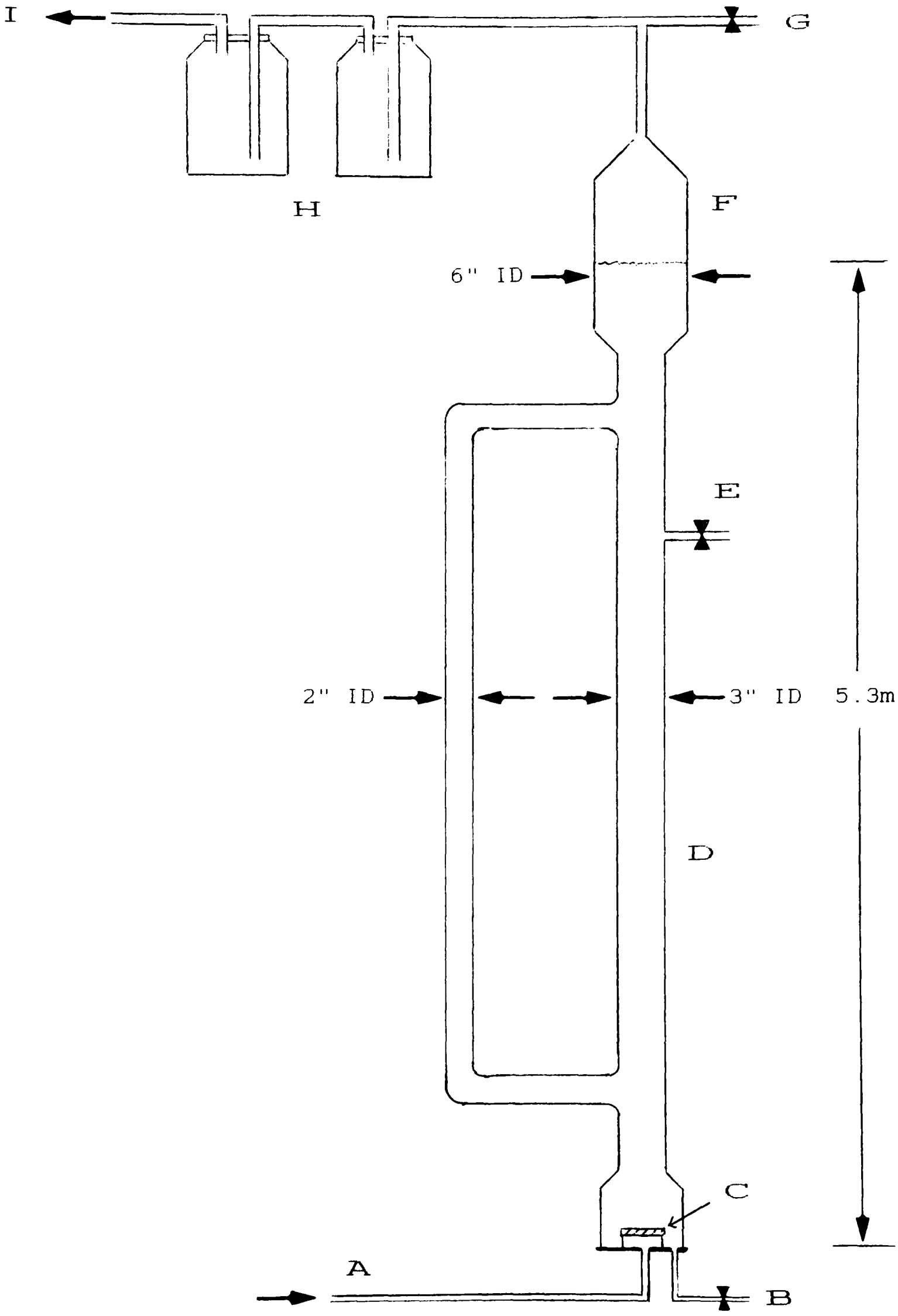


Plate 3. Ozone contactor



A PTFE Ozonised Air Inlet Pipe
B Drain Valve
C Sintered Stainless Steel Disc
D Pyrex Glass Contactor Vessel
E Sample Port
F Gas Detrainer
G Off Gas Sample Port
H Gas Scrubber Bottles
I To Ozone Destructor

Figure 38. Schematic Diagram of the Ozone Contactor

After acidification, the liberated iodine was titrated with sodium thiosulphate according to:-

$$I_2 + 2Na_2S_2O_3 \xrightarrow{H_2SO_4} Na_2S_4O_6 + 2NaI$$

An iodine indicator was used to determine the 'end point'.

2.11.3.1 Potassium Iodide Solution

In 1 litre of distilled water:

Potassium iodide	20.0 g
Disodium hydrogen orthophosphate	7.3 g
Potassium dihydrogen orthophosphate	3.5 g

2.11.3.2 Ozone Assay Procedure

A one litre wash bottle was completely filled (1.2L) with potassium iodide solution, sealed and inverted. The ozonised air sample (approximately 800ml) was then introduced into the top of the bottle and the potassium iodide solution displaced into a graduated measuring cylinder.

The bottle was then sealed, the volume of potassium iodide solution displaced noted, and the bottle shaken vigorously for two minutes. The solution, now straw coloured, was placed into a white enamel dish (400ml) to which 2ml of 25% sulphuric acid was added. The solution was then titrated against 0.1N sodium thiosulphate solution until a pale yellow colour was obtained. A small amount of BDH iodine indicator was then added and the titration was continued until complete

decolouration had occurred, when the final level in the burette would be read.

2.11.3.3 Calculation of Ozone Concentration

Ozone concentration was determined from the following formula:-

$$O_c = \frac{24 \times S \times N \times (T + 273) \times 1013}{V \times 273 \times P} \quad gO_3/m_n^3$$

where O_c is ozone concentration under normal conditions

(i.e. S.T.P. with pressure of 1013mbar at 0^oC)

S is the consumption of thiosulphate (ml)

N is the strength of the thiosulphate (0.1N)

T is the ozonised air temperature (^oC)

V is the measured volume of gas collected (L)

P is the pressure at which V is measured (mbar)

2.11.4 Ozone Safety

Since ozone is such a toxic gas (Section 1.4.6.1) it is imperative that adequate safety measures are taken. For this reason an Otrameter was utilised. This device continuously samples the ambient air and measures the ozone concentration. If the concentration of ozone exceeds 0.1ppm. (100nbar partial pressure) then an alarm sounds thus warning of a potential health hazard. In addition to this, all pipe fittings were tested for ozone leakage by compressing a piece of moist

tissue, impregnated with starch and potassium iodide, around the joint for several seconds. On exposure to ozone a blue/black colouration develops. Leakage of ozone, no matter how slight, was not permissable and remedial work was carried out.

2.12 STERILISATION

Liquid media, agar, old cultures and polypropylene pipette tips were all sterilised for 15 minutes at 121^{o}C, 15psig.

The media used for the activated sludge plants were not sterilised since the K10 wash water effluent components appeared intolerant to elevated temperatures and also because of the risk of toxic vapour generation.

2.13 GROWTH MEASUREMENTS

Viable counts are often used for estimation of viable cells present in a culture. However, this technique is dependent on a mono-disperse solution of cells, otherwise the number of cells will be underestimated. Since activated sludge organisms have a tendency to flocculate, it is hard, if not impossible, to obtain a mono-disperse solution without damaging viable cells. For this reason, growth was monitored by optical density, although this method includes measurement of both viable and non-viable cells. A Cecil CE505 spectrophotometer using 1cm path length cuvettes was used at a wavelength of 650nm with distilled water as the reference.

2.14 ANCILLARY INSTRUMENTS

Autoclave- Boxer 360 Minor (Boxer Laboratory Equipment Ltd., Ware, Hertfordshire).

Incubator- Gallenkamp Cooled Orbital Incubator (A. Gallenkamp and Co. Ltd., London) which was used for shake flask experiments.

pH Control- Gallenkamp Modular Fermenter Controllers were used for pH control of the activated sludge plants.

Balance- Mettler PE 160 (Mettler Instruments Ltd., High Wycombe) was used for the weighing of chemicals and for dry weight determinations.

Stirrer- Corning PC 351 Hot Plate Stirrer (Corning Glass Works, Corning, N.Y.) which was used for agar preparation and the stirring in the oxygen respirometer vessel.

Pipettes- Gilson (supplied by Anachem Ltd., Luton). The sizes 0-20, 0-200, 0-1000 and 0-5000µl were used with disposable polypropylene tips.

2.15 CHEMICALS USED

All chemicals used for analyses were of analytical grade, and at least of general purpose grade for use in growth media or as nutrient supplements. The chemicals were obtained from a variety of sources, including:-

B.D.H. (British Drug House Ltd.), Poole, Dorset.

Fisons Reagents Ltd., Loughborough, Leicestershire.

Oxoid Ltd., Basingstoke, Hampshire.

Aldrich Chemical Company Ltd., Gillingham, Dorset.

CHAPTER 3

RESULTS

3.1 SHAKE FLASK STUDIES - PRELIMINARY WORK

Experience has shown that TNT red water is toxic to microbial systems, and it was therefore advisable to find if K10 wash water effluent behaves similarly. Shake flask experiments were therefore devised using the simple salt medium described in Section 2.2.1, supplemented with one of six carbon sources and dosed with either 0.1, 1.0 or 10% (v/v) of K10 wash water effluent. Three control flasks (which had had the carbon supplement omitted) were also prepared. The flasks were inoculated with organisms obtained both from domestic sewage and from activated sludge from a phenolic waste treatment plant (Section 2.3), incubated in an orbital shaker at 20^{o}C and 100 rpm, and examined daily by microscopy, by spectrophotometry (using absorbance measurements at 650nm) and, finally, for general physical appearance of the contents.

After 24 hours incubation the carbon-supplemented flasks were observed to contain healthy cultures even at 10% K10 wash water effluent concentration. This rapid growth was unexpected since it was anticipated that the waste would be toxic to microbial systems. The flasks were examined daily for a further four weeks and it was noted that the diversity of organisms was some what reduced when compared with the spectrum of organisms in the initial inoculum. At the 0.1%

dilution of K10 wash water effluent, bacteria, fungi, ciliates and ameobae were observed; the latter were found only at this dilution. Bacteria, fungi and a greatly reduced number of ciliates were observed at the 1.0% dilution of K10 wash water effluent, whereas at 10% only bacteria were found. Clearly, then, as K10 wash water effluent will permit microorganisms to grow, it is not a total bacteriocide or bacteriostat at these concentrations as had been feared. Nevertheless, there is considerable toxicity towards the ameobae and some toxicity towards the ciliate inocula from the activated sludge. Moreover, no growth was observed in any of the three control flasks, suggesting that the carbon present in the K10 wash water effluent was unavailable to the microbes.

Thus attention was turned to the possibility of setting up a system which would allow the adaptation and enrichment of suitable microbial cultures. The aerated activated sludge process was chosen for reasons previously discussed (Section 1.5).

3.2 AEROBIC ACTIVATED SLUDGE PROCESS

The activated sludge process was developed by Arden and Lockett (1914) and is essentially a continuous fermentation with recycle of flocculating microorganisms. The effluent is mixed and aerated with the organisms which oxidise the waste matter. The primary degrading organisms in such a system are bacteria, as already discussed (Section 1.4.8), which derive nourishment directly from the raw waste in order to synthesise proteins and to satisfy energy requirements. Although one organism may be able to degrade a variety of compounds the necessary enzymes may not be expressed continuously but need to be induced i.e. produced only when the particular compound is present. Thus a period of acclimatisation may be required for the induction of and subsequent manufacture of the required enzymes. Further to this, it is unlikely that all organisms within the inoculum would possess the necessary genetic information to produce such enzymes and thus enrichment of the degrading organisms would be necessary. Such enrichment is enhanced by recycling of organisms within a waste treatment process, as occurs in activated sludge processes.

Other organisms which also contribute to this process include ciliates and other protozoa which feed on free-swimming bacteria and so assist in producing a more clarified final effluent. In general, the number and types of protozoa have been used to indicate the condition of activated

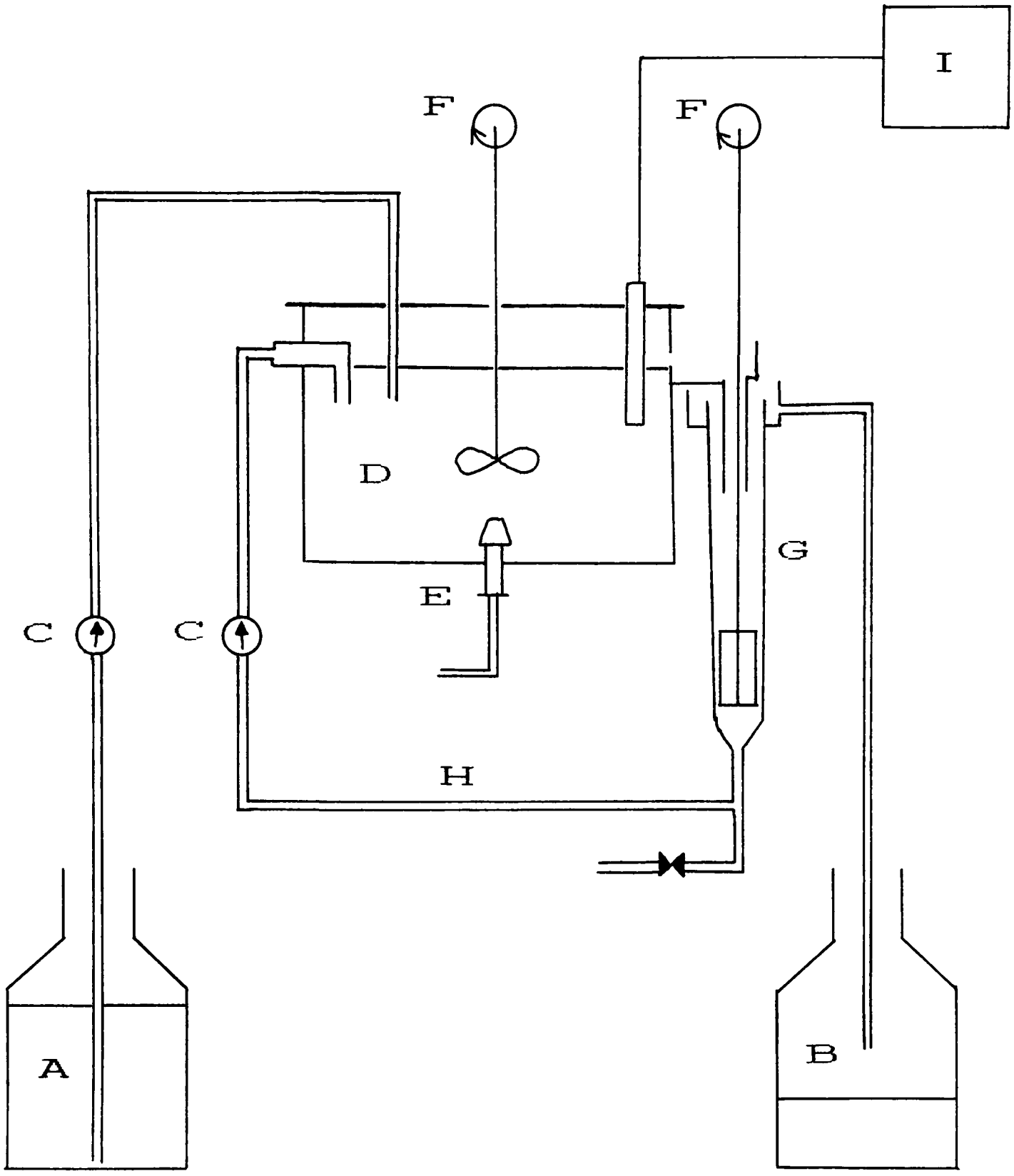
sludge processes (Curds, 1975), however, in the particular case of K10 wash water treatment, such a procedure is likely to be of little value for two reasons. Firstly the waste was clearly toxic towards protozoa during the shake flask experiments, killing all ameobae and reducing the number of ciliates. Secondly, whilst in some cases, protocidal compounds might be diluted and metabolised in a continuous activated sludge plant later experiments indicated clearly that there was very little degradation of these compounds in such situations.

There are several types of activated sludge process which have been discussed in detail by Forster (1977). The extended aeration process was chosen for the treatment of K10 wash water.

3.3 ACTIVATED SLUDGE PLANT START UP

Two identical laboratory scale activated sludge plants were constructed as shown in Figure 39 and Plate 4. Each plant comprised a five litre rectangular aeration tank and a one litre sedimentation tank. The aeration tank lid was fitted with ports for a pH electrode and stirrer motor shaft, and entry points for acid/alkali and feed.

The contents of the reactor were constantly aerated by sparging air at one litre per minute and stirred using a paddle stirrer at forty rpm. The overflow from the reactor was collected in the settling tank where the organisms readily formed flocs. The settling tank was constructed with a 45^{o} conical base to encourage settling and a slow rotating rubber paddle stirrer which scraped the sludge from the side walls causing the flocs to fall. The settled sludge was continuously recycled using a Watson-Marlow peristaltic pump and the clarified effluent passed over the weir (final effluent). The pH of the aeration tank was maintained between 6.8 and 7.2 by an automatic pH controller fitted with a Russel pH electrode. 2.0M sodium hydroxide solution was initially used to maintain the pH but was changed to a potassium hydroxide solution since potassium ions are less detrimental to microbes (Wase, 1987) Temperature control was not practicable; temperature therefore fluctuated with room temperature (temperature is, in any case, not controlled in full scale activated sludge plants as it is economically prohibitive).



A	Influent Reservoir	F	Stirrer
B	Collected Effluent	G	Settler
C	Peristaltic Pump	H	Sludge Recycle
D	5 litre Reactor	I	pH Controller
E	Sparger		

Figure 39. Laboratory Scale Activated Sludge Plant

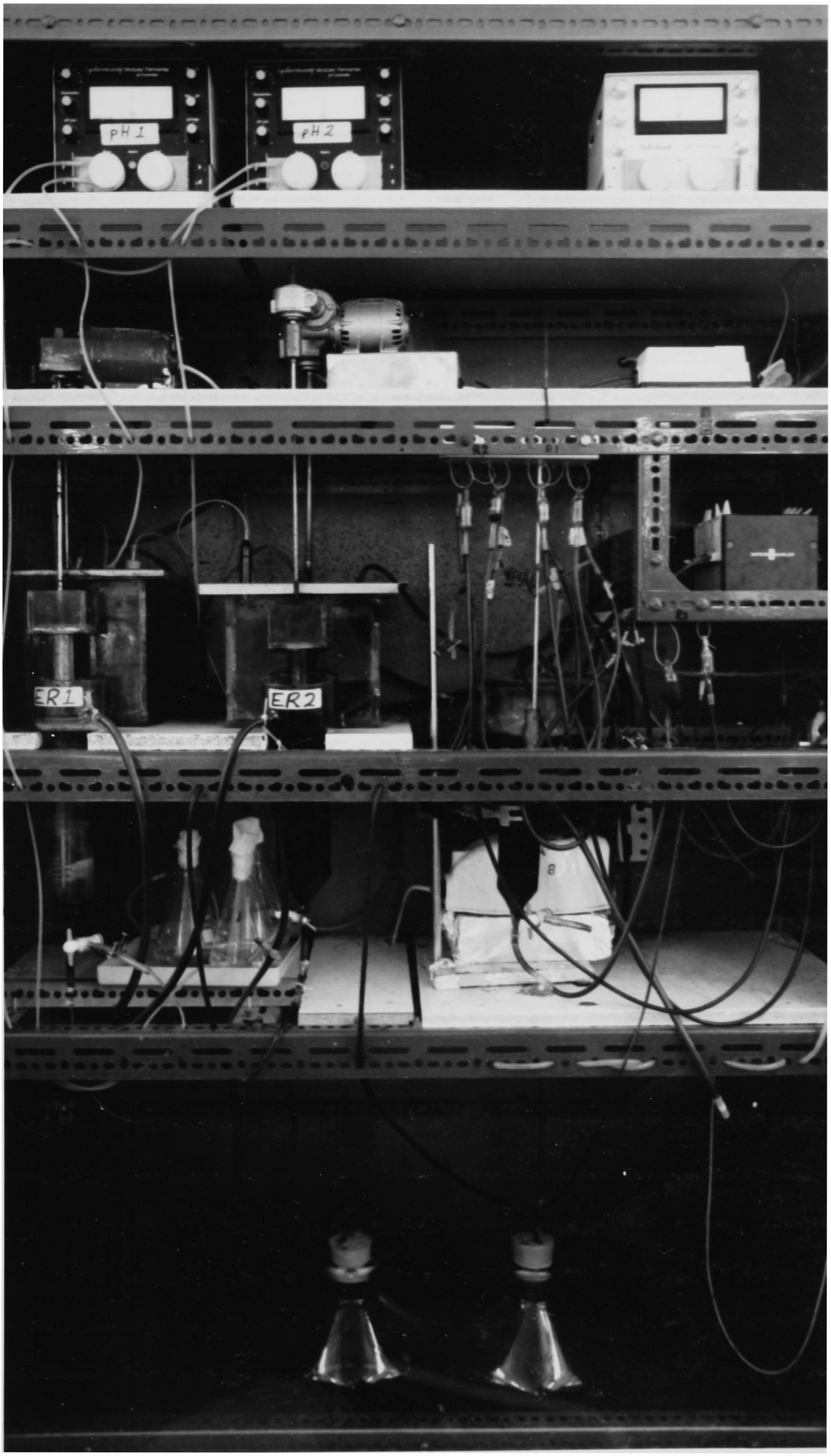


Plate 4. Laboratory scale activated sludge plant

3.4 EXPERIMENTAL STRATEGY: A GENERAL OVERVIEW

For the initial start-up, both activated sludge plants were fed on mixed phenolic medium (Section 2.2.2) with a retention time of two days. Several types of monitoring were employed to assess plant performance. These included frequent (3-5 day intervals) analyses for chemical oxygen demand (Section 2.4.2), total organic carbon (Section 2.4.3) and phenol (Section 2.4.5). Mixed liquor suspended solids were also monitored but less frequently, and usually with a microscopic examination, and this was supplemented with occasional photographic records.

Colonisation of a reactor was observed to follow a general pattern; after inoculation bacteria would proliferate rapidly: this would be followed by the appearance of protozoa, including ameobae, ciliates and flagellates, nematodes, rotifers and fungi.

The next stage was to introduce K10 wash water effluent to one of the reactors, which was designated the experimental reactor, at a concentration of 1% (v/v) (Section 2.2.4.1) on day 128. On day 196 this was increased to 2.5% (Section 2.2.4.2). Many of the protozoa disappeared with the introduction of K10 wash water as was observed in the shake flask experiments.

A third, smaller reactor (2 litre) was later commissioned

in order to assess the effect of increasing the K10 wash water effluent concentration still further without risk to the stability of the experimental plant. The reactor was seeded with sludge from the experimental reactor and fed with 2.5% K10 medium until considered stable. The effect of phenol as a carbon source in replacement of mixed phenolic waste was investigated and so was the effect of increasing the K10 wash water effluent concentration to 12.5% K10 medium in which the K10 wash water effluent was the sole source of carbon. The latter was not successful and the reactor was sterilised and re-seeded with sludge from the experimental reactor and fed on 2.5% K10 medium.

The experimental reactor had been operating on 2.5% K10 medium for over 12 months without sign of detriment due to feed concentration or accumulation of toxic/inhibitory materials. As a result it was decided to re-inoculate the control reactor with sludge from the experimental reactor and switch the feed to 2.5% K10 medium.

The degradation of individual components of the K10 wash water effluent was assessed by HPLC analysis (Section 4.3.2). Although K10 wash water effluent 2.5% K10 waste did not appear toxic at low concentrations, neither was it significantly degraded. In the final biological treatment assessment it was considered best to use the small reactor as a reserve source of inoculum and to use both larger reactors for further experimentation.

The aim of this experiment was to increase the selective pressure placed on the sludge microorganisms to metabolise K10 wash water effluent components by increasing the concentration of the waste in the feed of both reactors. However, the concentration of carbon supplement (phenol) fed to the two reactors (now Reactor 1 and Reactor 2) differed. In reactor 1 the phenol concentration was maintained constant whilst the concentration of K10 wash water effluent was increased, thus the COD value of the medium also increased (Static Phenol Medium, section 2.2.5.1). The other reactor also had increasing K10 wash water effluent concentration but the phenol content of the feed was reduced with increasing K10 wash water effluent concentration in order to maintain a fairly constant COD value (Decreasing Phenol Medium, Section 2.2.5.2)

3.5 RESULTS

3.5.1 Phenol analysis

The control plant was fed on mixed phenolic medium whilst the experimental plant was fed on a combination of mixed phenolic medium and K10 wash water effluent. Samples were not taken until the sixth day after inoculation. This was designated day 1. Figures 40a to 40f display the feed, effluent and removal efficiencies of phenol for the control reactor. The experimental reactor was treated similarly except that on day 128 K10 medium was introduced at 1% (v/v) and on day 196 this was replaced with 2.5% (v/v) K10 medium. Figures 41a to 41f display the results for this reactor.

Several deductions can be made from examination of the results. These are:

i) For the majority of the operational period, the phenol removal efficiencies were above 90% with a final effluent containing less than 10mg/l phenol and occasionally as low as 0.2mg/l, the limit of detection for the sample volume and type of assay utilised (Section 2.4.5).

ii) The introduction of K10 wash water effluent to the experimental plant had to be deferred from the anticipated day 30. This was due to the possibility of interruptions in electrical power supply and also a laboratory relocation involving dismantling and

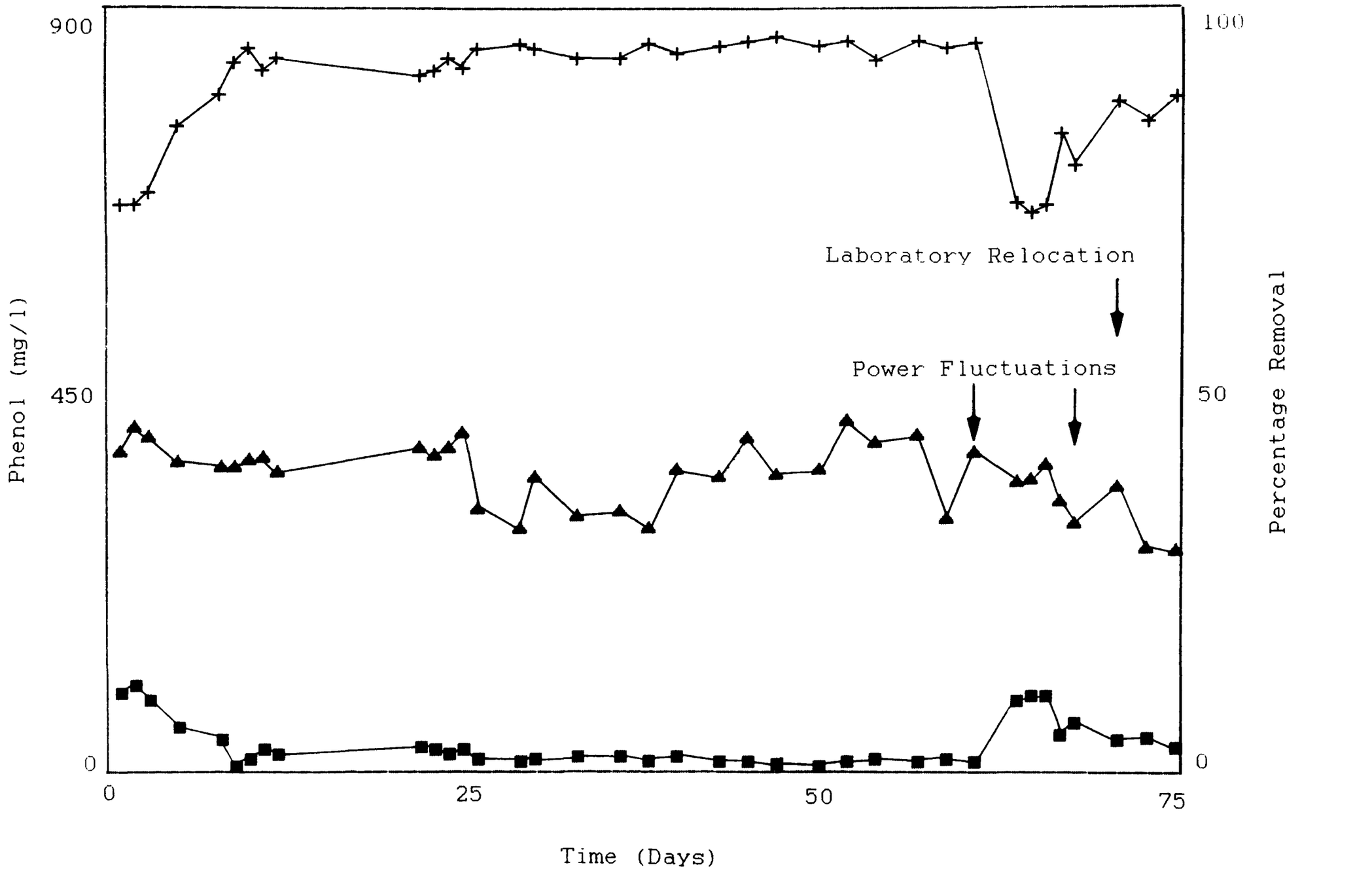


Figure 40a. Phenol Analysis of the Control Reactor

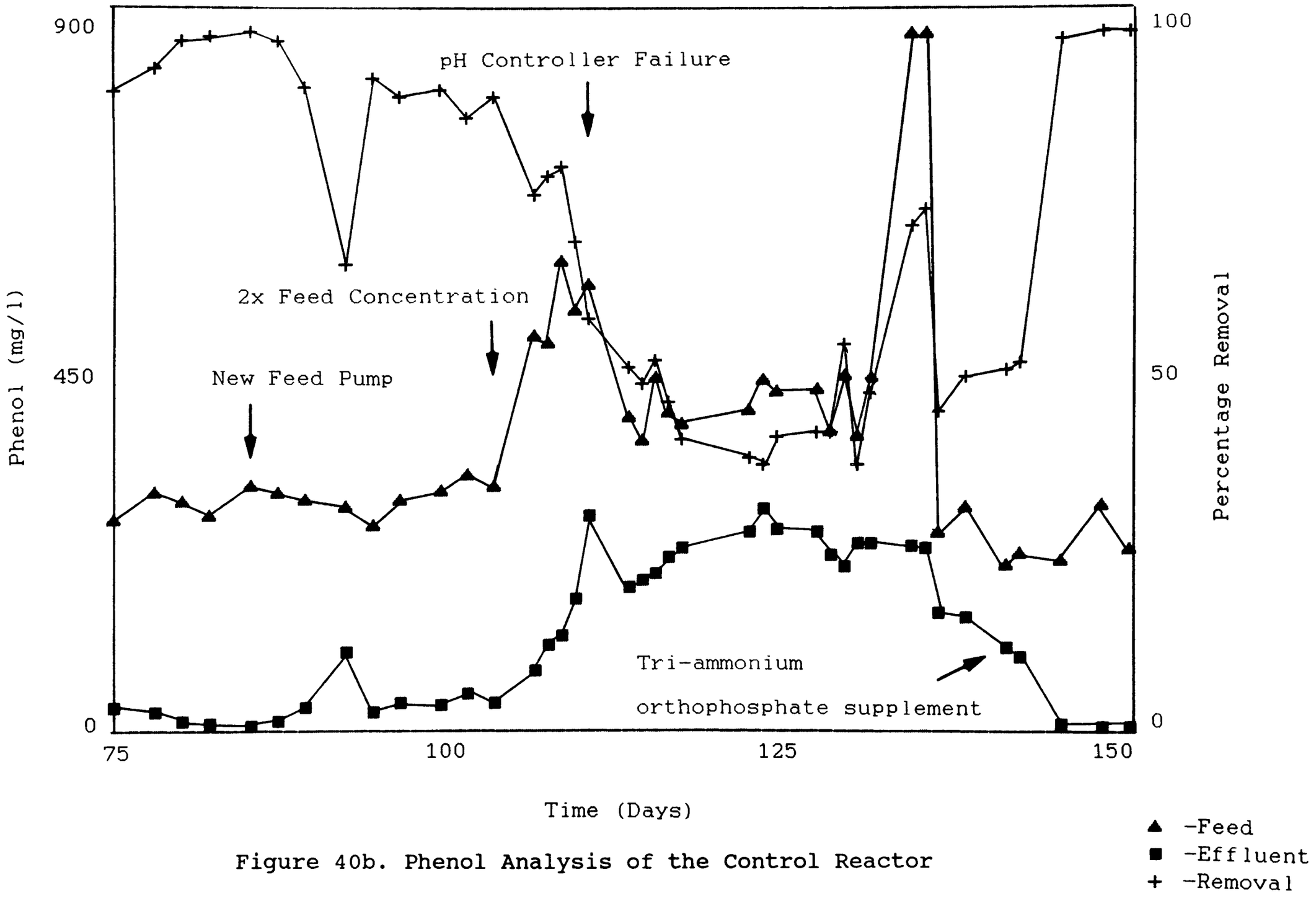


Figure 40b. Phenol Analysis of the Control Reactor

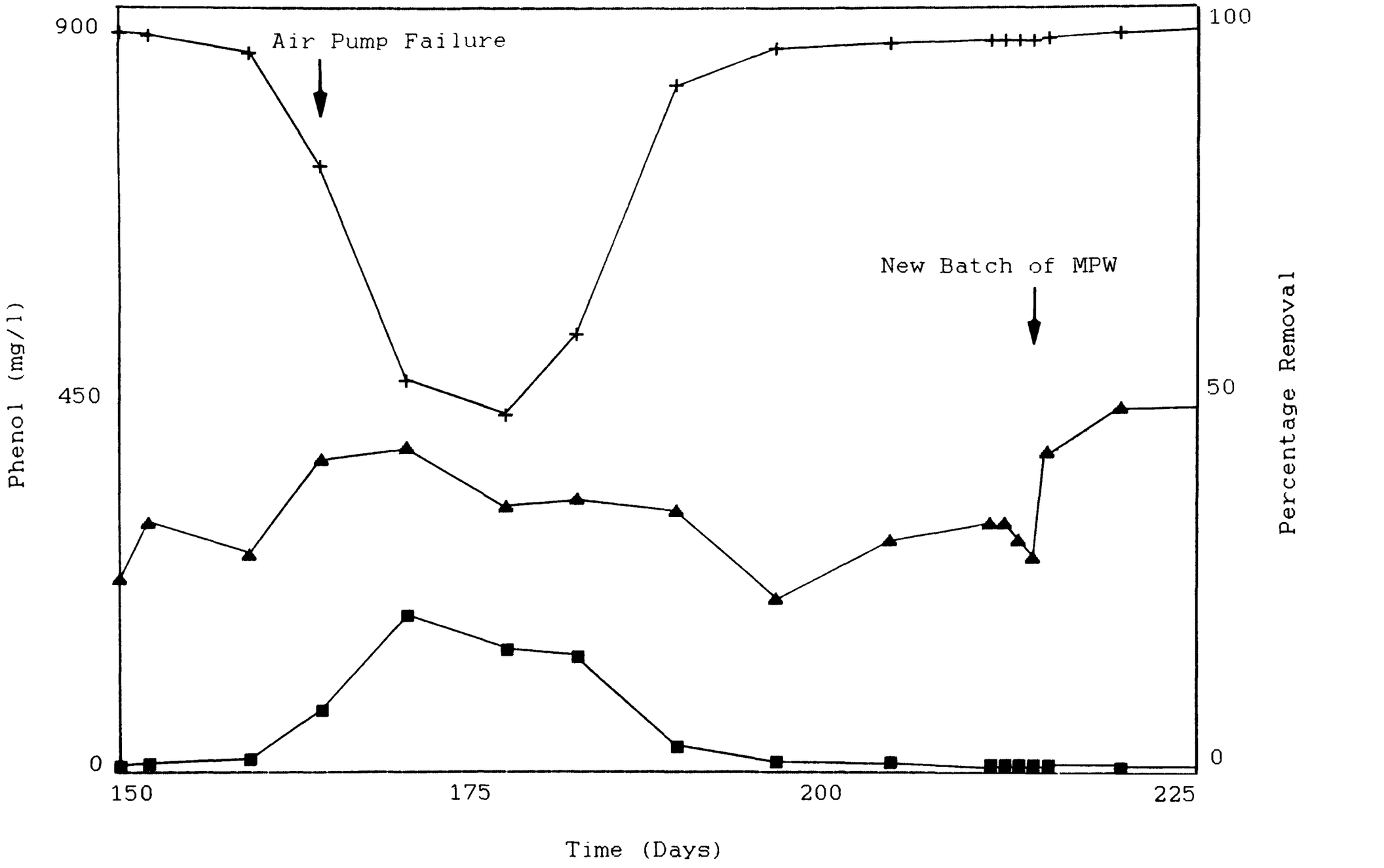


Figure 40c. Phenol Analysis of the Control Reactor

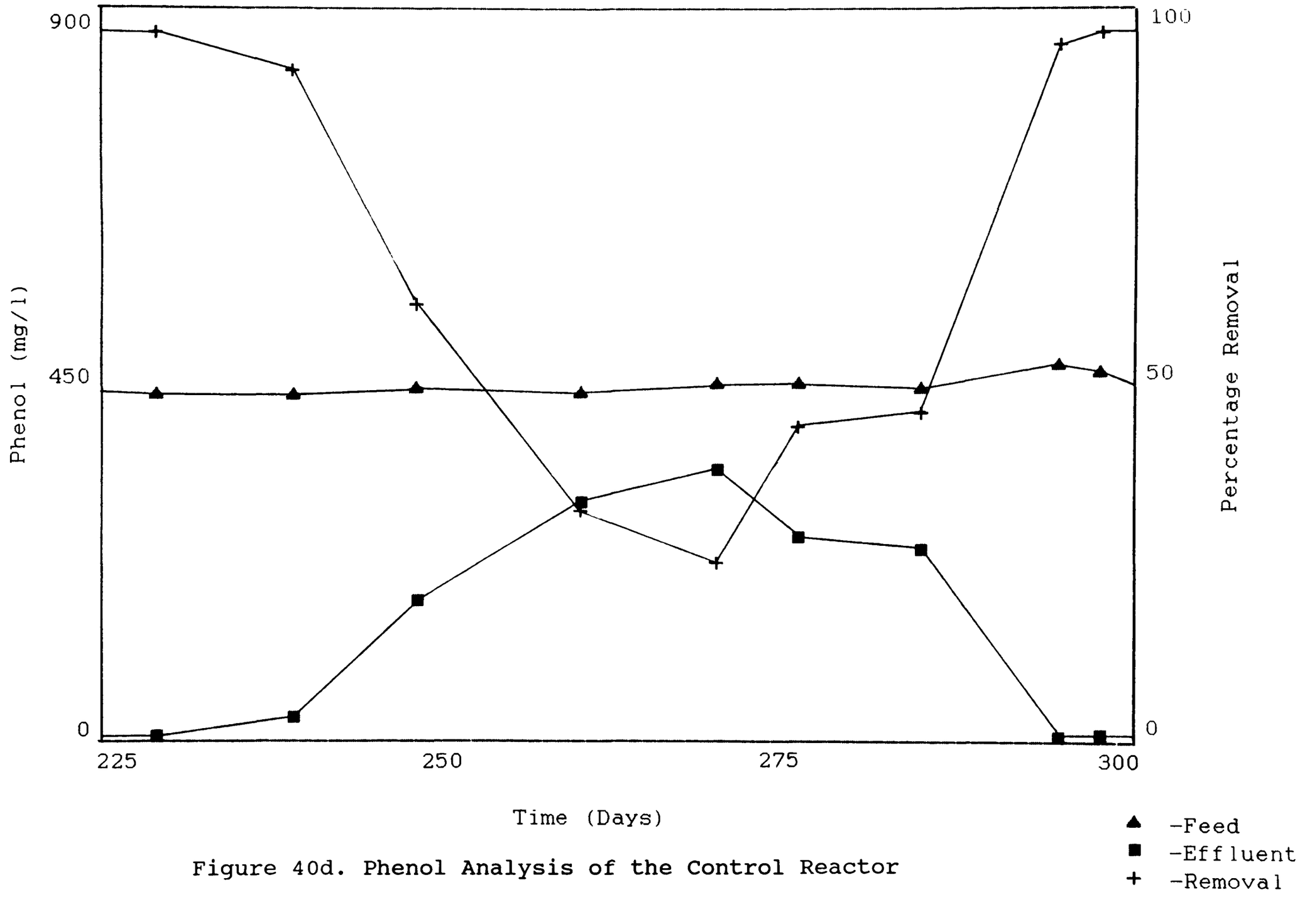


Figure 40d. Phenol Analysis of the Control Reactor

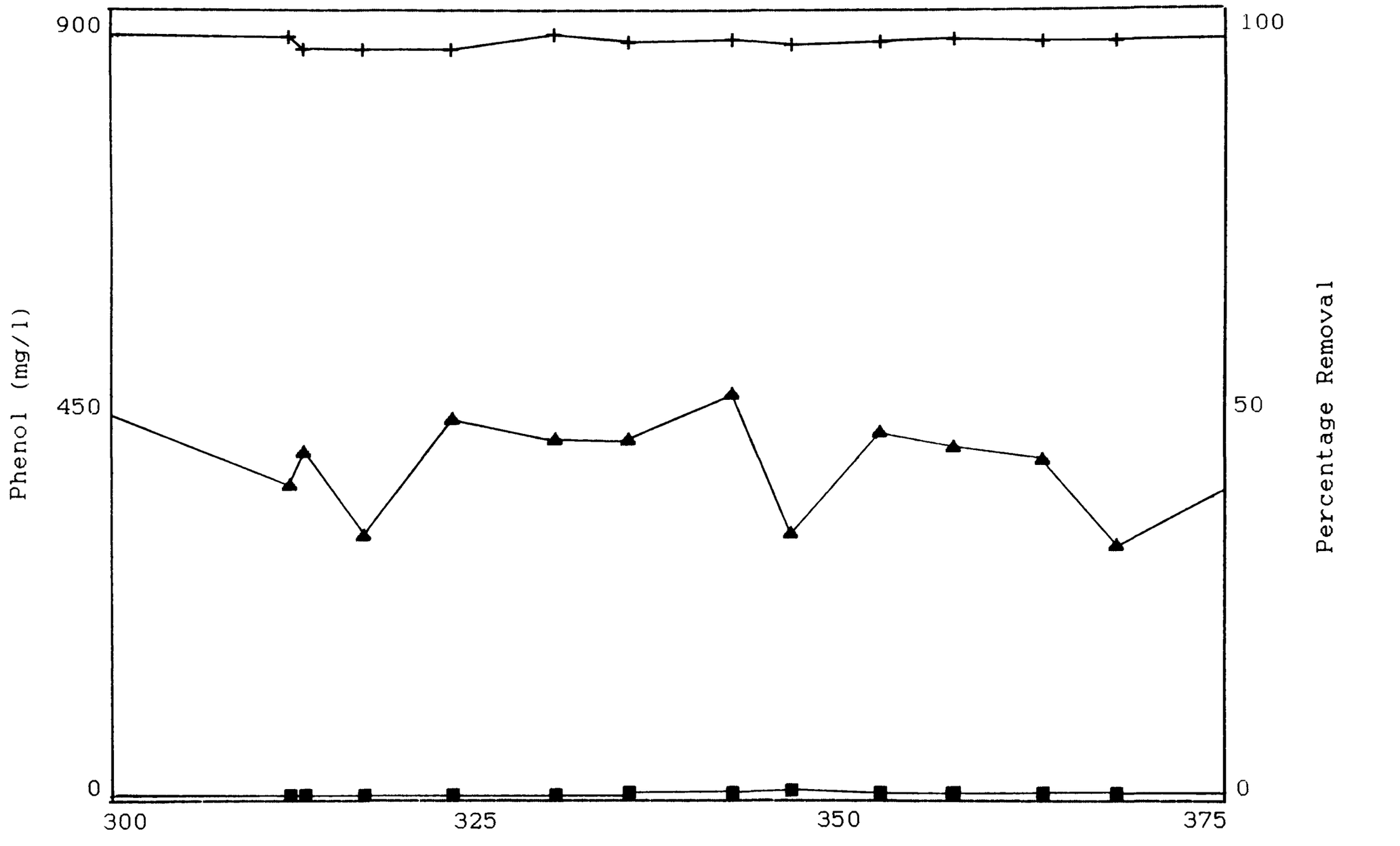


Figure 40e. Phenol Analysis of the Control Reactor

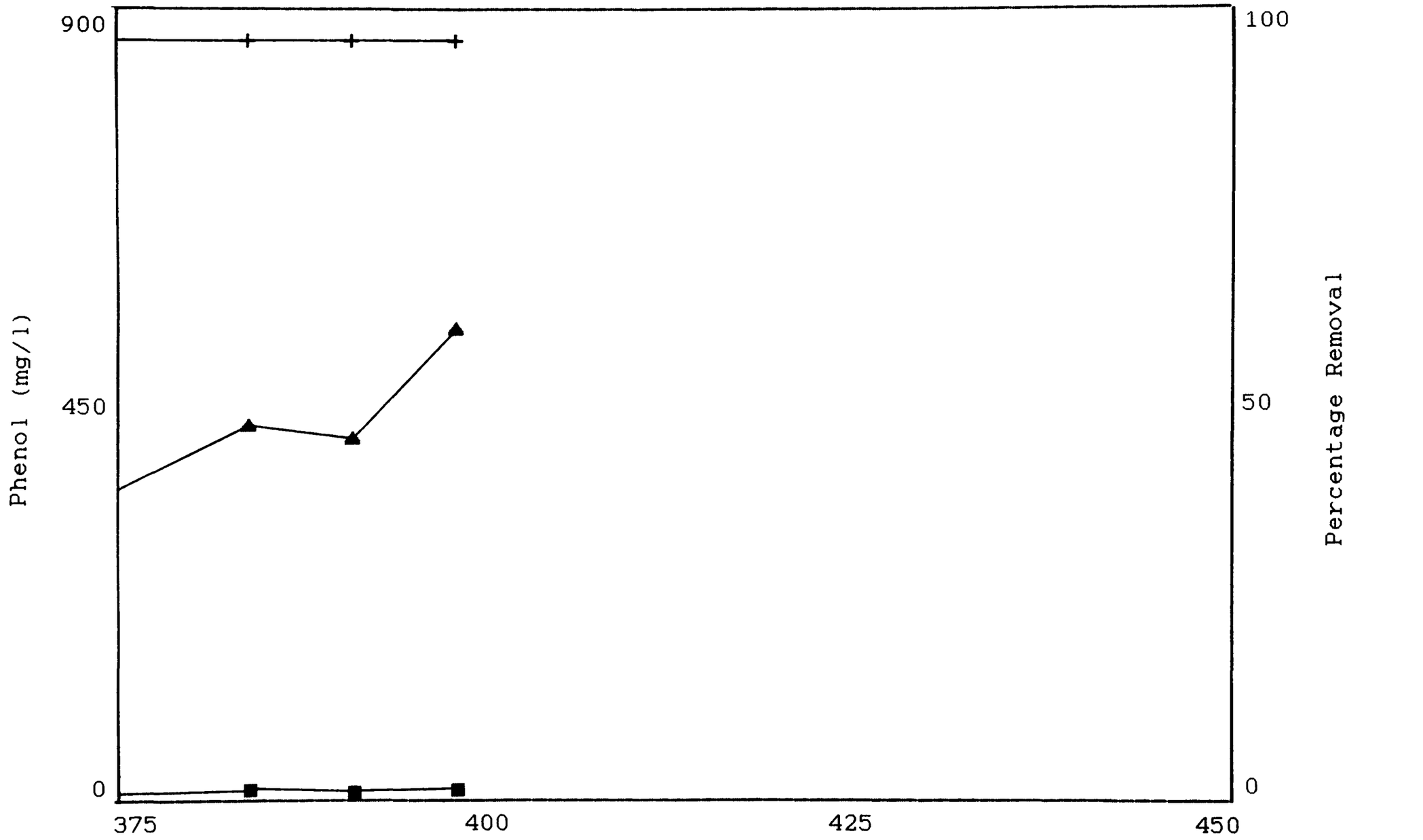


Figure 40f. Phenol Analysis of the Control Reactor

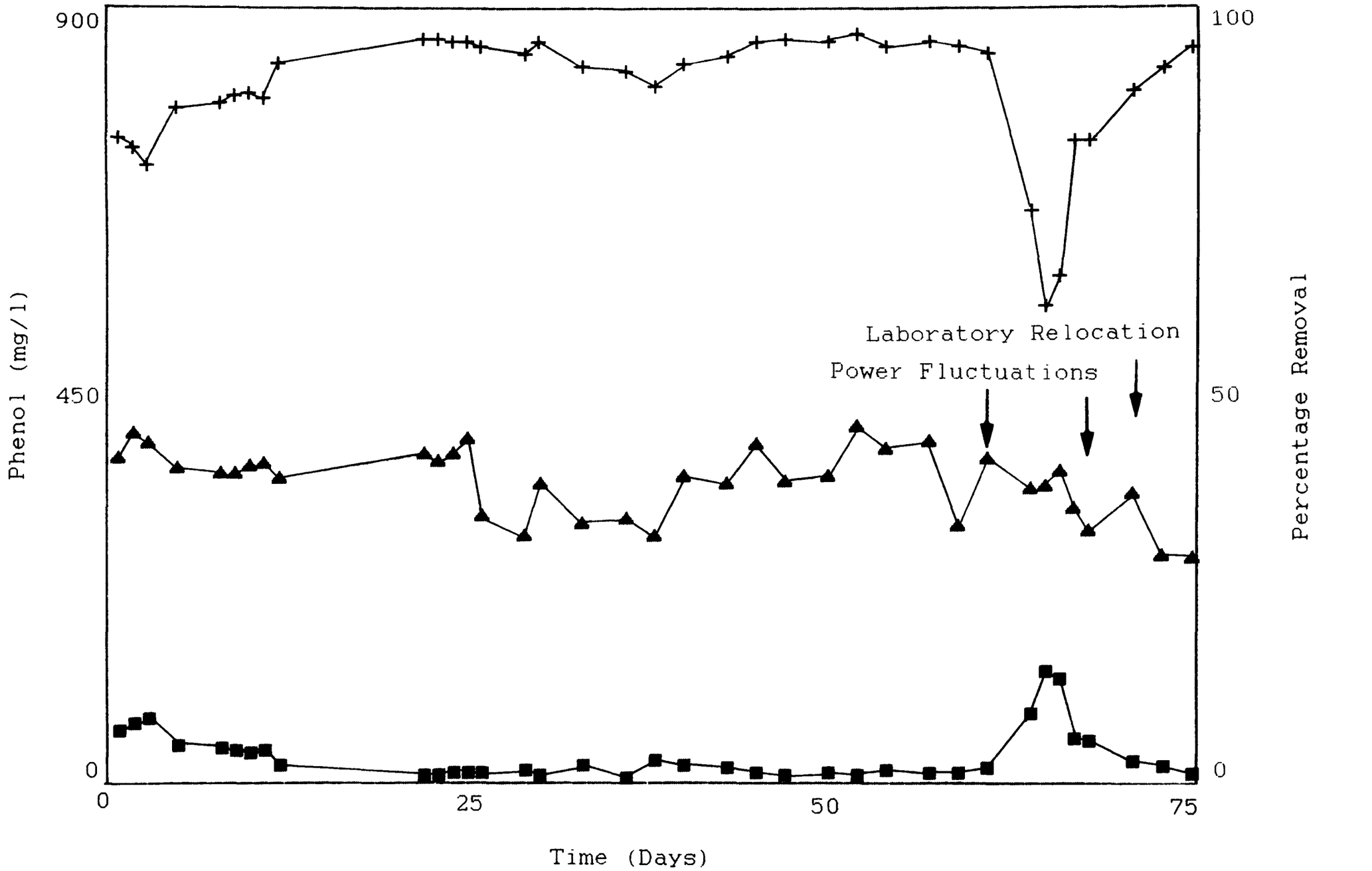


Figure 41a. Phenol Analysis of the Experimental Reactor

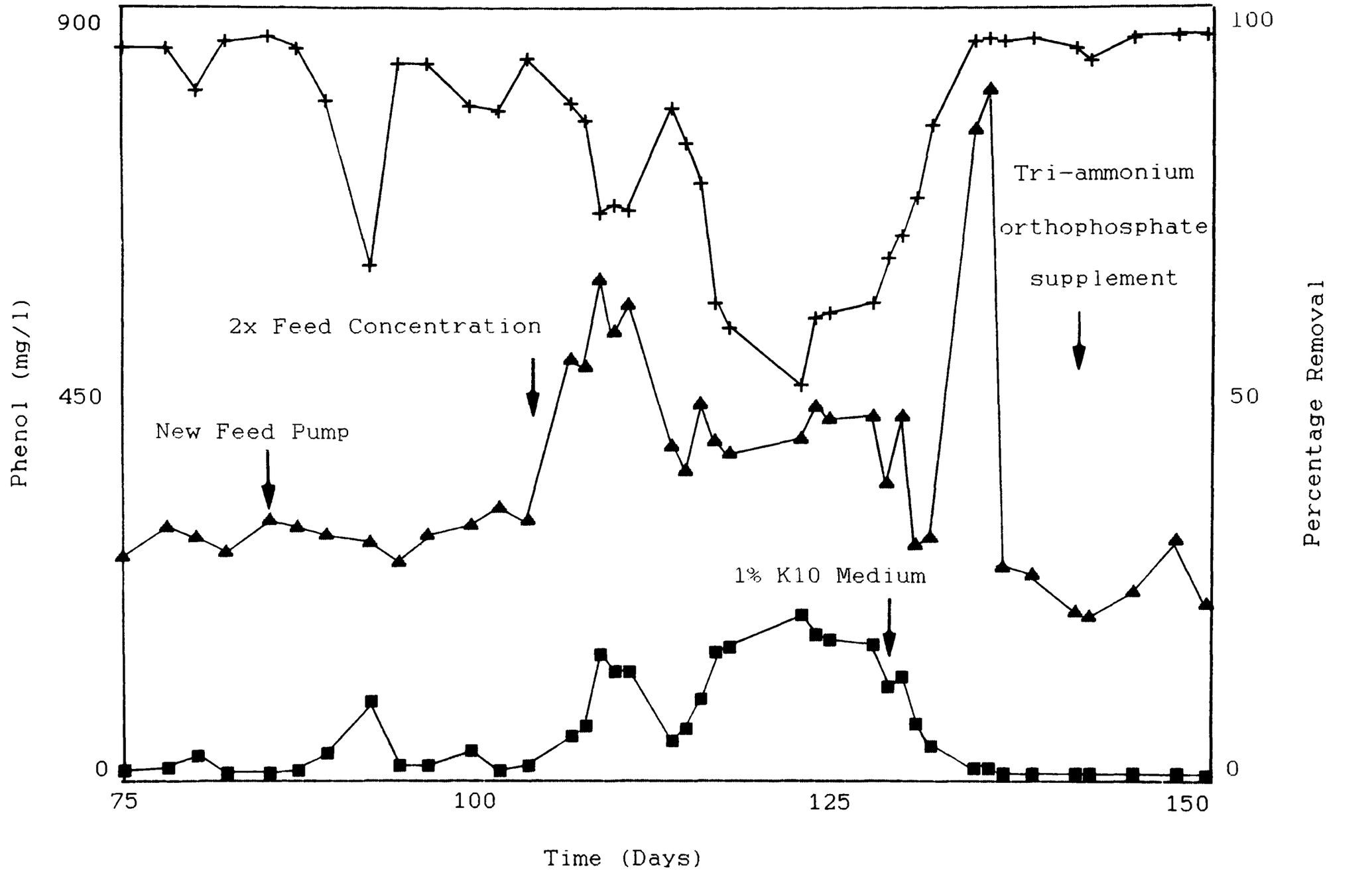


Figure 41b. Phenol Analysis of the Experimental Reactor

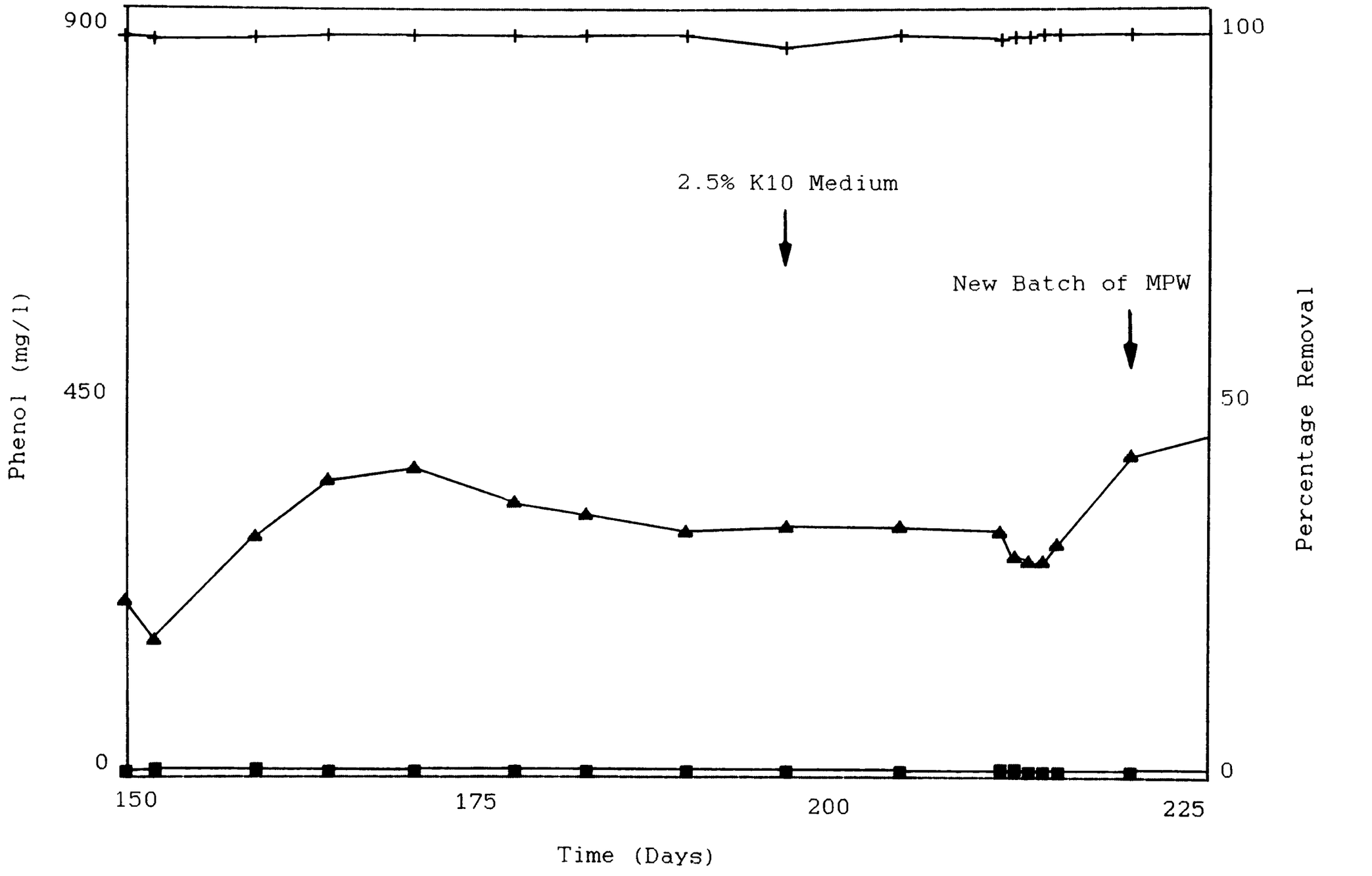


Figure 41c. Phenol Analysis of the Experimental Reactor

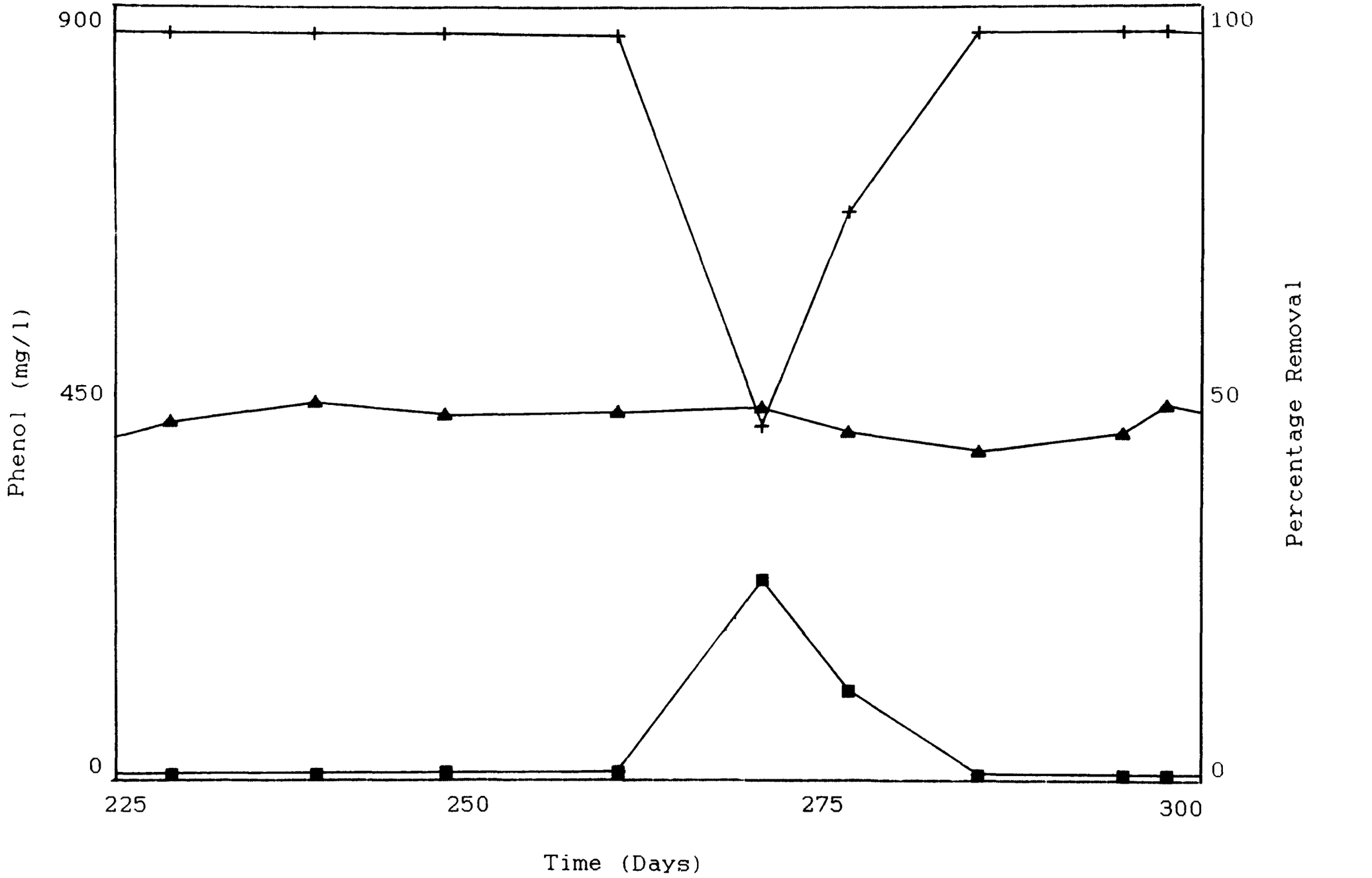


Figure 41d. Phenol Analysis of the Experimental Reactor

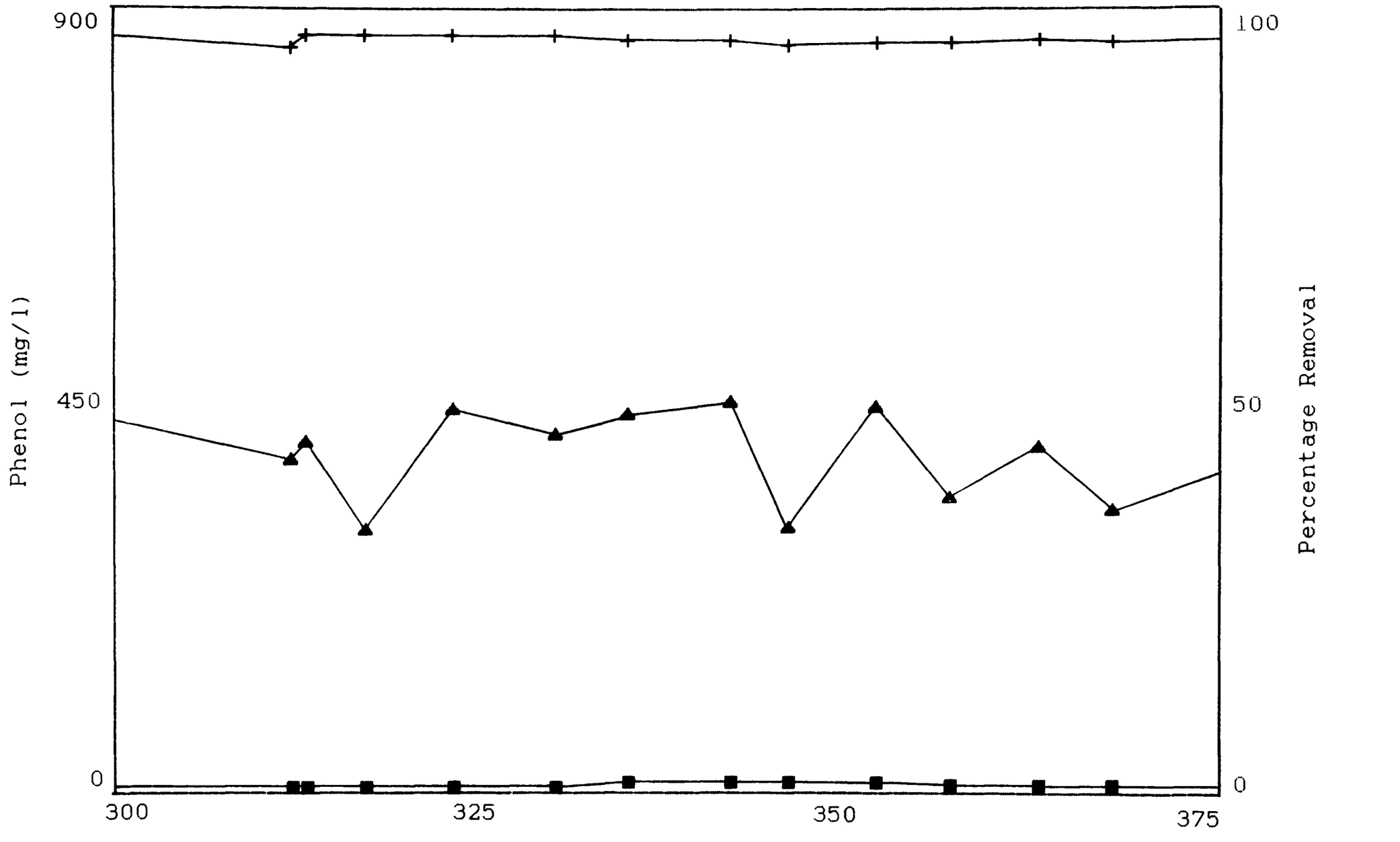


Figure 41e. Phenol Analysis of the Experimental Reactor

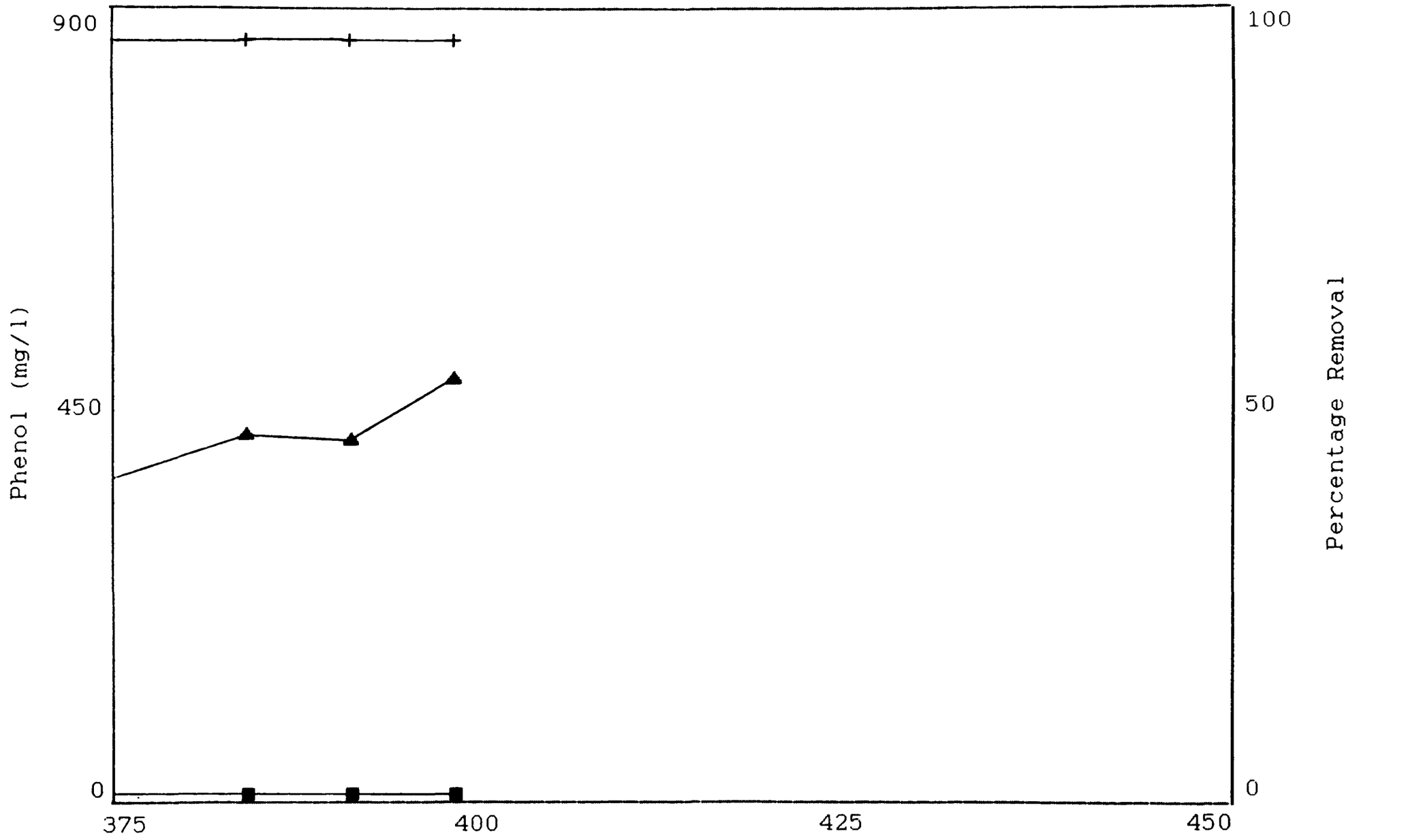


Figure 41f. Phenol Analysis of the Experimental Reactor

reconstruction of the entire experimental apparatus. Compressed air supplies were used when possible but reactors most certainly became anoxic when the air supply failed for periods of six hours or more. The onset of these disruptions had a marked effect on the phenol removal efficiency, however, the reactors were quick to recover to original levels within a week after the final disruption.

On day 164, the air pump (which fed air to the control reactor) was found to have failed. This may have lasted several days, and it is interesting to note that the reactor took two weeks to recover, the length of time required for a plant to establish itself after seeding.

A further, more minor, disruption was the installation of a new and more accurate feed pump. Both feed and sludge recycle were disturbed for short periods and this is the only explanation available for the decrease in performance occurring a few days later.

iii) Raw mixed phenolic waste, used to make media, was deficient in essential nutrients (nitrogen and phosphorus) which accounts for the drop in phenol removal efficiency noticeable by day 131 which affected both reactors. This sudden decrease in performance may have been aggravated by the doubling of the feed rate on day 103. A further problem concerned a fault in the pH controller to the control reactor which resulted in excess sodium hydroxide addition, the pH reaching 9.5 on

day 110. The supplementation of the feed with tri-ammonium orthophosphate rapidly reversed the nitrogen and phosphorus starvation.

iv) The introduction of 1% K10 wash water effluent (day 128) as a supplement to mixed phenolic waste medium did not appear to have any detrimental effect to the experimental reactor. On the contrary, its effects were largely beneficial and were attributed to the nitrogen content of K10 wash water effluent. The later use of 2.5% K10 medium as a feed to the experimental reactor (day 196) did not significantly affect the reactor phenol removal efficiency. It is worth mentioning that the K10 wash water effluent does not contain phenols or interfere with their assay: thus the phenol analysis provides a good technique for monitoring general carbon metabolism since it excludes the K10 wash water effluent components, unlike the TOC and COD assays which measure both mixed phenolic waste and K10 wash water effluent components.

v) A new batch of mixed phenolic waste (used for media preparation) was introduced to the control plant on day 214. A week was considered adequate as a check to ensure that no detrimental effects had arisen. Nothing untoward had happened during this week and consequently on day 221 the new mixed phenolic waste batch was introduced into the feed of the experimental plant. However, three weeks after the introduction of the new mixed phenolic waste to

the control reactor its phenol removal efficiency dropped dramatically and required more than forty days to recover. Microscopic examination of the sludge during this period did not reveal anything untoward regarding the health of the sludge. The effect of the new waste might have been due to the presence of a new component which required a period of selection and enrichment of the bacterial population. The effect on the experimental reactor was far less marked, perhaps due to the expression of different organisms within the population.

vi) For the final period (days 300 to 450) both plant performances were stable and had equal removal efficiencies and no inhibition appeared to occur.

3.5.2 COD analysis

COD analysis was not utilised during the early part of the experimentation. This was because a COD apparatus was unavailable for general use and so a set of apparatus was constructed within the departmental workshop to the Author's design.

Figures 42a to 42d illustrate the COD input, output and removal efficiencies for the control plant and Figures 43a to 43d are those for the experimental reactor.

The results were similar to those of the phenol analysis with certain exceptions. These are as follows:

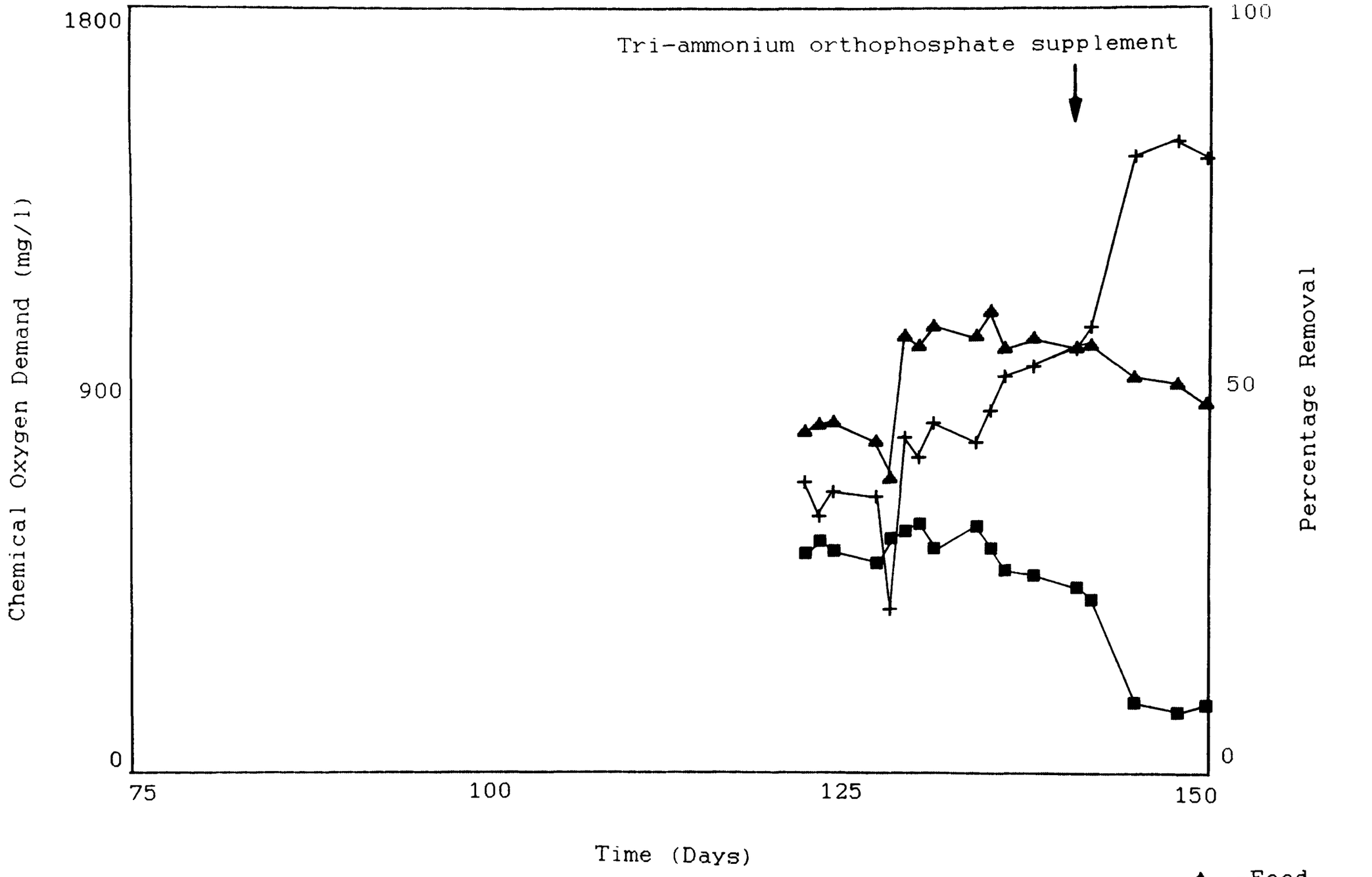


Figure 42a. COD Analysis of the Control Reactor

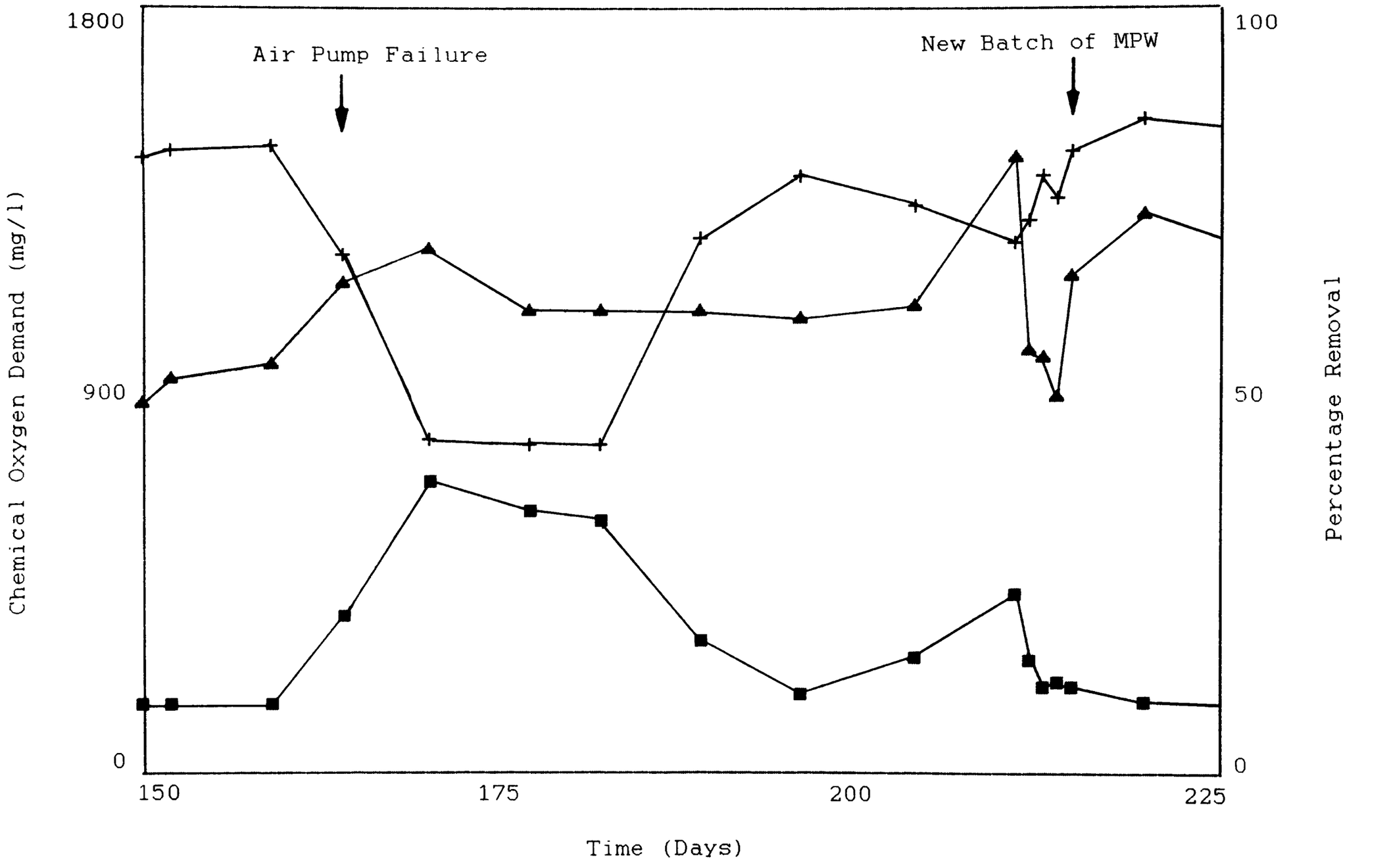


Figure 42b. COD Analysis of the Control Reactor

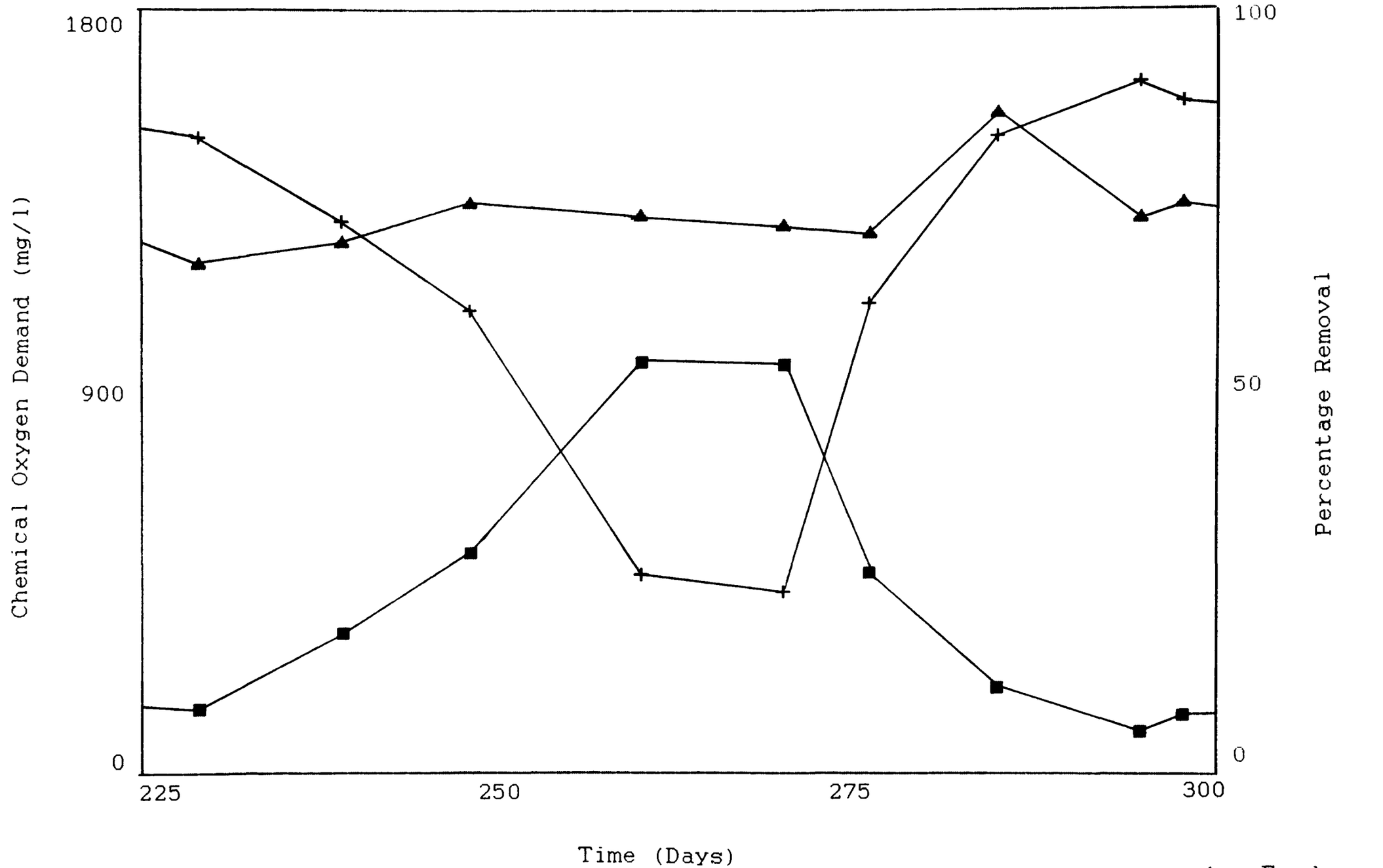


Figure 42c. COD Analysis of the Control Reactor

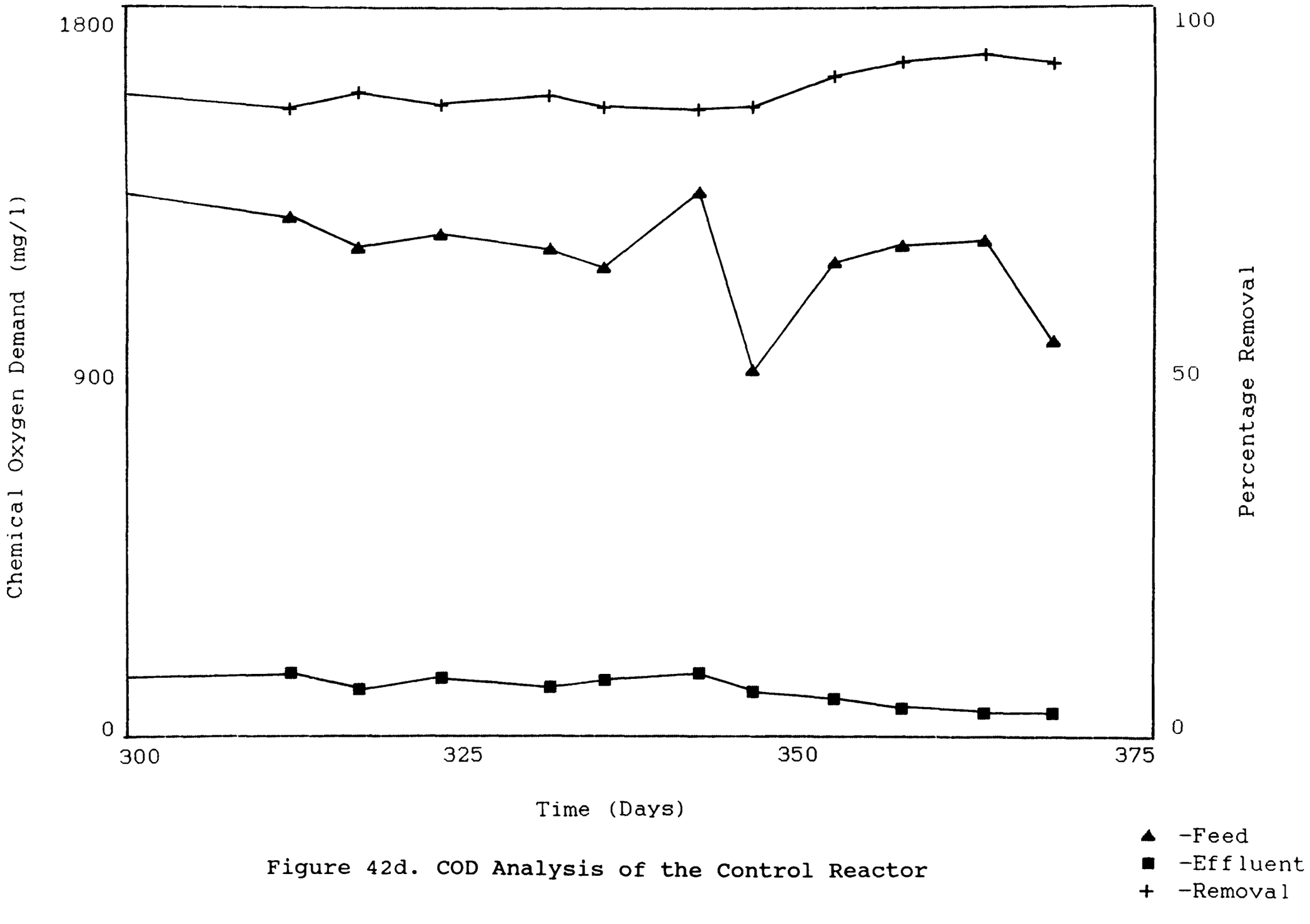


Figure 42d. COD Analysis of the Control Reactor

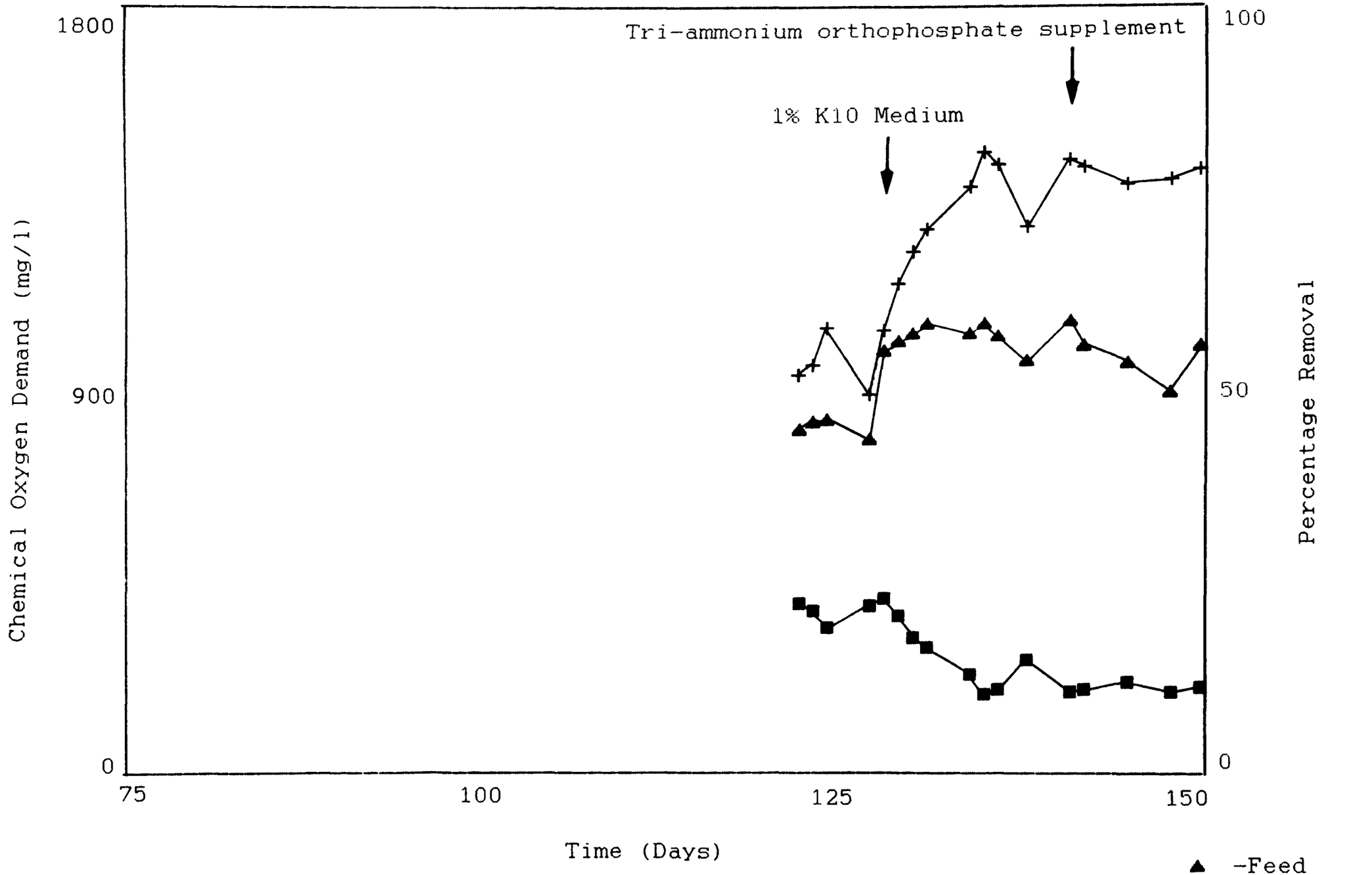


Figure 43a. COD Analysis of the Experimental Reactor

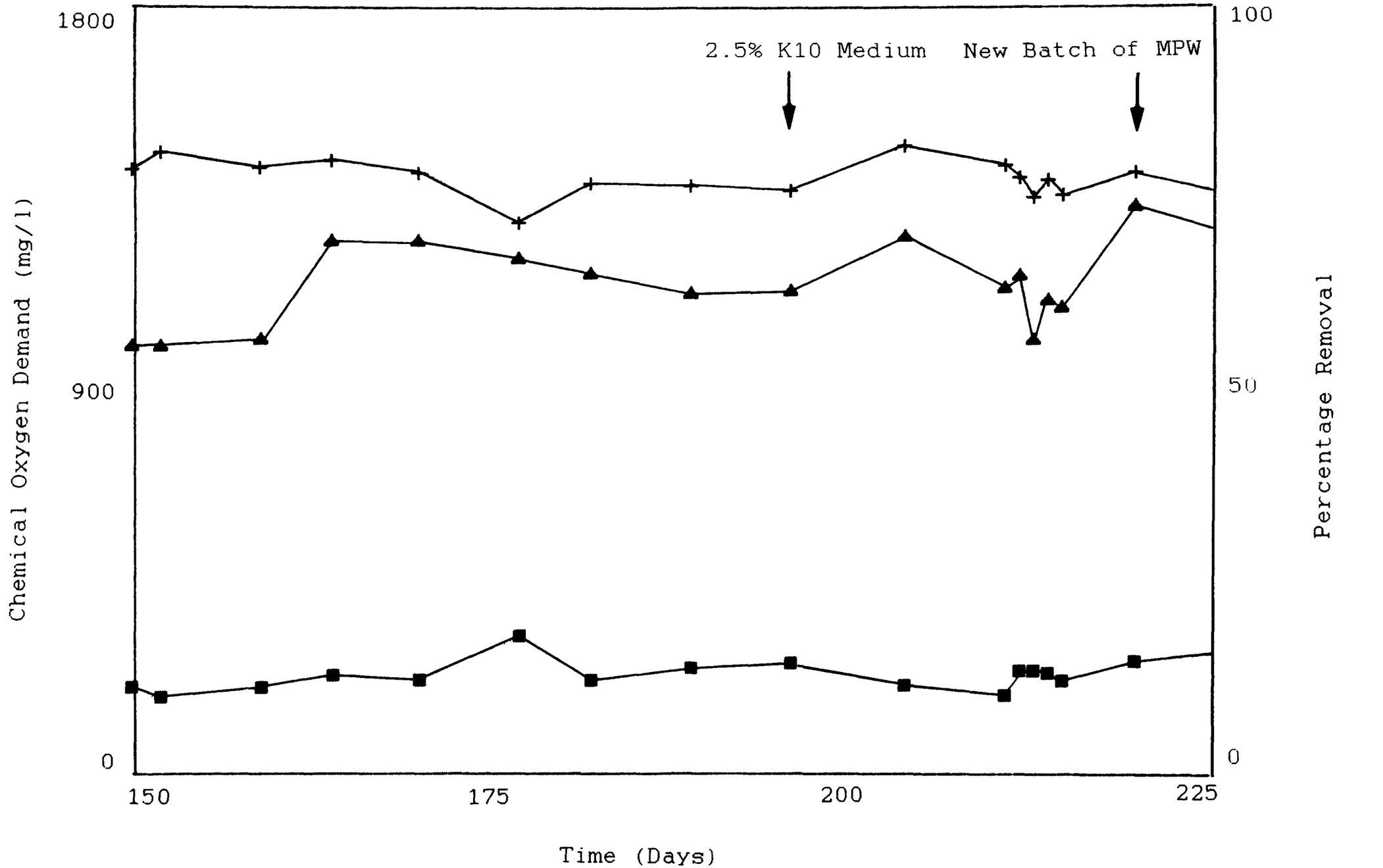


Figure 43b. COD Analysis of the Experimental Reactor

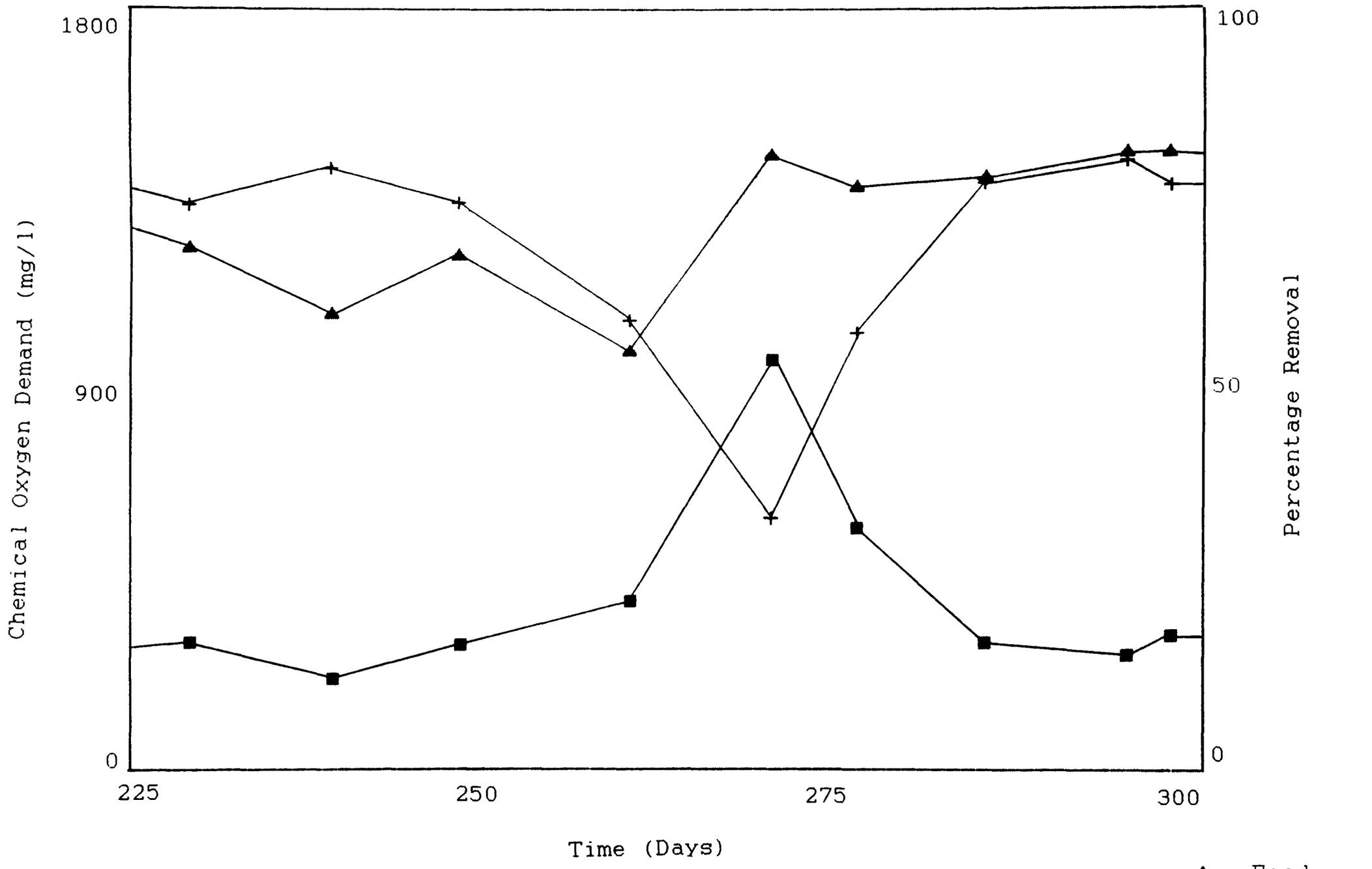


Figure 43c. COD Analysis of the Experimental Reactor

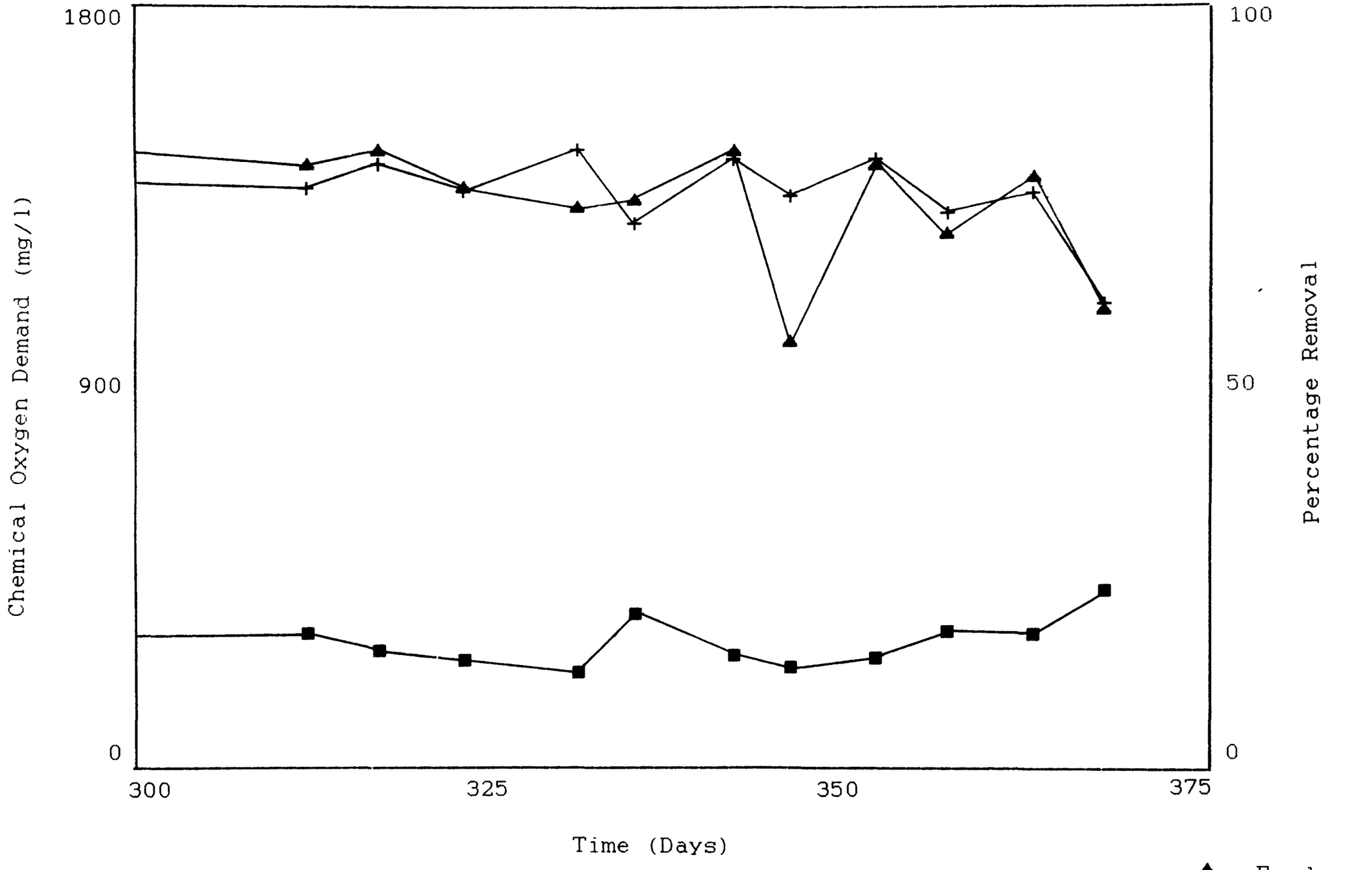


Figure 43d. COD Analysis of the Experimental Reactor

The removal efficiencies were not generally as high. In the case of the control reactor, which was fed only with phenolic waste, removal efficiency was generally 85 to 95% when the reactor was considered to be in a stable condition. The efficiency for the experimental reactor, fed on phenolic waste, plus 2.5% K10 medium, was lower, ranging from 75 to 85%. This was attributed to K10 wash water effluent components which were not mineralised, since the phenol removal efficiencies of the two reactors were equal. However the increase in the COD of the effluent from the experimental reactor (compared with that of the control) was not equal to the contribution to the COD of the K10 wash water effluent components of the experimental reactor feed (200mg/l at 2.5% K10 waste). In many instances the observed increase was as low as 100mg/l. Thus it seems likely that some of the K10 wash water effluent components were mineralised. However, the K10 wash water effluent did contain urea and sodium carbonate and the overall contribution of this component to the COD of the waste was unknown.

3.5.3 TOC analysis

Figures 44a to 44e show the TOC content of the feed and effluent together with the percentage removal for the control reactor. Figures 45a to 45e show these for the experimental reactor.

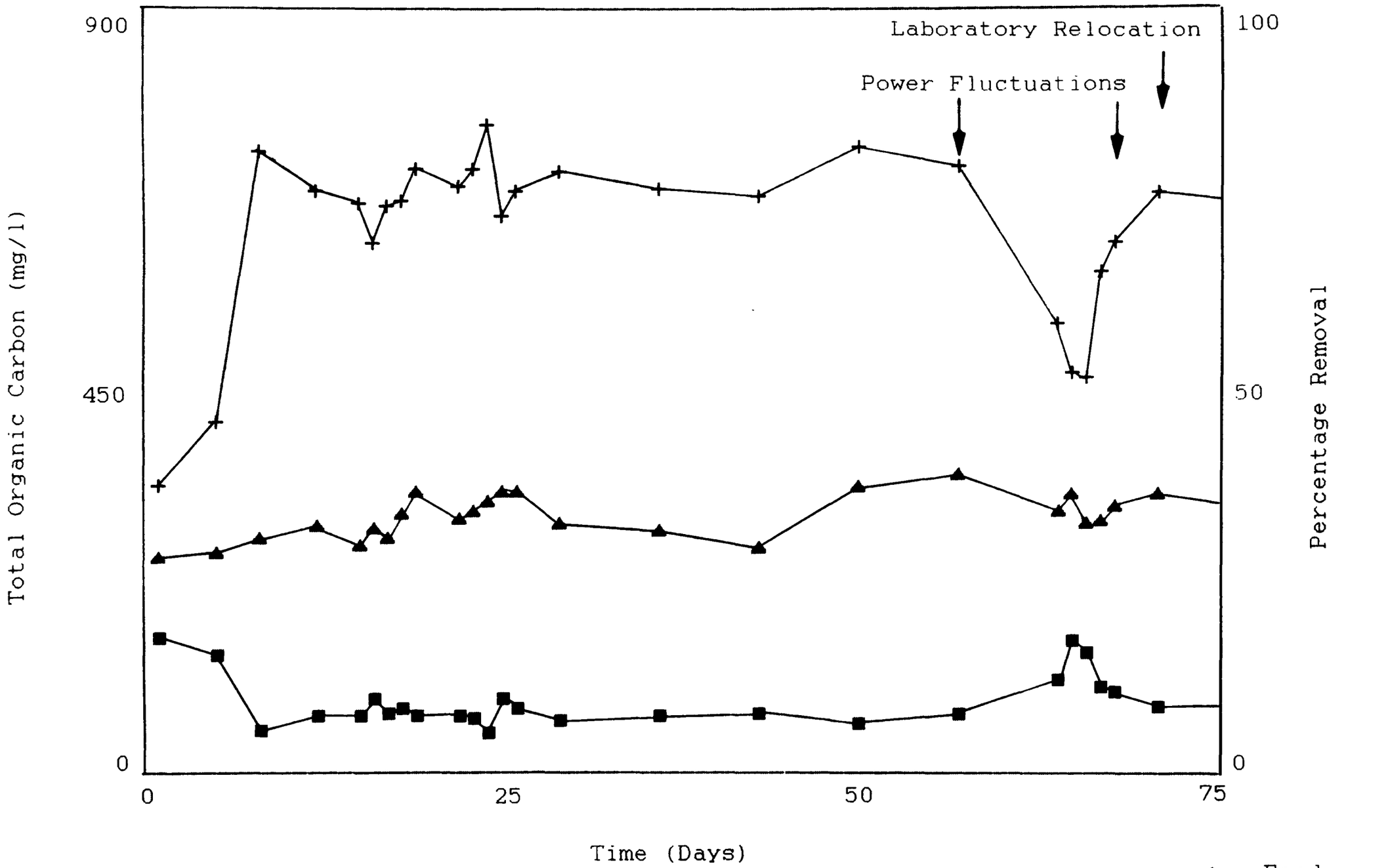


Figure 44a. TOC Analysis of the Control Reactor

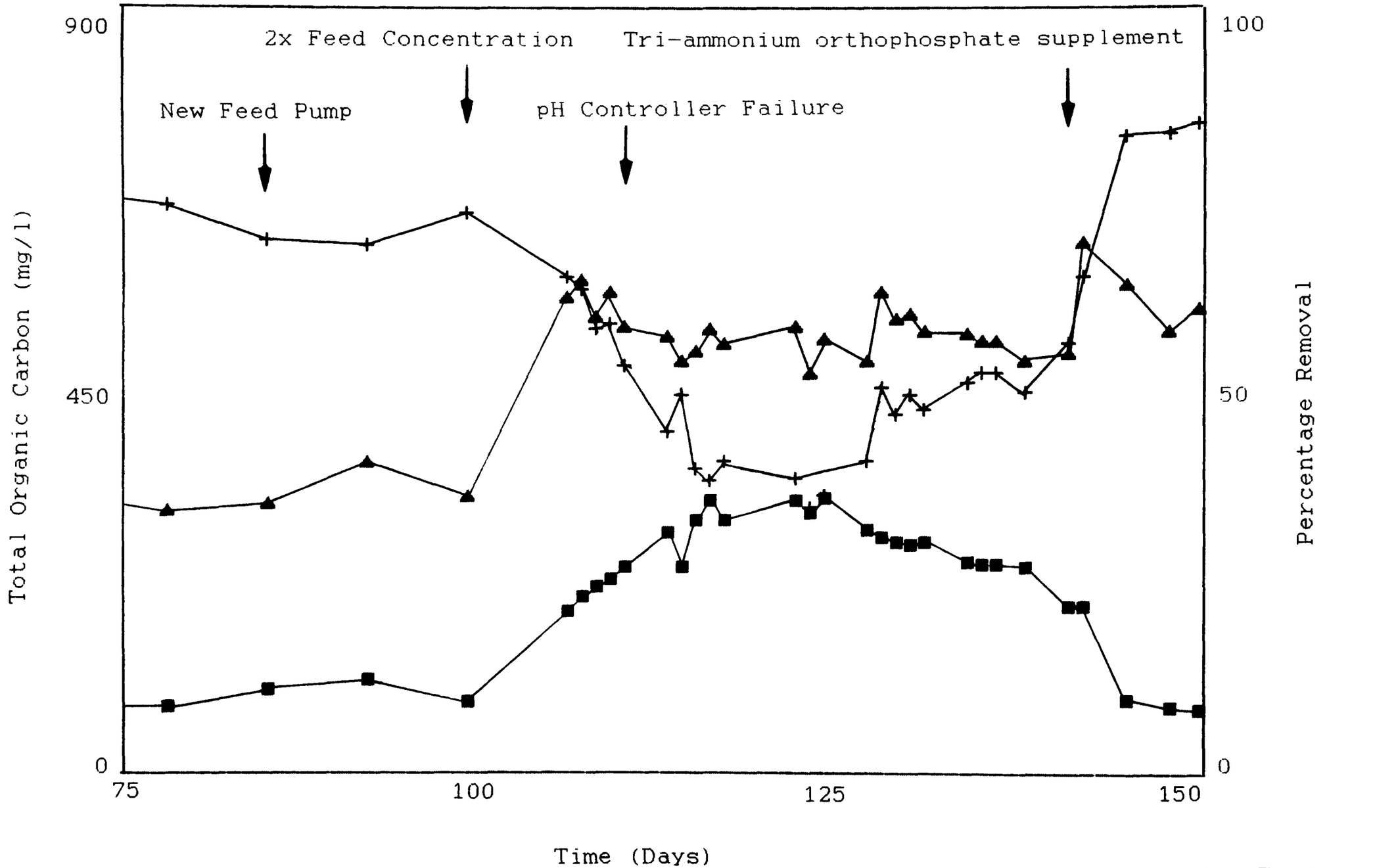


Figure 44b. TOC Analysis of the Control Reactor

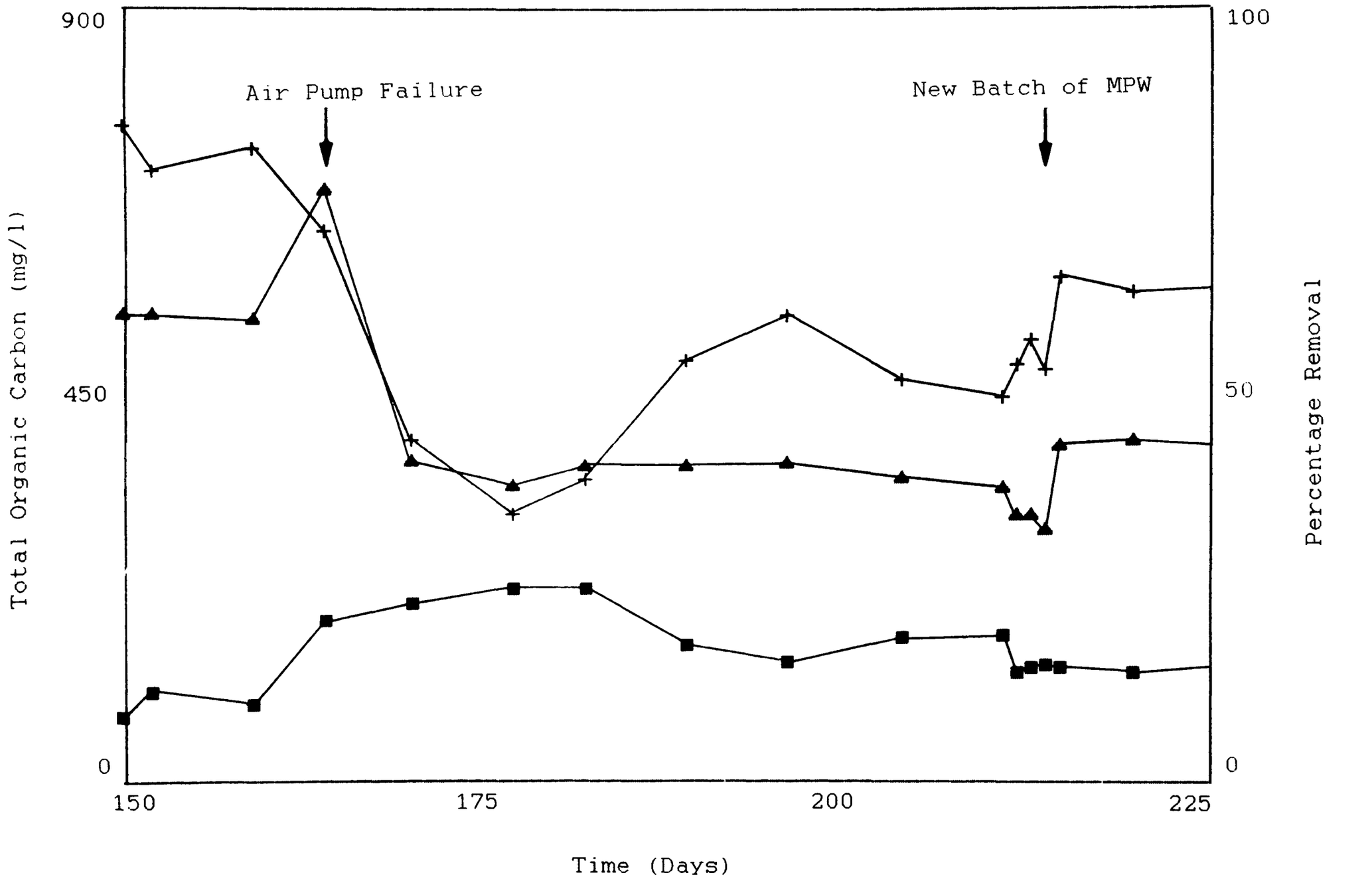


Figure 44c. TOC Analysis of the Control Reactor

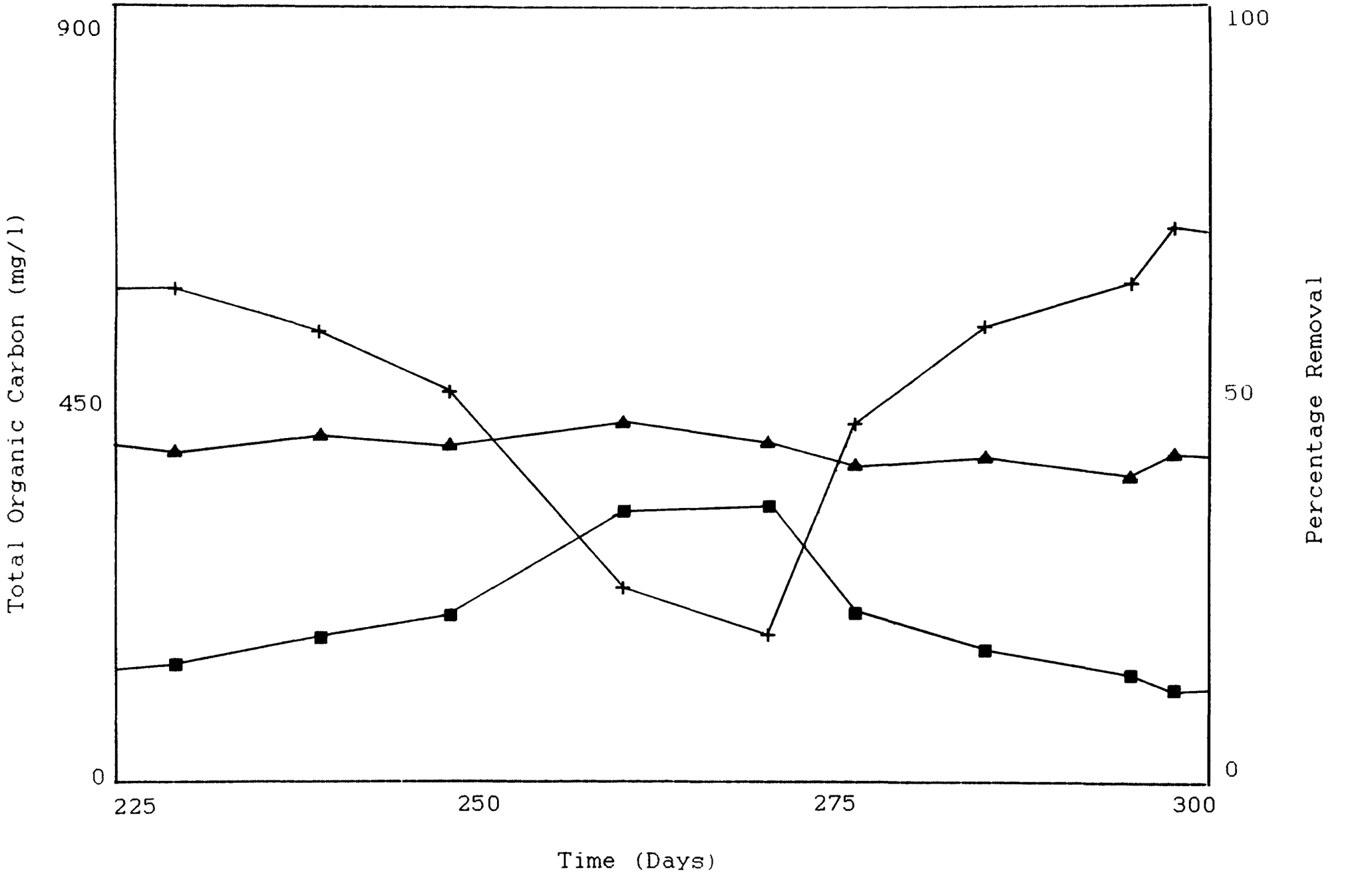


Figure 44d. TOC Analysis of the Control Reactor

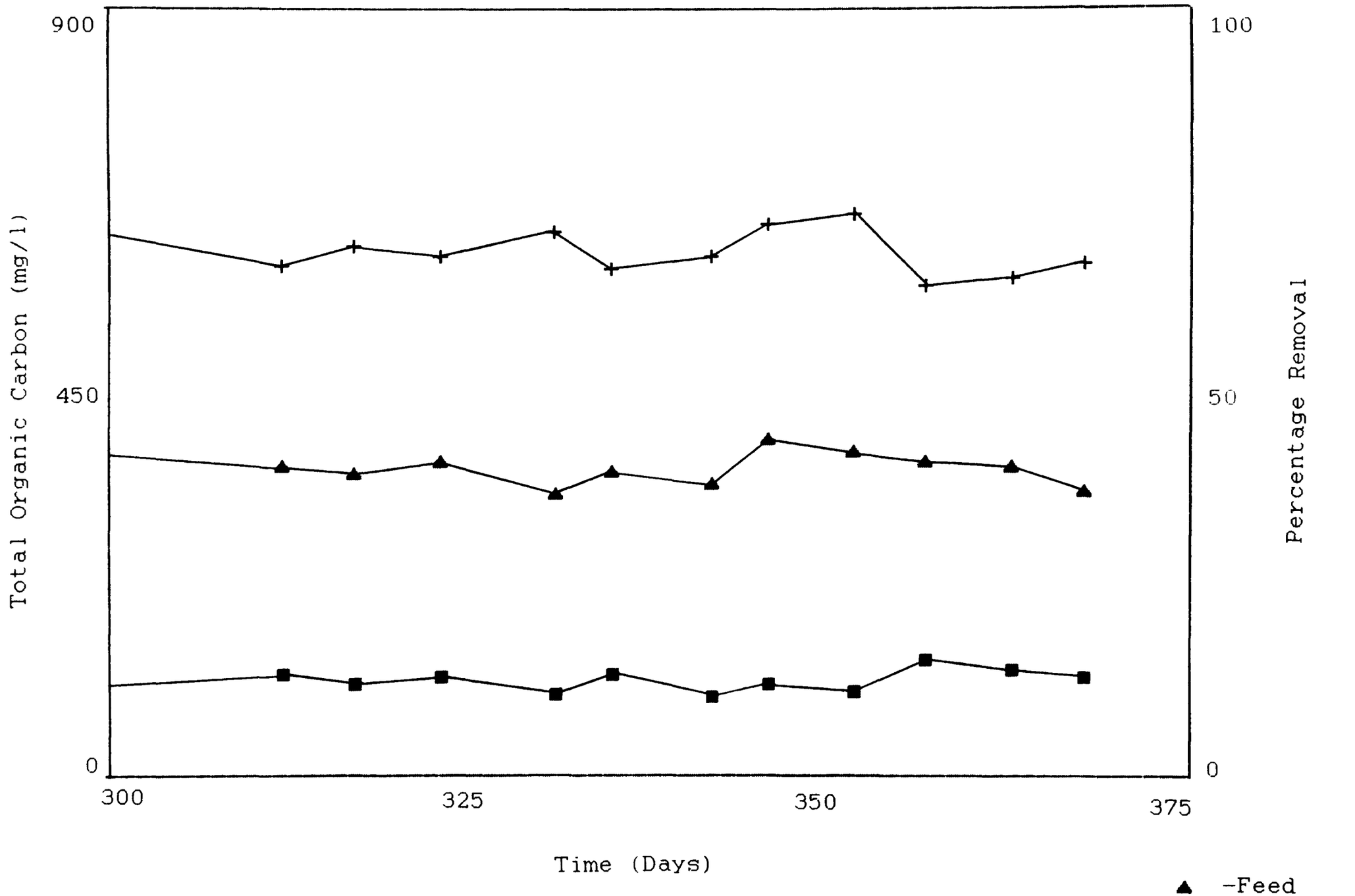


Figure 44e. TOC Analysis of the Control Reactor

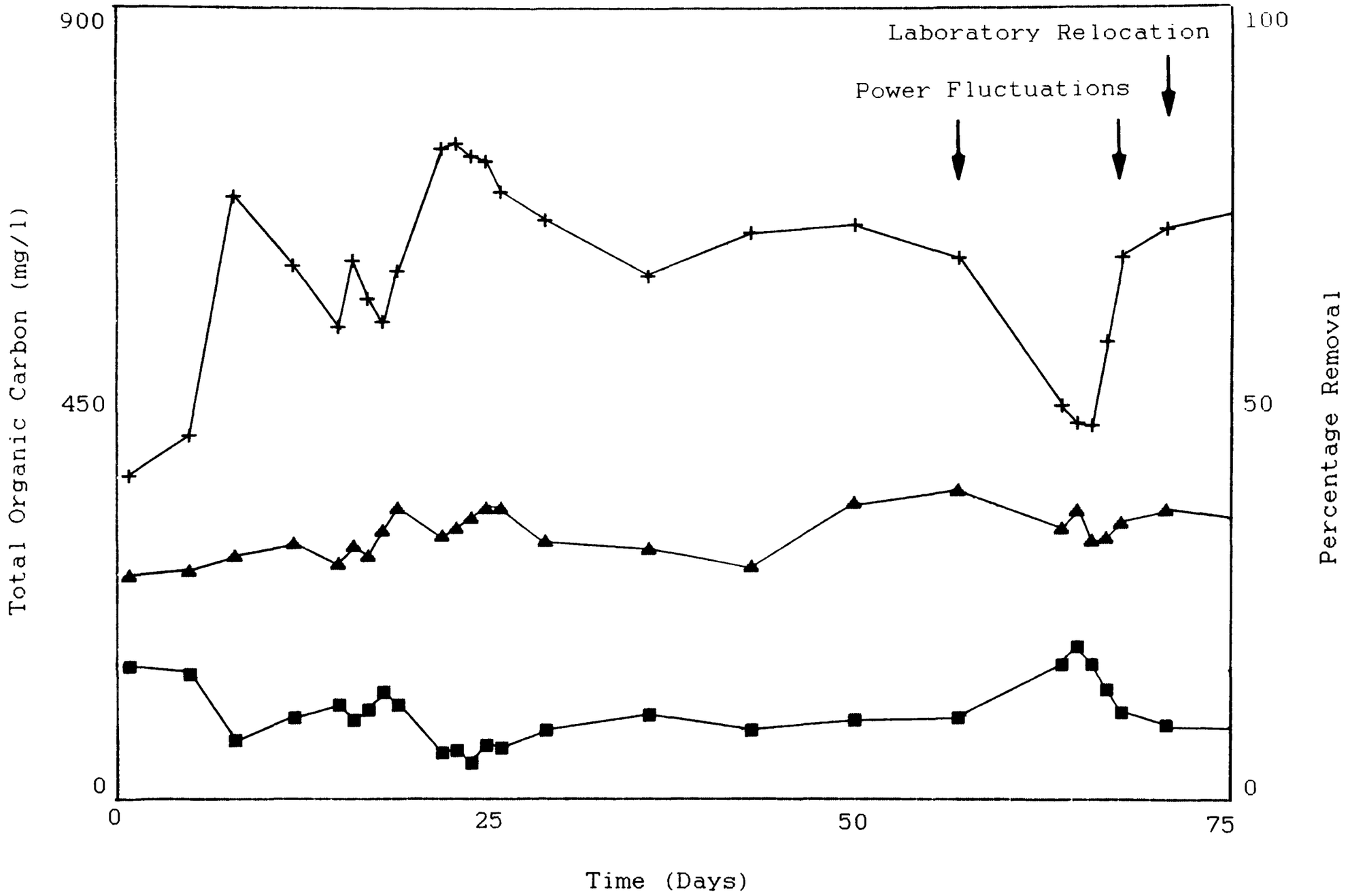


Figure 45a. TOC Analysis of the Experimental Reactor

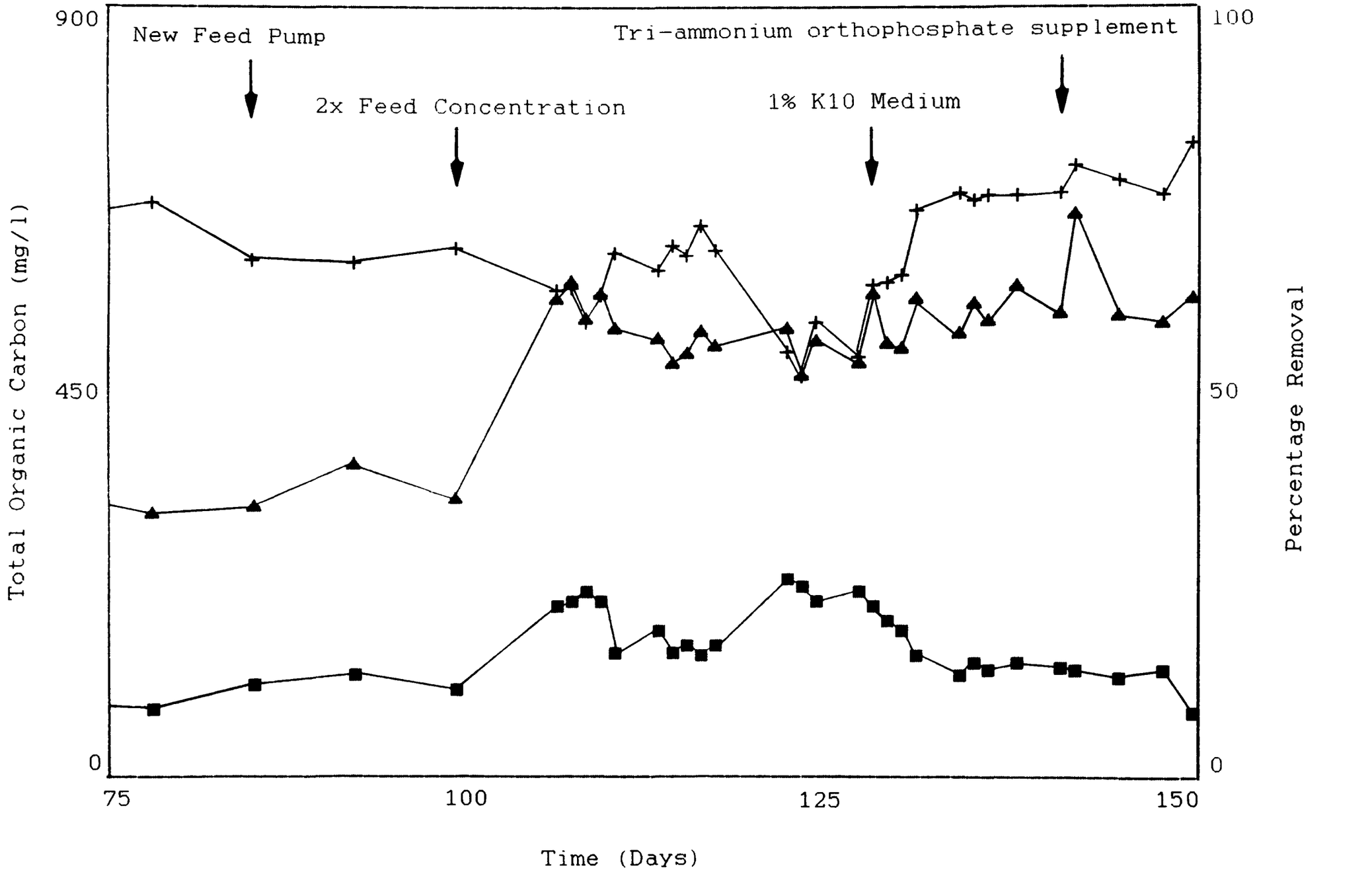


Figure 45b. TOC Analysis of the Experimental Reactor

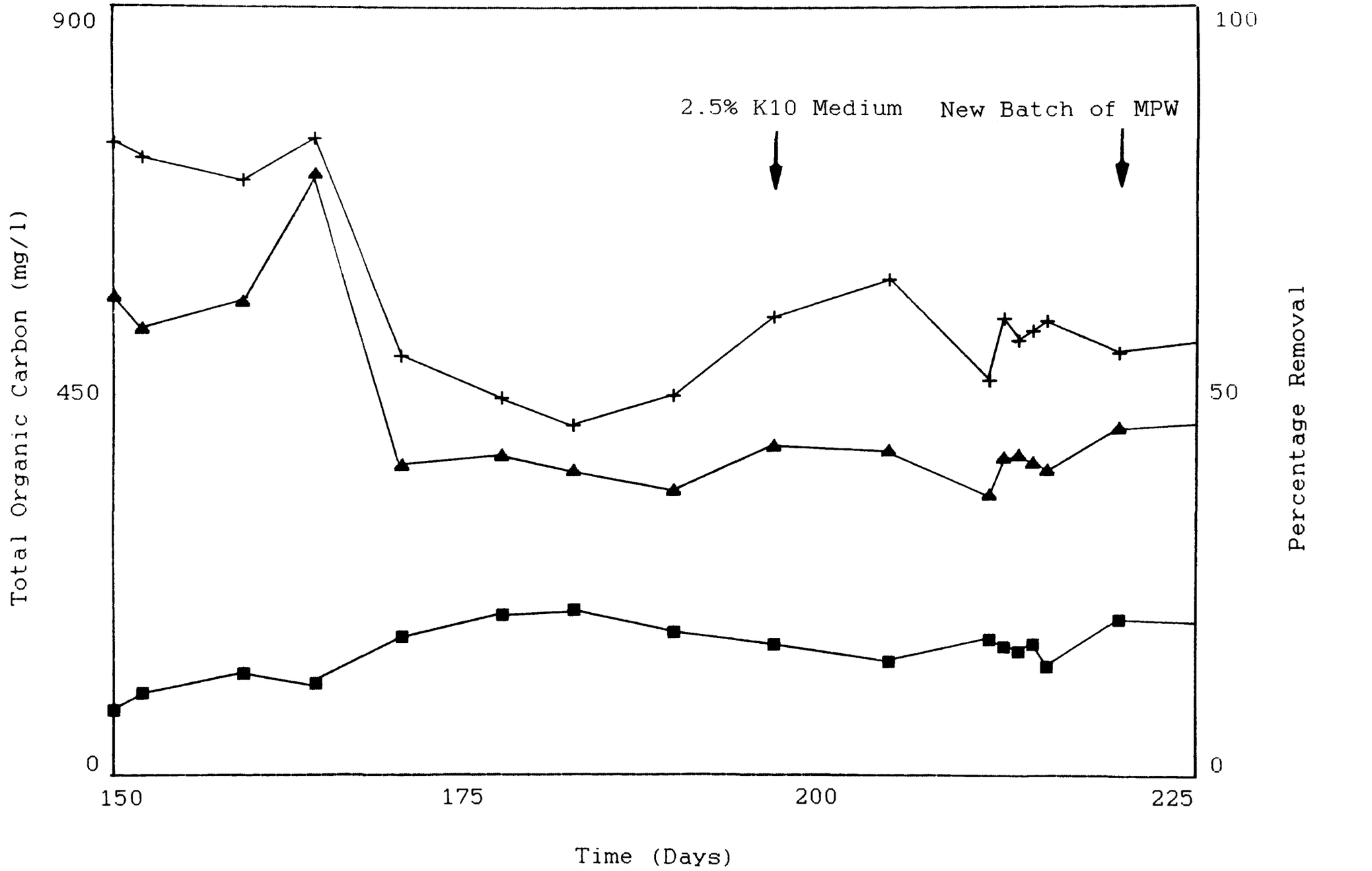


Figure 45c. TOC Analysis of the Experimental Reactor

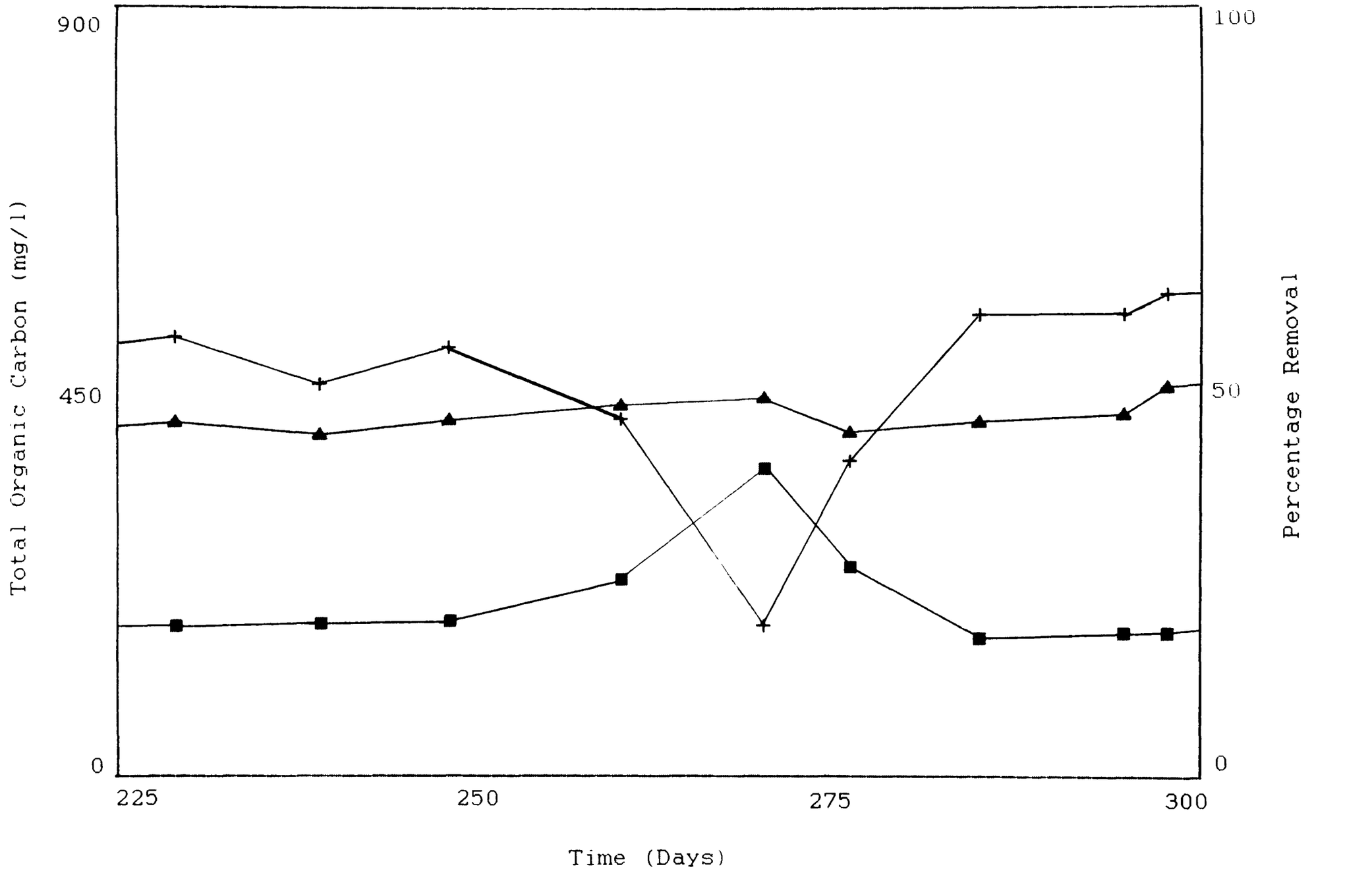


Figure 45d. TOC Analysis of the Experimental Reactor

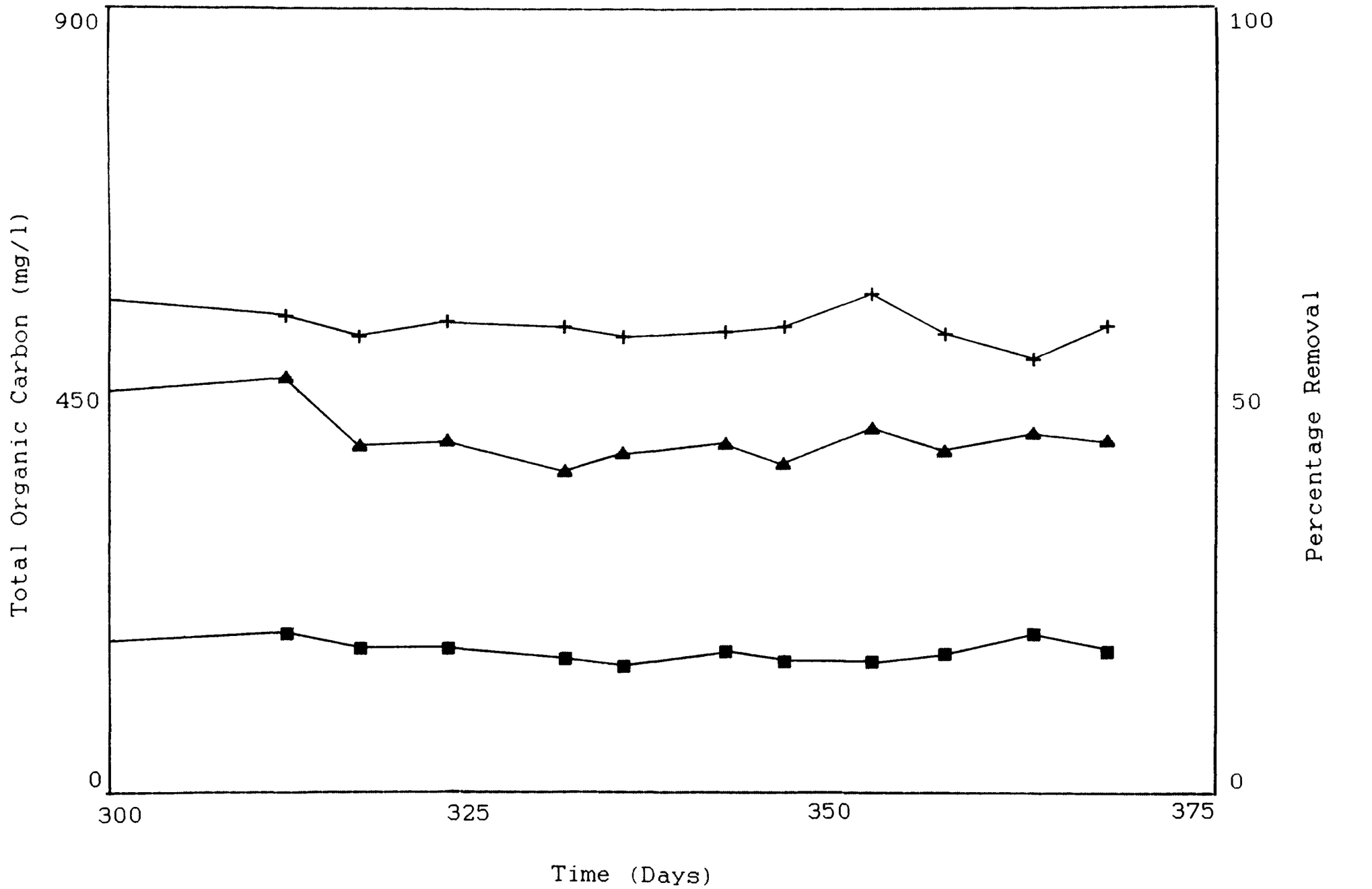


Figure 45e. TOC Analysis of the Experimental Reactor

The overall patterns of the TOC results are identical with those of the COD analyses (Section 3.5.2), however, the removal efficiencies were generally lower; 65 to 75% for the control and 55 to 65% for the experimental reactor.

3.5.4 Control and Experimental Plants - General Comments

Whilst, in general, the plants both worked well on the feed provided, there were one or two points which deserve comment and emphasis. As previously mentioned, for optimum performance, mineral supplements and a continuous air supply are necessary: this type of waste (phenolic/aromatic) appears particularly susceptible to shortages of dissolved oxygen, hardly surprising as the first steps in degrading many of these compounds involve direct incorporation of oxygen by means of oxidase enzymes (Section 1.6.4). Comparisons between the control and experimental reactors indicate, as already pointed out, a discrepancy between the COD and TOC removal efficiencies; since the phenol removal is around 100% in both reactors, not all the K10 wash water effluent could have been degraded, although, equally, a proportion was removed. Thus in over a year's operation, biological acclimatisation to all the available carbon still had not been achieved. Clearly it was desirable to examine the situation more closely.

3.6 PILOT REACTOR RESULTS (2L)

This reactor was initially constructed for preliminary investigations so as to reduce the possibility of inadvertantly killing off the population of the experimental reactor. If the pilot reactor population was killed off it could be re-seeded with a large inoculum from the experimental reactor.

Figures 46a, 46b and 47a, 47b show the COD and phenol analysis results respectively. It can be seen that this reactor required only a short period of time to become established since a large inoculum was used from the experimental reactor which was utilising the same type of feed.

The first phase of experimentation was to observe the effect of utilising phenol as a sole carbon source, replacing the mixed phenolic waste. However this medium was supplemented with additional nutrients (Section 2.2.3) and it was envisaged that these might also contribute to any change in plant performance. In order to avoid this, the nutrients were added to the mixed phenolic waste K10 medium from day 17 onwards and they had no observable effect. When, on day 32, the synthetic phenolic K10 medium was introduced any observed effects were due solely to the carbon source change. It was found that there was an increase in COD removal efficiency with no change in the phenol removal efficiency. It would seem that not even

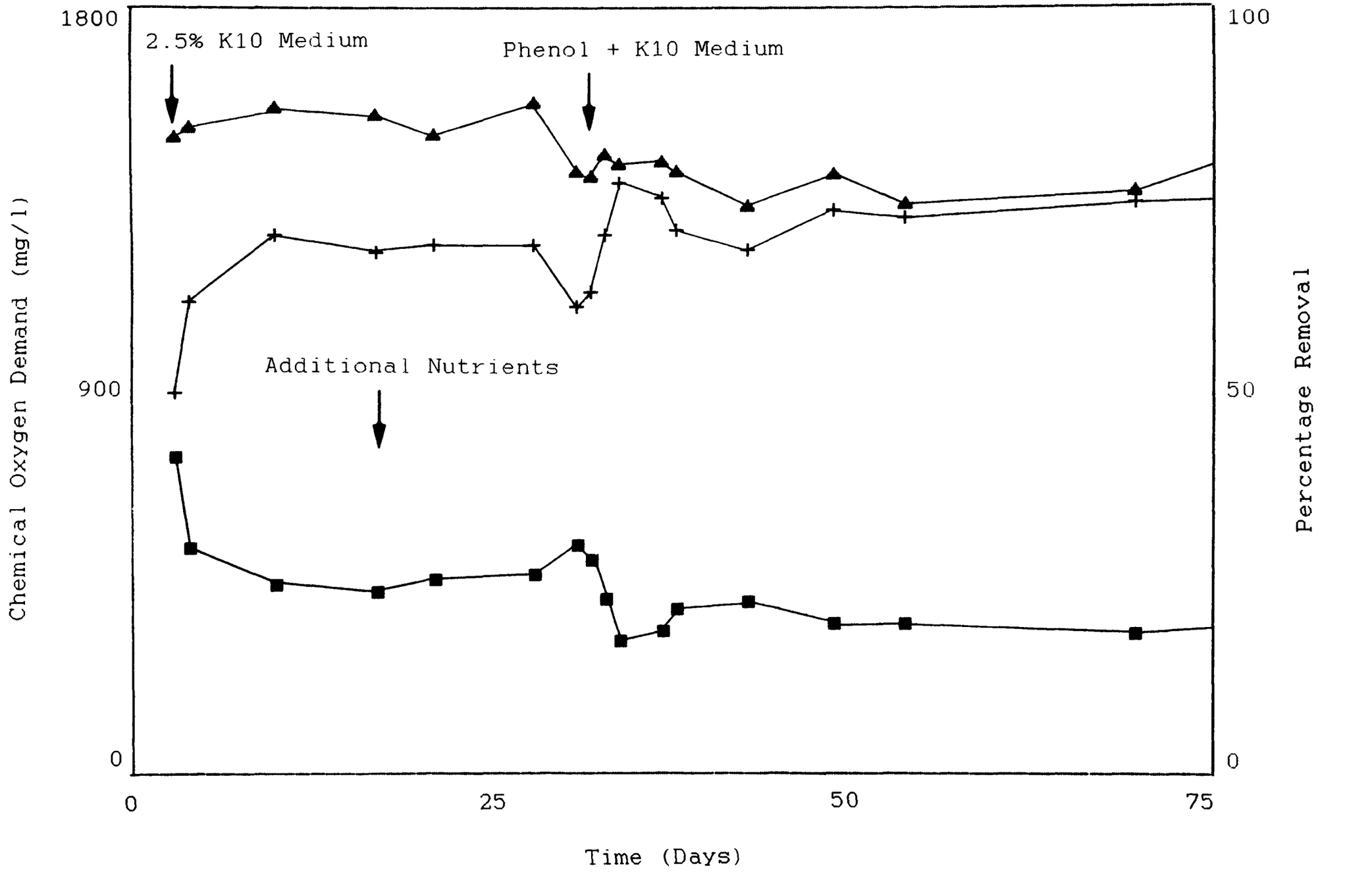


Figure 46a. COD Analysis of the 2L Pilot Reactor

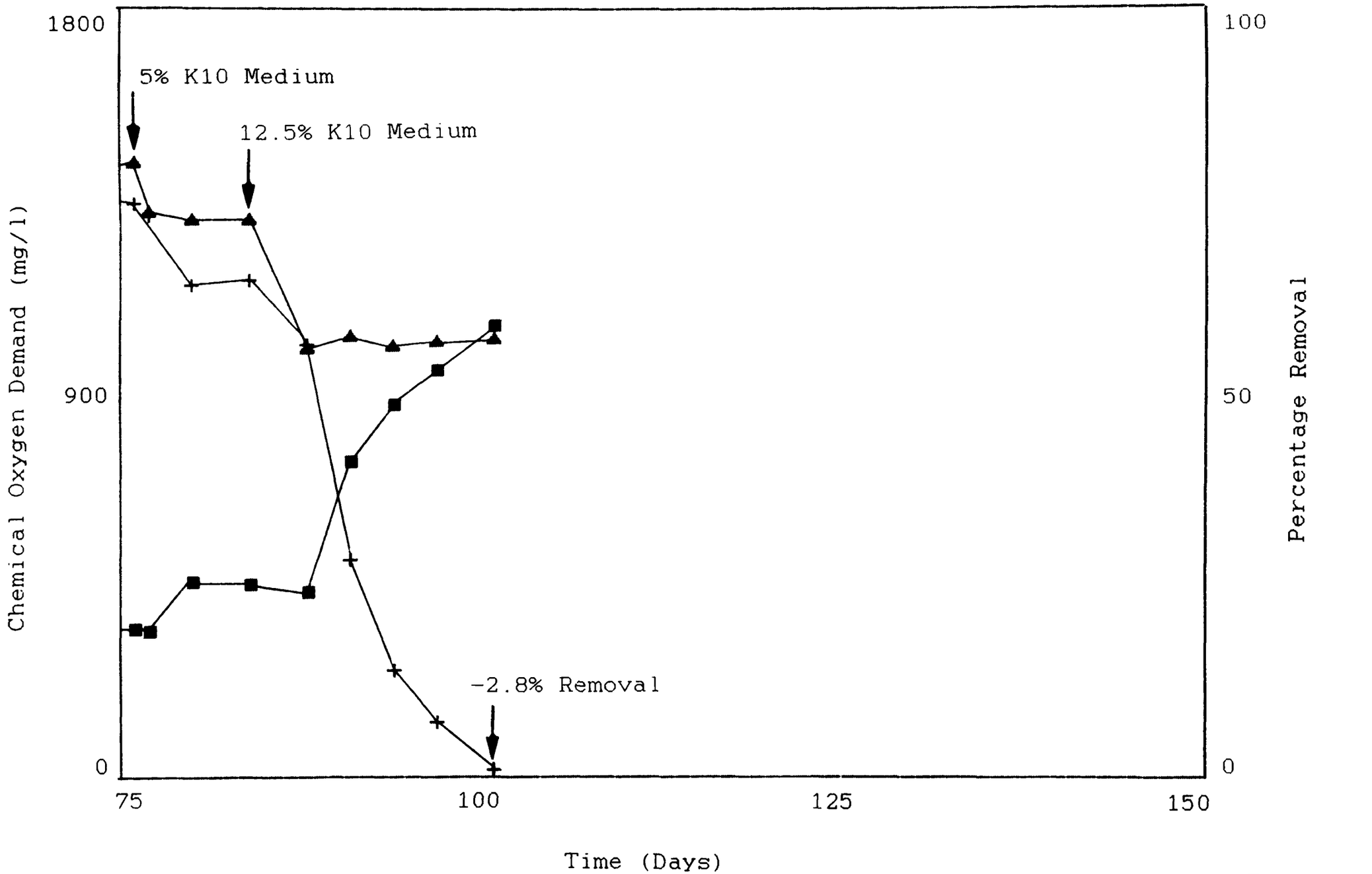


Figure 46b. COD Analysis of the 2L Pilot Reactor

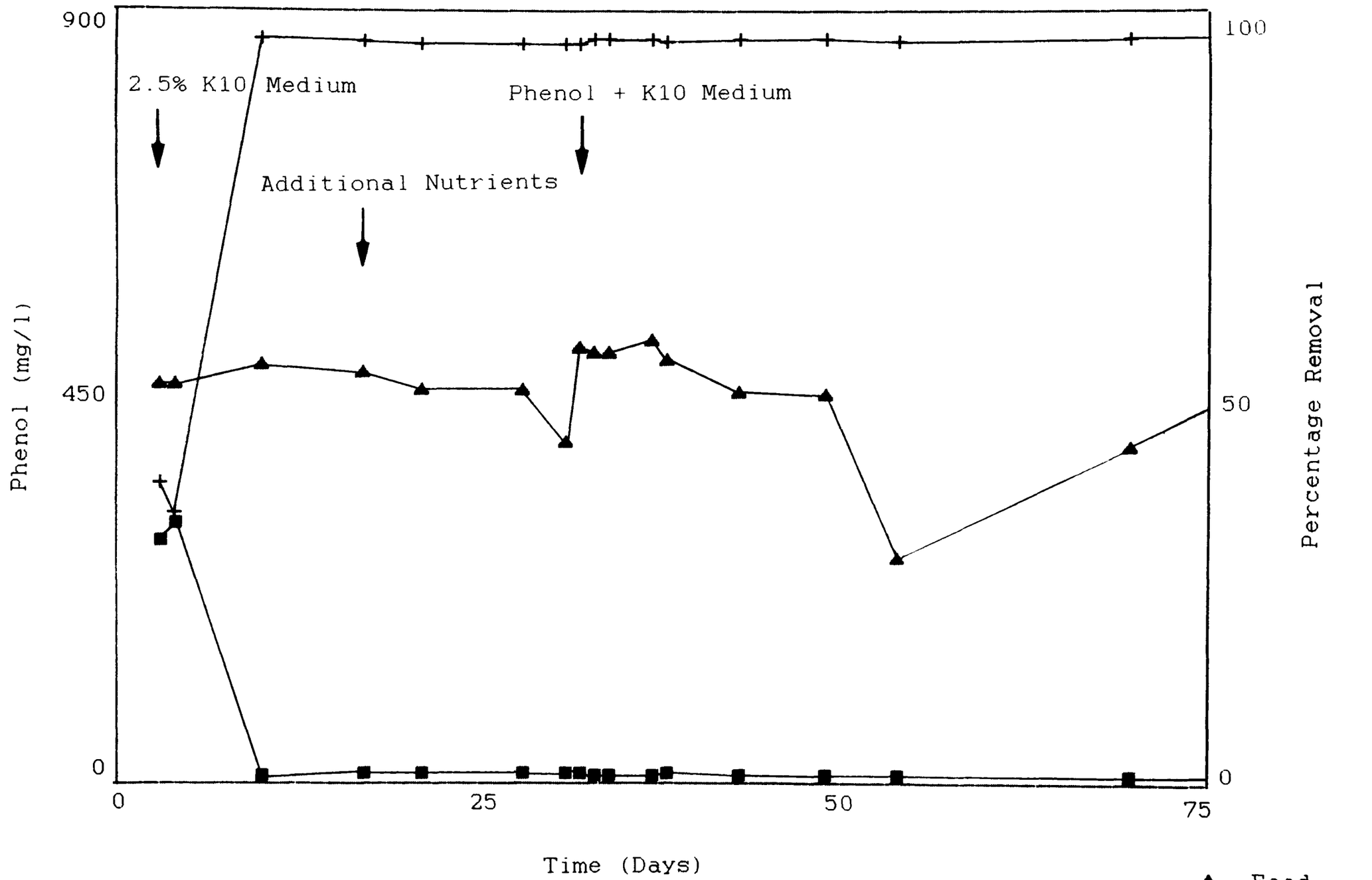


Figure 47a. Phenol Analysis of the 2L Pilot Reactor

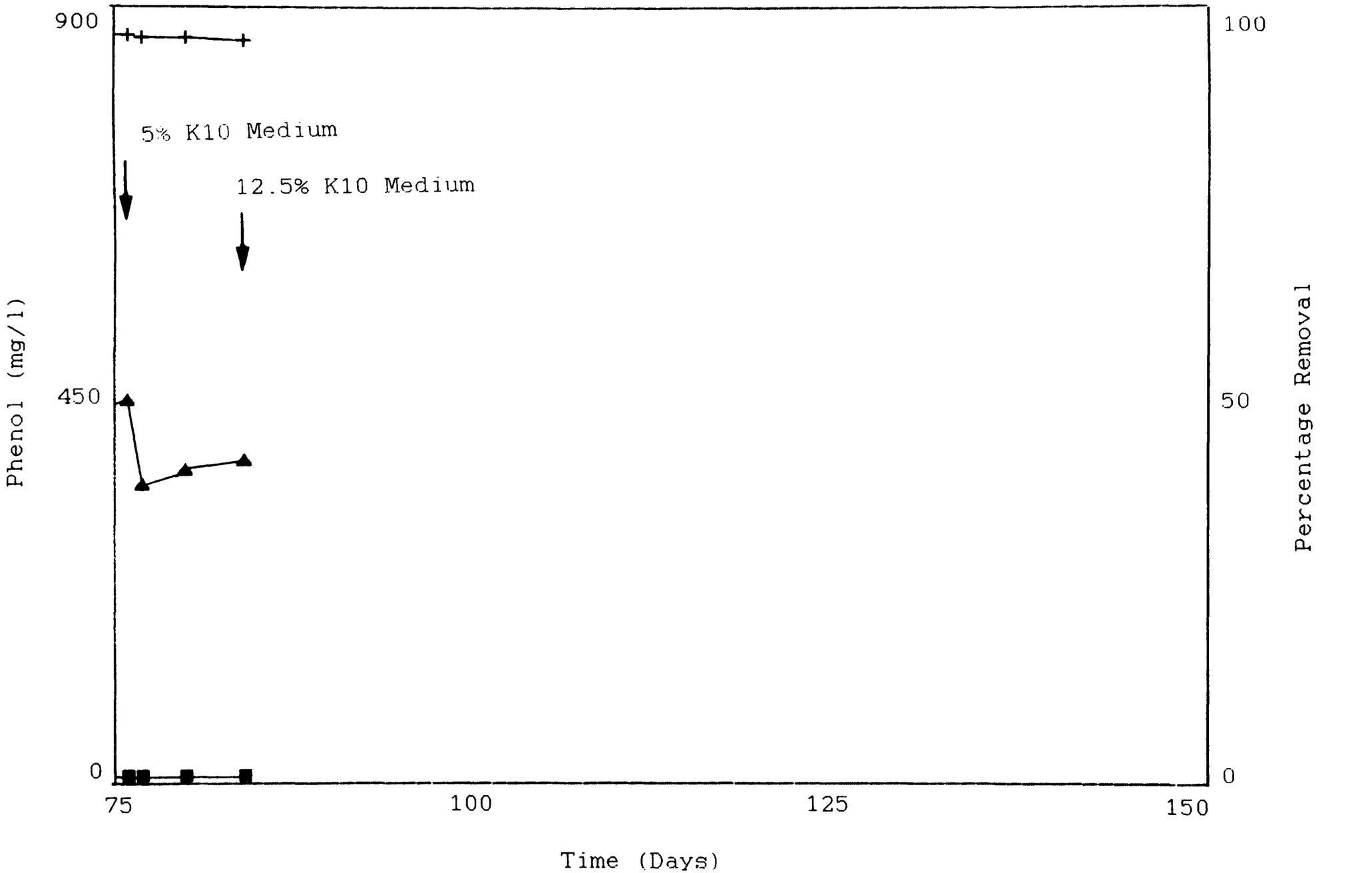


Figure 47b. Phenol Analysis of the 2L Pilot Reactor

all of the mixed phenolic waste components were mineralised completely: these may or may not have been phenolic.

On day 77 the feed was changed to 5% K10 medium (Section 2.2.4.3) with phenol remaining as the additional carbon source. This was maintained for one week during which the phenol removal efficiency was unchanged. The COD removal efficiency decreased by 10%, although the contribution of the additional 2.5% K10 waste COD to that of the effluent, if completely recalcitrant, would have been nearer 20%. These results confirmed the point that some of the K10 wash water effluent components are partially degraded or mineralised by the activated sludge organisms.

The next phase was to increase the concentration of the K10 wash water effluent to 12.5% and remove the phenol carbon supplement although the additional nutrients were maintained. This had drastic effect. The COD removal efficiency dropped rapidly and within two weeks there was no COD removal. Microscopic examination during this period revealed only bacteria which rapidly depleted in number. By day 101 few bacteria could be seen and the negative COD value was probably due to bacterial debris in the reactor effluent. The cause of this devastation could have been due to the toxicity of the waste at this concentration or possibly that the organisms were unable to utilise K10 wash water effluent as a sole carbon source.

3.7 FINAL BIOLOGICAL ASSESSMENT

The reader will recall that reactor 1 was fed on static phenol medium (Section 2.2.5.1) while reactor 2 was fed on decreasing phenol medium (Section 2.2.5.2). The concentration of K10 wash water effluent in the feed of the reactors was 2.5%. At this K10 wash water effluent concentration the two media have equal phenol concentrations and thus the reactors had identical feed composition from day 0 to day 8. During the course of the experiment the concentration of K10 wash water effluent in the feed was increased to 5.0%, then 7.5% and finally to 10%. The concentration of phenol was maintained or adjusted in accordance with the respective medium.

3.7.1 COD Analysis

The COD value results are shown in Figures 48a and 48b for reactor 1 and 49a and 49b for reactor 2. COD removal efficiencies dropped with the addition of 5% K10 wash water effluent and continued to do so when 7.5% K10 wash water effluent was introduced with the feed. A feeding error on day 39, involving a three fold increase in feed rate, caused a sudden drop in the efficiency of both reactors. However, recovery occurred within two days. The COD removal appeared stable from day 84 to 109 and the mean efficiencies were 59.0 and 44.7% for reactors 1 and 2 respectively. The greater efficiency of reactor 1 can be attributed to the composition of the feed to this reactor since it contains higher

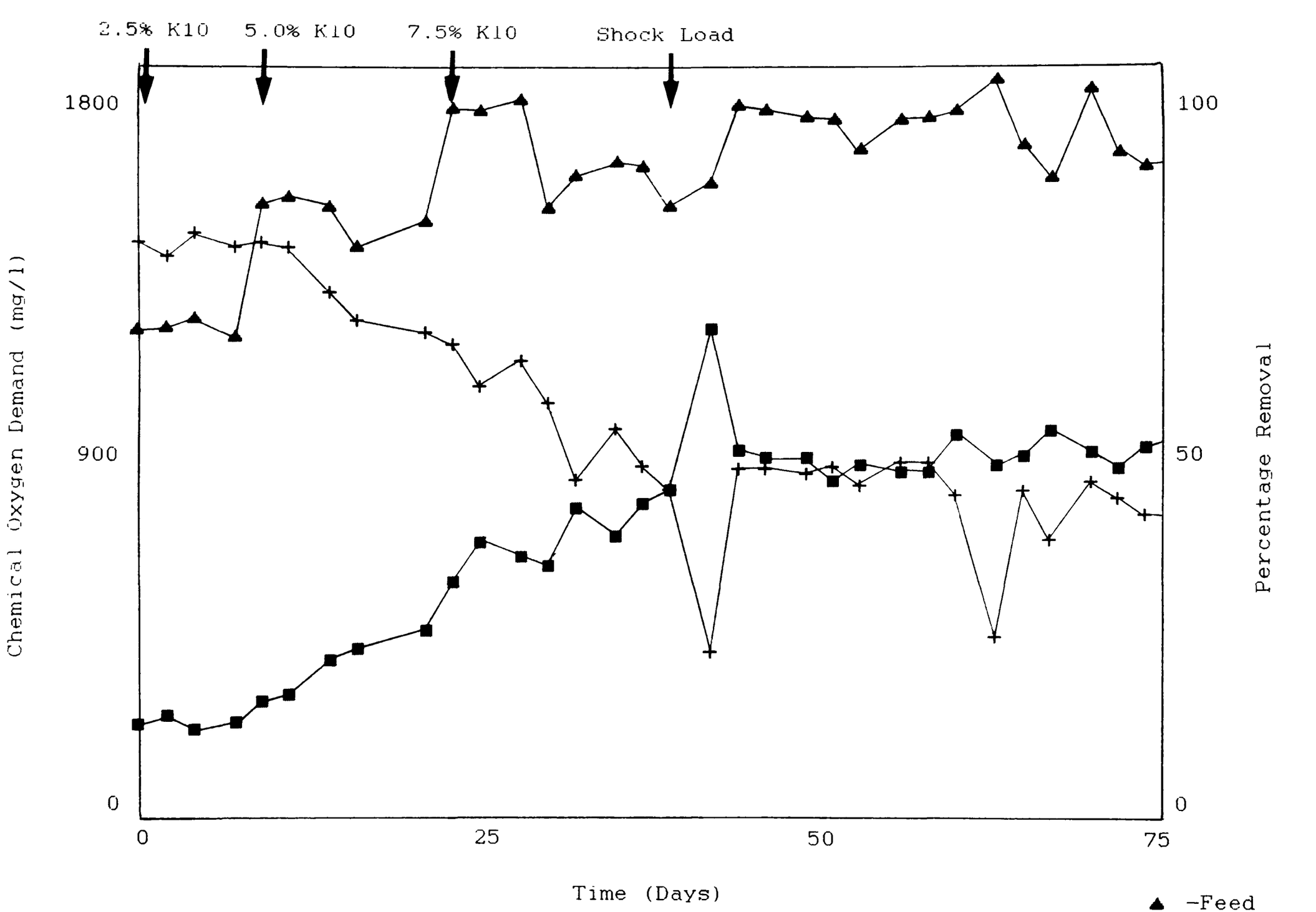


Figure 48a. COD Analysis of Reactor 1. Static Phenol Media

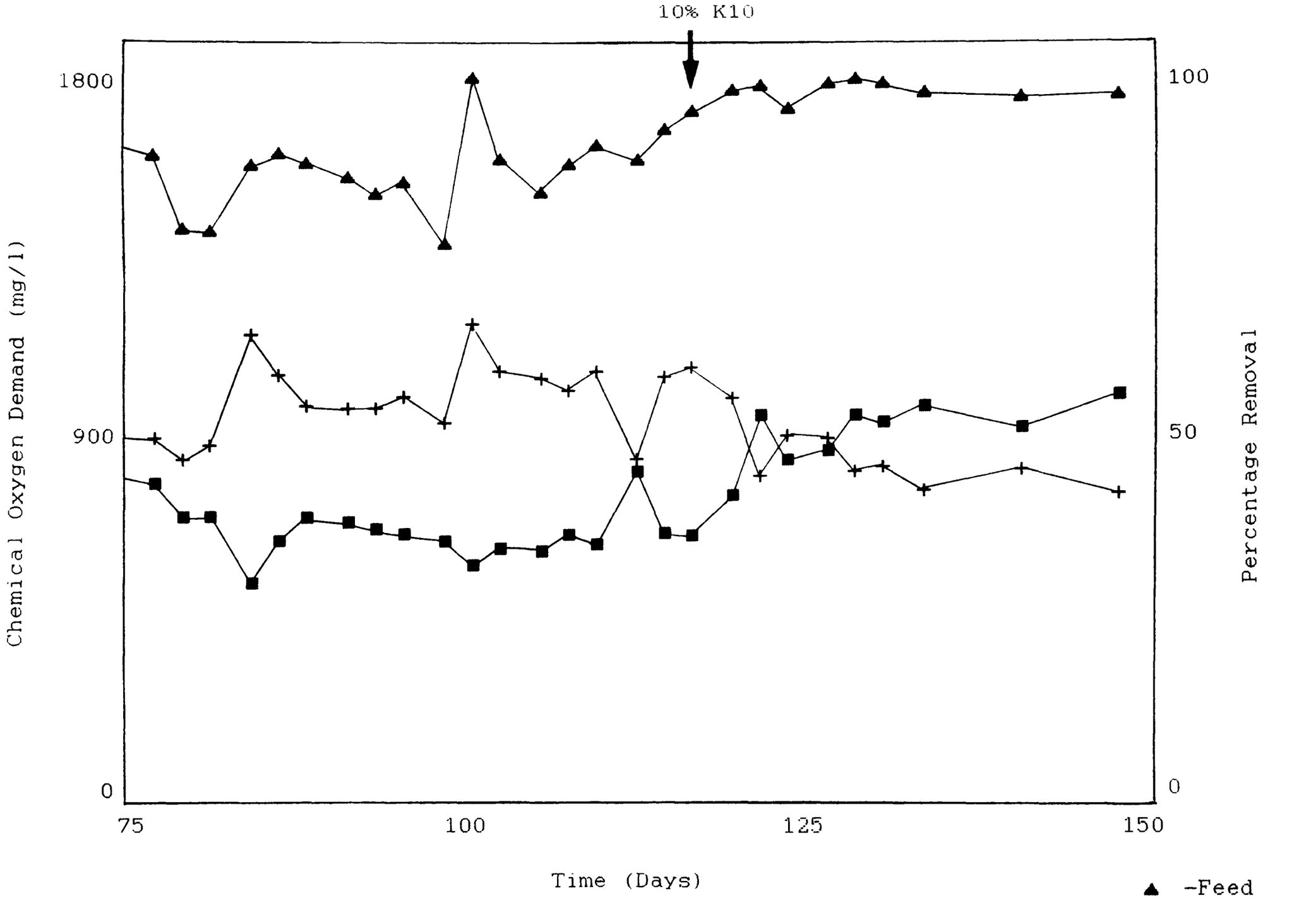


Figure 48b. COD Analysis of Reactor 1. Static Phenol Media

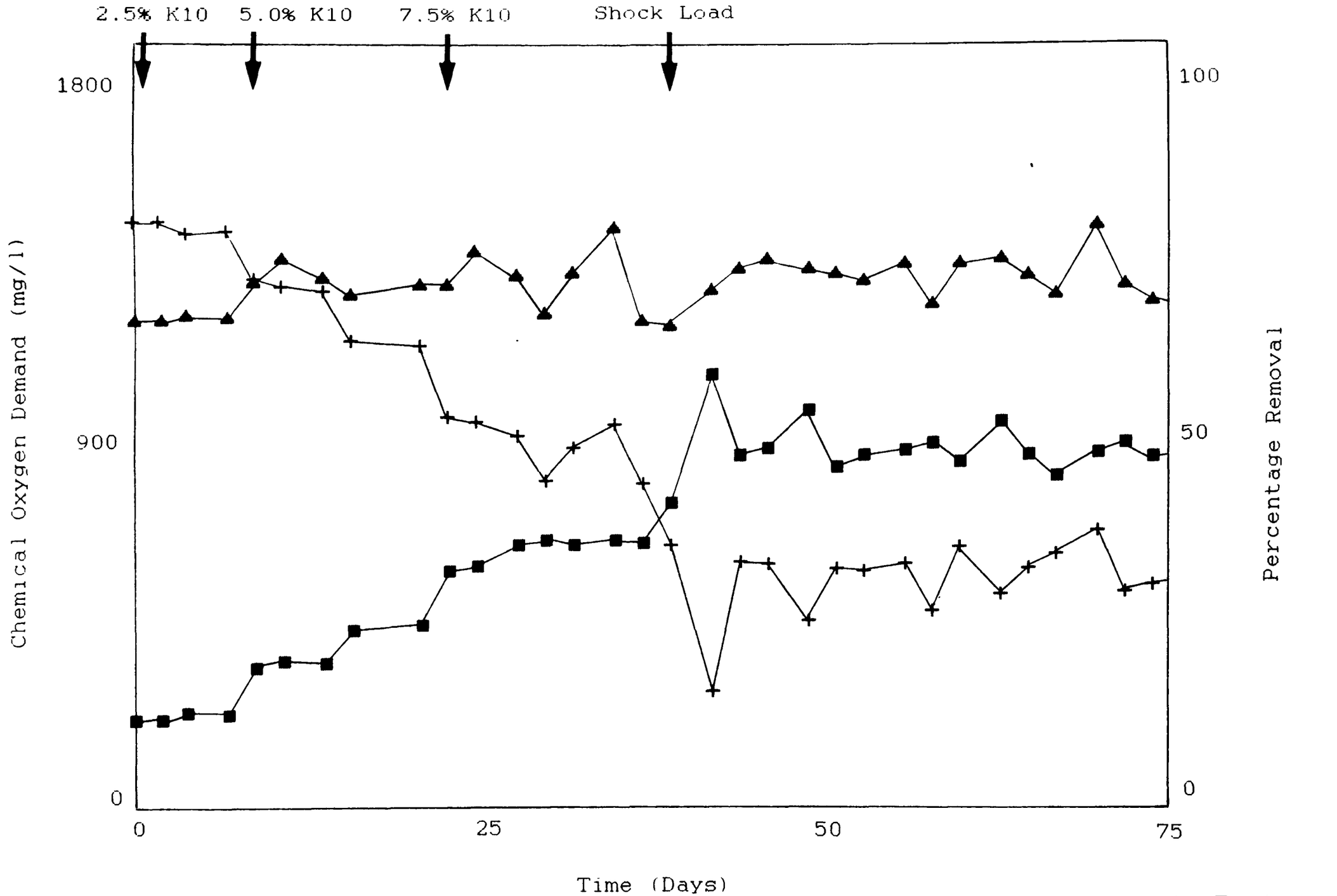


Figure 49a. COD Analysis of Reactor 2. Decreasing Phenol Media

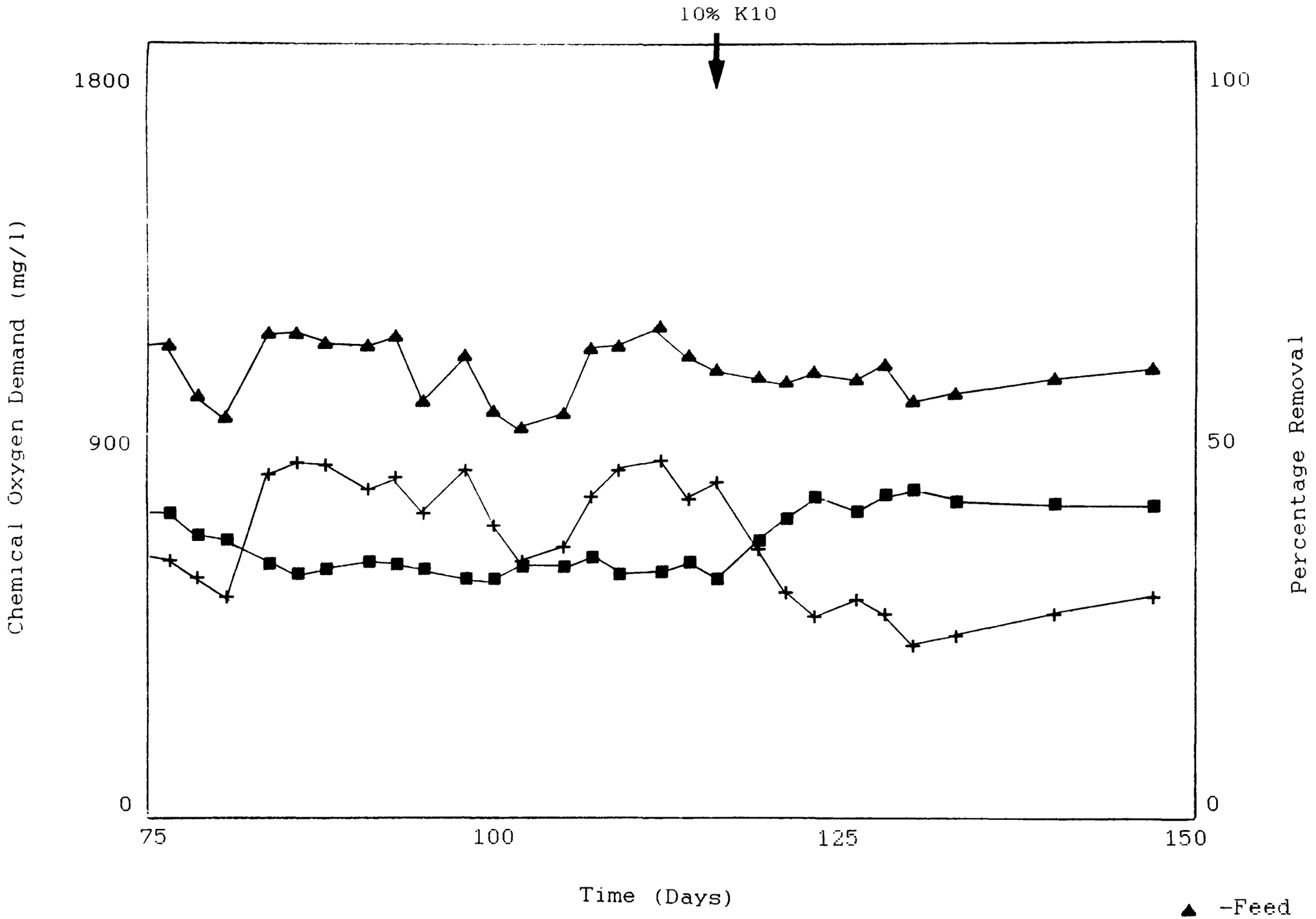


Figure 49b. COD Analysis of Reactor 2. Decreasing Phenol Media

concentrations of phenol which is normally metabolised readily by these sludge microorganisms. Examination of the mean COD values of the treated effluent over the same period gave cause for concern. The mean value for reactor 1 was 657mg/l whilst for reactor 2 it was 624mg/l. Although the difference is slight it may indicate that not all of the phenol carbon source was being metabolised to completion.

The introduction of 10% K10 waste, on day 116, to the feed of the reactors gave rise to a further increase in the COD value of the treated effluent leaving the reactors. The mean values from day 121 to 147 were 961mg/l and 789mg/l for reactors 1 and 2 respectively. This was an increase of 304mg/l for reactor 1 whilst the increase for reactor 2 was only 165mg/l. This is quite a large difference and adds further evidence to the suggestion that the phenol may not be completely metabolised by the sludge microorganisms under these conditions.

3.7.2 Phenol Analysis

The phenol analysis results are shown in Figures 50a and 50b for reactor 1 and 51a and 51b for reactor 2. Phenol removal efficiencies did not alter as significantly with the addition of increasing K10 wash water effluent concentrations as did the COD efficiencies. The mean phenol removal efficiencies were 95.5% for reactor 1 and 93.3% for reactor 2 from day 84 to 109 (7.5% K10). The mean phenol concentrations in the treated effluent over the same period were 20.2mg/l and 19.4mg/l for reactors 1 and 2 respectively. Clearly, then, most of the phenol was at least partially metabolised during this period to a degree that it would no longer react in the phenol assay (Section 2.4.5). The introduction of 10% K10 waste caused a further increase in phenol concentrations in the treated effluent with means of 27.4mg/l and 27.0mg/l for reactors 1 and 2 respectively from day 121 to 147. This small increase is not nearly as large as that which occurred with the COD analysis.

Phenol levels in the treated effluent only reached high levels after the shock load on day 39 where the concentration rose to 228mg/l (56.7% efficiency) in reactor 1 and 150mg/l (59.1% efficiency) in reactor 2.

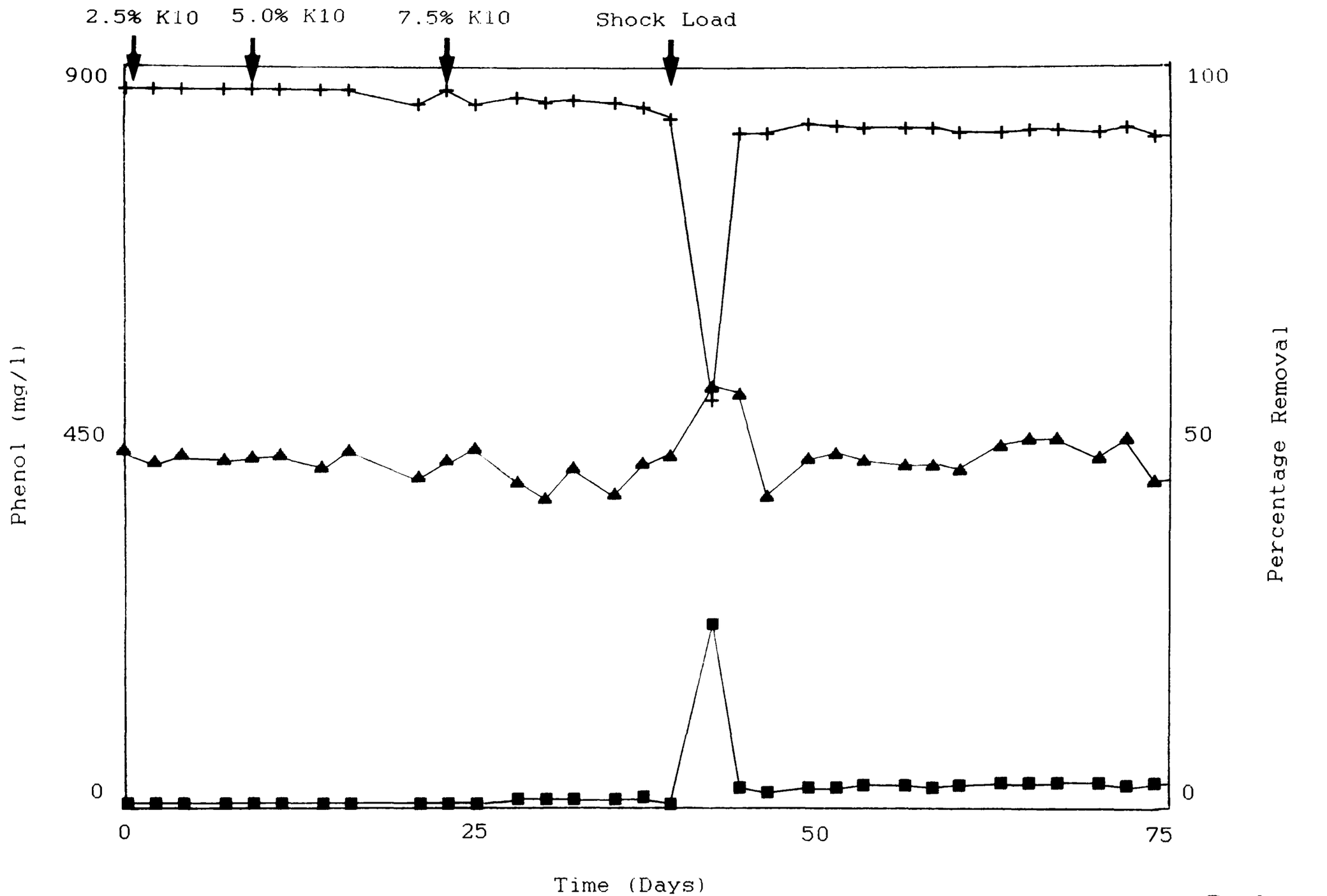


Figure 50a. Phenol Analysis of Reactor 1. Static Phenol Media

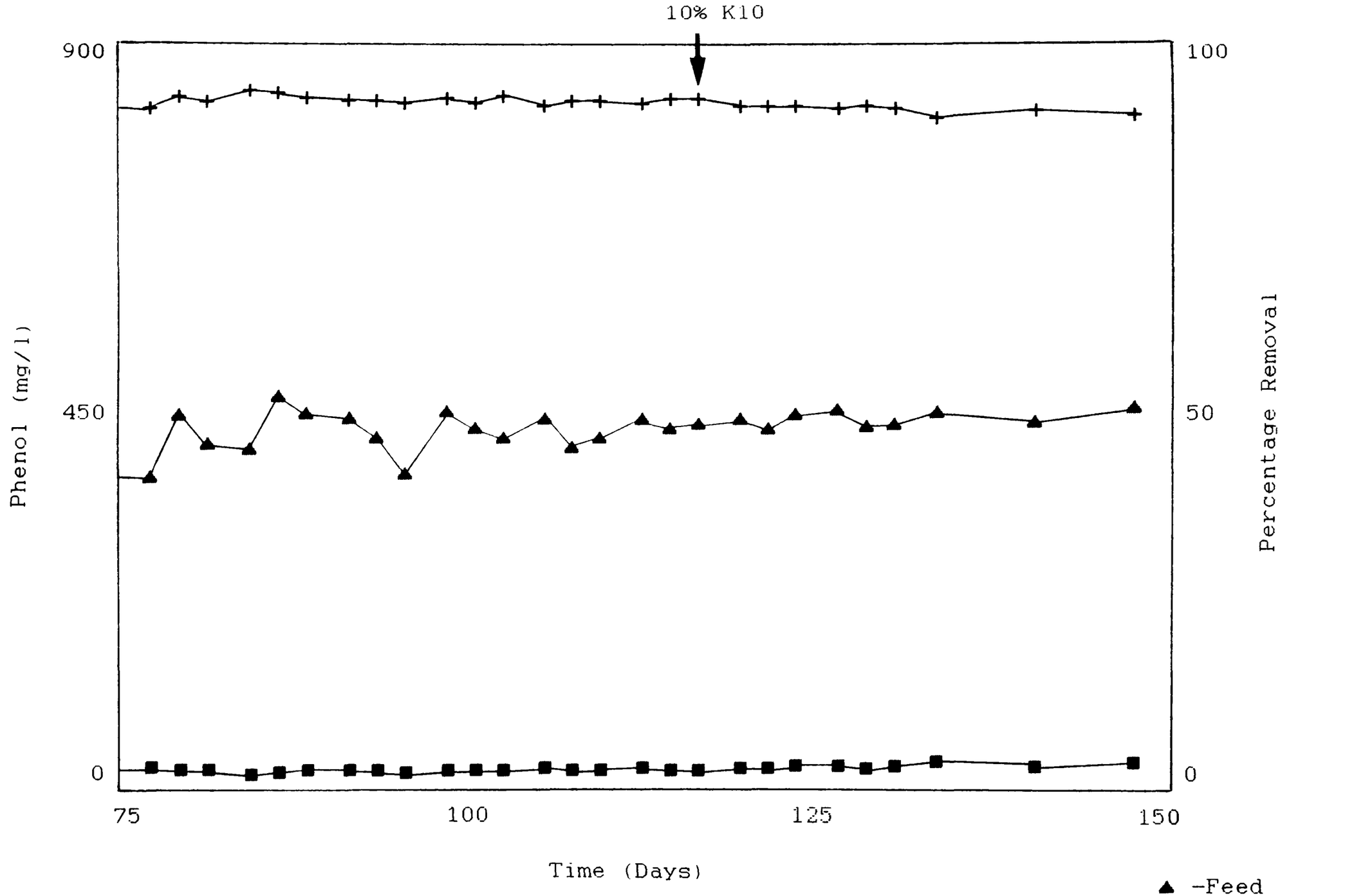


Figure 50b. Phenol Analysis of Reactor 1. Static Phenol Media

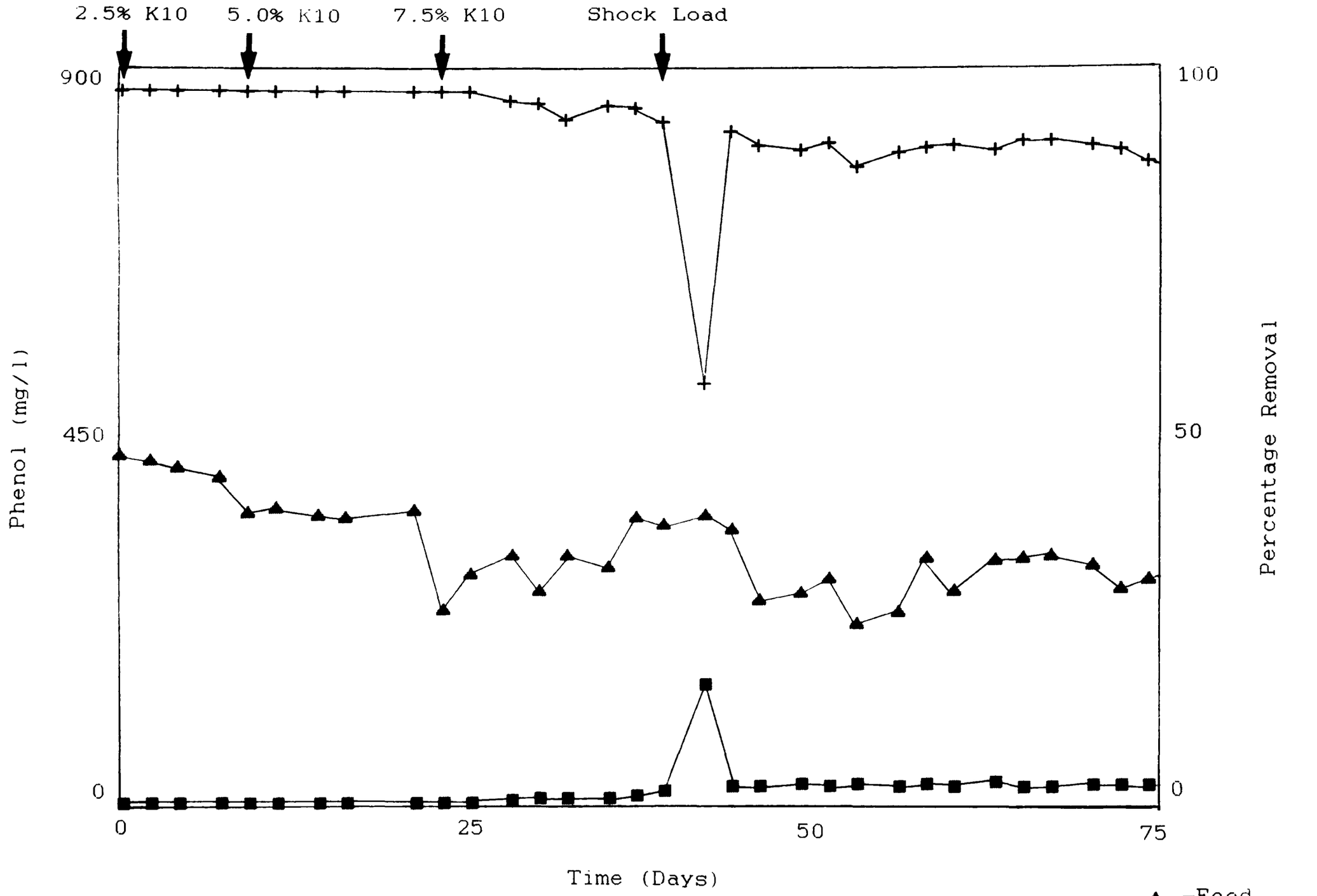


Figure 51a. Phenol Analysis of Reactor 2. Decreasing Phenol Media

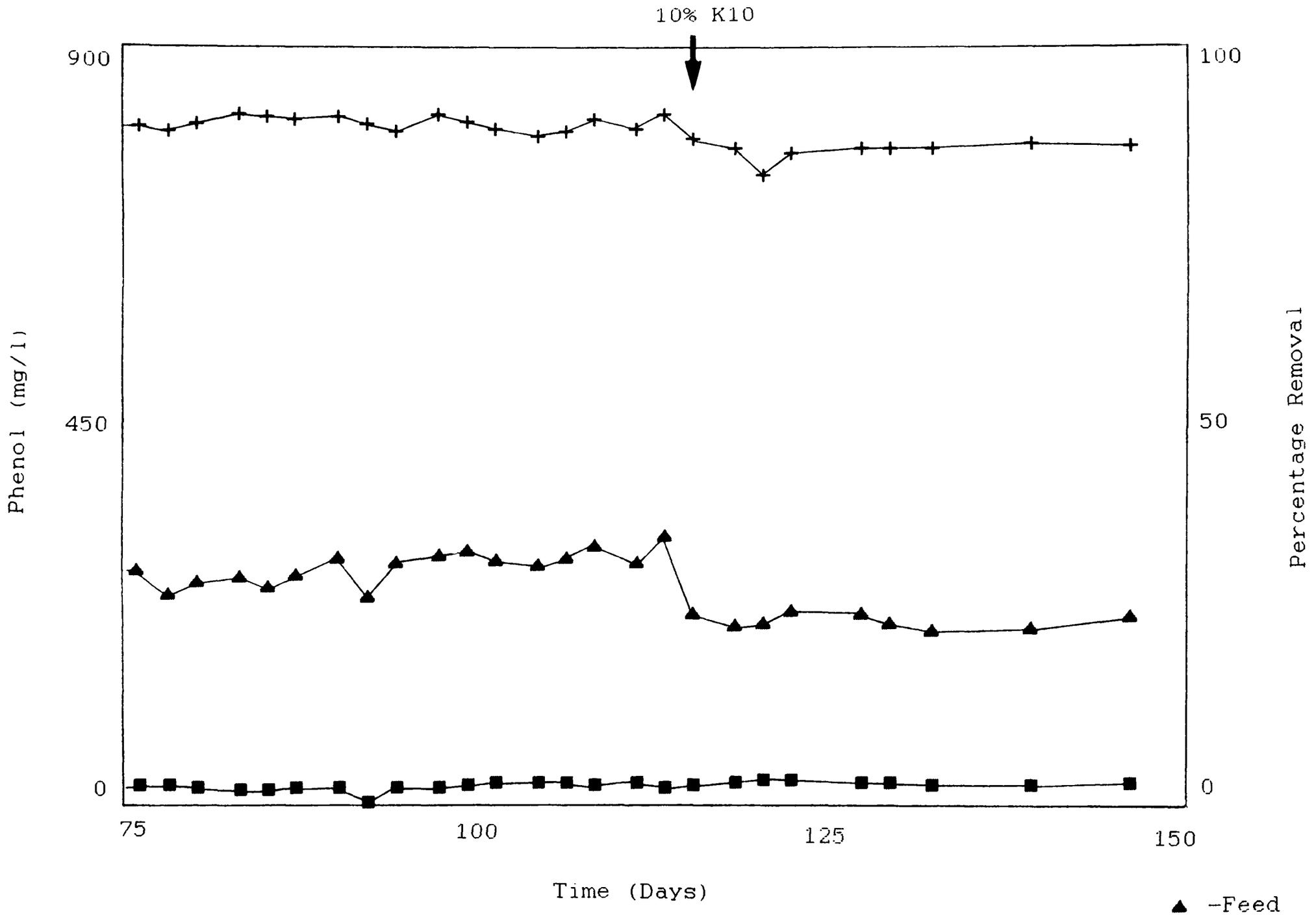


Figure 51b. Phenol Analysis of Reactor 2. Decreasing Phenol Media

3.7.3 TOC Analysis

The TOC analysis results are shown in Figures 52a and 52b for reactor 1 and 53a and 53b for reactor 2. TOC removal efficiencies dropped rapidly in both reactors with the addition of 5% K10 waste and further still with 7.5% K10 waste concentrations. The shock load on day 39 did not cause as dramatic change in efficiency as for the COD and phenol determinations since the TOC removal was already less than 25% efficient for both reactors.

The mean TOC values of the treated effluent from day 84 to 109 (7.5% K10 waste concentration) were 301mg/l (29.9% efficiency) and 288mg/l (20.8% efficiency) for reactors 1 and 2 respectively. The addition of 10% K10 waste in the feed of these reactors resulted in a further decrease in efficiency. The mean TOC values of the treated effluent from day 121 to 147 were 535mg/l (10.2% efficiency) and 413mg/l (7.8% efficiency) for reactors 1 and 2 respectively. Over the same period the mean feed TOC values were 596mg/l for reactor 1 and 448mg/l for reactor 2. Thus the difference between reactor feed and effluent was 61mg/l for reactor 1 and 35mg/l for reactor 2. This very low removal, much less than the contribution to the TOC value made by the phenol component in the reactor feed, further supports, in fact confirms, the incomplete metabolism of phenol which is caused by elevated K10 wash water effluent concentrations.

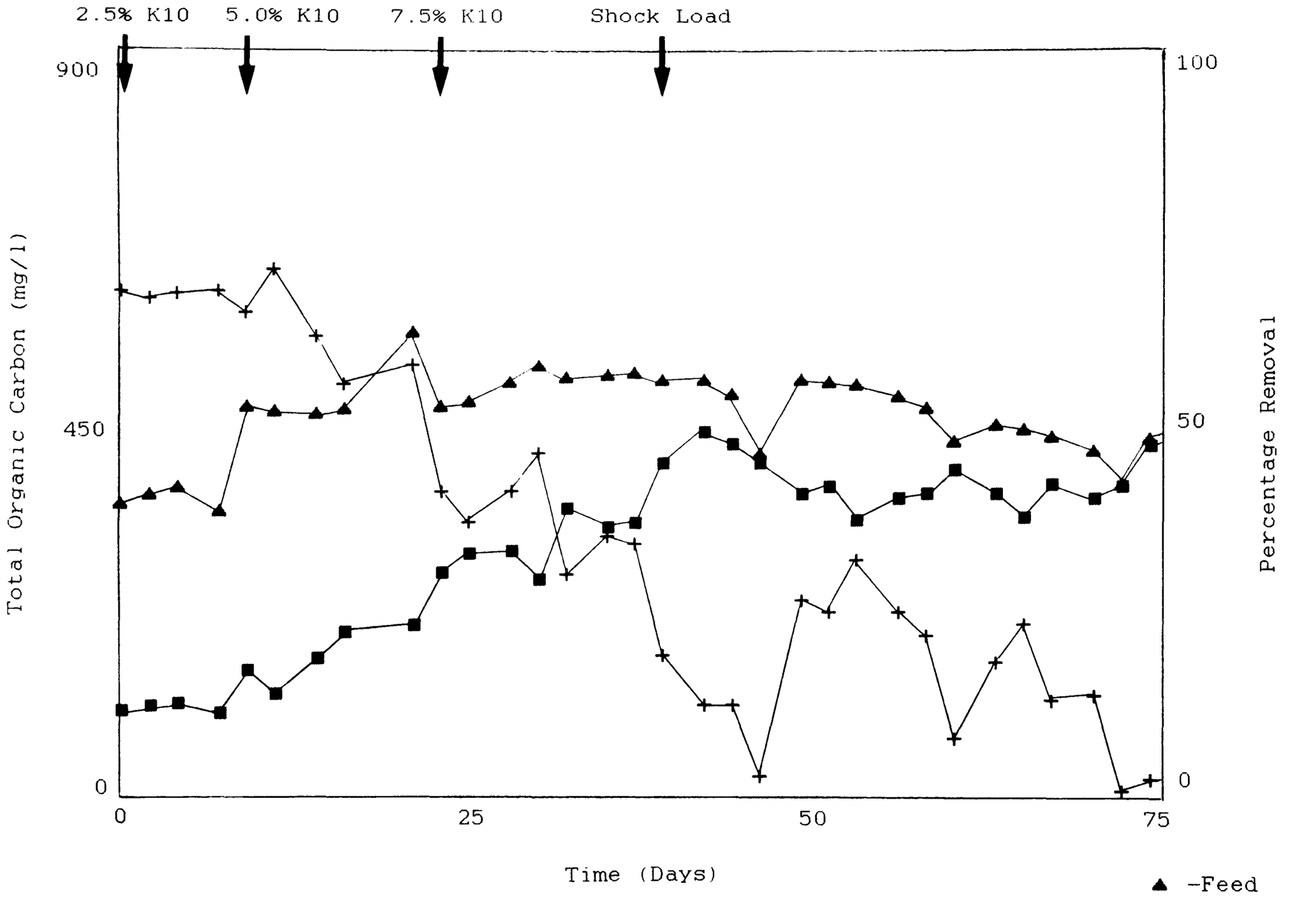


Figure 52a. TOC Analysis of Reactor 1. Static Phenol Media

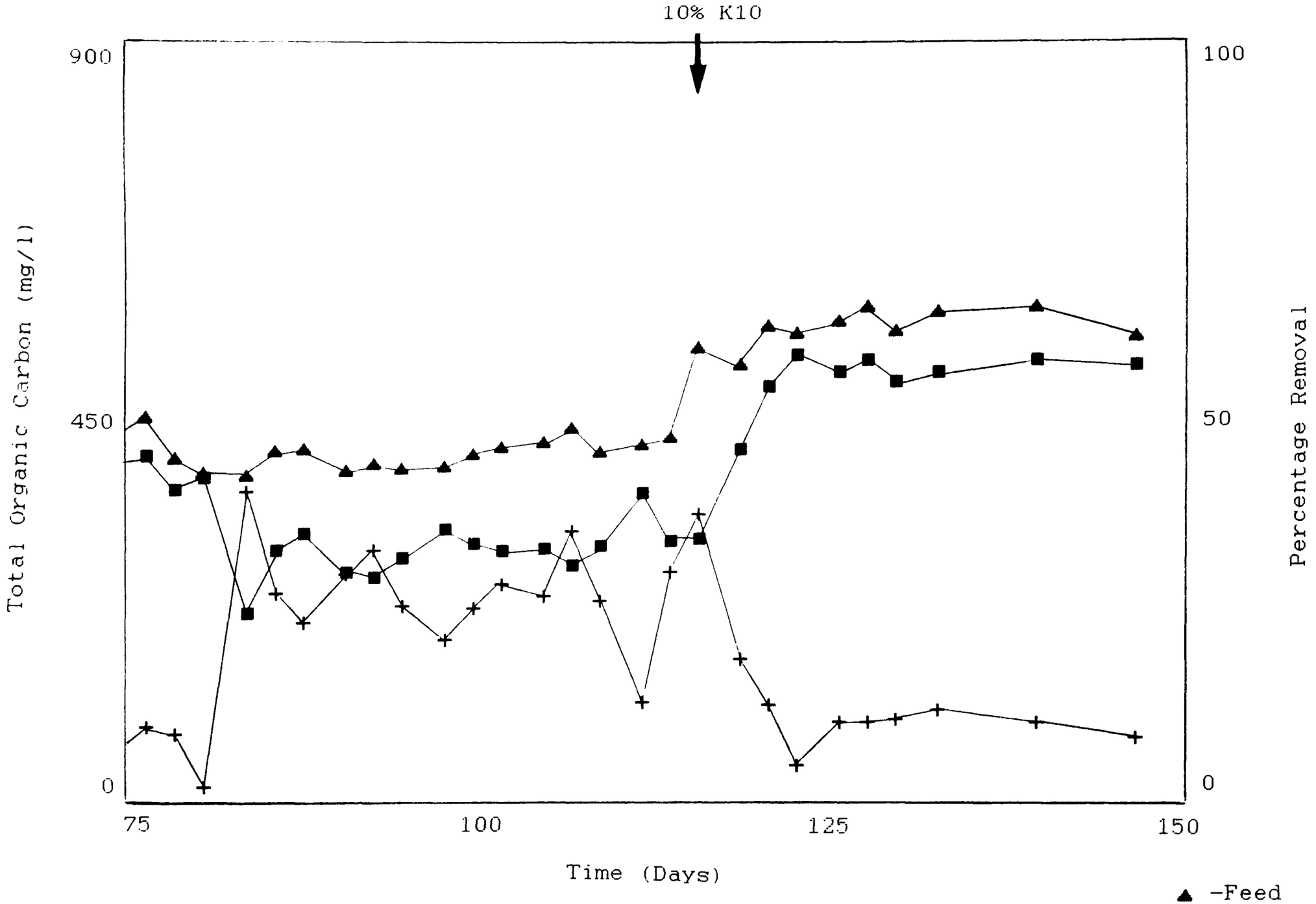


Figure 52b. TOC Analysis of Reactor 1. Static Phenol Media

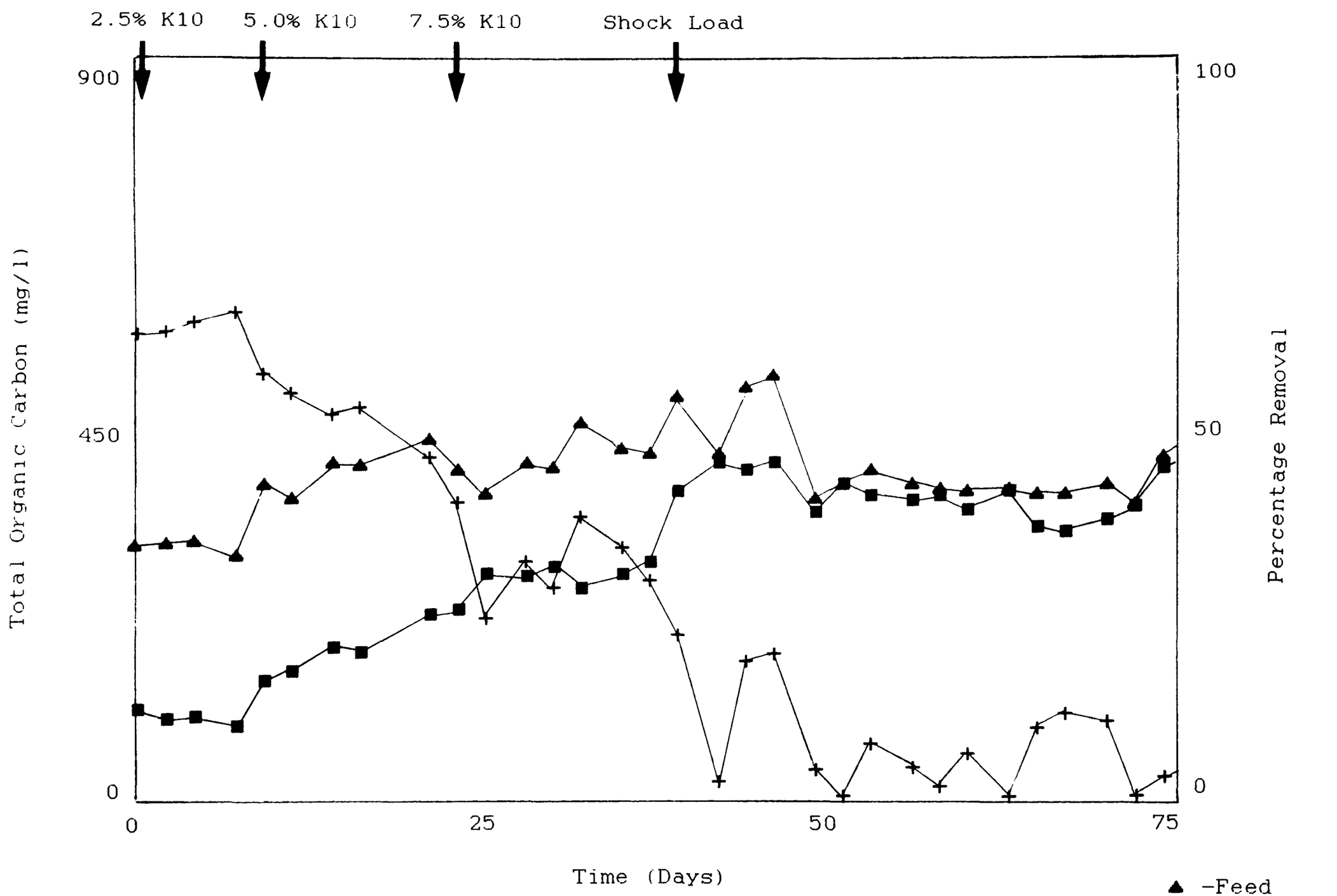


Figure 53a. TOC Analysis of Reactor 2. Decreasing Phenol Media

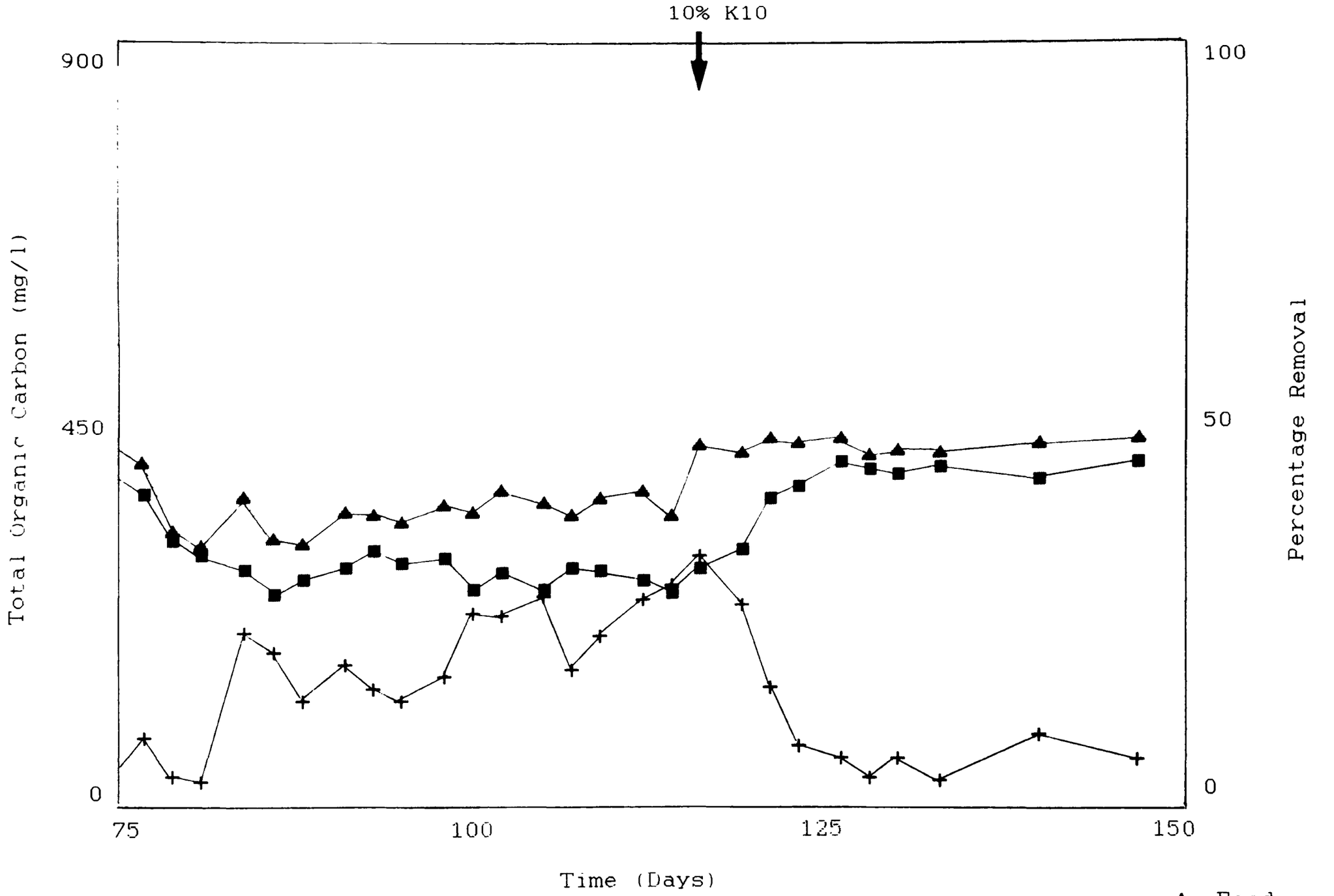


Figure 53b. TOC Analysis of Reactor 2. Decreasing Phenol Media

3.7.4 Final Biological Assessment - General Comments

The reactors were maintained for several months on 10% K10 wash water effluent feed after day 147. Occasional sampling during this period did not reveal any change in the status of the reactors. It was thus concluded that whilst 10% K10 waste was not totally bactericidal it was most certainly inhibitory causing incomplete metabolism of a carbon substrate (phenol) which would ordinarily have been readily metabolised to completion.

During the course of this experiment microscopic examinations with photographic records of the sludge were made. Changes were observed and these are discussed in Section 4.1.

CHAPTER 4

FURTHER INVESTIGATIONS AND ANALYSES

The results of analyses for phenol, COD and TOC on feed and effluent, discussed previously, are those which would normally be monitored in most sewage treatment laboratories. However, they provide no further detailed information as to the toxic nature of the waste, nor do they indicate which, if any, of the K10 wash water effluent components are mineralised or partially degraded. Consequently, more detailed work was carried out to investigate these matters further - photo-microscopy, HPLC, ultra-violet wavelength scans and oxygen uptake studies (Chapter 5).

4.1 MICROSCOPIC SLUDGE EXAMINATION

Plates 5 to 7 show typical samples taken from the activated sludge plants before introduction of K10 wash water effluent. There was a wide selection of protozoa including ciliates, rotifers, nematode worms and higher organisms such as arachnids. The bacterial flocs did not contain filamentous fungi or large quantities of cysts which are often associated with sludge in poor health.

The introduction of 2.5% K10 wash water effluent caused the disappearance of many vorticella and flagellates and all ameobae. This was also accompanied by a slight increase in the number of cysts within the sludge flocs. Plate 8a shows a

Vorticella sp. which is obviously under stress since adverse conditions cause certain species to break free from the stalk and swim away to settle elsewhere. During this process the mouth region closes and a ring of aboral cilia form in the region of the stalk. The organism then breaks free, swims off, and settles in an environment which has more suitable conditions. In the free swimming stage (the teletroch stage) it is not possible to identify the organism since the nature of the stalk is used in identification, Plate 8b shows the teletroch stage of a Vorticella sp.

5.0% and 7.5% K10 wash water effluent concentrations caused further decreases in the relative number of protozoal organisms essentially leaving only a very sparce sprinkling of protozoa, together with some nematode worms and a much reduced number of higher organisms.

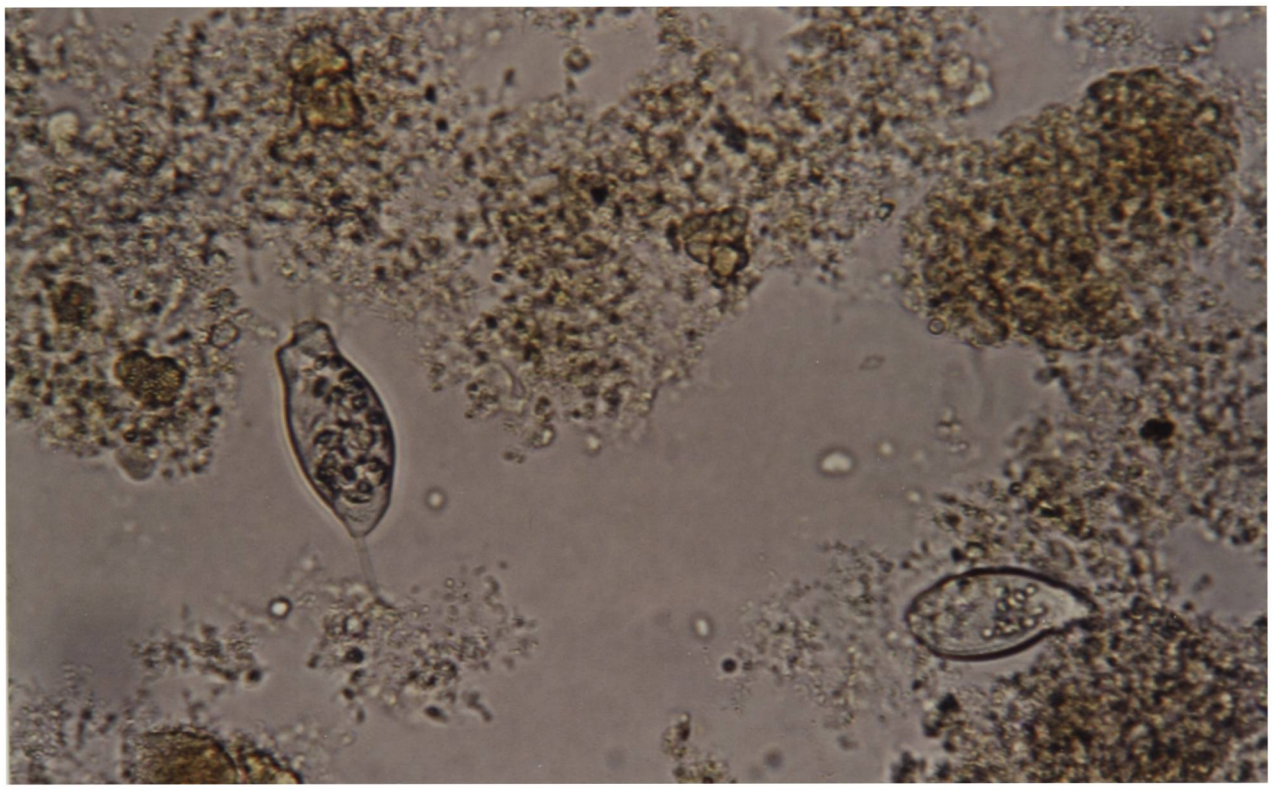
10% K10 wash water effluent introduction resulted in the complete disappearance of all the remaining protozoa. Nematode worms still remained, however. Plates 9 and 10 show a very obvious increase in the relative numbers of nematode worms and also stages of egg development. These changes were also accompanied by dramatic changes in sludge floc composition - an increase in filamentous organisms (Plate 11) and also large numbers of cysts as in Plates 9 (with the nematode worms) and also Plate 12. The reason for the proliferation of nematodes might have been due to the absence of competition, or the warmer weather (Dosanjh, 1985) although

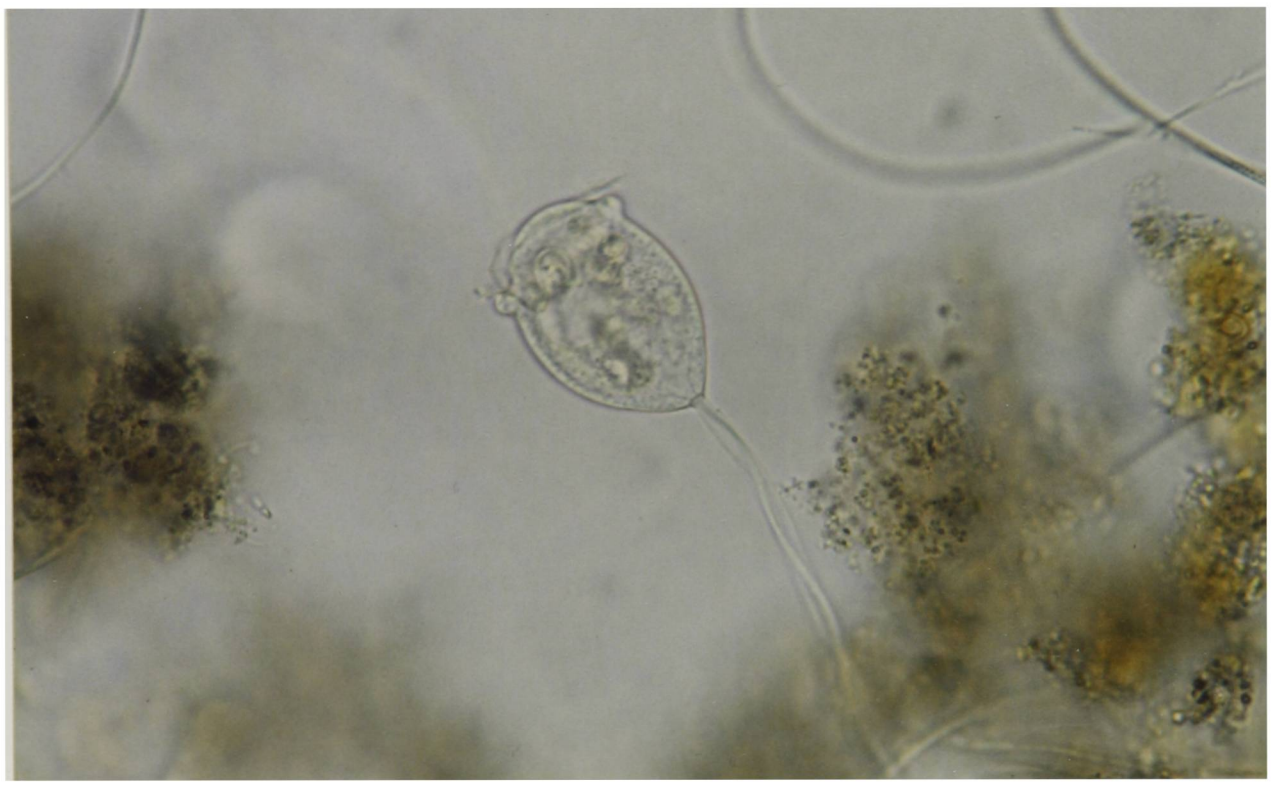
Chaudhuri et al. (1965) found that nematode population densities increased with decreasing temperatures and population maxima occurred, in aerobic treatment plants, between 17-18^{o}C. The operating temperatures of the activated sludge plants used in this experimentation, during the summer months, ranged from 23-29^{o}C when the population explosion occurred. However, the absence of higher organisms at the 10% K10 wash water effluent concentration is another possible factor influencing the nematode population increase since many of the arachnids are the only important predators of worms within percolating filters, the more common habitat of the arachnids. Plate 13 shows the mouth-parts of an arachnid alongside an almost fully developed nematode worm egg. It is not hard to visualise such an organism eating this egg or newly hatched worms. However, Baker (1975) states that arachnids are not found in activated sludge plants although Evans and Hyatt (1960) found Platyseius italicus (an arachnid) at Minworth Sewage Works, Birmingham. This is an interesting point since part of the domestic sewage sludge used initially to seed the sludge reactors was obtained from this source.

It was quite obvious that K10 wash water effluent caused dramatic changes within the population of an established activated sludge plant. However, after four months continual exposure of sludge to 10% K10 wash water effluent (post Section 3.7 experimentation) a return to 2.5% K10 wash water effluent concentration resulted in the return of almost all

the flora and fauna previously observed at the start of the work. However, there were just a few exceptions such as ameobae, which did not return, and have not been observed since, in over two years of operation. Rotifers, too, only seemed to survive with very low concentrations of K10 wash water effluent in the feed.

The presence of large numbers of cysts, the appearance of filamentous fungi, and the loss of most protozoa (either permanently or temporarily) may perhaps indicate the somewhat toxic nature of K10 wash water effluent.





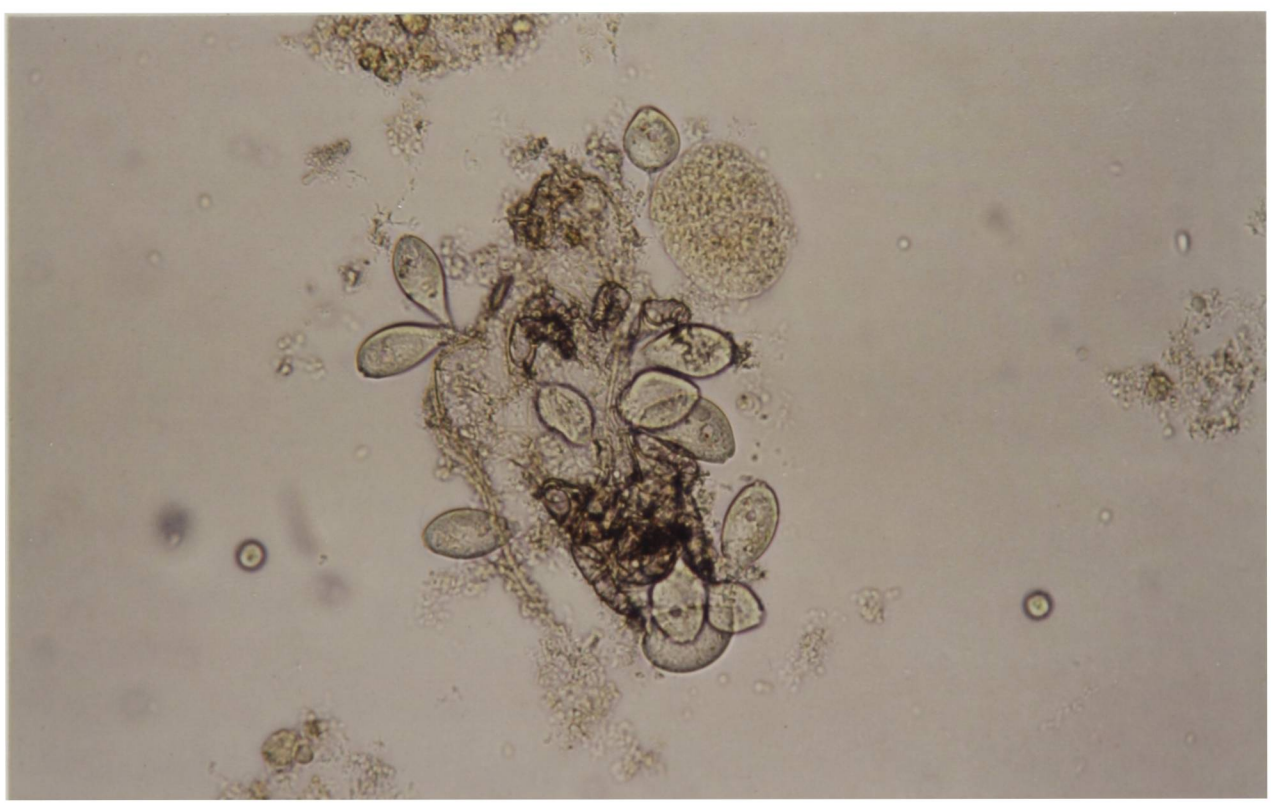
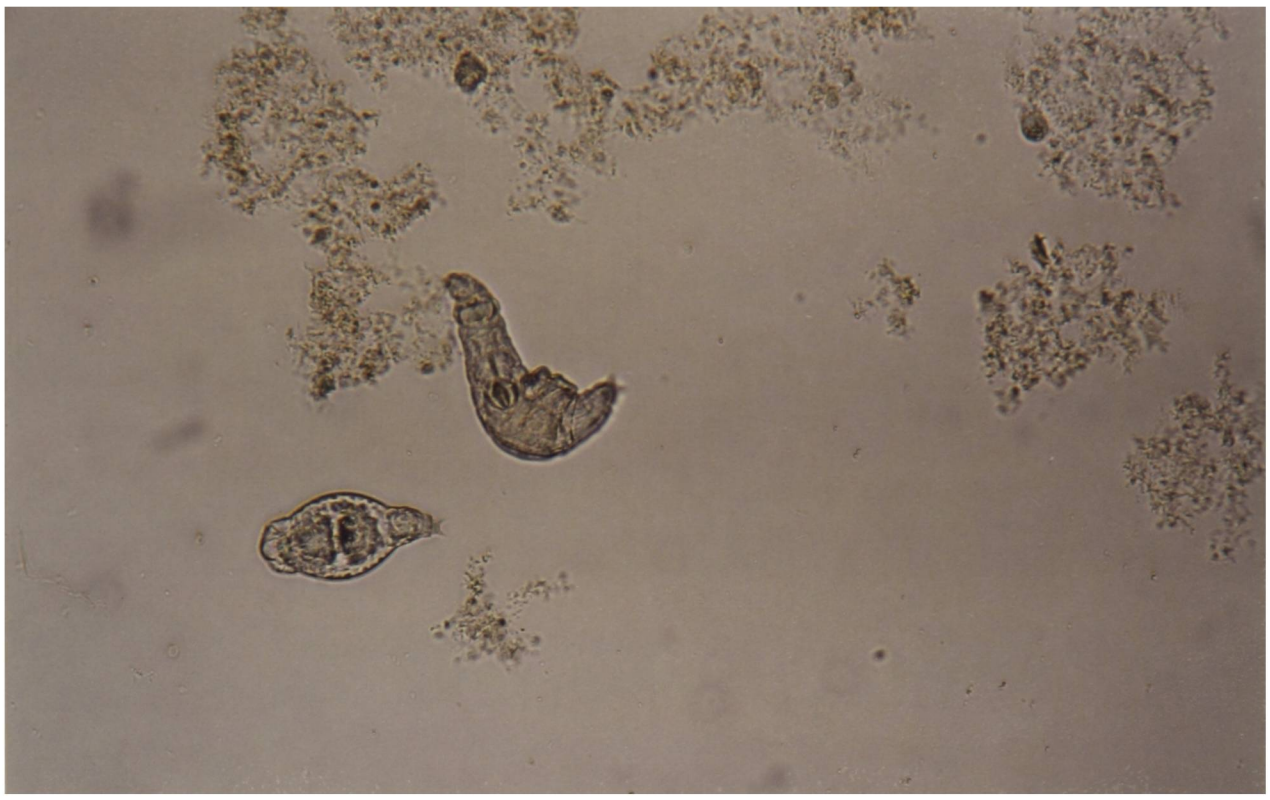
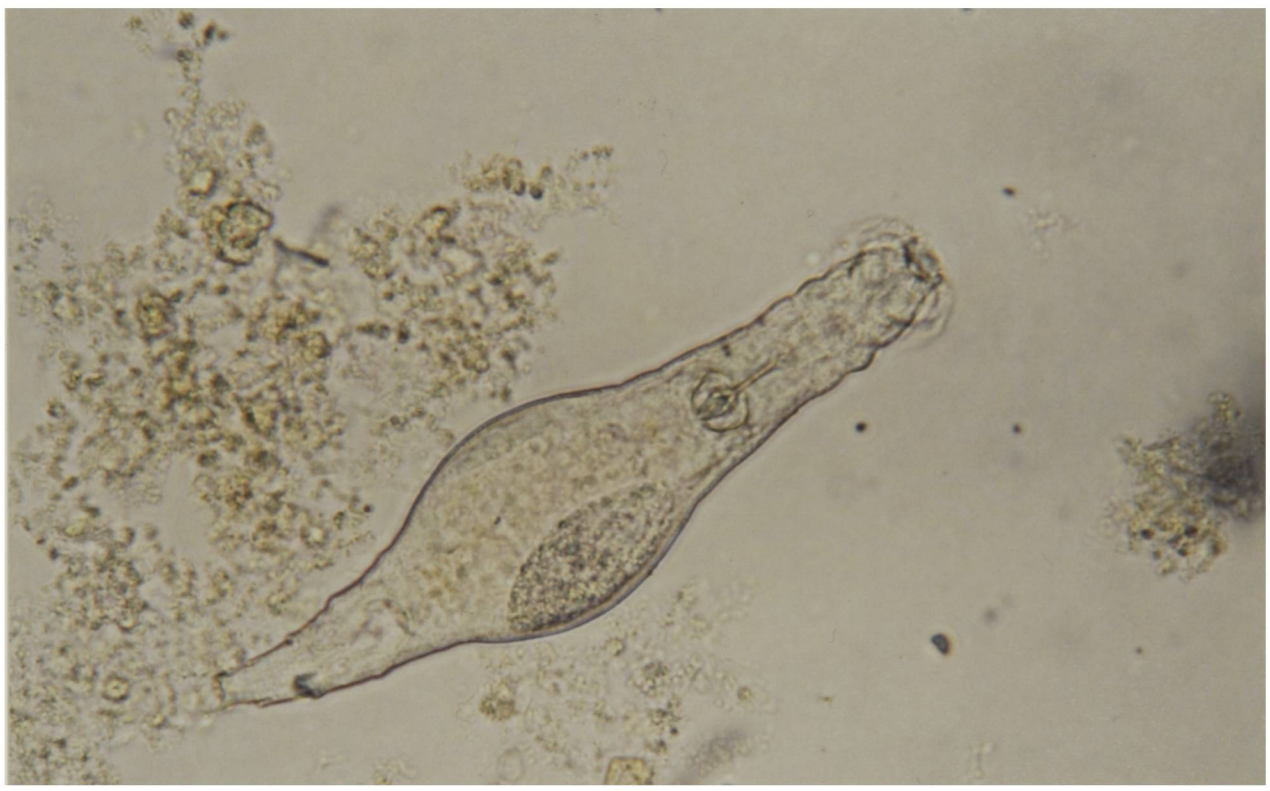


Plate 5. Examples of <u>Vorticella</u> <u>sp</u>. found in activated sludge





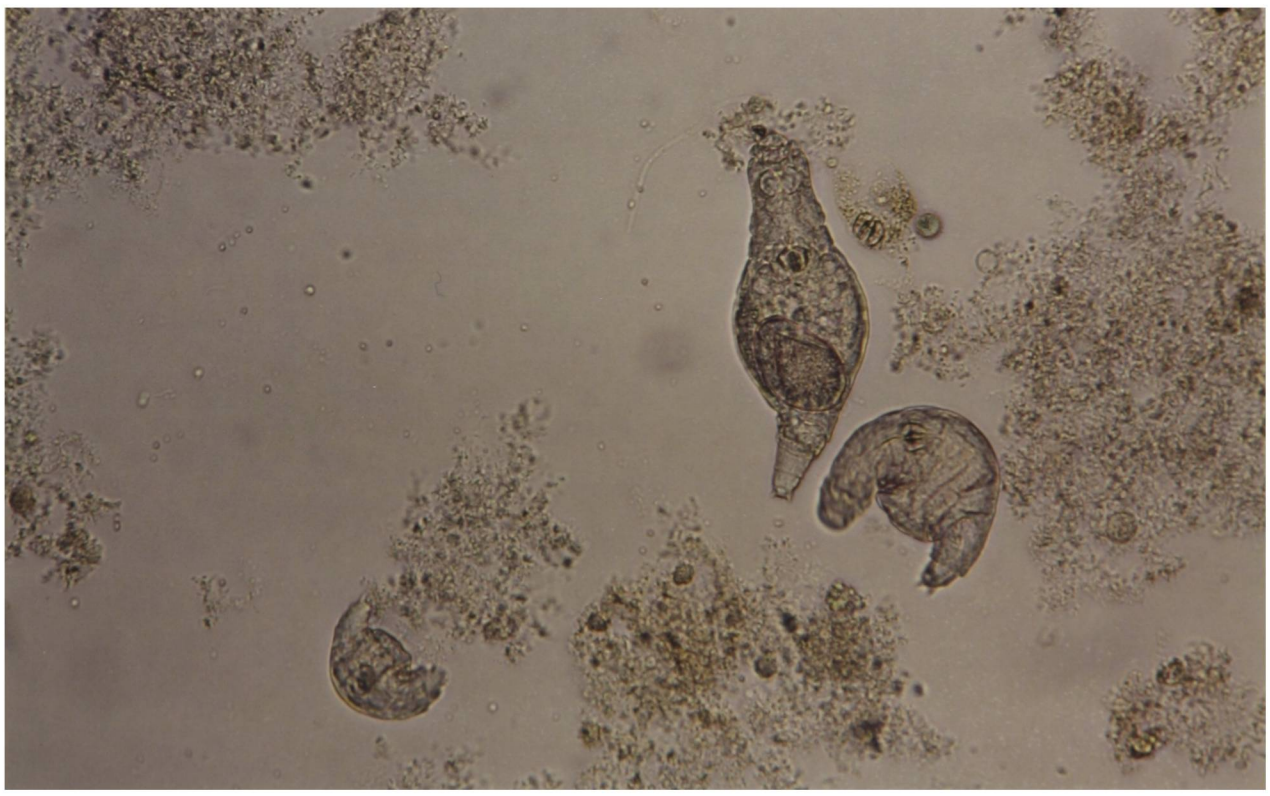


Plate 6. Examples of rotifers found in activated sludge

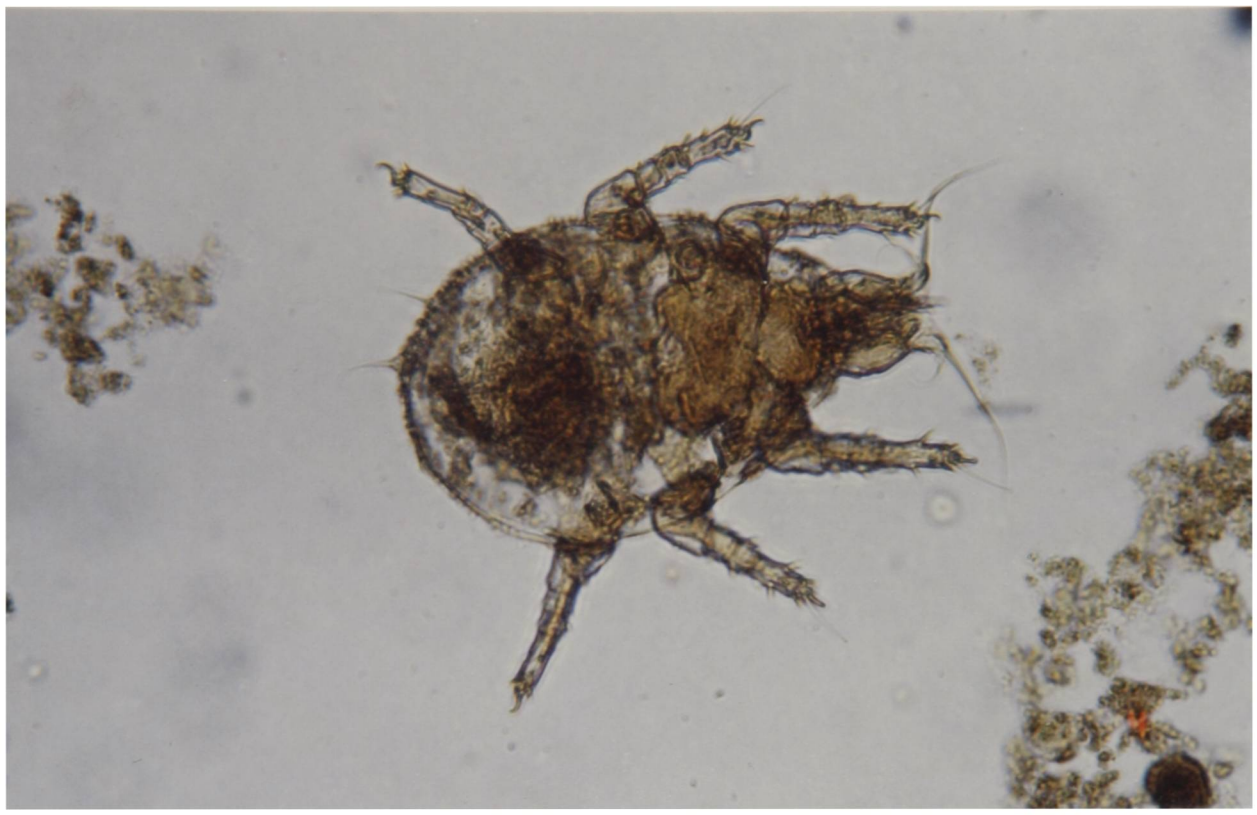
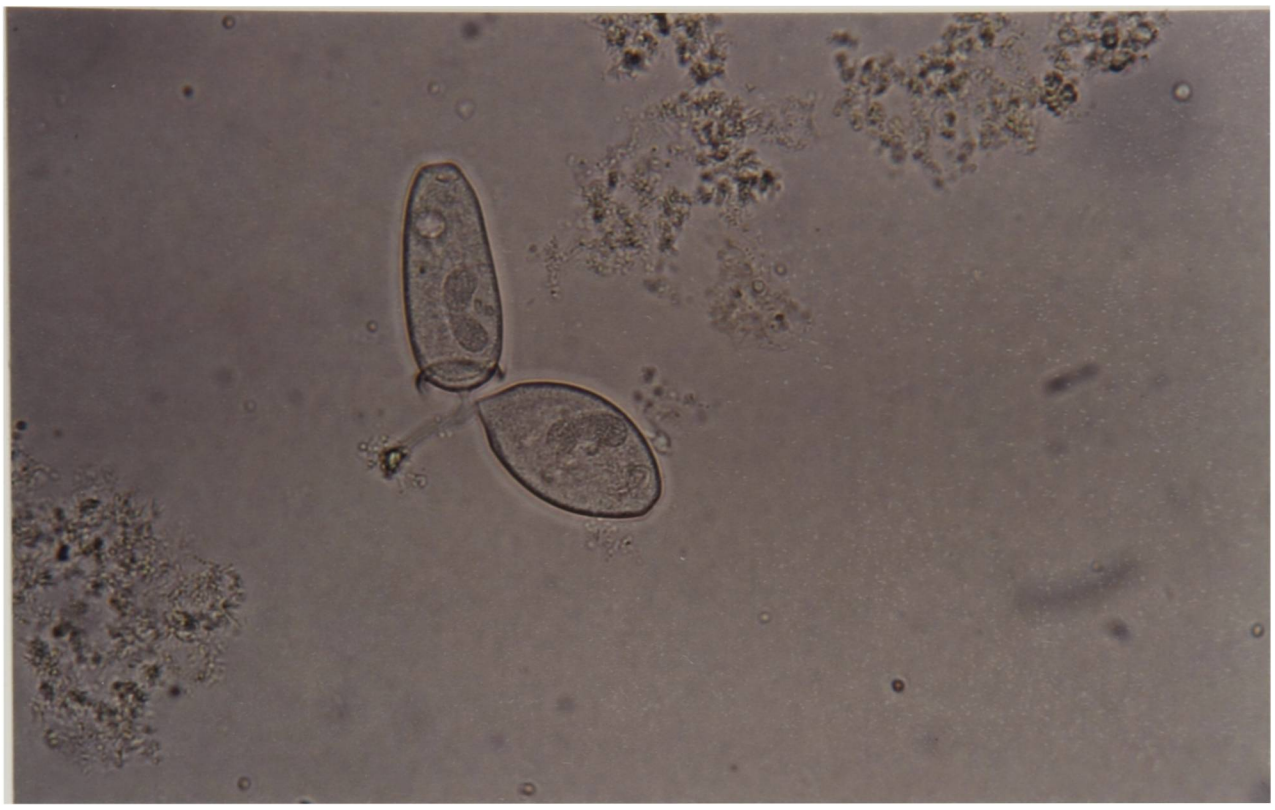
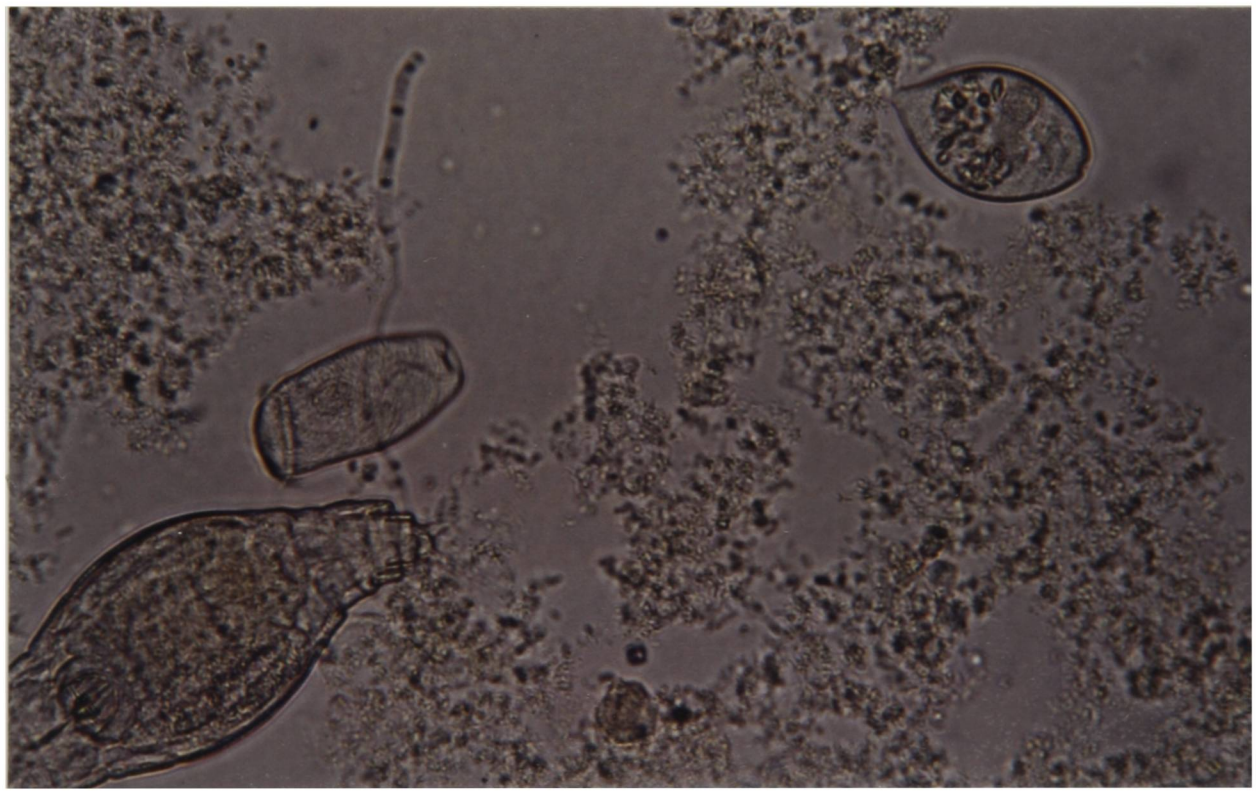


Plate 7. Examples of higher organisms found in activated sludge

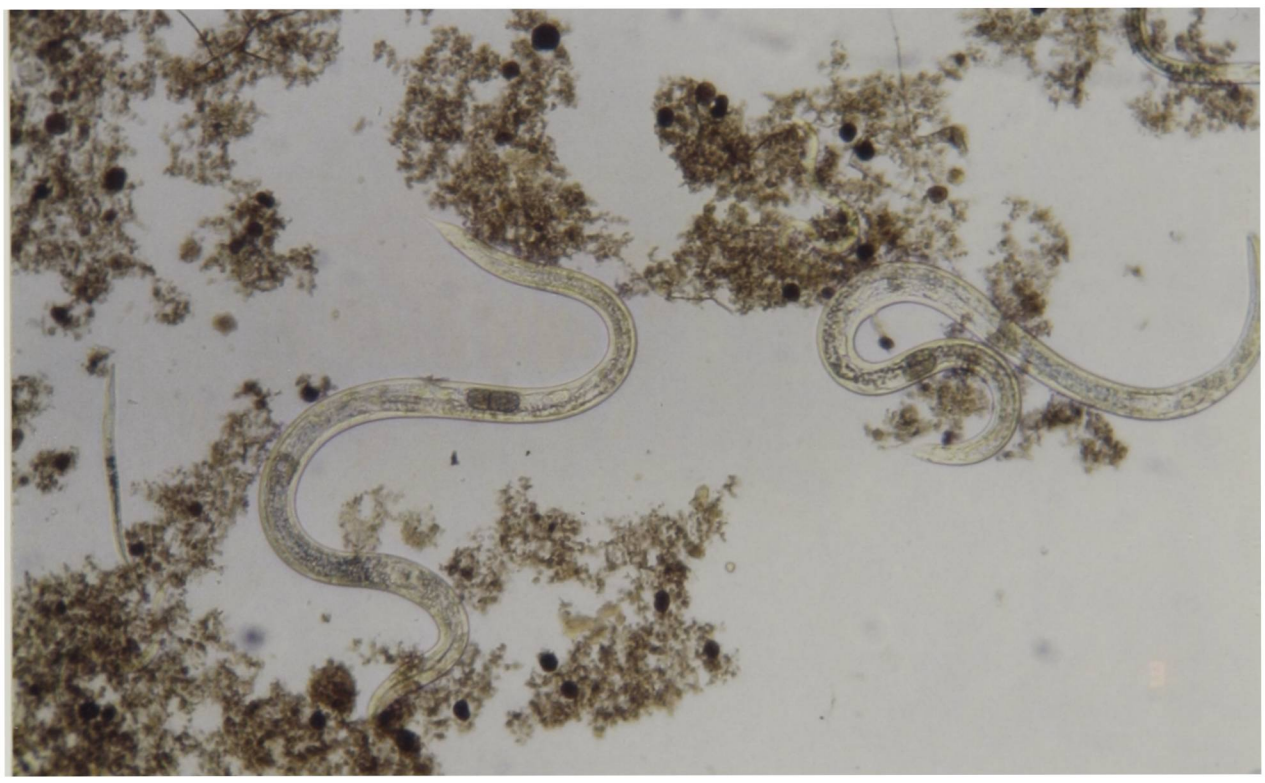


A - Vorticella sp. forming a ring of aboral cilia



B - Vorticella sp. in teletroch stage

Plate 8. Vorticella sp. found in sludge exposed to 2.5% K10 wash water effluent



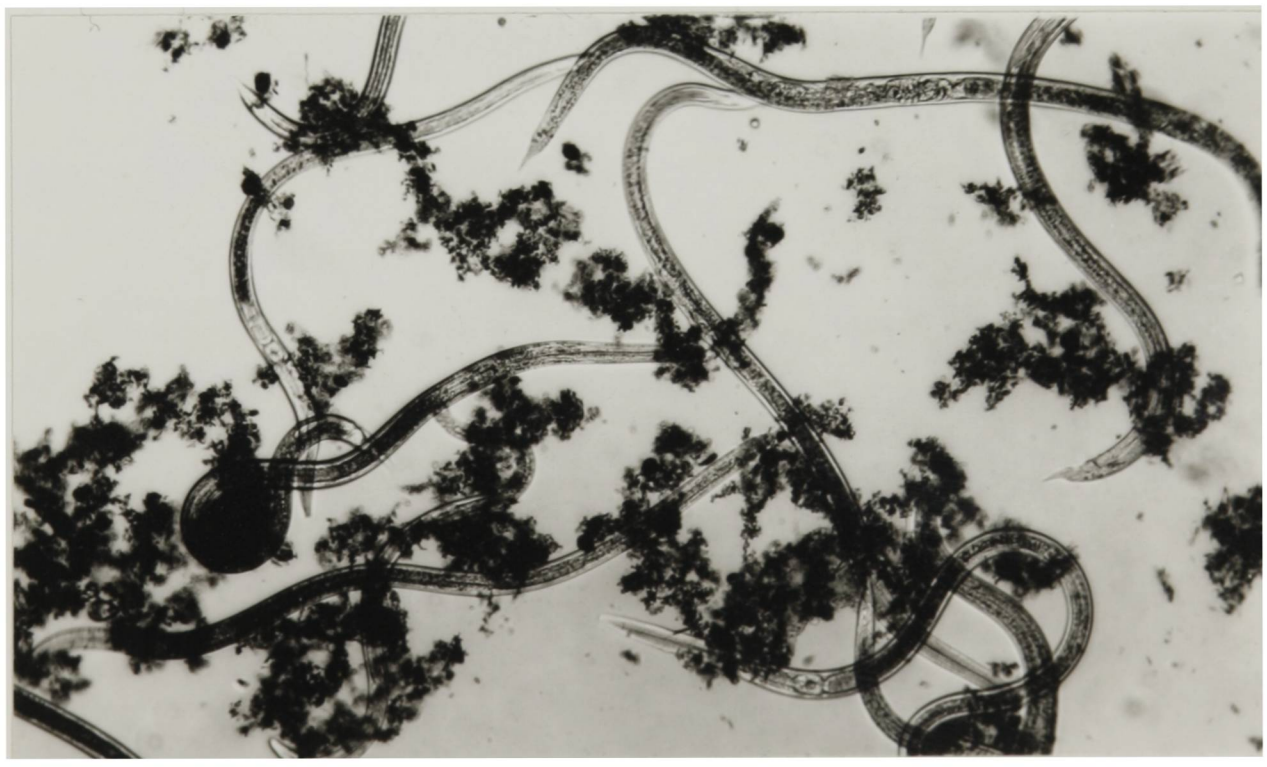
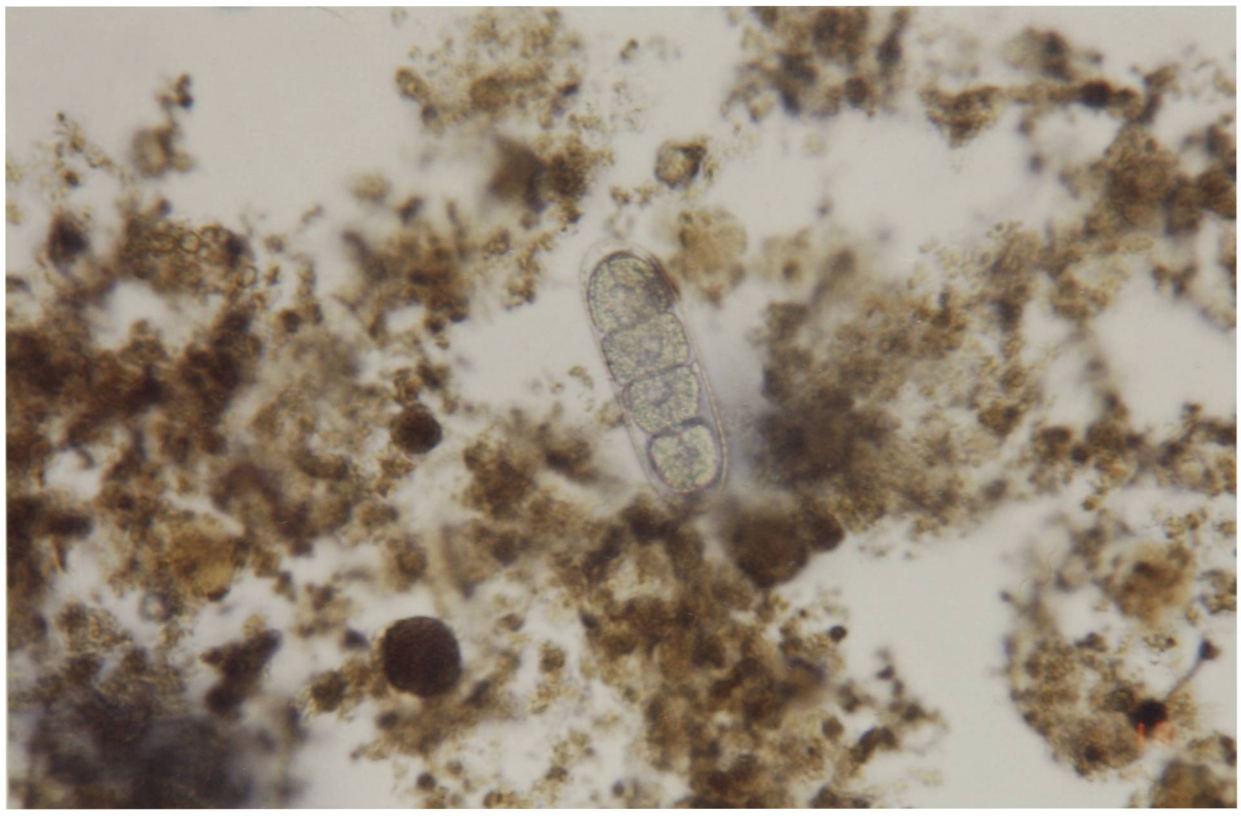


Plate 9. Dramatic increase in numbers of nematode worms in activated sludge exposed to 10% K10 wash water effluent



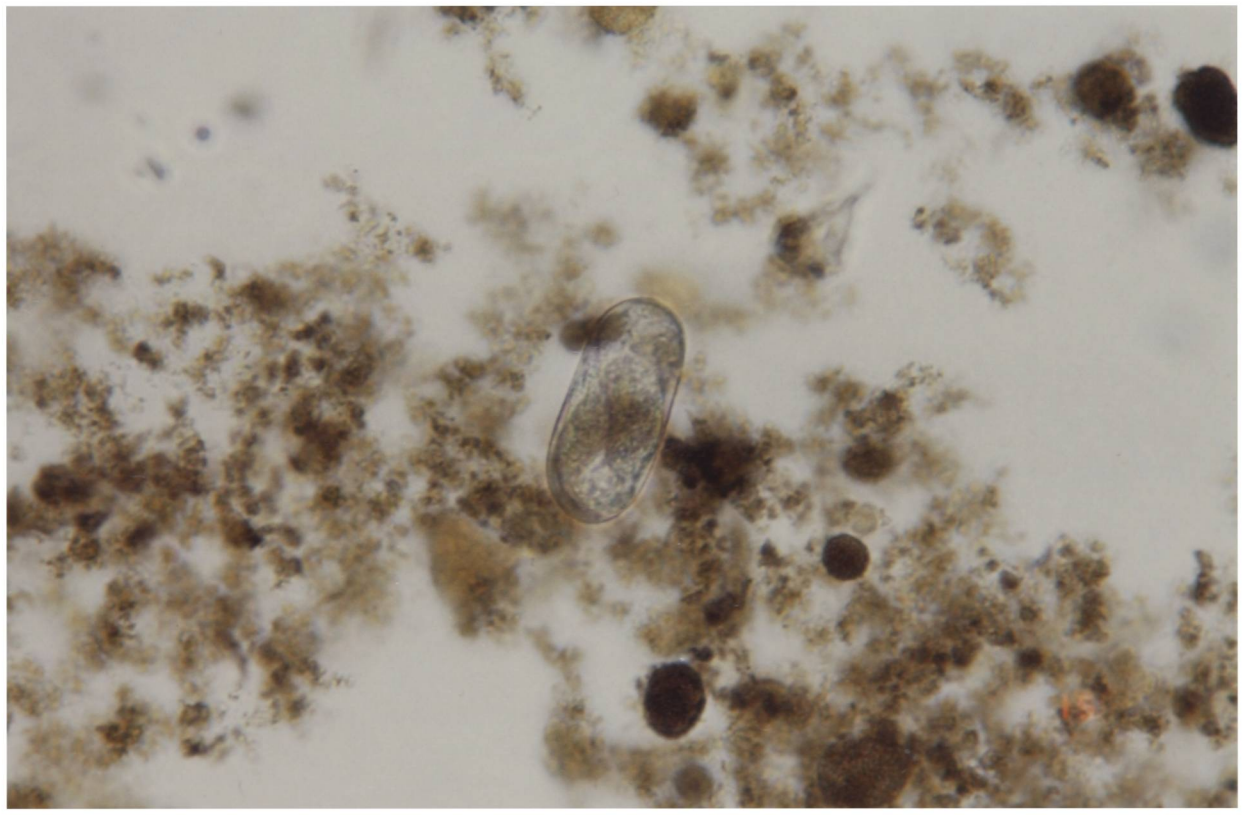


Plate 10. Nematode worm eggs found in activated sludge exposed to 10% K10 wash water effluent

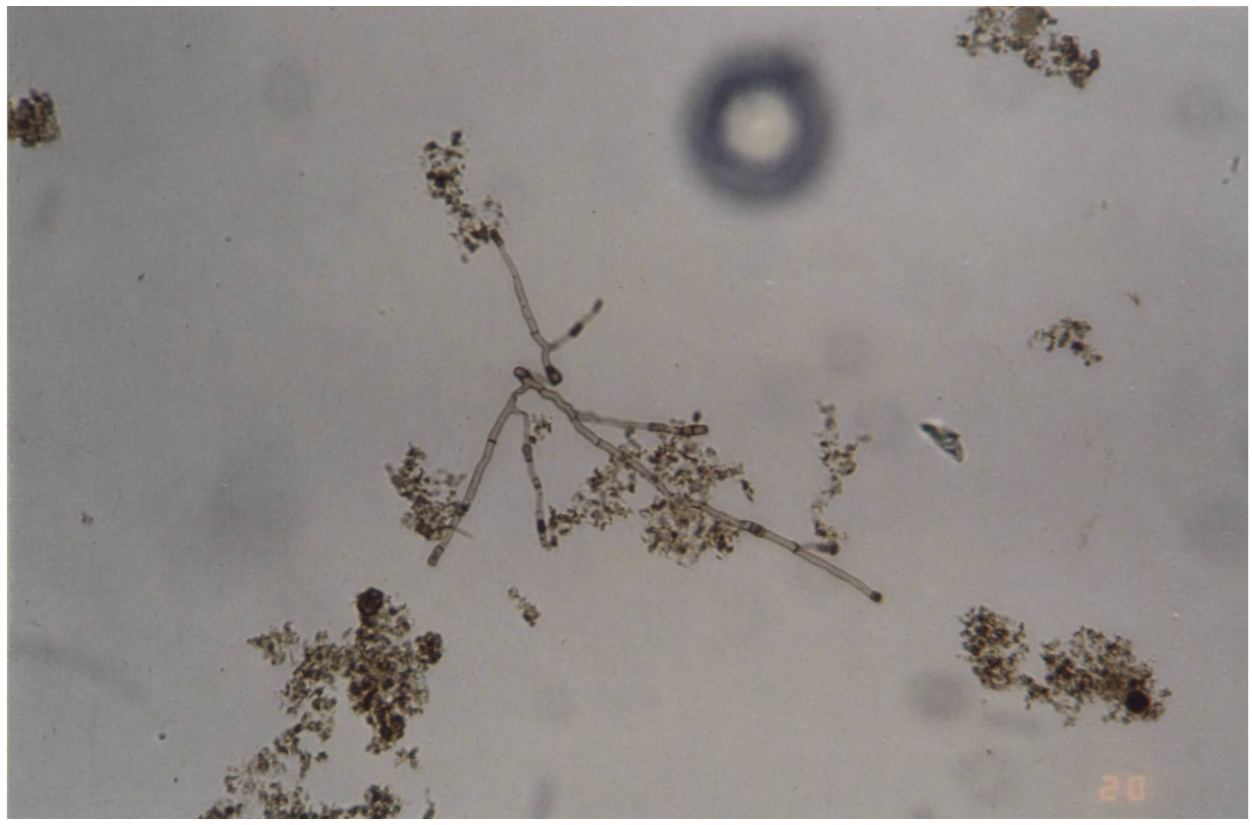
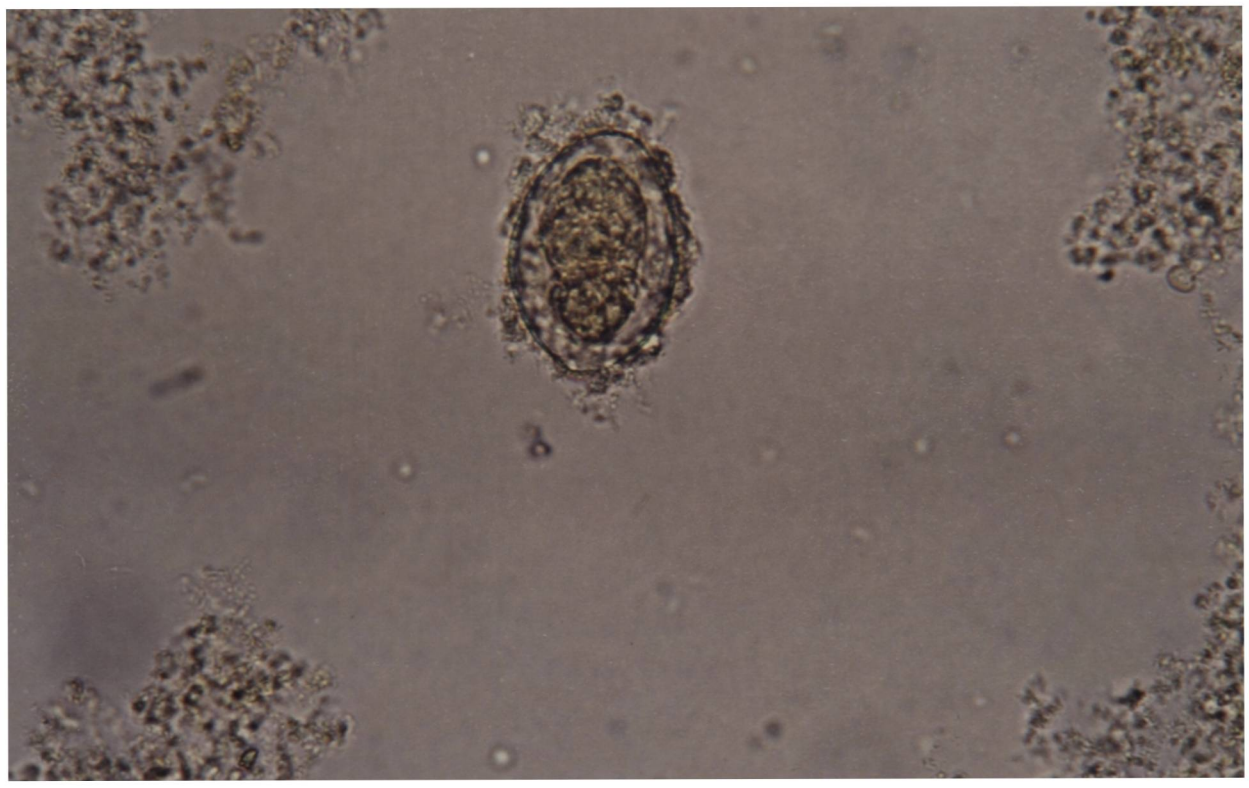
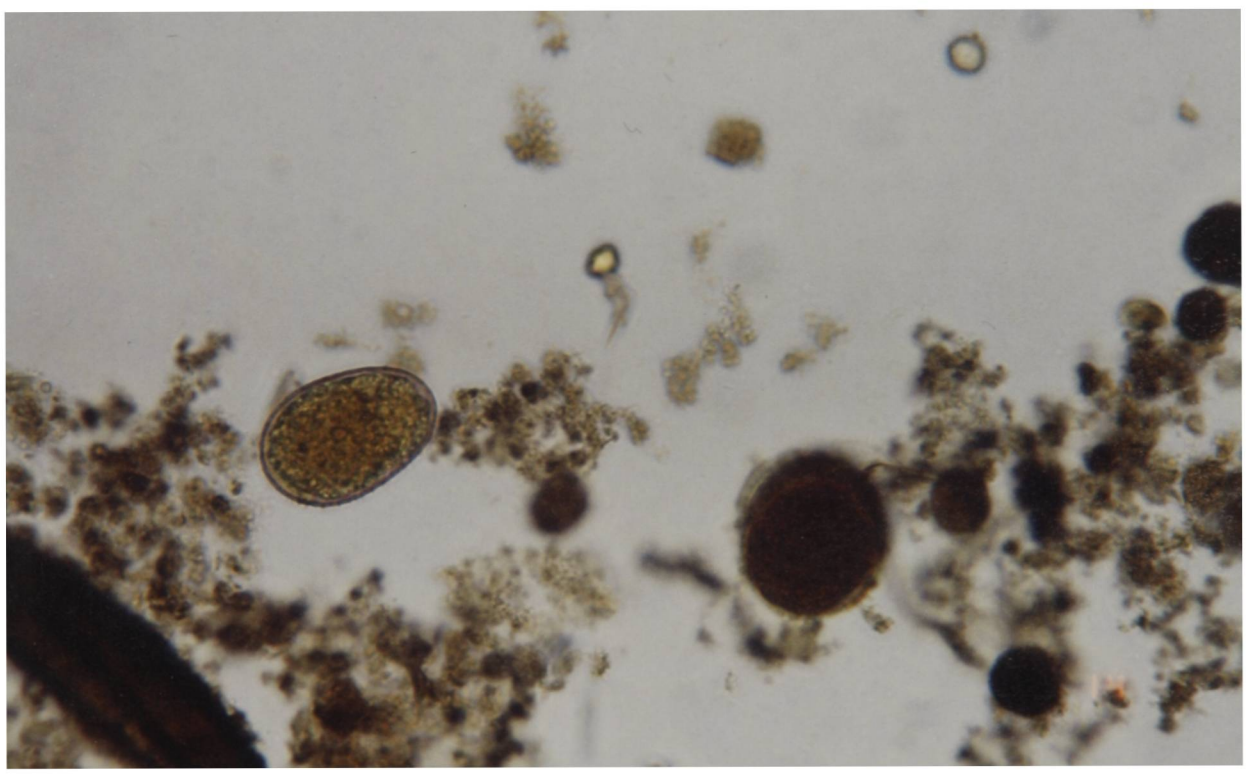


Plate 11. Filamentous organisms found in activated sludge exposed to 10% K10 wash water effluent





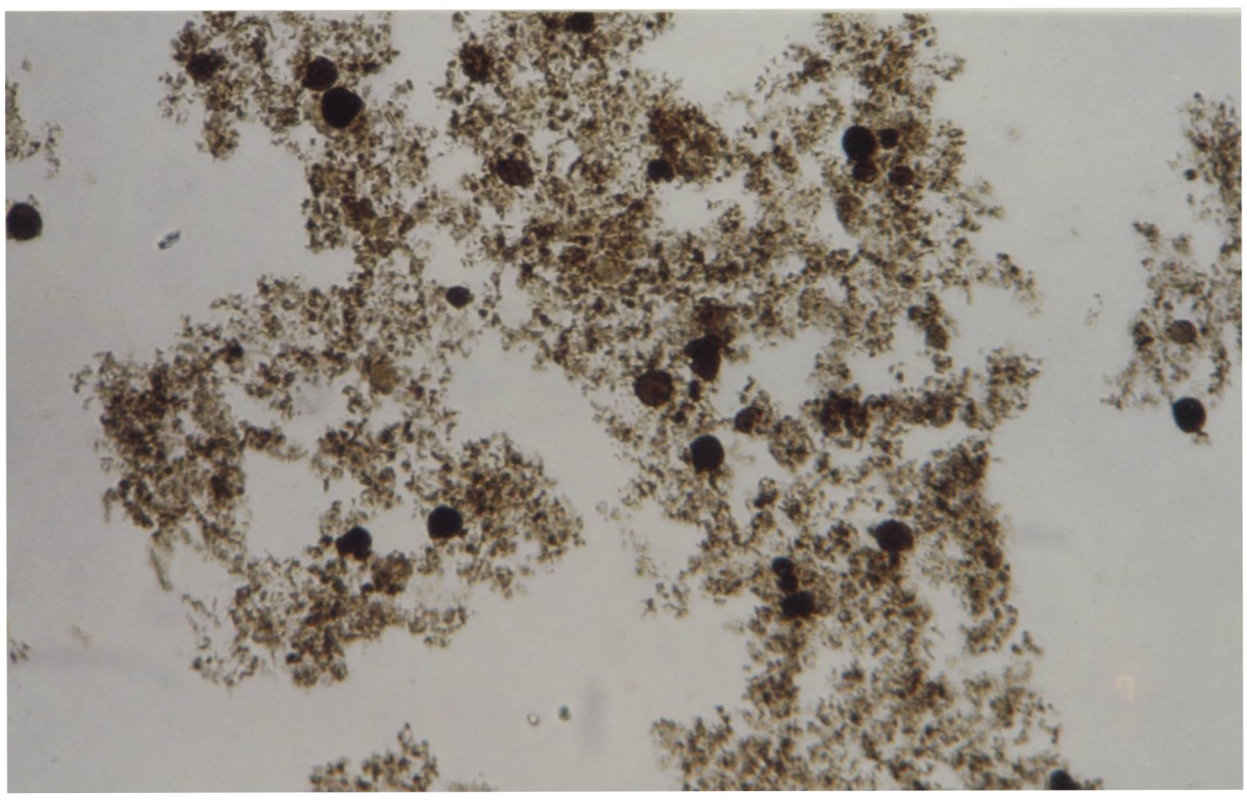


Plate 12. Examples of cysts found in activated sludge exposed to 10% K10 wash water effluent

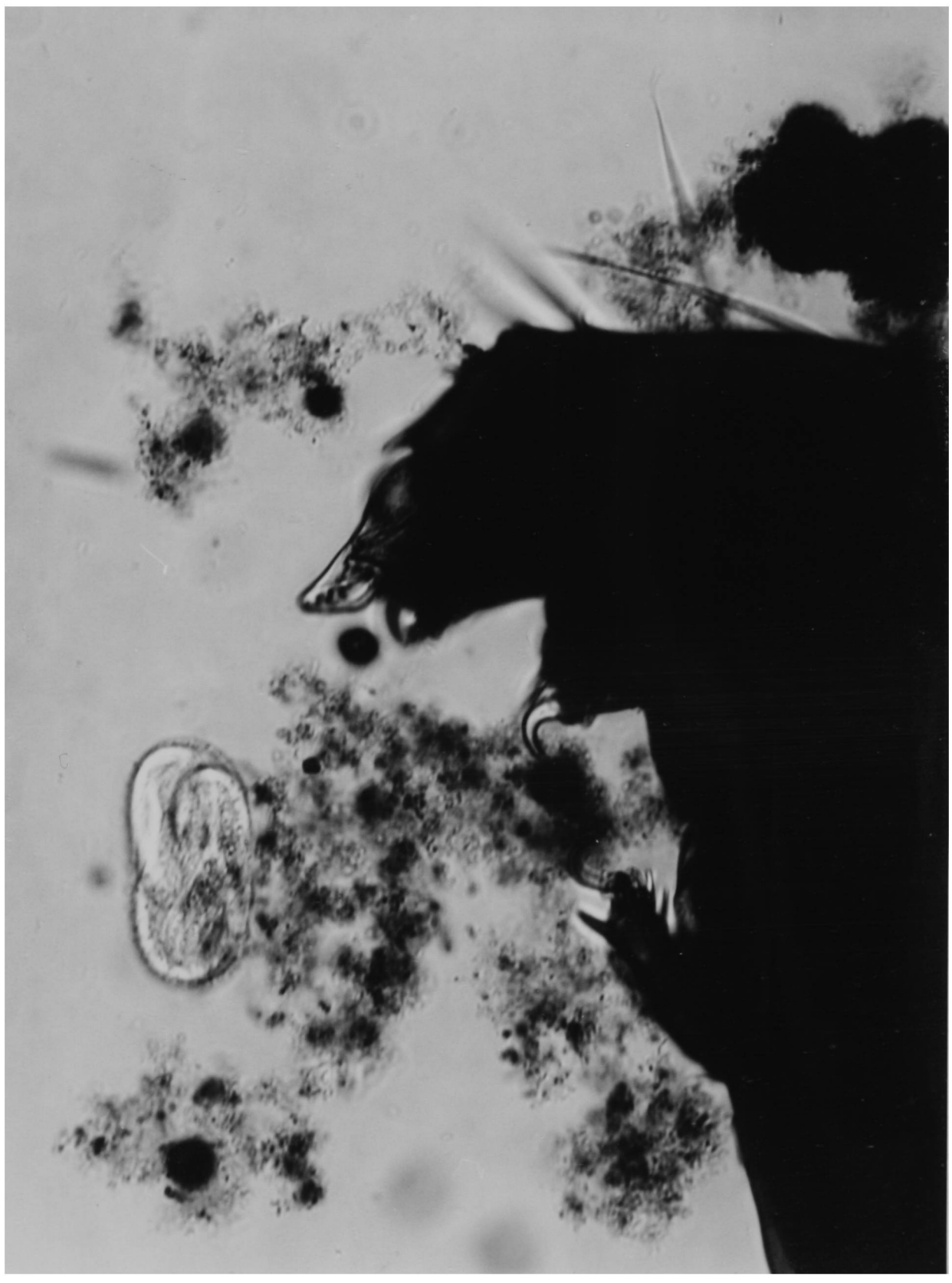
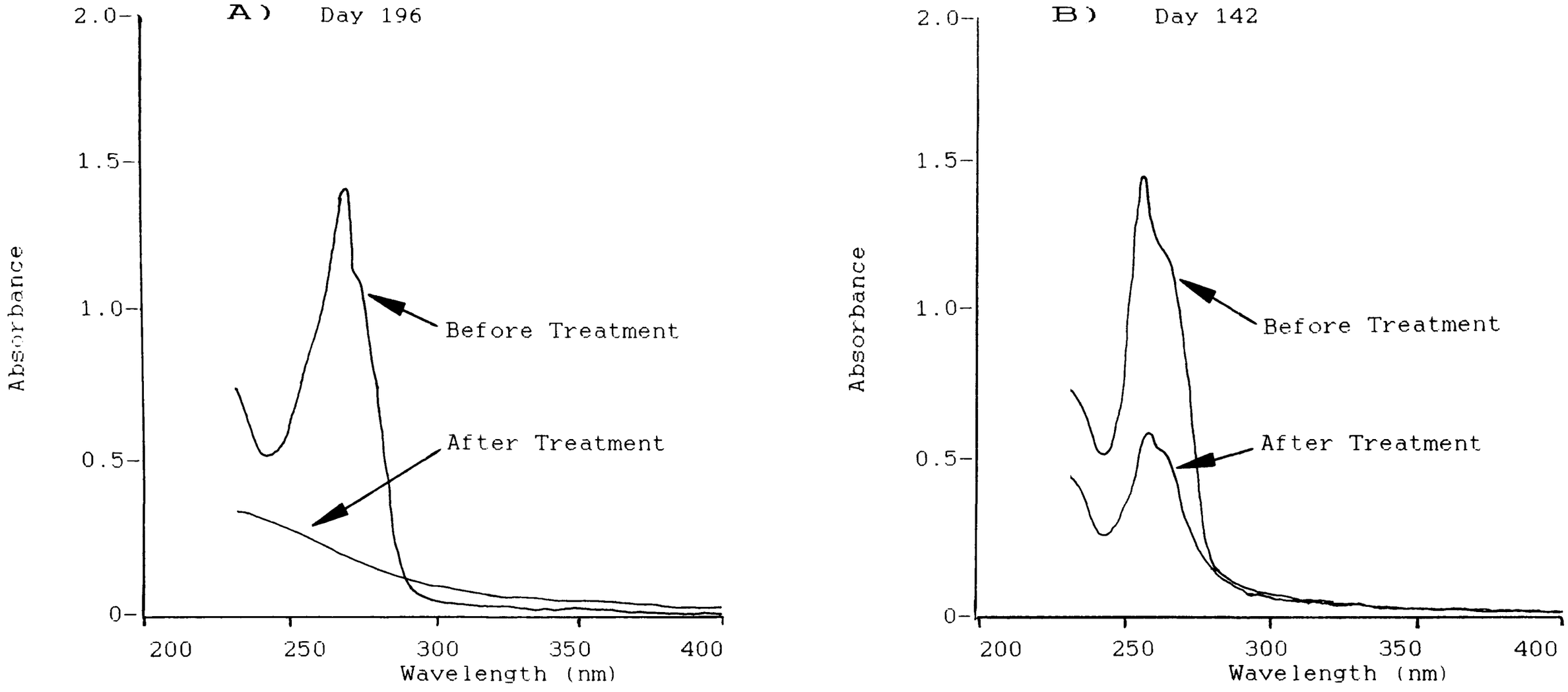


Plate 13. An arachnid beside a nematode worm egg in activated sludge exposed to 7.5% K10 wash water effluent

4.2 ULTRA-VIOLET SCANS

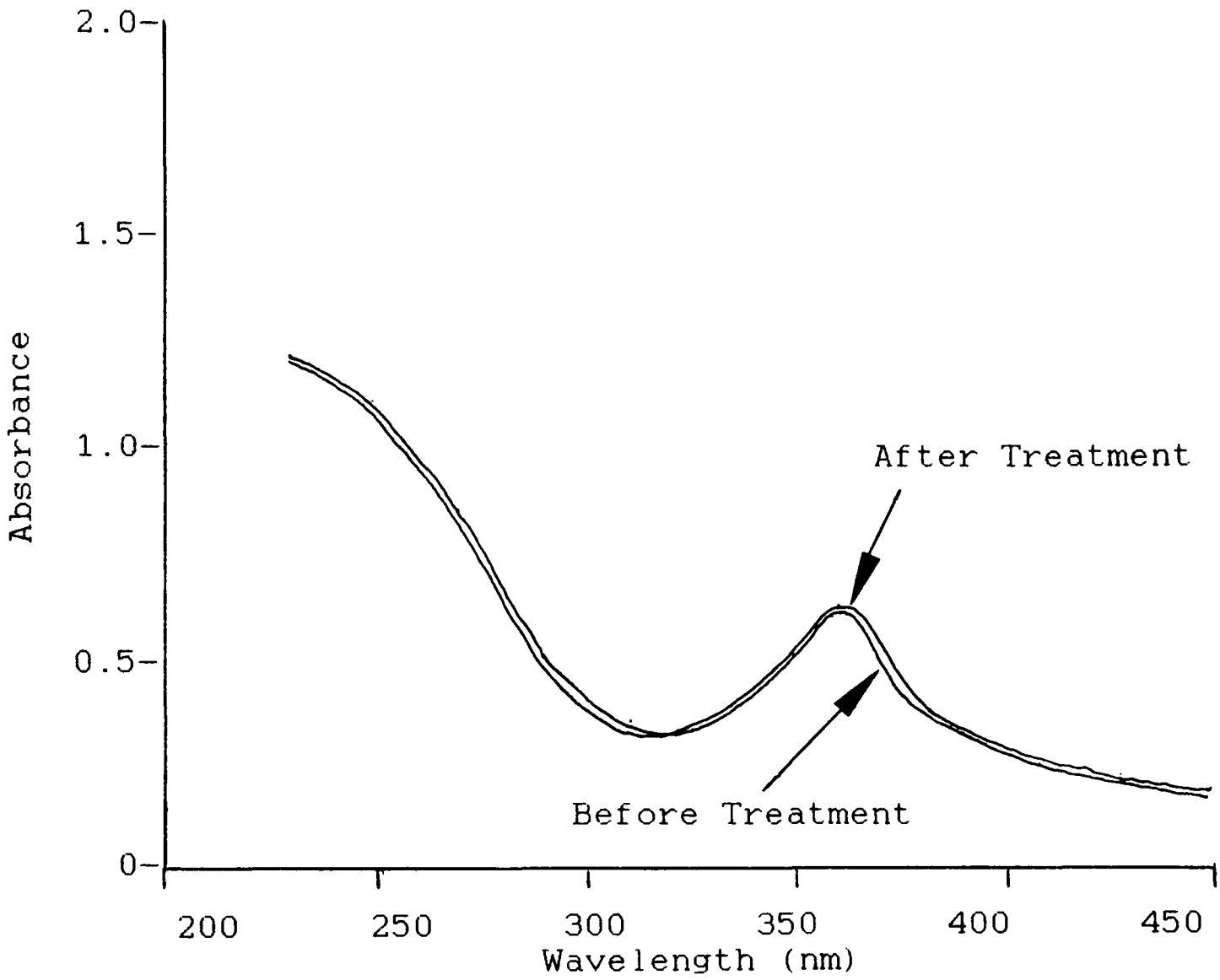
Ultra-violet wavelength scans were useful for rapid assessment of plant performance although the data were not used quantitatively. Wavelength scans of the feed and effluent of the control reactor are given in Figure 54b for day 146 (reactor efficiency low, phenol removal 52%) and in Figure 54a for day 196 (efficiency high, phenol removal 98%). A clear difference can be seen. In Figure 54b there is an absorbance peak at 270nm which was not present in the reactor effluent on day 196. The absorbance peak at this wavelength is characteristic of mixed phenolic waste and not found in scans of K10 wash water effluent as can be seen from Figure 55. Clearly, the reactor is operating satisfactorily, all the material in the phenolic waste which absorbs at 270nm is being degraded. There was no absorbance peak at 270nm for K10 wash water effluent whilst there was a peak at 350nm arising from the orange colouration of the waste. This colouration was pH dependent and also subject to both photochemical and temperature enhancement. This coloured peak also exhibited small and not always reversible wavelength shifts (depending on physical factors such as heat, light and pH) and was not removed by the experimental reactor as can be seen from Figure 56.

Wavelength scans were also used to determine the most suitable wavelength for HPLC analysis which is subject to two considerations. The first, most obvious, is that the compounds



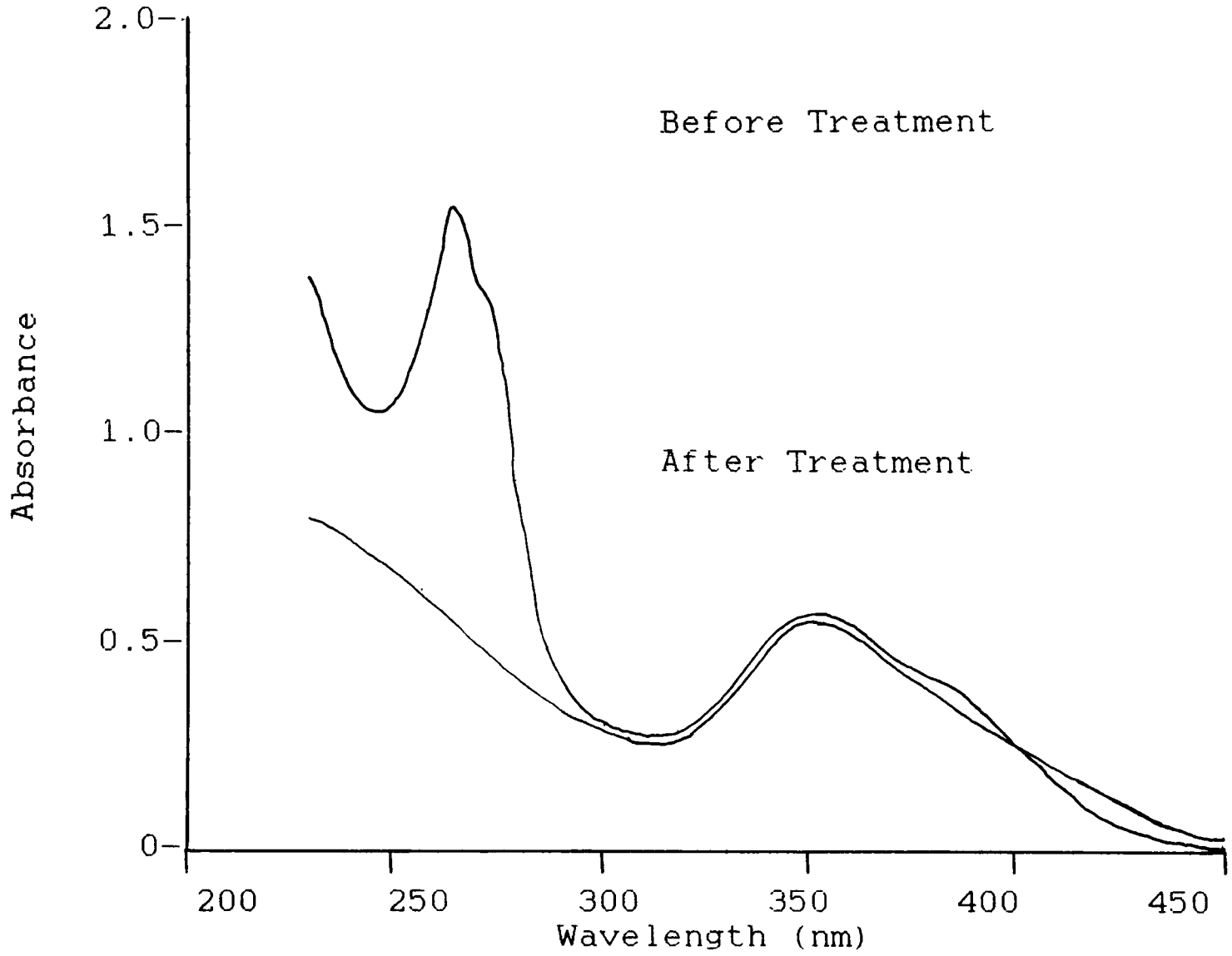
Scan 230-400nm
Scan Speed 2.5nm/sec
Scale Expansion 20nm/cm
Time Constant 2sec

Figure 54. Use of Wavelength Scans for Rapid Assessment of Plant Performance



Scan 230-450nm
Scan Speed 2.5nm/sec
Scale Expansion 20nm/cm
Time Constant 2sec
Dilution 1% (v/v) K10 waste

Figure 55. Wavelength Scan of K10 Effluent



Scan 200-450nm
Scan Speed 2.5nm/sec
Scale Expansion 20nm/cm
Time Constant 2sec

Figure 56. Wavelength Scan of 1% K10 Medium

to be detected must absorb light at the wavelength used, which, as is clear from Figure 55, is as short a wavelength as possible. The minimum wavelength that the equipment would reliably detect was 190nm, but at wavelengths shorter than 230nm the K10 wash water effluent adsorbed radiation too strongly. Thus this consideration was satisfied choosing 230nm for the main UV absorbance and 350nm for the coloured peak. However, the second consideration was that the HPLC solvents used must not absorb light too intensely at the selected wavelength otherwise they will mask the absorbance of the compound to be detected. The solvents used for HPLC analysis absorbed light (transmitted poorly) at 230nm (Table 5). However, at 260nm the solvent absorbance was greatly reduced whilst the K10 wash water effluent still absorbed quite strongly and so this wavelength was taken as a compromise.

Solvent	Percentage Transmission (vs Water)	
	230nm	260nm
Acetonitrile (Far UV)	95	98
Methanol	80	98
Tetrahydrofuran	20	90

Table 5. Transmission Properties of some Solvents used for HPLC Analysis (1cm Path Length).

4.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

4.3.1 The system utilised and results obtained

The equipment utilised is described in section 2.7. A reverse phase column was recommended by Kelly (1987) with methanol, water and tetrahydrofuran, at a ratio of 2:2:1 respectively, as an isocratic mobile phase. A C_{18} guard column was utilised which was replaced every twenty-five injections and a pre-injection solvent filter, packed with preparatory grade silica, was used to saturate the mobile phase with silica so protecting the main analytical column from dissolving. Sample injection was via a Rheodyne valve fitted with a 20µl sample loop which was always used fully filled with sample.

An HPLC analysis of K10 wash water effluent is shown in Figure 57. The separation of the colourless compounds is satisfactory, but the coloured components of the waste are not retained, or separated, particularly well under the conditions used. These coloured components are eluted from the column within a few minutes, almost certainly just behind, or with, the solvent front. The remaining four component peaks are retained and separated quite well. However, the heights of these peaks are much smaller (less than 10%) than the orange component peaks, although this does not necessarily have any bearing on the proportions of these compounds in the waste.

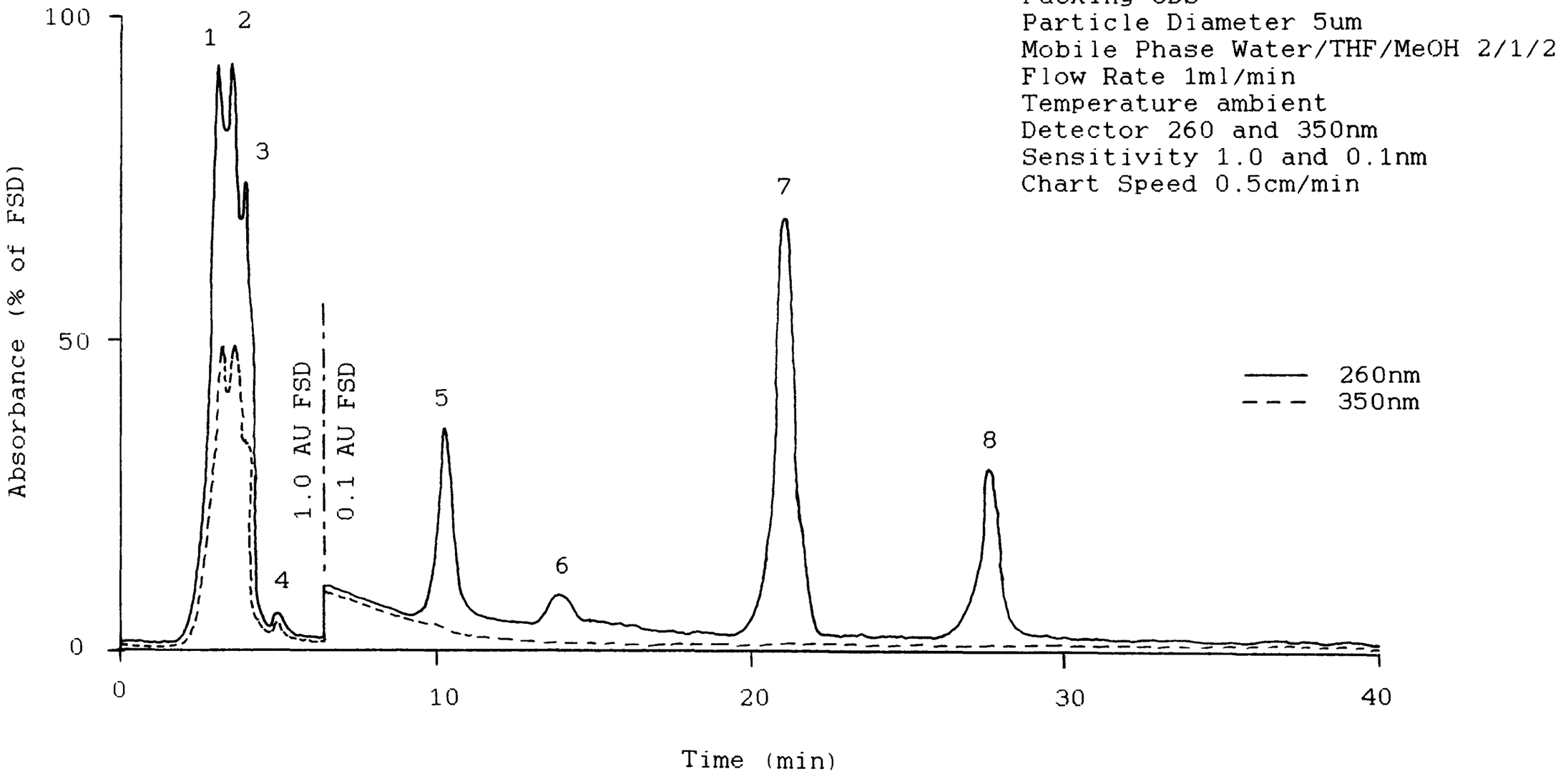


Figure 57. HPLC Analysis of K10 Waste

4.3.2 HPLC Analysis of K10 Degradation

Many analyses using C_{18} as the stationary phase were performed on K10 wash water effluent both before and after incubation with activated sludge organisms. A typical situation is as follows. Figure 58 shows the analysis of K10 after incubation and accompanies Figure 57, a comparable analysis of a K10 wash water effluent sample before incubation. A comparison of these reveals several differences and similarities. These are:

i) Peaks 1,2 and 4 which absorb at 350nm, being coloured, have not been degraded by the microbes.

ii) Peak 3, which also absorbs at 350nm (indicated by the shoulder on peak 2), has been partially degraded during incubation.

iii) The remaining peaks, numbers 5,6,7 and 8, do not absorb at 350nm and have all been significantly degraded during incubation with the complete disappearance of peak 6.

iv) Two new peaks have emerged, 5a and 8a. These could be recalcitrant transformation products or secondary metabolites.

Due to the lack of retention and hence poor separation of peaks 1,2 and 3 they might be combinations of fairly large peaks or be masking smaller ones. It is also worth mentioning that peak height is not a good method for quantitative analysis of an individual compound. Peak area measurements are far more reliable since they consider both the height and

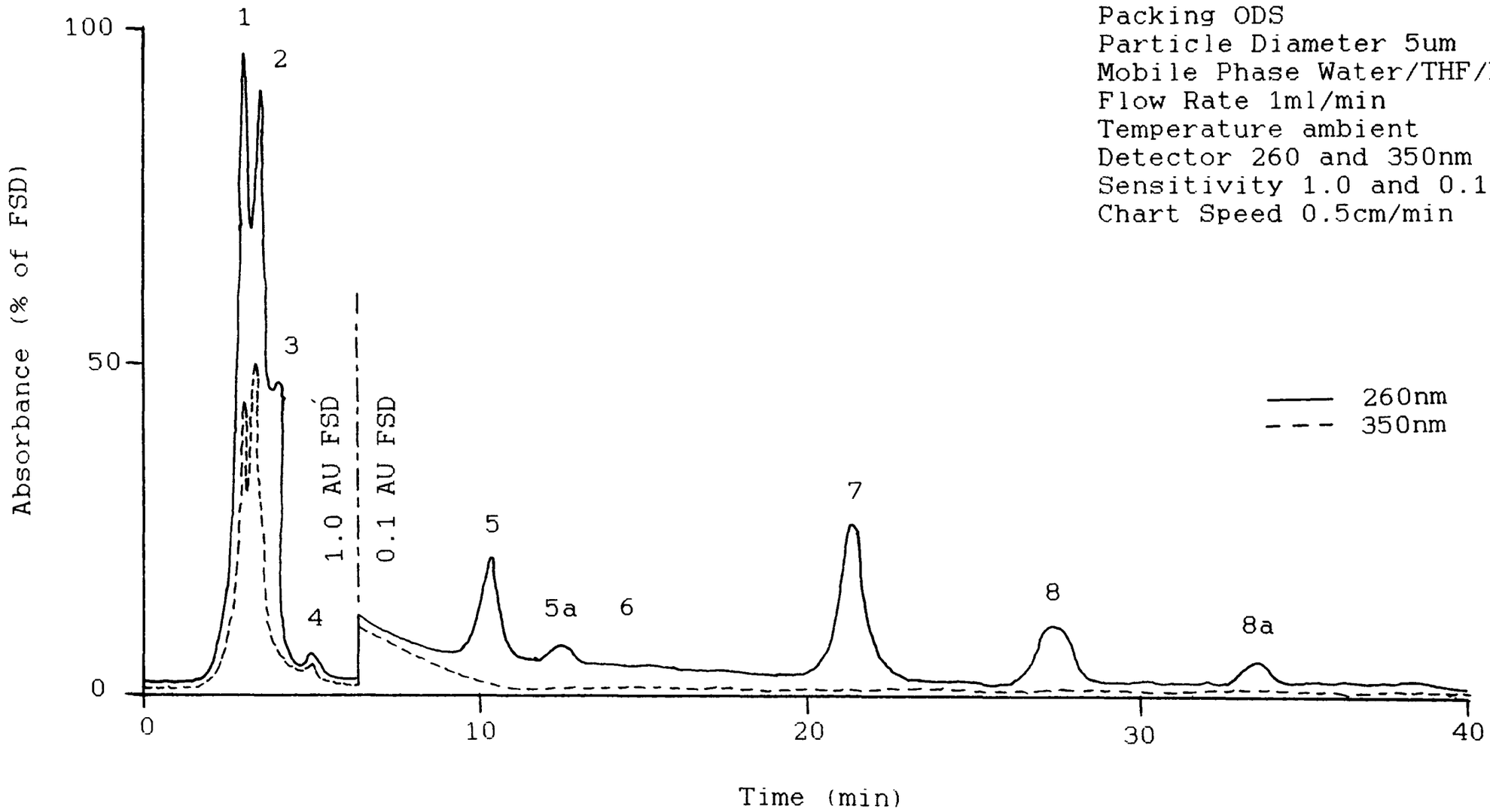


Figure 58. HPLC Analysis of K10 Waste After Treatment

breadth of the peak.

The results of HPLC analysis show that some of the organic components of K10 wash water effluent are metabolised by activated sludge. However, it is likely that these compounds are only a minority of the total waste since they have very small absorbance peaks at 260nm. The fact that coloured waste components are not significantly removed ties in with work by Nay et al. (1972) who found that the coloured components of the analogous TNT red water effluent were very refractory and also toxic.

4.3.3 Stationary and Mobile Phase Selection

Many analyses were carried out on the K10 wash water effluent with varying types and combinations of mobile phase solvents on a range of stationary phases. The aim was to retain (strong enough in that 20 bed volumes of solvent washing do not elute the isolate) and then elute (no more than 5 bed volumes of solvent required) the K10 wash water effluent components. A thorough investigation was directed towards HPLC stationary phase properties. These can be separated into four categories: polar, non-polar, anionic and cationic interactions.

Non-polar interactions (reverse phase) are those which occur between the carbon-hydrogen bonds of the sorbent functional groups and the carbon-hydrogen bonds of the isolate

molecules ("van der Waals forces"). Since most organic molecules have some non-polar structure, non-polar interactions are very effective for isolation of groups of compounds dissimilar in structure. Retention on these stationary phases is facilitated by polar solvents and elution by non-polar solvents since these disrupt the non-polar isolate/sorbent interactions.

As previously discussed (Section 4.3.1) K10 wash water effluent separation was initially achieved using a cocktail of relatively polar solvents (water, tetrahydrofuran and methanol) in conjunction with a C_{18} reverse phase column. This stationary phase is considered as the least selective of the non-polar interacting matrices since it retains almost anything as well as being the most non-polar sorbent available.

An attempt to use water as the mobile phase with a C_{18} column for the retention of K10 wash water effluent components ought to be successful since water is obviously highly polar, and polar solvents facilitate retention on reverse phase columns. However, the use of water was unsuccessful in retaining the coloured K10 wash water effluent components whilst the non-coloured components were retained too strongly so that they were not eluted. It was thus concluded that the coloured components must be highly polar in character.

Since the use of non-polar stationary phases was

unrewarding, the next step was to examine polar interactions. These might well be more suitable for the retention of the coloured components since the retention principle is opposite to that of reverse phase. Polar interactions include hydrogen bonding and pi-pi bonding. Groups that are retained by these sorbents include hydroxyls, amines, carbonyls, aromatic rings, double bonds and groups containing atoms such as oxygen, nitrogen, sulphur and phosphorus. Retention on these stationary phases is facilitated by non-polar solvents and elution by polar solvents.

However, K10 wash water effluent is a water based effluent and the addition of highly polar solvents into normal phase columns is not recommended. Furthermore the waste contains salts (sodium carbonate and urea) which are insoluble in non-polar solvents and thus precipitate within the non-polar solvent system causing column or frit blockage. Thus the waste would need to be de-salted. To do this C_{18} cartridges are generally used to bind the organics to the column which is then flushed with water to remove the salts, which are not retained. The isolates are eluted with a non-polar solvent. This eliminates the problem of injecting water, since the isolate is now in a less polar organic solvent, and also the problem of injecting salts, into a normal phase system. The flaw in this strategy obviously lies in the desalting. Coloured K10 wash water effluent components are not retained by C_{18} and are lost in the flushing water.

The last possible consideration was that of ionic interactions. These can be cationic (positively charged) or anionic (negatively charged). However, these groups can appear as either cationic or anionic (rather than simply one or the other) since most potentially ionic groups can be charged or not depending on the pH of the solvent used. In order to obtain effective retention of isolates on a sorbent two conditions must be met. The first is that the solvent is at a pH where both isolate and sorbent are charged and the other that the solvent does not contain high concentrations of a strongly competing ionic species. Thus water or low strength ionic buffers (<0.1M) can be used for retention at a suitable pH. In the case of anion exchange a solvent pH is required which is below the pKa of the sorbent but above the pKa of the anionic isolates, thus they are both charged. The converse regarding pH applies for cation exchange.

Elution can be achieved by several methods or combinations of them. Buffers containing high strength anions (>0.1M) or those containing high selectivity anions (in the case of anion exchange) will facilitate elution since these will compete with the anionic isolate for the sorbent. Alternately, a buffer with a pH 2 units higher than the pKa of the sorbent will cause elution since it will neutralise the charge on the anionic sorbent or a pH 2 units lower than the pKa of the isolate since it will neutralise the charge of the isolate. The converse applies to cation exchange systems.

Attempts to utilise ion exchange systems for the analysis of K10 wash water effluent components were unsuccessful. In several cases the coloured components were so strongly bound that they proved to be impossible to remove. An example of this was the use of an SAX (trimethylaminopropyl (chloride form)) sorbent which is primarily an anion exchange sorbent but also has polar and non-polar secondary interactions. The sample was applied in water and the coloured components were seen to bind to the top of the column. Various elution solvents were applied to the column at a range of pH values from pH2 to 11. Organic solvents, singularly and in combination with each other, and including water were utilised, as were high strength counter ions including citrate and iodide ions which are two of the strongest counter ions. These were also mixed with organic solvents and at various pH values. All were unsuccessful in eluting the coloured components. The column was discarded.

Fortunately, Fox (1989) recommended that 0.05% TFA (trifluroacetic acid) in water should be tried with the C_{18} column. This was very successful and elution was achieved using a gradient to acetonitrile with 0.05% TFA. The Author is, of course, aware of the danger of dissolving the stationary phase with this reagent. However this was the only solvent and stationary phase combination that actually worked. Since a system capable of producing a reliable gradient was not made available until the end of this project (the Gilson system (Section 2.7)) this analysis technique could only be applied to the ozonolysis experimentation (Chapter 6).

CHAPTER 5

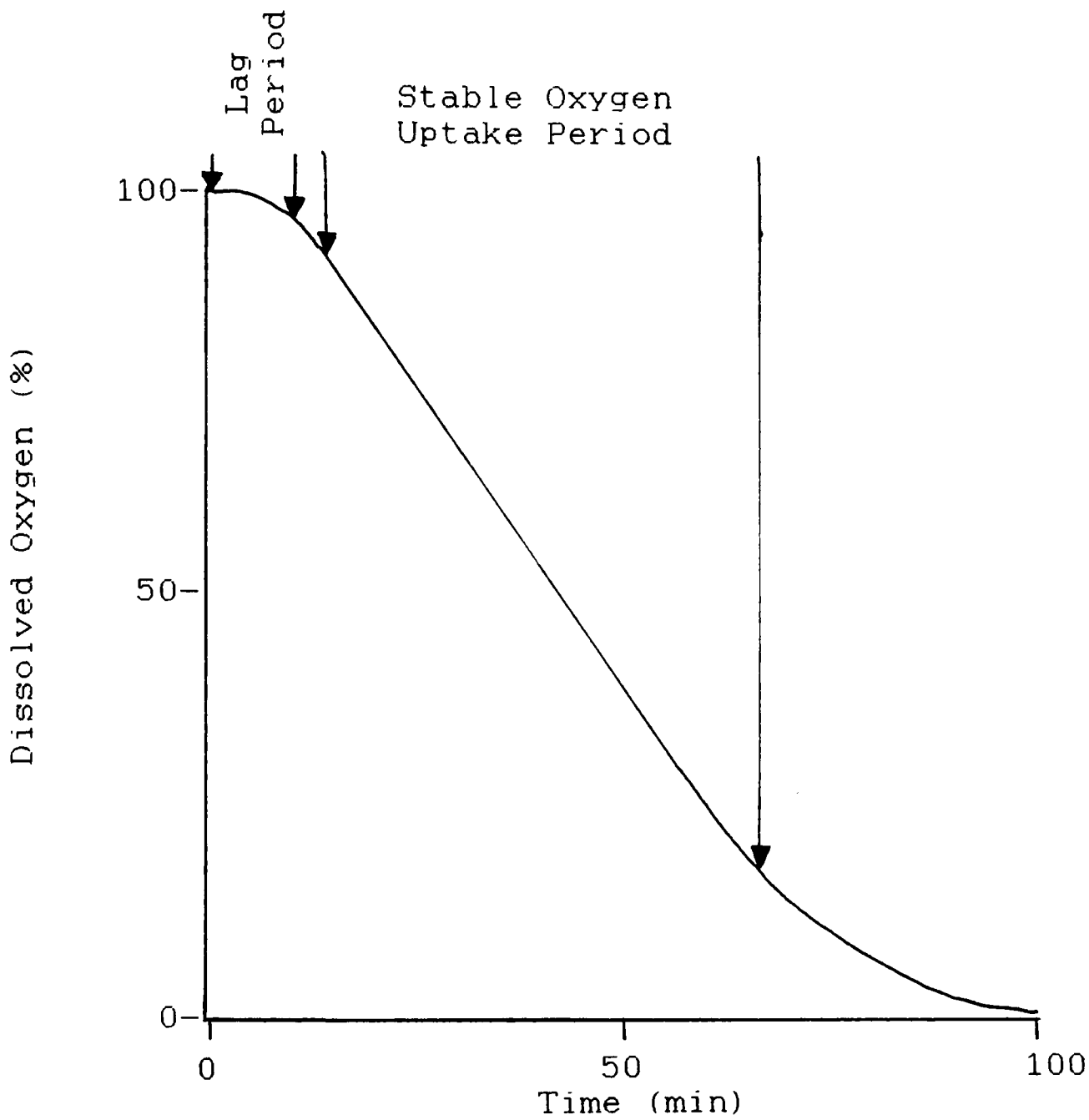
OXYGEN UPTAKE STUDIES

Information about waste treatability can often be obtained rapidly by oxygen utilisation tests. There are many different versions of this test but all are fundamentally similar - measuring the rate of oxygen utilisation by microorganisms in response to the waste to be treated. However, accurate evaluation of a waste requires extensive laboratory analysis and two questions raise concern. The first is the ability of microorganisms to degrade the waste components and the other the possible deleterious effects of the waste on biological systems.

5.1 OXYGEN UPTAKE BY SLUDGE IN RESPONSE TO K10 EXPOSURE

The equipment used and the sludge preparation for this assay are described in section 2.8. The MLSS value for the sludge within the respiration chamber was 1120mg/l and Figure 59 shows a typical oxygen uptake response. After the initial lag period there was an increase in the respiration rate which quickly became stable and it is this stable respiration period which is used to assess the effect of the waste component on the organisms.

In use, the gradient of the recorder trace is defined as the percentage oxygen (expressed as a percentage saturation of the liquor) consumed in unit time. Since, at 21^{o}C, there are



10% (v/v) K10 Waste with
adapted organisms

Figure 59. Typical Oxygen Uptake Response

8.272 milligrams of dissolved oxygen per litre in air saturated water and since the liquor in the vessel is not going to cause a large deviation in the value, the percentage dissolved oxygen value can be easily converted to milligrams of dissolved oxygen per litre.

Figure 60 shows the oxygen uptake rate for sludge from the experimental reactor (fed on 2.5% K10 medium) with varying concentrations of K10 wash water effluent. From this analysis one might conclude that 10% K10 wash water effluent is the highest exposure concentration for the microorganisms, above which it becomes toxic or inhibitory, since the uptake rate peaks at 10% concentration and falls off after this value. It would also appear that K10 wash water effluent was readily metabolised by microorganisms. However, exposure of organisms from the control reactor to K10 wash water effluent (Figure 61) gave results which were contradictory to that which might have been expected when comparing carbon source utilisation between adapted and unadapted organisms. The unadapted organisms had a much higher oxygen uptake rate throughout, which was maximal at 0.45% K10 wash water effluent concentration. This was tentatively attributed to uncoupling of oxidative phosphorylation by some of the aromatic compounds contained within the K10 wash water effluent. This process entails the breakdown of electrochemical membrane potential and prevents the phosphorylation of ADP to ATP. This, in turn, provokes an increase in the rate of oxygen uptake. Many such uncoupling agents have been described; most are lipid soluble,

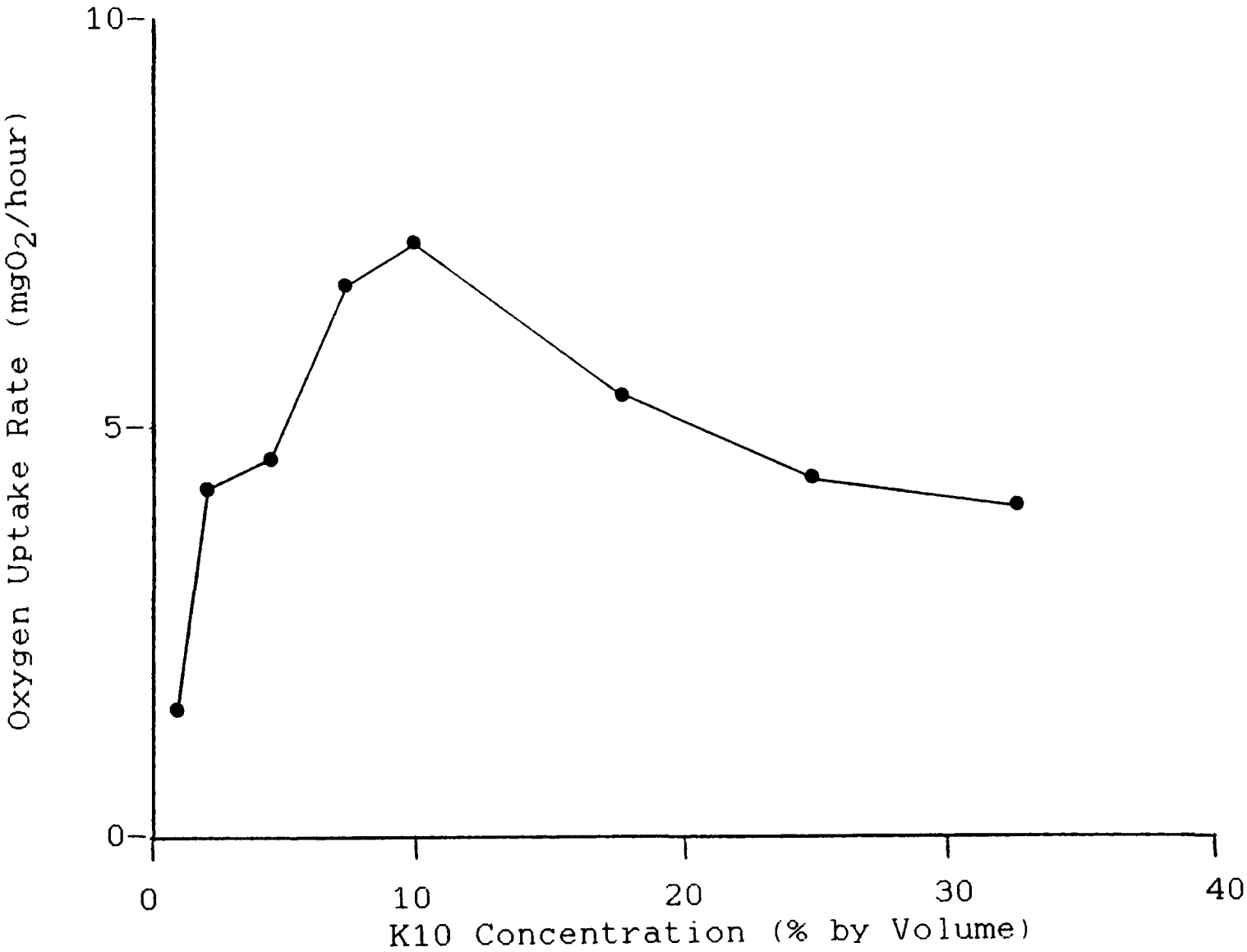


Figure 60. Affect of K10 Wash Water Effluent on the Oxygen Uptake Rate of Activated Sludge from the Experimental Reactor

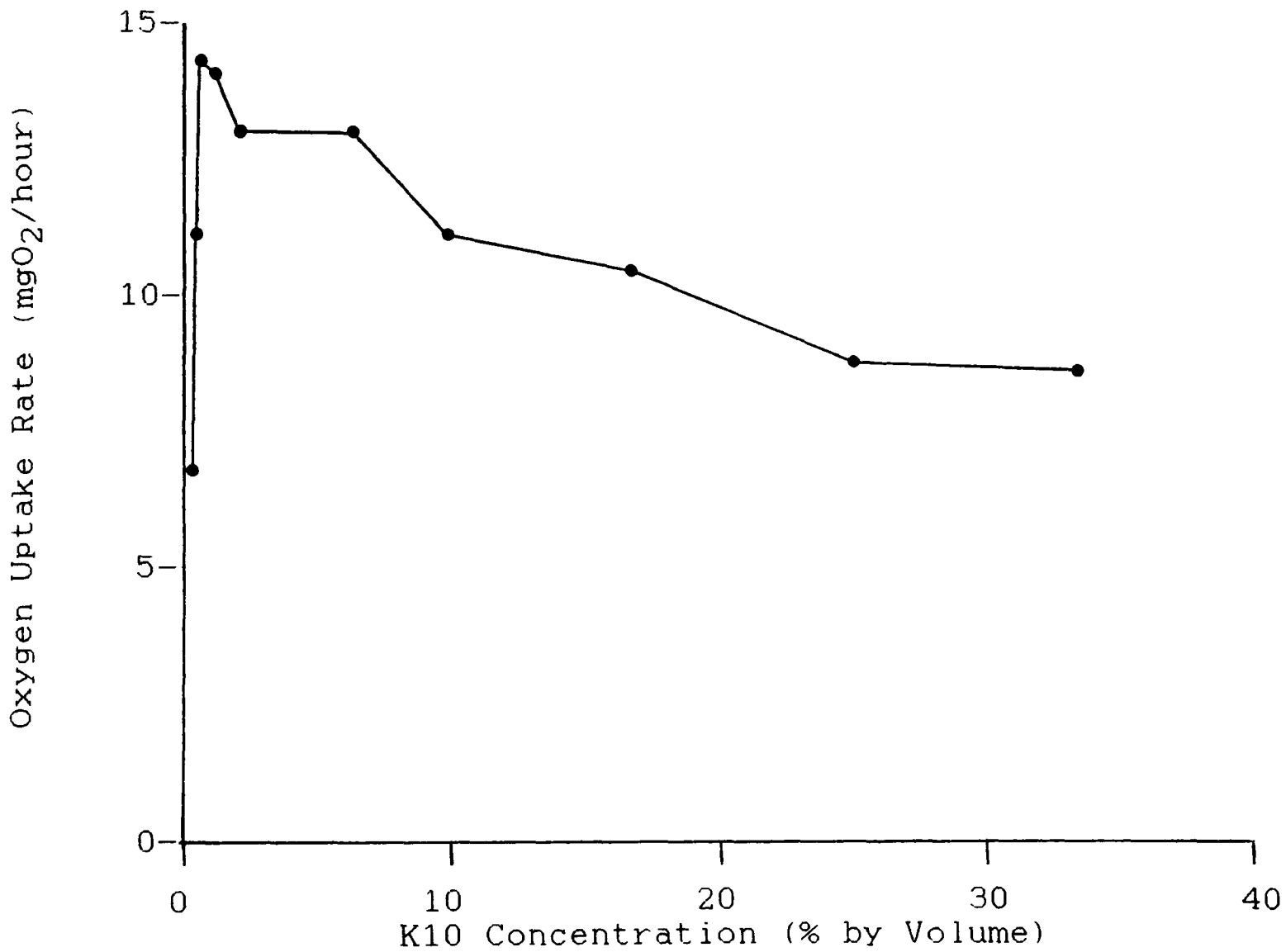


Figure 61. Affect of K10 Wash Water Effluent on the Oxygen Uptake Rate of Activated Sludge from the Control Reactor

have an acidic group and an aromatic ring. Dinitrophenol is a classical uncoupling agent (Lehninger, 1977) and has obvious similarities with K10 wash water effluent components. Clearly the adapted organisms must have developed a mechanism, possibly involving modifications to the cell-wall/cell-membrane system, which enabled them to tolerate uncoupling agents of this nature, and thus survive and proliferate. It might be useful at this stage to define an uncoupling agent. It is a compound that can cause an accelerated dissipation of proton motive forces by transporting, or allowing movement, of protons across a membrane.

5.2 FURTHER CONFIRMATION OF UNCOUPLING ACTIVITY

The technique used for detection of uncoupling activity is described in Section 2.9 and the resultant chart recorder traces are shown in Figures 62a and 62b. In general, the absorbance decrease after the pulse of light follows a half life curve (Jackson, 1988). Thus a plot of log absorbance versus time should yield a straight line. This was true for the control sample and for the carbonyl cyanide-p--trifluoromethoxyphenyl-hydrazone (FCCP) uncoupler analyses. However K10 wash water effluent results (Figure 63) did not follow such a half life curve. Instead it appeared more as a gate effect, in that the majority of the membrane potential was dissipated within the first tenth of a second (for the 2.0 and 5.0% K10 concentration). As the test progressed further,

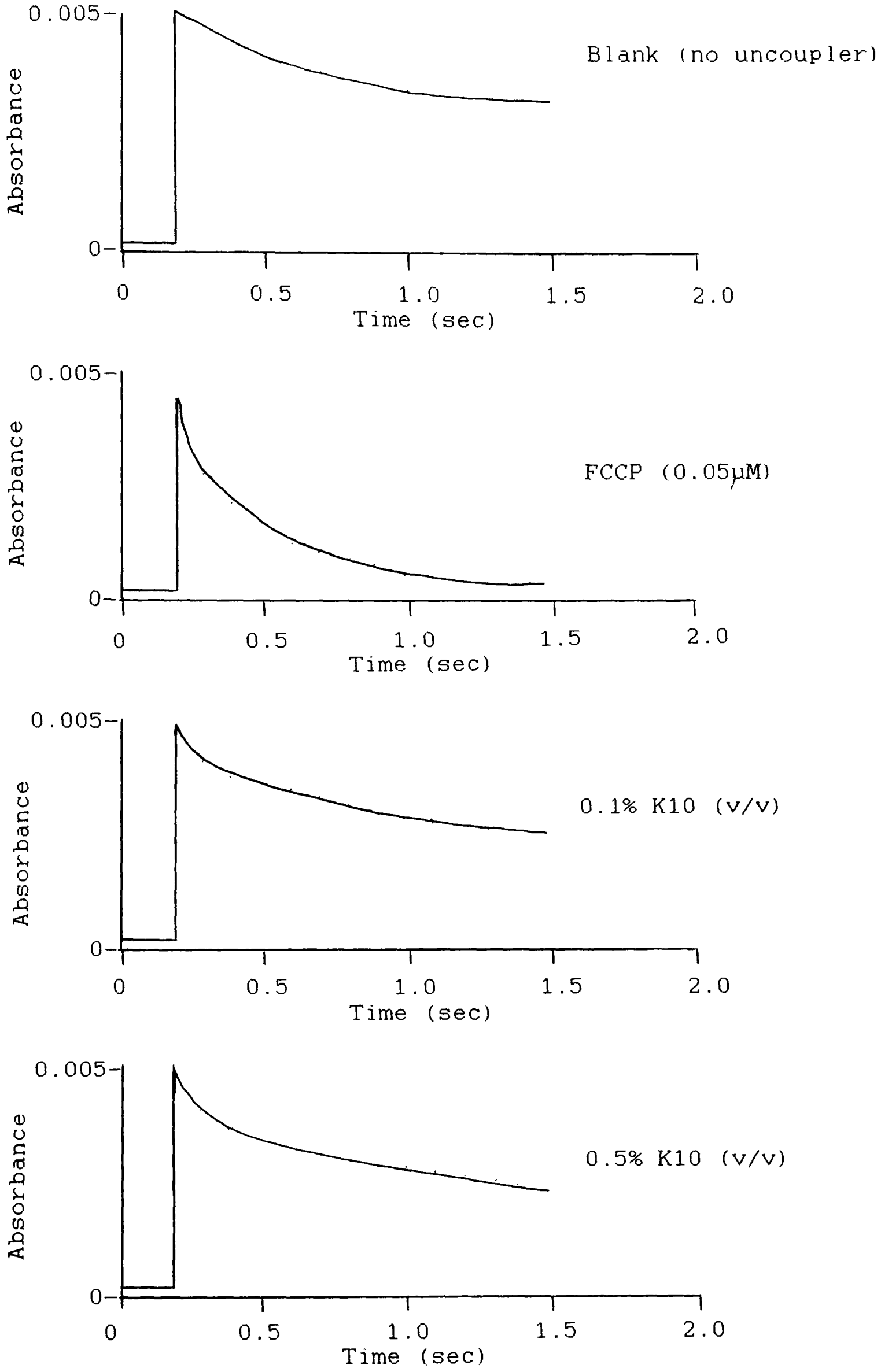
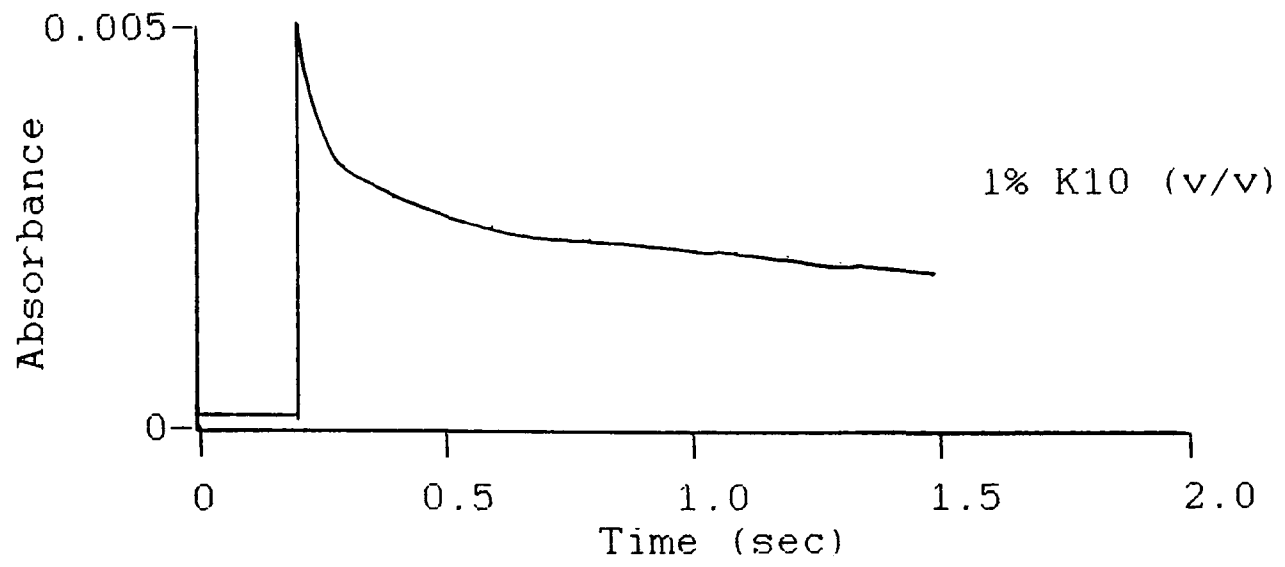
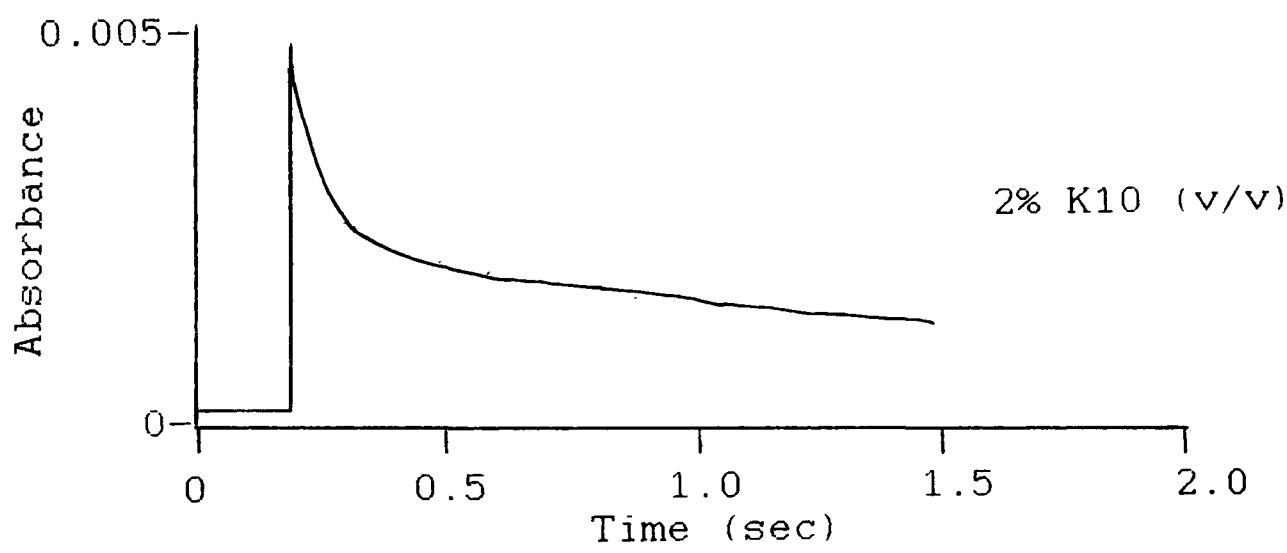


Figure 62a. Affect of K10 Wash Water Effluent on Absorbance Decay Rate in Membrane Vesicles from Rhodobacter capsulatus due to Dissipation of Membrane Potential





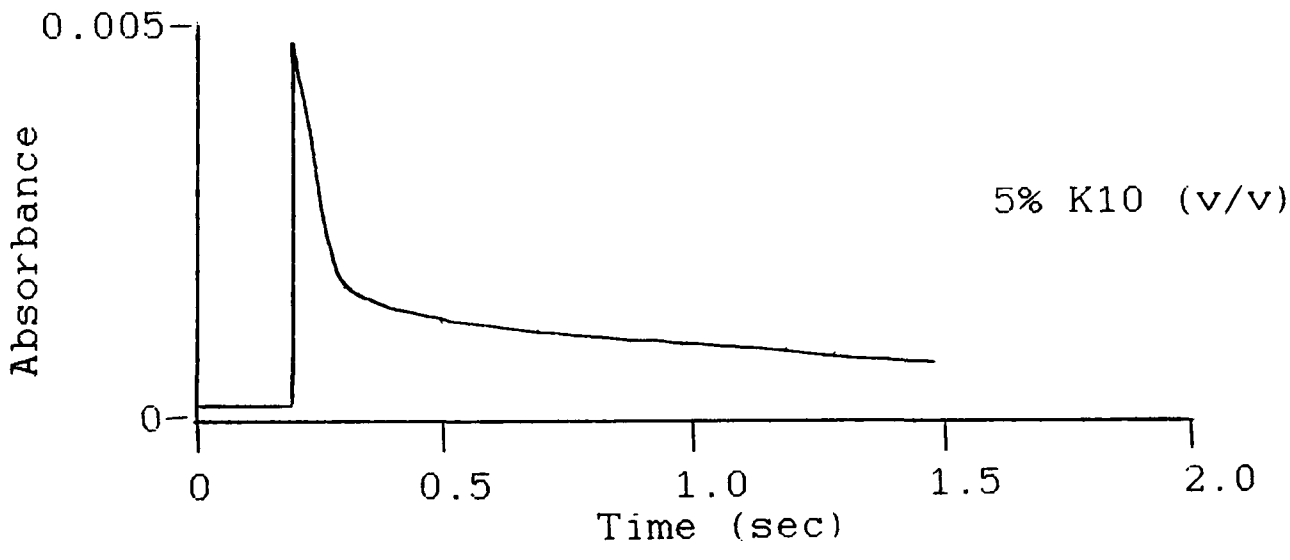


Figure 62b. Affect of K10 Wash Water Effluent on Absorbance Decay Rate in Membrane Vesicles from Rhodobacter capsulatus due to Dissipation of Membrane Potential

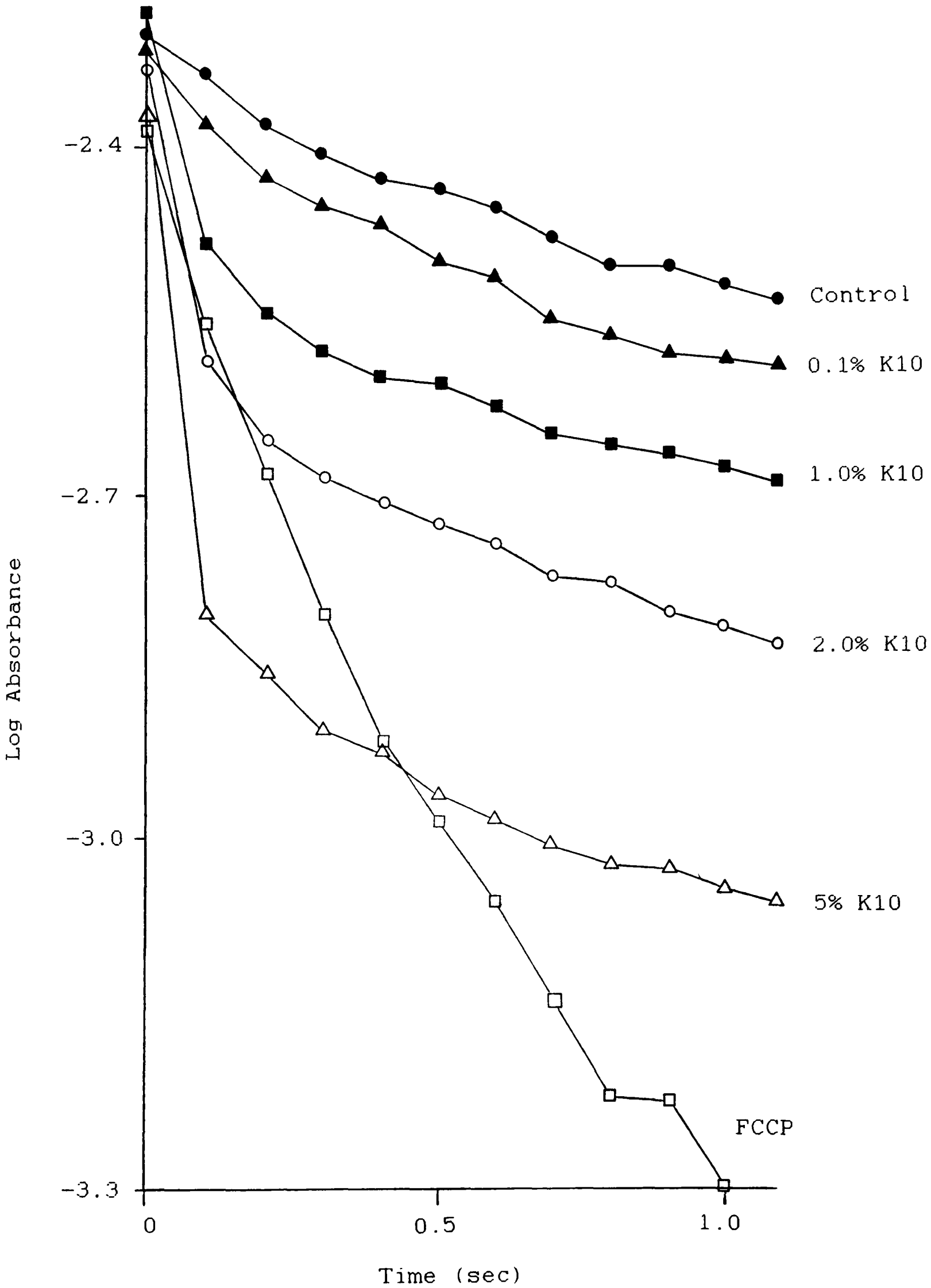


Figure 63. Log Absorbance vs Time for Several K10 Wash Water Effluent Concentrations

the membrane potential dissipation rate then roughly matched that of the control; so this system did not behave in the same way as FCCP.

This experiment therefore showed that membrane potential was dissipated by K10 wash water effluent but did not throw light on the mechanisms involved. It would seem probable that K10 was a true uncoupling agent since it also increased oxygen uptake rates. The abnormal uncoupling response was considered to occur in one of two ways, the explanation of either being highly speculative. The first possibility is that the uncoupling agent might be inactivated by its own uncoupling activity. However, repeated light pulse exposure to the same sample yielded similar results each time although this could be attributed to the diffusion of new active uncoupler into the membrane in the period (several minutes) between light pulses. Another possibility is that the protonated form of the carrier molecule might move quickly through the membrane, thus transporting a proton, whilst the ionic form travels more slowly across the membrane and thus cannot repeat the process within the short duration of the experimental period (<2 seconds). Both mechanisms would give a rapid initial response followed by a slower decrease in membrane potential.

5.3 REJECTION OF HYPOTHESES

An experiment was devised which would either support or totally reject the hypotheses proposed in the previous section. However a new batch of K10 wash water effluent was now being utilised for experimentation and so the initial electro-chemical membrane potential dissipation experiments were repeated using this new batch of waste. The results were identical and this confirmed that the observed uncoupling effect was not an anomaly associated with one single manufacturing batch.

This experiment involved the adaptation of the experimental equipment (Section 2.9) to allow a second light flash. The reader will recall that the first light flash was at time = 0. The second one came between 0.7 and 0.8 seconds after the first. The overall duration of the test was 2 seconds as before. K10 wash water effluent (5.0%) was used in this experiment and the results shown in Figure 64. The rapid dissipation of membrane potential after both light flashes, at equivalent rates, means that both hypotheses explaining this process must be rejected. Other theories as to the mode of action of this previously unencountered form (Jackson, 1988) of uncoupling effect have been elusive.

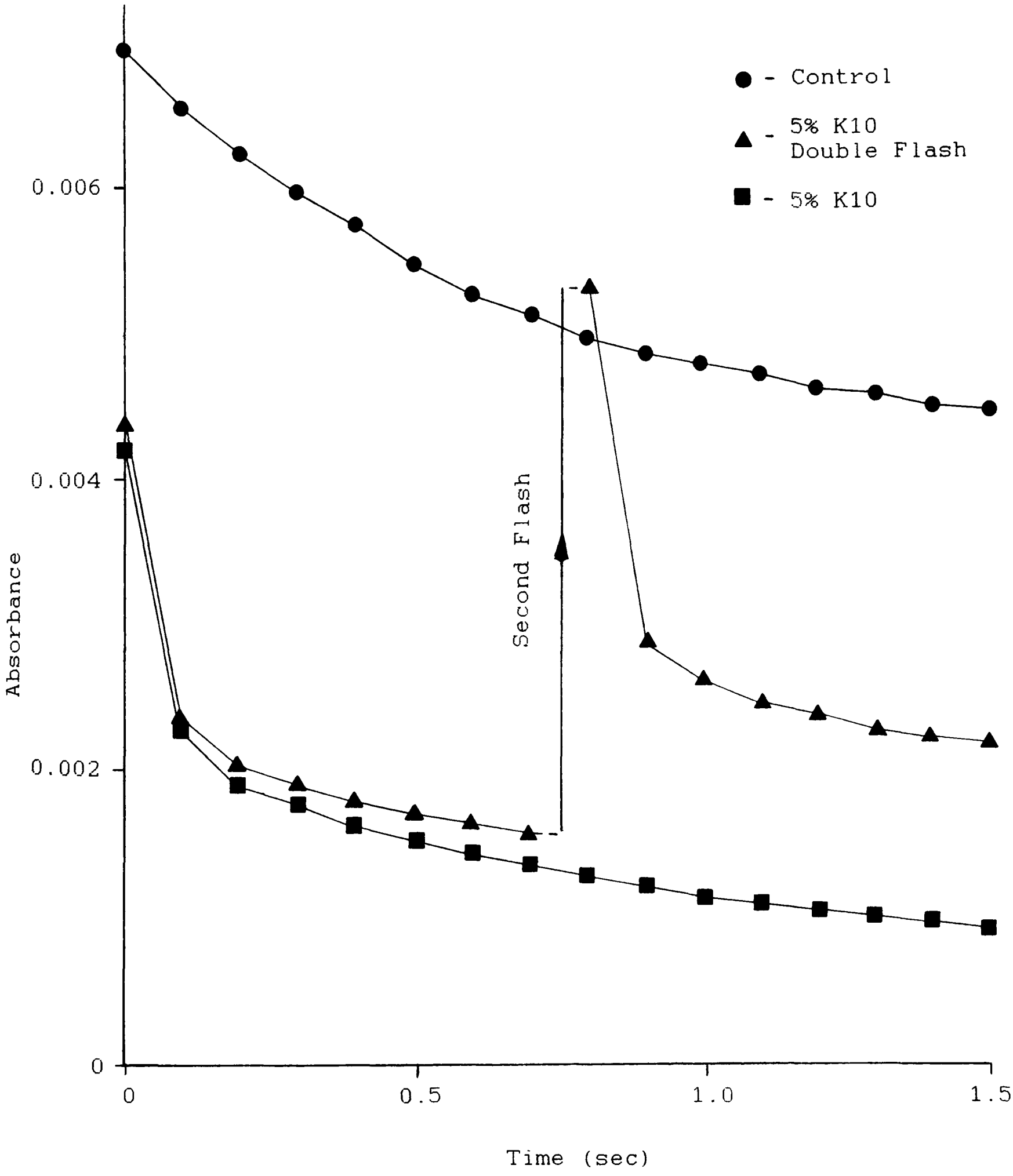


Figure 64. Affect of Double Light Flash on Absorbance Decay Rate in Membrane Vesicles from Rhodobacter capsulatus due to Dissipation of Membrane Potential

5.4 DISCUSSION

ATP has probably been with us since the primordial broth, and as proton motive forces are required in all living organisms including anaerobes, the treatment of K10 wash water effluent by biological means obviously has some serious implications. It is unlikely that a new organism will suddenly evolve that does not require membrane potentials and so adaptation of existing organisms is necessary. This might, quite simply, involve selective exclusion of the molecule from the cell, via the cell wall. Alternatively, it could involve the secretion of an enzyme which inactivates or degrades the uncoupling agent.

Organisms may currently exist within the environment which are capable of mineralising K10 wash water effluent components. Likely places include the open soil-lined effluent ditches (rhines) located in Bridgwater, Somerset, which carry the analogous TNT red water. However soil samples were obtained from this site and introduced to the activated sludge plants without any improvement in plant performance (though this does not imply that organisms do not exist at this site, merely that active cultures were not isolated).

5.5 TNT - MEMBRANE POTENTIAL DISSIPATION

Since K10 wash water effluent was capable of dissipating electro-chemical membrane potentials it was a point of interest to examine the analogous TNT red water for similar properties.

The testing of TNT red water was carried out in the same manner as for K10 wash water effluent (Section 2.9). The resultant chart recorder traces are shown in Figures 65a and 65b. These resemble those of a classical uncoupling agent such as FCCP. A log plot of absorbance verses time shows this more clearly (Figure 66). Thus TNT red water is capable of dissipating electro-chemical membrane potential but it would not necessarily behave as an uncoupling agent when presented to activated sludge organisms. This would require further investigations such as those performed on K10 wash water effluent in Section 5.1. However, this, in turn, would have required larger quantities of red water than had been made available, since some organisms would have needed to have been exposed to TNT red water for periods of several months, and the investigation was beyond the scope of this research project.

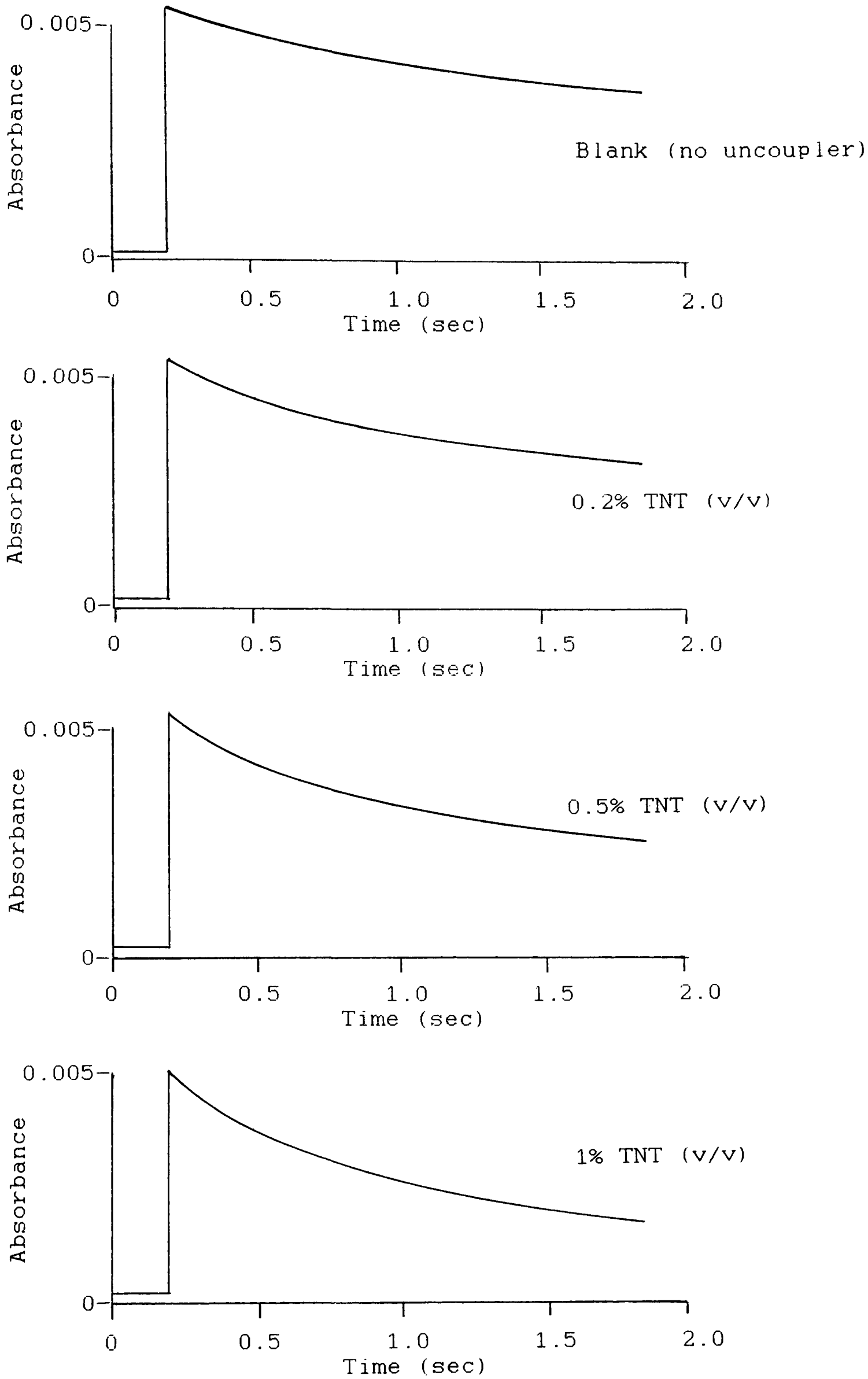


Figure 65a. Affect of TNT Red Water Effluent on Absorbance Decay Rate in Membrane Vesicles from Rhodobacter capsulatus due to Dissipation of Membrane Potential

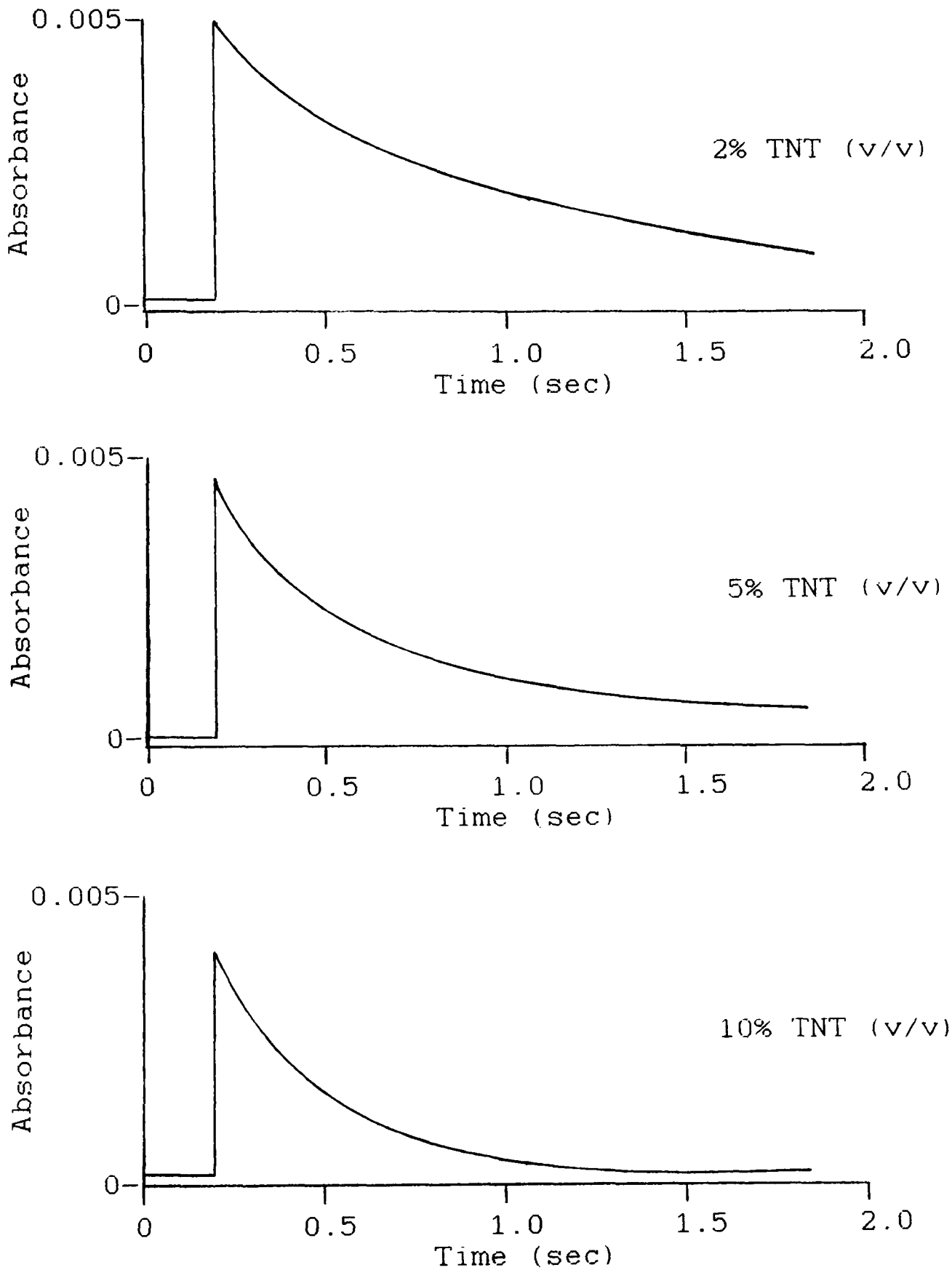


Figure 65b. Affect of TNT Red Water Effluent on Absorbance Decay Rate in Membrane Vesicles from Rhodobacter capsulatus due to Dissipation of Membrane Potential

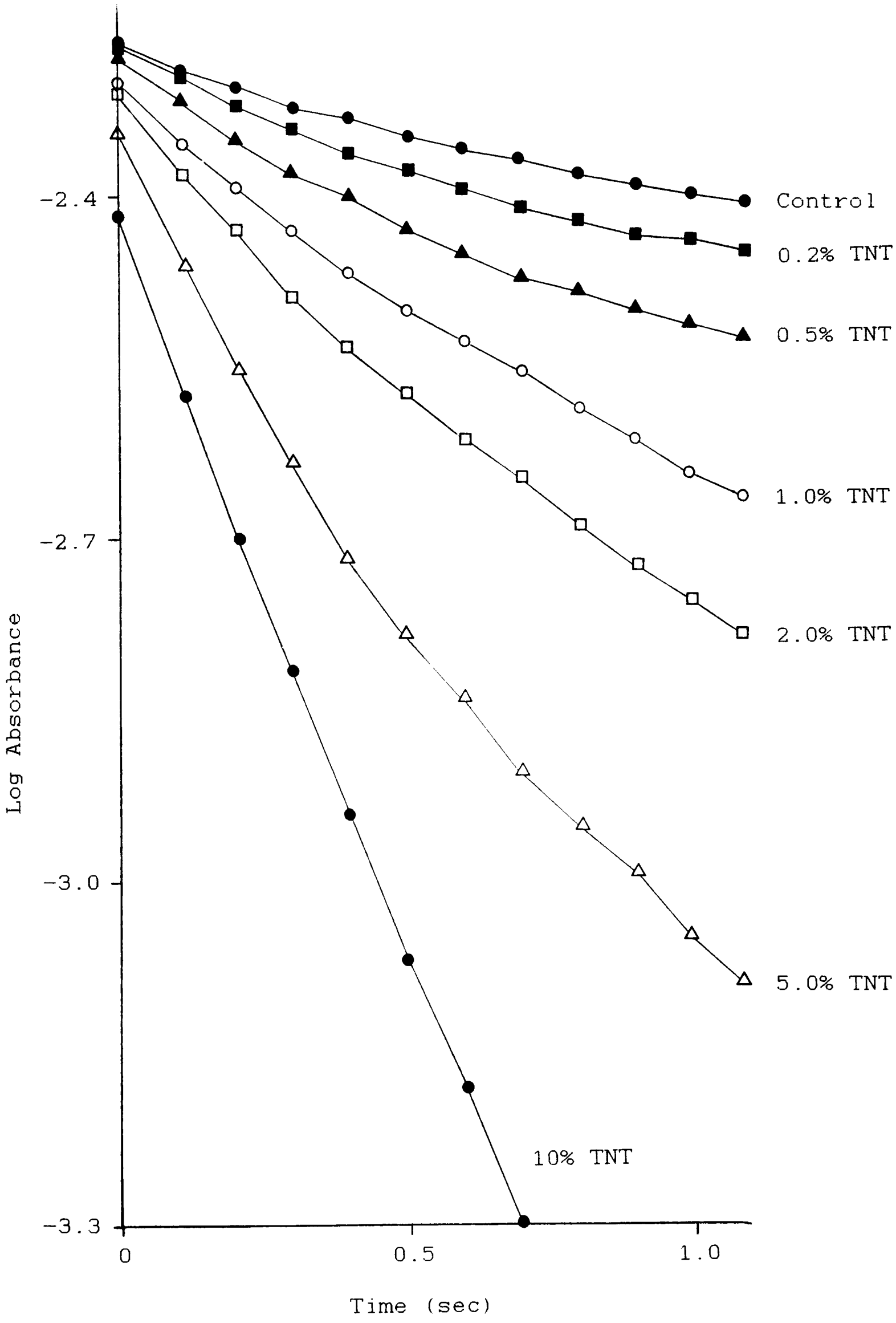


Figure 66. Log Absorbance vs Time for Several TNT Red Water Effluent Concentrations

CHAPTER 6

CARBON AND OZONE TREATMENT RESULTS

6.1 ACTIVATED CARBON

The equipment used to assess the feasibility of activated carbon treatment is described in Section 2.10. The carbon column packing had a dead volume of 36.5ml and K10 wash water effluent was applied to the column at a rate of 0.93ml/min with a retention time of 39.3min. Schulte (1973) described 22.2 minutes as a long retention period in his experimentation during the treatment of a TNT manufacturing waste stream while using granular carbon. The reader will recall from Section 1.4.1 that powdered carbon will have a higher rate, and possibly capacity, for adsorption of this coloured material over that of granular carbon. Thus powdered carbon was chosen for the work on K10 wash water effluent: a much extended contact time was used in order that considerable adsorption could occur. The results of this experiment are shown in Figure 67 and the basic trend is similar to the results of Schulte (1973). However, the absorbances at both 260nm and 350nm increase simultaneously, suggesting that the coloured compounds contain unsaturated bonds. In this connection, Schulte (1973) has already mentioned that in his opinion, coloured compounds from TNT wastewater are eluted before nitroaromatics. He does not however indicate the nature of such compounds, and, indeed, the present Author finds difficulty in giving them a structure if it is not nitroaromatic. There is thus a situation of considerable

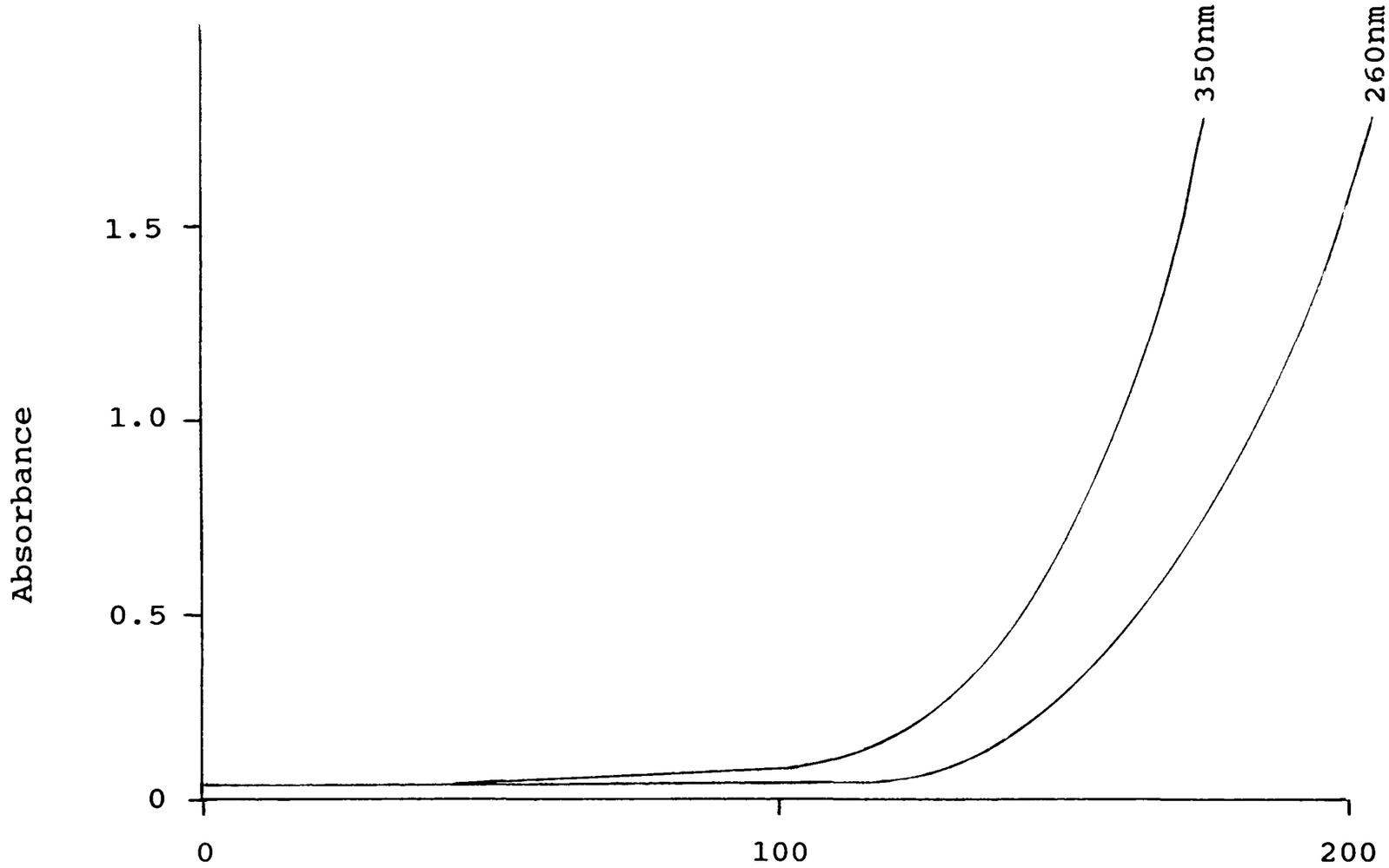


Figure 67. Monitoring of Activated Carbon Treatment of K10 Wash Water Effluent by Continuous Absorbance Measurement

confusion. The analogous K10 situation seems to be equally clouded: if some of the coloured compounds are not nitroaromatic (originally suggested to be Meisenheimer complexes (Section 1.3.3)), then this Author has no idea at all as to their identity.

6.1.1 Costing of Carbon Treatment

The carbon appeared saturated (retention capacity lost) after about 140ml of K10 wash water effluent was applied. However, the column was originally filled with water, and so a dead volume of 36.5ml must also be taken into account. 20g of carbon can therefore treat approximately 100ml of raw K10 wash water effluent, and thus 200kg carbon is required per cubic metre of K10 wash water effluent.

Based on a production figure of two tonnes of K10 per annum, an estimate of the treatment cost for the wash water effluent can be made using activated carbon. Since K10 has a density of 1.34Kg/l, two tonnes of K10 has a volume of $1.49m^3$. During manufacture approximately six volumes of wash water are produced per volume of K10 and so $8.96m^3$ of wastewater will be produced per annum. Thus, from the results of our experimentation, 1.791t of activated carbon would be required for successful treatment of K10 wash water effluent generated in one year at a cost of £5400 (FSA Laboratory Supplies at £3/Kg).

The next obvious step is carbon regeneration. However for the analogous TNT red water adsorption process, regeneration is not a feasible option (Section 1.4.1) and the spent carbon has to be discarded. This may also be the case for K10 wash water effluent and thus the purchase of carbon would be a recurrent expenditure. Moreover, further costs will be incurred in the disposal of the spent carbon.

6.2 OZONOLYSIS

Many ozone treatment assessments were carried out on K10 wash water effluent. Preliminary investigations involved the use of a 5L vessel with K10 wash water effluent at 10%(v/v) in distilled water. Sample analysis was of COD, TOC and colour with no measurement of the ozone entering or leaving the contactor and thus it was not possible to estimate the quantity of ozone required for treatment. However, these experiments demonstrated the potential of ozone for the oxidation of K10 wash water effluent since the relative reduction of all parameters monitored was high.

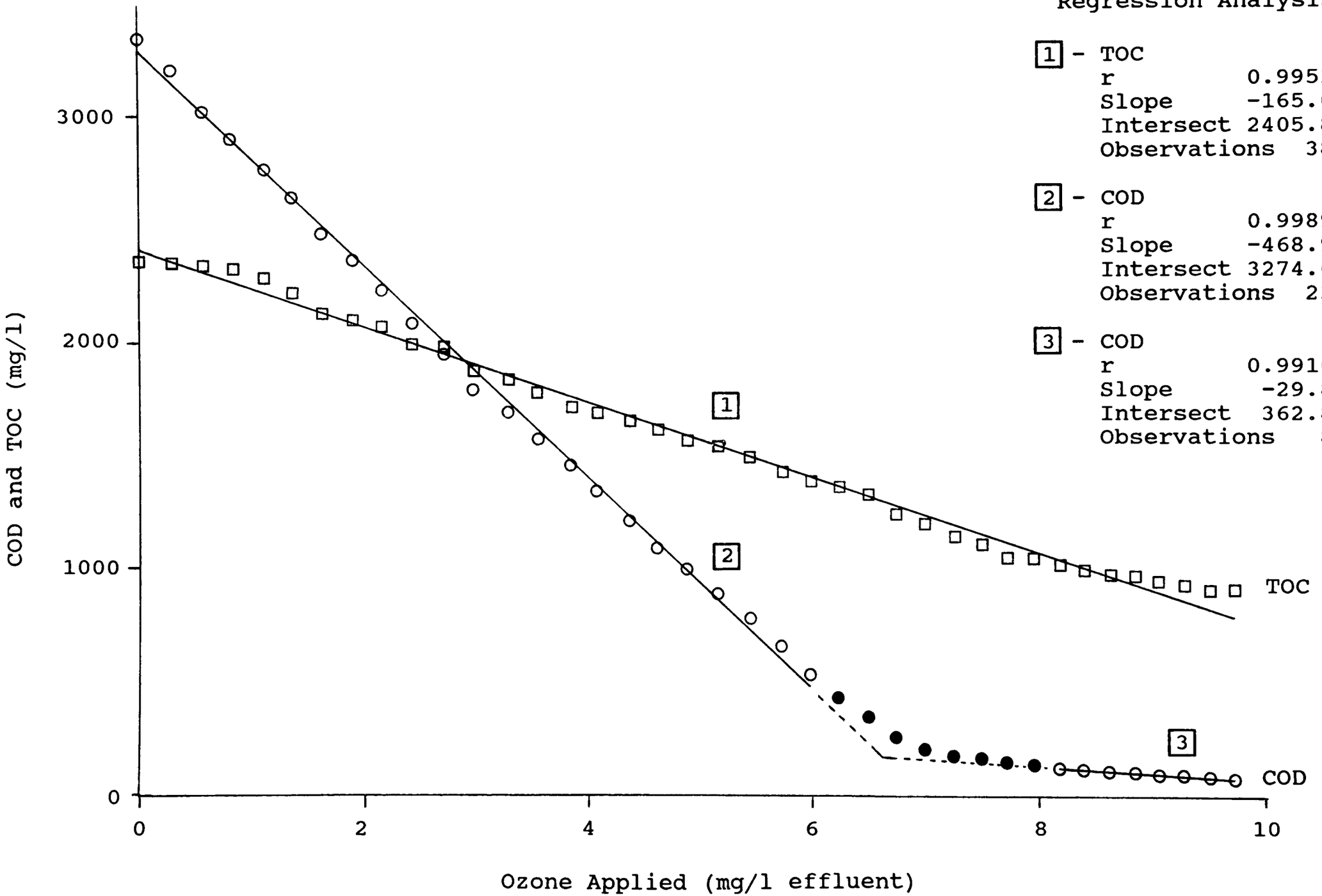
Secondary investigations were carried out at Ozotech Limited, Burgess Hill, Sussex. 5%(v/v) K10 wash water effluent in distilled water was ozonised in a column over 3m tall with a capacity of 6.5L. In these investigations the parameters monitored included COD, TOC, full wavelength scans (200-500nm) and also the measurement of ozone entering and leaving the contactor. Hence the amount of ozone required to oxidise K10 wash water effluent could be calculated. However, the reductions of COD and TOC were not as consistent with the amount of ozone applied as was hoped and this was attributed to the carry over of differing amounts of the previous sample through the sample port.

Finally a 36L capacity contactor, over five metres in height, was constructed as described in Section 2.11. The

sample port was filled with air between sample periods and then flushed thoroughly before sampling. Ozonolysis in this contactor was performed at 5%, 10%, 25% and 40%(v/v) K10 wash water effluent in distilled water. The pattern of results was similar at all concentrations and only the 40% K10 wash water effluent treatment assessment will be discussed further since analysis at this concentration included high performance liquid chromatography (Section 4.3.3) as well as analysis of COD, TOC, full wavelength scans (200-500nm) and also the measurement of ozone entering and leaving the contactor.

The ozone generator was operated at a power of 1.2A and the air ozonised at a pressure of 0.75bar gauge. The ozonised air was supplied to the contactor at a rate of 8.5 l/min (STP) giving an ozone doseage rate of 9.75gO_3/h. The efficiency of ozone utilisation during ozonolysis of 40% K10 wash water effluent was greater than 99% during the first 20 hours of ozonolysis and after 38 hours 80% of the ozone was being utilised. The amount of ozone utilised was assessed every hour during ozonolysis and the results of COD, TOC and wavelength scans (Figures 68 and 69) are plotted against ozone utilised rather than time. The COD value decreased rapidly with a slope of -468.88 thus 1g of ozone removed 468.88mg of COD. However, once 90% of the COD had been removed the the rate of COD removal dropped to reach a value of 29.81mg of COD removed per gramme of ozone applied. If both lines are extrapolated they intersect at 165mg/l and this point could be taken as the COD value at which ozonolysis was no longer efficient at removing

Figure 68. Reduction of COD and TOC Values versus Ozone Applied



Regression Analysis

1 - TOC

r	0.9951
Slope	-165.0
Intersect	2405.8
Observations	38

2 - COD

r	0.9989
Slope	-468.9
Intersect	3274.0
Observations	23

3 - COD

r	0.9910
Slope	-29.8
Intersect	362.8
Observations	8

Figure 69. Changes in Absorbance versus Ozone Applied

COD. However, ozone was still being utilised, and even at the end of this ozonolysis assessment, 80% of the ozone applied did not leave the contactor. Examination of the TOC analysis results shows a continuous removal of carbon throughout the ozonolysis period at a rate of 164.95g carbon removed per gramme of ozone applied, probably as carbon dioxide. Since the TOC removal rate did not decrease as dramatically as the COD analysis, it would imply that the ozone continued to oxidise compounds which were not available to the COD analysis procedure.

Figure 70 shows the relative reduction in COD, TOC and absorbance at 250nm against the amount of ozone utilised. The calculation of the sample correlation coefficients (r) show virtually linear relationships of these parameters with ozone applied (the minimum value of r is equal to 0.9951 for the TOC analysis (very significant indeed)). Since the correlations between the values of TOC, COD and absorbance at 250nm and the ozone applied decrease linearly it would therefore follow that they also have a linear relationship one with an other. For instance the relationship between relative change in absorbance at 250nm and COD has a sample correlation coefficient of 0.9979 (Figure 71). This therefore enables one to monitor closely the way in which COD and TOC change during ozonolysis merely by simple absorbance measurements.

The removal of colour was very rapid (Plate 14), as would be expected, since colour is due to resonance of unsaturated

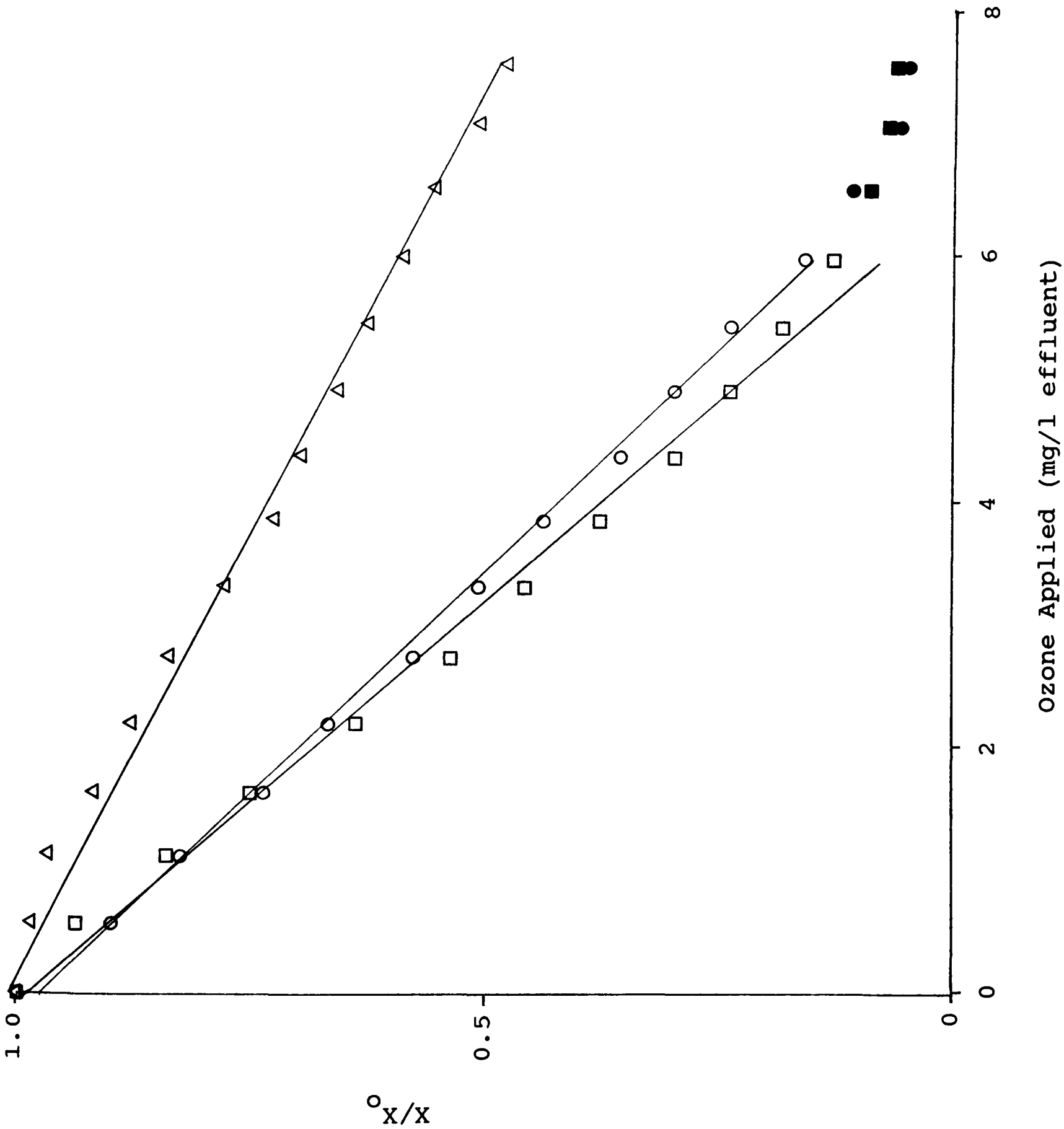


Figure 70. The Reduction of COD, TOC and Absorbance, versus Ozone Applied, relative to the Initial Value prior to Ozonolysis

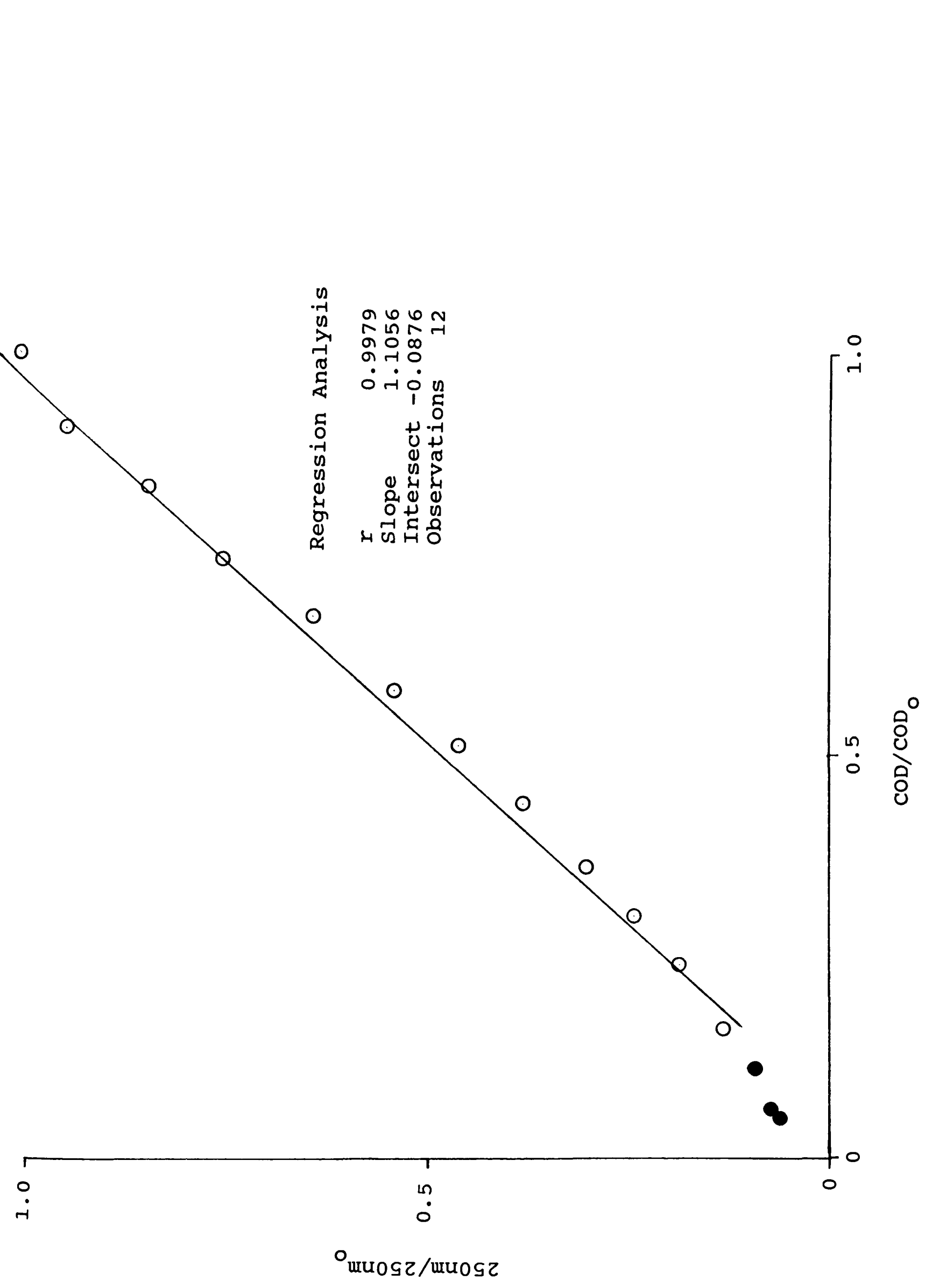


Figure 71. Comparison of COD and Absorbance values during Ozonolysis

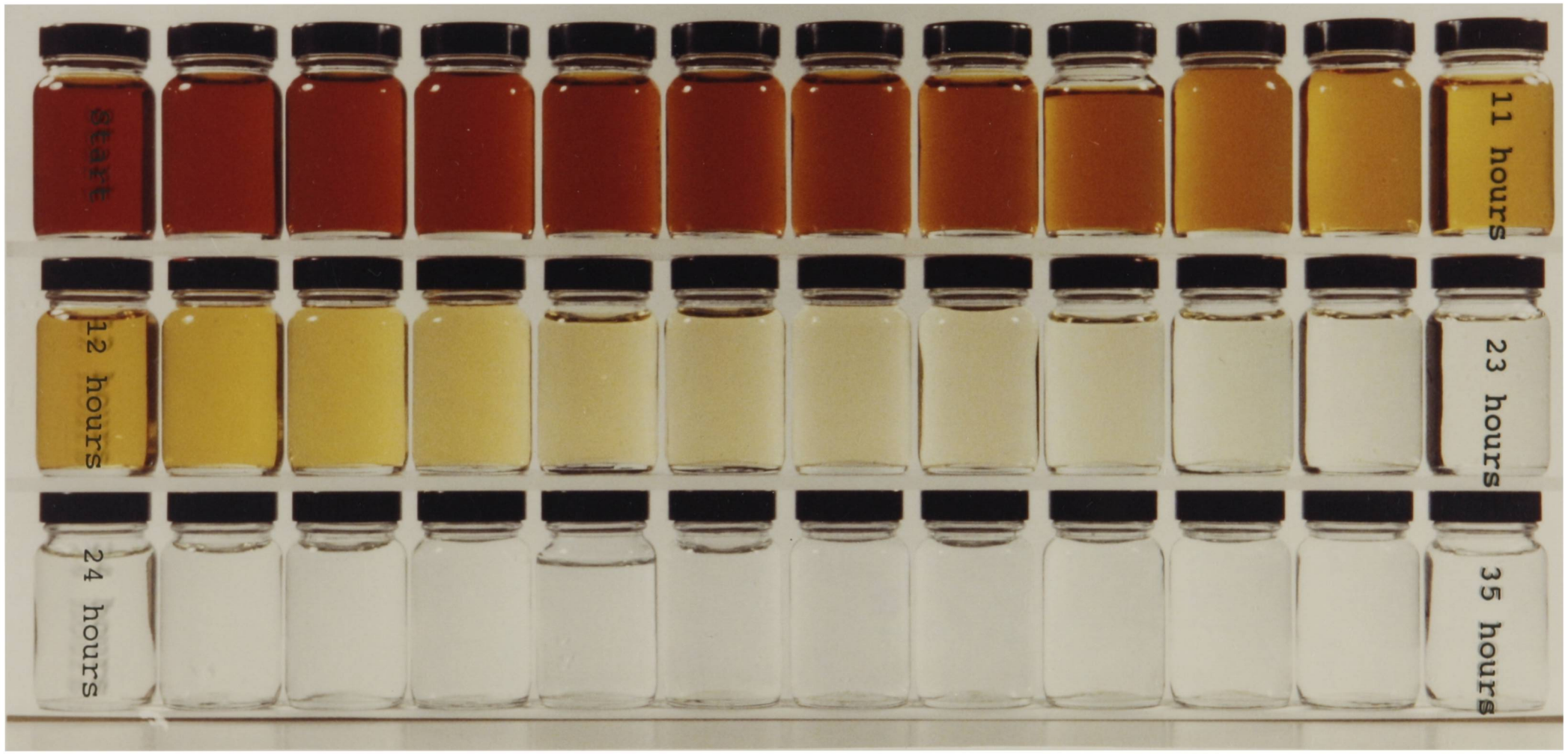


Plate 14. Colour reduction during the ozonolysis of 40% K10 wash water effluent

bonds which ozone preferentially attacks. The decrease in absorbance at 250nm was somewhat slower since this reflected the total number of unsaturated bonds which still remained after the colour-forming resonating bonds had been disrupted. In contrast, the absorbance at 200nm increased during ozonolysis, probably due to the production of organic acids which form when aromatics are broken down by ozone (Sections 1.4.6.3 and 1.4.6.8).

The increase in compounds which absorbed light at 215nm was observed using HPLC analysis (the shortest wavelength possible for reliable analysis under the mobile phase conditions) whilst at 260 (unsaturated bonds) and 350nm (orange colouration) almost all peaks were reduced substantially in terms of height and area during ozonolysis. Figures 72a to 74b and 75 to 77 show the HPLC results for the start, middle and end of the ozonolysis of 40% K10 wash water effluent at 260 and 215nm respectively (all results Appendix 5). Peaks which absorbed light at 350nm (and therefore orange coloured) are shown in Figure 78.

The peaks which eluted rapidly are considered to be organic acids which increase in concentration during ozonolysis. They absorb light at 215nm more strongly than at 260nm and have a retention time of less than two minutes. This further confirms their identity since under the conditions utilised (Section 4.3.3) these compounds would need to be highly polar to elute rapidly, and organic acids are highly polar compounds.

Figure 72a. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 250nm

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	0.0g/l effluent

Figure 72b. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 250nm (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	0.0g/l effluent

Figure 73a. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 250nm

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	3.2508g/l effluent

Figure 73b. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 250nm (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	3.2508g/l effluent

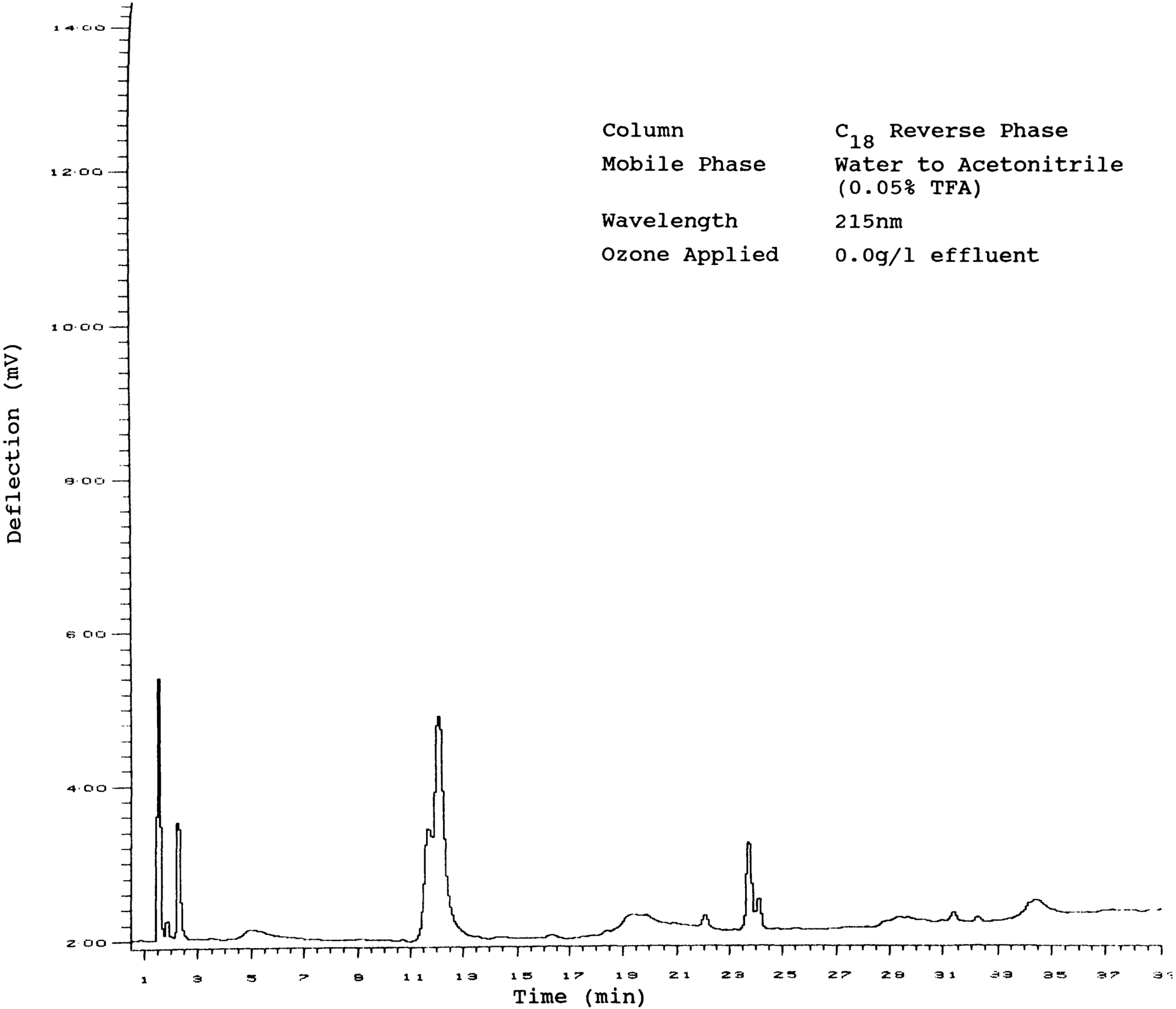
Figure 74a. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 250nm

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	8.4047g/l effluent

Figure 74b. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 250nm (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	8.4047g/l effluent

Figure 75. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 215nm



Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	215nm
Ozone Applied	0.0g/l effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	215nm
Ozone Applied	3.2508g/l effluent

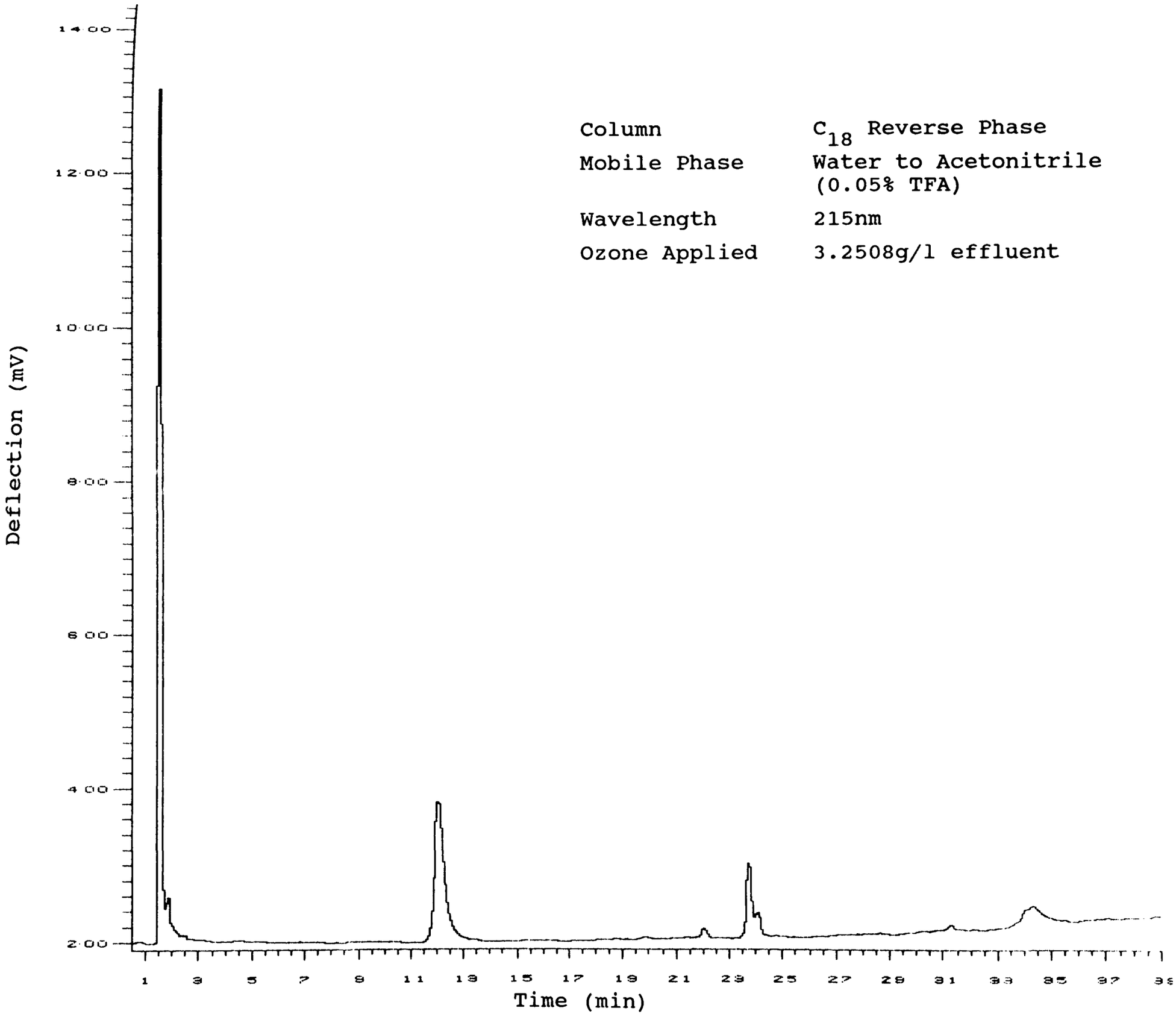


Figure 76. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 215nm

Figure 77. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 215nm

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	215nm
Ozone Applied	9.3000g/l effluent

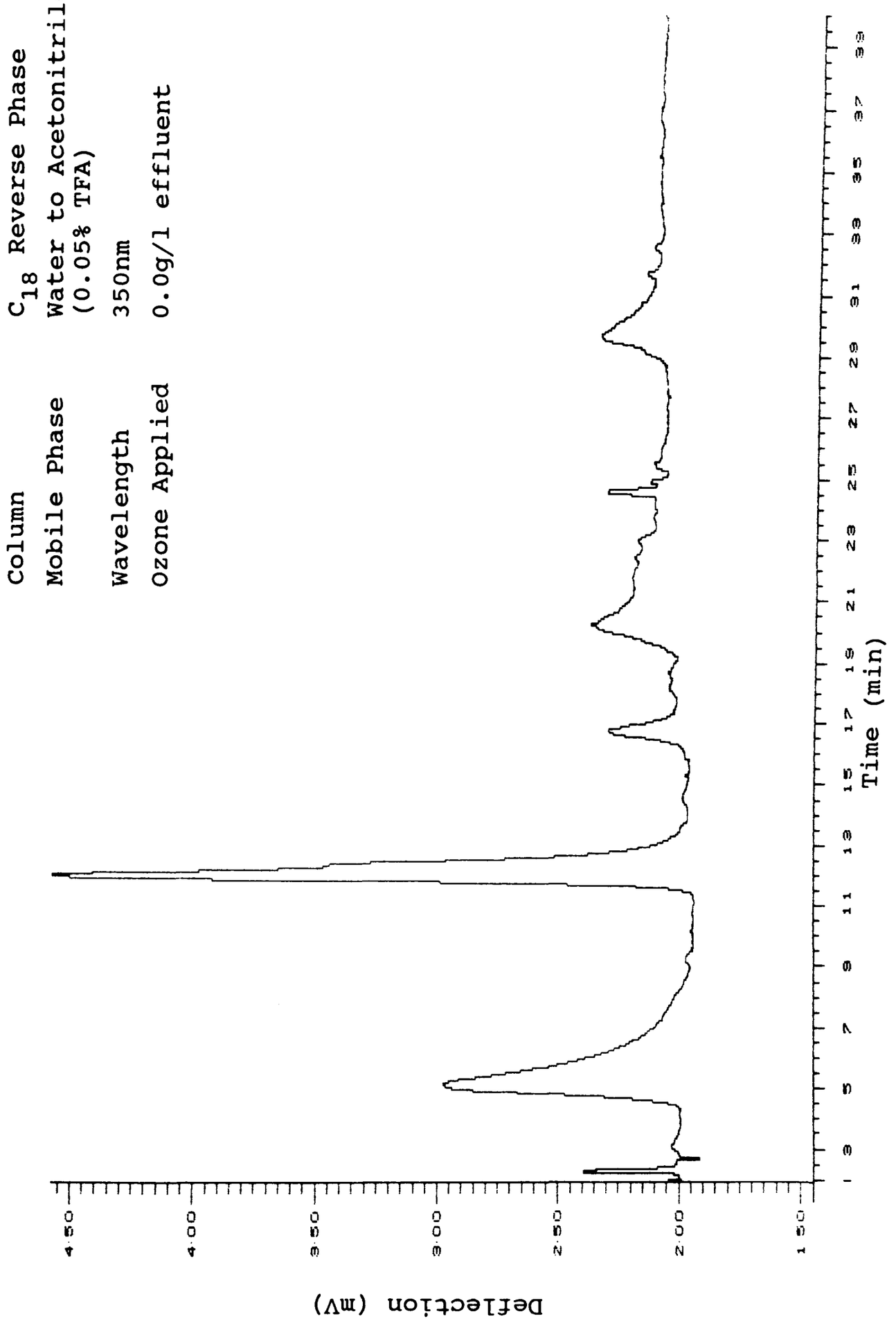


Figure 78. HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent - 350nm

6.2.1 Ozonolysis Costing

The costs in terms of capital investment are discussed in Section 1.4.6.9. From this cost assessment, one of the smaller ozone generators capable of producing $72gO_3/h$, would supply sufficient ozone to treat the volume of K10 wash water effluent which is anticipated to be produced per annum. This unit would cost approximately £25,000 which includes an ambient ozone monitor but not an ozone contactor. However, once the capital costs of this treatment process have been met then the only further expenditure is that of operating costs for which electricity and maintainance are the main considerations.

In Section 6.1.1 the volume of K10 wash water effluent was calculated to be $8.96m^3$ per annum. If ozone is applied to a 40% K10 wash water effluent concentration until the COD value is reduced to 165mg/l (the intersect of the lines from the fast and slow COD removal rates (Figure 68)), then 6.6g of ozone would have been utilised. This is equivalent to 16.5g of ozone per litre of undiluted K10 wash water effluent. However, if the COD was to have been reduced to less than 75mg/l then 9.7g of ozone would have been utilised, equivalent to 24.4g per litre of undiluted K10 wash water effluent.

The size and type of ozone generator utilised has a very significant bearing on the cost of ozone gas production. Large generators can produce 50g of ozone, from air, using one kWh

of electricity (Appendix 1) while Rice et al. (1981) claim an ozone generating plant in Indianapolis to be capable of producing almost 80g of ozone from air using only one kWh of electricity. With smaller generators this output value may be less than 10g. However, Anderson (1989) stated that 33g of ozone per kWh can be used as a reasonable value for ozone production in terms of energy requirements. Using this value the electricity requirement to treat K10 wash water effluent calculated from experiments at 40% concentration and extrapolated to undiluted waste would be from 0.5 to 0.74kWh per litre of waste depending on the degree of treatment. Since, from Section 6.1.1, the volume of K10 wash water effluent is expected to be 8.96m^3 per annum, then, between 4480 and 6630kWh of electricity would be used, depending on the degree of treatment. The cost of electricity at the Royal Ordnance site is 4.46p per kWh. Thus the cost of electricity for ozonolysis of K10 wash water effluent ranges from £199.80 per annum for lower level treatment to £295.70 for a higher degree of treatment.

6.2.2 Summary

Ozonolysis is a very effective and efficient treatment process for K10 wash water effluent. The COD value can be reduced by over 95% and the orange colouration of the waste can be removed completely. In fact, no colour was visible to the naked eye after 24 hours of ozonolysis of 40% K10 waste at which stage over 350mg/l COD still remained. It is therefore particularly effective in colour reduction since it disrupts unsaturated bonds which are often the causative agent of colour.

The use of absorbance measurements at 250nm for monitoring of the ozonolysis treatment process would allow for rapid estimation of the COD and TOC values.

6.3 OZONOLYSIS OF TNT RED WATER

From the literature, the ozonolysis of TNT red water and associated effluents would not seem a plausible process (Section 1.4.6.8). However, the amenability of K10 wash water effluent to treatment by ozonolysis prompted an assessment of TNT red water ozonolysis utilising the same conditions as for the ozonolysis of 40% K10 wash water effluent.

The TNT red water sample was obtained from Royal Ordnance, Bridgwater and had a COD of 3042mg/l. 36L of this effluent was applied to the contactor and ozonised air was applied at a rate of 8.5 l/min. The generator power setting was 1.2A at an air pressure of 0.75 bar gauge. This gave a mean ozone application rate of 9.35gO_3/h.

The parameters monitored were those of absorbance at 200, 250 and 327nm, and the COD value. The ozone applied to and leaving the contactor was also measured at one hour intervals while TOC and HPLC analyses were not carried out. This was due to the labour intensive nature of these analyses since the ozonolysis of TNT red water is not directly relevant to this research project.

The results are shown in Figures 79 (COD) and 80 (200, 250 and 327nm). The efficiency of the contacting process was not as high as for ozonolysis of K10 wash water effluent. Figure 81, a comparison of contactor efficiency between K10

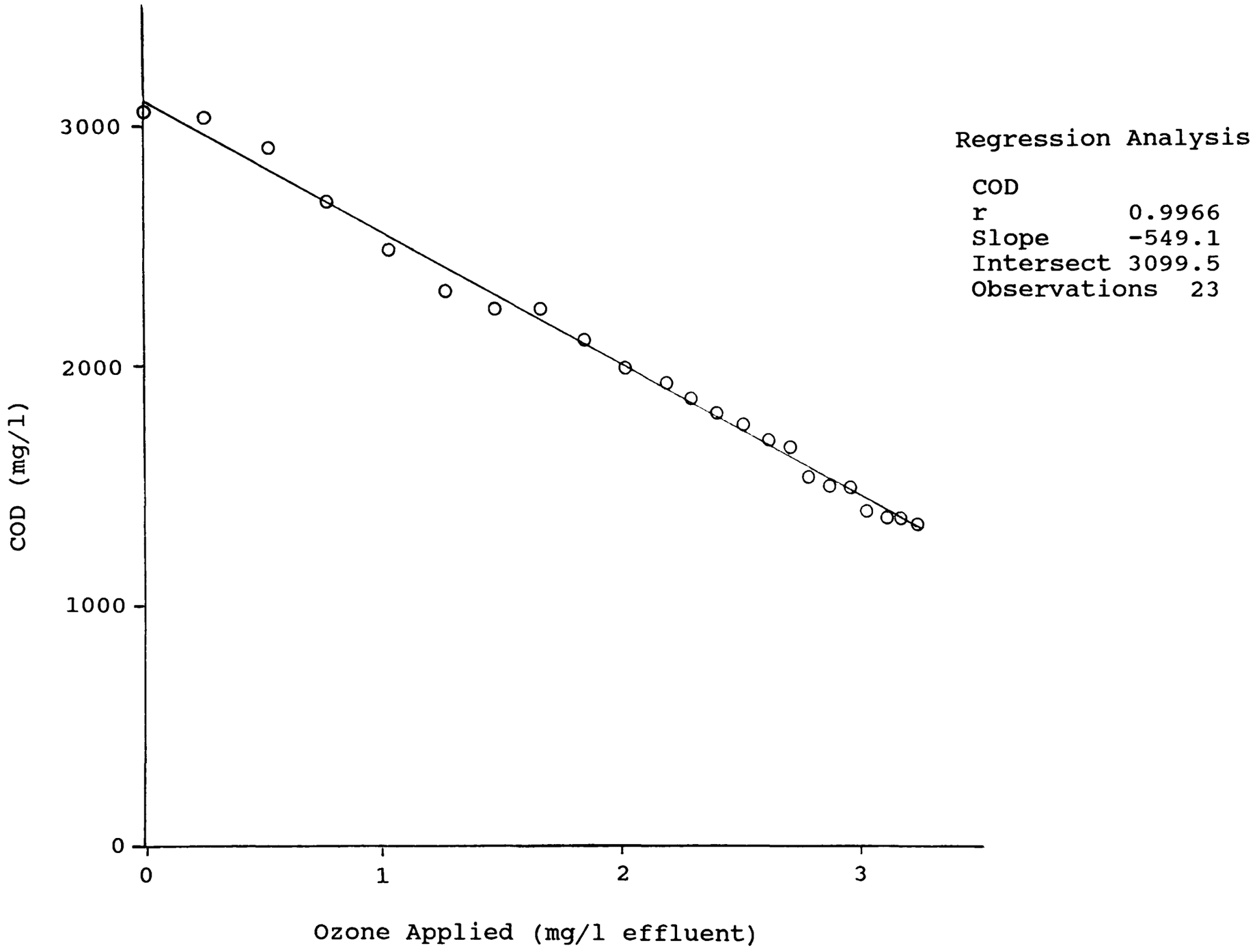


Figure 79. Reduction of COD versus Ozone Applied

Figure 80. Changes in Absorbance versus Ozone Applied

Figure 81. Contacting Efficiency during the Ozonolysis of both K10 Wash Water Effluent (40%) and TNT Red Water

wash water effluent and TNT red water treatments, shows that after 22 hours of continuous operation the efficiency of the contacting process was such that 75% of the ozone applied to the TNT red water was not utilised and thus was present in the contactor off gas. At this point the generator was shut down since a fault had developed in the ambient ozone monitor and the Author was not confident about the carrying of high levels of ozone in the PVC contactor-to-ozone destructor pipe since PVC can be attacked by ozone.

The inefficiency of the contactor for TNT red water treatment is clearly due to the very low reaction rate between ozone and TNT red water components. The contactor, however, operated quite adequately for K10 wash water effluent under the same conditions.

The low contactor efficiency did not cause deviation from the predicted results of ozonolysis of TNT red water. The reduction in COD versus the ozone applied was clearly linear (correlation coefficient (r) of 0.9966 and that of absorbance at 250nm was 0.9986). Absorbance measurements are thus suitable for estimation of the residual COD during ozonolysis as was found for the K10 waste treatment. Figure 82 shows the relationship between absorbance decrease with the decrease in COD value. The sample correlation coefficient was 0.9963 and the slope 1.16. Thus the absorbance decreased at a faster rate than COD (as was found with K10 wash water effluent where the slope was 1.11).

Figure 82. Comparison of COD and Absorbance values during Ozonolysis of TNT Red Water

Suprisingly, it was found that the amount of COD removal per gramme of ozone applied was higher for TNT red water than for K10 wash water effluent. One gramme of ozone removed 549mg of COD as compared to 469mg for K10 wash water effluent. The extent to which COD can be removed from TNT red water by ozone cannot be derived from this experiment since insufficient ozone was applied. The use of lower ozonised air flow rates to the contactor would probably improve the ozone utilisation since this would increase the contact time and thus assist the reaction rate limitation problem. It is clear that the possibility of using ozone to treat Royal Ordnance TNT red water should not be completely discounted.

The relative rate of colour removal was, as with K10 wash water effluent, much more rapid than for the other parameters monitored and absorbance at 200nm increased, probably due to organic acid production.

Further investigation of TNT red water ozonolysis was not possible since only a small quantity of red water was obtained and, in any case, this assessment was only of incidental interest and is not directly part of this research project. However, the ozonolysis of TNT red water may well be a feasible process and further investigation is recommended.

6.4 BIOLOGICAL TREATMENT OF POST-OZONOLYSIS K10 EFFLUENT

As the reader will recall, the destruction of K10 wash water effluent components using ozone was expected to yield organic acids. Such compounds should be readily assimilated by microorganisms via the glyoxylate shunt pathway. Organisms which are metabolising phenol as a carbon source will have an operative glyoxylate shunt mechanism.

After adding mineral salts to the post-ozonolysis K10 wash water effluent it was fed to an activated sludge plant which had previously been metabolising phenol. Unfortunately, due to the high efficiency of the ozonolysis treatment, the waste did not contain sufficient oxidisable material for successful treatment (COD value <75mg/l). This resulted in the loss of the sludge from the reactor.

Shake flask experiments were thus devised in which the samples taken towards the end of the ozonolsis experimentation (30 to 38 hours) were mixed together. The COD of this mixture was 105mg/l. This was then concentrated further by rotary evaporation to a COD value of 226mg/l. Absorbance scans were also carried out on the samples before and after evaporation. The flasks were to be inoculated with phenol degrading activated sludge which had been freeze-dried. However, reactivation of this sludge using 400mg/l phenol solution supplemented with mineral salts as for medium 2.2.3 was unsuccessful. This was suprising since previously the

sludge had been reactivated successfully although it had now been stored, in a freezer, for a period of two and a half years and the last reactivation of a sample of this sludge had been after one year of storage. Thus another source of organisms had to be found. It was decided to inoculate using activated sludge which was not adapted to the metabolism of phenol to which a phenol degrading Pseusomonas sp. had been added. This pseudomonad had been isolated by Dosanjh (1985) and stored since this time (over 5 years) on a nutrient agar slope at 5^{o}C and was successfully reactivated in a phenol based medium.

After inoculation of the flasks, absorbance at 203nm decreased by over 10% within 24 hours and continued to decrease by various amounts until it reached a value of only 44% of the original absorbance. Although inorganic salts added to the medium did not cause an increase in absorbance, as found from the control flask (sludge plus mineral salts), the post-ozonolysis waste did contain over 3g/l of residue after evaporation of the water. These salts may have contributed to the absorbance value at 203nm, but this was not investigated further.

Microorganisms clearly grew in this concentrated post-ozonolysis effluent since absorbance at 650nm increased from a starting value of 0.345 to a final value of 1.05. At the same time, the COD value decreased from the starting value of 226mg/l to 82mg/l (the limit of the COD analysis range).

Thus metabolism of post-ozonolysis effluent is clearly a viable proposition and the discharge of the post-ozonolysis effluent to receiving waters would not have any lasting detrimental effect. The components of the waste would be readily metabolised by activated sludge organisms at a sewage treatment works since the glyoxylate shunt mechanism is utilised in the metabolism of a wide range of compounds and so no further treatment of this effluent would be necessary.

Overall, this treatment is effective, producing an effluent from ozonolysis which is readily biodegradable, and could be dealt with in a standard treatment plant. As there is no doubt that K10 wash water effluent is treatable, the process can now be run without fear of environmental damage from this cause.

6.5 SUMMARY OF OZONOLYSIS

Ozone readily attacks both K10 wash water effluent and TNT red water although more slowly for the latter. In both cases the absorbance (250nm) correlates with the COD and also for TOC in the case of K10 wash water effluent treatment (no TOC was measured in TNT red water treatment). These correlations allow rapid estimation of the COD and TOC parameters which are labour intensive from simple absorbance measurements.

The ozone oxidises unsaturated bonds at a rate almost equal to the relative rate of COD removal for both wastes and in the case of K10, the carbon level continued to decrease at a constant rate even when the COD and absorbance at 250nm had been entirely removed.

The "add nothing" feature of the ozonolysis process in combination with the strong oxidising ability of ozone forms an attractive combination for wastewater treatment. In the case of K10 wash water effluent the post-ozonolysis liquor is clearly metabolised by microorganisms and thus can be discharged to local sewage treatment works where complete metabolism should occur.

Clearly ozonolysis has been very successful in the treatment of K10 wash water effluent. Literature reports are less encouraging for TNT effluent, but the red water in this

study was degraded by ozone, and the ratio between ozone applied and COD reduction was comparable with K10 wash water effluent. These results would suggest that the limited success of work reported in the literature is due to the inefficiencies in contactor design and operation. This process evidently merits reinvestigation.

CHAPTER 7

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 DISCUSSION AND CONCLUSIONS

An initial assessment of the toxicity of K10 wash water effluent showed that in shake flask experiments the waste was toxic to many of the microorganisms present in activated sludge. However, this was somewhat reduced in the activated sludge plants. Many wastes can be treated successfully in continuous stirred reactor systems (Smith et al., 1982 and Nay et al., 1974) due to the dilution factor inherent in this equipment, since organisms continually metabolise compounds within the reactor. Thus, provided there is some degree of mixing, the concentration of these compounds is lower within the reactor than in the incoming effluent. This of course does not occur in shake flasks and also if the compounds in question cannot be metabolised by the microorganisms within the continuous stirred reactor system. The latter was the case for K10 wash water effluent treatment as HPLC analysis revealed no detectable change in the concentration of coloured components after biological treatment although there was a reduction in the compounds which were not colour forming.

A further reason for the tolerance of the activated sludge microorganisms is possibly the nature of continuous stirred reactor systems which have a sludge recycle. Sludge

recycle *per se* allows for the selection and adaptation of microorganisms and therefore the few higher organisms present which were relatively more resistant to the action of the K10 wash water effluent would be able to survive and proliferate: they would not, of course, be able to do this in the shake flasks.

The failure of biological systems to degrade the coloured components of K10 wash water effluent coupled with its toxicity at elevated concentrations has shown that treatment of this waste using a biological process is likely to be unsuccessful. That is not to say that biological treatment of this waste is impossible, rather that suitable degrading organisms were not isolated or adapted to the treatment of this waste after prolonged experimentation. Suitable organisms which might be capable of metabolising such compounds may currently exist within the environment; the problem is finding them.

The lack of success of biological treatment ties in with treatment studies on the analogous TNT red water which has been widely investigated (Carpenter *et al.*, 1978; Hallas and Alexander, 1983; Kaplan and Kaplan, 1982b and Won *et al.*, 1974 and 1976). The general opinion is that TNT red water is difficult, if not impossible, to degrade completely by biological means. Correlation of chemical structure with microbial degradation rate (Pitter, 1985) further confirms that TNT red water, and therefore , by analogy, also K10 wash

water effluent, should be difficult to degrade since the enzymatic attack of the aromatic nucleus is electrophilic in character and electron withdrawing substituent groups of the type known to be present in these effluents will make the nucleus more resistant to enzyme attack. Electron withdrawing groups such as nitro and chloro, and also amino, are often found in the explosives industry and make treatment of such effluents difficult or impossible.

The rate of oxygen utilisation by activated sludge organisms either previously exposed or unexposed to K10 wash water effluent in response to various concentrations of K10 wash water effluent prompted further investigation. This was because organisms which had never been exposed to K10 wash water effluent had a higher oxygen uptake rate when mixed with the waste than organisms which had been exposed to K10 wash water effluent for a period of several months and which were similarly treated. This is contrary to what would have been expected. Further examination of this situation revealed that K10 wash water effluent was able to dissipate electro-chemical membrane potential and thus was an oxidative phosphorylation uncoupling agent. The uncoupling action was also unusual since it was biphasic in that most of the uncoupling effect occurred within one tenth of a second after a membrane potential had been created, after which the dissipation rate assumed a value equivalent to the control sample. Hypotheses for this effect were postulated but were rejected after further experimentation and further theories to explain this unusual

action have been elusive.

Electro-chemical membrane dissipation experiments were also carried out on the analogous red water which also demonstrated the ability dissipate membrane potential. In this instance the behaviour was similar to a classical uncoupling agent such as dinitrophenol (Lehninger, 1977) or FCCP (Nicholls, 1982) in that the membrane potential was dissipated at a rate which gave a straight line on a log plot, the gradient of which was dependent on the concentration of the waste. However, this experiment does not show whether TNT can uncouple oxidative phosphorylation, by dissipation of membrane potential, when presented to microorganisms. In order to uncouple, the component or components of the TNT red water responsible would need to be able to access the cell membrane. Studies between adapted and unadapted organisms were not possible since insufficient TNT red water had been obtained.

The use of activated carbon as a single treatment for K10 wash water effluent is most certainly a feasible process. However, the carbon loading capacity was not very high and 200g of carbon is necessary per litre of waste. In the analogous TNT red water treatment, spent carbon is discarded and this would also be likely to be the case for K10 wash water effluent treatment. Thus carbon would be a recurrent expenditure costing in excess of £5000 per annum and additional costs will be incurred for the disposal of the spent carbon.

The use of ozone for the treatment of K10 wash water effluent was also successful. Ozone removed all of the orange coloration from the waste and also oxidised unsaturated bonds since absorbance at 250nm was also removed; thus the waste was no longer aromatic. The COD value of the waste was also significantly removed (over 95%) and so was carbon (over 60%). The carbon was probably converted to carbon dioxide since the only means by which carbon could leave the reactor was as a gas. In contrast the absorbance at 215nm increased during ozonolysis and this absorbing material eluted very rapidly during HPLC analysis either with or just behind the solvent front. Under the analysis conditions such compounds would have to be extremely polar in character and are most likely to be organic acids such oxalic, formic and glyoxylic acids. These were identified by Caprio *et al*. (1984) after the ozonolysis of nitrobenzene. Organic acids are readily metabolised by microorganisms but attempts to treat these using a conventional activated sludge plant was unsuccessful since the loss of organisms from the reactor was greater than the rate at which they could grow on this substrate. This was attributed to the very low amount of oxidisable material remaining after treatment since the COD value was only 74mg/l. However, after concentration of post-ozonolysis K10 wash water effluent, biological treatment using shake flasks, inoculated with activated sludge containing a phenol degrading *Pseudomonas sp*., was very successful. Thus discharge of post-ozonolysis K10 wash water effluent to receiving waters

would not have any detrimental effect since the waste is no longer toxic and it does not contain sufficient oxidisable material to cause serious anoxic effects.

Since the absorbance, COD and TOC values all decreased with a virtually linear relationship with ozone applied, it follows that they will have a linear relationship with one another. Thus absorbance measurements could be used as a rapid estimate of COD and TOC values during treatment, therefore eliminating the need to perform difficult and time consuming analyses.

Ozonolysis is environmentally far sounder than the activated carbon process and treatment costs in terms of electricity to drive the ozone generator are less than £300 per annum; much lower than for activated carbon treatment although capital expenditure will be much higher. Nevertheless, this is clearly the most suitable treatment process for K10 wash water effluent.

Ozonolysis was also carried out on the analogous TNT red water. The COD value decreased at a rate higher than that in the K10 wash water effluent assessment with the amount of ozone applied. However, the reaction rate between ozone and TNT red water components was lower than for K10 wash water effluent, and ozone was present in the contactor off gas. For safety reasons, the assessment had to be terminated when 75% of the ozone applied was no longer utilised. Thus assessment

of TNT red water was incomplete and sufficient red water was not available to reassess this treatment process. Essentially a change in conditions to longer contact times would have improved the results. Nevertheless, the colour of the waste was substantially reduced as was the absorbance at 250nm and also the COD value. Once again the absorbance and the COD value had a virtually linear relationship with ozone applied and thus with one another meaning that absorbance measurements can be used to estimate the remaining COD during treatment.

It is the author's opinion that the ozone contacting devices used in work reported in the literature on TNT effluent were not particularly efficient. In particular they were often far too small for adequate contacting to occur (see Section 1.4.6). The actual ozone utilisation efficiency in terms of ozone applied with COD removal for the TNT effluent treatment was comparable with that of K10 wash water effluent under similar conditions. It is clear, therefore, that treatment of TNT red water by ozonolysis could well also be a viable proposition.

7.2 RECOMMENDATIONS

Ozonolysis should be used for the treatment of K10 wash water effluent. The degree of ozonolysis required will depend on the levels of COD, BOD and TOC that can be discharged and is dependent on the local water authority regulations. No post-ozonolysis biological treatment is necessary on site since the compounds can be readily metabolised by bacteria present in activated sludge.

However, if biological treatment of the post-ozonolysis waste is to be carried out then a suitable phenol degrading Pseudomonas sp. could be stored for many years on nutrient agar slopes and reactivated using a phenolic based medium. These organisms could then be added to activated sludge and thus reduce the period required for organisms to be adapted and selected for this treatment process.

Further work on ozonation of the analogous TNT red water is required with particular attention to the contacting process such that an extended contact time is utilised. The treatment of such an effluent after ozonolysis is likely to be similar to that for the K10 wash water effluent situation.

Finally, it would be of interest, now that suitable HPLC analysis conditions have been devised, to isolate the component or components responsible for the uncoupling of oxidative phosphorylation. Such a substance may, due to its novel mode of action, have potential uses within the field of biochemistry and thus be of marketable value.

CHAPTER 8

REFERENCES

Alexander, M.
Biodegradation: Problems of Molecular Recalcitrance and Microbial Fallibility
Adv. Appl. Microbiol. (1965) 7, 35-80

Alexander, M.
Biodegradation of Chemicals of Environmental Concern
Science (1981) 211, 132-138

Alexander, M.
Biodegradation of Organic Chemicals
Environ. Sci. Technol. (1985) 19, No.2, 106-111

Anderson, G. K., Saw, C. B. and Howarth, C. R.
Ozonolysis of Wastewaters using a Spinning Disc Reactor
39th Industrial Waste Conference (1984) 163-172
Purdue University, USA.

Anderson, R.
Personal Communication (1989)
Ozotech Ltd., 116 Station Road, Burgess Hill, Sussex

Anon.
"Chemical Oxygen Demand (Dichromate Value)" (1977)
Her Majesty's Stationary Office (HMSO), London

Anon.
Ozone: Health Hazards and Precautionary Methods.
HMSO., Environmental Hygiene Series EH38 (1983) July

Anon.
In "Standard Methods for Examination of Water and Wastewater" (1985) 16th Ed., American Public Health Association (APHA); American Water Works Association (AWWA); Water Pollution Control Federation (WPCF)

Anon.
"Chemical Oxygen Demand" (1986) 2nd Ed.
Her Majesty's Stationary Office (HMSO), Oxford

Arden, E. and Lockett, W. T.
Experiments on the Oxidation of Sewage without the aid of Filters
J. Soc. Chem. Ind. London (1914) 33, 523-539

Augustine, R. L.
Catalytic Hydrogenation of alpha and beta unsaturated Ketones. The Effect of Quantity and Type of Catalyst.
J. Org. Chem. (1963) 28, 152-155

Bailey, P. S.
"Ozonation in Organic Chemistry" (1978) Volume 1
Academic Press, N.Y., USA.

Bailey, P. S.
"Ozonation in Organic Chemistry" (1982) Volume 2
Academic Press, N.Y., USA.

Bailey, P. S., Bath, S. S. and Ashton, J. B.
Initial Attack of Ozone on an Unsaturated System
Ozone Chemistry and Technology: Advances in Chemistry Series No. 21
(1959) 143-148

Baker, R. A.
In "Ecological Aspects of Used Water Treatment" (1975) 375-392
Ed. Curds, C. R. and Hawkes, H. A.
Academic Press, London

Barberis, K. and Howarth, C. R.
The Ozonolysis of Low, Moderate and Highly Reactive Pollutants using a Perforated Spinning Disc
International Ozone Association, London Conference (1988)

Bartha, R. and Pramer, D.
Metabolism of Acyclanilide Herbicides
Adv. Appl. Microbiol. (1970) 13, 317-341

Besselierre, E. B. and Schwartz, M.
In "The Treatment of Industrial Wastes" (1976) 2nd Edition
McGraw-Hill Book Company, USA.

Brabets, R. I. and Marks, G. E.
Ozonolysis of Pink Water
IITRI Report No. C6275 (1974)

Braun, K. and Gibson, D. T.
Anaerobic Degradation of 2-Aminobenzoate (Anthranilic Acid) by Denitrifying Bacteria
Appl. Environ. Microbiol. (1984) 48, No.1, 102-107

Brown, D. and Laboureur, P.
The Aerobic Biodegradability of Primary Aromatic Amines
Chemosphere (1983) 12, No.3, 405-414

Bull, A. T.
Biodegradation: Some Attitudes and Strategies of Microorganisms and Microbiologists
In "Contemporary Microbiology" (1980) 107-136
Ed. Ellwood, Hedger, Latham, Lynch and Slater

Burns, D. E. and Shell, G. L.
Carbon Treatment of a Municipal Wastewater
Journal WPCF (1974) 46, No 1, 148-164

Callely, A. G., Forster, C. F. and Stafford, D. A. (Editors)
In "Treatment of Industrial Effluents" (1977)
Hodder and Stoughton, London

Campbell, R.
In "Basic Microbiology" (1966) Vol. 5
Ed. Wilkinson, J. F.
John Wiley and Sons, N.Y., USA.

Caprio, V., Insola, A. and Volpicelli, G.
Ozonation of Aqueous Solutions of Nitrobenzene
Ozone: Science and Engineering (1984) 6, 115-121
Pergamon Press Ltd., USA.

Carpenter, D. F., McCormick, N. G., Cornell, J. H. and Kaplan, A. M.
Microbial Transformation of 14C Labelled 2,4,6-Trinitrotoluene in an Activated Sludge System
Appl. Environ. Microbiol. (1978) 35, No.5, 949-954

Channon, H. J., Mills, G. T. and Williams, R. T.
The Metabolism of 2,4,6-Trinitrotoluene (TNT)
Biochem. Journ. (1944) 38, 70-85

Chaudhuri, N., Englebrecht, R. S. and Austin, J. H.
J. Am. Wat. Wks. Ass. (1965) 57, 1561

Cooper, J. C. and Hager, D. G.
Water reclamation with activated carbon
Chem. Eng. Progr. (1966) 62, 85-90

Curds, C. R.
In "Ecological Aspects of Used Water Treatment" (1975)
Vol. 1, 203-268
Ed. Curds and Hawkes
Academic Press, London

Dagley, S.
Microbial Degradation of Aromatic Compounds
Dev. Indust. Microbiol. (1983) 25, 53-68

De Renzo, D. J.
Unit Operations for the Treatment of Hazardous Industrial Wastes (1978)
Pollution and Technology Review, No.47, Noyes Data Corp., N.Y.

Dosanjh, M. K.
Ph.D. Thesis (1985)
Chemical Engineering, University of Birmingham

Duguet, J. P., Anselme, C., Mazounie, P. and Mallevialle, J.
Application of Combined Ozone/Hydrogen Peroxide for the Removal of Aromatic Compounds from a Groundwater
International Ozone Association, London Conference (1988)

Dutton, P. L. and Evans, W. C.
The Photometabolism of Benzoic Acid by Rhodopseudomonas palustris: A New Pathway of Aromatic Ring Metabolism
Biochem. J. (1968) 109, 5P

Eckenfelder, W. W.
Principles of Water Quality Management (1980)
CBI Publishing Company, USA.

Evans, G. O. and Hyatt, K. H.
Bull. Br. Mus. nat. Hist. zool (1960) 6 (2), 27-101

Evans, W. C.
The Microbial Degradation of Aromatic Compounds
J. Gen. Microbiol. (1963) 32, 177-184

Evans, W. C.
Biochemistry of the Bacterial Catabolism of Aromatic Compounds in Anaerobic Environments
Nature (1977) 270, 17-22

Faber, M. D.
Microbial Degradation of Recalcitrant Compounds and Synthetic Aromatic Polymers
Enzyme Microb. Technol. (1979) 1, No.10, 226-232

Farmer, E. H. and Galley, R. A. E.
Catalytic Hydrogenation of Olefinic Compounds
Nature (1933) 131, 60

Fewson, C. A.
In "Microbial Degradation of Xenobiotics and Recalcitrant Compounds" (1981) 141-179
Ed. Leisinger, Cook, Hutter and Nuesch
Academic Press, London

Fochtman, E. G. and Huff, J. E.
Ozone-Ultraviolet Light Treatment of TNT Wastewaters
Proc. Sec. Intl. Symp. on Ozone Technology (1975) 211-223
Montreal

Forster, C. F.
"Biotechnology and Wastewater Treatment" (1985)
Cambridge University Press, London

Forster, C. F.
In "Treatment of Industrial Effluents" (1977) 65-83
Ed. Callely, Forster and Stafford
Hodder and Stoughton, London

Fox, J. (1989)
Personal Communication
Biochemistry, University of Birmingham

Gale, E. F.
In "The Chemical Activities of Bacteria" (1947)
Academic Press, N.Y.

Guittonneau, S., De Latt, J., Duguet, J. P., Bonnel, C. and Dore, M.
Oxidation of Parachloronitrobenzene in Diluted Aqueous Solution by Ozone and UV and by Hydrogen Peroxide and UV: A Comparative Study
International Ozone Association, London Conference (1988)

Hallas, A. E. and Alexander, M.
Microbial Transformation of Nitroaromatic Compounds in Sewage Effluent
Appl. Environ. Microbiol. (1983) 45, No.4, 1234-1241

Hardisty, D. M. and Rosen, H. M.
Industrial Wastewater Ozonation
32nd Industrial Waste Conference (1977) 294-302
Purdue University, USA.

Hemphill, L., Ramaiah, V. and Valentine, M.
Thermal Regeneration of Activated Carbon
32nd Industrial Waste Conference (1977) 127-140
Purdue University, USA.

Holiday, A. D. and Hardin, D. P.
Activated Carbon removes Pesticides from Wastewater
Chem. Eng. (1981), 88 (6), 88-89

Horvath, R. S.
Microbial Co-Metabolism and the Degradation of Organic Compounds in Nature
Bact. Rev. (1972) 36, No.2, 146-155

Jackson, J. B.
Personal Communication (1988)
Biochemistry, University of Birmingham

Jones, M. K. and Jennelle, E. M.
Water Resources Bulletin (1972) 8, 312

Kaplan, D. L. and Kaplan A. M.
Composting Industrial Wastes - Biochemical Consideration
Biocycle (1982a) May/June, 42-44

Kaplan, D. L. and Kaplan, A. M.
2,4,6-Trinitrotoluene - Surfactant Complexes: Decomposition, Mutagenicity and Soil Leaching Studies
Environ. Sci. Technol. (1982b) 16, No.9, 566-571

Kaplan, D. L. and Kaplan, A. M.
US. Govt. Reps. (1984) AD-A135, 512/2

Kaufman, D. D., Plimmer, J. R. and Klingebiel, U. I.
Journal of Agricultural and Food Chemistry (1973) 21, 127-132

Knackmuss, H-J.
In "Microbial Degradation of Xenobiotics and Recalcitrant Compounds" (1981) 190-213
FEMS Symposium No. 12

Kelly, P
Personal Communication (1987)
Royal Ordnance, Waltham Abbey, Essex

Kobayashi, H. and Rittmann, B. E.
Microbial Removal of Hazardous Organic Compounds
Environ. Sci. Technol. (1982) 16, No.3, 170A-183A

Koziorowski, B. and Kucharski, J.
In "Industrial Waste Disposal" (1972)
Pergamon Press, Oxford

Kul'skii, L. A. and Shabolina, A. G.
Adsorption of DDT from colloidal solutions on the Iodine KAD activated carbon
Chem. Abstr. (1967) 67, No. 14686y

Kuo, C-J. and Sierka, R. A.
Catalytic Oxidation of Munitions Wastewater
38th Industrial Waste Conference (1983) 377-385
Purdue University, USA.

Latorre, J., Reineke, W. and Knackmuss, H-J.
Microbial Metabolism of Chloroanilines: Enhanced Evolution by Genetic Exchange
Arch. Microbiol. (1984) 140, 159-165

Leh, F.
Ozone. Properties, Toxicity and Applications
Journal of Chemical Education (1973) 50, 404-405

Lehninger, A. L.
"Biochemistry" (1977) 2nd Edition
Worth Publishers, Inc., N.Y., USA.

Liu, D., Thomson, K. and Anderson, A. C.
Identification of Nitroso Compounds from Biotransformation of 2,4-Dinitrotoluene
Appl. Environ. Microbiol. (1984) 47, No.6, 1295-1298

Lyons, C. D., Katz, S. and Bartha, R.
Mechanisms and Pathways of Aniline Elimination from Aquatic Environments
Appl. Environ. Microbiol. (1984) 48, No.3, 491-496

March, J.
In "Advanced Organic Chemistry" (1985) 3rd Edition
A Wiley-Interscience Publication

Mason, H. S.
Adv. Enzymol. (1957) 19, 79-223

McCormick, N. G., Cornell, J. H. and Kaplan, A. M.
Identification of Biotransformation Products from 2,4-Dinitrotoluene
Appl. Environ. Microbiol. (1978) 35, No.5, 945-948

McCormick, N. G., Feeherry, F. E. and Levinson, H. S.
Microbial Transformation of 2,4,6-Trinitrotoluene and Other Nitroaromatic Compounds
Appl. Environ. Microbiol. (1976) 31, No.6, 949-958

Metzer, A. and Miyamoto, H. K.
The Biotreatability of Industrial Dye Wastes Before and After Ozonation and Hypochlorination - Dechlorination
30th Industrial Waste Conference (1975) 804-815
Purdue University, USA.

Mitchell, W. R. and Dennis, W. H.
Biodegradation of 1,3-Dinitrobenzene
J. Environ. Sci. Health (1982) A17, 837-853

Montgomery, H. A. C.
The Determination of Biochemical Oxygen Demand by Respirometric Methods
Water Research (1967) 1, 631-662

Nay, M. W., Randall, C. W. and King, P. H.
Factors Affecting Colour Development during the Treatment of TNT Waste
27th Industrial Waste Conference (1972) 983-993
Purdue University, USA.

Nay, M. W., Randall, C. W. and King, P. H.
Biological Treatability of Trinitrotoluene Wastewater
Journal W.P.C.F. (1974) 46, No.3, 485-497

Nebel, C., Gottsching, R. D. and O'Neill, H. J.
"Ozone Decolourization of Pulp and Paper Mill Secondary Effluents
Proc. First Intl. Sypm. of the Intl. Ozone Institution
Washington D.C. December 2-5, 1973

Nemerow, N. L.
In "Liquid Waste of Industry" (1971)
Addison-Wesley Publishing Company, USA.

Nicholls, D. G.
"Bioenergetics" (1982)
Academic Press, London

Painter, H. A.
Biodegradability
Proc. Royal Soc. London Series B (1974) 185, 149-158

Patterson, J. W., Shapira, N. I. and Brown, J.
Pollution Abatement in the Military Explosives Industry
31st Industrial Wastewater Conference (1976) 385-395
Purdue University, USA.

Pereira, W. E., Short, D. C., Manigold, D. B. and Roscio, R. K.
Bull. Environ. Contam. Toxicol. (1979) 21, 554-652

Pitter, P.
Correlation of Microbial Degradation Rate with Chemical Structure
Acta Hydrochim. Hydrobiol. (1985) 13, No 4, 453-460

Rice, R. G., Robson, C. M., Miller, G. W. and Hill, A. G.
Uses of Ozone in Drinking Water Treatment
Journal A.W.W.A. (1981) 73, No.1, 44-57

Rylander, P. N.
In "Catalytic Hydrogenation over Platinum Metals" (1967)
Academic Press, N.Y., USA.

Saleh, F. Y., Lee, G. F. and Wolf, H. W.
Selected Organic Pesticides; Behavior and Removal from Domestic Wastewater by Chemical and Physical Processes
Water Res. (1982) 16, 479-488

Saw, C. B., Anderson, G. K., Howarth, C. R. and Porter, J. E.
Application of the Spinning Disc Reactor as an Ozone Contactor
40th Industrial Waste Conference (1985) 813-823
Purdue University, USA.

Sax, N. I.
In "Dangerous Properties of Industrial Materials" (1963)
Ed. Rheingold, N.Y., USA.

Sayler, G. S., Breen, A., Blackburn, J. W. and Yagi, O.
Predictive Assessment of Priority Pollutant Bio-oxidation Kinetics in Activated Sludge
Environ. Progress (1984) 3, No.3, 153-163

Schulte, G. R., Hoehn, R. C. and Randall, C. W.
The Treatability of a Munitions-Manufacturing Waste with Activated Carbon
28th Industrial Waste Conference (1973) 150-162
Purdue University, USA.

Shaw, J. A.
Determination of Phenols in Aqueous Wastes from Coke Plants
Analytical Chemistry (1951) 23, No.12, 1788-1792

Shelby, S. E., Lankford, P. W. and McCollum, R. W.
A Case Study for the Treatment of an Explosives Wastewater from an Army Ammunitions Plant
39th Industrial Waste Conference (1984) 821-836
Purdue University, USA.

Sierka, R. A.
The High Temperature Treatment of Trinitrotoluene (TNT) and Cyclotrimethylene-triamine (RDX) with Ozone and Ultrasound
Ozone: Science and Engineering (1985) 6, 275-290
Pergamon Press Ltd., USA.

Slater, J. H. and Bull, A. T.
Environmental Microbiology: Biodegradation
Phil. Trans. Royal Soc. London B (1982) 297, 575-597

Smith, L. L., Carazza, J. and Wong, K.
Biological Treatment for Waste Streams from Propellants and Explosives Manufacturing
J. Haz. Materials (1982) 5, 227-296

Sotelo, J. L.
Application of Gas-Absorption Theories to O-Cresol Ozonation in Water
International Ozone Associaion, London Conference (1988)

Spain, J. C. and Van Veld, P. A.
Adaption of Natural Microbial Communities to Degradation of Xenobiotic Compounds: Effects of Concentration, Exposure Time, Inoculum and Chemical Structure
Appl. Environ. Microbiol. (1983) 45, No.2, 428-435

Spanggord, R. J., Gibson, B. W., Keck, R. G., Thomas, D. W. and Barkley, J. J.
Effluent Analysis of Wastewater Generated in the Manufacture of 2,4,6-Trinitrotoluene. 1. Characterization Study
Environ. Sci. Technol. (1982) 16, No.4, 229-232

Symons, J. M., McKinney, R.E., Smith, R. M. and Donovan, E. J.
Degradation of Nitrogen-Containing Organic Compounds by Activated Sludge
Int. J. Air and Water Poll. (1961) 4, 115-138

Tabak, H. H., Quave, S. A., Mashni, C. I. and Barth, E. F.
Biodegradability studies with Organic Priority Pollutant Compounds
Journal W.P.C.F. (1981) 53, No.10, 1503-1517

Wase, D. A. J.
Personal Communication (1987)
Chemical Engineering, University of Birmingham

Wheatstone, K. C.
In "Treatment of Industrial Effluents" (1977) 30-65
Ed. Callely, Forster and Stafford
Hodder and Stoughton, London

Won, W. D., DiSalvo, L. H. and NG, J.
Toxicity and Mutagenicity of 2,4,6-Trinitrotoluene and its Microbial Metabolites
Appl. Environ. Microbiol. (1976) 31, No.4, 576-580

Won, W. D., Heckley, R. J., Glover, D. J. and Hoffsommer, J. C.
Metabolic Disposition of 2,4,6-Trinitrotoluene
Appl. Microbiol. (1974) 27, No.3, 513-516

Wyman, J. F., Guard, H. E., Won, W. D. and Quay, J. H.
Conversion of 2,4,6-Trinitrophenol to a Mutagen by Pseudomonas aeruginosa
Appl. Environ. Microbiol. (1979) 37, No.2, 222-226

ABBREVIATIONS

MNEB - Mononitoethylbenzene

DNEB - Dinitroethylbenzene

TNEB - Trinitroethylbenzene

K10 - A mixture of DNEB and TNEB

TNT - Trinitrotoluene

COD - Chemical Oxygen Demand

TOC - Total Organic Carbon

HPLC - High Performance Liquid Chromatography

FCCP - Carbonyl cyanide-p-trifluromethoxyphenol hydrazone

APPENDICES

APPENDIX 1

OZOTECH
O3 Engineers and suppliers for ozone systems

OZOTECH LTD.
116 STATION ROAD
BURGESS HILL
SUSSEX RH15 9EN
ENGLAND

Telephone: (0444) 235411
Telex: 877919
Fax: (0444) 242187

Mr. I. Clarke,
The University of Birmingham,
School of Chemical Engineering,
P.O. Box 363,
BIRMINGHAM. B15 2TT

30th November, 1989

Ref. RA/CAW/1278

Dear Ian,

RE. NITROBENZENE EFFLUENT

Following our telephone conversation of yesterday regarding costs, I trust the following will be useful.

The Bridgewater plant will only produce approximately 6m³ effluent per year. I have assumed 4 batches x 1.5m³ that would be treated batchwise over several weeks, using an 18g/h ozoniser.

I would propose a packagesd plant consisting of:-

* Holding/Recirculation tank
* Recirculation pump
* Stainless steel contact columns
  Compressor/Ozoniser
  Instrumentation
* All as a skid mounted package

Budget price £45,000 - £50,000

General Costs

It is very difficult to generalise on costs of ozonation of effluents because each will have a different ozone demand and flow rate. These two factors determine the capital cost of the ozone plant itself; however civil costs again will vary for each effluent according to contact time, flow variations etc.

The following are budget prices for packaged ozonisers with compressor and ozone ambient monitor, but excluding contacting etc.

g O_3/h	£	kW Max
18	20,000	2.1
36	23,000	2.5
72	25,000	3.2
160	35,000	8
320	40,000	12
450	45,000	16.4
700	50,000	24
1080	75,000	38.6
1500	85,000	57
2000	95,000	68
2500	105,000	84
3000	115,000	105

Large scale plants would use low pressure drying systems which are far more energy efficient. For example a 3kg/h low pressure ozone stream would only require 60kW compared to 105kW for the high pressure one.

Best regards,

R. ANDERSON

APPENDIX 2

CHAPTER 2 RESULTS

Calibration curve for Total Organic Carbon (TOC)

Total Carbon (mg/l)	Deflection
5	8
10	9.5
15	13
25	20
50	38
75	58
100	73
150	104
200	133
250	155

Calibration curve for Total Organic Carbon (TOC)

Inorganic Carbon (mg/l)	Deflection
5	8
10	12
15	16
25	24
50	45
75	68
100	91
150	130
200	161

Calibration curve for Phenol

Phenol (mg/l)	Absorbance (500nm)
0	0
1	0.14
2	0.27
3	0.40
4	0.54
5	0.67
6	0.80
7	0.94
8	1.07
9	1.20
10	1.33

APPENDIX 3

CHAPTER 3 RESULTS

Phenol Analysis (Section 3.5.1)

Date	Day	Experimental Reactor			Control Reactor			Comments
		Feed	Eff	%Rem	Feed	Eff	%Rem	
09.02.87	1	386	56	85.6	386	91	76.3	
10.02.87	2	417	65	84.3	417	98	76.4	
11.02.87	3	404	73	82.0	404	81	79.9	
13.02.87	5	375	39	89.5	375	48	87.1	
16.02.87	8	370	36	90.3	370	32	91.3	
17.02.87	9	368	32	91.2	368	17	95.5	
18.02.87	10	379	31	91.7	379	9.1	97.6	
19.02.87	11	381	34	91.0	381	21	94.5	
20.02.87	12	363	16	95.7	363	14	96.2	
02.03.87	22	392	4.0	99.0	392	23	94.1	
03.03.87	23	385	4.2	98.9	385	21	94.5	
04.03.87	24	392	5.9	98.5	392	14	96.3	
05.03.87	25	412	5.2	98.7	412	20	95.1	
06.03.87	26	317	6.0	98.1	317	7.6	97.6	
09.03.87	29	295	8.6	97.1	295	4.7	98.4	
10.03.87	30	356	4.3	98.8	356	8.9	97.5	
13.03.87	33	308	14	95.4	308	11	96.4	
16.03.87	36	314	17	94.5	314	12	96.2	
18.03.87	38	295	21	92.7	295	5.3	98.2	
20.03.87	40	365	16	95.6	365	11	97.0	
23.03.87	43	356	12	96.6	356	6.7	98.1	
25.03.87	45	404	5.3	98.7	404	5.3	98.7	
27.03.87	47	377	3.4	99.1	377	3.0	99.2	
30.03.87	50	366	4.8	98.7	366	0.4	99.9	
01.04.87	52	426	1.7	99.6	426	5.1	98.8	
03.04.87	54	398	8.0	98.0	398	8.4	97.9	
06.04.87	57	407	6.1	98.5	407	5.3	98.7	
08.04.87	59	307	5.8	98.1	307	7.7	97.5	
10.04.87	61	388	11	97.1	388	6.6	98.3	Power Fluctuations
13.04.87	64	351	78	77.9	351	82	76.7	
14.04.87	65	355	130	63.3	355	88	75.2	
15.04.87	66	371	121	67.4	371	88	76.3	
16.04.87	67	326	48	85.4	326	39	87.9	
17.04.87	68	299	44	85.2	299	55	81.5	Power Fluctuations
20.04.87	71	344	21	93.9	344	34	90.2	Laboratory Move
22.04.87	73	287	14	95.0	287	36	87.6	
24.04.87	75	268	6.7	97.5	268	24	91.0	
27.04.87	78	304	7.6	97.5	304	18	94.2	
29.04.87	80	291	23	92.1	291	4.9	98.3	
01.05.87	82	273	3.3	98.8	273	2.5	99.1	
04.05.87	85	311	2.5	99.2	311	1.2	99.6	
06.05.87	87	302	6.9	97.7	302	4.8	98.4	New Feed Pump
08.05.87	89	294	28	90.6	294	24	91.7	
11.05.87	92	286	90	68.7	286	97	66.2	
13.05.87	94	261	11	95.6	261	18	93.0	
15.05.87	96	294	13	95.7	294	29	90.2	
18.05.87	99	307	30	90.1	307	27	91.3	
20.05.87	101	328	5.9	89.2	328	42	87.2	
22.05.87	103	312	11	96.4	312	30	90.3	2x Feed Conc'n
25.05.87	106	507	49	90.3	507	73	76.2	
26.05.87	107	498	59	88.1	498	104	79.1	
27.05.87	108	602	147	75.6	602	118	80.4	
28.05.87	109	540	126	76.6	540	164	69.7	
29.05.87	110	573	127	77.9	573	237	58.6	Con React pH 9
01.06.87	113	401	41	89.7	401	197	51.7	
02.06.87	114	373	56	85.0	373	189	49.3	
03.06.87	115	453	92	79.6	453	215	52.6	
04.06.87	116	409	149	63.5	409	219	46.5	
05.06.87	117	393	156	60.4	393	231	41.2	

Phenol Analysis (Section 3.5.1)

Date	Day	Experimental Reactor			Control Reactor			Comments
		Feed	Eff	%Rem	Feed	Eff	%Rem	
10.06.87	122	411	194	52.7	411	252	38.7	
11.06.87	123	450	172	61.8	450	281	37.6	
12.06.87	124	435	164	62.3	435	254	41.6	
15.06.87	127	438	159	63.7	438	253	42.2	
16.06.87	128	358	109	69.6	383	221	42.3	1% K10 to Exp React
17.06.87	129	437	119	72.8	457	206	54.9	
18.06.87	130	283	63	77.7	379	236	37.7	
19.06.87	131	292	37	87.3	454	236	48.0	Nitrogen Limitation?
22.06.87	134	783	10	98.7	896	234	73.9	
23.06.87	135	831	7.5	99.1	897	230	74.4	
24.06.87	136	254	3.2	98.7	269	147	45.5	
26.06.87	138	246	2.5	99.0	284	142	50.2	
29.06.87	141	201	4.3	97.8	209	102	51.2	Tri-ammon ortho phos
30.06.87	142	194	4.0	97.9	224	107	52.3	
03.07.87	145	224	2.5	98.9	216	2.7	98.7	
06.07.87	148	289	1.7	99.4	289	1.0	99.7	
08.07.87	150	209	1.2	99.4	231	1.2	99.5	
10.07.87	152	179	2.0	98.9	299	2.2	99.3	
17.07.87	159	289	1.7	99.4	261	7.9	96.9	
22.07.87	164	357	1.1	99.7	379	70	81.6	Air Pump Failure to
28.07.87	170	371	1.1	99.7	393	186	52.7	Con React
04.08.87	177	329	1.0	99.7	321	161	49.9	
09.08.87	182	314	1.4	99.5	329	136	58.9	
16.08.87	189	293	0.5	99.8	314	23	92.6	
23.08.87	196	300	0.5	99.9	207	4.5	97.8	2.5% K10 to Exp React
31.08.87	204	300	0.7	99.8	279	4.8	98.3	
07.09.87	211	293	3.0	99.0	300	4.3	98.6	
08.09.87	212	264	2.0	99.3	300	3.8	98.8	
09.09.87	213	257	1.4	99.4	279	3.2	98.8	
10.09.87	214	257	0.5	99.8	257	3.2	98.8	New MPW to Con React
11.09.87	215	279	1.4	99.5	386	3.4	99.1	
16.09.87	220	386	1.1	99.7	442	0.7	99.8	New MPW to Exp React
25.09.87	229	430	1.3	99.7	439	1.0	99.8	
05.10.87	239	453	1.1	99.8	438	25	94.2	
14.10.87	248	437	2.3	99.5	446	173	61.2	
26.10.87	260	442	3.0	99.3	442	300	32.1	
05.11.87	270	447	237	47.0	454	343	24.6	
11.11.87	276	416	102	75.5	454	254	44.1	
20.11.87	285	393	1.4	99.7	447	241	46.1	
30.11.87	295	431	1.2	99.7	477	0.6	99.9	
03.12.87	298	447	1.2	99.7	485	1.5	99.7	
17.12.87	312	393	0.6	99.9	370	1.0	99.7	
18.12.87	313	431	0.8	99.8	408	0.4	99.9	
22.12.87	317	308	1.0	99.7	308	0.2	99.9	
28.12.87	323	454	0.8	99.8	447	0.6	99.9	
04.01.88	330	424	0.8	99.8	424	0.2	100	
08.01.88	335	447	4.7	98.9	424	4.2	99.0	
15.01.88	342	462	5.3	98.9	477	4.0	99.2	
19.01.88	346	311	5.1	98.4	311	4.5	98.5	
25.01.88	352	455	5.1	98.8	432	4.2	99.0	
30.01.88	357	348	4.4	98.7	414	3.3	99.2	
05.02.88	363	407	4.3	99.0	400	3.9	99.0	
10.02.88	368	333	3.9	98.8	296	3.7	98.9	
26.02.88	384	429	3.9	99.1	437	5.0	98.9	
04.03.88	391	422	4.1	99.0	422	4.4	98.9	
11.03.88	398	496	4.3	99.1	551	5.2	98.9	

Chemical Oxygen Demand Analysis (Section 3.5.2)

Date	Day	Experimental Reactor			Control Reactor			Comments
		Feed	Eff	%Rem	Feed	Eff	%Rem	
13.02.87	5	389	65	83.1	389	73	81.1	
10.06.87	122	863	404	53.2	863	527	38.9	
11.06.87	123	844	383	54.6	844	555	34.3	
12.06.87	124	852	344	59.6	852	531	37.6	
15.06.87	127	805	397	50.7	805	506	37.1	
16.06.87	128	1021	416	59.3	716	561	21.5	1% K10 to Exp React
17.06.87	129	1078	370	65.7	1059	584	44.9	
18.06.87	130	1059	317	70.1	1040	599	42.4	
19.06.87	131	1083	291	73.1	1083	575	46.9	Nitrogen Limitation?
22.06.87	134	1063	228	78.6	1063	591	44.4	
23.06.87	135	1083	181	83.3	1122	575	48.8	
24.06.87	136	1054	194	81.6	1034	484	53.2	
26.06.87	138	995	265	73.4	1054	476	54.8	
29.06.87	141	1093	186	82.3	1034	445	57.0	Tri-ammon ortho phos
30.06.87	142	1029	191	81.4	1038	415	60.0	
03.07.87	145	989	207	79.1	960	163	83.0	
06.07.87	148	920	187	79.7	940	140	85.1	
08.07.87	150	1030	196	81.0	895	157	82.5	
10.07.87	152	1030	173	83.2	953	157	83.5	
17.07.87	159	1049	196	81.3	992	157	84.2	
22.07.87	164	1289	227	82.4	1221	371	69.6	Air Pump Failure to
28.07.87	170	1289	250	80.6	1270	703	44.6	Con React
04.08.87	177	1250	324	74.1	1123	629	44.0	
09.08.87	182	1210	251	79.3	1122	607	45.9	
16.08.87	189	1161	243	79.1	1122	314	72.0	
23.08.87	196	1171	255	78.2	1102	215	80.4	2.5% K10 to Exp React
31.08.87	204	1304	206	84.2	1136	269	76.3	
07.09.87	211	1176	213	81.8	1492	427	71.4	
08.09.87	212	1206	241	80.0	1028	265	74.2	
09.09.87	213	1049	239	77.2	1010	200	80.2	
10.09.87	214	1147	231	79.8	912	208	77.2	New MPW to Con React
11.09.87	215	1127	251	77.7	1206	196	83.7	
16.09.87	220	1375	266	80.7	1355	162	88.0	New MPW to Exp React
25.09.87	229	1271	300	76.4	1236	177	85.7	
05.10.87	239	1108	208	81.2	1291	332	74.3	
14.10.87	248	1255	294	76.6	1388	525	62.2	
26.10.87	260	1020	400	60.8	1353	996	26.4	
05.11.87	270	1500	996	33.6	1333	988	25.9	
11.11.87	276	1422	584	58.9	1314	482	63.3	
20.11.87	285	1447	299	79.4	1608	195	87.9	
30.11.87	295	1507	267	82.3	1347	91	93.3	
03.12.87	298	1507	315	79.1	1387	131	90.6	
17.12.87	312	1472	318	78.4	1317	151	88.5	
22.12.87	317	1505	278	81.5	1242	110	91.1	
28.12.87	323	1418	285	79.9	1279	137	89.3	
04.01.88	331	1370	223	83.7	1240	115	90.7	
08.01.88	335	1387	364	73.8	1191	129	89.1	
15.01.88	342	1504	266	82.3	1388	151	88.7	
19.01.88	346	1037	236	77.2	932	102	89.0	
25.01.88	352	1470	260	82.3	1207	83	93.2	
30.01.88	357	1299	325	75.0	1250	59	95.3	
05.02.87	363	1440	320	77.8	1260	45	96.4	
10.02.88	368	1150	427	62.8	1000	50	95.0	

Total Organic Carbon Analysis (Section 3.5.3)

Date	Day	Experimental Reactor			Control Reactor			Comments
		Feed	Eff	%Rem	Feed	Eff	%Rem	
09.02.87	1	259	151	41.6	259	160	38.3	
13.02.87	5	264	140	47.1	264	139	46.9	
16.02.87	8	283	62	78.2	283	46	83.7	
20.02.87	12	298	91	69.4	298	64	78.4	
23.02.87	15	274	106	61.3	274	64	76.5	
24.02.87	16	293	88	70.1	293	84	71.4	
25.02.87	17	281	98	65.0	281	66	76.3	
26.02.87	18	312	119	62.0	312	72	76.9	
27.02.87	19	338	106	68.6	338	63	81.4	
02.03.87	22	306	47	84.6	306	64	79.0	
03.03.87	23	316	52	83.5	316	59	81.2	
04.03.87	24	327	53	83.7	327	41	87.4	
05.03.87	25	340	58	82.9	340	85	75.1	
06.03.87	26	338	71	79.0	338	73	78.3	
09.03.87	29	301	75	75.2	301	57	81.0	
16.03.87	36	290	93	68.1	290	62	78.6	
23.03.87	43	287	75	73.7	287	65	77.5	
30.03.87	50	344	87	74.7	344	54	84.3	
06.04.87	57	361	107	70.4	361	66	81.8	Power Fluctuations
13.04.87	64	314	154	51.0	314	125	60.2	
14.04.87	65	336	173	48.5	336	156	53.7	
15.04.87	66	299	154	48.4	299	141	52.9	
16.04.87	67	304	123	59.4	304	99	67.4	
17.04.87	68	321	95	70.3	321	92	71.3	Power Fluctuations
20.04.87	71	337	81	75.9	337	74	78.1	Laboratory Move
27.04.87	78	315	74	76.5	315	74	76.4	
04.05.87	85	341	106	69.0	341	97	71.5	New Feed Pump
11.05.87	92	376	118	68.6	376	109	71.0	
18.05.87	99	334	98	70.7	334	82	75.3	2x Feed Conc'n
25.05.87	106	576	202	65.0	576	191	66.8	
26.05.87	107	597	207	65.4	597	209	65.0	
27.05.87	108	553	218	60.6	553	222	59.8	
28.05.87	109	581	207	64.3	581	231	60.3	
29.05.87	110	540	161	70.1	540	245	54.7	Con React pH 9
01.06.87	113	529	171	67.7	529	288	45.6	
02.06.87	114	497	144	71.0	497	245	50.8	
03.06.87	115	510	154	69.8	510	303	40.5	
04.06.87	116	536	142	73.5	536	326	39.1	
05.06.87	117	519	154	70.4	519	303	41.7	
10.06.87	122	540	235	56.5	540	327	39.4	
11.06.87	123	483	225	53.4	483	313	35.2	
12.06.87	124	525	206	60.8	525	331	37.0	
15.06.87	127	499	219	56.1	499	291	41.7	
16.06.87	128	583	201	65.5	583	281	51.8	1% K10 to Exp React
17.06.87	129	539	183	66.0	549	275	49.9	
18.06.87	130	515	170	67.0	555	273	50.8	
19.06.87	131	577	140	75.7	535	275	48.6	Nirogen Limitation?
22.06.87	134	533	117	78.1	530	253	52.3	
23.06.87	135	571	131	77.1	539	250	53.6	
24.06.87	136	549	122	77.8	539	249	53.8	
26.06.87	138	592	132	77.7	499	245	50.9	
29.06.87	141	575	126	78.1	507	215	57.6	Tri-ammon ortho phos
30.06.87	142	677	123	81.8	642	215	66.5	

Total Organic Carbon Analysis (Section 3.5.3)

Date	Day	Experimental Reactor			Control Reactor			Comments
		Feed	Eff	%Rem	Feed	Eff	%Rem	
03.07.87	145	556	113	79.7	592	85	85.6	
06.07.87	148	545	122	77.6	535	75	86.0	
08.07.87	150	577	89	84.6	561	72	87.2	
10.07.87	152	537	93	82.7	561	103	81.2	
17.07.87	159	571	117	79.5	555	87	84.3	
22.07.87	164	725	106	85.4	711	189	73.4	Air Pump Failure to
28.07.87	170	371	163	56.1	383	209	45.4	Con React
04.08.87	177	383	190	50.4	353	228	35.4	
09.08.87	182	367	196	46.6	379	227	40.1	
16.08.87	189	343	169	50.7	378	159	57.9	
23.08.87	196	396	153	61.4	382	138	63.9	2.5% K10 to Exp React
31.08.87	204	390	131	66.4	363	169	53.4	
07.09.87	211	337	160	52.5	350	172	50.9	
08.09.87	212	381	149	60.9	319	143	55.2	
09.09.87	213	384	161	58.1	317	131	58.7	
10.09.87	214	375	153	59.2	299	136	54.5	New MPW to Con React
11.09.87	215	365	143	60.8	402	133	66.9	
16.09.87	220	417	182	56.4	409	143	65.0	New MPW to Exp React
25.09.87	229	426	176	58.7	392	136	65.3	
05.10.87	239	412	197	52.2	415	167	59.8	
14.10.87	248	429	183	57.3	401	194	51.6	
26.10.87	260	448	234	47.8	429	320	25.4	
05.11.87	270	457	368	19.5	404	327	19.1	
11.11.87	276	431	250	42.0	378	199	47.4	
20.11.87	285	427	163	61.8	387	154	60.2	
30.11.87	295	436	168	61.5	362	123	66.0	
03.12.87	298	469	167	64.4	391	104	73.4	
17.12.87	312	489	184	62.4	371	118	68.2	
22.12.87	317	408	164	59.8	362	105	71.0	
28.12.87	323	431	166	61.5	378	115	69.6	
04.01.88	331	395	154	61.0	340	92	72.9	
08.01.88	335	399	161	59.6	367	117	68.1	
15.01.88	342	412	163	60.4	351	107	69.5	
19.01.88	346	387	151	61.0	404	105	74.0	
25.01.88	352	430	149	65.3	391	97	75.2	
30.01.88	357	401	160	60.1	395	135	65.8	
05.02.88	363	423	184	56.5	372	124	66.7	
10.02.88	368	415	162	61.0	359	113	68.5	

2L Pilot Reactor Analysis (Section 3.6)

Date	Day	Phenol Assay			COD Analysis			Comments
		Feed	Eff	%Rem	Feed	Eff	%Rem	
21.12.87	3	477	289	39.5	1544	759	50.9	2.5% K10 Medium
22.12.87	4	477	308	35.5	1564	575	63.3	
28.12.87	10	501	3.9	99.2	1613	449	72.2	
04.01.88	17	493	4.8	99.0	1593	465	70.1	Additional Nutrients
08.01.88	21	470	5.9	98.7	1583	461	70.9	
15.01.88	28	470	5.9	98.7	1628	473	71.0	
18.01.88	31	409	6.3	98.5	1458	547	62.5	
19.01.88	32	523	6.4	98.8	1445	512	64.6	Phenol + 2.5% K10
20.01.88	33	515	4.0	99.2	1497	414	72.3	
21.01.88	34	515	3.2	99.4	1510	313	79.3	
24.01.88	37	530	4.0	99.3	1484	336	77.4	
25.01.88	38	508	4.7	99.1	1459	392	73.1	
30.01.88	43	469	3.0	99.4	1374	407	70.4	
05.02.88	49	466	2.6	99.4	1453	356	75.5	
10.02.88	54	266	2.4	99.1	1382	352	74.5	
26.02.88	70	403	2.4	99.4	1407	328	76.7	
03.03.88	76	455	3.0	99.3	1483	346	76.7	
04.03.88	77	355	3.5	99.0	1364	341	75.0	5% K10
07.03.88	80	371	4.1	98.9	1341	463	65.5	
11.03.88	84	385	4.4	98.8	1344	455	66.2	
15.03.88	88	---	---	---	1032	438	57.6	12.5% K10
18.03.88	91	---	---	---	1064	758	28.8	
21.03.88	94	---	---	---	1036	893	13.8	
24.03.88	97	---	---	---	1047	978	6.6	
28.03.88	101	---	---	---	1057	1087	-2.8	NEGATIVE EFFICIENCY!

Chemical Oxygen Demand Analysis (Section 3.7.1)

Date	Day	Reactor 1			Reactor 2			Comments
		Feed	Eff	%Rem	Feed	Eff	%Rem	
03.04.89	0	1245	229	81.6	1233	219	82.2	2.5% K10
05.04.89	2	1255	254	79.8	1238	220	82.2	
07.04.89	4	1280	216	83.1	1250	240	80.8	
10.04.89	7	1230	232	81.1	1240	236	81.0	
12.04.89	9	1572	290	81.5	1366	352	74.2	5% K10
14.04.89	11	1590	304	80.9	1390	372	73.2	
17.04.89	14	1565	398	74.6	1341	366	72.7	
19.04.89	16	1463	427	70.8	1301	447	65.6	
24.04.89	21	1529	472	69.1	1323	463	65.0	
26.04.89	23	1826	597	67.3	1327	597	55.0	7.5% K10
28.04.89	25	1816	701	61.4	1407	645	54.2	
01.05.89	28	1846	665	65.1	1347	665	52.2	
03.05.89	30	1567	643	59.0	1251	675	46.0	
05.05.89	32	1647	825	49.9	1353	667	50.7	
08.05.89	35	1687	754	55.3	1468	675	54.0	
10.05.89	37	1673	805	51.9	1235	669	45.8	
12.05.89	39	1574	837	46.8	1225	773	36.9	Shock Load
15.05.89	42	1633	1251	23.4	1314	1100	16.4	
17.05.89	44	1870	939	49.8	1370	896	34.6	
19.05.89	46	1826	922	49.5	1391	913	34.4	
22.05.89	49	1804	922	48.9	1370	1009	26.3	
24.05.89	51	1802	866	51.9	1356	899	33.7	
26.05.89	53	1721	907	47.3	1336	891	33.3	
29.05.89	56	1802	887	50.8	1377	903	34.4	
31.05.89	58	1804	890	50.6	1276	924	27.6	
02.06.89	60	1825	983	46.1	1382	873	36.8	
05.06.89	63	1909	907	52.5	1392	975	30.0	
07.06.89	65	1735	927	46.6	1347	892	33.8	
09.06.89	67	1649	996	39.6	1304	841	35.5	
12.06.89	70	1886	944	49.9	1476	901	39.0	
14.06.89	72	1718	934	45.6	1327	926	30.2	
16.06.89	74	1677	951	43.3	1286	885	31.2	
19.06.89	77	1636	802	50.9	1183	761	35.7	
21.06.89	79	1452	755	48.0	1058	705	33.3	
23.06.89	81	1445	722	50.1	1002	696	30.5	
26.06.89	84	1614	553	65.8	1213	637	47.5	
28.06.89	86	1642	657	60.0	1212	614	49.3	
30.06.89	88	1620	721	55.5	1223	622	49.1	
03.07.89	91	1586	710	55.2	1184	643	45.7	
05.07.89	93	1543	692	55.2	1205	636	47.2	
07.07.89	95	1571	676	57.0	1077	621	42.4	
10.07.89	98	1413	660	53.3	1156	597	48.4	
12.07.89	100	1843	602	67.3	1017	602	40.8	
14.07.89	102	1631	644	60.5	975	627	35.7	
17.07.89	105	1581	638	59.6	1011	630	37.7	
19.07.89	107	1622	680	58.1	1173	651	44.5	
21.07.89	109	1667	656	60.6	1183	612	48.3	
24.07.89	112	1631	842	60.6	1229	619	49.7	
26.07.89	114	1710	684	60.0	1156	644	44.3	
28.07.89	116	1755	677	61.4	1121	600	46.5	10% K10
31.07.89	119	1813	778	57.1	1105	693	37.3	
02.08.89	121	1824	983	46.1	1092	751	31.2	
04.08.89	123	1799	870	51.6	1116	804	28.0	
07.08.89	126	1830	891	51.3	1097	765	30.3	
09.08.89	128	1841	983	46.6	1131	810	28.4	
11.08.89	130	1827	964	47.2	1079	819	24.1	
14.08.89	133	1803	1008	44.1	1061	793	25.3	
21.08.89	140	1796	951	47.0	1098	788	28.2	
28.08.89	147	1833	1036	43.5	1123	781	30.5	

Phenol Analysis (Section 3.7.2)

Date	Day	Reactor 1			Reactor 2			Comments
		Feed	Eff	%Rem	Feed	Eff	%Rem	
03.04.89	0	447	0	100	441	0	100	2.5% K10
05.04.89	2	433	0	100	436	0	100	
07.04.89	4	442	0	100	427	0	100	
10.04.89	7	435	0	100	431	0	100	
12.04.89	9	438	0	100	370	0	100	5% K10
14.04.89	11	442	0	100	375	0.1	100	
17.04.89	14	427	0	100	367	0.1	100	
19.04.89	16	446	0.1	100	363	0	100	
24.04.89	21	431	0.3	99.9	371	0	100	
26.04.89	23	435	0.2	100	245	0.1	100	7.5% K10
28.04.89	25	450	0.3	99.9	292	0.1	100	
01.05.89	28	409	4.5	98.9	315	4.3	98.6	
03.05.89	30	386	6.7	98.3	287	5.1	98.2	
05.05.89	32	425	6.5	98.5	315	6.7	97.9	
08.05.89	35	394	5.9	98.4	299	5.9	98.0	
10.05.89	37	449	10	97.7	362	7.9	97.8	
12.05.89	39	441	17	96.1	354	15	95.8	Shock Load
15.05.89	42	528	228	56.7	366	150	59.1	
17.05.89	44	520	21	95.9	347	20	94.3	
19.05.89	46	391	16	95.9	258	20	92.3	
22.05.89	49	438	20	95.4	266	23	91.5	
24.05.89	51	445	22	95.1	285	21	92.6	
26.05.89	53	434	23	94.6	227	24	89.2	
29.05.89	56	430	24	94.5	242	21	91.2	
31.05.89	58	428	22	94.8	313	25	92.1	
02.06.89	60	422	25	94.0	287	22	92.4	
05.06.89	63	453	27	94.1	309	26	91.5	
07.06.89	65	461	26	94.4	313	21	93.1	
09.06.89	67	463	27	94.2	314	22	92.9	
12.06.89	70	439	26	94.1	303	23	92.4	
14.06.89	72	462	24	94.7	273	23	91.7	
16.06.89	74	409	28	93.2	284	23	91.9	
19.06.89	77	386	23	94.1	288	21	92.6	
21.06.89	79	466	21	95.5	258	20	92.1	
23.06.89	81	428	21	95.1	273	19	93.1	
26.06.89	84	424	14	96.6	280	16	94.2	
28.06.89	86	489	19	96.2	268	16	94.1	
30.06.89	88	485	21	95.7	282	18	93.8	
03.07.89	91	462	21	95.4	303	18	94.1	
05.07.89	93	439	20	95.3	254	17	93.1	
07.07.89	95	394	19	95.1	296	18	93.9	
10.07.89	98	470	20	95.8	307	18	94.2	
12.07.89	100	451	22	95.1	311	21	93.2	
14.07.89	102	438	20	96.1	299	23	92.3	
17.07.89	105	462	25	94.6	295	24	91.3	
19.07.89	107	427	20	95.3	304	24	92.1	
21.07.89	109	437	21	95.2	317	20	93.7	
24.07.89	112	461	23	95.0	296	23	92.2	
26.07.89	114	450	20	95.6	330	19	94.2	
28.07.89	116	455	20	95.6	234	21	91.0	10% K10
31.07.89	119	462	25	94.6	219	23	89.5	
02.08.89	121	451	24	94.7	223	27	87.9	
04.08.89	123	469	26	94.5	236	26	89.0	
07.08.89	126	474	27	94.3	231	27	88.3	
09.08.89	128	453	24	94.7	235	24	89.8	
11.08.89	130	455	26	94.3	221	23	89.6	
14.08.89	133	471	33	93.0	214	22	89.7	
21.08.89	140	460	27	94.1	217	21	90.3	
28.08.89	147	477	32	93.3	231	23	90.0	

Total Organic Carbon Analysis (Section 3.7.3)

Date	Day	Reactor 1			Reactor 2			Comments
		Feed	Eff	%Rem	Feed	Eff	%Rem	
03.04.89	0	369	109	70.5	341	117	65.7	2.5% K10
05.04.89	2	381	115	69.8	327	105	67.9	
07.04.89	4	389	116	70.2	329	108	67.2	
10.04.89	7	360	106	70.6	311	97	68.8	
12.04.89	9	491	159	67.6	402	153	61.9	5% K10
14.04.89	11	486	129	73.5	385	164	57.4	
17.04.89	14	484	173	64.3	428	195	54.4	
19.04.89	16	489	208	57.5	426	190	55.4	
24.04.89	21	586	233	60.2	459	237	48.4	
26.04.89	23	491	281	42.8	419	243	42.0	7.5% K10
28.04.89	25	497	306	38.4	389	289	25.7	
01.05.89	28	539	309	42.7	430	285	33.7	
03.05.89	30	543	272	49.9	424	297	30.0	
05.05.89	32	528	364	31.1	479	287	40.1	
08.05.89	35	532	339	36.3	448	289	35.5	
10.05.89	37	534	346	35.2	441	304	31.1	
12.05.89	39	525	421	19.8	512	392	23.4	Shock Load
15.05.89	42	524	458	12.5	440	429	2.5	
17.05.89	44	506	443	12.5	525	421	19.8	
19.05.89	46	432	421	2.5	540	428	20.7	
22.05.89	49	526	382	27.3	384	367	4.4	
24.05.89	51	522	389	25.5	403	401	0.5	
26.05.89	53	519	348	32.9	420	386	8.1	
29.05.89	56	505	375	25.7	401	382	4.7	
31.05.89	58	490	381	22.2	396	388	2.0	
02.06.89	60	448	412	8.0	394	368	6.6	
05.06.89	63	469	382	18.5	396	394	0.5	
07.06.89	65	462	351	24.0	389	349	10.3	
09.06.89	67	453	393	13.2	391	343	12.3	
12.06.89	70	436	375	14.0	401	356	11.2	
14.06.89	72	394	391	0.8	379	375	1.1	
16.06.89	74	451	441	2.2	439	423	3.6	
19.06.89	77	473	425	10.1	424	384	9.4	
21.06.89	79	422	384	9.0	340	326	4.1	
23.06.89	81	406	399	1.7	321	310	3.4	
26.06.89	84	403	232	42.4	381	291	23.6	
28.06.89	86	433	310	28.4	330	261	20.9	
30.06.89	88	436	330	24.3	325	279	14.2	
03.07.89	91	407	281	31.0	364	294	19.2	
05.07.89	93	418	275	34.2	377	316	16.1	
07.07.89	95	412	301	26.6	351	301	14.2	
10.07.89	98	431	336	22.0	372	306	17.7	
12.07.89	100	430	317	26.3	363	267	26.4	
14.07.89	102	439	308	29.8	391	289	26.1	
17.07.89	105	445	312	29.9	375	267	28.8	
19.07.89	107	461	291	36.9	361	293	18.8	
21.07.89	109	432	314	27.3	380	291	23.4	
24.07.89	112	440	381	13.4	391	280	28.4	
26.07.89	114	467	321	31.3	377	263	30.2	
28.07.89	116	561	341	39.2	448	294	34.4	10% K10
31.07.89	119	540	436	19.3	439	317	27.8	
02.08.89	121	588	512	12.9	456	381	16.4	
04.08.89	123	579	551	4.8	451	413	8.4	
07.08.89	126	595	532	10.6	457	427	6.6	
09.08.89	128	613	547	10.8	436	418	4.1	
11.08.89	130	583	519	11.0	441	412	6.6	
14.08.89	133	606	532	12.2	437	421	3.7	
21.08.89	140	612	547	10.6	451	406	10.0	
28.08.89	147	593	541	8.8	455	425	6.6	

APPENDIX 4

CHAPTER 5 RESULTS

Comparison of Respiration Rates of K10 Adapted and Unadapted Activated Sludge in Response to Varying Concentrations of K10 Wash Water Effluent

Concentration of K10 Wash Water Effluent (% K10 (v/v)	Specific Respiration Rate (mgO_2/h)	
	Adapted Sludge	Unadapted Sludge
0	1.59	2.97
0.1	----	6.80
0.189	----	11.11
0.45	----	14.34
1	4.31	14.04
2.5	4.66	12.90
7	6.72	13.15
10	7.30	11.11
17.5	5.38	10.42
25	4.40	8.73
33	4.14	8.63

MLSS for Assay = 1120mg/l

APPENDIX 5

CHAPTER 6 RESULTS

Ozonolysis of 40% K10 Wash Water Effluent (Section 6.2)

TIME	O_3/l	COD	COD/COD	TOC	TOC/TOC	200	200/200	250	250/250	358	358/358
0	0	3360	1.0	2371	1.0	1.35	1.0	0.87	1.0	0.44	1.0
1	0.2709	3195	0.95	2371	1.0						
2	0.5418	3029	0.90	2350	0.99	1.54	1.14	0.82	0.94	0.41	0.92
3	0.8127	2905	0.86	2340	0.99						
4	1.0836	2780	0.83	2299	0.97	1.63	1.21	0.73	0.84	0.33	0.75
5	1.3545	2656	0.79	2224	0.94						
6	1.6254	2490	0.74	2147	0.92	1.72	1.27	0.65	0.75	0.25	0.56
7	1.8963	2365	0.70	2122	0.89						
8	2.1672	2241	0.67	2090	0.88	1.80	1.33	0.56	0.64	0.175	0.40
9	2.4381	2095	0.62	2007	0.85						
10	2.7090	1950	0.58	1986	0.84	1.84	1.36	0.47	0.54	0.110	0.25
11	2.9799	1805	0.54	1889	0.80						
12	3.2508	1701	0.51	1846	0.78	1.89	1.40	0.40	0.46	0.070	0.16
13	3.5217	1587	0.47	1785	0.75						
14	3.7926	1463	0.44	1732	0.73	1.91	1.41	0.33	0.38	0.040	0.09
15	4.0635	1359	0.40	1700	0.72						
16	4.3344	1219	0.36	1661	0.70	1.94	1.44	0.26	0.30	0.033	0.08
17	4.6053	1094	0.33	1626	0.69						
18	4.8762	1001	0.30	1576	0.66	1.94	1.44	0.21	0.24	0.028	0.06
19	5.1471	887	0.26	1552	0.65						
20	5.4180	794	0.24	1505	0.63	1.95	1.44	0.15	0.17	0.025	0.06
21	5.6889	659	0.20	1443	0.61						
22	5.9571	532	0.16	1396	0.59	1.96	1.45	0.11	0.13	0.021	0.05
23	6.2253	438	0.13	1373	0.58						
24	6.4935	354	0.11	1335	0.56	1.98	1.47	0.08	0.09	0.019	0.04
25	6.7617	258	0.08	1252	0.53						
26	7.0203	204	0.06	1212	0.51	1.99	1.47	0.06	0.07	0.017	0.04
27	7.2615	183	0.05	1149	0.48						
28	7.4945	158	0.05	1128	0.48	2.00	1.48	0.05	0.06	0.015	0.03
29	7.7247	147	0.04	1066	0.45						
30	7.9536	139	0.04	1071	0.45						
31	8.1785	118	0.04	1041	0.44						
32	8.4047	116	0.03	1010	0.43						
33	8.6268	104	0.03	992	0.42						
34	8.8517	99	0.03	987	0.42						
35	9.0738	89	0.03	956	0.40						
36	9.3000	87	0.03	937	0.40						
37	9.5222	78	0.02	924	0.39						
38	9.7389	74	0.02	928	0.39						

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	215nm
Ozone Applied	0.0g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	215nm
Ozone Applied	1.0836g/l effluent

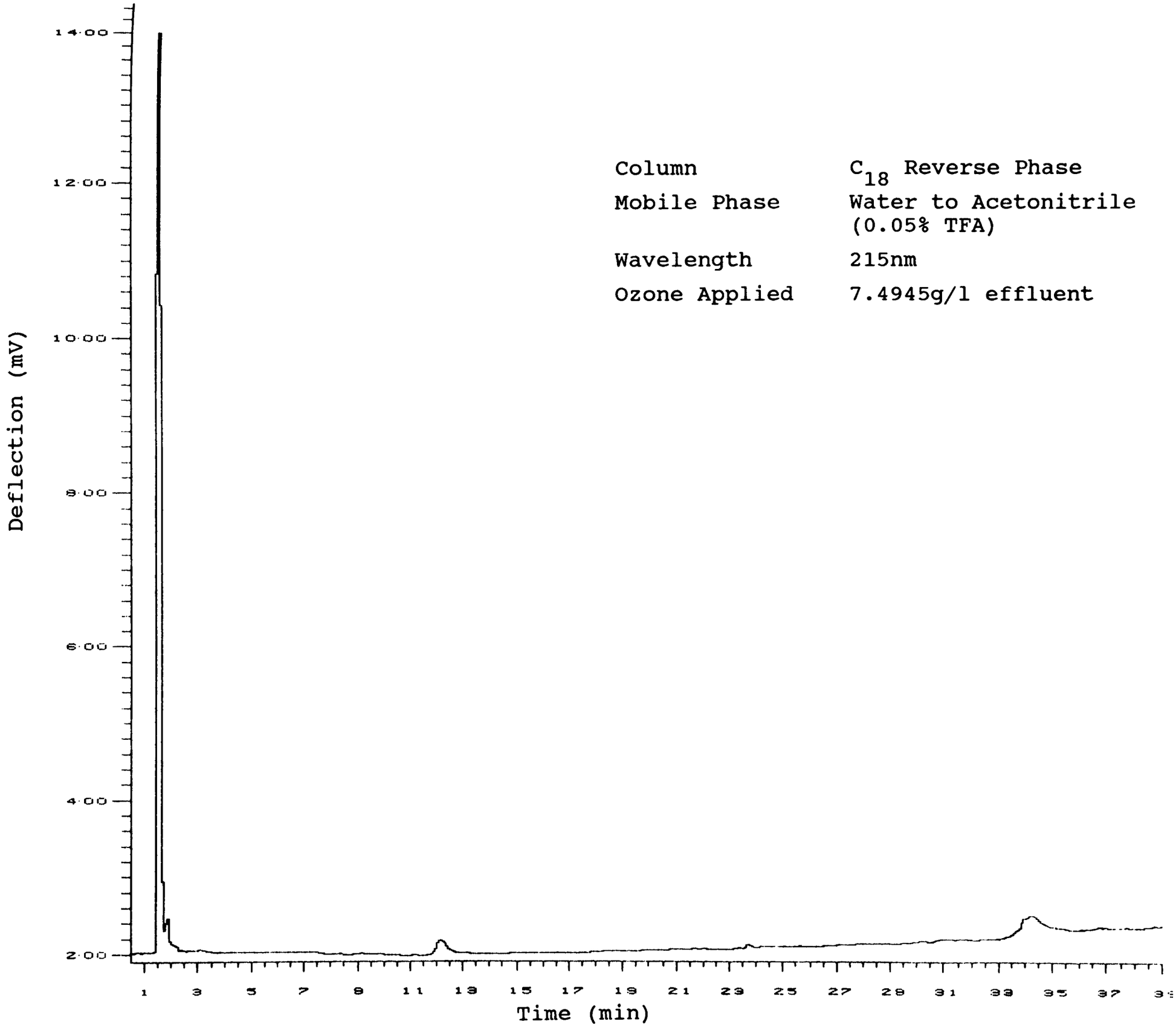
HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	215nm
Ozone Applied	3.2508g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	215nm
Ozone Applied	5.4180g/l effluent

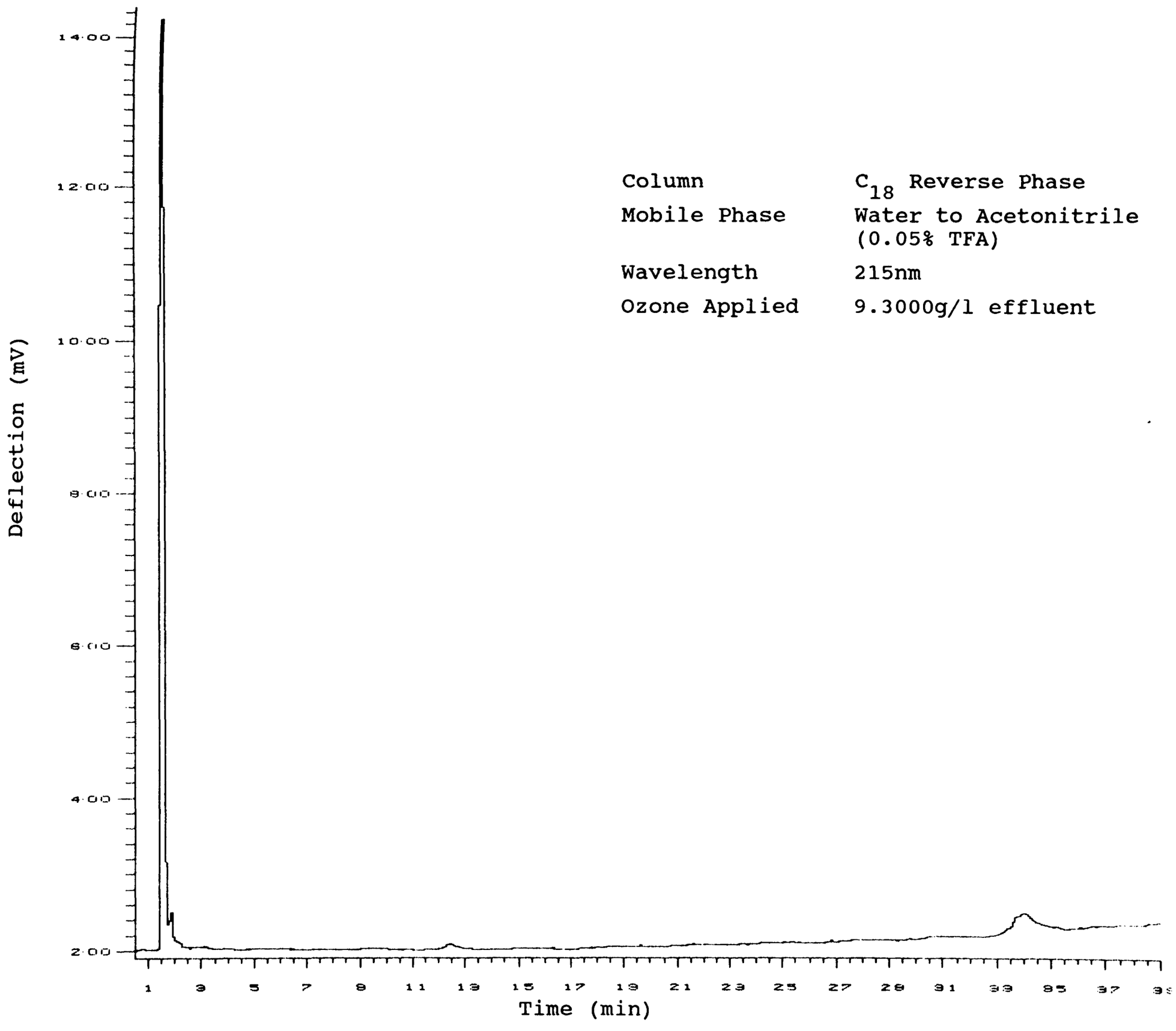
HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent



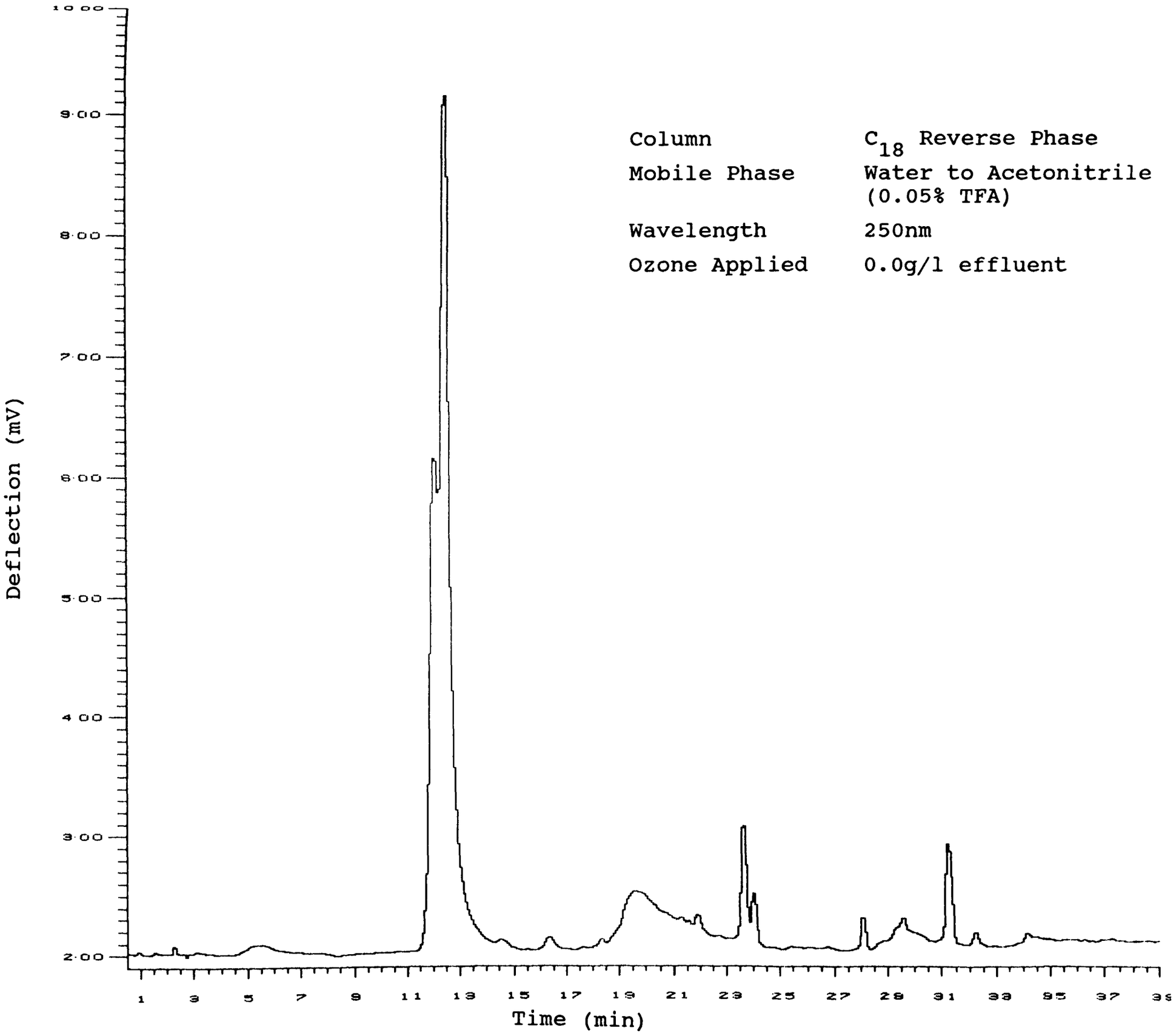
Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	215nm
Ozone Applied	7.4945g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	215nm
Ozone Applied	9.3000g/l effluent



HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent



Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	0.0g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

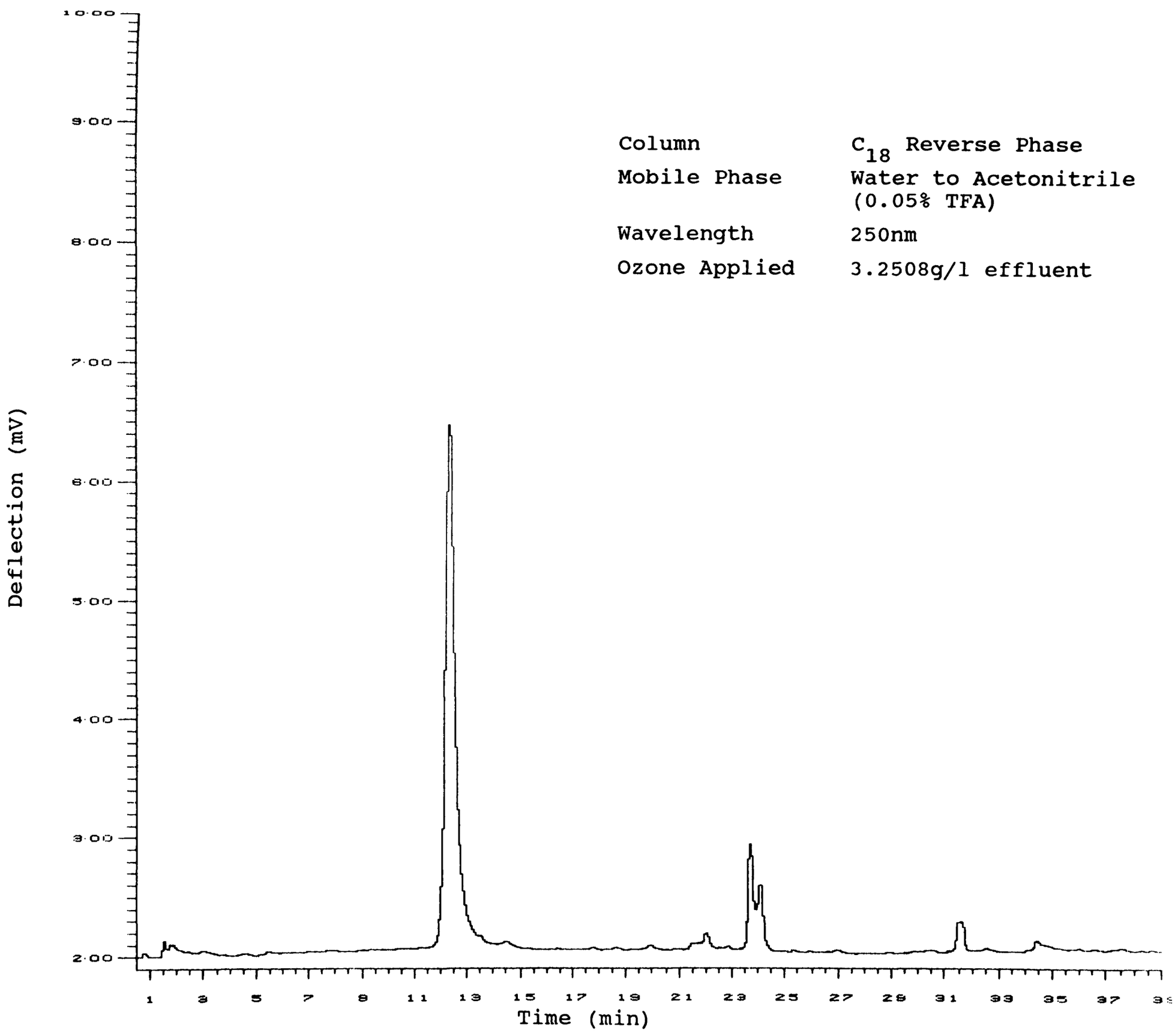
Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	1.0836g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	2.1672g/l effluent

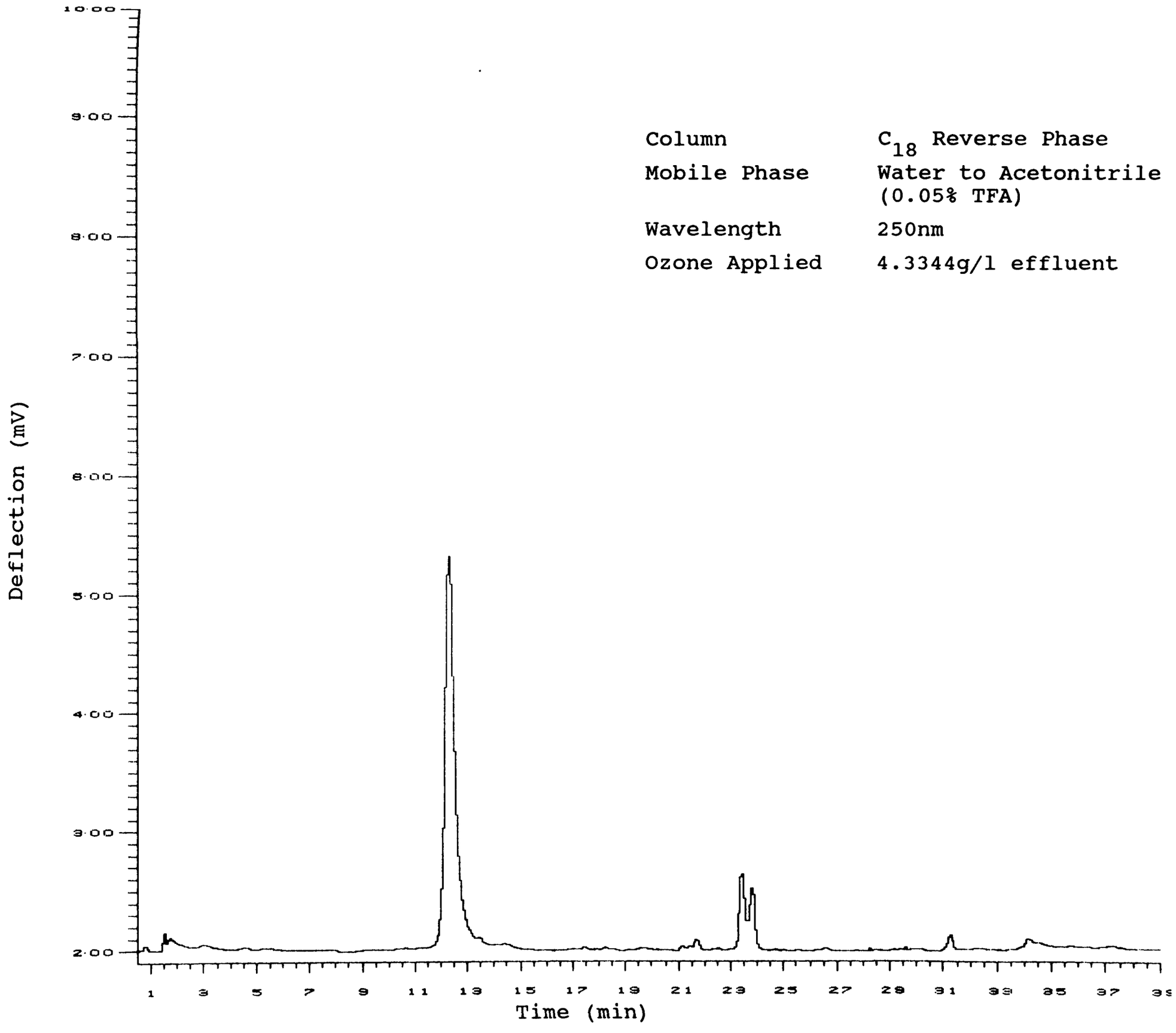
HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	3.2508g/l effluent



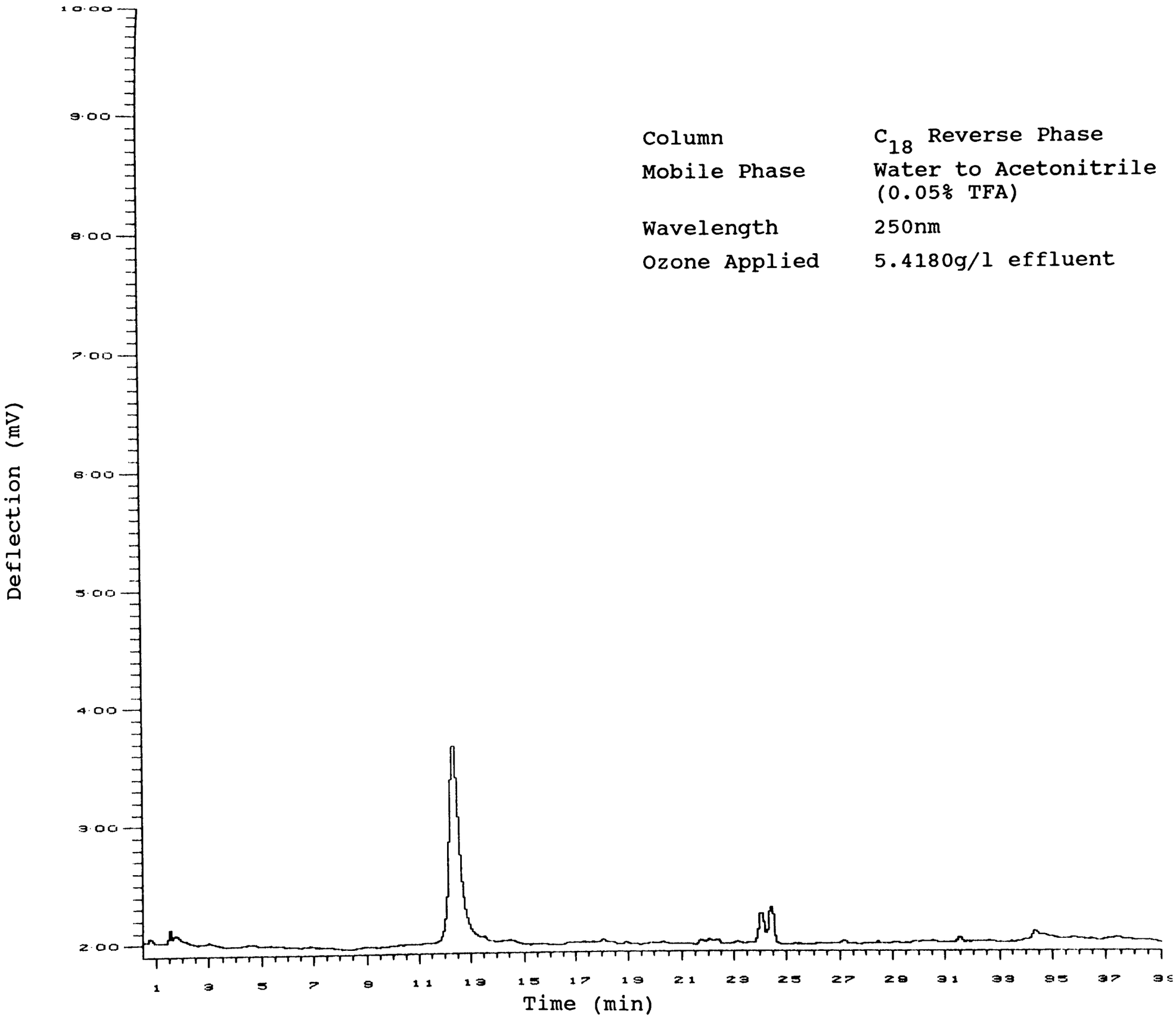
HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	4.3344g/l effluent



HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	5.4180g/l effluent

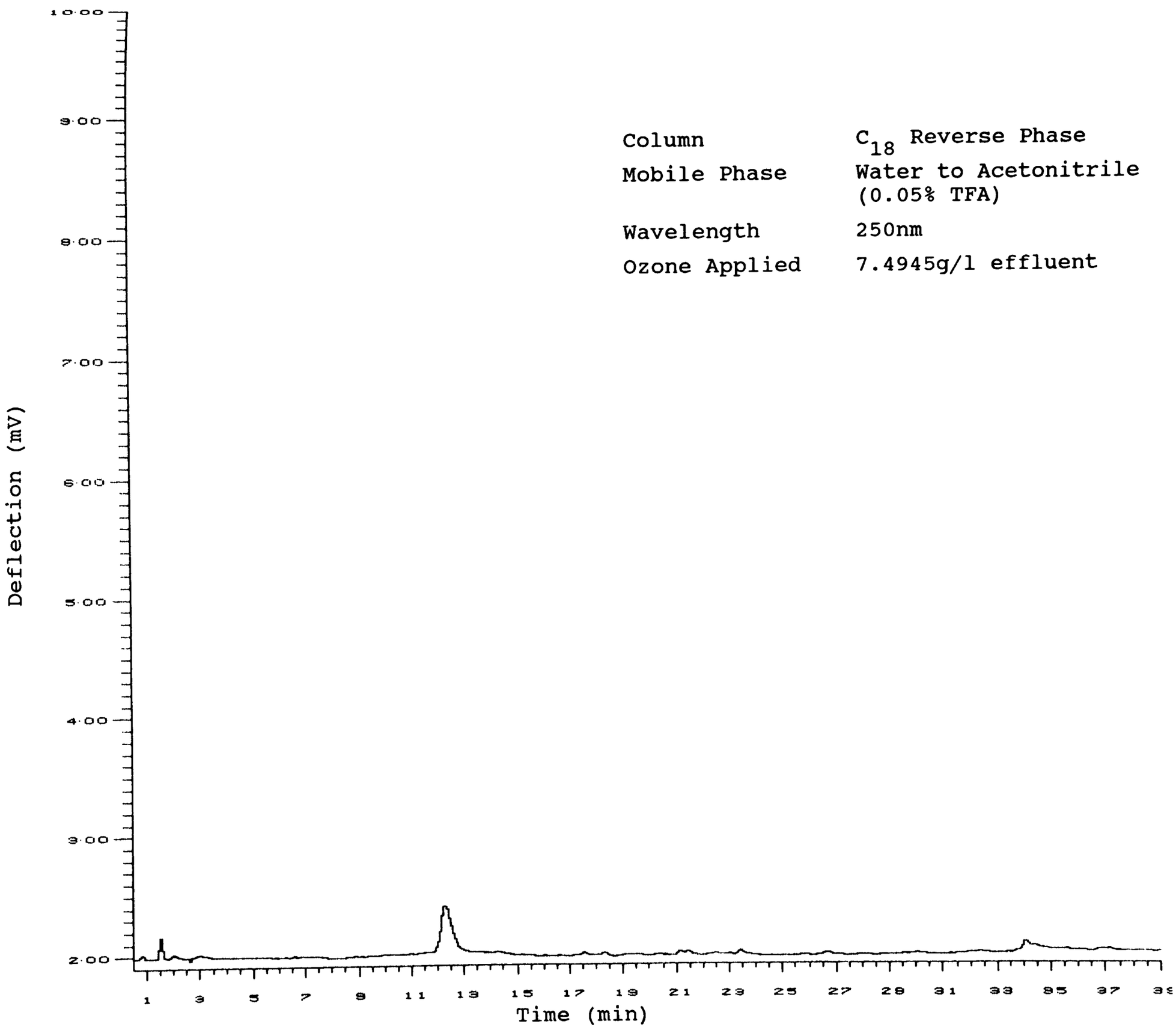


HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	6.4935g/l effluent

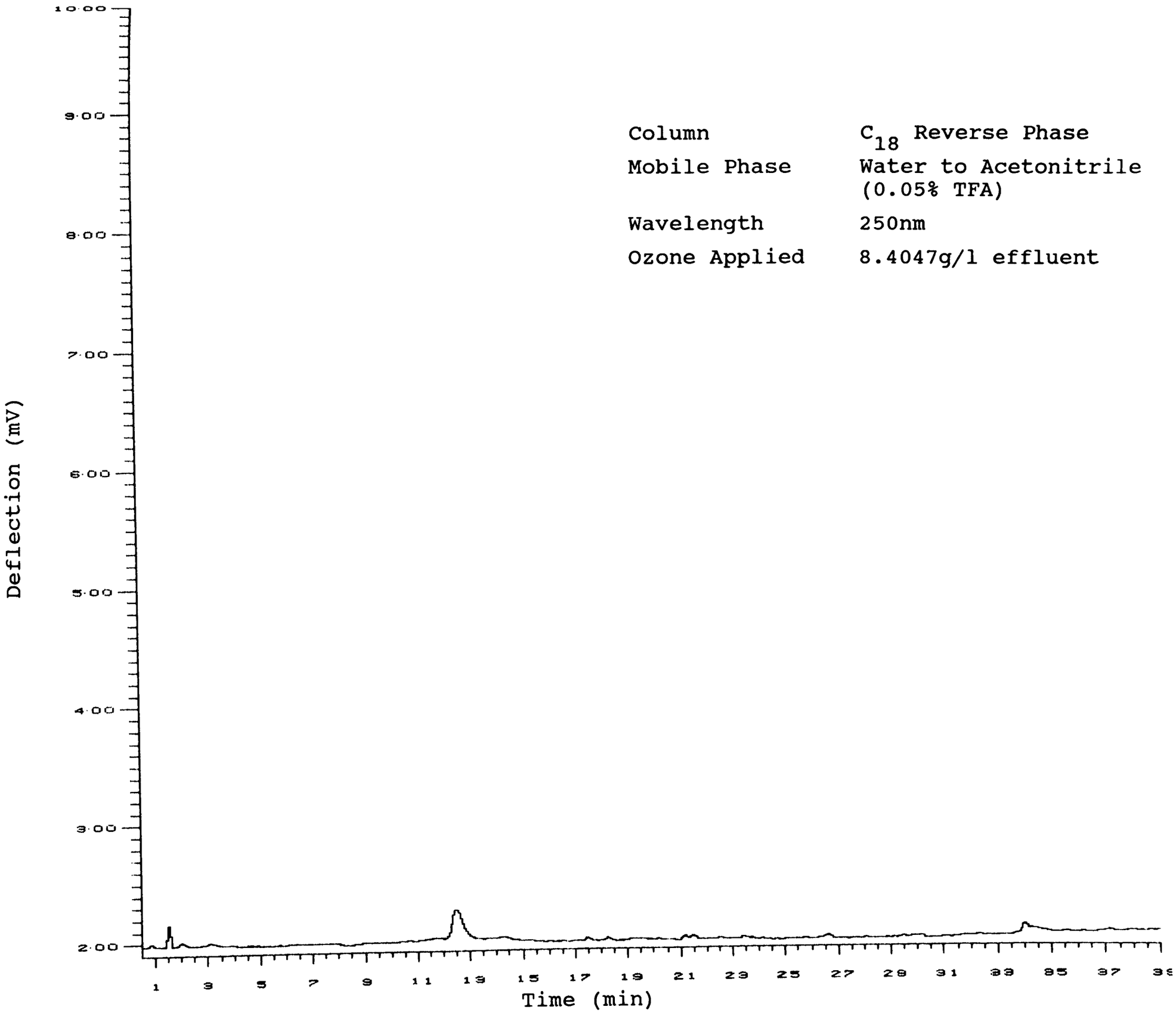
HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	7.4945g/l effluent



HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	8.4047g/l effluent



HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	0.0g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	1.0836g/l effluent

Deflection (mV)

9.40
9.20
9.00
2.80
2.60
2.40
2.20
2.00

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39

Time (min)

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	2.1672g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	3.2508g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	4.3344g/l effluent

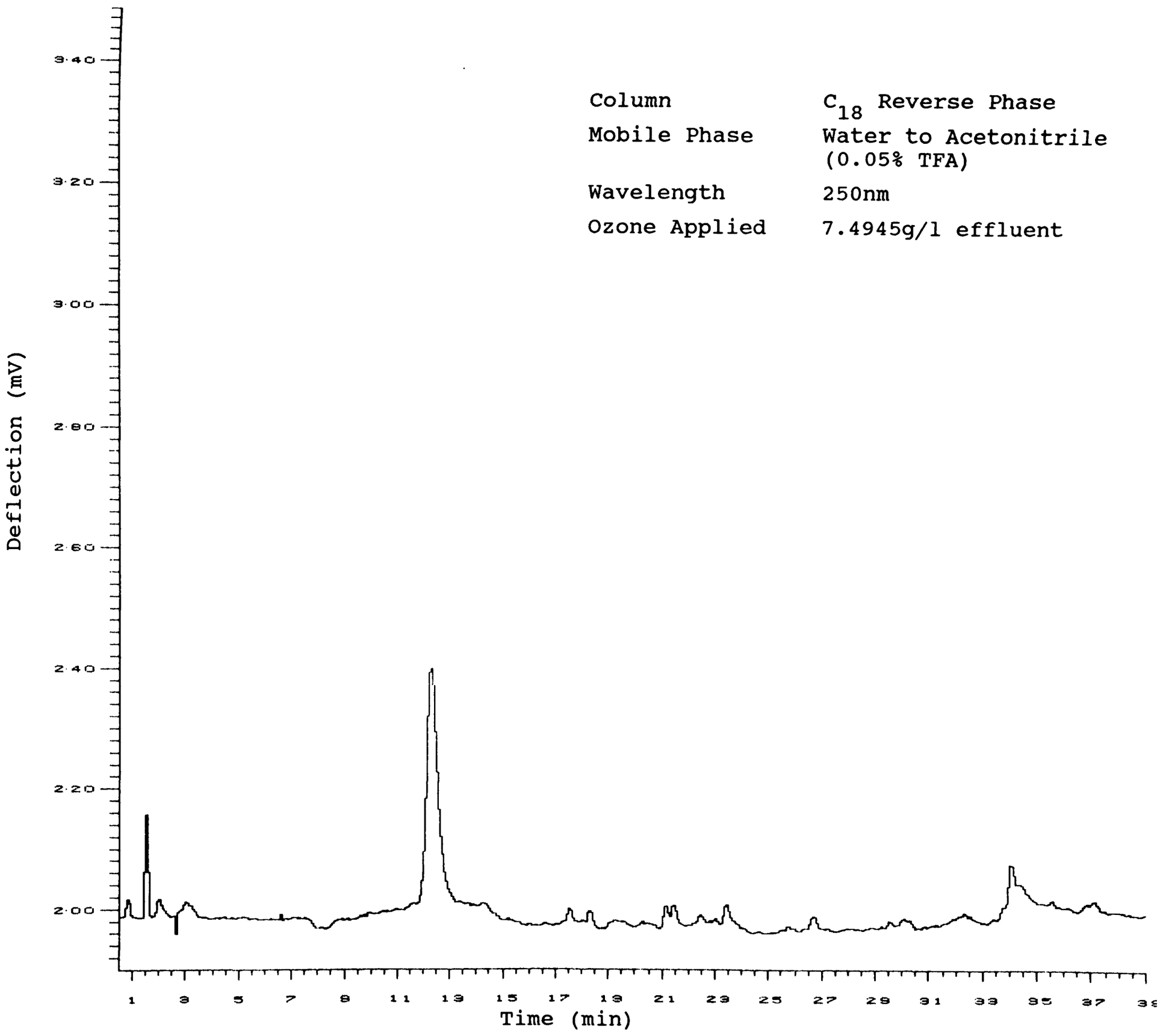
HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	5.4180g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	6.4935g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent (Expanded Absorbance Scale)



Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	7.4945g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent (Expanded Absorbance Scale)

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	250nm
Ozone Applied	8.4047g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	350nm
Ozone Applied	0.0g/l effluent

HPLC Analysis Results from the Ozonolysis of 40% K10 Wash Water Effluent

Column	C_{18} Reverse Phase
Mobile Phase	Water to Acetonitrile (0.05% TFA)
Wavelength	350nm
Ozone Applied	1.0836g/l effluent

Ozonolysis of TNT Red Water (Section 6.3)

TIME	O_3/L	COD	COD/COD	200	200/200	250	250/250	327	327/327
0	0	3042	1.0	1.47	1.0	0.79	1.0	0.30	1.0
1	0.2600	3021	0.99						
2	0.5199	2896	0.95	1.52	1.03	0.70	0.89	0.20	0.67
3	0.7773	2687	0.88						
4	1.0243	2479	0.81	1.53	1.04	0.60	0.76	0.15	0.50
5	1.2582	2312	0.76						
6	1.4662	2240	0.74	1.56	1.06	0.53	0.67	0.115	0.38
7	1.6638	2240	0.74						
8	1.8433	2115	0.70	1.57	1.07	0.47	0.59	0.090	0.30
9	2.0019	1990	0.65						
10	2.1500	1927	0.63	1.59	1.08	0.41	0.52	0.075	0.25
11	2.2748	1865	0.61						
12	2.3865	1795	0.59	1.65	1.12	0.38	0.48	0.065	0.22
13	2.4931	1753	0.58						
14	2.5920	1691	0.56	1.66	1.13	0.35	0.44	0.060	0.20
15	2.6829	1670	0.55						
16	2.7733	1520	0.50	1.68	1.14	0.32	0.41	0.052	0.17
17	2.8544	1499	0.49						
18	2.9351	1494	0.49	1.68	1.14	0.30	0.38	0.050	0.17
19	3.0130	1468	0.48						
20	3.0911	1371	0.45	1.73	1.18	0.28	0.35	0.046	0.15
21	3.1664	1355	0.45						
22	3.2366	1345	0.44	1.73	1.18	0.26	0.33	0.043	0.14

APPENDIX 6

ADDITIONAL REFERENCES

ADDITIONAL REFERENCES

Although these references were not cited in this thesis, they may be of interest to the reader.

Albert, R. C., Hoehn, R. C. and Randall, C. W.
Treatment of a Munitions Manufacturing Waste by the Fixed Activated Sludge Process
27th Engineering Extension Series (1972) 458-472
(Proc. Int. Waste Water Conf.)

Andren, R. K., Nystron, J. M., McDonnell, R. P. and Stevens, B. W.
Explosives Removal from Munitions Wastewater
30th Industrial Wastewater Conference (1975) 816-825
Purdue University, USA.

Bollag, J-M. and Loll, M. J.
Incorporation of Xenobiotics into Soil Humus
Experientia (1983) 39, 1221-1331

Boyd, S. A., Shelton, D. R., Berry, D. and Tiedje, J. M.
Anaerobic Biodegradation of Phenolic Compounds in Digested Sludge
Applied and Environmental Microbiology (1983) 46, No. 1, 50-54

Chambers, C. W., Tabak, H. H. and Kabler, P. W.
Degradation of Aromatic Compounds by Phenol Adapted Bacteria
Journal W.P.C.F. (1963) 35, No.12, 1517-1528

Cook, A. M., Grossenbacher, H. and Hutter, R.
Isolation and Cultivation of Microbes with Biodegradative Potential
Experientia (1983) 39, 1191-1197

Cooper, R. L. and Wheatstone, K. C.
The Determination of Phenols in Aqueous Effluents
Water Research (1973) 7, 1375-1384

Cullivan, B. M.
Industrial Toxics Oxidation: an Ozone-Chlorine Comparison
33rd Industrial Waste Conference (1978) 903-910
Purdue University, USA.

Curry, N. A.
Guidelines for Landfill of Toxic Industrial Sludges
28th Industrial Waste Conference (1973) 329-339
Purdue University, USA.

Czekalowski, J. W. and Skarzynski, B.
The Breakdown of Phenols and Related Compounds by Bacteria
J. Gen. Microbiol. (1948) 2, 231-238

Dagley, S.
Microbial Catabolism and the Carbon Cycle
Biochemical Society Transactions (1976) 4, 455-466
562nd Meeting Bangor

Eisenhauer, H. R.
The Ozonation of Phenolic Wastes
Journal W.P.C.F. (1968) 40, No.11, 1887-1899

Evans, R. A., Parr, W. H. and Evans, W. C.
The Bacterial Oxidation of Aromatic Compounds
Biochem. J. (1949) 44, viii

Evans, W. C., Smith, B. S. W., Linstead, R. P. and Elvidge, J. A.
Chemistry of the Oxidative Metabolism of Certain Aromatic Compounds by Microorganisms
Nature (1951) 168, 772-775

Finn, R. K.
Use of Specialised Microbial Strains in the Treatment of Industrial Waste and in Soil Decontamination
Experientia (1983) 39, 1231-1236

Forsten, I.
Pollution Abatement in a Munitions Plant
Environ. Sci. Technol. (1973) 7, No.9, 806-810

Fox, R. D. and Himmelstein, K. J.
Recovery or Destruction - New Developments for Industrial Wastes
29th Industrial Waste Conference (1974) 445-455

Freeman, D. J.
Continuous Fixation and Removal of Explosive Wastes from Pink Water using Surfactant Technology
40th Industrial Waste Conference (1985) 659-676
Purdue University, USA.

Ghisalba, O.
Microbial Degradation of Chemical Waste, an Alternative to Physical Methods of Waste Disposal
Experientia (1983) 39, 1247-1257

Guy, S.
Effect of Cross Treatment on the Subsequent Breakdown of 2,4-D, MCPA and 2,4,5-T in Soil
Chemosphere (1983) 12, No. 7/8, 1101-1106

Happold, F. C.
The Oxidation of Aromatic Rings by Microorganisms in Metabolism
Biochem. Soc. Symposia (Brit) (1950) 5, 85-96

Harder, W.
Enrichment and Characterization of Degrading Organisms
In "Microbial Degradation of Xenobiotics and Recalcitrant Compounds" (1980) 77-96
FEMS Symposium No. 12

Irvine, R. L. and Busch, A. W.
Factors Responsible for Non-Biodegradability of Industrial Wastes
24th Industrial Waste Conference (1969) 903-913
Purdue University, USA.

Jennett, J. C. and Dennis, D. D.
Anaerobic Filter Treatment of Pharmaceutical Waste
Journal W.P.C.F. (1975) 47, No.1, 104-121

Kearney, P. C., Plimmer, J. R., Wheeler, W. B. and Konston, A.
Persistence and Metabolism of Dinitroaniline Herbicides in Soil
Pesticide Biochemistry and Physiology (1976) 6, 229-238

Kiff, R. J.
Water Pollution Control in the Fertilizer Manufacturing Industry
In "Surveys in Industrial Wastewater Treatment"
3. Manufacturing and Chemical Industries
Ed. Barnes, D., Forster, C. F. and Longman Scientific and Technical

Kincannon, D. F., Weinert, A., Padorr, R. and Stover, E. L.
Predicting Treatability of Multiple Organic Priority Pollutant Wastewaters from Single-Pollutant Treatability Studies
37th Industrial Waste Conference (1982) 641-650
Purdue University, USA.

Klausmeier, R. E., Osmon, J. L. and Walls, D. R.
The Effect of Trinitrotoluene on Microorganisms
Dev. Ind. Microbiol. (1973) 15, 309-317

Lal, R., Lal, S. and Shivaji, S.
Use of Microbes for Detoxification of Pesticides
CRC Critical Reviews in Biotechnology (1986) 3, 1-16

Lamb, J. C., Westgarth, W. C., Rogers, J. L. and Vernimmen, A. P.
A Technique for Evaluating the Biological Treatability of Industrial Wastes
13th SMIWC (1964) 62-75

Leisinger, T.
Microorganisms and Xenobiotic Compounds
Experientia (1983) 39, 1183-1191

Mallik, M. A. B. and Tesfai, K.
Transformation of Nitrosamines in Soil and in Vitro by Soil Microorganisms
Environmental Contamination and Toxicology (1981) 27, 115-121

Marion, C. V. and Malaney, G. W.
Ability of Activated Sludge Microorganisms to Oxidise Aromatic Organic Compounds
18th Industrial Waste Conference (1963) 297-308
Purdue University, USA.

Miller, R. A., Johnson, J. D., Helsel, R. W. and Pitts, D. M.
Destruction of Toxic Compounds by Catalysed Wet Oxidation
35th Industrial Waste Conference (1980) 425-429
Purdue University, USA.

Mulbarger, M. C.
Nitrification and Denitrification in Activated Sludge Systems
Journal W.P.C.F. (1971) 43, No.10, 2059-2070

Ochs, L. D., Keller, P. J., Weatherspoon, J. V., Blick, C. and Cole, C. A.
Hydrogen Peroxide as a Supplemental Oxygen Source in Industrial Waste Treatment
28th Industrial Waste Conference (1973) 447-453
Purdue University, USA.

Okamoto, Y. and Wang, J. Y.
Micelliar Effects on the Reaction of 2,4,6-Trinitrotoluene with Amines
J. Org. Chem. (1977) 42, No.7, 1261-1262

Osmon, J. L. and Klausmeier, R. E.
The Microbial Degradation of Explosives
Dev. Ind. Microbiol. (1972) 14, 247-252

Parrish, F. W.
Fungal Transformation of 2,4-Dinitrotoluene and 2,4,6-Trinitrotoluene
Applied and Environmental Microbiology (1977) 34, No. 2, 232-233

Patterson, J. W. and Minear, R. A.
Pollution Control in the Explosives Industry
29th Industrial Waste Conference (1974) 808-831
Purdue University, USA.

Pillis, L. J. and Davis, L. T.
Process for Treating Wastewater Containing Phenolics and Microorganisms Capable of Degrading Phenolics
U.S. Patent 4,352,886
Oct. 5, 1982

Rogonska, T. I.
The Effect of Trinitrotoluene on the Microorganisms and Biochemical Processes of Self Purification in Water
Microbiologiya (1951) 20, 265-272
Translated by Dr. Alexandr Urvakin, Polytecnic Institute, Kursk

Ruchhoft, C. C., LeBosquet, M. and Meckler, W. G.
TNT Wastes from Shell-Loading Plants
Industrial and Engineering Chemistry (1945) 37, No.10, 937-943

Schmidt, E., Hellwig, M. and Knackmuss, H-J.
Degradation of Chlorophenols by a Defined Mixed Microbial Community
Appl. Environ. Microbiol. (1983) 46, No.5, 1038-1044

Schott, S., Ruchhoft, C. C. and Megregian, S.
TNT Wastes
Industrial and Engineering Chemistry (1943) 35, No.10, 1122-1127

Semmens, M. J., Barnes, D. and O'Hara, M.
Treatment of an RDX-TNT Waste from a Munitions Factory
39th Industrial Waste Conference (1984) 837-842
Purdue University, USA.

Shelton, D. R. and Tiedje, J. M.
General Method for Determining Anaerobic Biodegradation Potential
Applied and Environmental Microbiology (1984) 47, No. 4, 850-857

Simpson, J. R. and Evans, W. C.
The Metabolism of Nitrophenols by Certain Bacteria
Biochem. J. (1953) 55, xxiv

Slater, J. H. and Godwin, D.
Microbial Adaption and Selection
In "Contemporary Microbiology" (1980) 137-151
Ed. Ellwood, Hedger, Latham, Lynch and Slater

Slater, J. H. and Somerville, H. J.
Microbial Aspects of Waste Treatment with Particular Attention to the Degradation of Organic Compounds
29th Symposium of the Society of General Microbiology, Cambridge (1979)
Ed. Bull and Ellwood

Smith, L. L. and Zeigler, E.
Biological Treatment of a Munitions Manufacturing Facility Wastewater
33rd Industrial Waste Conference (1978) 432-439
Purdue University, USA.

Smith, L. L.
Evaluation of an Anaerobic Rotating Surface System for Treatment of a Munition Wastewater containing Organic and Inorganic Nitrates
34th Industrial Waste Conference (1979) 628-634
Purdue University, USA.

Stoneburner, D. L.
Biological Studies in Military-Industrial Waste Surveys
28th Industrial Waste Conference (1973) 841-848

Stover, E. L. and McCartney, D. E.
BOD Results that are Believable
Water/Engineering Management (1984) April, 37-66

Strier, M. P. and Gallup, J. D.
Removal Pathways and Fate of Organic Priority Pollutants in Treatment Systems: Chemical Considerations
37th Industrial Waste Conference (1982) 813-824
Purdue University, USA.

Vaidya, M. V.
Effluent Treatment of Phenolic Waste - Our Experience
Indian Chemical Manufacturer (1979) 17, No. 8/9, 45-49

Wilhelmi, A. R. and Ely, R. B.
The Treatment of Toxic Industrial Wastewaters by a Two Step Process
30th Industrial Waste Conference (1975) 288-295
Purdue University, USA.

Wolff, J. B.
Industrial Waste Disposal via Municipal Sewers
7th Annual Symposium on Industrial Waste Control (1966) 21-28
John Hopkins University, Baltimore

Wood, D. K. and Tchobanoglous, G.
Trace Elements in Biological Waste Treatment with Specific Reference to the Activated Sludge Process
29th Industrial Waste Conference (1974) 648-661
Purdue University, USA.

Yasuda, S. K.
Identification of 1,3,5-Triamino-2,4,6-Trinitrobenzene Impurities by Two Dimensional Thin-Layer Chromatography
Journal of Chromatography (1972) 71, 481-486