Activated Sludge; Surface Properties and Settlement

by

Kevin Morgan Evenett

Thesis submitted in accordance with the requirements of the University of Liverpool for the Degree of Doctor in Philosophy.

March, 1986

Activated Sludge; Surface Properties and Settlement

(Kevin M. Evenett)

The factors affecting adsorption by activated sludge of the polycationic dyes Alcian blue and Ruthenium red were investigated. Adsorption isotherms for the two dyes were found to fit Langmuir and mass action equations. A simple test for dye adsorption was developed and this was correlated with plant data at three activated sludge plants.

Dye adsorption was also compared with sludge extracellular polymer production. Sludge polymers were assessed at various stages throughout activated sludge treatment. Pertinent changes were found to occur at the sludge/sewage mixing stage with only small changes in quantity and composition on further aeration. Similar results were found when sludges and sewage were mixed in the laboratory. Polymers in the sludge supernatant and sewage were also analysed. Sewage polymer was found to make up only 1.2 to 37.5% of the total chemical oxygen demand for a range of sewages.

Aerobic, heterotrophic bacterial populations in activated sludge were studied after isolation on casitone glycerol yeast extract agar. Isolates were tentatively divided into taxonomic groups. When bulking sludge populations were compared to those of non-bulking sludges, they were characterized by an increase in the number of isolates capable of anaerobic growth. These changes were accompanied by an increase in growth of filamentous microorganisms. The nature and identification of filamentous microorganisms in activated sludges is also reported.

ACKNOWLEDGEMENTS

I would like to thank Dr. M.V. Jones for his help and constructive criticism throughout the duration of this project. I would also like to thank the Science and Engineering Research Council and North West Water for their financial support during my research studentship.

I am indebted to Mr. E. Clark of North West Water and all staff at Runcorn and Warrington South Effluent Treatment Works.

I would also like to acknowledge my supervisor Professor H.R. Perkins and colleagues; Dr. P. Vojak and Dr. I. Oborska for their support and friendship. I also thank Mrs. L. Marsh for her patient typing of the manuscript.

Finally, I would like to thank my wife, Bridget, without whom completion of this thesis would have been impossible.

CONTENTS

Page

CHAPTER 1 : Introduction 1

1.1 Introduction 2

1.2 Types of Settlement Problem 3

1.3 Factors Giving Rise to Sludge Bulking 4

1.4 Treatment of Builking 5

1.5 Activated Sludge Composition and Viability 6

1.6 Microorganisms in Activated Sludge 7

1.6.1 Floc-Forming, Unicellular Bacteria 7

1.6.2 Filamentous Bacteria 9

1.6.3 Protozoa 10

1.7 Sludge Extracellular Polymers 11

1.7.1 Factors Affecting Polymer Production 13

1.8 Sludge Characteristics and Settlement 14

1.8.1 Filament Length and Floc Size 14

1.8.2 Surface Charge 14

1.8.3 Extracellular Polymer Production 15

1.9 Hypotheses for Activated Sludge Filamentous Bulking 15

1.9.1 The Importance of Mixing Regime 16

1.9.2 Biosorption and Substrate Accumulation 19

1.10 Activated Sludge and Adsorption Isotherms 22

1.10.1 Adsorption of Dyes by Activated Sludge 28

1.11 Summary 31

1.12 Aims and Objectives 32

CHAPTER 2 : Materials and Methods 34

2.1 Samples from Activated Sludge Plants 35

2.2 Wastewater and Sludge Analysis 36

2.3 Microbiological Methods for Activated Sludge Investigation 37

2.3.1 Floc Structure 37

2.3.2 Filamentous Bacteria 38

2.3.3 Staining Techniques 38

2.3.4 Sudan B Test for Fat Deposits 39

2.3.5 Sulphur Storage Test 39

2.3.6 Protozoa, Rotifers and Nematodes 39

2.4 Isolation and Characterization of Activated Sludge Bacteria 40

2.4.1 Colony Morphology 40

2.4.2 Physiological Characteristics 41

2.5 Measurement of Sludge Activity 41

2.5.1 Oxygen Uptake 41

2.5.2 Triphenyl Tetrazolium Chloride Reduction 42

2.5.3 Sludge Viability 42

2.6 Adsorption of Cationic Dyes by Activated Sludge 43

2.6.1 Dye Solutions 44

2.7 Parameters Affecting Dye Adsorption 46

2.7.1 pH 46

2.7.2 Temperature 46

2.7.3 Sonication 47

2.7.4 Inhibitors and Other Compounds 47

2.8 Adsorption of Metabolic Substrates by Activated Sludge 47
2.8.1 Glucose, Pyruvate and Urea 47
2.8.2 Adsorption of Amino Acids 49
2.9 Adsorption of Alcian Blue by Pure Bacterial Cultures 49
2.10 Extraction of Sludge Extracellular Polymers 50
2.10.1 Total Material 50
2.10.2 Soluble Material 51
2.10.3 Bound Material 51
2.10.4 Sewage Polymer 52
2.11 Investigation of Sewage and Sludge Mixing 52
2.12 Biochemical Analysis 53
2.12.1 Carbohydrates 53
2.12.2 Protein 53
2.12.3 Hexuronic Acids 53
2.12.4 DNA 54

CHAPTER 3 : Adsorption of Cationic Dyes by Activated Sludge; Development of a Standard Procedure 55
3.1 Description of Study Sites 56
3.1.1 Runcorn E.T.W. 56
3.1.2 Warrington South E.T.W. 57
3.1.3 Sludge Disposal 58
3.1.4 Effluent 58
3.2 Adsorption of Nutrients by Activated Sludge 58

3.3 Cationic Dye Adsorption by Activated Sludge 59
3.3.1 Time Taken to Reach Equilibrium 60
3.3.2 Effect of Dye Concentration on Dye Adsorption (sludge solids concentration approximately 0.1 $g\,l^{-1}$) 61
3.3.3 Effect of Sludge Solids Concentration on Dye Adsorption (0.5-10% sludge) 62
3.3.4 Effect of Dye Concentration on Dye Adsorption (sludge solids concentration approximately 1.0 $g\,l^{-1}$) 63
3.3.5 Effect of Sludge Solids Concentration on Dye Adsorption (10-80% sludge) 65
3.3.6 Effect of pH on Dye Adsorption 67
3.3.7 The Effect of Sonication on Dye Adsorption, Sludge Viability and Sludge Activity 68
3.3.8 The Effect of Temperature on Sludge Viability, Dye Adsorption and Activity 69
3.3.9 The Effect of Metabolic Inhibitors and Biocides 70
3.3.10 The Effect of Solutes on Alcian Blue Adsorption 71
3.4 Adsorption of Alcian Blue by Pure Bacterial Cultures 71
Discussion 72
Summary 78

CHAPTER 4 : Adsorption of Cationic Dyes and Activated Sludge Plant Operation 80
4.1 Non-bulking Activated Sludges 81
4.2 Bulking Activated Sludge 88

Discussion 89

Summary 96

CHAPTER 5 : Heterotrophic Aerobic Bacterial Populations of Activated Sludge 97

Discussion 102

Summary 107

CHAPTER 6 : Effects of Sewage Addition on Activated Sludge Extracellular Polymers 108

Discussion 119

Summary 126

CHAPTER 7 : General Discussion 128

REFERENCES 138

CHAPTER 1

Introduction

1.1 Introduction

There are two requirements for the successful operation of an activated sludge plant. Firstly, that the sludge microorganisms are capable of breaking down the organic matter within the sewage in a given time, and secondly that the sludge can be separated from the effluent by settlement. Problems with the latter stage are not uncommon, occurring in 52% of plants in the United Kingdom (Tomlinson, 1976) and it is sludge settlement with which this project is primarily concerned.

Quantitative evaluation of sludge settlement has been measured traditionally by the sludge volume index (SV1):

$$SV1 = \frac{SV}{MLSS} \text{ ml g}^{-1}$$

where

SV = settlement of sludge in a 1 litre measuring cylinder after 30 minutes (ml l^{-1})

MLSS = mixed liquor suspended solids (g l^{-1})

Much criticism has been levelled at the SV1 test, particularly in view of its unpredictable dependence on solids concentration (Dick and Vesilind, 1969; White, 1975a, b; Rachwal *et al.*, 1982). Despite recent developments such as the stirred sludge volume index (White, 1975a, b) and the diluted SV1 (Stobbe, 1964; Lee *et al.*, 1982), the SV1 is still the most widely used, routine settlement test.

1.2 Types of Settlement Problem

Pipes (1967) describes three types of settlement problem within the activated sludge treatment process.

(i) Floc formation problems

This situation is characterized by a turbid effluent above the settled sludge mass. Four classes of problem fall within this group; dispersed growth, deflocculation, pin point floc and billowing sludge.

(ii) Density problems

Here the sludge is less dense than water and therefore rises to the surface in the final clarifier. This may be caused by the presence of gas bubbles in the sludge. If there is nitrification in the aeration tank and denitrification in the settlement tank, then bubbles of nitrogen are formed. Anaerobic conditions can lead to the formation of carbon dioxide or hydrogen sulphide. Overaeration may result in the formation of air bubbles.

A second type of density problem is floating sludge. This is generally a temporary problem associated with a small number of buoyant particles within the sludge. These may be stalked ciliates, killed rotifers or nematodes and sometimes saprophytic fungi with large diameter hyphae and vacuoles containing lipids.

(iii) Compaction problems

Sludges of this type are known as bulking sludges and are characterized by slow settlement and poor compaction. There is a clear supernatant. Pipes recognizes two types of bulking sludges; filamentous bulking, where poor settlement is associated

with a proliferation of filamentous organisms, and zoogloeal bulking, where filaments are not present in large numbers.

1.3 Factors Giving Rise to Sludge Bulking

(i) Wastewater composition

Generally speaking a wastewater containing large amounts of carbohydrate results in bulking (Pipes, 1967; Metcalfe and Eddy, 1972; Kiff, 1978; Schwartz *et al.*, 1980). Similarly, high carbon to nitrogen, or carbon to phosphorus ratio, along with nitrogen or phosphorus deficiency may lead to poor settlement (Genetelli and Heukelekian, 1964; Jones, 1964; Wagner, 1982; Wu *et al.*, 1984a, b).

The presence of particular nutrients may also give rise to bulking sludge. Sulphide in the sewage often leads to the growth of *Thiothrix* spp. (Farquhar and Boyle, 1972; Voelkel *et al.*, 1974; Merkel, 1975). A deficiency of trace elements may lead to bulking (Wood and Tchobanoglous, 1974, 1975; Forster and Dallas-Newton, 1980). Low pH leads to the development of filamentous fungi (Jones, 1964; Strom and Jenkins, 1984).

(ii) Sludge loading

Activated sludge plants are usually designed to operate at a loading of 0.5 Kg BOD Kg MLSS^{-1} day^{-1} and there is generally a range of loading values at which settlement is good. Deviation from this range results in settlement problems. In particular, the detrimental effect of high loading has been well documented (Orford *et al.*, 1961; Ganczarczyk, 1970; Chudoba *et al.*, 1974; Magara *et al.*, 1976; Kiff, 1978; Barahora and Eckenfelder, 1984).

The optimum loading range may be dependent upon the nature of the influent (Genetelli and Heukelekian, 1964; Ford et al., 1967).

Sludge age has also been shown to affect settlement. Lovett et al., (1983, 1984) reviewed the literature in this area and concluded that an increase in sludge age improved settlement. As with loading, the range of optimum sludge age is a function of the nature of the influent.

(iii) Dissolved oxygen concentration

Many authors have found that low oxygen levels give rise to bulking (Pipes, 1967; Adamse, 1968; Houtmeyers et al., 1977; Palm et al., 1978; 1981; Sezgin et al., 1978; Pitman, 1980; Schwartz et al., 1980), although contrary reports have also appeared (Bhatla, 1967, Benefield et al., 1975; Chudoba et al., 1982). It is possible that low dissolved oxygen levels may be an incidental feature of bulking and will in any case be a direct result of high loads and the subsequent increase in respiration rates of microorganisms.

1.4 Treatment of Bulking

Bulking may be dealt with by a manipulation of the plant in direct response to the above factors. Other treatments are temporary and include addition of weighting agents such as lime, or coagulants like alum, ferric salts (Rensink, 1979; Rensink et al., 1979) and polyelectrolytes (Walker and Wheale, 1981). The addition of toxic substances such as hydrogen peroxide (Cole et al., 1973; Houtmeyers et al., 1977; Jenkins et al., 1982) or

chlorine (Jenkins *et al.*, 1982) improve settlement by destruction of exposed filamentous bacteria.

1.5 Activated Sludge Composition and Viability

Activated sludge consists of a mass of microorganisms and their products, adsorbed or trapped organic material as well as inorganic matter. The organic component is quantified as the mixed liquor volatile suspended solids (MLVSS) which is given by the weight loss of a dry sample after ignition at 550°C. This is normally 60-90% of the MLSS (Tebbut and Paraskevopoulos, 1981; Roy *et al.*, 1983).

Reported values for the proportion of viable microorganisms in activated sludge have ranged from 10-20% (Weddle and Jenkins, 1971) and 1-2% (Water Pollution Research Laboratory, 1970). Such figures have little value however as no medium can fully support growth of all activated sludge organisms. Thus Walker and Davies (1977) found that 80% of sludge metabolic activity was due to "non-viable" bacteria. Droste and Sanchez (1983) used a method of observing active cells through their reaction with 2:3:5 triphenyl tetrazolium chloride (TTC) and found 28-53% of bacteria in sludge to have metabolic activity.

Methods of estimating "sludge activity" include analysis of DNA (Carlson, 1965; Genetelli, 1967), ATP (Weddle and Jenkins, 1971; Roy *et al.*, 1983) and dissolved oxygen uptake rate (Weddle and Jenkins, 1971; Haas, 1979; Edwards and Sherrard, 1982). The presence of dehydrogenase enzymes and their ability to reduce colourless compounds to coloured ones has also been utilized.

TTC has been extensively used (Lenhard *et al.*, 1964; Bucksteeg, 1966; Ford *et al.*, 1966; Jones and Prashad, 1969; Klapwijk *et al.*, 1974; Ryssov-Nielsen, 1975; Logue *et al.*, 1983; Miksch, 1983, 1984; Gabbita and Hwang, 1984a, b) and also resazurin (Lui, 1983) and methylene blue (Halasz, 1972; Jorgensen, 1984). A technique was developed by Bitton and Koopman (1982) to estimate the activity of filamentous bacteria. This combined TTC reduction and malachite green staining.

1.6 Microorganisms in Activated Sludge

These can be divided into five categories as follows:

Floc-forming, unicellular bacteria.

Filamentous bacteria, including cyanobacteria.

Fungi.

Algae.

Protozoa.

1.6.1 Floc-Forming, Unicellular Bacteria

Organisms of this group are difficult to study quantitatively due to the nature of activated sludge flocs. Any attempt at enumeration or isolation firstly requires an adequate method of disruption, and secondly a medium which can support a maximum proportion of isolated bacteria. A variety of methods has been successfully used for floc disruption including the Kerry ultrasonic cleaning bath (Pike *et al.*, 1972), the Waring blender (Heukelekian and Gurbaxani, 1949; Morand, 1964) and sonication (Fung and Kraft, 1968; Banks and Walker, 1977; Hall,

1981; 1982a, b). Dispersion may be increased by addition of sodium pyrophosphate, sodium citrate (Gayford and Richards, 1970) or EDTA (Hall, 1982a). When isolation media were reviewed and compared, (Department of the Environment 1971; Pike *et al*., 1972; Pike and Carrington, 1972) casitone glycerol yeast extract agar (CGY) was found to be most efficient.

Further problems are encountered in the identification of floc-forming bacteria from activated sludge. These are discussed in Chapter 3. Table 1.1 shows the organisms found in sludge by a variety of workers. Many have been shown to form flocs in pure culture, for example *Pseudomonas* spp. (McKinney and Weichlein, 1953; Deinema and Zevenhuisen, 1971; Tago and Aida, 1977; Lau *et al*., 1980), and *Flavobacter* spp. (Tezuka, 1969; Kato *et al*., 1971; Endo *et al*., 1976).

There is evidence that the microbial population of activated sludge is influenced by both wastewater composition (Seiler and Blaim, 1982) and plant design (Brodisch and Joyner, 1983). Several authors (Takii, 1977; Seiler *et al*., 1984) have shown that Gram-positive coryneform bacteria are associated with a high carbohydrate content in the sewage feed. Seiler and Blaim (1982) found that the efficiency of a chemical waste activated sludge plant correlated well with the presence of *Zoogloea ramigera* in the sludge, although it is unlikely that this organism is present in significant numbers in sludge to affect treatment (Williams and Unz, 1983).

CONTAINS
PULLOUTS

Table 1.1 Bacteria Isolated from Activated Sludge

Author	Source of Sludge	Isolation Medium	BACTERIAL GENERA																																									
			Achromobacter	Acinetobacter	Aerobacter	Aeromonas	Alcaligenes	Alginobacter	Arthrobacter	Bacillus	Bacterium	Bordetella	Brevibacterium	Caulobacter	Chromobacter	Comomonas	Corynebacterium	Cytophaga	Enterobacteriaceae	Flavobacterium	Gram positive	Hyphomicrobium	Hyphomonas	Lactobacillus	Microbacterium	Micrococcus	Microcyclus	Moraxella	Neisseria	No cardia	Paracolobacter	Pasteurella	Pseudomonas	Rhodococcus	Seliberia	Sarcina	Spirillum	Spirosoma	Streptococcus	Streptomyces	Vibrio	Yersinia	Zoogloea	Yeasts	Fungi
Adamse (1968)	Laboratory plant (dairy waste)	Tryptone glucose extract agar	+										+	+			D							+									+						+						
	Oxidation ditch (dairy waste)	plus skimmed milk	+										+	+			D		+														+												
Tezuka (1969)	Domestic	Sakurai's medium																		D													+										+		
Ministry of Technology (1969)	Domestic	Yeast extract agar																		+													+										+		
Austin and Forster (1969)	Domestic	Nutrient agar	+		+		+	+		+									+	+						+				+	+		+							+					
Lighthart and Oglesby (1969)	Hospital and domestic	Tryptone glucose extract agar plus vitamins	+		+													+		+													+												
Benedict and Carlson (1971)	Laboratory plant	Tempered sewage agar		+			+						D	+		+		+		+					+								+												
	Municipal			+			+	+					+			+		+		+		+											D												
Ueda and Earle(1972)	Domestic	Proteose peptone yeast extract agar		+			+		+				+						+												+		D										+		
Takii (1977)	Kraft pulp waste	Sakurai's medium															+			+				+									+												
	Brewery waste (1)																			+													+												
	Brewery waste (2)					+							+				+																+												
	Confectionary waste																+			+																									
	Fruit juice waste																+																+												
Bisz-Konarzewska (1978)	Laboratory plant (oil refinery waste)	Not given			+		+												+	+													+										+		
Rawlings and Woods (1978)	Laboratory plant (fellmongery waste)	Activated sludge extract agar		D																													D												
Brodisch and Joyner (1983)		Casitone glycerol yeast extract	+	+		+	+					+							+	+	+											+	+												
		agar (CGY)	+	+		+	+					+							+	+	+											+	+												
			+	+			+					+							+	+	+											+	+									+			
Baker et al., (1983)	Reactor treating cutting fluids	Nutrient agar		+		+	+						+				+			+			+				D	+		+			D		D			+							
Seiler et al., (1984)	Chemical Combine	Peptone meat extract agar					D										+			+									+				+									+	+		
Allen (1944)	Laboratory plant	Nutrient agar	+												+					+																									
	Domestic	Nutrient agar	+								+				+																		+	+											
McKinney and Weichlein (1953)	Laboratory plant	Nutrient agar	+				+												+							+							+										+		
	Domestic	Nutrient agar	+				+			+									+	+				+					+				+										+		
	Toxic	Nutrient agar					+																			+					+		+												
Jasewichz and Porges (1956)	Laboratory plant; assimilation	Nutrient agar + 0.1%	+				D			+										D					+	+							+												
	Laboratory plant; endogenous	skimmed milk								D	D						+			+						+							+												
Rogovskayaand Lazareva (1959)	Domestic sewage									+																+							+	+										+	
	Shale effluent									+																+							+	+											
	Brown coal									+																+							+	+											+
	Brown coal plus domestic									+																+							+	+											
	Blended sewage									+																+							+	+											+
	Fatty acid manufacture									+																+							+	+											+
	Rubber reclaimation									+																+							+	+											+
Anderson and McCoy (1963)	Municipal and dairy waste	Not given																															D										+		
Dias and Bhat (1964)	Domestic	Proteose peptone yeast											+			D				+					+								+				+						D		
	Domestic	extract medium					+			+						D				+					+																		D		
	Laboratory fill and draw		+													D									+								+										D		
	Laboratory fill and draw		+		+		+									D									+								+										D		
	Sludge formed by sewage aeration		+				+			+						D	+								+								+										D		
	Sludge formed by sewage aeration		+													D	D								+								+										D		
	Sludge formed by sewage aeration															D	+			+													+										D		
Van Gils (1964)	Municipal	Tryptone glycerol agar	+				D		+											D													+												
		Sewage agar	+				D		+											D													+												
	Laboratory plant	Tryptone glycerol agar	+				+		+											D													+												
		Sewage agar	+				+													+													+												

KEY:

+ = Organism present.

D = Organism dominant or codominant.

Sludge source refers to full scale activated sludge plant unless stated.

1.6.2 Filamentous Bacteria

The literature in this area has been extensively reviewed by Pipes (1978). The most important of recent studies was that of Eikelboom (1975). This worker studied 110 activated sludge samples, mainly from oxidation ditches in the Netherlands. Twenty six types of filamentous bacteria were identified and divided into 7 groups according to their physiological properties. Eikelboom provided a key for identification, which has been reviewed and updated (Eikelboom, 1977, 1981; Strom and Jenkins, 1984). A number of filamentous microorganisms have been studied in pure culture, namely *Sphaerotilus natans* (Stokes, 1954), *Haliscomenobacter hydrossis* (Van Veen *et al.*, 1971, 1973, 1982), *Microthrix parvicella* (Slijkhuis and Deinema, 1982; Slijkhuis, 1983; Slijkhuis *et al.*, 1984), *Bacillus mycoides* and *B.cereus* (Trick *et al.*, 1984), *Heterosiphon* spp. (Trick and Lingens, 1984), *Thiothrix* spp., *Beggiatoa* spp. and Eikelboom Type 021N (Williams and Unz, 1985), and *Trichococcus flocculiformis* (Scheff *et al.*, 1984). In addition a number of studies have been performed on mixed cultures of filamentous and non-filamentous organisms. These include *H.hydrossis* and *Z.ramigera* (Krul, 1977), *S.natans* and a *Pseudomonas* sp. (Lau *et al.*, 1980; Lau *et al.*, 1984a, b), and *S.natans* and an *Arthrobacter* sp. (Mulder, 1964; Adamse, 1968; Houtmeyers, 1978; Van den Eynde *et al.*, 1983).

Strom and Jenkins (1984) related the occurrence of filamentous organisms in bulking activated sludge to plant operating parameters and nutrient conditions (Table 1.2). Their

Table 1.2 Occurrence of filamentous bacteria in bulking activated sludge

Likely cause of bulking problem	Organism
Low organic loading	*0041, 0092, *Microthrix parvicella*, probably 0803.
Low dissolved oxygen	1701, 021N, *Sphaerotilus natans*, 1864.
Low pH	Fungi
Sulphide	*Thiothrix*, *Beggiatoa*
Low nutrients (nitrogen or phosphorus)	perhaps *S.natans*

from Strom and Jenkins (1984)

* Numbers refer to the types of filamentous organisms described by Eikelboom (1975).

work agrees in the main with that of Eikelboom (1977) who reported similar results from oxidation ditches and carousel plants.

Fungi and algae

Cook and Pipes (1970) analysed samples from 19 treatment plants for fungi. The most common were yeast-like organisms, i.e. *Trichosporon*, *Geotrichum*, *Candida* and *Rhodotorula* along with many fungi imperfecti. One plant demonstrated fungal bulking with *Cephalosporium* growing at a pH of less than 4.0. *Geotrichum* has also been reported in fungal bulking incidents (Jones, 1964; Nash *et al*., 1977).

1.6.3 Protozoa

The role and occurrence of protozoa in the activated sludge process have been extensively reviewed (Curds, 1975, 1982). Activated sludge may contain 50,000 protozoa ml^{-1} and these may constitute 5% of the dry weight of the aeration tank. (Ministry of Technology, 1968). Five classes of protozoa have been reported in sludge; Phytomastigophorea, Zoomastigophorea, Rhyzopodea, Actinopodea and Ciliatea. Of these the ciliates are most numerous (Curds, 1975). Under protozoa-free conditions, laboratory activated sludge plants produce highly turbid effluents of inferior quality (Curds *et al*., 1968) and it seems that their major role in the activated sludge process is the removal of free-swimming bacteria.

1.7 Sludge Extracellular Polymers

The various biological elements of activated sludge exist together within a complex ecological framework. Of critical importance in the relationship between dispersed bacteria, flocculated bacteria, filamentous bacteria and protozoa is the nature of extracellular material within the sludge matrix. This is composed not only of bacterial and protozoal extracellular polymers, but also the products of cell lysis (Forster, 1976) and compounds adsorbed from the incoming sewage (Kiff, 1978). Most extracts of sludge extracellular material have revealed the presence of carbohydrate, protein and nucleic acids (Table 1.3) as well as ash and lipid. Hydrolysis of this material gives a range of monosaccharides and monosaccharide derivatives. Several studies have also been carried out on the structure of extracellular polymers of individual activated sludge bacteria (see Table 1.4).

The quantity and composition of sludge extracellular polymer is dependent upon the method of extraction. Alkali extraction methods (Sato and Ose, 1980; Takiguchi, 1968) give rise to large amounts of cell disruption (Brown and Lester, 1980), whereas extraction methods using high speed centrifugation (Busch and Stumm, 1968) do not remove extracellular polymer from activated sludge (Novak and Haugan, 1981). Removal of polymer with hot water or steam was found to be the best method by several workers (Brown and Lester, 1980; Carr and Ganczarczyk,1974) although composition of polymer is affected by incubation temperature, with lipid and protein fractions reduced in their relative

Table 1.3 Extracellular polymers extracted from activated sludge. (Updated from Brown & Lester, 1980; Thompson & Forster, 1983)

Author	Method of extraction	Composition of extract
Busch and Stumm (1968)	High speed centrifugation. Ethanol precipitation.	Carbohydrate. (Protein and DNA absent.)
Takaguchi (1968; 1972)	Cold water, warm water, acetate buffer, HCl,NaOH. No precipitation.	Mainly polysaccharide and protein (Ash 35%). Monomers of glucuronic acid in bulking sludge only, glucose, mannose, rhamnose, maltose, galactose, xylose, lactose, hexosamine.
Nishikawa and Kuriyama (1968)	EDTA and alkaline extraction. Ethanol precipitation.	Polysaccharide, DNA, RNA.
Coackley (1969)	Not reported.	Polysaccharide containing 85% glucose, 2% mannose, 4% xylose, 4% ribose, 5% rhamnose.
Water Pollution Research Laboratory (1971)	Boiling or auto-claving. Acetone precipitation.	8-12% ash. Glucose, galactose, fucose, mannose, arabinose. Sometimes rhamnose, ribose, galacturonic acid.
Forster (1971, 1976)	Heating at 100°C. Precipitation in ethanol:acetone 1:1.	Galactose, glucose, fucose, mannose, xylose, rhamnose, ribose, glucuronic acid, glucurono-lactone.
Pavoni *et al.*, (1972)	High speed centri-fugation. Ethanol precipitation.	Polysaccharide, protein, DNA, RNA.
Wallen and Davis (1972)	Boiling benzene and boiling water extraction. Ethanol precipitation.	Glucose, mannose, rhamnose, galactose, maltose, lactose. Glucosamine or galactosamine.
Ueda and Earle (1972)	Ammonium hydroxide or EDTA extraction. Ethanol precipitation.	Glucose, pentose, glucuronic acid.
Tenney and Verhoff (1973)	Sodium hyroxide and blending. Ethanol precipitation.	Polysaccharide, protein, DNA, RNA.

Table 1.3 (continued)

Carr and Ganczarczyk (1974)	Boiling or autoclaving, trichloroacetic acid, sulphuric acid, ultra-sound action, sodium hydroxide, boiling benzene. Ethanol and acetone precipitation.	Polysaccharide, protein.
Farrah and Unz (1976)	Blending. Precipitation with cetyltrimethylammonium bromide (CTAB).	Glucosamine, a D-methyl pentose amine, uronic acids.
Kiff and Thompson (1979)	High speed centrifugation, heat extraction, sonicatiton, homogenization, extrusion under pressure. Ethanol precipitation.	Not reported.
Wase and Balasind (1980)	Continuous centrifugation. Ethanol precipitation.	Not reported.
Sato and Ose (1980)	Ammonium hydroxide extraction. Precipitation in 60% ethanol, 90% acetone, ethyl ether.	Protein, DNA, carbohydrate, RNA, water, ash. Glucose, galactose, arabinose, mannose, glucosamine or galactosamine.
Novak and Haugan (1981)	High speed centrifugation. Ethanol or acetone precipitation.	Not reported.
Brown and Lester (1980)	High speed centrifugation, ultrasonication, sodium hydroxide, EDTA, steaming. Ethanol precipitation.	Carbohydrate protein, DNA, RNA.
Rudd _et al_., (1983)	Ion exchange resin, ultrasonication, dialysis, steaming, sodium hydroxide.	Carbohydrate, protein.
Gehr and Henry (1983)	High speed centrifugation, boiling, H_2SO_4 and HCl, sodium hydroxide, blending. Ethanol precipitation.	DNA, RNA, carbohydrate, protein.

Table 1.3 (continued)

Forster and Clarke (1983)	0.1 N and 2.0N H_2SO_4; 0.1 N and 2.0N NaOH with acetone and ethanol precipitation. Ethanol only extraction technique	Carbohydrate, protein, lipid.
Kakii *et al.*, (1984)	As for Nishikawa and Kuryama (1968).	Carbohydrate, protein, DNA, RNA. Carbohydrate contained rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose.
Goodwin and Forster (1985)	Heat and solvent extraction.	Carbohydrate, protein, two lipid types, one of which was thought to be a tri-glyceride.

Table 1.4 Composition of extracellular polymers produced by bacteria isolated from activated sludge.

Author	Organism	Composition of extracellular polymer
Gaudy and Wolfe (1962)	*Sphaerotilus natans*	Fucose, galactose and glucuronic acid in the ratio 1:0.77: 0.77:0.8.
Anderson and McCoy (1963)	*Zoogloea ramigera*	Polymer containing a pentose and a hexosamine.
Crabtree *et al.*, (1966)	*Zoogloea ramigera 1-16-M.*	Polymer containing a hexosamine.
Friedman *et al.*, (1968) Friedman and Dugan (1968)	*Zoogloea ramigera 115.*	Polyglucose.
Deinema and Zevenhuisen (1971)	Bacteria of the genera *Pseudomonas*, *Alcaligenes*, *Aerobacter* and *Achromobacter*.	Polymer susceptible to cellulase.
Parsons and Dugan (1971)	*Zoogloea ramigera 115.*	Polymer of glucose and galactose. (Glucose predominant.)
Wallen and Davis (1972)	*Zoogloea ramigera NRPL B-3669M.*	Polymer containing mannose, glucose and galactose.
	Undesignated Gram-negative rod.	Polymer containing rhamnose, mannose, galactose.
Tezuka (1973)	*Zoogloea ramigera (tentative)*	N-acetyl glucosamine and N-acetyl fucosamine in the ratio 1:2.
Zevenhuisen and Ebbink (1973)	*Achromobacter* sp.	Polymer containing glucose, galactose, pyruvate and O-acetyl in the ratio 1:1:1: 0.5.
Farrah and Unz (1976)	*Zoogloea ramigera MP6.*	Amino sugars (17%), hexoses (2%), uronic acid (1%).
Williams and Unz (1983)	*Zoogloea ramigera MP6.*	Amino sugars.

concentrations above 80°C (Goodwin and Forster, 1985). Recently, some authors have successfully extracted sludge extracellular polymer by the removal of cations. This may be achieved by dilution with distilled water (Novak and Haugan, 1981) or addition of Dowex resin (Rudd et al., 1983).

Most often, precipitation of extracted material is carried out by ethanol addition at 4°C, although Novak and Haugan (1981) found that acetone gave a slightly higher yield. Using X-ray diffraction analysis, ethanol insoluble material extracted from sludge was shown to contain large quantities of calcium, phosphorus and silicon (Carr and Ganczarcyk, 1974). Similarly, Sato and Ose (1980) found up to 17% ash in their extracted material. Thus it appears that large quantities of inorganic salts may be precipitated by ethanol along with microbial polymers.

Several reports have been published dealing with the size of extracellular polymers from activated sludge. Forster (1976, 1985) passed heat extracted polymer from three different sludges through Sephadex gels. All extracts contained a fraction of molecular weight greater than 100,000 and another between 100,000 and 10,000. The major fraction under 100,000 increased in size with the $SSVI_{3.5}$. Novak and Haugan (1981) found that the supernatant liquor of a poorly denaturing industrial waste sludge and a municipal sludge contained two major fractions of molecular weight 800 to 1,500 and 3,000 to 5,000. Finally, polysaccharides extracted with sodium hydroxide were found to have a molecular weight of at least 100,000 (Kakii et al., 1984).

1.7.1 Factors Affecting Polymer Production

Kiff (1978) and Clarke and Forster (1983) found a direct relationship between plant loading and extracellular polymer production. The latter workers calculated the correlation between polymer yield obtained using a heat extraction technique and a variety of plant operating parameters. Best correlation was found between yield, MLSS and influent BOD. Clarke and Forster concluded that not unexpectedly, nutrition was important in determination of polymer production. Even better correlation was found using an ethanolic extraction technique (Forster and Clarke, 1983). Sewage deficient in nitrogen or phosphorus may also give rise to the production of large amounts of extracellular polymers (Wu, 1978). In disagreement with these findings, Chao and Keinath (1979) reported a decrease in extracellular polymer concentration with an increase in sludge loading, and Brown and Lester (1982b) found that polymer production remained constant over a sludge age range of 3-18 days. Saunders (1975) found such large variations in extracellular polymer occurred that he could not relate these to MLSS or to sludge age.

Extracellular polymer content of activated sludge was estimated as a measure of the optimal dose of cationic polymer required for filtration by Novak *et al*. (1977). It was found that a sludge which had recently been subjected to anaerobic conditions responded to aeration by a depletion of the extracellular polymer concentration. Conversely, aeration of an aerobic sludge led to an increase in polymer concentration.

1.8 Sludge Characteristics and Settlement

1.8.1 Filament Length and Floc Size

Several authors have demonstrated a direct relationship between total filament length and SVI (Finstein and Heukelekian, 1965; Pipes, 1979). Although tedious, such measurements have been used to predict bulking problems (Walker, 1982; Green, 1982). Sezgin *et al*., (1980) and Sezgin (1980, 1982) found that sludge settlement properties decreased sharply above a filament length of 1 x 10^7 µm ml^{-1}. Below this level, filament length had no affect on settlement although SVI was related directly to floc size. Where the filament length was above 1 x 10^7 µm ml^{-1} floc size had no affect on settlement.

Barahona and Eckenfelder (1984) and Magara *et al*., (1976) found a linear relationship between floc size, SVI and sludge loading. The latter workers also demonstrated that large flocs were less dense than small flocs.

1.8.2 Surface Charge

Forster (1968) found that the SVI of a non-filamentous sludge was linearly related to the average electrophoretic mobility and therefore the charge of the floc surface. Similar results were found by Goodwin and Forster (1985), Wu *et al*. (1984b) and Steiner *et al*. (1976). Forster (1971) suggested that the main ionogenic material at the sludge surface was glucuronic acid. However, the relationship between glucuronic acid and polysaccharide in extracellular polymers from activated sludge

was found to be constant at approximately 1:6 for all SVI values. This worker proposed that some other constituent was making a contribution to the surface charge at higher SVI's.

No relationship was found between surface charge and SVI by Aiba et al. (1970) and Goodwin and Forster (1985) failed to find a correlation between surface charge and $SSVI_{3.5}$. Clearly surface charge is not the only factor in sludge bulking.

1.8.3 Extracellular Polymer Production

Most workers have found that settlement properties deteriorate as sludge polymer production increases (Biosogni and Lawrence, 1971; Magara et al., 1976; Rideaux and Morfaux, 1976; Kiff, 1978; Beccari et al., 1980) although Wu et al. (1984b) reported that in a nitrogen deficient sludge, settlement improved with polymer concentration as seen under the light microscope. Composition of polymer may also be a factor in settlement. Kiff (1978) suggested that polymers at high SVI's were different from those at low SVI's. Recent interest has arisen with regard to the lipid content of extracellular polymer. Goodwin and Forster (1985) found that as a general trend, lipid concentration increased as settlement deteriorated.

1.9 Hypotheses for Activated Sludge Filamentous Bulking

Early work in this area has been summarized by Pipes (1967) and Dallas-Newton and Forster (1980).

1.9.1 The Importance of Mixing Regime

Most recent hypotheses have arisen from the realisation that plug-flow activated sludge plants have superior settling properties to their completely mixed counterparts (see Fig. 1.1). Pasveer (1969) reported that filamentous bulking in an oxidation ditch could be cured by changing the continuous feeding pattern to an intermittent one. He concluded that the temporarily high concentration of nutrients were favourable to the development of flocculant bacteria (Heide and Pasveer, 1974). Similar high nutrient concentrations are found in the first pocket of plug-flow plants, the concentration gradient along the system being determined by the pattern of the aeration lane.

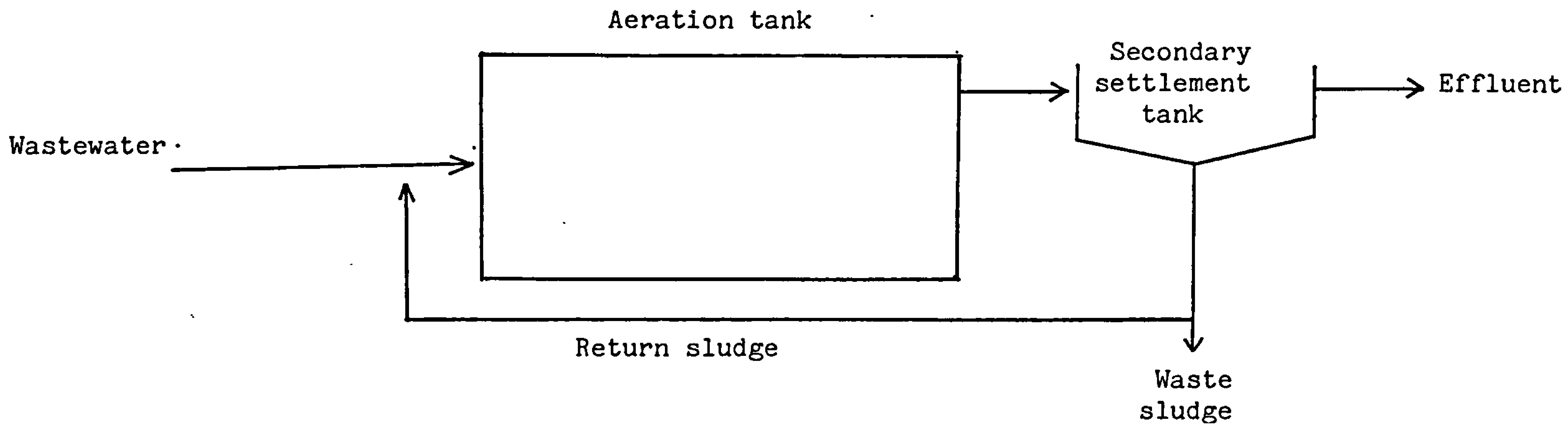
Chudoba *et al*., (1973a, b, 1974) investigated aeration patterns in laboratory scale activated sludge plants. These plants were characterized by different degrees of mixing. A Dispersion Number was used to quantify mixing characteristics, havinga value of 0 for ideal plug-flow plants, and ∞ for completely mixed plants. Chudoba *et al*. were able to demonstrate an inverse relationship between Dispersion Number and SVI. Tomlinson and Chambers (1979a) and Chambers (1982) showed a similar relationship for 24 full scale activated sludge plants throughout the British Isles. Chudoba *et al*., felt that the primary factor in microorganism selection was a high COD in the initial aeration pocket. They put forward a theory to explain their results based on the Monod equation. This describes the relationship between growth rate and substrate concentration in a microbial culture:

Figure 1.1

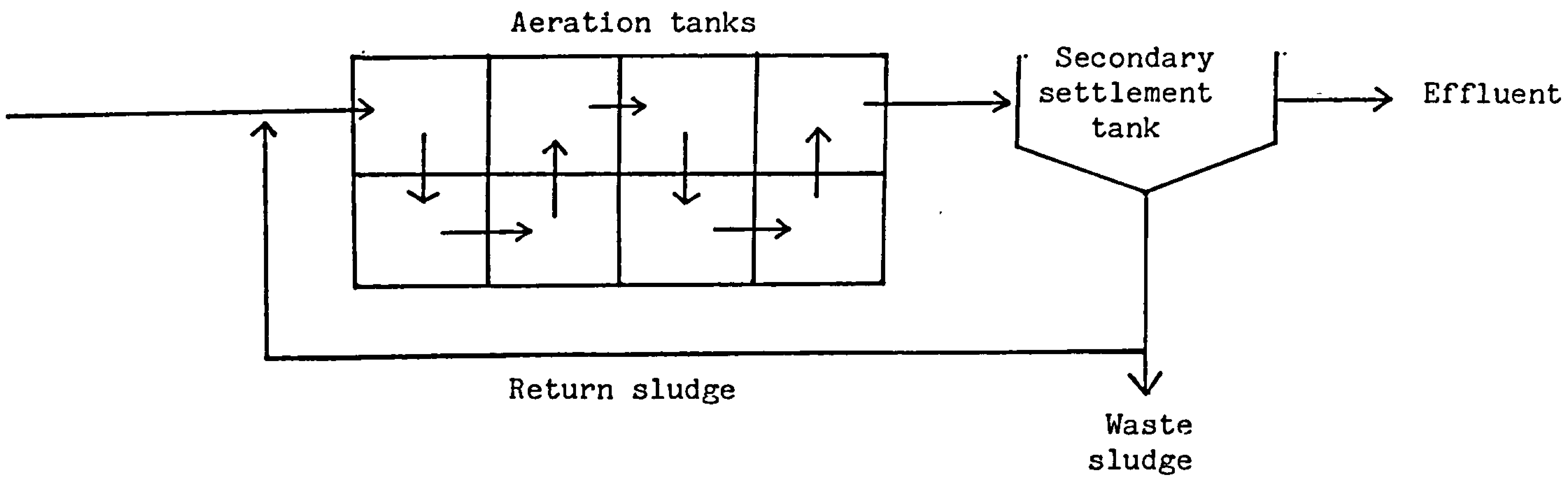
Completely mixed (a) and plug-flow (b) activated sludge plants.

Figure 1.1

(a) Completely mixed



(b) Plug-flow



$$\mu = \mu m \frac{S}{Ks + S}$$

Where:

μ = specific growth rate hr^{-1}

μm = maximum growth rate hr^{-1}

Ks = rate constant mg l^{-1}

S = concentration of growth limiting substrate mg l^{-1}

Ks is defined as the substrate concentration at which the specific growth rate is half maximal. Thus the lower the value of Ks, the higher affinity of an organism for the substrate.

According to Chudoba *et al*., floc-forming and filamentous microorganisms possess different values of Ks and µm. The former are characterized by a high µm and a high Ks. Conversely, filaments possess a low µm and a low Ks. Thus where the concentration of growth limiting substrate is low filaments have a competitive advantage. If the concentration is high, as in the first pocket of a plug-flow system, then floc-forming organisms predominate. Chudoba *et al*. (1985) later verified this theory experimentally.

Lau *et al*. (1980) found that the above kinetic relationships existed between pure cultures of *S.natans* and a *Pseudomonas* sp. isolated from activated sludge. However, kinetic determinations were performed on the *Pseudomonas* sp. in dispersed

culture only, and any values obtained would certainly differ if growing in the form of flocs.

The hypothesis of Chudoba et al. was extended by Sezgin et al. (1978). These workers made allowance for the diffusion properties of the microbial floc. For a good settling sludge they proposed that the rate of growth of filamentous microorganisms would be greater than that of flocculant bacteria inside the flocs. However, at the outside of the floc there would be a much higher growth rate of floc-forming microorganisms. A certain amount of filamentous growth was thought to be important in forming a rigid backbone to which flocculant microorganisms attach like "flesh on a bone".

Chiesa and Irvine (1985) further extended the model of Chudoba et al. (1973a) by proposing two types of filamentous bacteria, i.e. fast growing starvation susceptible and slow growing starvation resistant. The former group were also proposed to have a high affinity for oxygen. These organisms therefore proliferate under conditions of high organic loading and low dissolved oxygen concentration. Conversely, the latter group would dominate where nutrients are in short supply as proposed by Chudoba et al. (1973a).

Chiesa and Irvine explain the success of plug-flow systems due to the fact that slow growing starvation resistant filamentous organisms are at a competitive disadvantage due to the high nutrient content in the first pocket of the aeration lane. At the other extreme fast growing starvation susceptible

microorganisms to make use of their high substrate uptake rate there must be a sufficiently long 'endogenous phase' where nutrients have been removed from the medium. The superiority of intermittently fed systems over continuous feeding was further verified for a variety of carbon sources (Verachtert et al., 1980) and also for industrial wastes (Van den Eynde et al., 1982).

Additional evidence for a higher substrate uptake rate in floc-forming bacteria was provided by experiments with pure and mixed cultures of an Arthrobacter sp. and S.natans (Van den Eynde et al., 1983; Houtmeyers, 1978).

Eikelboom (1982) used the term biosorption to describe the combination of biological and physiochemical processes by which nutrients are removed by activated sludge. Biosorption was measured as a function of the COD removed from the medium. A number of experiments were carried out in which the biosorption of a range of activated sludges from both pilot plants and full scale plants was measured. Several important points may be derived from the results:

(a) There is a large variation in the biosorption capacity of sludges obtained from different sources.

(b) Biosorption is directly related to floc loading, this being defined as the total COD in the mixing tank divided by the MLSS. It is a measure of instantaneous sludge loading. Although a decrease in floc loading is accompanied by an increase in biosorption, there is a decrease in the percentage of substrate adsorbed.

(c) Biosorption is largely instantaneous, this being unaffected by oxygen concentration. However, later stages of biosorption are restricted by anoxic conditions.

(d) The biosorption capacity decreases as the sludge loading increases. This is attributed to the short endogenous phase which results from high sludge loads.

(e) Biosorption capacity is a function of the sludge, and not the sewage.

Eikelboom concluded that the prevention and control of bulking sludge by means of a high floc loading, i.e. the incorporation of a sewage/sludge mixing zone, was only possible if the sludge had a high biosorption capacity. Where sludge was of a lower biosorption capacity, then filamentous organisms would develop due to their relatively large surface area.

Chudoba *et al*. (1982) refer to the accumulation capacity (AC) and storage capacity (SC) of the activated sludge microbial population. AC is defined as the quantity of substrate that can be accumulated in a unit weight of sludge. SC is the quantity of substrate transformed into storage and cell products prior to replication. Kinetic studies revealed that for laboratory scale activated sludge systems and glucose substrate the AC was approximately 0.3 to 0.4 g g^{-1}, and the SC 2.0 g g^{-1}. As the F:M ratio in activated sludge plants is generally in the region of 0.1 to 0.5 g g^{-1} it was felt that AC rather than SC is important in microbial selection.

In systems where the initial F:M ratio is high, Chudoba *et al*. proposed that organisms of high AC are selected. Conversely,

where the initial F:M ratio is low such as in a completely mixed system, organisms of low accumulation capacity have an advantage. Filamentous organisms were thought to fall into the latter category. Further work (Cech and Chudoba, 1983) revealed that not only was the accumulation capacity higher in sludge developed semi-continuously, but the rate of accumulation capacity saturation was also higher. Chudoba et al. (1982) proposed that a period of regeneration, or reaeration in the absence of substrate was necessary in order to return the accumulation capacity to its full value. A model was developed in order to predict the time of regeneration required.

1.10 Activated Sludge and Adsorption Isotherms

The nature of recent research suggests that the adsorption properties of activated sludge are critical in the selection of the dominant microflora. The relationship between an adsorbent (in this case activated sludge) and adsorbate is described as the adsorption isotherm. The nature of adsorption isotherms at a variety of interfaces has been reviewed by Kipling (1965). The most common form of isotherm for adsorption of a solid from solution is shown in Figure 1.2.

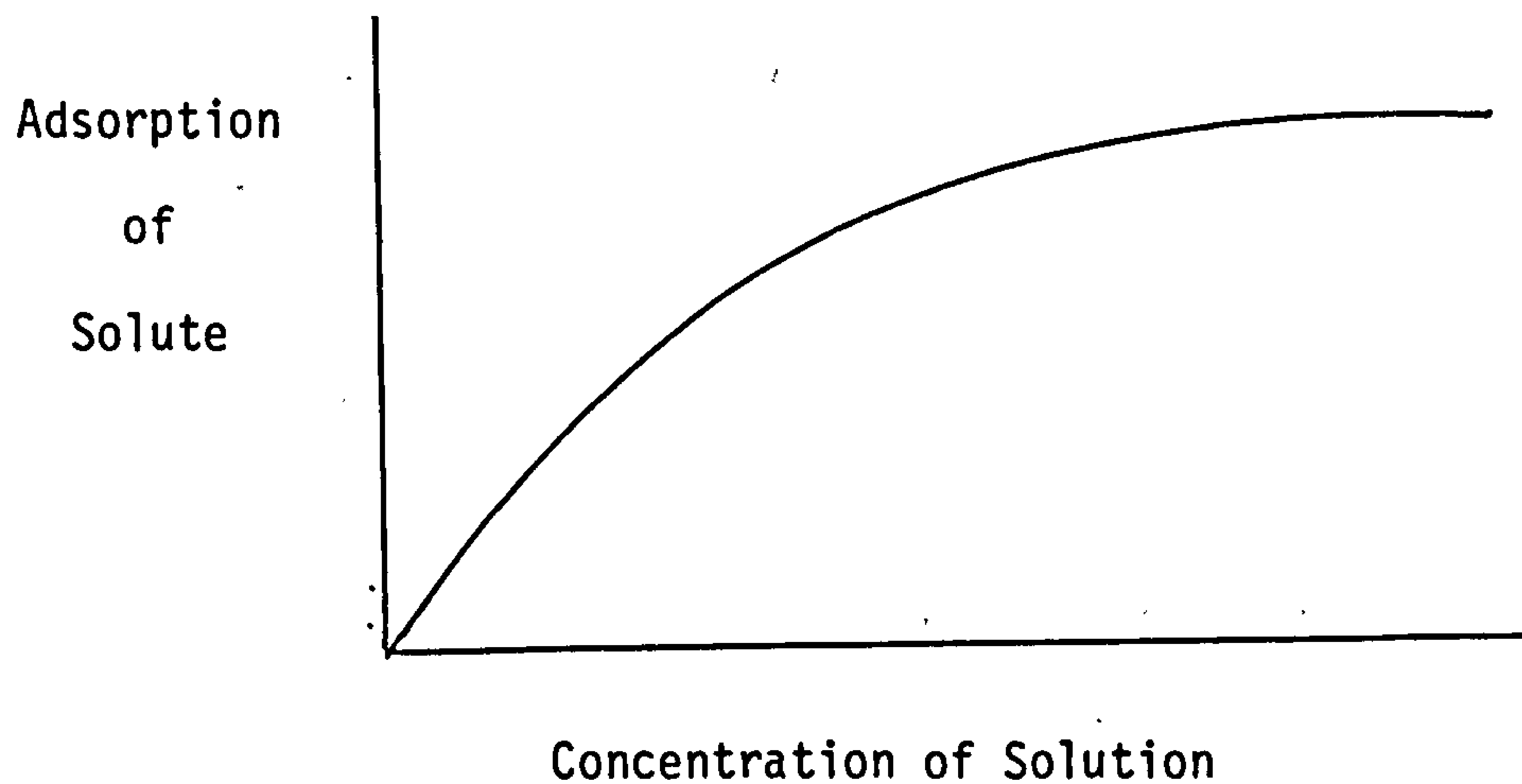


Figure 1.2 The most common isotherm for adsorption of solids from solution.

At low concentrations, this isotherm can generally be fitted to the Freundlich equation (Kipling, 1965) which has the form:

$$m = K_3 C^{\frac{1}{n_1}}$$

Where:

m = the amount of solute adsorbed per unit weight of adsorbent (mg mg^{-1})

C = the concentration of solute remaining in solution (mg l^{-1})

K_3 = a constant. This is an indication of adsorption intensity.

$1/n_1$ = a constant. This is an indication of adsorption capacity.

Data are usually fitted to the logarithmic form of the equation:

$$\log_{10} m = 1/n_1 \log_{10} C + \log_{10} K_3$$

If the data conforms to the Freundlich equation then a straight line is obtained by plotting $\log_{10}$ m against $\log_{10}$ C. The constants $1/n_1$ and K_3 can be calculated from the slope and the intercept respectively.

A second type of equation to which adsorption data is often fitted is the Langmuir equation (Kipling, 1965). This has the form:

$$m = \frac{K_1 K_2 C}{(1 + K_2 C)}$$

where K_1 is a constant which reflects the adsorption capacity, and K_2 is a constant related to the energy or net enthalpy of adsorption. If expressed in the form:

$$\frac{C}{m} = \frac{1}{K_1} C + \frac{1}{K_1 K_2}$$

then by plotting C/m against C a straight line will be obtained if the data conforms to the Langmuir equation. The constants K_1

and K_2 can be calculated from the slope and the intercept respectively.

Dye adsorption may also be analysed using the law of mass action (Hall, 1982b). This assumes dye-sludge interactions to occur as follows:

$$\text{Sludge solids + Dye} \overset{K_B}{\rightleftharpoons} \text{Sludge-Dye complex}$$

where K_B is the binding constant (in l Equivalents^{-1}) given by

$$K_B = \frac{C_{SD}}{C_D \; C_S}$$

where

C_{SD} = concentration of sludge-dye complex at equilibrium (Equivalents l^{-1})

C_S = concentration of free dye-binding sites on sludge at equilibrium (Equivalents l^{-1})

C_D = concentration of free dye at equilibrium (Equivalents l^{-1})

it follows that

$$\frac{(SS)}{(C_I - C_D)} = \frac{1}{K_B} \cdot \frac{1}{C_D} + \frac{1}{\sigma}$$

where

C_I = initial concentration of free dye (Equivalents l^{-1})

and

σ = total concentration of dye binding sites on the sludge per unit weight of solids (Equivalents mg^{-1})

Thus a plot of $(SS)/C_I - C_D$ against $1/C_D$ should be linear. Values for K_B and θ can be calculated from the slope and intercept respectively. When using dye concentrations of mg l^{-1}, Hall fitted the data to the following equation:

$$1/m = \beta \, 1/D_{30} + 1/\theta'$$

Where:

m = the amount of solute bound per unit weight of adsorbent (mg mg^{-1})

D = the concentration of solute remaining in solution, in this case after 30 minutes (mg l^{-1})

A plot of 1/m against 1/D should be linear with the slope equal to the constant β, and the intercept to $1/\theta'$. Hall proved that θ' (mg dye bound mg suspended solids^{-1}) is related to θ (Equivalents dye bound mg suspended solids^{-1}) and can be converted to the latter by dividing by the molecular weight of adsorbent and multiplying by the number of charges per molecule. β (mg suspended solids l^{-1}) can then be converted to K_B (l Equivalents^{-1}) according to the equation:

$$\beta\theta = 1/K_B$$

Shapes of isotherms have been obtained other than that in Fig. 1.2 and these have been classified by Giles *et al.*, (1960)

depending on the slope of the initial stage of the isotherm. These are described as S, L, H, or C isotherms. Using this classification, the nature of the adsorption reaction can be determined.

Several workers have studied the adsorption of substrates by activated sludge (Crombie-Quilty and McLoughlin, 1983; Banerji _et al_., 1968a; Stephenson _et al_., 1983). Banerji _et al_. failed to fit data for starch adsorption to the Freundlich equation, whereas Crombie-Quilty and McLoughlin failed to fit data for protein adsorption to either Langmuir or Freundlich equations. The latter workers therefore developed an activated sludge adsorption equation' of the form:

$$m = \frac{K_4 C^{1/n_2}}{b} \qquad \text{(b = mass of adsorbent mg ml}^{-1}\text{)}$$

where K_4 and N_2 are constants. Data for both protein and starch adsorption were found to fit this equation.

A great deal more research has been carried out on the adsorption of metals by activated sludge or by activated sludge extracellular polymers. This has been extensively reviewed by Brown and Lester (1979). Isotherms for metal adsorption were fitted to the Giles classification by Steiner _et al_. (1976). _Zoogloea_ 1-16, soluble activated sludge polymer and polygalacturonic acid all fitted L-type curves, whilst _Z.ramigera_ 115, solid sludge polymer and activated sludge showed the S-type configuration. It was suggested that carboxyl groups were the adsorption sites for the L-type isotherms, and that the hydroxyl

units of the hexose ring were responsible for the S-type of isotherm. It was proposed that the L-type of binding was irreversible, whilst the S-type was of a weak electrostatic nature. As the hydroxyl groups would be hydrated, adsorption of metal ions would result in a decrease of the bound water content of the polymer. Similar S-type adsorption isotherms were found for chromium, lead and zinc by Forster, (1983). The results of Steiner *et al.* (1976) also demonstrate that soluble extracellular polymer binds cations in a different way than extracellular polymers bind to sludge.

1.10.1 Adsorption of Dyes by Activated Sludge

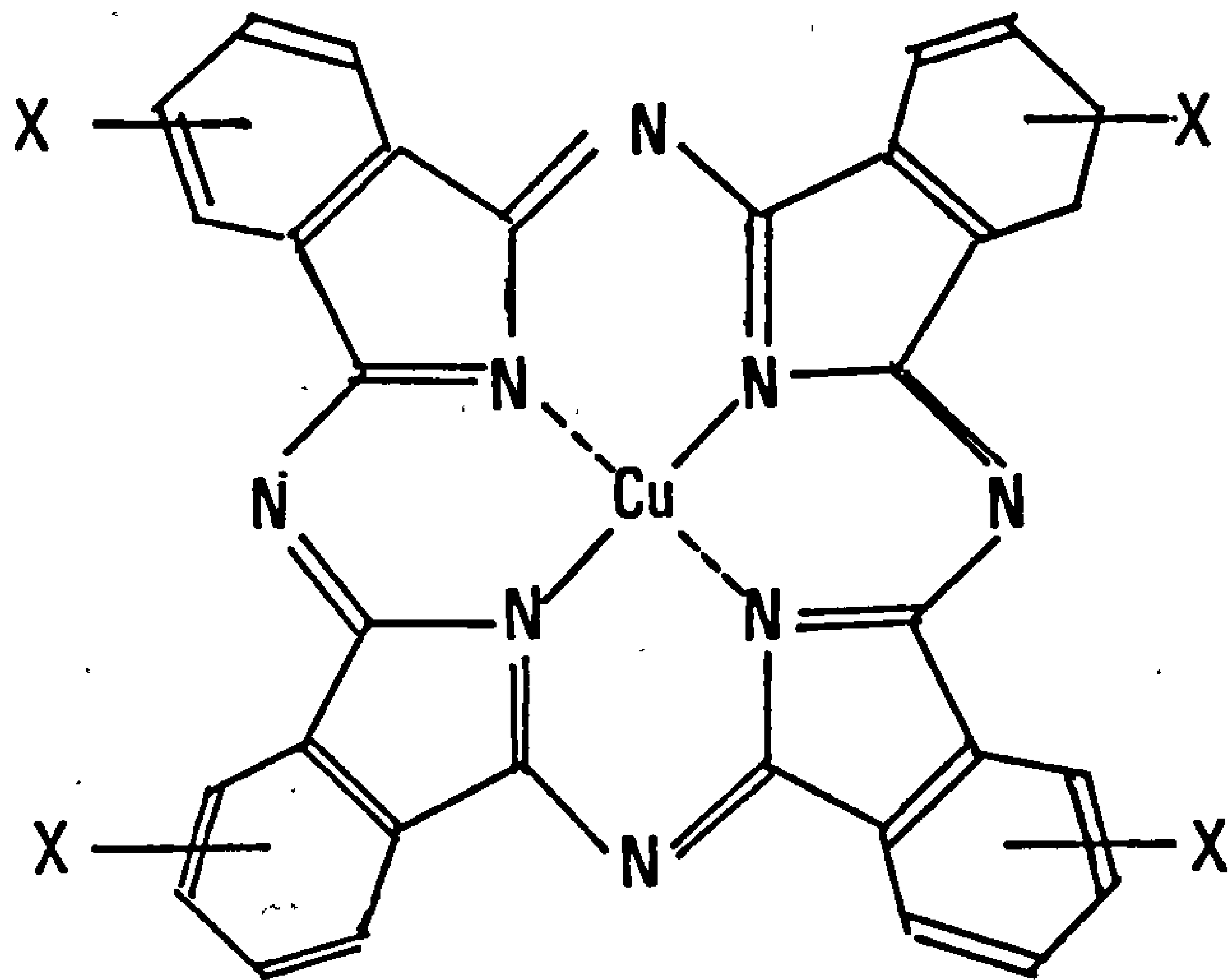
The adsorption of dyes by activated sludge is less well documented, although the reactions between dyes and bacterial cells have been intensively investigated (Daniels, 1980). Hitz *et al.* (1978) measured the adsorption by activated sludge of a range of different dyes with regard to their removal in wastewater treatment. They found that acid and reactive dyes were poorly adsorbed to the sludge surface, but that there was high adsorption of direct, diverse and basic dyes.

Andreadakis (1978) and Smith and Coackley (1983) measured the adsorption by activated sludge of Lissamine Scarlet 4R. As an anionic dye, it was necessary to firstly acidify the incubation mixture to facilitate binding of the dye and it is possible that this altered the surface properties of the sludge. The amount of dye adsorbed was used to estimate the surface area

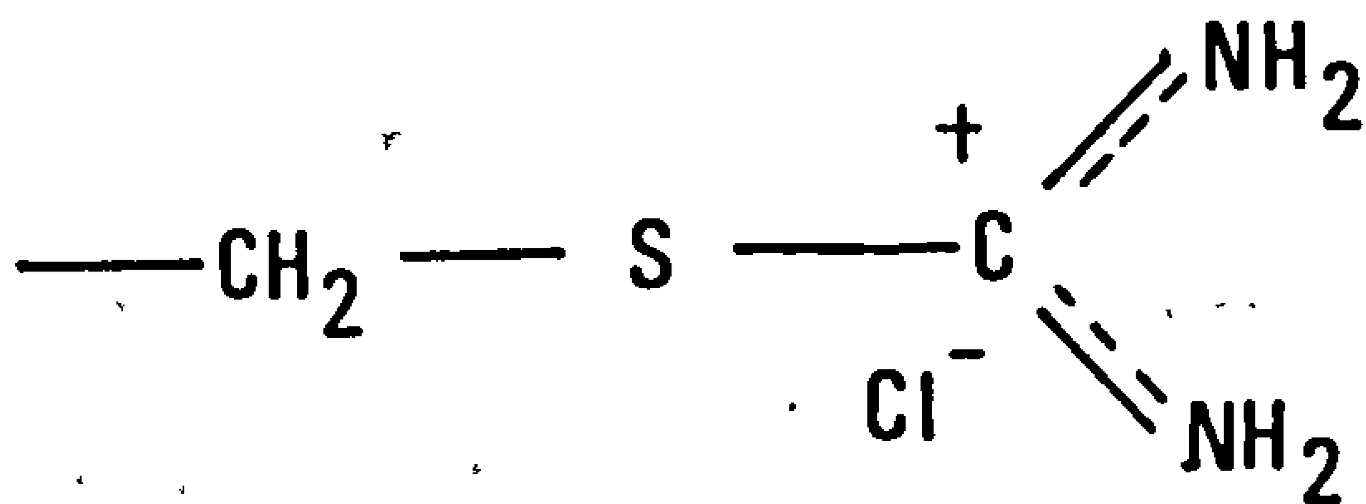
of sludge particles. A relationship was found between dye adsorption and SVI (Andreadakis, 1978).

The polycationic dye Alcian blue has been used to stain polysaccharide material at the activated sludge surface (Wu, 1978) and also in the quantitative determination of capsular polysaccharides (Powell et al., 1982). Several workers have studied the binding of Alcian blue to activated sludge (Banks et al., 1976; Hall, 1982b; Devloo et al., 1983) and there may be useful relationships with plant operational parameters. It is possible that the relationship between sludge charge, extracellular polymer production and dye adsorption could lead to a useful indication of sludge settlement characteristics.

The structure and properties of Alcian blue have been well defined (Quintarelli et al., 1964; Schenk, 1981; Scott, 1973a; 1973b; 1972a; 1972b; Scott et al., 1972; 1964; Scott and Dorling, 1965). Its chemical structure is as follows:



where X is the isothiouronium group;



The positive charge is shared between both nitrogen atoms and a carbon atom. Alcian blue 8GX has between 2.2 and 2.9 positive charges (Scott, 1973b) and would therefore be expected to be strongly held in the negative electrostatic field associated with polymers such as DNA and acid glycoproteins. It is likely that the only binding mechanism of Alcian blue is that of electrostatic attractions leading to the formation of salt links.

Like Alcian blue, the cationic dye Ruthenium red has been used to stain capsular polysaccharides. The structure and properties of Ruthenium red have been reviewed by Luft (1971) and its structural formula given as follows:

$$[(NH_3)_5 Ru - O - Ru(NH_3)_5 - O - Ru(NH_3)_5]^{6+}$$

Binding is of a similar nature to Alcian blue in that electrostatic forces are involved although some differences are described in that the charge distribution is in a different form, and higher in Ruthenium red. The dye was shown to bind a range of polyanions including polygalacturonic acid, pectin and DNA.

1.11 Summary

Research into the activated sludge wastewater treatment process has been characterized by two distinct approaches, that of the engineer and that of the microbiologist. The former has generally dealt with the sludge as an amorphous mass and attempted to fit its behaviour to mathematical models. Such an approach has its limitations due to the biological and thus often unpredictable nature of the sludge microorganisms. Nevertheless, large steps have been made by the engineer in the control of problems such as sludge bulking. In particular the development of plug-flow plants has led to a reduction in the number of bulking problems (Tomlinson, 1976).

In contrast, the microbiologist sees the sludge biomass as a host of different species of microorganism , each with its own

place within a complex ecological framework. Some success may also be attributed to this approach. Certain filamentous organisms are known to be associated with factors leading to filamentous bulking (Strom and Jenkins, 1984; Eikelboom, 1977) and can thus point the way towards a remedy. Studies of protozoan populations can be used to predict effluent quality (Curds and Cockburn, 1970).

Studies on extracellular polymer production and the floc surface fall midway between the realms of the microbiologist and engineer, and there are indications that this work may be useful in bulking control (Forster and Clarke, 1983). However, although advances have been made in the development of cures for bulking there is currently no way in which a plant operator can predict a problem in advance and thus take steps to avoid solids loss in the effluent. Too little information is available with regard to the activity and viability of activated sludge, its microbial composition, and the nature of the floc surface.

1.12 Aims and Objectives

The aims and objectives of the project were as follows:

1. To gain more knowledge of the sludge surface by studying factors affecting polycationic dye adsorption by activated sludge.
2. To develop a standard procedure for the routine estimation of dye adsorption by activated sludge.
3. Adsorption of substrates and sludge surface charge are both clearly important in either selection of filamentous

organisms or sludge settlement. By studying the relationship between dye adsorption and plant operational parameters it was hoped to produce a test giving plant operators a useful indication of sludge settlement problems.

4. To investigate the changes in the sludge surface throughout the course of plug-flow activated sludge plants. This was to take the form of dye adsorption measurements as well as studies on extracellular polymer production.
5. To investigate the role of sludge extracellular polymers in adsorption of polymers from sewage and to find the extent to which sewage polymers contribute to the nature of the sludge surface.
6. To provide a further insight into the bulking problem by establishing the nature of changes in both filamentous and non-filamentous bacterial populations during bulking incidents.

CHAPTER 2

Materials and Methods

2.1 Samples from Activated Sludge Plants

Activated sludge, sewage and effluent were obtained from either Warrington South or Runcorn Effluent Treatment Works (E.T.W.). Details of activated sludge plants are given at a later stage (Chapter 3). Daily routine analyses by North West Water Authority staff were carried out on raw sewage, settled sewage, mixed liquor, return activated sludge and final effluent. For this purpose sewage and effluent samples were composite, taken over a 24 hour period. These were obtained using Bestel Dean samplers removing 200 ml at hourly intervals. Mixed liquor was removed from the aeration lane and return activated sludge from the return line prior to re-entry to the aeration lane. Samples were collected at approximately 8.00 to 9.30 a.m. Raw and settled sewage were analysed for COD, BOD, ammonia nitrogen, and suspended solids, whilst additional analyses for nitrite and nitrate were carred out on plant effluents. Return activated sludge and mixed liquor samples were tested for suspended solids, sludge volume index and settled volume.

Return activated sludge samples for dye adsorption, sludge activity and polymer measurements were also removed from the sludge return line immediately prior to re-entry to sludge aeration. Mixed liquor samples were taken where possible at the midway point between aerating cone and the tank wall. Sewage samples were removed as close as possible to aeration lane entry. Sludge and sewage were removed in 5 litre plastic containers. Where these were carried to the laboratory at Liverpool there was

generally a transport time of 30 minutes. Samples were then stored at 4°C.

2.2 Wastewater and Sludge Analysis

Unless otherwise stated, suspended solids were estimated by passage of duplicate 10 ml samples through pre-weighed 7 cm diameter Whatman GF/C discs. The filter discs were then dried at 105°C for 3 hours, cooled under vacuum in a desiccator and reweighed.

Analyses of nitrate, nitrite and ammonia nitrogen were carried out according to the methods of the Department of the Environment (1977b). Nitrate was first reduced to nitrite by hydrazine and then treated with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride under acid conditions to form a pink azo-dye. This was subsequently measured spectrophotometrically. Nitrite alone was measured by omission of the reduction. Ammonia nitrogen was analysed using a Kent EIL ion selective electrode.

COD was estimated after oxidation of the sample with sulphuric acid and potassium dichromate. Residual dichromate was then assessed spectrophotometrically using a ferroin indicator (Dept. Environment 1977a). $BOD_{(5)}$ analysis was carried out using a seed of final effluent and a YSI dissolved oxygen electrode (Hammerton and Sherratt, 1972).

Sludge volume index was estimated according to the method of APHA-AWWA-WPCF (1975). The sludge settled volume (SV) was

given as the settlement of sludge solids in a 1 l measuring cylinder after 30 minutes.

$$\text{SVI (ml g}^{-1}) = \frac{\text{SV (ml l}^{-1})}{\text{Suspended solids (g l}^{-1})}$$

Where sewage and sludge mixing was carried out in the laboratory (Chapter 6) SV was estimated by the settlement of sludge solids in a 100 ml measuring cylinder after 30 minutes and corrected.

2.3 Microbiological Methods for Activated Sludge Investigation

Activated sludge was analysed microscopically using the methods of Eikelboom (1981, 1975) and detailed below.

2.3.1 Floc Structure

Unstained sludge samples were examined using phase contrast microscopy and floc sizes estimated under low power with the aid of a curtain micrometer (Graticules Ltd.). Initially the diameter of ten, randomly selected flocs were measured, but later flocs were classified according to the scheme of Eikelboom (1981). This involved estimation of the percentage of flocs within certain size ranges (Table 2.1).

Floc structure was also described with respect to firmness, shape and density. Firmness could be established by the extent to which disruption occurred when the coverslip was pressed.

Description	Size range (μm)
Large flocs	>500
Middle size flocs	150 - 500
Small flocs	<150

Table 2.1 Classification of floc size (Eikelboom, 1981)

2.3.2 Filamentous Bacteria

Activated sludge was divided into five categories according to the criteria shown in Table 2.2 (Eikelboom, 1981).

In order to identify filamentous bacteria fresh activated sludge samples were examined using a phase contrast microscope and magnification X1250. Filament size was estimated using a curtain micrometer (Graticules Ltd.) and presence or absence of the following characteristics were noted; mobility, cell inclusions, septa, filament shape, attached growth, constrictions, branching or false branching, "false" cells and sheath.

Sheath presence was confirmed using the staining technique of Farquhar and Boyle (1971). With the sample under the phase contrast microscope, a drop of crystal violet solution (Table 2.3) was added at one side of the coverglass. This was then drawn across the sample by placing a piece of absorbent paper at the opposite edge. As the staining solution enveloped the filament the sheath became more visible.

2.3.3 Staining Techniques

The Gram stain was carried out according to the method of Lillie (1928). The Neisser stain was performed according to the method of Gurr (1965). Preparation of staining solutions is shown in Table 2.3. Procedure for the Neisser stain was as follows:-

(a) A heat fixed smear of activated sludge was prepared.

(b) Reagent D was added to the smear for 10 to 15 seconds.

Filament Category	Description
0	No filaments
1	Small numbers of filaments
2	Moderate numbers of filaments
3	Large numbers of filaments
4	Excessive numbers of filaments

Table 2.2 Description of filament categories (Eikelboom, 1981)

Test	Reagents
Sheath detection	Dissolve 20 g crystal violet in 200 ml ethanol. Make up to 1 L with 1% aqueous ammonium acetate
Neisser stain	Reagent A : Methylene blue 0.1g acetic acid, glacial 5 ml absolute alcohol 5 ml water 100 ml Reagent B : Crystal violet, 10% in absolute alcohol 3.3 ml absolute alcohol 6.7 ml distilled water 100 ml Reagent C : Chysoidin Y, 1% aqueous 33.3 ml distilled water 66.7 ml Reagent D : 2 parts reagent A to one part of reagent B
Fat deposits	Sudan black B 0.3g 70% ethanol 100 ml

Table 2.3 Staining reagents used in sludge investigation

(c) Reagent C was applied for 45 seconds.

(d) The slide was rinsed with distilled water and dried.

Neisser positive filaments stain dark blue whilst negative organisms stain yellow or brown.

2.3.4 Sudan B Test for Fat Deposits (Farquhar and Boyle, 1971)

A fresh activated sludge sample was observed using the phase contrast microscope. A drop of Sudan Black B solution (Table 2.3) was added to one side of the coverglass and drawn across the sample with a piece of absorbent paper. The microscope was then changed to bright field illumination. Fat globules were unstained whilst the remainder of the cell was stained black.

2.3.5 Sulphur Storage Test (Farquhar and Boyle, 1971)

To 20 ml of activated sludge were added a similar volume of 0.2% sodium sulphide ($Na_2S.7H_2O$). The mixture was then shaken at 200 r.p.m. at room temperature for 10 minutes. Filaments were observed under the phase contrast microscope, sulphur droplets appearing as bright specs.

2.3.6 Protozoa, Rotifers and Nematodes

The number of protozoa, rotifers and nematodes was estimated by counting their presence in each of ten randomly selected microscope fields (magnification X400). Results were expressed as mean numbers per field.

2.4 Isolation and Characterization of Activated Sludge Bacteria

Studies on the aerobic heterotrophic bacterial populations in activated sludge were carried out as follows. Activated sludge was diluted to 50% using 1/100 CGY medium containing (g l^{-1}) casitone 5, glycerol 5, and yeast extract 1. A volume of 50 ml of this solution was then sonicated for 20 seconds using an MSE sonicator and a 10 mm probe. After sonication the sample was cooled with an ice bath. Bacterial suspensions were then serially diluted in 1/100 CGY and 0.2 ml of each dilution spread onto CGY agar. Plates were incubated aerobically at 20°C for 5 days after which plates containing approximately 100 isolates were selected for further investigation.

Each bacterial colony from the selected plates was then subcultured onto CGY medium, and purified by repeated subculture. Purity was checked using Gram stain and colony morphology. Once this was established bacterial isolates were subjected to a number of physiological and biochemical investigations.

2.4.1 Colony Morphology

Colony morphology was examined on CGY agar. The size, shape and appearance of colonies were recorded as well as an estimation of slime production. Each bacterial isolate was given a slime production index of between 0 and 5, 0 representing no production and 5 excessive production.

2.4.2 Physiological Characteristics

The primary classification scheme of Cowan (1974) was applied to all pure strains of activated sludge bacteria. This required performance of tests for Gram stain, motility, catalase, oxidase and oxidative or fermentative metabolism. Motility was observed using a hanging drop preparation after growth in peptone water (peptone 10 g l^{-1}, NaCl 5 g l^{-1}). Catalase and oxidase tests were performed according to the methods of Cowan (1974). Oxidative or fermentative (O/F) metabolism of glucose was assessed as follows: Two glass tubes of diameter 16 mm and length 12 cm were half filled with sterile O/F medium (g l^{-1} peptone 2, NaCl 5, K_2HPO_4 0.3, agar 3, glucose 0.1 and 0.03 bromothymol blue) and inoculated with the test organism. Sterile liquid paraffin was then overlayed to a depth of 1 cm in one tube and both tubes incubated for 5 days at 20^oC. Oxidation of glucose and acid production were identified by a yellow colour in the absence of paraffin. Fermentative organisms were identified by acid production in both tubes.

Anaerobic growth was determined by growth in the anoxic zone of brewer modified thioglycollate medium (Difco).

2.5 Measurement of Sludge Activity

2.5.1 Oxygen Uptake

A Rank Oxygen Electrode was used for the measurement of oxygen uptake by activated sludge samples. Endogenous sludge respiration rates were assessed using 1 ml of sludge plus 1 ml

0.05 M Tris HCl buffer at pH 7.0. Measurement of respiration rates in the presence of substrate was carried out after the addition of 0.1 ml 0.1 M glucose or 0.1 ml 10% w/v glycerol. After equilibration for 10 minutes oxygen uptake was measured at 20°C.

2.5.2 Triphenyl Tetrazolium Chloride (TTC) Reduction

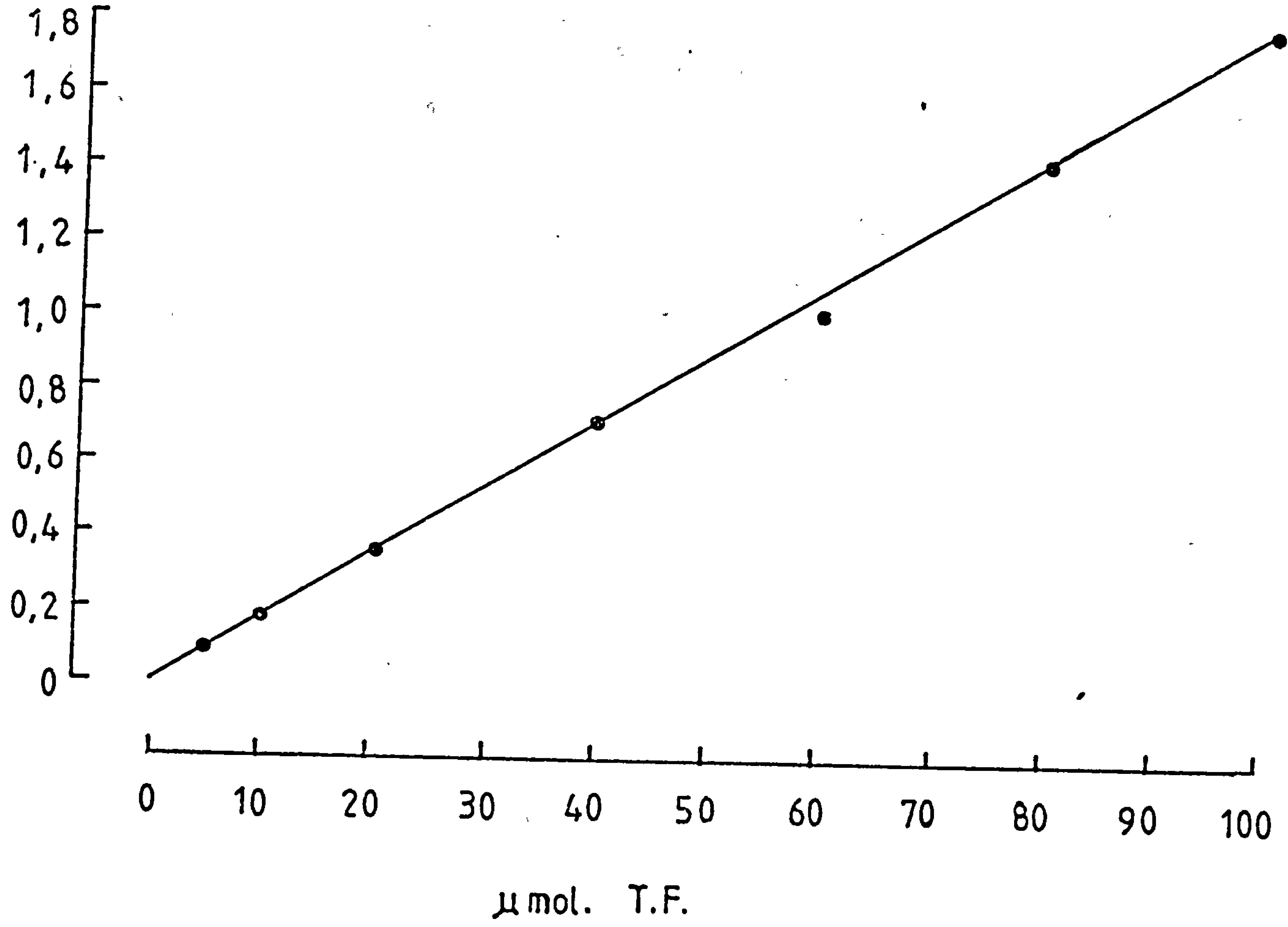
Sludge activity was measured using a modification of the method of Coackley and O'Niell (1975). Into a glass tube was added 2 ml of activated sludge, followed by 1 ml 0.05 M Tris HCl buffer pH 7.0, and 0.2 ml of a reagent containing 0.5% 2:3:5 triphenyl tetrazolium chloride (TTC) and 1% w/v glucose in aqueous solution. The suspensions were then mixed and incubated in the dark at 20°C for 15 minutes, after which 2 ml of ethanol were added in order to stop the reaction and extract any triphenyl formazan produced. After centrifugation at 4,250 g for 5 minutes in a MSE bench centrifuge the supernatant was transferred to a clean tube. Extraction with 2 ml ethanol was repeated twice. The absorbance at 484 nm of the combined extracts was then measured in a Cecil Spectrophotometer. Production of triphenyl formazan (TF) was determined from a calibration curve using pure TF in ethanol (Fig. 2.1). Sludge activity was expressed as μmol TF produced gram sludge^{-1}.

2.5.3 Sludge Viability

Activated sludge samples were diluted to 50% in 1/100 CGY medium and sonicated for 20 seconds using a 10 mm diameter probe.

Figure 2.1

Calibration graph for triphenyl formazan (TF) production.


A 484
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0
0
10
20
30
40
50
60
70
80
90
100
µmol. T.F.

Samples were then serially diluted in 1/100 CGY, duplicate 0.2 ml of the appropriate dilutions spread onto CGY agar, and incubated at 20°C for 5 days before counting. Results were expressed as colony forming units (c.f.u.) gram sludge^{-1}.

2.6 Adsorption of Cationic Dyes by Activated Sludge

Dye adsorption was measured by adding appropriate mixtures of dye, activated sludge and water (total volume 50 ml) to a 250 ml conical flask and incubating on a rotary shaker at 200 r.p.m. and 20°C. Equilibration of sludge and water was allowed for 10 minutes after which 0.5 ml of dye solution was added (t = 0). Samples (2 ml) were removed after 10 minutes and centrifuged in glass tubes at 2,000 g for 2 minutes in a MSE bench centrifuge. This sedimented flocs but not free bacterial cells (Eikelboom, 1981). The absorbance of supernatants was measured at the appropriate wavelength in a Cecil Spectrophotometer. Dilution was often necessary with the higher dye concentrations (see below). Control flasks in which either sludge or dye were omitted were prepared simultaneously to determine glass adsorption of dye and the contribution made to absorbance by free bacterial cells. Dye adsorption by sludge was calculated as a function of the amount of dye available after glass adsorption. An example of calculation of Alcian blue adsorption in dye adsorption tests (using 5% activated sludge) is shown in Figure 2.2.

Figure 2.2

Example of calculation of Alcian blue adsorption in dye adsorption tests.

Sludge suspended solids = 2.5 g l^{-1}

Sludge dilution in test = 5%

Stock dye solution = 10 g l^{-1} manufacturers preparation

(a) Preparation of flasks

	Control A	Control B	Test Sample
Water (ml)	49.5	47.5	47.0
Alcian blue soln. (ml)	0.5	0	0.5
Activated sludge (ml)	0	2.5	2.5
Absorbance at 602 nm (after incubation and centrifugation)	0.800	0.015	0.550

(b) Calculation of percentage Alcian blue bound by activated sludge.

i) Subtract control B in order to account for any free swimming bacteria.

$$0.550 - 0.015 = 0.535$$

ii) Subtract from control A to find absorbance of dye bound to sludge.

$$0.800 - 0.535 = 0.265$$

iii) Calculate percentage

$$\frac{0.265}{0.800} \times 100 = 33.125\%$$

(c) Calculation of g Alcian blue bound g sludge^{-1}.

i) Find dye concentration in control A using extinction coeficient; $E^{1\%}_{1cm} = 425$ (Scott 1972a).

$$= \frac{0.800}{425} \times 10 = 1.882 \times 10^{-2} \text{ g l}^{-1}$$

ii) Find sludge solids concentration in sample

$$= \frac{5}{100} \times 2.5 = 0.125 \text{ g l}^{-1}$$

iii) Calculate mass of dye bound using value obtained in (b, iii).

$$\frac{1.882 \times 10^{-2} \times 33.125}{100 \times 0.125} = 0.050 \text{ g dye g sludge}^{-1}$$

2.6.1 Dye Solutions

Commercially prepared Alcian blue and Ruthenium red (Sigma Chemical Company) were added to distilled water at 10 g l^{-1} and 2 g l^{-1} respectively. After mixing for a minimum of 2 hours using a magnetic stirrer, the solutions were centrifuged at 12,000 g on a Beckman J2-21 centrifuge for 15 minutes to remove undissolved dye particles. The supernatant was retained and dye concentration estimated using the following extinction coefficients.

Alcian blue	$E^{1\%}_{1cm}$ = 425 at A602nm
Ruthenium red	$E^{1\%}_{1cm}$ = 769 at A538nm

The extinction coefficient of Ruthenium red is that of Luft (1971) given for solutions in 0.1 M ammonium acetate. There was no significant difference between absorption spectra of Ruthenium red in 0.1 M ammonium acetate and fresh distilled water (Fig. 2.3).

Scott (1972a) gives the extinction coefficient of Alcian blue as $E^{1\%}_{1cm}$= 425 at A 625 nm. Absorption spectra of Alcian blue in distilled water obtained in this study are shown in Fig. 2.4. The absorption maximum was found not at 625 nm but at 602 nm. This discrepancy is probably due to the different sources of Alcian blue. Purification of commericially prepared Alcian blue was carried out according to the method of Scott (1972a). The

Figure 2.3

Absorption spectra for Ruthenium red.

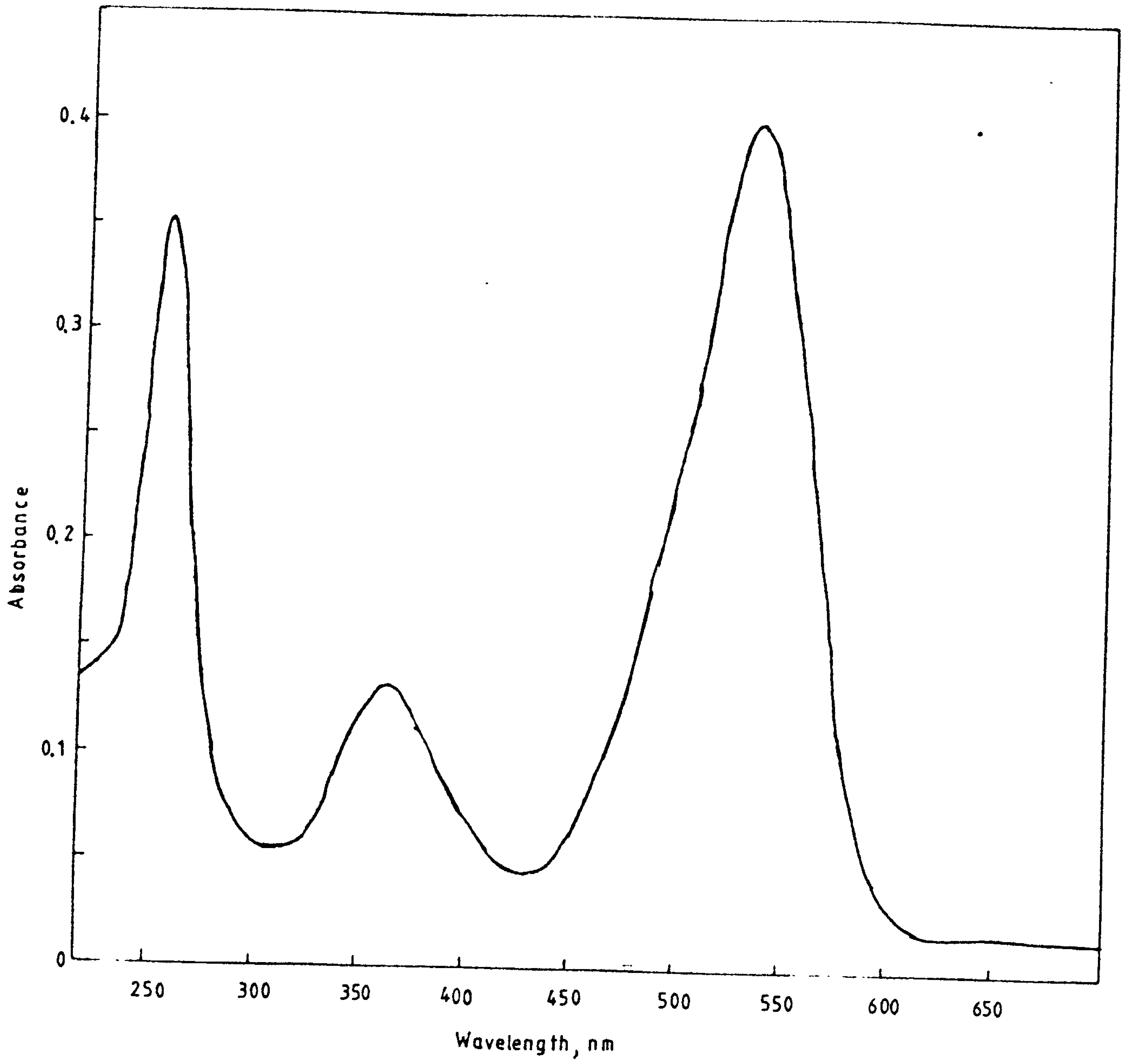
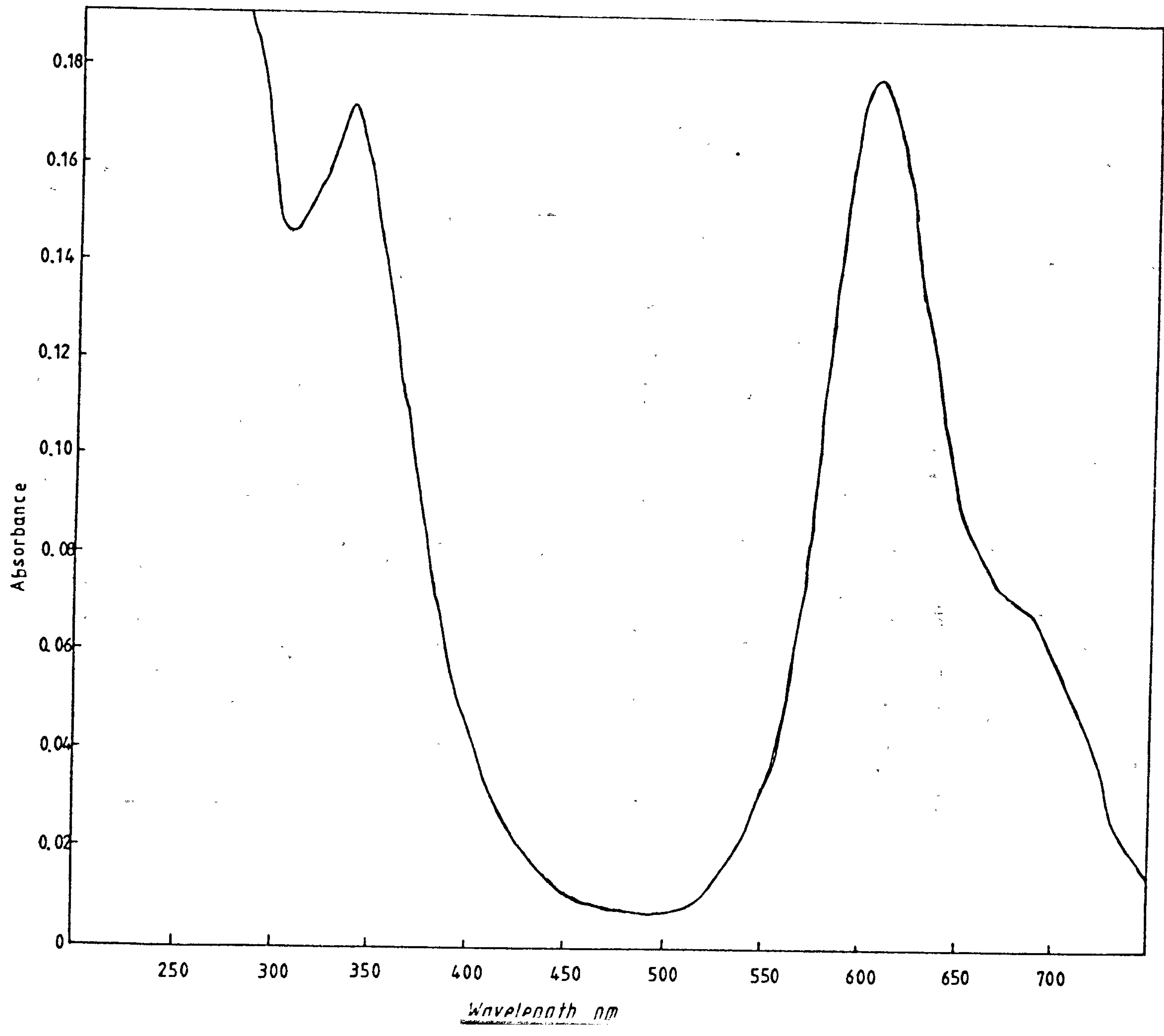

0.4
0.3
0.2
0.1
0
Absorbance
250
300
350
400
450
500
550
600
650
Wavelength, nm

Figure 2.4

Absorption spectra for Alcian blue.

Text cut off in original


0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
Absorbance
250
300
350
400
450
500
550
600
650
700
Wavelength nm

dye was washed in acetone, centrifuged at 4,250 g for 30 minutes on a bench MSE centrifuge after which the supernatant was discarded and the residue dried. Purified Alcian blue preparations also possessed an absorption maxima of 602 nm. Scotts extraction coefficient of $E^{1\%}_{1cm} = 425$ was used to calculate dye concentrations, though absorbance was measured at 602 nm. Although this may introduce an error into Alcian blue measurement, it was considered that an index of dye concentration was required rather than the absolute molecular value.

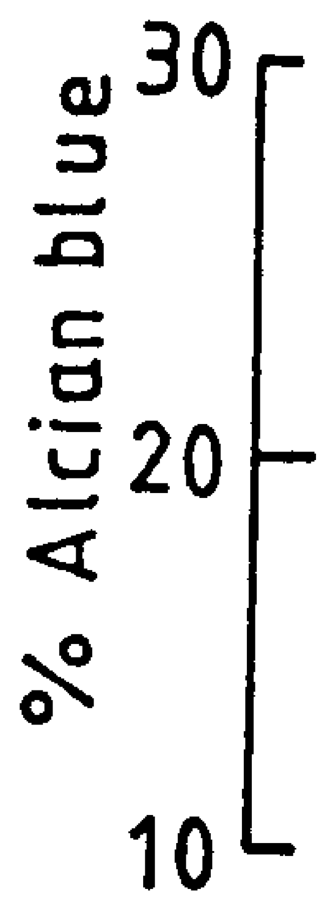
In studies where sludge concentration ranged from 0-10%, stock solutions of Alcian blue and Ruthenium red were adjusted to 10 g l^{-1} and 2 g l^{-1} respectively. Where higher sludge concentrations were used, stock solutions were made up at ten times these concentrations. A volume of 2.5 ml was then added to the test flasks. Dye solutions were kept in darkness at $4^{o}C$ for a maximum of 1 week.

Ruthenium red preparations were also examined for the presence of Ruthenium violet and Ruthenium brown using a chromatographic method (Luft, 1971). A strip of Whatman No. 1 filter paper (approximately 1 cm x 5 cm) was positioned with one end submerged in a solution of 0.1 M ammonium acetate in a petri dish. A drop of Ruthenium red in 0.1 M ammonium acetate was then placed above the solvent front and the chromatogram allowed to develop for 15 minutes. No Ruthenium brown or violet were visible and therefore contamination with these dyes was considered to be insignificant.

The relationship between absorbance and the concentration of commercial dye preparations are shown in Figs. 2.5 and 2.6.

Figure 2.5

Variation of absorbance with Alcian blue concentration. "Alcian blue" indicates commercially prepared dye powder.


% Alcian blue
30
20
10

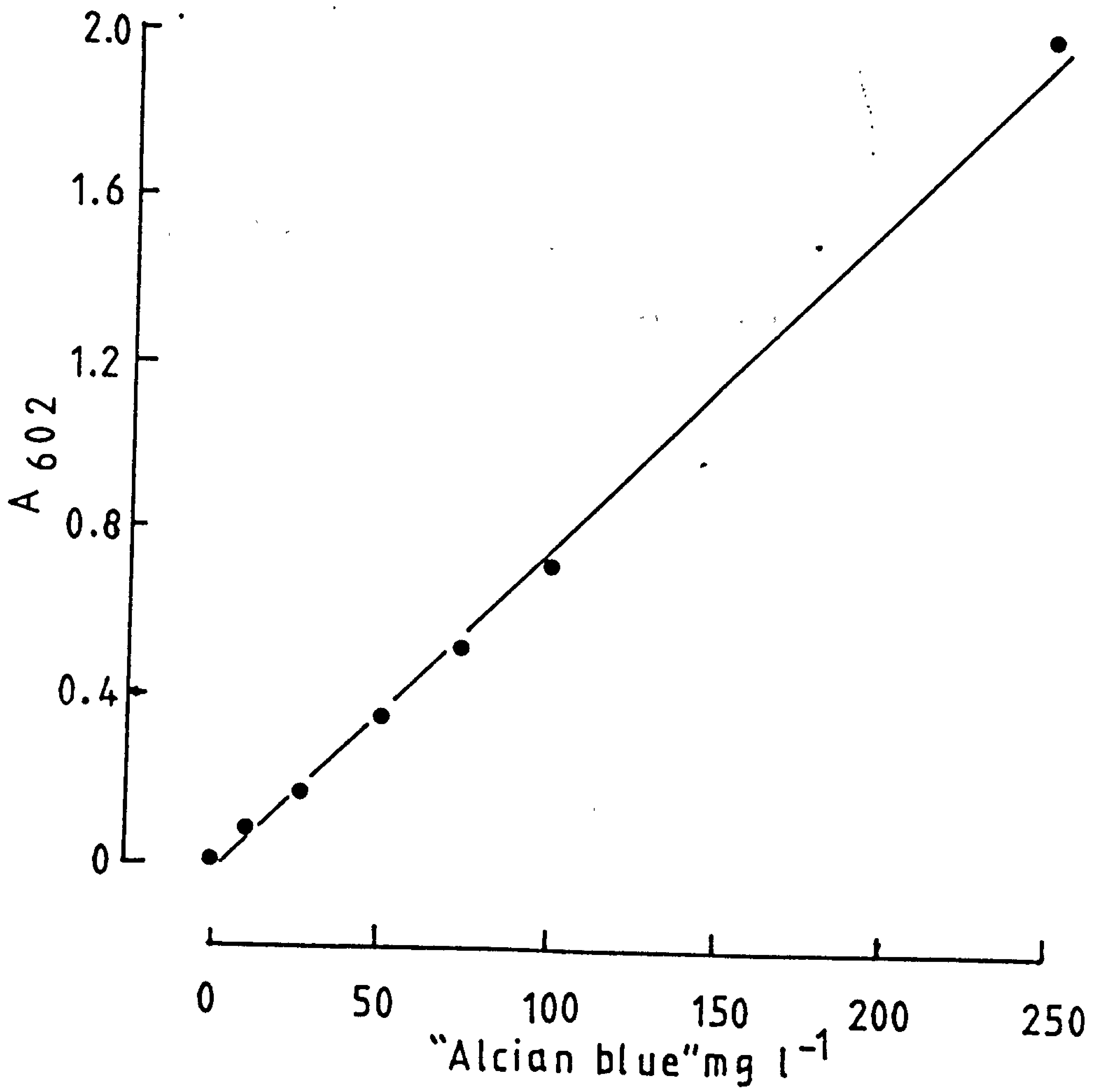
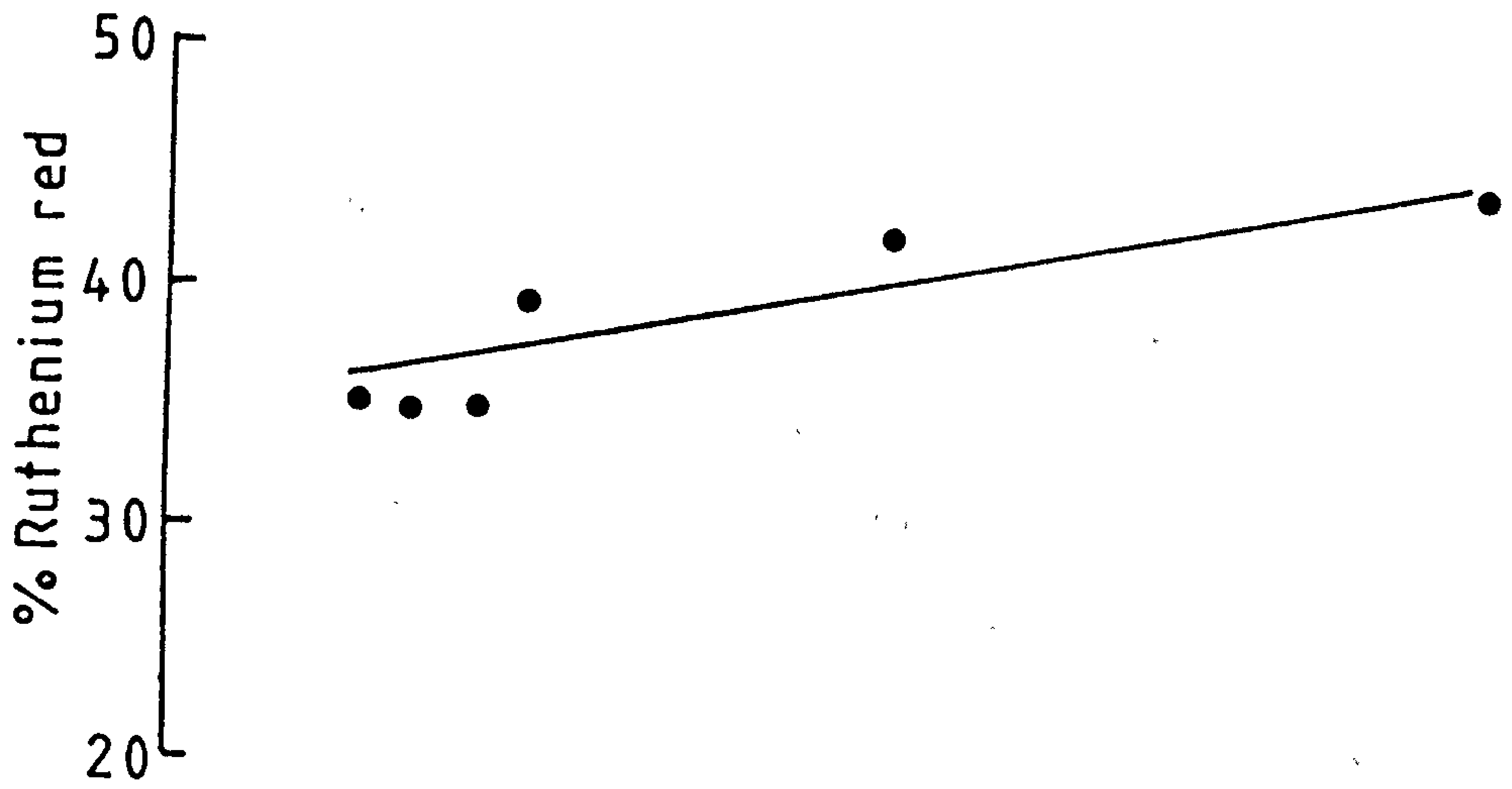
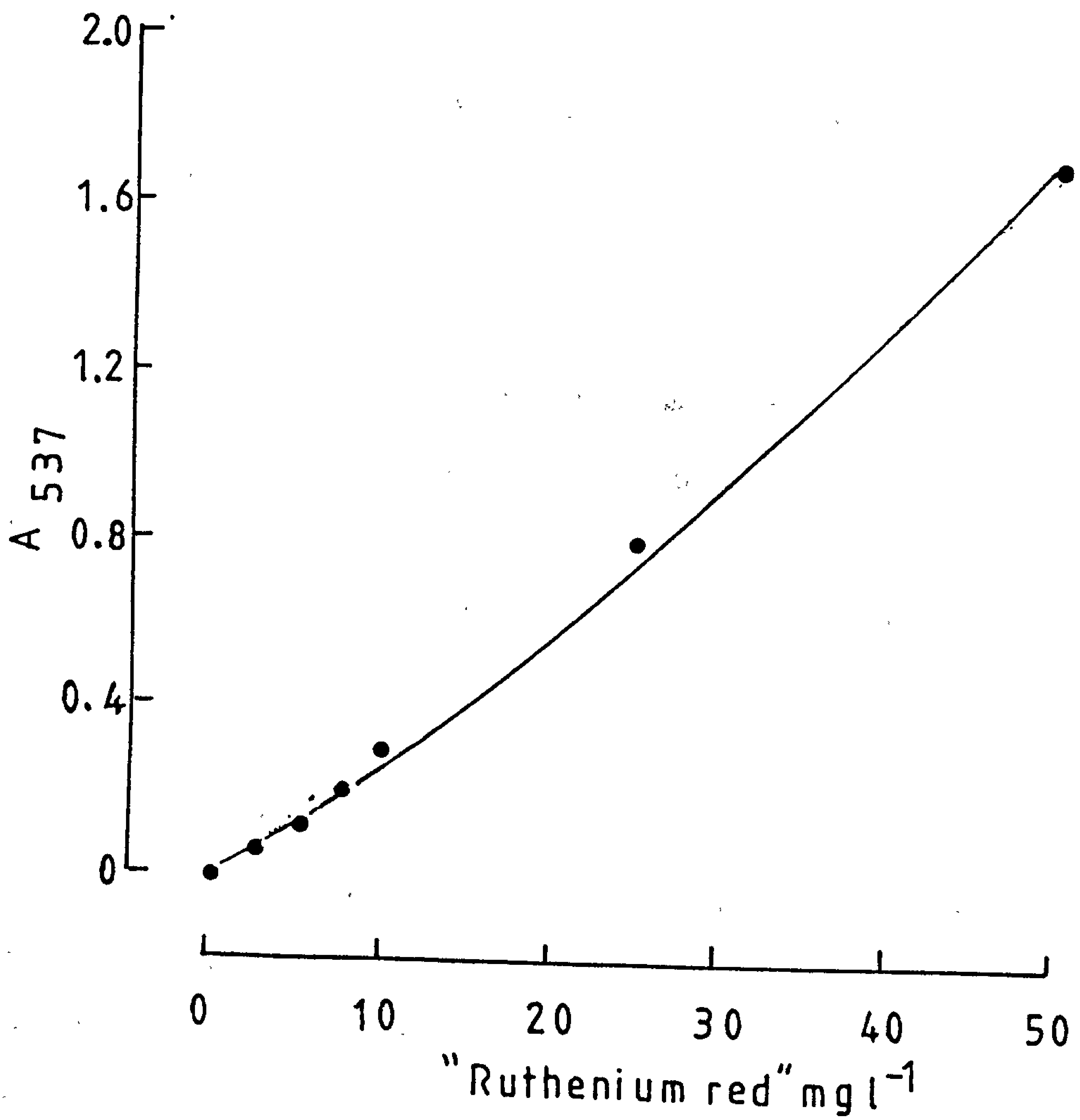

2.0
1.6
1.2
0.8
0.4
0
A 602
0
50
100
150
200
250
"Alcian blue" mg l⁻¹

Figure 2.6

Variation of absorbane with Ruthenium red concentration. "Ruthenium red" indicates commercially prepared dye powder.


% Ruthenium red
50
40
30
20


A 537
2.0
1.6
1.2
0.8
0.4
0
0
10
20
30
40
50
"Ruthenium red" mg l-1

Dye concentration is expressed as a percentage of the manufacturers preparation as calculated from the extinction coeficient. There is some variation in the calculated percentage dye with an increase in dye concentration. Alcian blue concentration shows a linear relationship with absorbance ($r = 1.00$), Ruthenium red also obeys Beers law ($r = 0.77$, $P < 0.005$) although there is some deviation at high dye concentrations.

2.7 Parameters Affecting Dye Adsorption

The standard method above was used throughout with the following modifications.

2.7.1 pH

A range of pH values from 4 to 9 was obtained using three buffer systems. Stock buffer solutions were made up at twice the final concentration required and added in a volume of 25 ml. Buffers used and variation in absorption of dye solutions with pH are shown in Table 2.4.

2.7.2 Temperature

Equilibration for 10 minutes and the dye adsorption test were carried out at the appropriate temperature in a shaking water bath. Temperature had no significant effect on the optical density or glass adsorption of Alcian blue solutions.

pH	Buffer system	Alcian blue A602	Ruthenium red A538
4.0	0.05 Citrate 0.1 M disodium	0.886	0.70
5.0	hydrogen orthophosphate	0.871	0.819
6.0	" "	0.828	0.822
7.2	" "	0.884	0.792
7.2	0.1 M Tris HCl	0.869	0.825
8.0	"	0.883	0.817
9.0	"	0.819	0.827
6.0	0.1 M Sodium phosphate	0.880	0.812
6.6	" "	0.890	0.812
7.2	" "	0.876	0.829
8.0	" "	0.850	0.826
4.75	Distilled water	0.886	0.837
6.8	Tap water	0.866	0.804
Mean		0.868	0.810
SD		0.0217 (2.5%)	0.034 (4.16%)

Table 2.4 Effect of pH and buffers on the absorbance of Alcian blue and Ruthenium red. Concentration of manufacturers preparation 0.1 g l^{-1} Alcian blue, 0.02 g l^{-1} Ruthenium red.

2.7.3 Sonication

Activated sludge was equilibrated with water for 10 minutes followed by sonication for the appropriate time using a 10 mm diameter probe on a MSE sonicator. After incubation with dye, samples were centrifuged at 4,250 g for 20 minutes in order to sediment all bacterial cells.

2.7.4 Inhibitors and Other Compounds

Stock solutions were made up in distilled water to ten times the final concentration required. Five ml were then added to the test flasks to give the standard test volume of 50 ml. Equilibration was carried out for 10 minutes in the presence of the compound prior to the addition of Alcian blue. Controls were also prepared with sludge omitted, and are shown in Tables 2.5 and 2.6. These were used in calculations of dye adsorption.

2.8 Adsorption of Metabolic Substrates by Activated Sludge

2.8.1 Glucose, Pyruvate and Urea

Return activated sludge (45 ml in a 250 ml conical flask) was shaken at 200 r.p.m. at 20°C. After 10 minutes, substrates (in 5 ml) were added to a final concentration of 0.2 g l^{-1}. Samples (5 ml) were removed 1, 10 and 30 minutes after substrate addition, transferred into glass tubes and centrifuged at 2,000 g for 2 minutes at 4°C. Supernatants were stored at 4°C until assayed for the appropriate compound. Control flasks in which substrates were replaced by water were run simultaneously.

Inhibitor	Absorbance at 602nm
None	40.625
Potassium cyanide (10 mM)	34.75
N-ethylmaleimide (1 mM)	36.38
Formaldehyde (4 g l^{-1})	36.50
Mercuric chloride (0.1 g l^{-1})	39.50

Table 2.5 The effect of inhibitors and biocides on the absorbance of Alcian blue. Concentration of manufacturers preparation approximately 5.0 g l^{-1}.

Compound	Absorbance at 602 nm
None	0.804
$NaNO_3$	0.795
$MgCl_2$	0.805
NaCl	0.815
KCL	0.810
Glycerol	0.802
Tween 80	0.831
Glucose	0.811
Casitone	0.804
Urea	0.800
Cooking oil	0.992

Table 2.6 The effect of possible sewage constituents (1 g l^{-1}) on the absorbance of Alcian blue. Concentration of manufacturers preparation approximately 0.1 g l^{-1}.

Glucose was assayed using a modification of the enzymatic method of Barton (Sigma Chemical Company, 1973). To 0.1 ml of sample or standard was added 1.0 ml of a reagent containing 1.6 ml of 2.5 mg ml^{-1} O-Dianisidine dihydrochloride plus 100 ml of PGO enzyme solution (Sigma Chemical Company). The mixture was shaken and then incubated at 37°C for 30 minutes after which the absorbance at 450 nm was measured against a reagent blank. The concentration of glucose in the sample was measured from a calibration curve of 0-150 mg l^{-1} glucose (Fig. 2.7).

Pyruvate was assayed using the following method (Dawes *et al.*, 1971). To 1.0 ml of sample or standard was added 0.33 ml of 0.1% 2-4 Dinitrophenyl hydrazine in 2.0 M HCl. Mixtures were then shaken and incubated at room temperature for 10 minutes after which 2.0 ml of 10% w/v NaOH were added. After a further 10 minutes the absorbance at 445 nm was measured in a Cecil Spectrophotometer. Concentration of pyruvate was estimated using a calibration curve of 0-50 mg l^{-1} (Fig. 2.8a).

Urea was estimated using a variation of the method (Sigma Chemical Company, 1980) of Marsh *et al.* (1965). To 0.1 ml of sample or standard was added 3 ml of BUN reagent and BUN colour reagent (Sigma Chemical Company). Mixtures were shaken and placed in a boiling water bath for exactly 10 minutes after which they were removed, cooled and the absorbance measured at 535 nm. The concentration of urea was then estimated using a calibration curve of 0-200 mg l^{-1} (Fig. 2.8b).

Figure 2.7

Calibration curve for measurement of glucose.

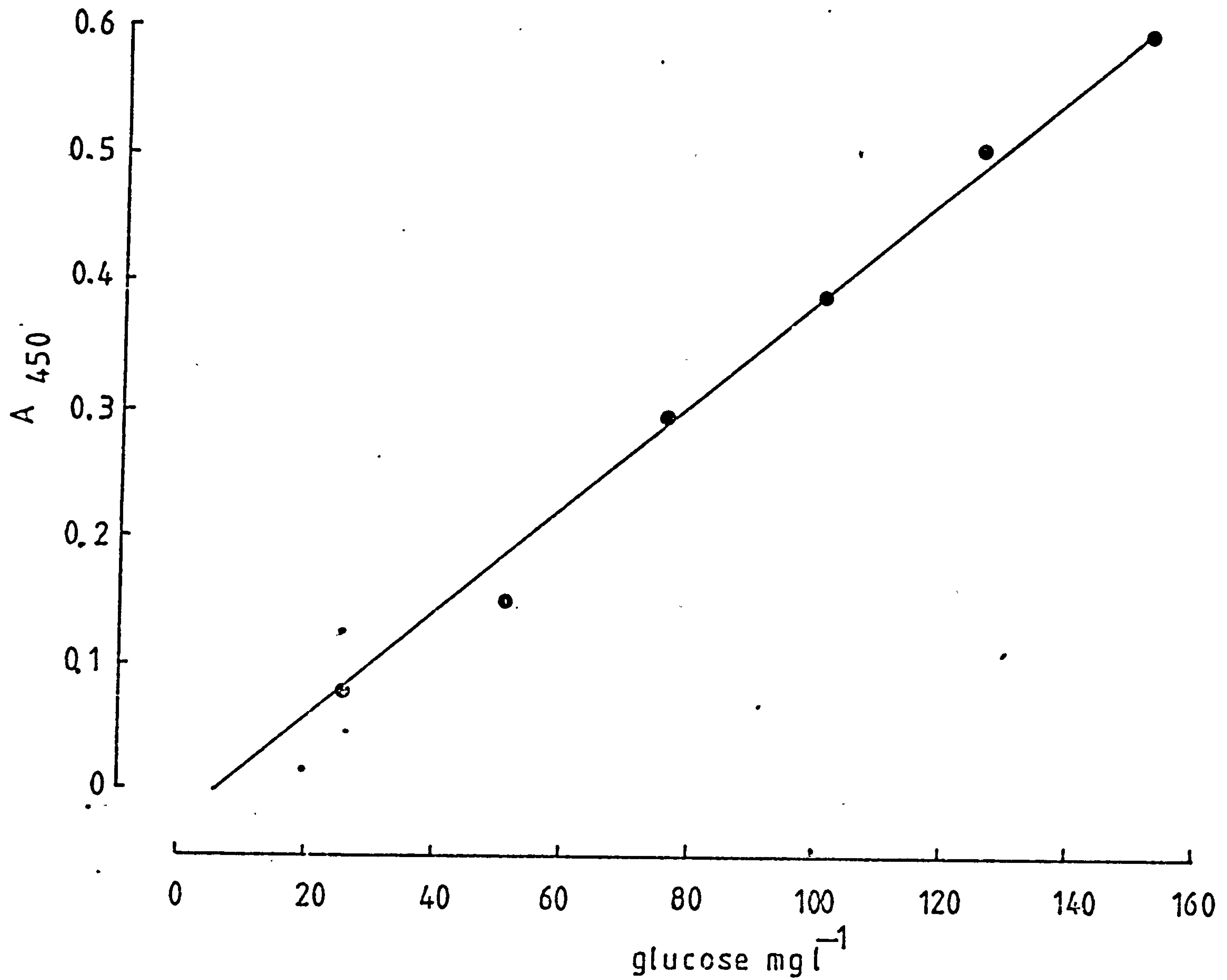
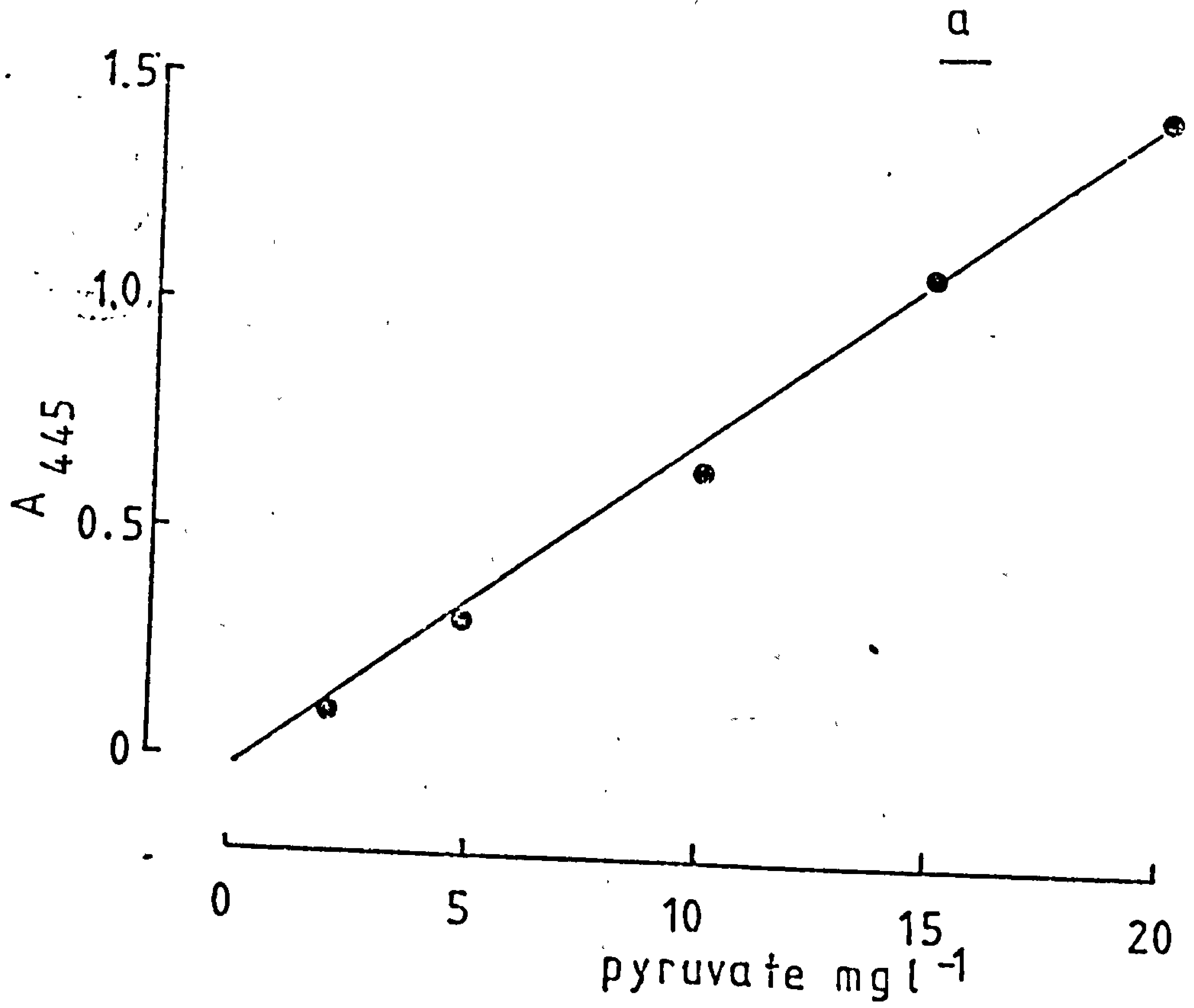
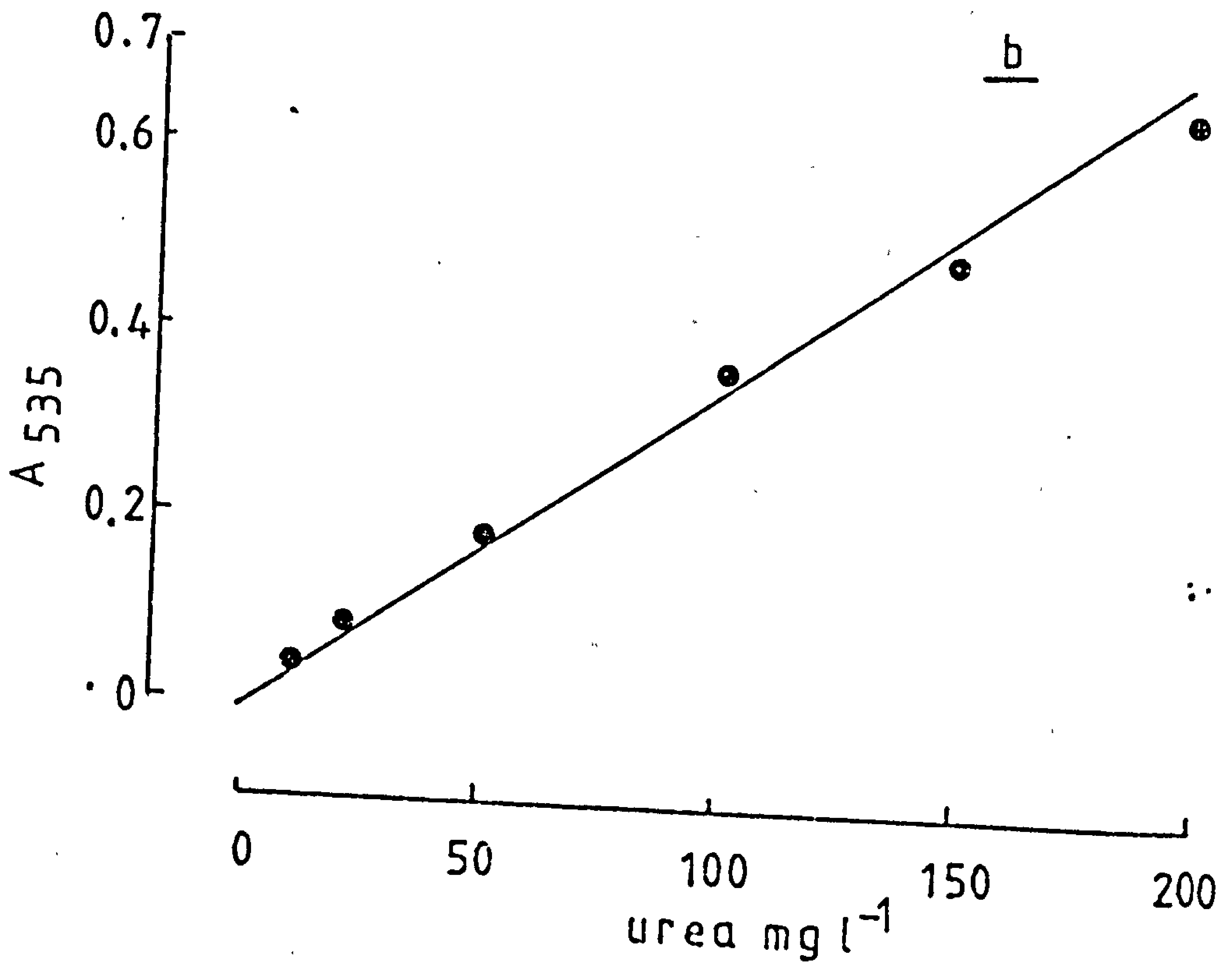

0.6
0.5
0.4
0.3
0.2
0.1
0
A 450
0
20
40
60
80
100
120
140
160
glucose mg l⁻¹

Figure 2.8

Calibration curves for measurement of pyruvate (a) and urea (b).


a
1.5
1.0
0.5
0
A 445
0
5
10
15
20
pyruvate mg l -1


b
0.7
0.6
0.4
0.2
0
A 535
0
50
100
150
200
urea mg l -1

2.8.2 Adsorption of Amino Acids

Radioactive (^{14}C) protein hydrolysate (Amersham International PLC) was diluted in distilled water to an activity of 1.0 μCi ml^{-1}. Aliquots (0.02 ml) of this solution were added to 10 ml glass tubes containing 1.78 ml of activated sludge and 0.2 ml of 2 g l^{-1} casitone solution. The mixture was shaken at 200 r.p.m. on a rotary shaker at 20°C. After 1, 10 and 30 minutes, samples were transferred into 1 ml Eppendorf tubes and centrifuged in a Jobling microcentrifuge for 2 minutes at approximately 2,000 g. Supernatant (25 μl) was added to 15 ml of Triton x 100/toluene/PPO scintillant and counted for 10 minutes in an Intertechnique SL30 liquid scintillation counter.

2.9 Adsorption of Alcian Blue by Pure Bacterial Cultures

Zoogloea ramigera (NCIB 10340) was grown at 30°C over 48 hours in 2 l conical flasks containing 500 ml of media of the following composition (g l^{-1}) arginine HCl 0.5, glucose 5, $MgSO_4.7H_2O$ 0.2, KH_2PO_4 1.0, $FeSO_4.5H_2O$ trace, cyanocobalamin 2 ng ml^{-1}, biotin 2 ng ml^{-1}. When the absorbance at 550 nm was approximately 1.0 the cells were harvested by centrifugation at 3,000 g for 20 minutes, washed twice with water and then made up to a volume of 50 ml in water. Dry weight was measured by filtration of duplicate 5 ml samples through preweighed Whatman GF/C filters. After drying at 105°C for three hours the filters were cooled in a desiccator and reweighed. The bacterial solution was then diluted to a solids concentration of 0.4 g l^{-1}.

Dye adsorption was then determined as in the standard method using 5 ml of dye solution and 45 ml of bacterial suspension.

Sphaerotilus natans (NCIB 11196) was grown at 30°C over 24 hours in 2 litre conical flasks containing 0.5 l of medium of the following composition (g l^{-1}); casitone 2, glycerol 5, proteose peptone 0.5, yeast extract 0.125, $MgSO_4.7H_2O$ 0.125. Harvesting of cells and preparation for dye adsorption measurement was as for Z.ramigera.

2.10 Extraction of Sludge Extracellular Polymers

The methods used were a modification of those of Forster (1976).

2.10.1 Total Material

Duplicate samples of 100 ml of activated sludge were poured into a 200 ml beaker, covered with aluminium foil and incubated at 100°C for exactly 1 hour. After cooling at room temperature for 15 minutes the sludge was transferred to 250 ml pots and centrifuged at 10,000 g for 40 minutes in a Beckman J2-21 centrifuge. Samples were allowed to cool to 15°C during centrifugation. To 1 volume of supernatant was then added 2 volumes of ethanol and the mixture left at 4°C overnight. Extracellular polymers were sedimented by centrifugation at 10,000 g for 40 minutes, resuspended in 20 ml ethanol and dried at 80°C in preweighed universal containers. Samples were allowed to cool in a desiccator under vacuum and then weighed. Dry

material was then resuspended in 5 ml water using a glass homogenizer for biochemical analysis.

2.10.2 Soluble material

Polymers in the liquid fraction of activated sludge were extracted using the following method. Duplicate samples (100 ml) of activated sludge were centrifuged at room temperature at 3,000 g for 15 minutes in a Beckman J2-21 centrifuge. Supernatant was decanted and filtered by vacuum through a Whatman GF/C filter followed by a Millipore cellulose acetate filter (pore size 0.45 μm). Ethanol insoluble material was then precipitated using the method described above.

The reproducibility of the above methods was assessed by extraction of 6 replicate samples of return activated sludge from Runcorn plant 1. The samples were processed in two batches of 3. Mean total extracted polymer was 20.66 mg ± a standard deviation of 1.22 or 6%. The mean soluble material was 18.12 mg ± a standard deviation of 1.16 or 6.4%. Soluble material was also estimated after heating for 1 hour at 100°C, giving a mean of 17.8 ± standard deviation of 0.55 or 3%. Using students t-test (Parker, 1979) there was no significant difference between heated and non-heated samples.

2.10.3 Bound Material

Where measurements were carried out on bound sludge polymers only, duplicate samples of activated sludge were centrifuged at 3,000 g for 15 minutes and the supernatant

discarded. The pellet was then made up to a volume of 100 ml in water before extraction of polymer as for total material above. Where extraction of total and soluble material was carried out, insoluble (bound) material was estimated by subtraction.

2.10.4 Sewage Polymer

Where sewage polymer was measured, one volume of settled sewage was added to 2 volumes of ethanol and stored overnight at 4°C. Polymer was then removed as above.

2.11 Investigation of Sewage and Sludge Mixing

Sewage and sludge were mixed using the following procedure. Volumes of 3 l of freshly collected return activated sludge were poured into a 10 l fermenter vessel and aerated by stirring at 250 r.p.m. After a period of 40 minutes a sample of 250 ml was removed, followed by addition of 2.75 l of fresh settled sewage. A further sample of 250 ml was immediately taken. Samples of 250 ml were then removed after 1, 2.5, 4, 5.5, 7 and 24 hours. The temperature of the vessel was maintained at 20°C. Samples were assessed for suspended solids, dye adsorption, polymer content and sludge activity. Supernatant COD was measured in 20 ml samples after centrifugation at 4,250 g for 15 minutes in a MSE bench centrifuge.

2.12 Biochemical Analysis

2.12.1 Carbohydrates

Total carbohydrate was measured by the anthrone method of Herbert *et al*. (1971). Samples and standards were pipetted into 10 ml test tubes, adjusted to a volume of 0.5 ml and placed on ice. This was followed by 2.5 ml of cold anthrone reagent (prepared by dissolving 200 mg of anthrone in 100 ml of 75% v/v H_2SO_4) and mixed rapidly by swirling in ice water for 5 minutes. The samples were then transferred to a boiling water bath and incubated for 10 minutes after which they were returned to an ice water bath. The absorption was measured at 625nm in a Cecil Spectrophotometer. Carbohydrate content was estimated from a calibration curve of 0-100 μg ml^{-1} glucose (Fig. 2.9a).

2.12.2 Protein

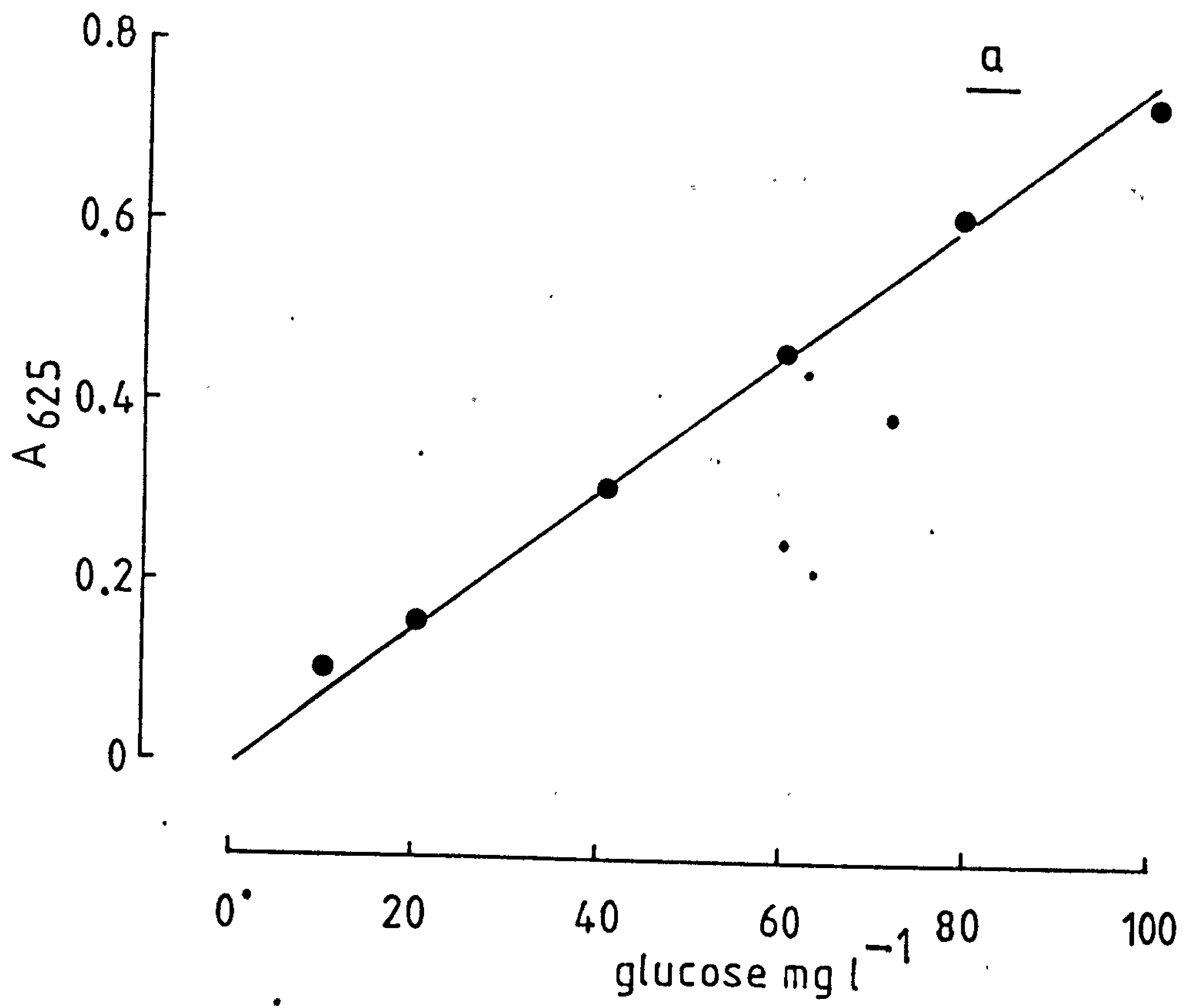
Protein content was determined using the method of Lowry *et al*. (1951). Absorbance values were converted to protein concentration by reference to a simultaneously prepared standard curve using bovine serum albumin.

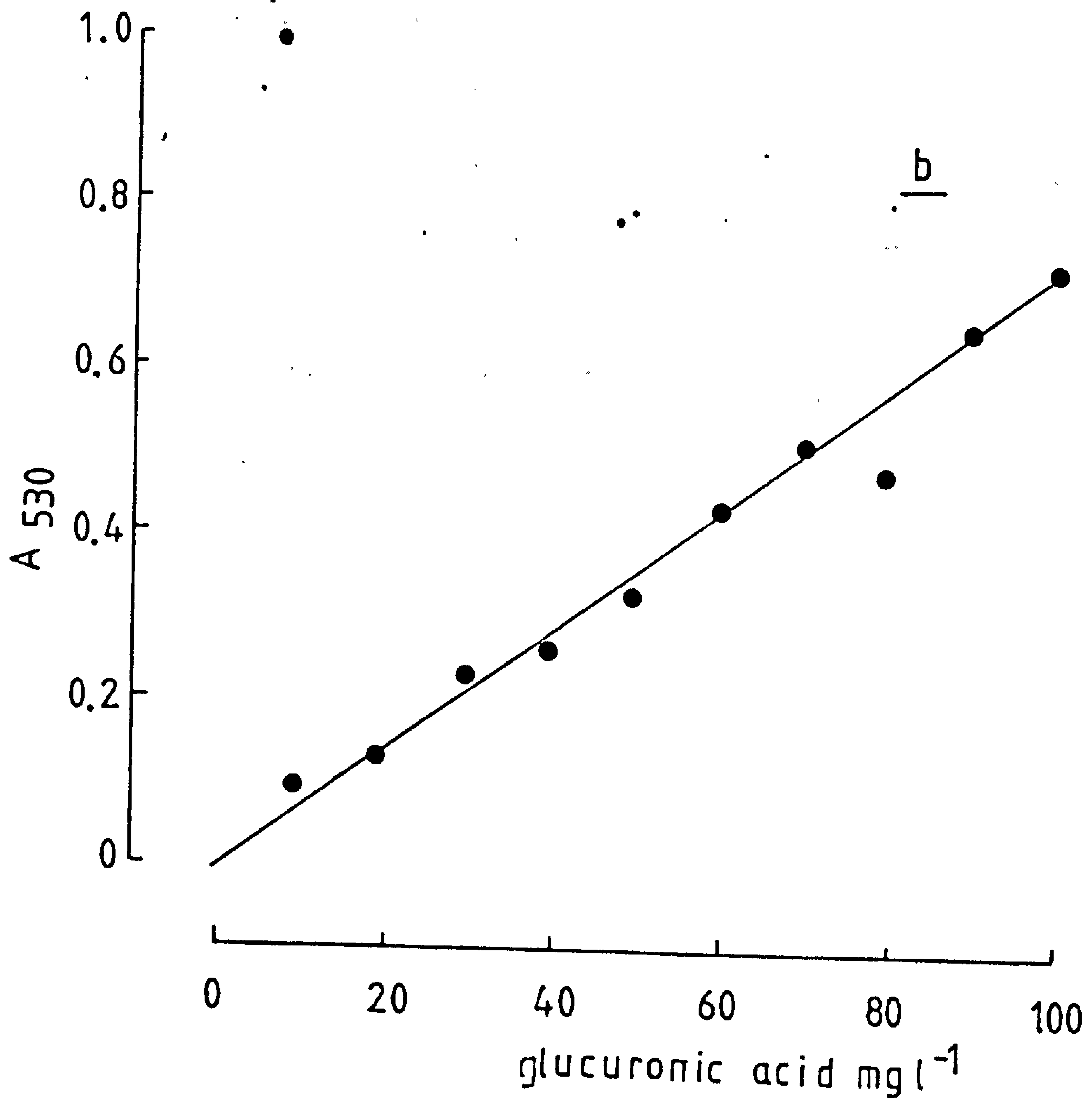
2.12.3 Hexuronic Acids

Hexuronic acids were measured using a modification of the method of Dische (1947). To 0.5 ml of sample or standard was added 2.5 ml of 0.025 M sodium tetraborate in concentrated sulphuric acid and the solutions placed in a boiling water bath for 10 minutes. After cooling to room temperature, 0.1 ml of

Figure 2.9

Calibration curve for measurement of hexose sugars (a) and uronic acids (b).


0.8
0.6
0.4
0.2
0
A 625
a
0
20
40
60
80
100
glucose mg l^{-1}


1.0
0.8
0.6
0.4
0.2
0
A 530
b
0
20
40
60
80
100
glucuronic acid mg l^{-1}

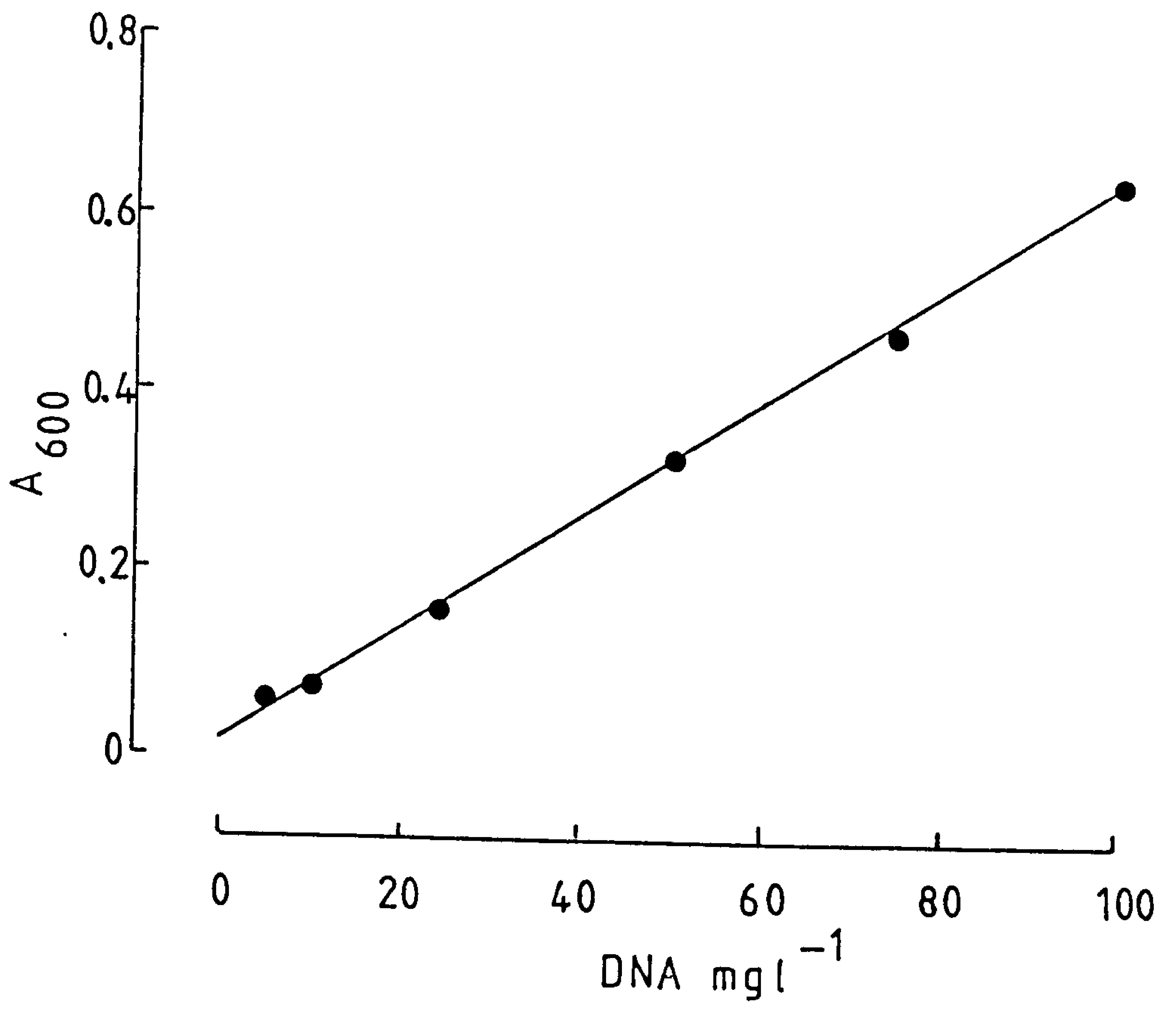
0.125% carbazole in ethanol was added, the solutions mixed and returned to the boiling water bath for a further 15 minutes. Samples were then cooled and the absorbance measured in a Cecil Spectrophotometer at 530 nm. Uronic acid content was estimated using a calibration curve of 0-100 mg l^{-1} glucuronic acid (Fig. 2.9b).

2.12.4 DNA

The method used was that of Burton (1956). To 0.5 ml of sample or standard was added 0.5 ml of 1 N perchloric acid followed by 2 ml of diphenylamine reagent (prepared by dissolving 1.5 g of diphenylamine in 100 ml glacial acetic acid and 1.5 ml concentrated sulphuric acid. Just before use 1 ml of aqueous acetaldehyde (16 mg ml^{-1}) was added). After incubation overnight at 30°C the absorbance at 600 nm was measured against a reagent blank. DNA content was determined from a calibration curve prepared using calf thymus DNA (Fig. 2.10).

Figure 2.10

Calibration curve for measurement of DNA.


0.8
0.6
0.4
0.2
0
A 600
0
20
40
60
80
100
DNA mg l −1

CHAPTER 3

Adsorption of Cationic Dyes by Activated Sludge : Development of a Standard Procedure

3.1 Description of Study Sites

3.1.1 Runcorn E.T.W.

At the Runcorn site there are two plants in operation both of which receive the same raw sewage. This is first comminuted and then passed through mechanically raked bar screens. Next the flow is passed through Dorr-Oliver detritors after which the sewage is split into plants 1 and 2. This is controlled automatically by a 'Peritol' system via two motorized penstocks. The split is approximately 60% to plant 1 and 40% to plant 2.

The major contributor to the plant influent is a large brewery which produces approximately 7.5 Ml waste per day, though this is subject to substantial variation. After pretreatment the BOD of the settled brewery discharge varies from 20 to 50 mg l^{-1}. This is much lower than the 200 mg l^{-1} for which the plant was designed. Consequently the Runcorn E.T.W. is underloaded.

Sludge from brewery pretreatment enters the works along with crude sewage and is removed in primary treatment. Fluctuations in brewery effluent volume may range from 0 to 70% of the sewage feed.

Flows in excess of 2 x Dry Weather Flow (D.W.F.) pass over a storm wier and into two storm tanks.

Details of primary settlement, aeration and final settlement tanks are given in Table 3.1. Mean performance and loading data for the period of April 1981 - March 1982 are shown in Table 3.2.

Table 3.1 Details of Activated Sludge Plants at Runcorn and Warrington South E.T.W.

	Runcorn Plant 1	Runcorn Plant 2	Warrington South
Primary settlement	Two circular tanks, diameter 33.5 m, capacity 2,920 m^3 each. Floor slope 7.5^o. Norstel Templewood Hawksley aluminium scrapers. Rotation speed 7 feet per minute. Moving half bridges.	One rectangular tank of 46.35 m x 16.5 m x 3.8 m (average depth). Total capacity 2,950 m^3. Scraper of Mieder type.	Two circular tanks diameter 24 m, capacity 1073 m^3 each. Floor slope 7.5^o. Templewood Hawksley scrapers. Moving half bridges.
Aeration Tanks	Two lanes of eight pockets, each of 9.75 m x 9.75 m x 3.9 m depth. Capacity of each pocket 343.1 m^3. Each pocket, fitted with Ames Crosta Mills Type "60" high intensity cone, individually driven by a 12.5 H.P. motor.	Two lanes of five pockets. The last three pockets in each lane have no dividing walls. Each pocket is 9 m x 9 m x 6.2 m deep. Capacity of each pocket is 468.7 m^3. Each pocket is fitted with an A.C.M. Type "80" high intensity cone, individually driven by a 20 H.P. motor.	Two lanes of three pockets. The final two pockets have no dividing wall. Each pocket is of 11 m x 11 m x 4.5 m deep, capacity 544.7 m^3. Each pocket is fitted with A.C.M. high intensity aeration cone driven by an 11 kw motor.
Final Sedimentation Tanks	Four tanks each of 19.6 m diameter, side wall depth 1.5 m. Maximum depth 6.8 m floor slope 30°. Total capacity 3972 m^3. N.T.H. aluminium half bridge chain scrapers. Rotation speed 4 feet per minute	Four tanks of similar dimension and capacity to plant 1. A.C.M. boom type scrapers.	Two circular tanks of radius 9.85 m, floor slope 15°, maximum depth 4.92 m Total capacity 2146 m^3. Templewood Hawkesley activated sludge half bridges with bladed scrapers.

Table 3.2 Plant Performance and Loading Data (April 1981 - March 1982)

	Runcorn E.T.W.		Warrington South E.T.W.
Population	70,000		22,000
Population equivalent (based on 0.06 Kg BOD $capita^{-1}$)	96,100		17,900
Design D.W.F. Ml d^{-1}	27.0		9.0
Actual D.W.F. Ml d^{-1}	18.9		3.3
Average flow to full treatment Ml d^{-1}	21.9		4.87
Crude sewage : BOD mg l^{-1}	264		275
Suspended solids mg l^{-1}	270		264
	Plant 1	Plant 2	
Primary Treatment			
Settled sewage output : BOD mg l^{-1}	131	-	131
Suspended solids mg l^{-1}	97	-	97
Detention time hrs	10.9	7.7	11.0
% Removal : BOD	50	-	42
Suspended solids	64	-	63
Aeration			
Hydraulic loading m^3 m^2 day^{-1}	4.7	4.9	3.02
Sludge loading Kg BOD Kg $MLSS^{-1}$ day^{-1}	0.23	0.27	0.15
Detention time hrs	5.1	4.9	8.5
Effluent BOD mg l^{-1}	10	14	10.2
Suspended solids mg l^{-1}	13	20	13
NH_4^+ mg l^{-1}	15	21	-
NO_3^- mg l^{-1}	4.9	3.0	-
% Removal BOD	9.2	89	92
Suspended solids mg l^{-1}	87	79	87
% of effluent samples outside consent (30:45)	2.7	7.2	4.6
Activated Sludge			
Mixed liquor suspended solids mg l^{-1}	2120	1923	1922
Return activated sludge solids mg l^{-1}	3302	2983	2590
Mixed liquor SV1 ml g^{-1}	311	421	328
Sludge age days	4.5	3.9	6.9
Final Tanks			
Detention time hrs	7.4	10.4	11.4

Sludge Aeration

Plant 1   Only one of the two aeration lanes is currently in operation. Until the Summer of 1982 the plant was operated as in Fig. 3.1a. However, due to an incident of sludge bulking, a step loading pattern was introduced with sewage entering at pockets 2, 3, 4, 5 and 6 depending upon the flow rate (Fig. 3.1b). The volume of sewage entering at each pocket was not measured.

Plant 2   The flow pattern of plant 2 is also shown in Fig. 3.1. Until the Summer of 1982 the plant was operated as in Fig. 3.1c. However, due to an incident of sludge bulking the pattern was changed to that of Fig. 3.1d. There is no return sludge reaeration in this plant. When operating as in Fig. 3.1d the majority of settled sewage enters at pocket 1, with smaller amounts in pockets 2 and 3. The volume of sewage entering at each aeration pocket was not measured.

3.1.2 Warrington South E.T.W.

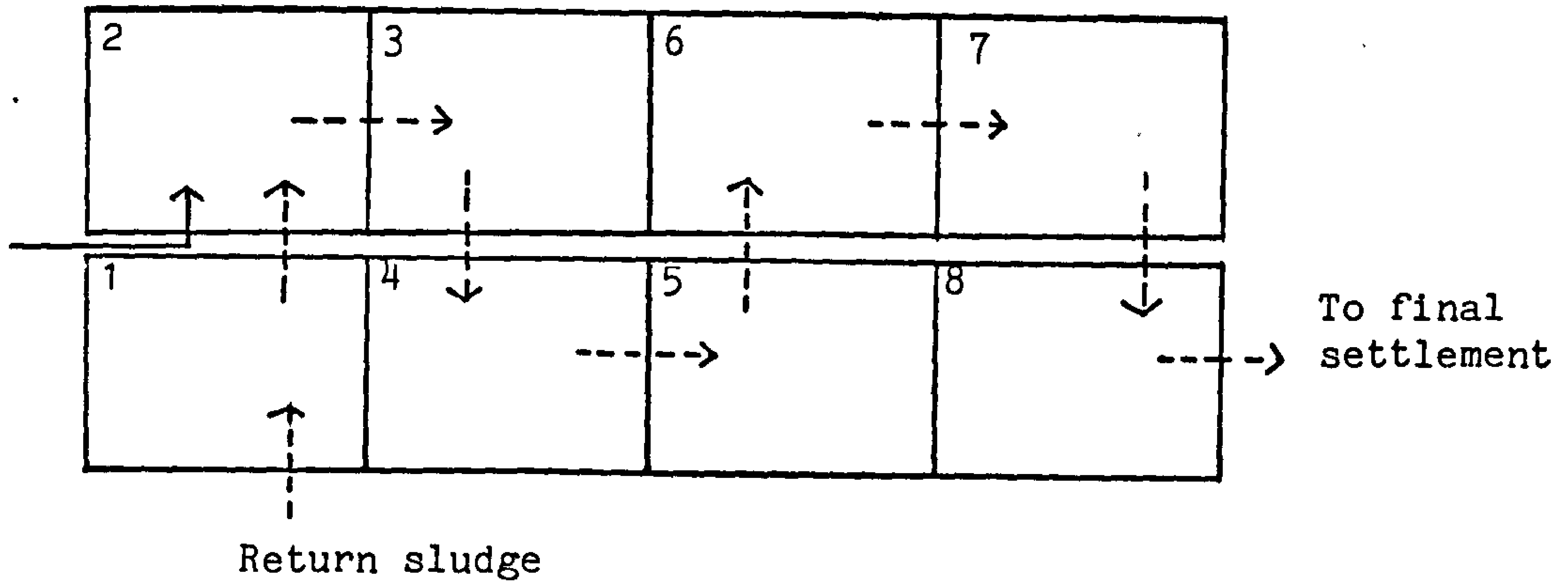
Sewage flow to this works is all pumped, arriving by way of one of two pumping stations. The works treats domestic sewage plus a limited quantity of trade effluents from the Warrington area. Details of primary settlement, aeration and final settlement tanks are given in Table 3.1. Mean performance and loading data for the period April 1981 to March 1982 are given in Table 3.2.

Figure 3.1

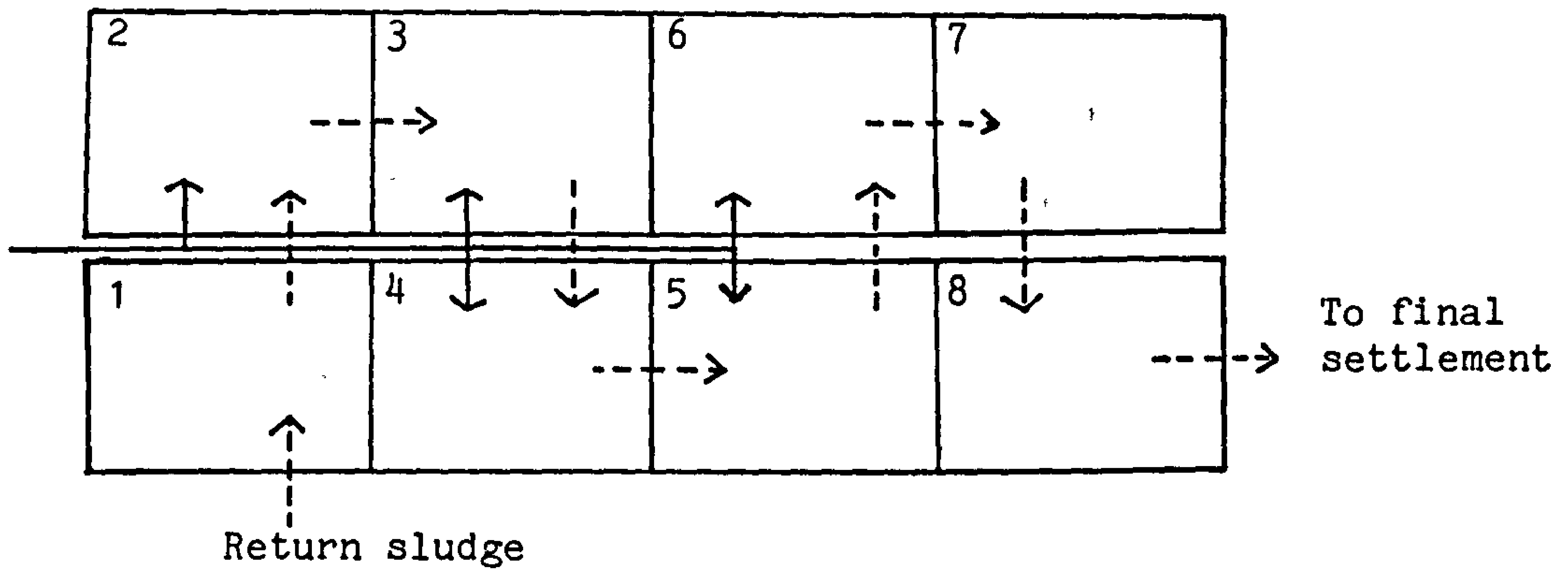
Patterns of aeration of activated sludge at Runcorn plant 1 (a and b) and plant 2 (c and d). Broken arrows (- - ->) show direction of sludge flow and solid arrows (——>) sewage flow. Broken lines between aeration tanks indicate hydraulic division.

Figure 3.1

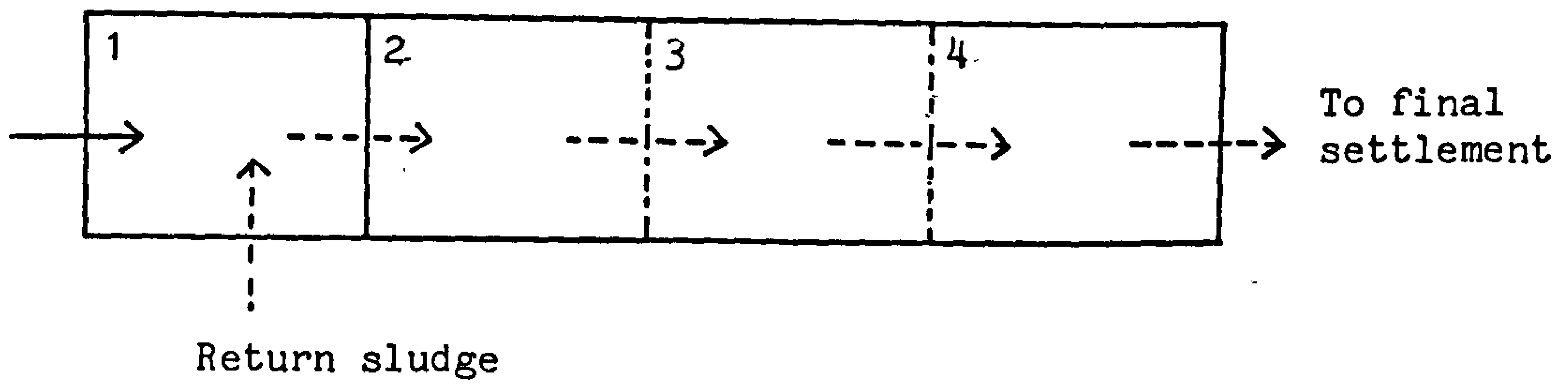
(a)



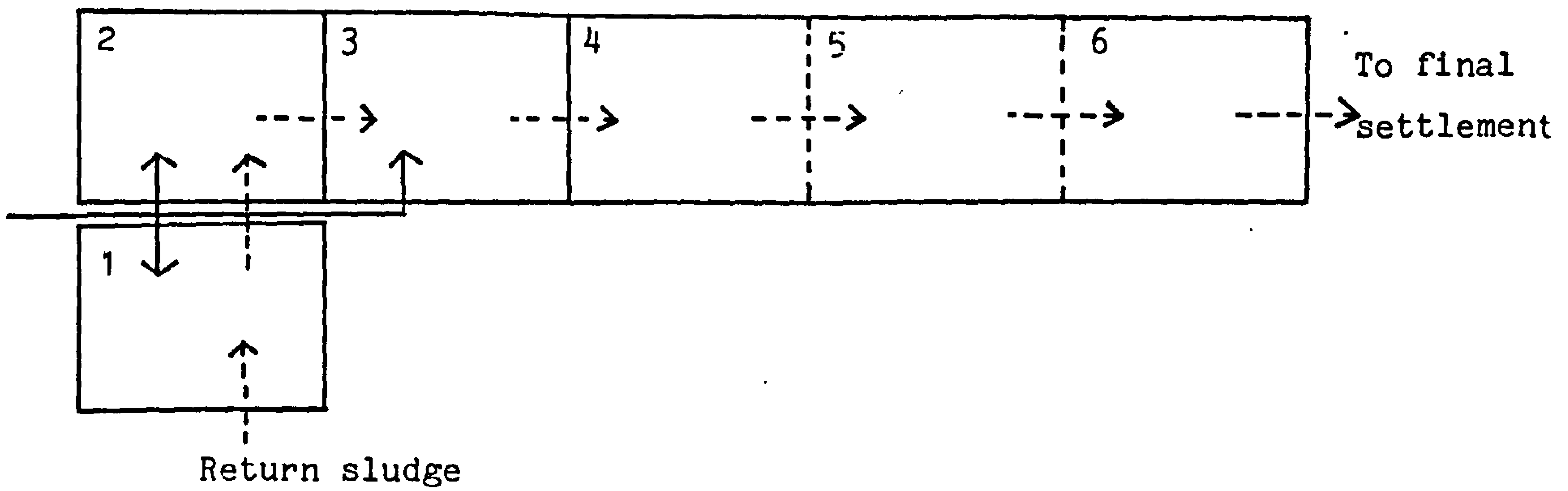
(b)



(c)



(d)



Sewage is first screened by automatically raked bar screens after which it passes through Door Detritors. The sewage then enters the primary sedimentation tanks.

The flow pattern in the aeration tanks is shown in Fig. 3.2.

3.1.3 Sludge Disposal

In all three works, activated sludge is returned by two Spaars screw pumps. Sludge is surplussed daily for about four hours at Warrington South and six hours at Runcorn.

At Warrington South E.T.W., waste sludge passes to one of two covered sludge consolidation tanks. Top water is decanted off and returned to the post-preliminary stage of treatment. Periodically, consolidated sludge passes to the storage tanks at Runcorn E.T.W. where the sludge is stored along with Runcorn sludge until ultimate sea disposal.

Top water from the storage tanks at Runcorn is also returned to the works, usually at night to prevent overloading.

3.1.4 Effluent

Purified effluent from all three plants passes into the Manchester Ship Canal.

3.2 Adsorption of Nutrients by Activated Sludge

Fig. 3.3 shows the adsorption and uptake by return activated sludge from Runcorn plant 1 and Warrington South of (a)

Figure 3.2

Pattern of aeration of activated sludge at Warrington South E.T.W. Broken arrows (- - ➤) show flow of activated sludge and solid arrows (⟶) sewage flow.

Figure 3.2

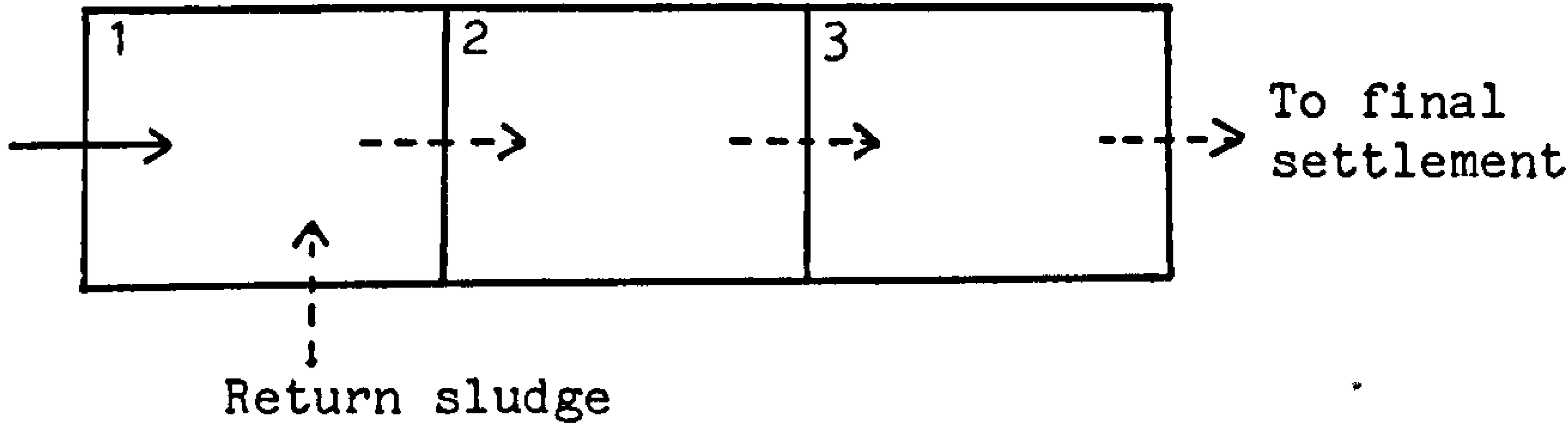
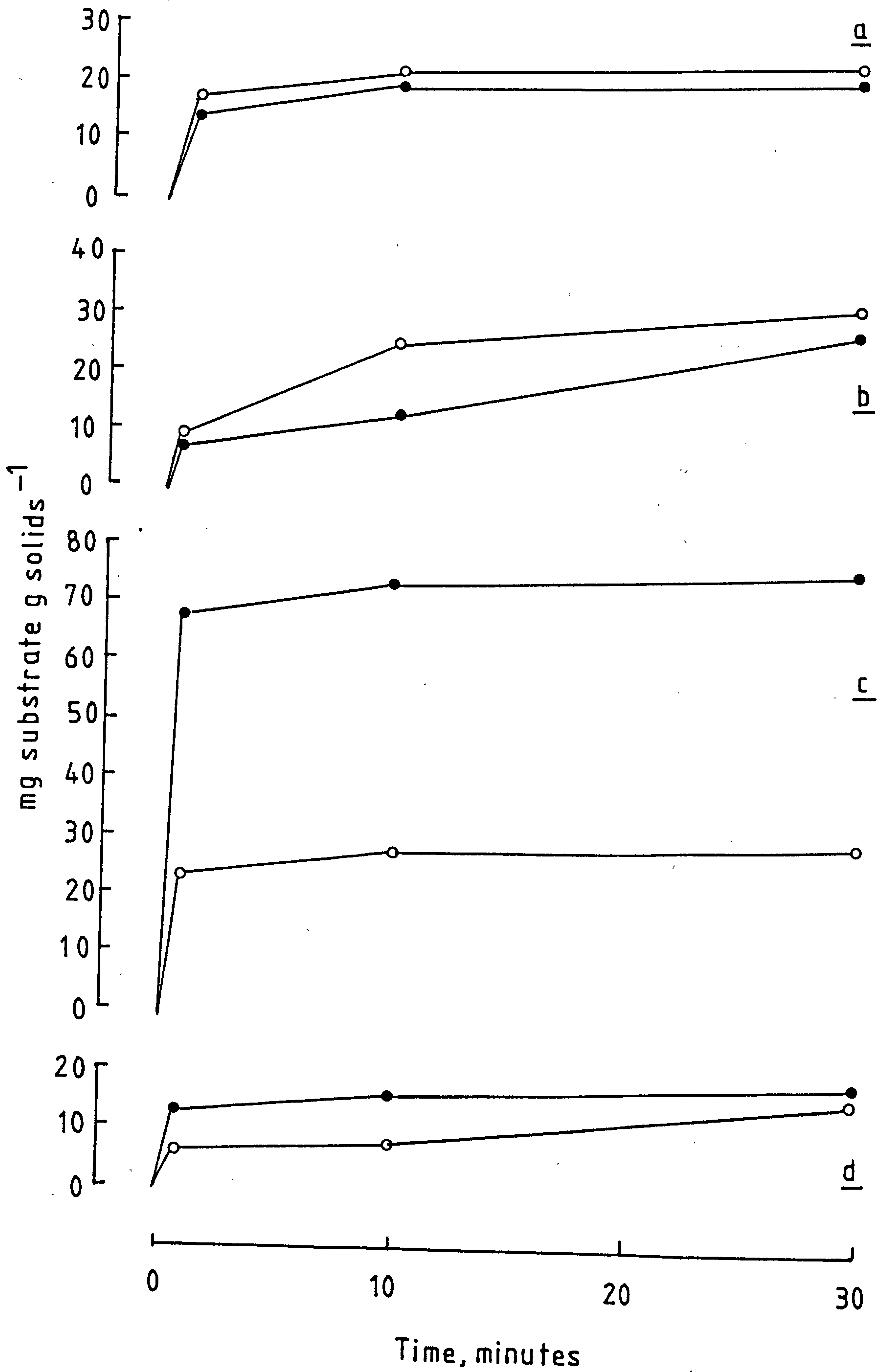


Figure 3.3

Adsorption of casitone (a), glucose (b), pyruvate (c) and urea (d) by return activated sludge. Initial substrate concentration was 200 mg l^{-1}. Suspended solids concentration was 2.74 g l^{-1} for Runcorn 1 sludge (o) and 1.47 g l^{-1} for Warrington South sludge. (•)



a
b
c
d
mg substrate g solids^{-1}
30
20
10
0
40
30
20
10
0
80
70
60
50
40
30
20
10
0
20
10
0
0
10
20
30
Time, minutes

casitone, (b) glucose, (c) pyruvate,and (d) urea. All nutrients were added at a concentration of 200 mg l^{-1}. In all cases there was a measurable amount of substrate adsorption after only 1 minute. This was highest for pyruvate and casitone. Clear differences exist between adsorption capacities of the two sludges particularly with regard to the adsorption of pyruvate.

Table 3.3 shows the above data as percentage substrate removal. In no case was more than 55.2% of substrate removed after 30 minutes.

The substrate removal occurring in one minute as a proportion of the uptake after 30 minutes was dependent upon the nature of the substrate. For casitone and pyruvate this value was high: 66.7% and 90.6% for Warrington South sludge and 75.4% and 79.4% for Runcorn 1 sludge respectively. Conversely, for substrates glucose and urea, values were comparatively low: 22% and 71.2% for Warrington South sludge and 24.8% and 39.8% for Runcorn 1 sludge respectively. These results suggests that for charged molecules, adsorption to the activated sludge surface is the primary means by which substrate is removed from the medium.

3.3 Cationic Dye Adsorption by Activated Sludge

The importance of adsorption in substrate uptake having been demonstrated, the adsorption of cationic dyes by activated sludge was evaluated as an indicator of adsorption capacity. Initially, the dynamics of the sludge/dye interaction were assessed.

Table 3.3 Adsorption of nutrients by activated sludge. Results are expressed as % substrate removal. In each case initial substrate concentration was 200 mg l^{-1}. Suspended solids concentration was 2.74 g l^{-1}, for Runcorn 1 sludge and 1.47 g l^{-1} for Warrington South sludge.

	Casitone			Glucose			Pyruvate			Urea		
Time (minutes) / Source of sludge	1	10	30	1	10	30	1	10	30	1	10	30
Warrington South E.T.W.	10.3	15.4	15.4	4.4	10.3	19.8	50.0	54.2	55.2	9.19	11.8	12.9
Runcorn E.T.W. plant 1	23.3	27.5	30.9	10.9	34.3	43.9	31.6	37.1	39.8	8.2	9.6	20.6

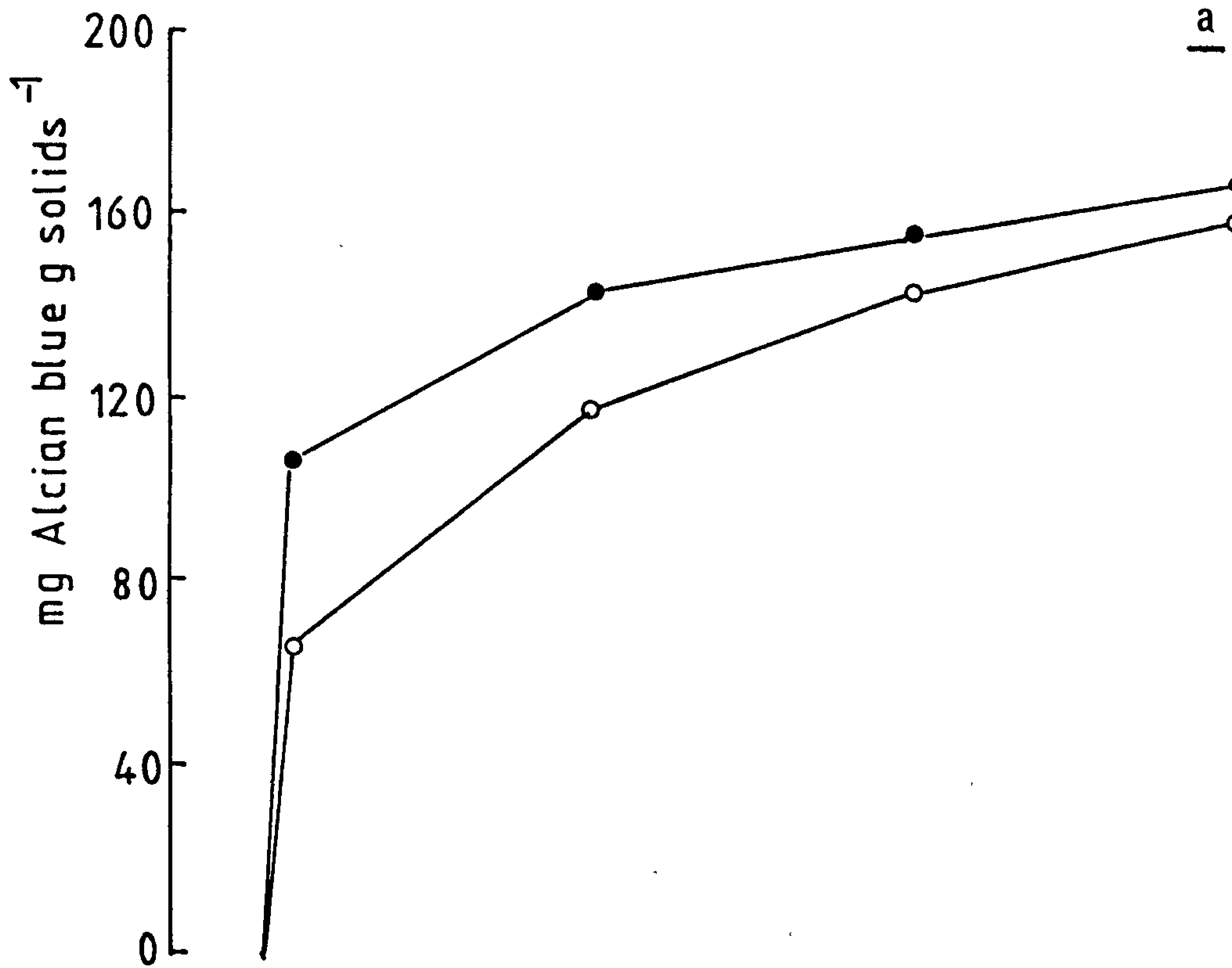
3.3.1 Time Taken to Reach Equilibrium

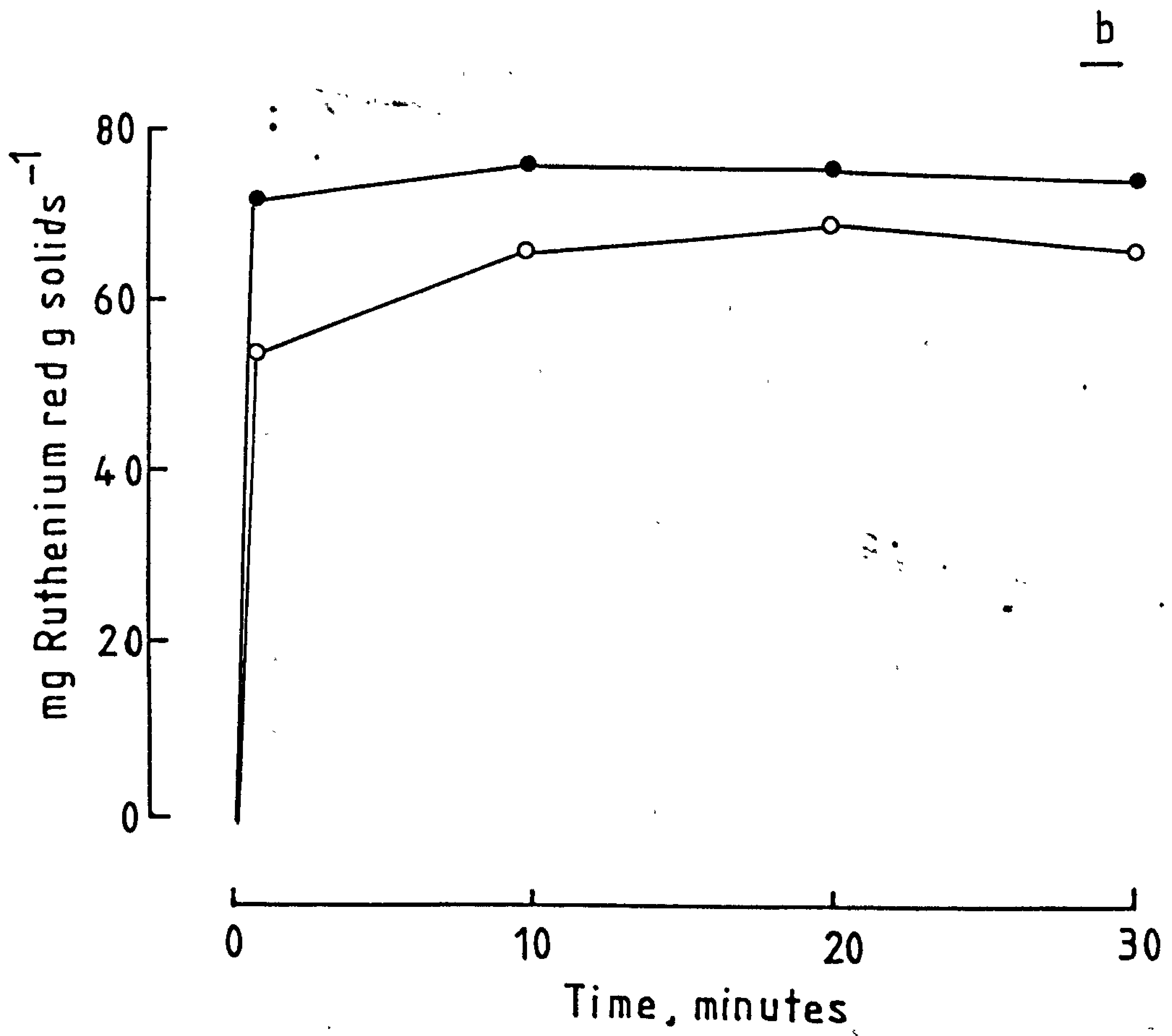
The adsorption of Alcian blue and Ruthenium red by Runcorn 1 and Warrington South return activated sludge was assessed. These results can be seen in Fig. 3.4. Initial suspended solids concentration was 2.03 g l^{-1} for Runcorn 1 sludge and 1.72 g l^{-1} for Warrington South sludge. Each sludge was diluted to 5% of the original suspended solids concentration for the adsorption study. Alcian blue concentration was 17.4 mg l^{-1} or 176.1 mg g^{-1} for Runcorn 1 sludge and 202.2 mg g^{-1} for Warrington South sludge. Ruthenium red concentration was 8.8 mg l^{-1} or 87.2 mg g^{-1} and 102.7 mg g^{-1} for each sludge respectively. After 30 minutes, 75.7% of Ruthenium red and 81.6% of Alcian blue were adsorbed by sludge from Warrington South. In all cases a large proportion of dye adsorption occurred within 1 minute, with values varying from 40.2% to 96.7% of the adsorption after 30 minutes. These results are comparable with values obtained for the adsorption by sludge of pyruvate and casamino acids.

Adsorption by Warrington South sludge was apparently more rapid than by Runcorn 1 sludge. In addition, binding of Ruthenium red occurred at a faster rate than Alcian blue with maximum adsorption of the former after 10 minutes. Alcian blue binding continued for at least 30 minutes. An incubation time of ten minutes was chosen for further experimental work.

Figure 3.4

Adsorption of Alcian blue (a) and Ruthenium red (b) by 5% return activated sludge from Runcorn plant 1 (o) and Warrington South (•).


a
mg Alcian blue g solids^{-1}
200
160
120
80
40
0


b
mg Ruthenium red g solids^{-1}
80
60
40
20
0
0
10
20
30
Time, minutes

3.3.2 Effect of Dye Concentration on Dye Adsorption (Sludge solids concentration approximately 0.1 g l^{-1})

The adsorption of Ruthenium red and Alcian blue by 5% return activated sludge is shown in Fig. 3.5. Initial suspended solids concentrations for sludges used in Ruthenium red adsorption were 2.21 g l^{-1}, 2.33 g l^{-1} and 1.69 g l^{-1} for Runcorn 1, Runcorn 2 and Warrington South sludges respectively. At maximum dye concentrations, 35.8, 47.7 and 33.5% of dye were removed. For Alcian blue adsorption, initial suspended solids concentrations were 3.31 g l^{-1} for Runcorn 1 sludge, 2.87 g l^{-1} for Runcorn 2 sludge and 2.68 g l^{-1} for Warrington South sludge. At maximum dye concentration, 53.9, 48.1 and 38.0% of dye was removed by each sludge respectively. Results were fitted to Langmuir, Freundlich (Table 3.4) and mass action equations (Table 3.5).

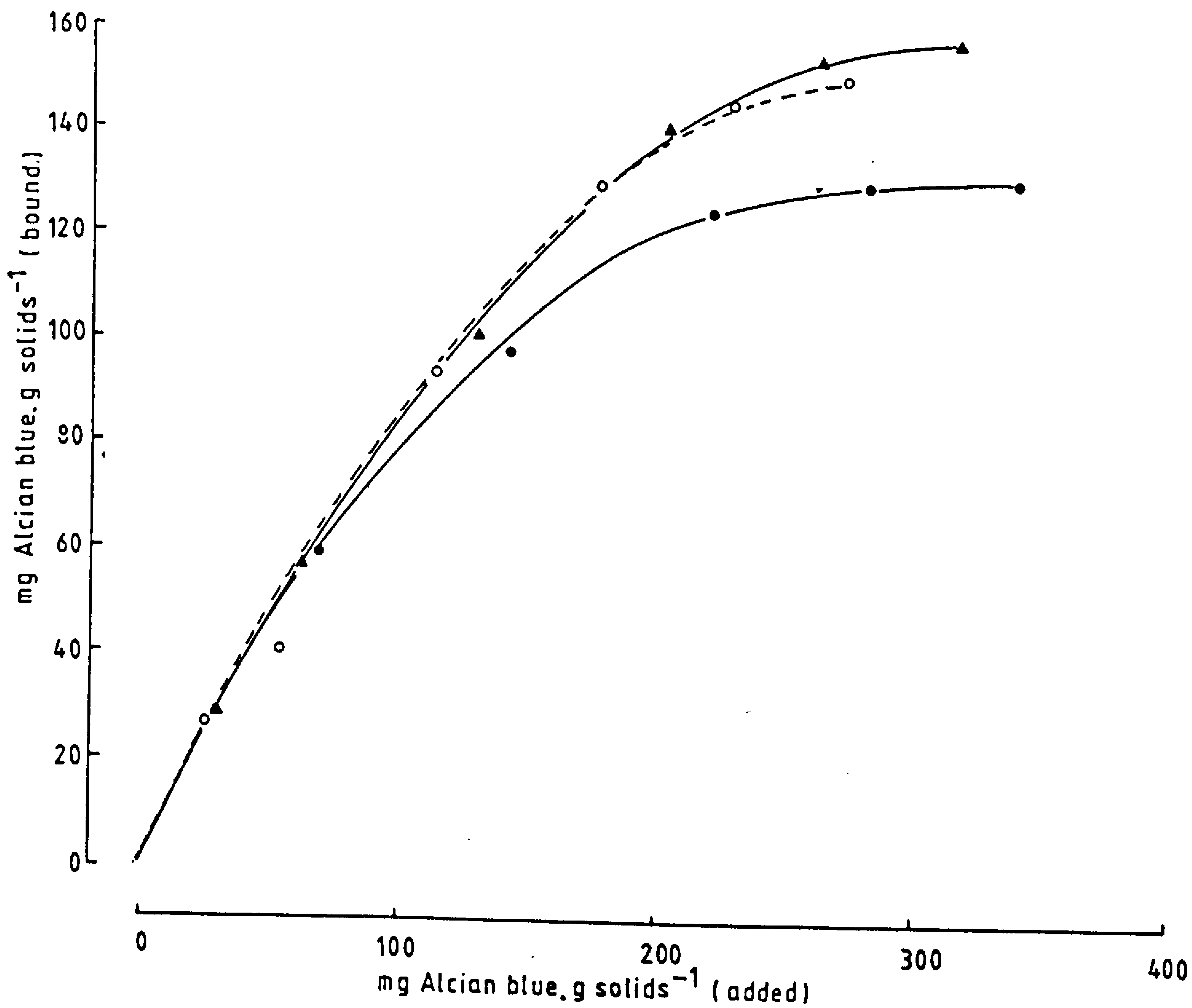
Clear differences in the adsorption capacity between sludges are evident. A plateau in adsorption was found above concentrations of 75 mg g sludge^{-1} for Ruthenium red and 250 mg g^{-1} for Alcian blue. Thus when using a solids concentration of 0.1 g l^{-1}, the minimum dye concentration required in a routine test is 7.5 mg l^{-1} for Ruthenium red and 25 mg l^{-1} for Alcian blue. In the case of Alcian blue, the adsorption plateau's were reached at approximately 280, 320 and 290 mg g^{-1} for Runcorn 1, Runcorn 2 and Warrington South sludges respectively. Taking the relative molecular mass of Alcian blue as 1301.9 (Scott, 1972a) this is equal to an adsorption capacity

Figure 3.5

Effect of dye concentration on dye adsorption (solids concentration approximately 0.1 g l^{-1}).

(a) Adsorption of Alcian blue by 5% return activated sludge. Initial suspended solids concentrations were 3.31 g l^{-1} for Runcorn 1 sludge (○), 2.87 g l^{-1} for Runcorn 2 sludge (▲) and 2.68 g l^{-1} for Warrington South sludge (●).

(b) Adsorption of Ruthenium red by 5% return activated sludge. Initial suspended solids concentrations were 2.22 g l^{-1} for Runcorn 1 sludge (○), 2.33 g l^{-1} for Runcorn 2 sludge (▲) and 1.70 gl^{-1} for Warrington South sludge (●).


a
160
140
120
100
80
60
40
20
0
mg Alcian blue.g solids^{-1} (bound)
0
100
200
300
400
mg Alcian blue.g solids^{-1} (added)

b

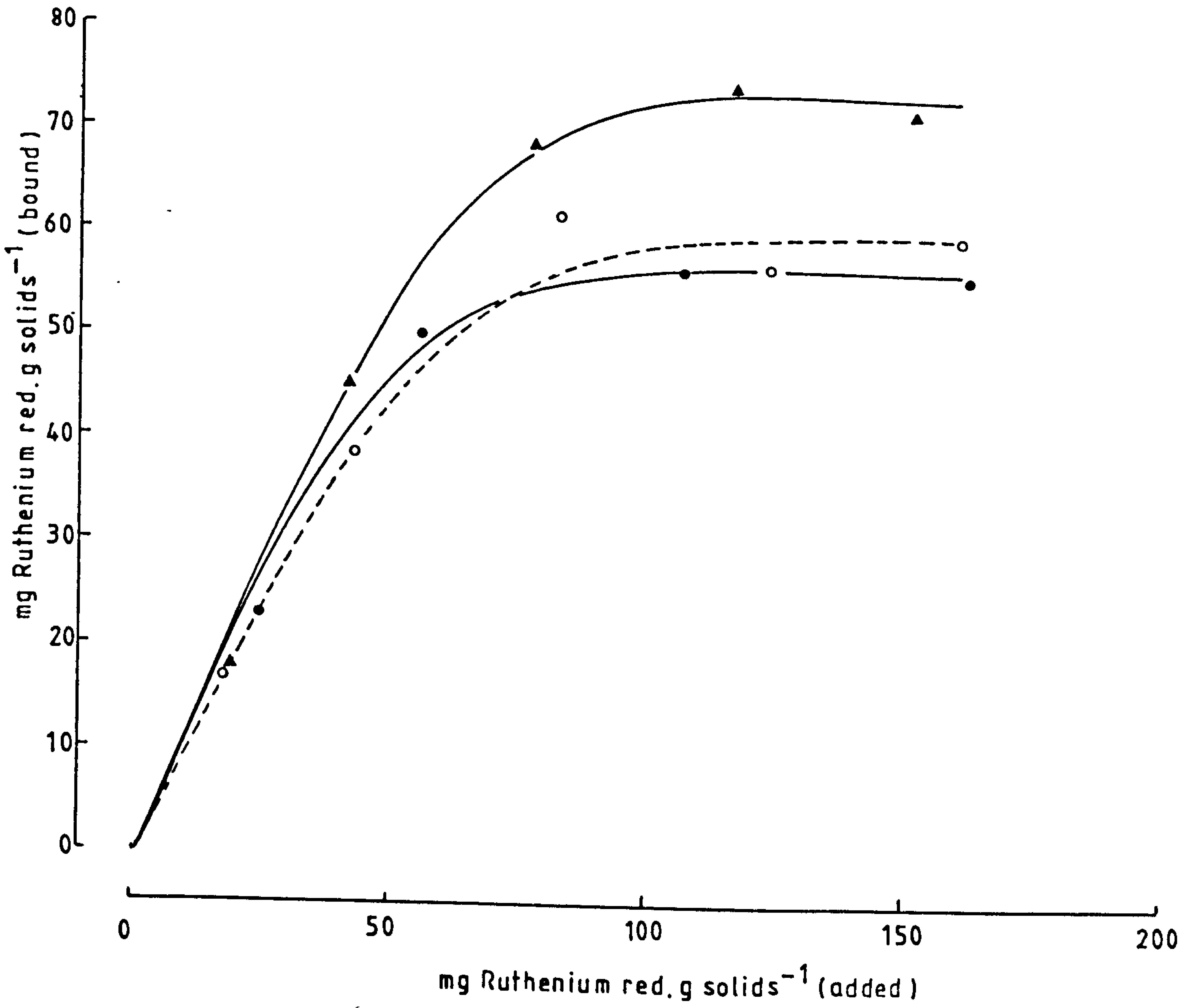


Table 3.4 Correlation of activated sludge and dye adsorption isotherms with Langmuir and Freundlich equations

			LANGMUIR EQUATION ($x = C$, $y = C/m$)					FREUNDLICH EQUATION ($x = \log_{10}C$, $y = \log_{10}m$)				
			Regression	r (df)	Signif.	Constants		Regression	r (df)	Signif.	Constants	
						K_1	K_2				$1/n_1$	K_3
ALCIAN BLUE	5% RAS	Runcorn 1	y = 5.138x + 27.9	0.972 (3)	<0.01	0.195	0.184	y = 0.503x - 1.42	0.887 (3)	<0.1	0.503	0.038
		Runcorn 2	y = 6.16x + 5.83	0.999 (4)	<0.001	0.162	1.057	y = 0.303x - 1.16	0.956 (4)	<0.005	0.303	0.069
		Warrington South	y = 7.30x + 11.52	0.998 (4)	<0.001	0.136	1.578	y = 0.268x - 1.23	0.994 (4)	<0.001	0.268	0.059
	50% RAS	Runcorn 1	y = 2.08x + 25.0	0.998 (2)	<0.005	0.481	0.083	y = 0.103x - 0.62	0.994 (2)	<0.01	0.103	1.239
		Runcorn 2	y = 3.68x + 37.7	0.999 (3)	0.001	0.271	0.098	y = 0.099x - 0.84	0.995 (3)	<0.005	0.099	0.144
		Warrington South	y = 3.15x + 40.4	0.999 (4)	<0.001	0.317	0.078	y = 0.168x - 0.95	0.987 (4)	<0.001	0.168	0.112
RUTHENIUM RED	5% RAS	Runcorn 1	y = 16.54x + 7.92	0.998 (3)	<0.001	0.060	2.088	y = 0.313x - 1.49	0.844 (3)	<0.1	0.313	0.032
		Runcorn 2	y = 13.71x + 1.31	0.999 (3)	0.001	0.072	10.46	y = 0.24x - 1.30	0.885 (3)	<0.05	0.240	0.050
		Warrington South	y = 17.83x + 2.24	0.999 (2)	0.001	0.056	7.96	y = 0.199x - 1.39	0.875 (2)	NS	0.199	0.041
	50% RAS	Runcorn 1	y = 15x + 51.5	1.0 (4)	<0.001	0.067	0.291	y = 0.176x - 1.57	0.955 (4)	<0.005	0.176	0.027
		Runcorn 2	y = 21.1x + 138.6	1.0 (4)	<0.001	0.047	0.152	y = 0.174x - 1.74	0.976 (4)	<0.001	0.174	0.018
		Warrington South	y = 22.31x + 38.9	1.0 (4)	<0.001	0.045	0.573	y = 0.102x - 1.57	0.923 (4)	<0.01	0.102	0.027

Table 3.5 Correlation of activated sludge and dye isotherms with the mass action equation. $x = 1/m$; $y = 1/D$

			Regression	r (df)	Signif.	β	σ'	K_B ($\times 10^5$)	σ ($\times 10^{-6}$)
Alcian blue	5% RAS	Runcorn 1	$y = 2.69x + 14.49$	0.87 (4)	<0.05	2.690	0.069	19.900	0.186
		Runcorn 2	$y = 2.28x + 8.13$	0.955 (4)	<0.005	2.280	0.123	13.171	0.333
		Warrington South	$y = 2.51x + 9.34$	0.958 (4)	<0.005	2.510	0.107	13.690	0.291
	50% RAS	Runcorn 1	$y = 0.319x + 2.70$	0.969 (2)	<0.05	0.319	0.370	31.285	1.002
		Runcorn 2	$y = 6.11x + 4.09$	0.947 (2)	<0.1	6.115	0.244	2.474	0.661
		Warrington South	$y = 3.53x + 3.86$	0.976 (3)	<0.05	3.529	0.259	4.036	0.702
Ruthenium red	5% RAS	Runcorn 1	$y = 14.96x + 12.66$	0.964 (3)	<0.01	14.957	0.079	1.619	0.413
		Runcorn 2	$y = 2.38x + 11.76$	0.967 (3)	<0.01	2.380	0.085	9.506	0.442
		Warrington South	$y = 3.89x + 16.67$	0.994 (2)	<0.01	3.890	0.060	8.187	0.314
	50% RAS	Runcorn 1	$y = 19.85x + 16.67$	0.984 (4)	<0.001	19.850	0.060	1.604	0.314
		Runcorn 2	$y = 34.4x + 7.09$	0.971 (4)	<0.005	34.400	0.141	1.358	0.214
		Warrington South	$y = 12.63x + 22.73$	0.994 (4)	<0.001	12.630	0.044	3.442	0.230

of 215.0, 245.7 and 222.7 μmol dye g sludge^{-1} for the three respective sludges. For Ruthenium red the adsorption plateau's occurred at approximately 59.0, 73.5 and 56.0 mg dye g sludge $^{-1}$ for Runcorn 1, Runorn 2 and Warrington South sludges respectively. This is equal to an adsorption capacity of 68.7, 85.6 and 65.2 μmol dye g sludge^{-1} for the three respective sludges. Sludge samples for the adsorption studies were taken on different days, thus comparisons between Ruthenium red and Alcian blue binding are not direct. However, it is clear from the results that the binding of Alcian blue is in the order of 3 to 4 fold that of Ruthenium red.

These results compare well with those of Smith and Coackley (1983) and Andreadakis (1978) who found that maximum adsorption of the anionic dye Lissamine scarlet 4R to be within the range of 70-180 μmol.g sludge^{-1}. However, values for Alcian blue binding were found to be lower than those of Banks _et al_. (1976) who found a range of values between 0.30 and 1.1 g g^{-1} for sludges from ten activated sludge works and also a range of similar order by Hall (1982b). Devloo _et al_. (1983) found still lower values for sludge from a laboratory activated sludge plant, ranging from 0.072 mg g^{-1} to 0.12 mg g sludge^{-1}.

3.3.3 Effect of Sludge Solids Concentration on Dye Adsorption (0.5 - 10% sludge)

Figs. 3.6 and 3.7 show the effect of solids concentration on the binding of Ruthenium red and Alcian blue at constant dye

Figure 3.6

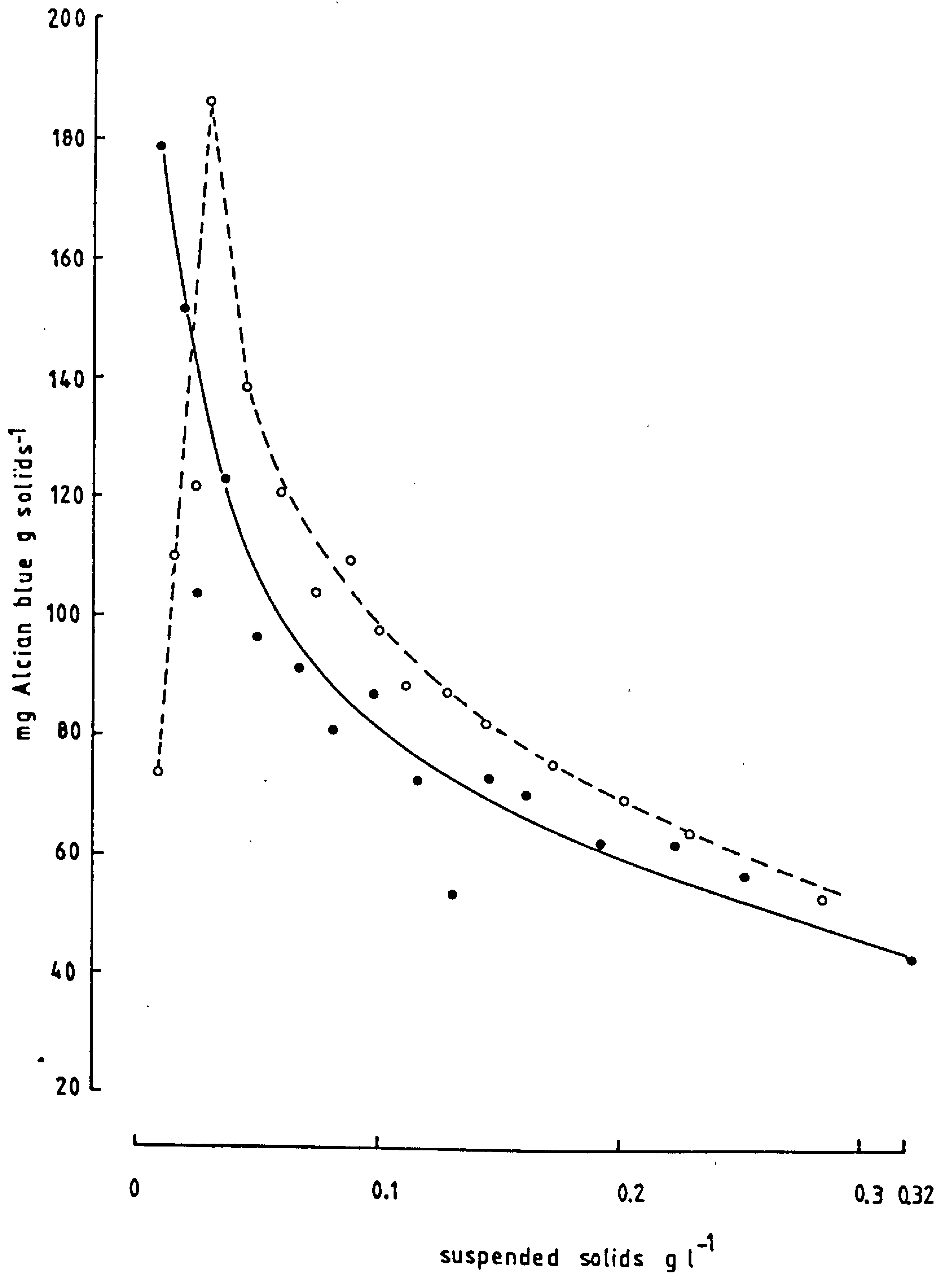
Effect of sludge solids concentration (0.5-10% sludge) on Alcian blue adsorption.

Initial sludge solids concentrations were 2.84 g l^{-1} for Runcorn 1 sludge (○), and 3.19 g l^{-1} for Warrington South sludge (●). Alcian blue concentration was 16.14 mg l^{-1} or 56.7 mg g^{-1} and 50.6 mg g^{-1} for the two respective sludges at the highest solids concentration.

(a) Results presented as mg dye adsorbed g $solids^{-1}$ against solids concentration.

(b) Results presented as % dye adsorbed against solids concentration.

a



b

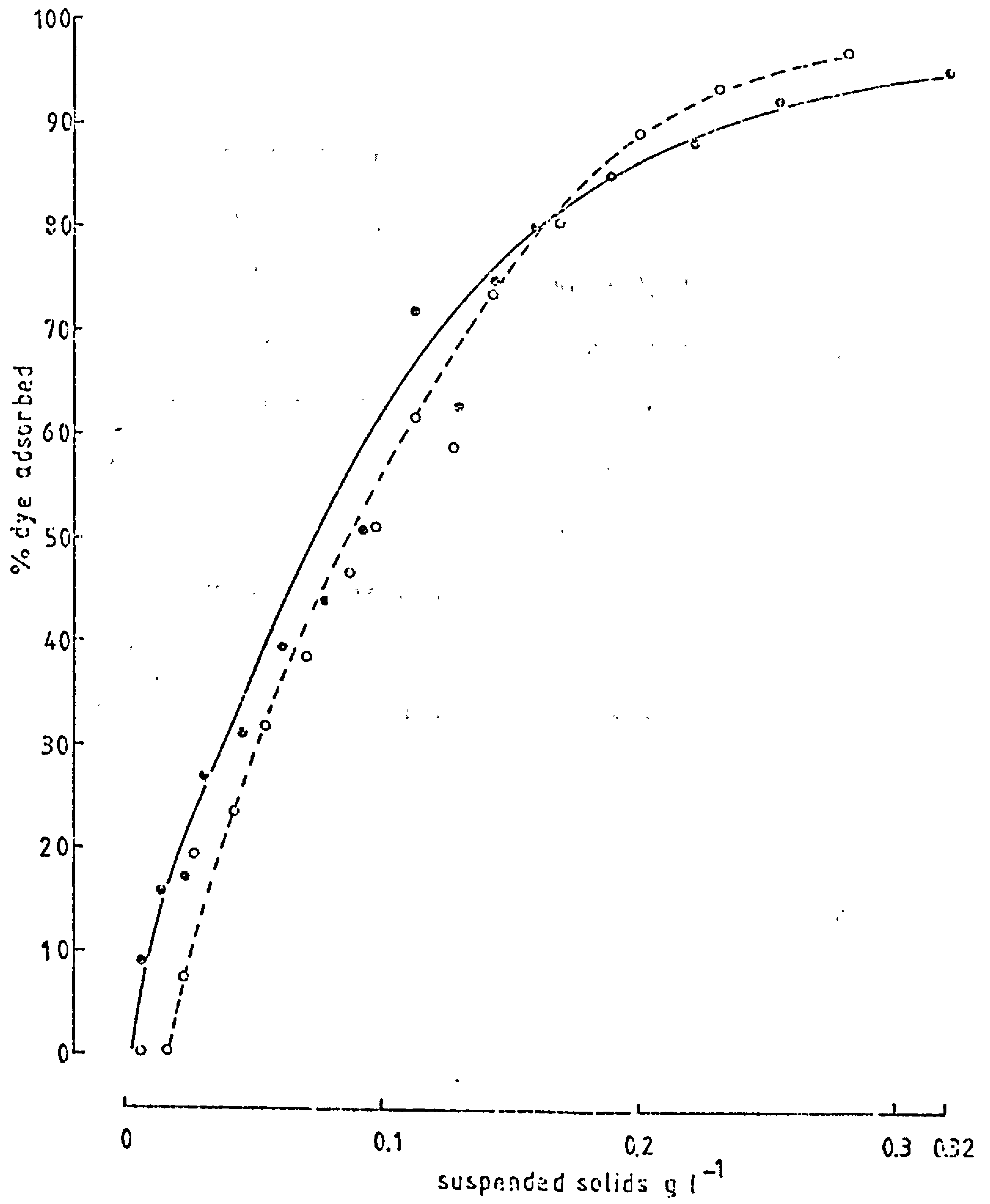


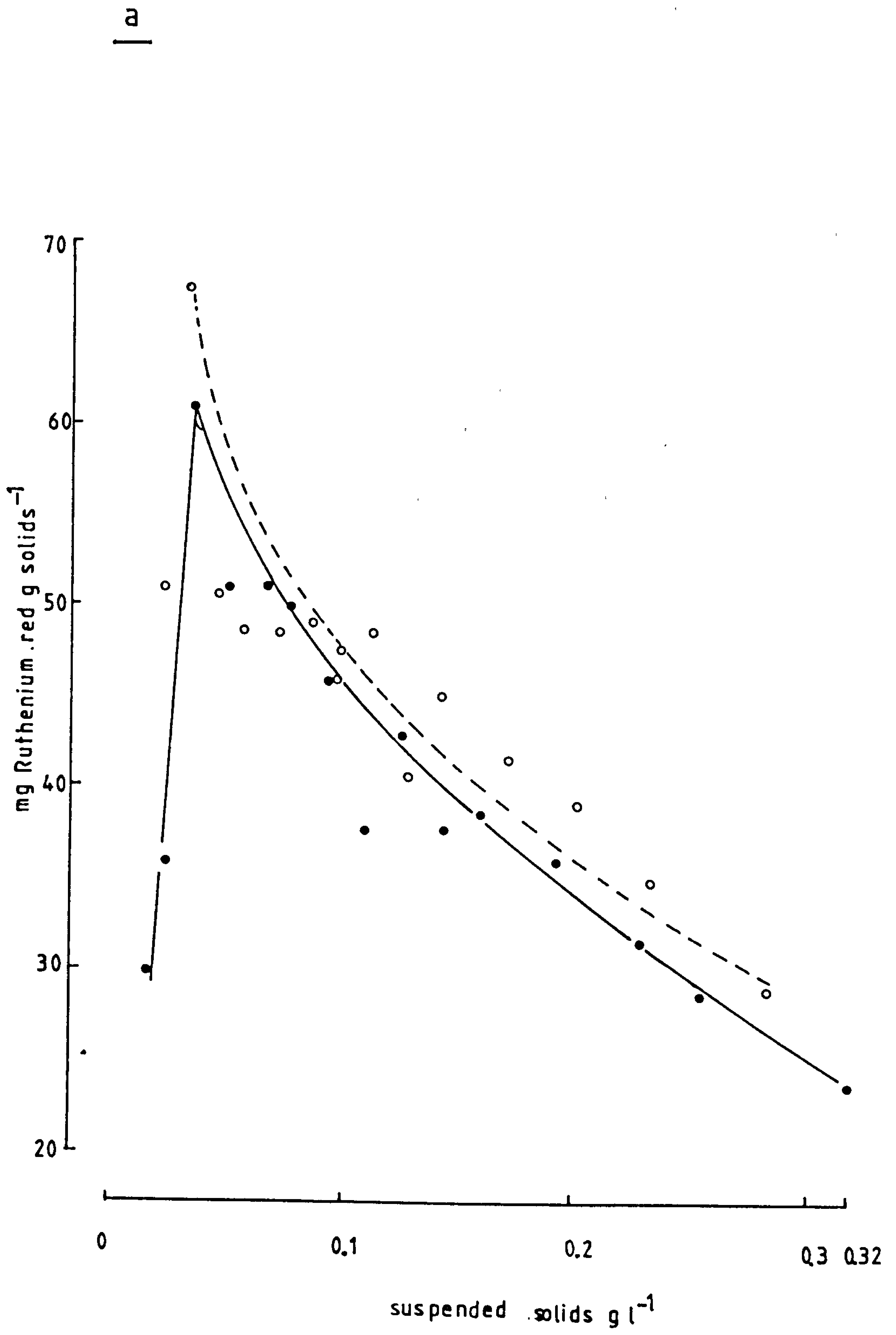
Figure 3.7

Effect of sludge solids concentration (0.5-10% sludge) on Ruthenium red adsorption.

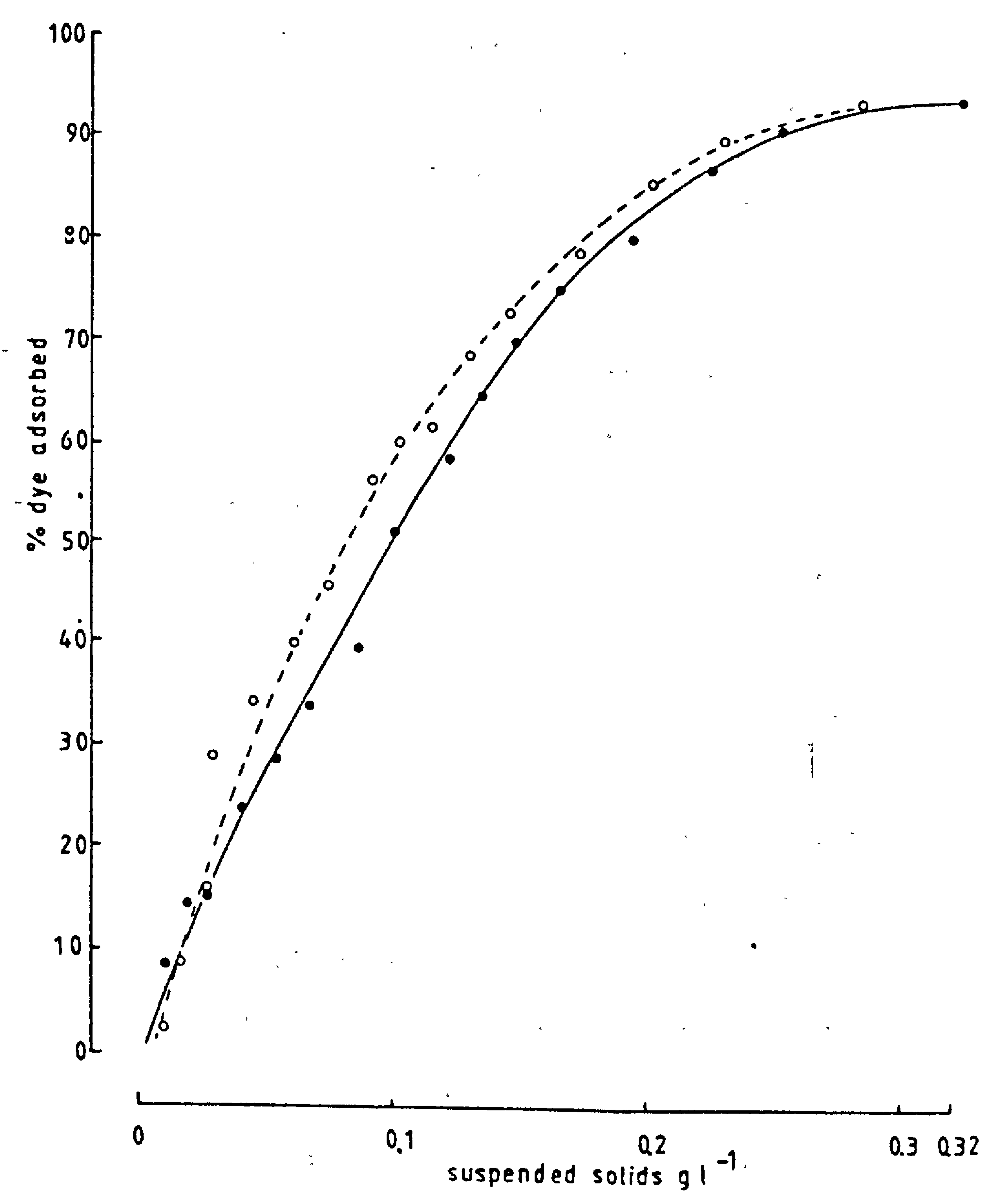
Initial sludge solids concentrations were 2.84 g l^{-1} for Runcorn 1 sludge (o), and 3.19 g l^{-1} for Warrington South sludge (●). Ruthenium red concentration was 8.75 mg l^{-1} or 30.76 mg g^{-1} and 27.43 mg g^{-1} for Runcorn 1 and Warrington South sludge respectively at the maximum solids concentration.

(a) Results presented as mg dye adsorbed g $solids^{-1}$ against solids concentration.

(b) Results presented as % dye adsorbed against solids concentration.


a
70
60
50
40
30
20
mg Ruthenium red g solids^{-1}
0
0.1
0.2
0.3
0.32
suspended solids g l^{-1}

b



concentrations. For studies with both dyes, initial sludge solids concentrations were 2.84 gl^{-1} for Runcorn 1 sludge and 3.19 g l^{-1} for Warrington South sludge. Ruthenium red concentration was 8.75 mg l^{-1} or 30.76 mg g^{-1} and 27.43 mg g^{-1} for Runcorn 1 and Warrington South sludge respectively at the maximum solids concentration tested. Alcian blue concentration was 16.14 mg l^{-1} or 56.7 mg g^{-1} and 50.6 mg g^{-1} for the two respective sludges at the highest solids concentration.

In all cases dye binding increased with sludge dilution, presumably due to the greater accessability of dye binding sites brought about by dilution and deflocculation. This is examined further in section 3.3.5. At extremely low solids concentrations below approximately 0.05 g l^{-1} a peak in adsorption was found which is difficult to explain in terms of dye adsorption kinetics. It is likely that at this point measurement of dye adsorption is beyond the resolution of the methods used.

The effect of dilution on binding is acute only below 0.1 g l^{-1}. This was the solids concentration used by Banks *et al.* (1976) and it was decided to use this in initial investigations. This is approximately equal to 5% return activated sludge.

3.3.4 Effect of Dye Concentration on Dye Adsorption (Sludge Solids Concentration Approximately 1.0 g l^{-1})

The adsorption of Ruthenium red and Alcian blue by 50% return activated sludge is shown in Fig. 3.8. In both cases,

Figure 3.8

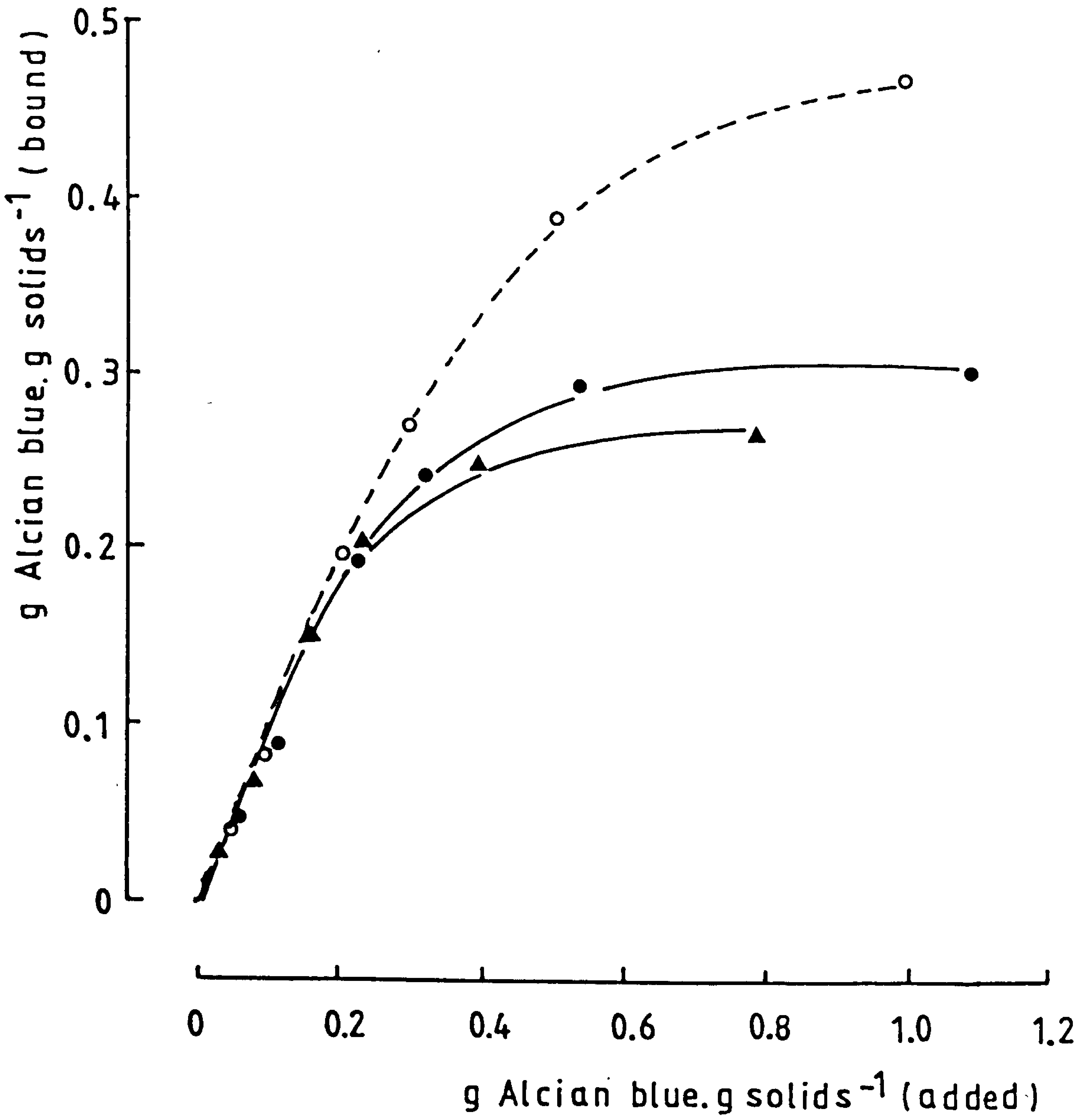
Effect of dye concentration on dye adsorption (solids concentration approximately 1.0 g l^{-1})

Initial suspended solids concentrations were 2.20 g l^{-1} for Runcorn 1 sludge (o), 2.78 g l^{-1} for Runcorn 2 sludge (▲) and 2.02 g l^{-1} for Warrington South sludge (●).

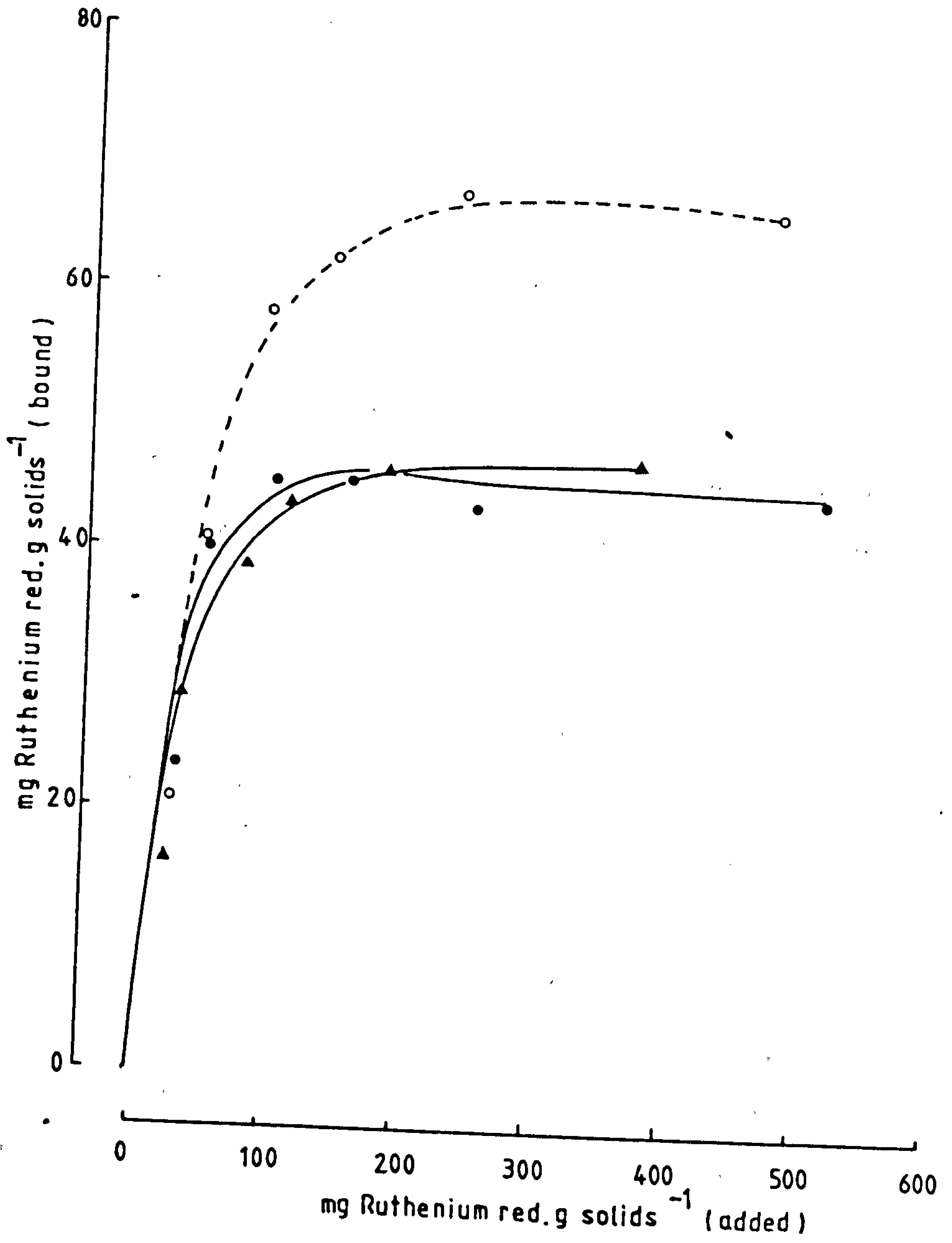
(a) Adsorption of Alcian blue.

(b) Adsorption of Ruthenium red.

a



b



initial suspended solids concentrations for Runcorn 1, Runcorn 2 and Warrington South sludges were 2.20 g l^{-1}, 2.78 g l^{-1} and 2.03 g l^{-1} respectively. In the study of Ruthenium red adsorption, 13.75, 12.2 and 8.4% of dye was removed by Runcorn 1, Runcorn 2 and Warrington South sludge at the maximum dye concentration. For Alcian blue adsorption these values were 46.0, 82.5 and 29.2% respectively.

Clear differences in dye adsorption by different sludges are evident. Maximum adsorption of Ruthenium red was 68.5, 45.8 and 45.8 mg g $sludge^{-1}$ for Runcorn 1, Runcorn 2 and Warrington South sludges respectively. This is equal to 79.8, 53.3 and 53.3 µmol dye g $sludge^{-1}$. In the case of Alcian blue, maximum adsorption capacity was 475, 272 and 312 g g $sludge^{-1}$, equal to 364.8, 208.9 and 240.0 µmol.g^{-1} for Runcorn 1, Runcorn 2 and Warrington South sludges respectively.

For Ruthenium red there was a plateau in adsorption above a dye concentration of approximately 150 mg g $sludge^{-1}$. In the case of Alcian blue this value was 400 mg g $sludge^{-1}$. Thus for measurement of dye adsorption capacity by 50% sludge these would ideally be the minimum dye concentrations required.

The ratio of Alcian blue binding to Ruthenium red binding was 4.6:1, 3.9:1, and 4.5:1 for Runcorn 1, Runcorn 2 and Warrington South sludge respectively. Assuming 2.9 charged groups per Alcian blue molecule (Scott, 1973b) and six per molecule of Ruthenium red (Luft, 1971) then these values are higher than the expected ratio of 2.06:1. The fact that the ratio

of binding remained fairly constant over a range of adsorption capacities indicates that binding sites on sludge for Alcian blue and Ruthenium red remain in constant proportions on different sludges.

According to the classification of Giles _et al._ (1960) isotherms for the binding of both Alcian blue and Ruthenium red by activated sludge fall into the category of L (Langmuir)2. This type of isotherm is found where there is no strong competition from the solvent for sites at the surface of the adsorbent. As more sites on the floc surface are filled it becomes increasingly difficult for the bombarding dye molecules to find a vacant site available.

Data was fitted to the Freundlich, Langmuir (Table 3.4) and mass action equations (Table 3.5). The data fits the Langmuir equation exceptionally well with rather less correlation with mass action and Freundlich equations. For adsorption of these dyes to activated sludge, the Freundlich equation can be rejected.

3.3.5 Effect of Sludge Solids Concentration on Dye Adsorption (10-80% sludge)

The effect of solids concentration on the adsorption of Ruthenium red and Alcian blue at a constant dye concentration is shown in Figs. 3.9 and 3.10. For both studies the initial solids concentrations were 3.09 g l^{-1} for Runcorn 1 sludge and 1.68 g l^{-1} for Warrington South sludge. Ruthenium red

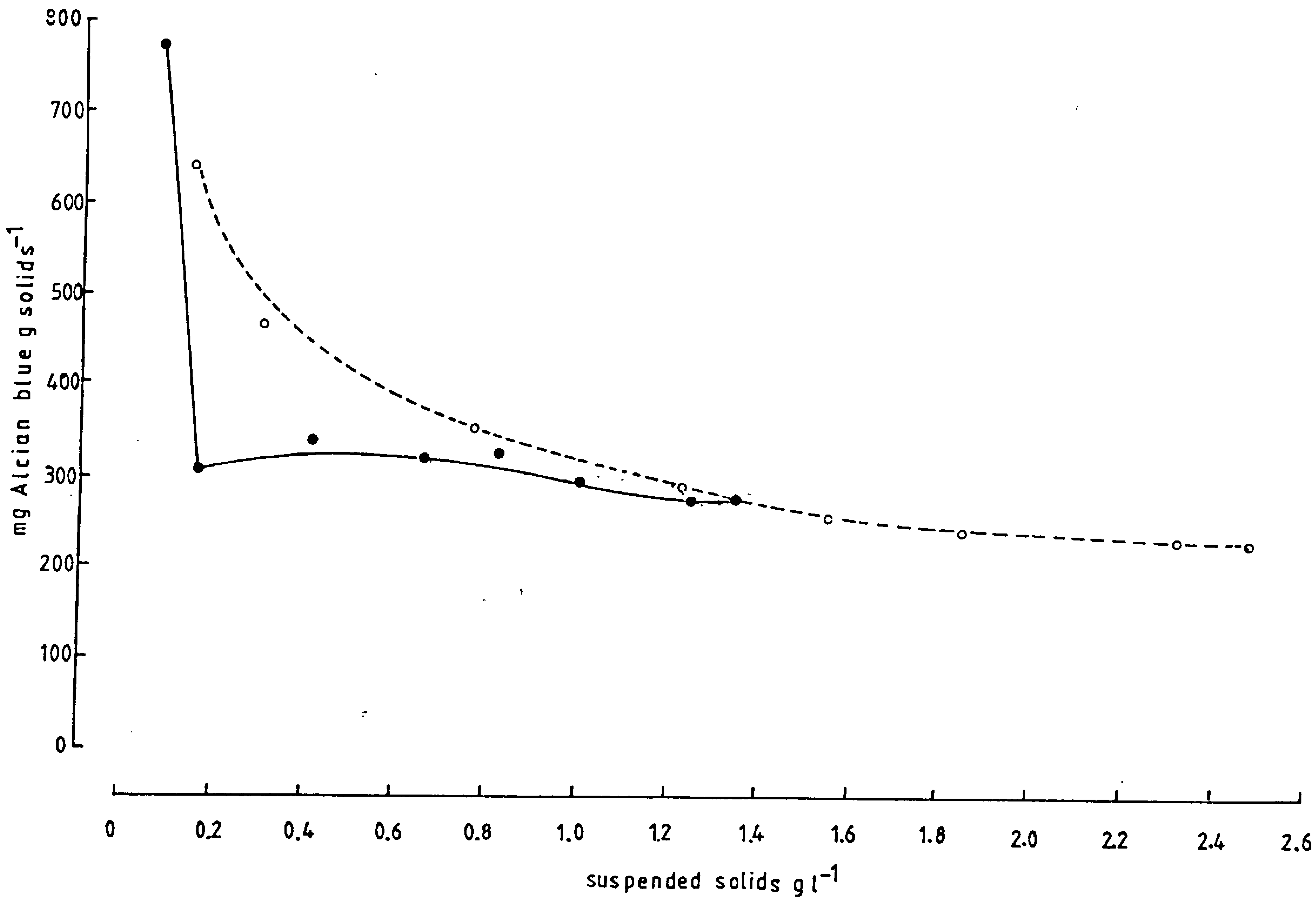
Figure 3.9

Effect of solids concentration (10-80% sludge) on Alcian blue adsorption.

Initial sludge solids concentrations were 3.09 g l^{-1} for Runcorn 1 sludge (○), and 1.68 g l^{-1} for Warrington South sludge (●). Alcian blue concentration was 611 mg l^{-1} or 247 and 453 mg g solids^{-1} for the respective sludges at the highest solids concentration.

(a) Results presented as mg dye adsorbed g solids^{-1} against solids concentration.

(b) Results presented as % dye adsorbed against solids concentration.


a
800
700
600
500
400
300
200
100
0
mg Alcian blue g solids^{-1}
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
suspended solids g l^{-1}

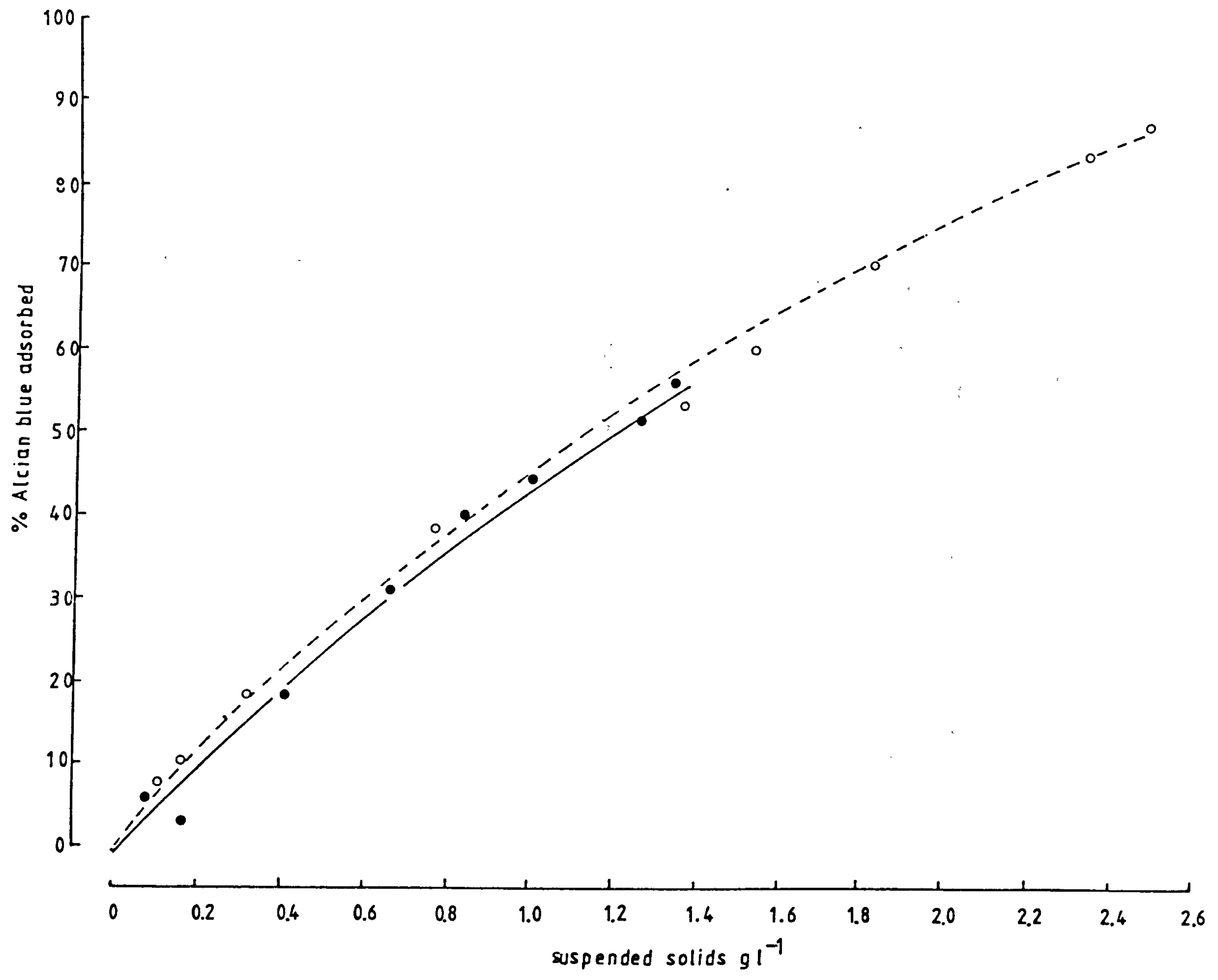

b
% Alcian blue adsorbed
100
90
80
70
60
50
40
30
20
10
0
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
suspended solids g l⁻¹

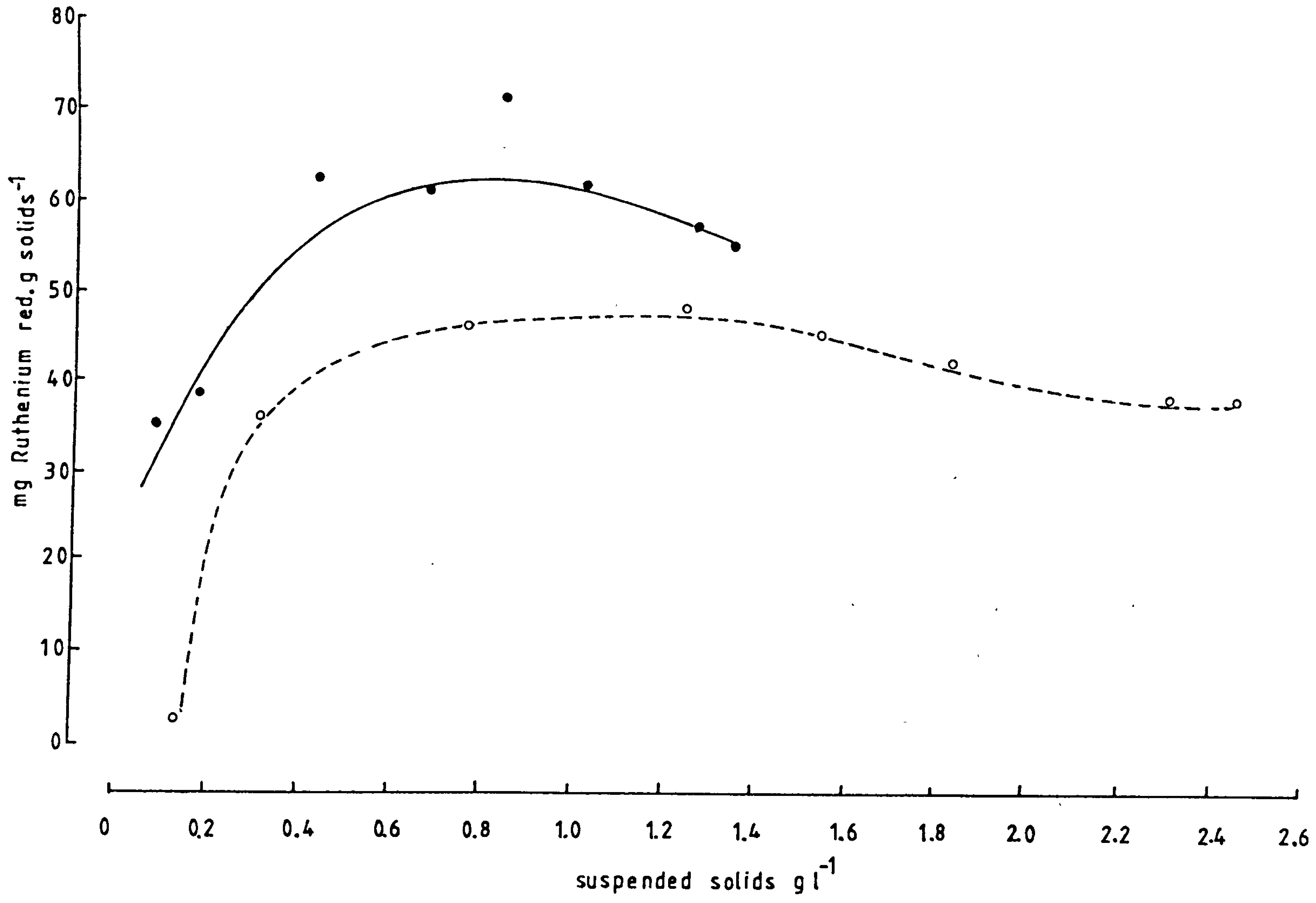
Figure 3.10

Effect of solids concentration (10-80% sludge) on Ruthenium red adsorption.

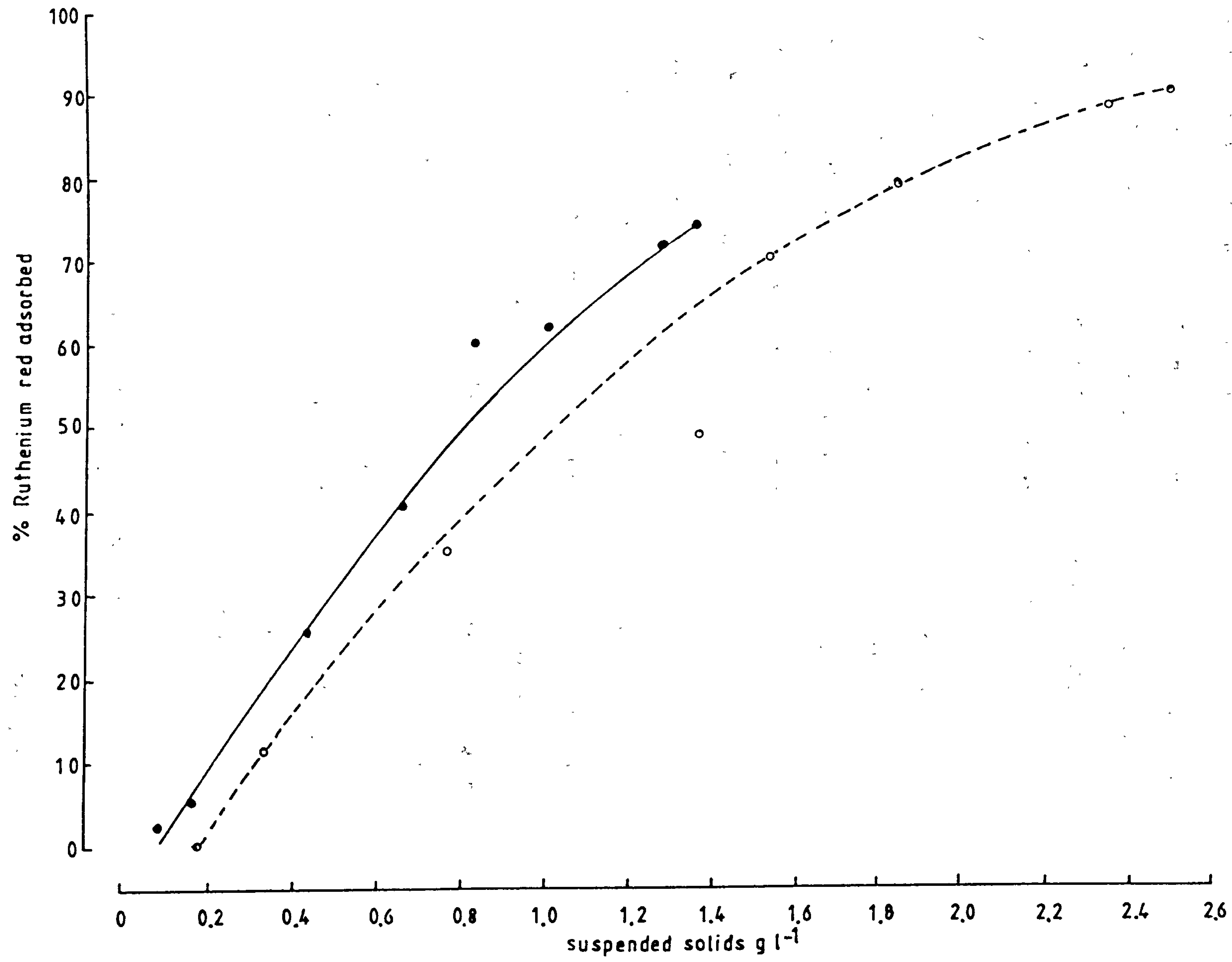
Initial sludge solids concentrations were 3.09 g l^{-1} for Runcorn sludge (o), and 1.68 g l^{-1} for Warrington South sludge (●). Ruthenium red concentration was 99.5 mg l^{-1} or 40.4 and 73.8 mg g solids^{-1} for Runcorn 1 and Warrington South sludge respectively at the highest solids concentration.

(a) Results presented as mg dye adsorbd g solids^{-1} against solids concentration.

(b) Results presented as % dye adsorbed against solids concentration.


a
mg Ruthenium red.g solids-1
80
70
60
50
40
30
20
10
0
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
suspended solids g l-1

b



concentration was constant at 99.5 mg l^{-1} or 40.4 mg g^{-1} and 73.8 mg g^{-1} solids for Runcorn 1 and Warrington South sludges at the maximum solids concentration tested. Alcian blue concentration was 611 mg l^{-1} or 247 mg g^{-1} and 453 mg g^{-1} respectively for Runcorn 1 and Warrington South sludge at the maximum solids concentration tested.

Binding remained constant above a solids concentration of 1.0 g l^{-1} for Alcian blue and 0.4 g l^{-1} for Ruthenium red. Below these values there was an increase in Alcian blue binding, but a decrease in Ruthenium red binding. At high solids dilution there would be expected to be an increase in adsorption due to deflocculation and floc breakage. That this is the case is supported by fitting the data to the mass action equation. Using the values of β from the data presented in Table 3.5, a plot of dye binding sites (σ equivalents dye mg solids^{-1}) against suspended solids reveals that the number of sites increases with sludge dilution. Examples of two such plots, for adsorption of Alcian blue by ranges of 0.5 to 10% and 10 to 80% Warrington South sludge are shown in Figures 3.11 and 3.12 respectively. The measured decrease in Ruthenium red adsorption at low solids concentration is probably beyond the resolution of the technique used.

For return activated sludge samples where solids concentration rarely falls below 2.0 g l^{-1} it was decided that a sludge dilution of 50% was an ideal procedure for routine dye adsorption analysis.

Figure 3.11

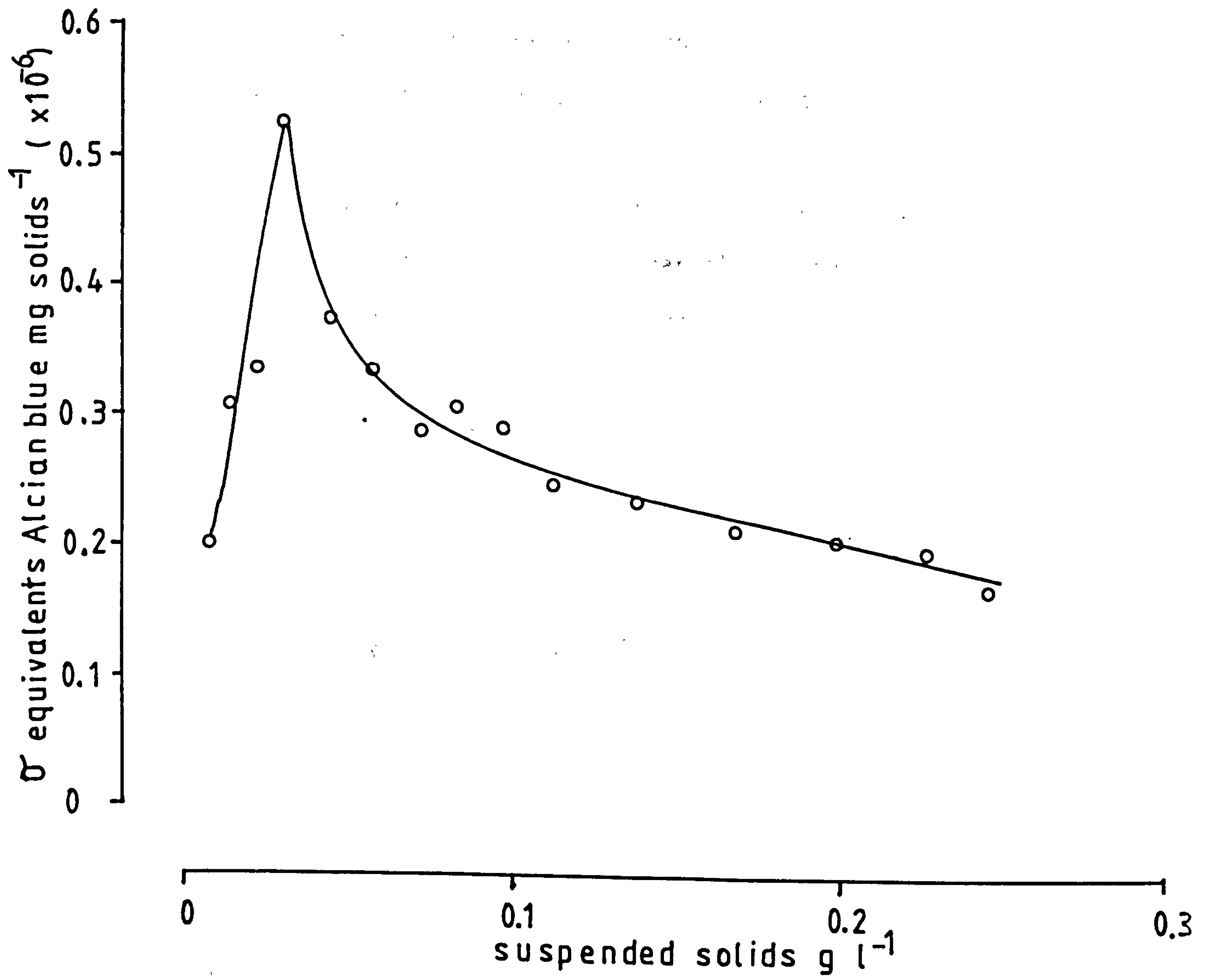
Effect of solids concentration (0.5 to 10% sludge) on available dye binding sites (θ equivalents Alcian blue mg solids^{-1}).

Sludge was taken from Warrington South with an initial solids concentration of 3.19 g l^{-1}. Alcian blue concentration was 16.14 mg l^{-1} or 50.66 mg g solids^{-1} at the highest sludge concentration.

The constant β = 2.51 where:

$$1/m = \beta\ 1/D + 1/\theta'$$

for Alcian blue $\theta = 2.71 \times 10^{-6}\ \theta'$



σ equivalents Alcian blue mg solids^{-1} (x10^{-6})
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
suspended solids g l^{-1}

Figure 3.12

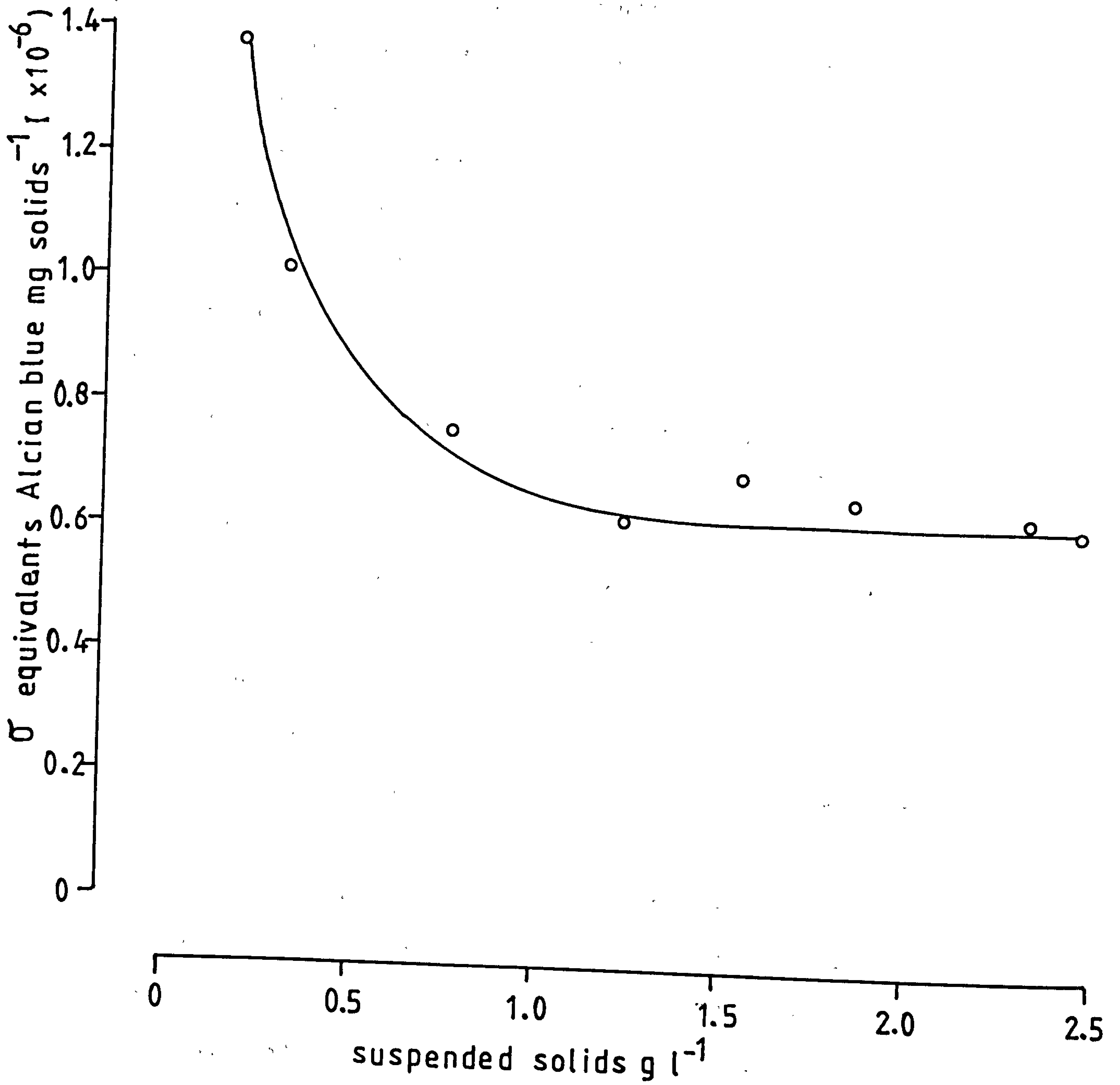
Effect of solids concentration (10 to 80% sludge) on available dye binding sites (θ equivalents Alcian blue mg solids^{-1}).

Sludge was taken from Warrington South with an initial solids concentration of 1.68 g l^{-1}. Alcian blue concentration was 6.11 mg l^{-1} or 453 mg g solids^{-1} at the highest sludge concentration.

The constant β = 3.529 where:

$$1/m = \beta \, 1/D + 1/\theta'$$

for Alcian blue $\theta = 2.71 \times 10^{-6} \theta'$


σ equivalents Alcian blue mg solids^{-1} (x10^{-6})
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
0.5
1.0
1.5
2.0
2.5
suspended solids g l^{-1}

3.3.6 Effect of pH on Dye Adsorption

The effects of pH on the adsorption of Alcian blue and Ruthenium red by sludges from three plants were evaluated. This study was carried out as two separate experiments and results can be seen in Figs. 3.13 and 3.14. Each sludge was diluted to 5% of the initial solids concentration. In the first experiment, pH ranges of 4.0 to 7.2 and 7.2 to 9.0 were established using citrate:phosphate and Tris-HCl buffers respectively. Initial suspended solids concentrations were 3.46 g l^{-1} for Runcorn 1 sludge, 2.36 g l^{-1} for Runcorn 2 sludge and 1.63 g l^{-1} for Warrington South sludge. Alcian blue concentrations were 20.8 mg l^{-1} or 120.5 mg g^{-1}, 176.7mg g^{-1} and 255.8 mg g^{-1} for the respective sludges. Ruthenium red concentrations were 10.88 mg l^{-1} or 62.9 mg g^{-1}, 91.5 mg g^{-1} and 133.5 mg g^{-1} for the three sludges.

Clearly the nature of the buffer used has a significant effect on adsorption of both Alcian blue and Ruthenium red. Binding of the latter is higher in Tris-HCl buffer than in citrate:phosphate buffer at a pH of 7.2. Conversely, Alcian blue binding was generally lower in Tris-HCl than in citrate:phosphate buffer. The reason for this is not clear.

In a second experiment, a pH range of 6.0 to 8.0 was established using sodium phosphate buffer. One flask containing citrate:phosphate buffer pH 6.0, and one of Tris-HCl buffer pH 8.0 were included for each sludge. Initial suspended solids concentrations were 2.07 g l^{-1} for Runcorn 1 sludge, 2.24 g l^{-1}

Figures 3.13 and 3.14

Effects of pH on adsorption of Alcian blue (Figure 3.13) and Ruthenium red (Figure 3.14) by sludges from Runcorn plant 1 (A), Runcorn plant 2 (B) and Warrington South (C).

In the first experiment, pH ranges of 4.0 to 7.2 and 7.2 to 9.0 were established using citrate:phosphate (●) and Tris-HCl (○) buffers respectively. Initial suspended solids concentrations were 3.46 g l^{-1} for Runcorn 1 sludge, 2.36 g l^{-1} for Runcorn 2 sludge and 1.63 g l^{-1} for Warrington South sludge. Alcian blue concentration was 20.8 mg l^{-1} and Ruthenium red 10.88 mg l^{-1}.

In the second experiment, a pH range of 6.0 to 8.0 was established using sodium phosphate buffer (▲). One flask containing citrate:phosphate buffer of pH 6.0 (■) and one of Tris-HCl of pH 8.0 (□) were included for each sludge. Initial suspended solids concentrations were 2.07 g l^{-1} for Runcorn 1 sludge, 2.24 gl^{-1} for Runcorn 2 sludge and 2.04 gl^{-1} for Warrington South sludge. Alcian blue concentration was 18.5 mg l^{-1} and Ruthenium red 7.84 mg l^{-1}.

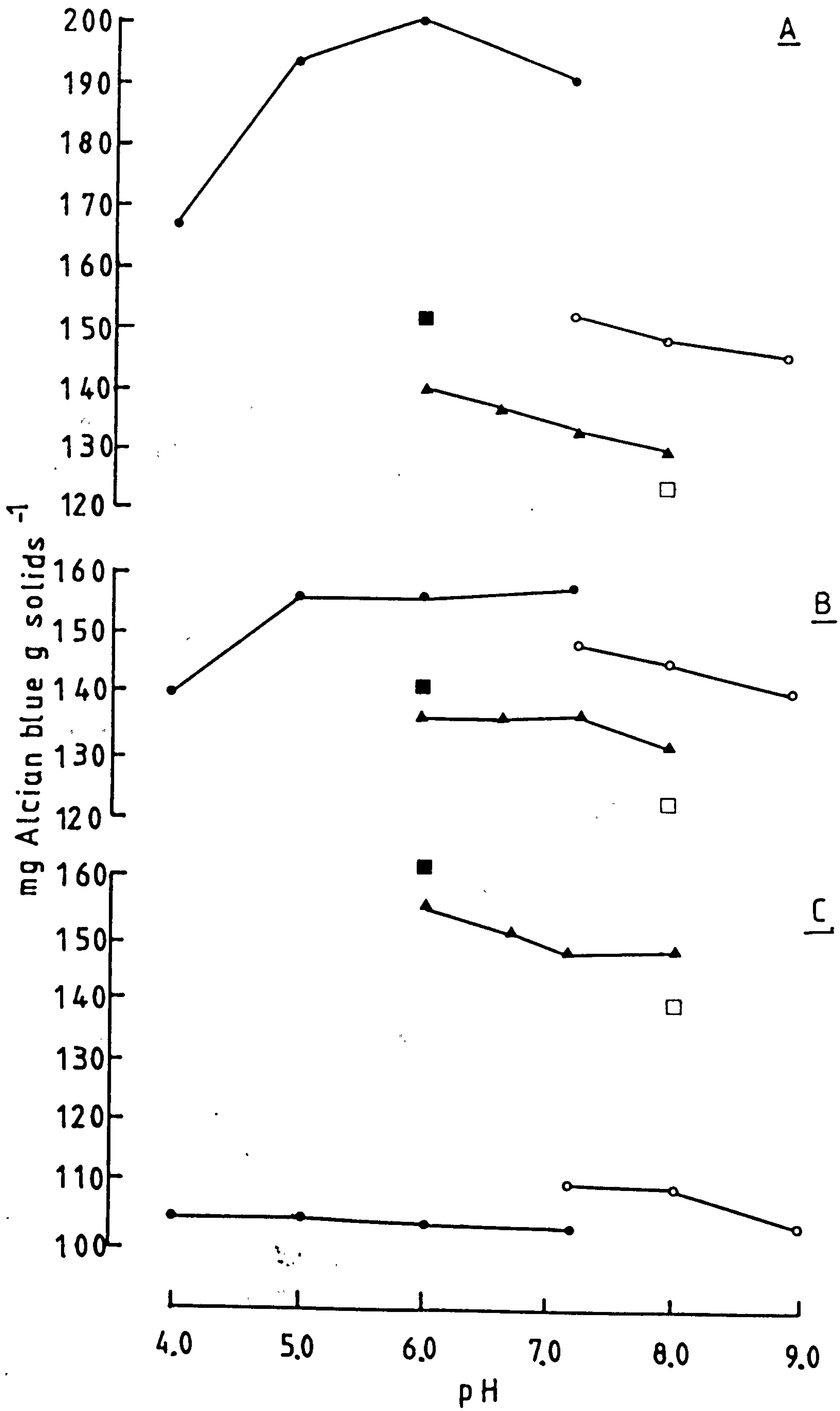


Fig.3.13

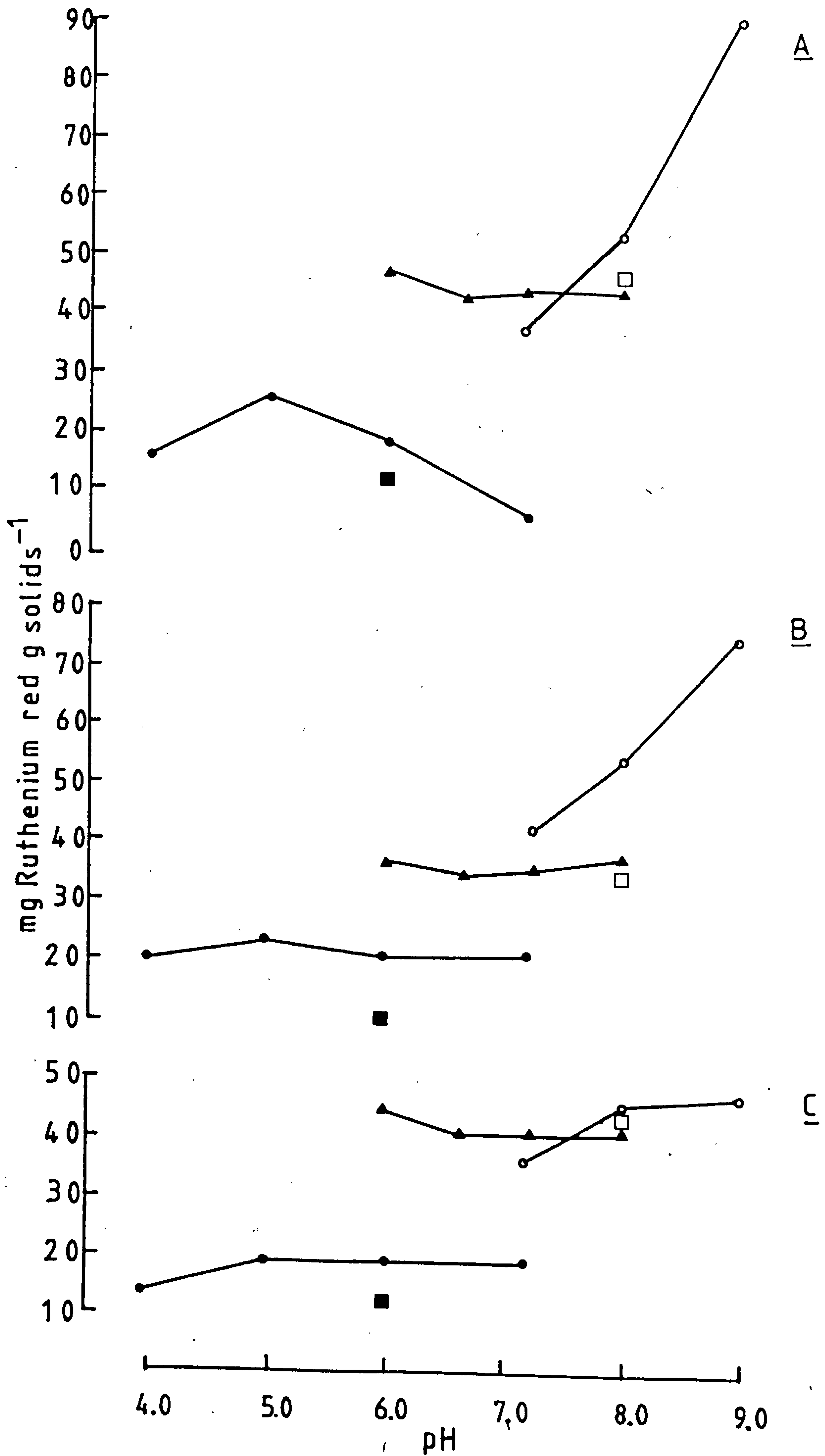


Fig. 3.14

for Runcorn 2 sludge and 2.04 g l^{-1} for Warrington South sludge. Alcian blue concentrations were 18.5 mg l^{-1} or 178.8 mg g^{-1}, 164.8 mg g^{-1} and 181.4 mg g^{-1} for each sludge respectively. For Ruthenium red these values were 7.84 mg l^{-1}, 75.9 mg g^{-1}, 69.9 mg g^{-1} and 77.0 mg g^{-1}. There was no significant affect on dye adsorption between the pH range 6.0 to 8.0. Where dye binding was low there was little affect of pH in the ranges 4.0 to 7.2 and 7.2 to 9.0. However, at high binding values, pH of less than 6.0 or greater than 8.0 had a marked affect on binding, particularly in the case of Ruthenium red.

As pH values of activated sludge are rarely outside the range 6.0 to 8.0 it was decided that no buffer was required in the test suspension.

3.3.7 The Effect of Sonication on Dye Adsorption, Sludge Viability and Sludge Activity

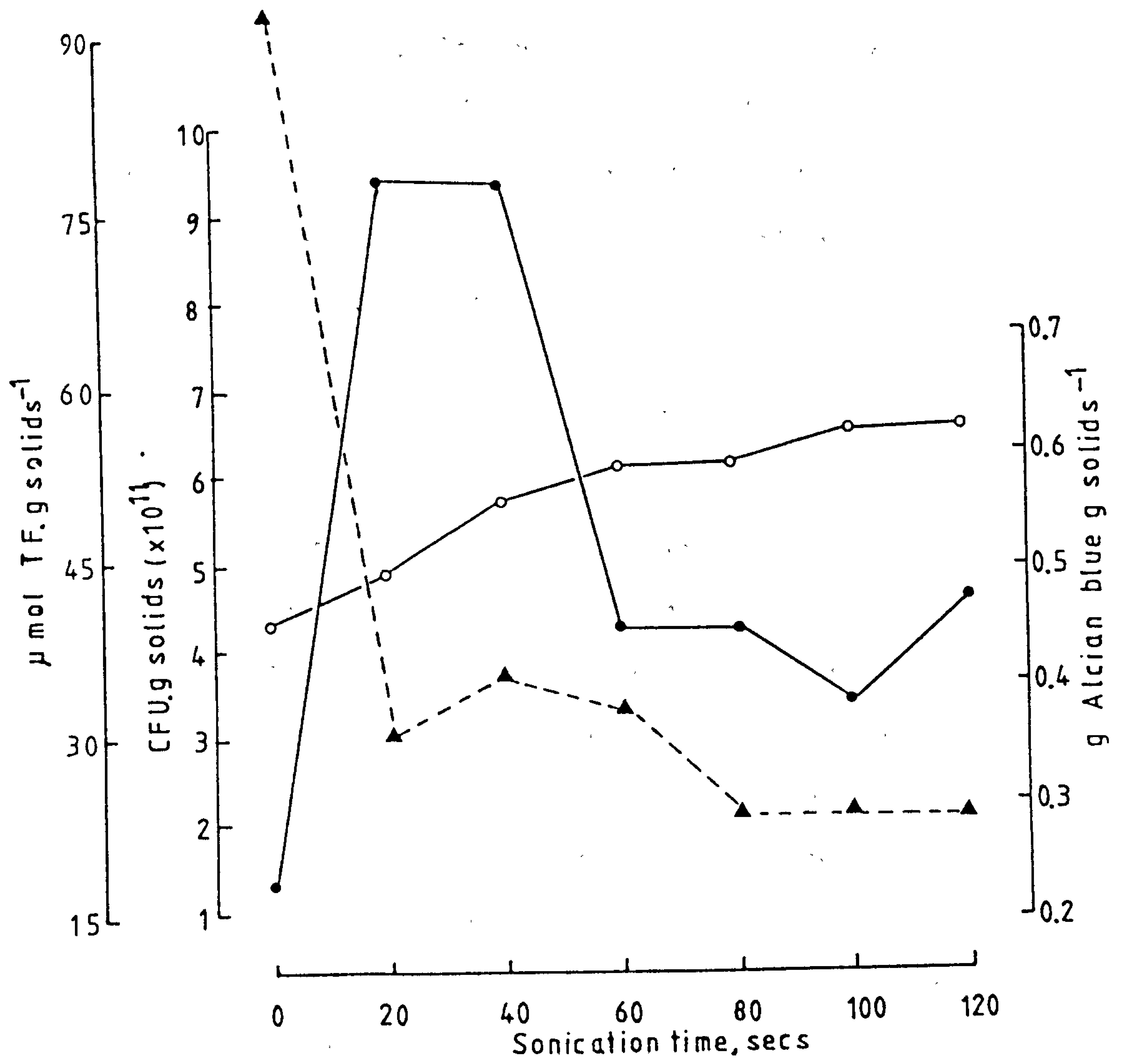
It can be seen from Fig. 3.15 that Alcian blue adsorption increases with sonication of 50% activated sludge. Initial sludge solids concentration was 1.88 g l^{-1} and this was obtained from Runcorn plant 1. Alcian blue concentration was 0.95 g l^{-1} or 1.01 g g^{-1} solids. Increase in dye adsorption was presumably due to the increase in surface area resulting from deflocculation.

The increase in sonication time was accompanied by a decrease in sludge activity as measured by triphenyl tetrazolium chloride (TTC) reduction. This was most apparent within the

Figure 3.15

Effect of sonication on Alcian blue adsorption (o), sludge activity (▲) and cell viability (●). Sludge was diluted by 50% and sonicated with a 10 mm probe. Initial sludge solids concentration was 1.877 g l^{-1} and Alcian blue concentration was 0.95 g l^{-1} or 1.01 g g $solids^{-1}$.

Sludge activity was measured by production of triphenyl formazan after triphenyl tetrazolium chloride reduction, and viability by recovery on CGY medium. Return activated sludge was obtained from Runcorn plant 1.



μ mol T.F. g solids^{-1}
90
75
60
45
30
15
CFU.g solids (x10^{11})
10
9
8
7
6
5
4
3
2
1
g Alcian blue g solids^{-1}
0.7
0.6
0.5
0.4
0.3
0.2
0
20
40
60
80
100
120
Sonication time, secs

first 20 seconds of sonication. Recovery of viable cells increased up to 40 seconds of sonication after which numbers of isolated bacteria decreased. The initial increase in recovery was due to dispersion of floc-forming bacteria. As sonication time increased this was outweighed by sonication induced lysis and thus recovery decreased. This phenomenon has been described by a number of workers (Pike et al., 1972).

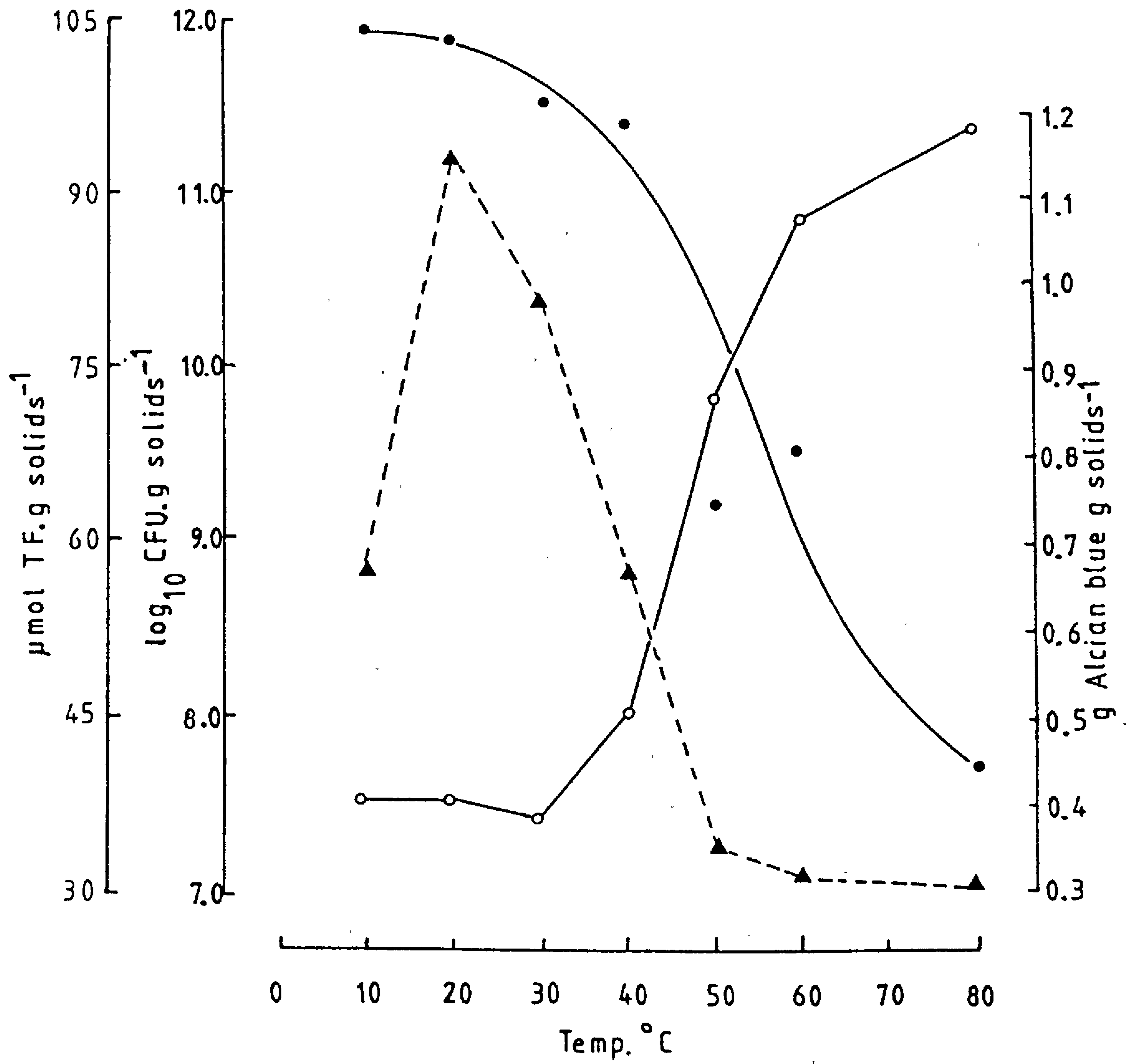
3.3.8 The Effect of Temperature on Sludge Viability, Dye Adsorption and Activity

The results of this investigation are shown in Fig. 3.16. Initial sludge suspended solids concentration was 1.88 g l^{-1} and obtained from Runcorn plant 1. Alcian blue concentration was 1.01 g l^{-1} or 1.08 g g $solids^{-1}$. Sludge activity reached a maximum at 20°C above and below which activity rapidly decreased. Alcian blue adsorption remained constant up to 30°C above which it rapidly increased. Usually with a rise in temperature an adsorption isotherm falls to a lower level, although it may have the same limiting value at high dye concentrations (Kipling, 1965). This explains the constant binding between 10 and 30°C. The increase in adsorption above 30°C is probably due to the detrimental affect of temperature on the bacterial membrane. There is substantial evidence (Brown and Melling, 1971) that membrane disruption and leakage of cell contents occurs with increased temperature. Possibly a release of cell lysis products would increase dye adsorption as large nucleic acid and protein

Figure 3.16

Effect of temperature on Alcian blue adsorption (○), sludge activity (▲) and cell viability (●). Flasks of sludge were heated in a water bath at the appropriate temperature for 10 minutes. Initial sludge suspended solids concentration was 1.877 g l^{-1} and Alcian blue concentration was 1.01 g l^{-1} or 1.08 g g-1 solids.

Sludge activity was measured by production of triphenyl formazan after triphenyl tetrazolium chloride reduction, and viability by recovery on CGY medium. Return activated sludge was obtained from Runcorn plant 1.



105
90
75
60
45
30
12.0
11.0
10.0
9.0
8.0
7.0
µmol TF.g solids-1
log10 CFU.g solids-1
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
g Alcian blue g solids-1
0
10
20
30
40
50
60
70
80
Temp. °C

molecules form insoluble complexes with Alcian blue molecules (Scott, 1972a).

3.3.9 The Effect of Metabolic Inhibitors and Biocides

The effects of a number of bacterial inhibitors on Alcian blue adsorption, sludge activity and sludge viability were investigated. Sludges were incubated with an appropriate inhibitor for ten minutes prior to adsorption, activity or viability tests. Sludge was obtained from Runcorn plant 1 with an initial suspended solids concentration of 1.88 g l^{-1}.

Each inhibitor gave rise to a characteristic decrease in sludge viability (Table 3.6) measured as recovery on CGY medium. This may have been due to not only cell death but also changes in the properties of floc strength. At the concentrations used, mercuric chloride proved to be the most lethal. Like both N-ethylmaleimide and formaldehyde solution this exerts its toxic effects through protein denaturation. In this respect there is evidence that each is selective. For example, formaldehyde solution gave rise to only an 8% decrease in sludge viability, but an 82.5% decrease in dehydrogenase enzyme activity as measured by the reduction of TTC.

Potassium cyanide exerts its lethal effects by inhibition of electron transport chains, especially of cytochrome oxidases. This toxic substance was unique in giving rise to a large increase in Alcian blue adsorption. The reason for this is unclear.

Table 3.6 The effect of inhibitors on return activated sludge. Sludge from Runcorn plant 1 was incubated at 20°C with the respective poison for 10 minutes prior to viability, activity and dye adsorption measurement. Viability was assessed by recovery on casitone glycerol yeast-extract agar after sonication for 20 seconds. Activity was measured by reduction of triphenyl tetrazolium chloride.

	Viability		Activity		Dye adsorption	
Inhibitor	C.F.U. g^{-1}	%	μmol T.F. g $solids^{-1}$	%	g Alcian blue g^{-1}	%
None	1.70×10^{11}	100	29.7	100	0.373	100
Potassium cyanide (10 mM)	1.03×10^{11}	60.9	11.1	37.4	1.020	273.5
N-ethylmaleimide (1 mM)	4.69×10^{10}	27.5	13.7	46.1	0.366	98.1
Formaldehyde (4 gl^{-1})	1.57×10^{10}	9.2	5.2	17.5	0.315	84.4
Mercuric chloride (0.1 gl^{-1})	3.99×10^{9}	2.3	3.2	10.8	0.338	90.6

3.3.10 The Effect of Solutes on Alcian Blue Adsorption

A number of low molecular weight substances, both organic and inorganic were analysed as to their effect on Alcian blue adsorption (Table 3.7). At concentrations tested (1 g l^{-1}) only casitone significantly competed with dye adsorption. This was further investigated and these results can be seen in Fig. 3.17. Clearly very low concentrations of amino acids can inhibit Alcian blue binding. However, this was deemed insignificant below 0.1 g l^{-1}. Concentrations of amino acids in sewage are normally below this level.

3.4 Adsorption of Alcian Blue by Pure Bacterial Cultures

Alcian blue adsorption was measured in pure cultures of two microorganisms commonly found in activated sludge; *Zoogloea ramigera* and *Sphaerotilus natans*. Dry weights were adjusted to 0.4 g l^{-1} before adsorption tests were performed. Alcian blue concentration was 14.35 mg l^{-1} or 35 mg g dry weight^{-1}. Results are presented in Fig. 3.18.

Clearly *S.natans* has a higher dye adsorption capacity than *Z.ramigera* under the conditions employed. The outside of *Z.ramigera* flocs possess a lower surface area than *S.natans* filaments and this is likely to be the major reason for differences in adsorption capacity. However, differences in the surface chemistry of the two species may also be a contributing factor.

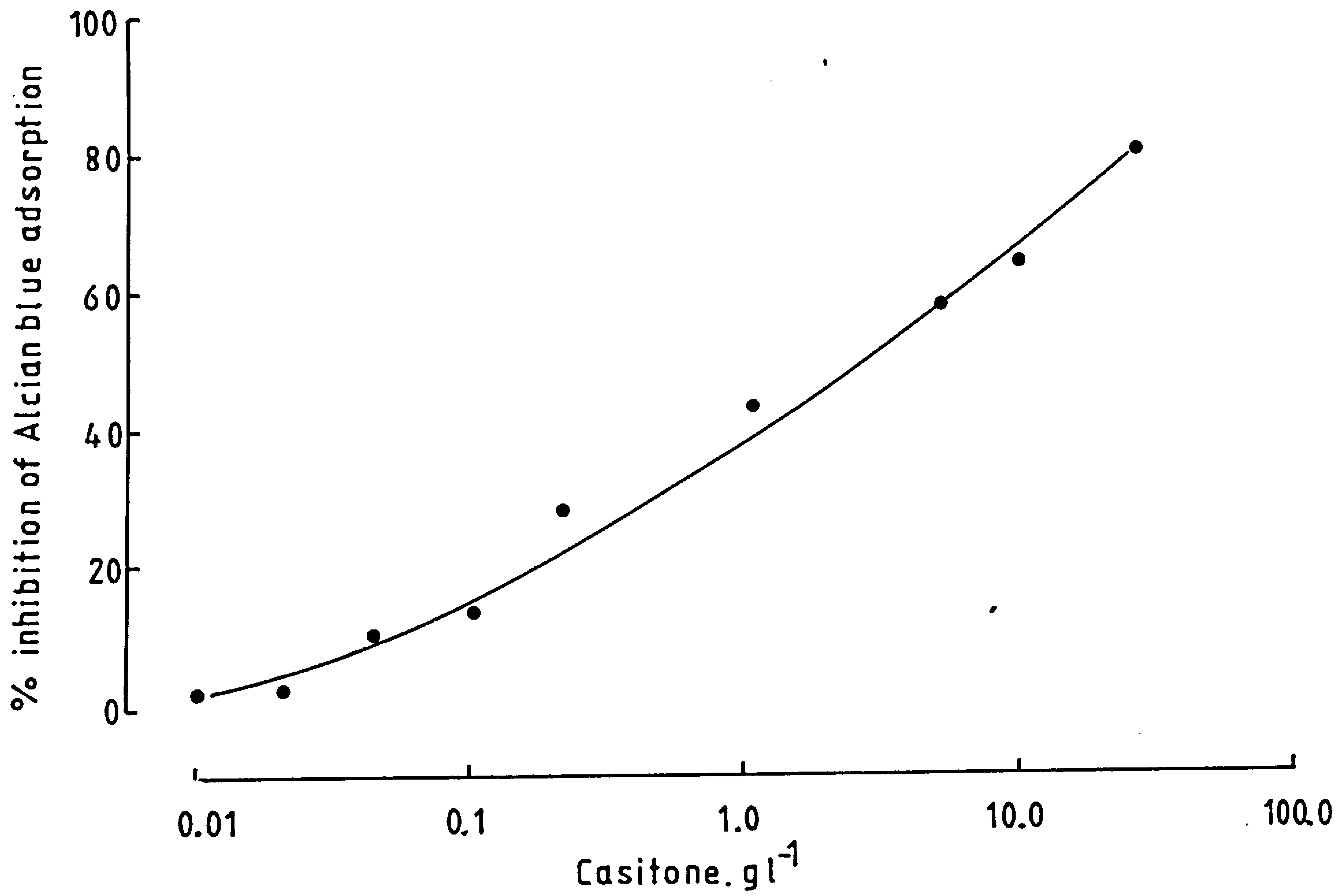
Table 3.7 Effect of possible sewage constituents on Alcian blue binding by 5% return activated sludge from Runcorn plant 1. All compounds were incubated with sludge for 10 minutes at a concentration of 1 g l^{-1} prior to the addition of Alcian blue.

Compound	Dye adsorption (mg Alcian blue g $solids^{-1}$)	Adsorption/Adsorption by control
None	96.3	1.0
$NaNO_3$	91.2	0.95
$MgCl_2$	93.3	0.97
NaCl	99.8	1.04
KCl	96.3	1.0
Glycerol	95.5	0.99
Tween 80	98.3	1.02
Glucose	94.9	0.98
Casitone	51.3	0.53
Urea	95.8	0.99
Cooking oil	92.2	0.95

Figue 3.17

Inhibition of Alcian blue binding by casitone.

Activated sludge was diluted by 50% and incubated with the appropriate concentration of casitone prior to performance of the dye adsorption test.

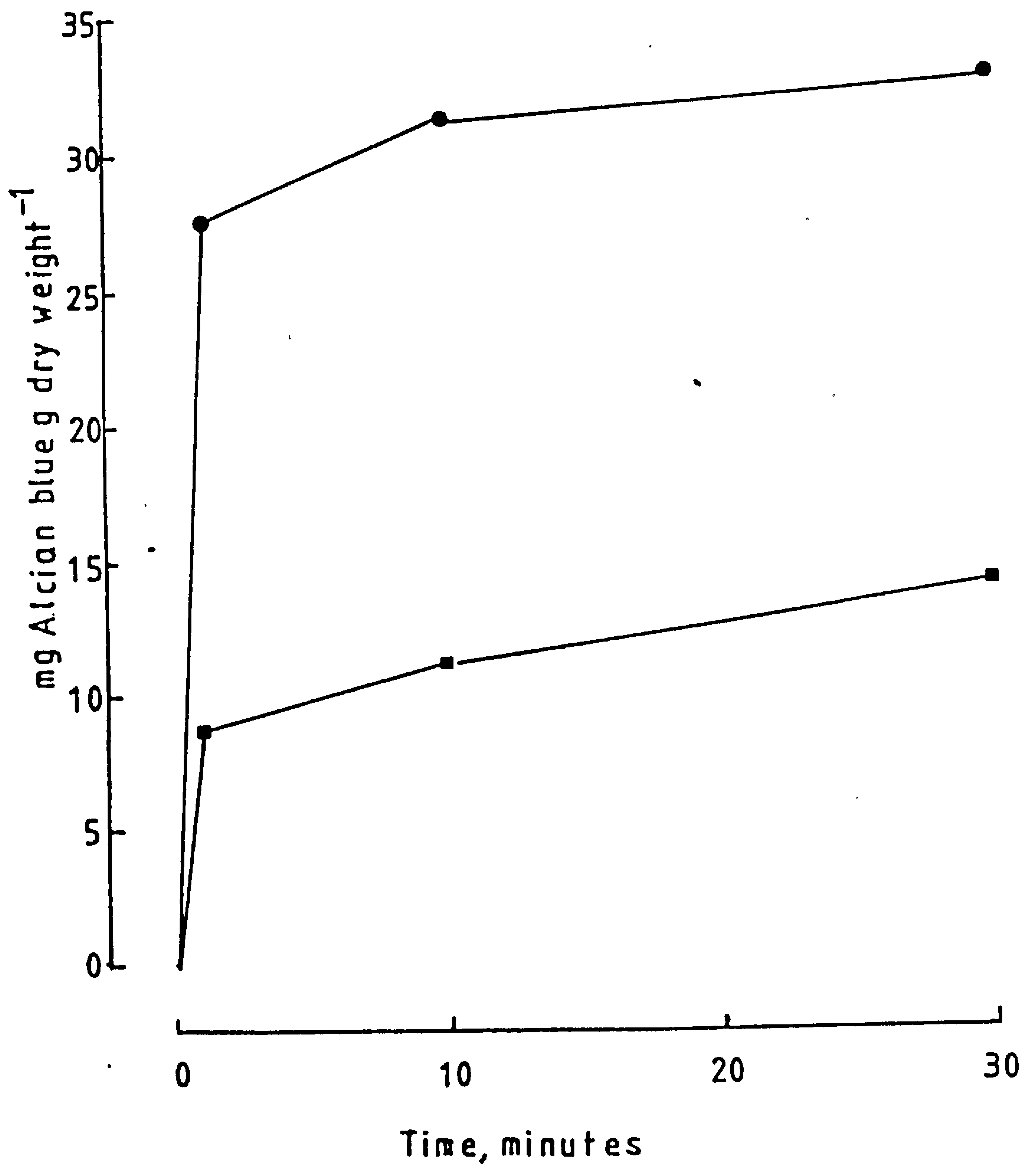


% inhibition of Alcian blue adsorption
100
80
60
40
20
0
0.01
0.1
1.0
10.0
100.0
Casitone. gl-1

Figure 3.18

Adsorption of Alcian blue by *Sphaerotilus natans* (●) and *Zoogloea ramigera* (■).

Dry weight of pure cultures of the appropriate organism were adjusted to 0.4 g l^{-1}. Alcian blue concentration was 143.5 mg l^{-1} or 35 mg g dry weight^{-1}.


35
30
25
20
15
10
5
0
mg Alcian blue g dry weight^{-1}
0
10
20
30
Time, minutes

DISCUSSION

There are currently a number of theories for the selection of filamentous microorganisms in activated sludge bulking. In each, emphasis is placed on the ability of the sludge to quickly remove nutrients from the medium at the sewage/sludge mixing stage. Although many factors may be involved in nutrient removal by activated sludge, undoubtedly the immediate adsorption has an important role. Thus a simple test for adsorption capacity may be of use in the prediction of bulking incidents.

Two polycationic dyes were chosen as indicators of sludge adsorption capacity. The nature of Alcian blue and Ruthenium red binding to a variety of organic polymers has been studied in some detail by Scott et al. (1964, 1972) and Luft (1971) respectively. Ruthenium red was initially thought to be a specific stain for pectins until Luft (1971) found it to precipitate a large number of polyanions including polygalacturonic acid, DNA and agar. He found that a high charge density was important in dye binding but that this alone was not sufficient for a reaction. It was also necessary for the carrier molecule to be of high molecular weight. Conversely, some highly polymeric substances do not combine with Ruthenium red even when highly charged. The dye does not combine with neutral polysaccharides, polypeptides and proteins.

In many respects, Alcian blue and Ruthenium red are similar. Both are metal containing polyvalent basic dyes, which precipitate similar polyanions. Similar substances are stained

under the light microscope. Scott et al. (1964) concluded that the major method of Alcian blue binding was electrostatic and the formation of salt links. Luft draws the same conclusions with regard to Ruthenium red. In addition Luft found that precipitation of polyanions was primarily related to the number of carboxylic acid groups available and it seems likely that binding of both dyes is due to a reaction with this ionic group. At the activated sludge surface, the majority of carboxylic acid groups would be expected in glucuronic acid residues and the relationship between these and dye adsorption is investigated at a later stage (see Chapter 6).

Despite their similarities there are some differences in the specificity of the two dyes. Structurally this is due largely to the nature of charge distribution around the dye molecules. In the case of Ruthenium red the charge distribution is spread uniformly over the molecule, whereas in Alcian blue charges are more localized.

The shape of the isotherms obtained for both Alcian blue and Ruthenium red were of the type L2 as classified by Giles et al., (1960). This type of isotherm is found where there is no strong competition from the solvent for sites at the surface of the adsorbent (it is also likely that the adsorbate molecules are aligned parallel to the surface of the adsorbent). The L-type isotherm shows that as more sites on the floc surface are filled it becomes increasingly difficult for the bombarding dye molecules to find a vacant site available.

Andreadakis (1978) found that the adsorption isotherm for activated sludge and the anionic dye Lissamine scarlet 4R was of the H type. This indicates that a second layer on the top of the chemosorped material was being formed due to physical adsorption of dye molecules. Clearly the mode of adsorption of this dye is of a different nature to that of Ruthenium red and Alcian blue. Lissamine scarlet 4R is only adsorbed to activated sludge at acid pH and this would be expected to affect the sludge surface. Andreadakis (1978) offers two explanations for the adsorption of Lissamine scarlet 4R at reduced pH. Firstly, he suggests that the anionic dye molecule is rejected by the negative charge of the floc surface at neutral pH. As the pH lowers, the surface charge approaches zero and may eventually become positive thus attracting the dye molecule. Secondly Andreadakis points out that initially the sludge is surrounded by strongly bound water molecules. These are disrupted by the presence of dissolved ions (e.g. H^+) allowing the dye to bind to the floc surface.

Adsorption data for both Alcian blue and Ruthenium red fitted both Langmuir and mass action isotherm equations, with best correlation with the former. The Freundlich isotherm was rejected although Hall (1982b) found Alcian blue binding to fit both mass action and Freundlich equations. Andreadakis found that the adsorption of Lissamine scarlet 4R by activated sludge fitted Freundlich and Langmuir equations equally well.

Isotherms have been applied to the adsorption of metal ions by activated sludge. Steiner _et al_. (1976) found that _Zoogloea_

1-16, soluble activated sludge polymer and polygalacturonic acid all fitted the L-type curve with regard to adsorption of calcium, cobalt and copper respectively. However, adsorption of cobalt by Zoogloea ramigera 115 and of calcium by activated sludge and activated sludge insoluble polymer all produced S-type isotherms. Steiner et al. suggested that carboxyl groups were the adsorption sites for the L-type of isotherm, and that the hydroxyl units of hexose residues were responsible for the S-type of binding. It would be expected that polycationic dyes would bind to sludge at similar sites to metal cations. However, Ruthenium red at least does not bind to neutral polysaccharides, indicating that the L-types of isotherm obtained for Ruthenium red and Alcian blue were due to binding with mainly carboxyl groups at the sludge surface.

The removal of metals by activated sludge has been extensively reviewed by Brown and Lester (1979). A variety of parameters have been shown to influence removal; SVl, sludge age, suspended solids concentration, dissolved oxygen concentration, temperature, pH, metal ion concentration, metal solubility, metal valency, use of complexing agents and particle size. The concentration of bacterial extracellular polymers appears to be the main biological factor. In addition, there is a variation in uptake between different metals. Iron, copper, chromium and zinc have the highest removal efficiencies with nickel, manganese, calcium and magnesium the lowest.

Brown and Lester propose three mechanisms for removal of metals by activated sludge:

i) Physical entrapment of metal ions by the floc.

ii) Binding of soluble material by sludge extracellular polymer.

iii) Accumulation of soluble metal by the cell.

iv) Volatilization of metal to the atmosphere.

It is likely that the second factor is the major one in dye adsorption. Physical entrapment is less likely with large dye molecules and the possibility of volatization is not encountered in the testing method.

From the results it appears that cell viability as measured by recovery on CGY medium and sludge activity measured as triphenyl tetrazolium chloride reduction have only a minor influence on dye adsorption. Protein denaturing agents formaldehyde, mecuric chloride and N-ethylmaleimide caused a slight reduction in dye adsorption. This was thought to be due primarily to disruption of the outer membrane of the predominantly Gram-negative microbial population. Though the method of exopolysaccharide attachment to the outer membrane is unclear, it is likely that protein anchorage is involved (Hammond _et al_., 1984). Thus treatment with the above reagents would be expected to give rise to a release of exopolymer and thus a reduction in dye adsorption.

The observed relationships between dye adsorption and temperature may have been due to two contributing factors. Fistly, the effect of temperature increase on adsorption may be complex and adsorption may increase, decrease or remain constant

depending upon the system (Kipling, 1965). Secondly, the disrupting effect of temperature on the cell surface must be taken into account. Severe disruption of bacterial membranes occurs above about 40°C with subsequent release of extracellular polymers to the medium (Brown and Melling, 1971).

Uptake of nutrients by activated sludge appeared to be a two-stage process. This consisted of an initial rapid adsorption phase followed by a slow uptake for up to thirty minutes of incubation. Adsorption was highest for charged compounds pyruvate and amino acids. Two-stage uptake by activated sludge has previously been reported for starch (Banerji et al., 1968a, b), bovine serum albumin (Crombie-Quilty and McLoughlin, 1983; McLoughlin and Crombie-Quilty, 1983) and metals (Steiner et al., 1976). It is likely that adsorbed nutrients such as metal ions and amino acids bind to the floc surface at similar sites to Alcian blue and Ruthenium red. Thus competion by sewage components may be expected to interfere with dye adsorption tests. High concentrations of $MgCl_2$ increase binding of Alcian blue to capsular polysaccharides (Powell et al., 1982). However, a variety of potential sewage components were found not to affect dye binding when added to the medium at a concentration of 1 g l^{-1}. An exception were amino acids added as casitone. Clearly at neutral pH these compounds have a high affinity for either dye or sludge.

The initial capacity of organisms to adsorb nutrients must be of prime importance in their selection in the activated sludge

wastewater treatment process. When pure cultures of Sphaerotilus natans and Zoogloea ramigera were subjected to dye adsorption tests, the former was found to have a much larger initial adsorption capacity. It is likely that this is due to the larger surface area of the filamentous organism compared to Z.ramigera when growing as flocs. However, if surface area was the prime factor in nutrient adsorption and organism selection then filamentous organisms would always dominate in activated sludge. Certainly the chemical nature of the sludge surface also plays a role. This is further investigated in Chapter 6.

Summary

1. The dynamics of adsorption by activated sludge of Alcian blue and Ruthenium red were studied with a view to establishing a routine test for dye adsorption capacity. Adsorption isotherms for both dyes were found to fit Langmuir and mass action isotherm equations.
2. Initially it was proposed to use 5% return activated sludge with concentrations of 7.5 mg l^{-1} Ruthenium red and 25 mg l^{-1} Alcian blue for routine dye adsorption tests A more accurate metod for adsorption measurement was to use 50% return activated sludge with Ruthenium red concentrations of 0.15 g l^{-1} and Alcian blue of 0.40 g l^{-1}.
3. Adsorption of possible substrates by activated sludge showed a similar pattern, though of a different order, to dye adsorption.

4. Binding of dyes was not affected by pH between the range of 6.0 to 8.0.
5. Binding of Alcian blue was not affected by temperature between 10°C and 30°C, above which it rapidly increased.
6. Binding of Alcian blue was only slightly influenced by the action of poisons on activated sludge. This was thought to be due to disruption of the sludge surface.
7. Dye adsorption was unaffected by low concentrations of salts and selected organic compounds, with the exception of amino acids.
8. Alcian blue adsorption by *Sphaerotilus natans* was higher than that of *Zoogloea ramigera*.

CHAPTER 4

Adsorption of Cationic Dyes and Activated Sludge Plant Operation

4.1 Non-bulking activated sludges

Studies were carried out on site to assess Alcian blue and Ruthenium red adsorption by activated sludge. This data was then correlated with plant operational parameters. Initially, dye adsorption tests were performed on 5% return activated sludge from Runcorn plants 1 and 2, and the plant at Warrington South. This data along with plant operating characters are shown in Figs. 4.1, 4.2 and 4.3 respectively. The mean values of plant data over the first study period (8th September 1981 to 2nd October 1981) are shown in Table 4.1. The two plants at Runcorn receive raw sewage of identical composition therefore any differences in performance or sludge characteristics result from variations in design or operating techniques. Over the first period of study, plant 2 produced a sludge of poor quality in terms of settleability when compared to plant 1. In addition the effluent from plant 2 contained higher concentrations of BOD, COD, ammonia nitrogen and suspended solids.

The load applied to the Warrington South E.T.W. was approximately half that of either Runcorn plants. Sludge settleability was poor although the final effluent was the best of the three plants studied. The sewage strength was lower at Warrington South than at Runcorn.

From Figs. 4.1, 4.2 and 4.3 there is no clear relationship between dye adsorption and plant operational parameters. Two peaks in Ruthenium red adsorption at Warrington South precede two

Figure 4.1

Plant characteristics throughout the first period of study at Runcorn plant 1 (8th September 1981 to 2nd October 1981).

COD final effluent mg l^{-1}
sludge loading gCODgMLSS^{-1}day^{-1}
SVI ml g^{-1}
MLSS gl^{-1}
RAS suspended solids gl^{-1}
mg Ruthenium red g solids^{-1}
mg Alcian blue g solids^{-1}
Day

Figure 4.2

Plant characteristics throughout the first period of study at Runcorn plant 2 (8th September 1981 to 2nd October 1981).

COD final effluent mg l-1
120
100
80
60
40
sludge loading g COD g MLSS-1 day-1
2.0
1.6
1.4
0.8
0.4
0
SVI ml g-1
600
500
400
300
200
100
MLSS g l-1
3.0
2.0
1.0
RAS suspended solids g l-1
4.5
3.5
2.5
1.5
0.5
mg Ruthenium red g solids -1
50
40
30
20
mg Alcian blue g solids-1
100
90
80
70
60
50
40
1
5
10
15
20
25
Day.

Figure 4.3

Plant characteristics throughout the first period of study at Warrington South (8th September 1981 to 2nd October 1981).

COD final effluent mg l⁻¹
sludge loading gCOD gMLSS⁻¹ day⁻¹
SVI ml g⁻¹
MLSS g l⁻¹
RAS suspended solids g l⁻¹
mg Ruthenium red g solids⁻¹
mg Alcian blue g solids⁻¹
Day

Table 4.1 Mean and range of plant data over first study period

	Runcorn plant 1		Runcorn plant 2		Warrington South	
	Mean	Range	Mean	Range	Mean	Range
Settled sewage						
BOD mg l^{-1}	16.9	80-250	16.9	80-250	132.4	90-194
COD mg l^{-1}	310.6	174-365	310.6	174-365	231	115-317
Ammonia nitrogen mg l^{-1}	31.1	17.6-79	31.1	17.6-79	24.9	15.0 - 38.2
Suspended solids mg l^{-1}	108.5	64-176	108.5	64-176	85.0	56-114
Aeration						
Load g COD g $MLSS^{-1}$ day^{-1}	0.79	0.34-1.74	0.84	0.34-1.98	0.42	0.20-1.03
SV1 ml g^{-1}	263	84-405	435.2	156-562	498	286-691
SV1 ml l^{-1}	62.5	15-96	86	21-98	85.4	31-96
MLSS g l^{-1}	2.077	1.74-3.24	1.888	1.31-2.43	1.76	1.39-2.4
RAS suspended solids g l^{-1}	3.15	2.36-3.62	2.96	2.20-3.69	2.52	0.81-4.27
Alcian blue adsorption mg g $solids^{-1}$	67.8	47.5-103	60.8	48-98	54.7	30-89
Ruthenium red adsorption mg g $solids^{-1}$	23.8	18.2-2.33	28.8	20-43	36.2	21-51
Effluent						
BOD mg l^{-1}	6.9	5-14	16.4	9-26	8.3	2-16
COD mg l^{-1}	52.3	37-93	67.8	40-112	40.2	17-54
Ammonia nitrogen mg l^{-1}	10.8	5.7-33.2	21.6	8.5-33.0	12.8	1.4-23.5
Nitrate nitrogen mg l^{-1}	6.1	1.32-12.3	1.2	0.33-3.83	3.3	2.6-4.6
Nitrite nitrogen mg l^{-1}	1.3	0.58-3.89	0.8	0.15-3.06	1.9	0.54-3.3
Suspended solids mg l^{-1}	10.2	7-20	20.0	7-29	12.5	5-20

peaks in SV1 on days 11 and 17 although this phenomenon was not repeated for Alcian blue, nor at other treatment works.

The correlation between the various plant characters and dye adsorption are shown in Table 4.2. In all cases there is a highly significant correlation between adsorption of Ruthenium red and Alcian blue. This relationship has been noted earlier (Chapter 3) and suggests that binding sites for each dye are in similar proportions on different sludges.

A significant inverse relationship was found in all but one case between dye adsorption and return activated sludge suspended solids concentration. This suggests that either the test is subject to floc dilution effects, or that sludge of low suspended solids concentration is capable of higher dye adsorption.

A significant correlation was also found between the adsorption of both dyes and ammonia in the effluent of the Warrington South plant. There was no such relationship between sludge solids concentration and ammonia nitrogen, so it is possible that some component in the effluent was enhancing dye binding. This is difficult to explain, as positively charged ammonium ions would be expected to compete with cationic dye for electrostatic binding sites on the sludge surface. A similar relationship was not found with Runcorn sludges.

There is no significant relationship between floc size as measured microscopically and dye adsorption. It would be expected that the smaller the size of the floc the larger the surface area and therefore higher dye binding would occur. That this was not

Table 4.2 Correlation between Ruthenium red and Alcian blue binding by 5% return activated sludge, and plant performance data

Plant operation parameter	Runcorn plant 1						Runcorn plant 2						Warrington South					
	Alcian blue adsorption			Ruthenium red adsorption			Alcian blue adsorption			Ruthenium red adsorption			Alcian blue adsorption			Ruthenium red adsorption		
	r	n	p	r	n	p	r	n	p	r	n	p	r	n	p	r	n	p
Load (BOD)	0.10	18	NS	-0.44	18	NS	0.37	18	NS	0.21	18	NS	0.01	17	NS	0.33	17	NS
Load (COD)	-0.36	17	NS	0.14	17	NS	-0.16	17	NS	0	17	NS	0.31	16	NS	-0.24	16	NS
BOD influent	0.44	18	NS	-0.32	18	NS	-0.29	18	NS	-0.5	18	NS	-0.02	17	NS	0.23	17	NS
COD influent	0.21	17	NS	-0.35	17	NS	0.24	17	NS	-0.21	17	NS	0.62	16	<0.01	0.25	16	NS
Nitrogen (NH_4) influent	0.05	18	NS	-0.19	18	NS	0.85	18	<0.001	0.29	18	NS	0.42	17	NS	0.18	17	NS
Suspended solids influent	0	18	NS	-0.08	18	NS	-0.11	18	NS	-0.37	18	NS	0.25	17	NS	0.20	17	NS
RAS suspended solids	-0.73	18	<0.001	-0.73	18	<0.001	0.24	18	NS	0.85	18	<0.001	-0.73	17	<0.001	-0.63	17	<0.01
Ruthenium red adsorption	0.80	18	<0.001	-	-	-	0.91	18	<0.001	-	-	-	0.79	17	<0.001	-	-	-
Floc size	0.42	18	NS	0.38	18	NS	0.27	18	NS	0.12	18	NS	0.42	17	NS	0.38	17	NS
BOD effluent	0.12	18	NS	0.45	18	NS	-0.31	18	NS	-0.05	18	NS	0.16	17	NS	0.02	17	NS
COD effluent	0.32	18	NS	-0.11	18	NS	-0.27	18	NS	-0.58	18	<0.01	0.16	17	NS	0.19	17	NS
Nitrogen (NH_4) effluent	-0.18	18	NS	0	18	NS	0.20	18	NS	0.14	18	NS	0.62	17	<0.01	0.43	17	<0.01
Nitrogen (NO_3) effluent	-0.14	18	NS	-0.39	18	NS	0	18	NS	-0.21	18	NS	-0.04	17	NS	0.07	17	NS
Nitrogen (NO_2) effluent	0.32	18	NS	0.04	18	NS	0.19	18	NS	-0.01	18	NS	-0.38	17	NS	0	17	NS

the case suggests that factors other than floc surface area are involved in dye adsorption. Floc morphology was examined over a monthly period (8th September 1981 to 2nd October 1981) and these results are shown in Table 4.3. Size estimations suffered from a large standard deviation indicating that large numbers of flocs need to be examined in order to give an accurate representation of their size.

Filamentous mircoorganisms in activated sludge were identified using the methods of Eikelboom (1981). Characteristics of the organisms observed are shown in Table 4.4 and the frequency of their appearance in Tables 4.5a, b and c. Only one organism could not be classified into any of Eikelboom's categories. This filament (Type O) was similar to one mentioned briefly by Eikelboom (1981) as resembling Type 0803 but which was Gram-positive. Characters of organisms I, J and M were all in agreement with the description by Eikelboom of Type 0041. It is possible, however, that these were different species.

Sludge from Runcorn plant 1 always contained a smaller number of filamentous organisms than that of Runcorn plant 2 or Warrington South. In each case the microflora was apparently stable. Microthrix parvicella was the dominant filament at Runcorn plant 1 and at Warrington South. Runcorn 2 activated sludge, however, was dominated by an organism identified as Eikelboom Type 0961. This organism was also present in large numbers at both of the other works. As both plants at Runcorn received sewage of identical composition, then clearly some plant

Table 4.3 Floc description and protozoal numbers in return activated sludge (first period of study)

Day	Runcorn Plant 1					Runcorn Plant 2					Warrington South				
	Filament category	Floc size		Protozoa 10 fields^{-1}	Description of floc	Filament category	Floc size		Protozoa 10 fields^{-1}	Description of floc	Filament category	Floc size		Protozoa 10 fields^{-1}	Description of floc
		Mean diameter mm	SD				Mean diameter mm	SD				Mean diameter mm	SD		
1	1	0.221	0.181	84	loose, irregular, compact.	2	0.180	0.138	48	loose, irregular, compact.	3	0.210	0.168	113	firm, irregular, compact.
2	0	0.200	0.142	62	"	2	0.162	0.161	106	"	3	0.177	0.138	101	"
3	1	0.164	0.143	92	loose, irregular, open.	2	0.063	0.021	141	"	3	0.114	0.065	68	"
4	0	0.200	0.121	132	loose, irregular, compact.	3	0.104	0.088	120	firm, irregular, compact.	3	0.156	0.115	92	"
7	1	0.115	0.120	61	firm, regular, compact.	2	0.165	0.043	108	loose, irregular compact.	1	0.169	0.146	104	loose, regular, compact.
8	1	0.155	0.092	74	"	2	0.109	0.094	114	loose, irregular, open.	1	0.116	0.080	83	firm, irregular, compact.
9	1	0.103	0.062	91	loose, irregular, open.	2	0.137	0.076	160	"	2	0.125	0.066	69	loose, irregular, compact.
10	0	0.127	0.083	93	firm, regular, compact.	2	0.087	0.088	92	"	2	0.147	0.103	127	firm, irregular, compact.
11	1	0.120	0.101	81	"	2	0.078	0.086	84	firm, regular, compact.	2	0.197	0.137	56	firm, irregular, open.
14	1	0.186	0.174	69	loose, regular, open.	3	0.220	0.141	113	loose, irregular, compact.	1	0.181	0.164	88	firm, irregular, compact.
16	1	0.130	0.102	120	firm, regular, compact.	3	0.122	0.102	113	"	1	0.121	0.105	107	"
17	1	0.181	0.084	116	"	3	0.179	0.114	102	firm, irregular, compact.	2	0.146	0.101	111	"

Table 4.3 continued over/. . .

Table 4.3 (continued)

18	0	0.121	0.141	74	firm, regular, compact.	3	0.123	0.068	90	firm, irregular, compact.	1	0.212	0.142	80	loose, irregular, open.
21	0	0.159	0.138	61	firm, regular, open.	3	0.121	0.082	59	firm, regular, compact.	1	0.124	0.084	89	"
22	0	0.159	0.122	96	"	3	0.126	0.042	111	firm, irregular, compact.	2	0.212	0.184	114	firm. regular, compact.
23	0	0.152	0.062	103	loose, irregular, open.	2	0.184	0.091	121	"	2	0.180	0.112	115	firm, irregular, compact.
24	0	0.210	0.109	111	firm, regular, compact.	2	0.166	0.138	69	"	1	0.087	0.022	67	"
25	0	0.124	0.131	43	"	2	0.118	0.072	80	"	1	0.172	0.104	118	"

Table 4.4 Filamentous microorganisms observed in activated sludge

Organism	Branching	Motility	Filament shape	Filament length	Attached, unicellular bacteria	Sheath	Crosswalls clearly visible	Cell width	Cell length	Cell shape	Granules	Gram stain	Neisser stain	Comments	Designation (Eikelboom, 1975)
A	-	-	Straight, or slightly bent	<1.0 mm	-	-	+	1.0 - 2.5 µm	1.0 - 2.5 µm	Rectangular	-	-	-	"False" cells occasionally present. May be overlap of sheath at filament tip.	0961
B	-	-	"	<0.5 mm	-	+	+	1.2 µm	1.0 - 2.5 µm	"	+	-	-	Large spaces visible between adjoining cells under Gram-stain.	Sphaerotilus natans
C	-	-	Pin like, straight filaments protruding from floc.	<0.05 mm	-	+(?)	-	0.25 - 0.75 µm	-	-		-	-	Sheath detection difficult due to small filament diameter, filament length also difficult to measure as partly embedded in floc.	Haliscomenobacter hydrosis
D	-	-	Straight or slightly bent. Uneven thickness.	<2.0 mm	-	-	+	>1.0 µm	>1.0 µm	Square or circular	-	-	-	Filaments taper at tips.	021N
E	-	-	Straight or slightly bent.	<0.05 mm	-	+	+	0.5 - 1.0 µm	1.0 µm	Rectangular	-	-	-	Large spaces visible between adjoining cells under Gram stain.	1701
F	-	-	Coiled and tangled filaments.	-	±	-	+	0.6 µm	1.2 µm	Cocci or diplococci	-	+	+	Filament length difficult to determine due to excessive coiling.	Nostocoida limicola I
G	-	-	"	-	±	-	+	0.8 - 1.2 µm	1.6 - 2.0 µm	"	-	+	+	" " "	Nostocoida limicola II
H	-	-	"	-	±	-	+	1.5 - 2.0 µm	2.0 - 3.5 µm	"	-	+	+	" " "	Nostocoida limicola III
I	-	-	Short, straight filaments	<0.150 mm	-	?	+	1.5 - 2.0 µm	2.0 - 2.5 µm	Rectangular	-	±	-	Spore strain negative. Filaments mainly Gram-positive with some Gram-negative areas.	0041 (I)
J	±	-	Tangled filaments with characteristic "kink".	<0.4 mm	-	±	+	1.0 - 1.5 µm	2.0 - 2.5 µm	"	-	±	-	Similar to organism I but longer and more tangled. May be true branching. Spore stain negative.	0041 (J)
K	-	-	Narrow twisting filaments.	<200 µm	±	-	-	0.25 - 0.75 µm	-	-	-	+	Granules	Possibly two types; those strains with and without attached unicellular bacteria.	Microthrix parvicella
L	F	-	Straight or slightly bent filaments with branches.	<0.25 mm	-	+	+	1.0 - 1.5 µm	1.0 - 2.5 µm	Rectangular	+	-	-	Large spaces visible between adjoining cells under Gram stain in similar manner to organism B.	Sphaerotilus natans
M	-	-	Straight or slightly bent.	<0.2 mm	+	?	±	1.5 µm	0.5 - 2.5 µm	"	-	+ "speckled"	-	"Speckled" appearance under Gram stain predominantly Gram-positive. Cells of non-uniform length, uniform width.	0041 (M)

Table 4.4 Continued over/. . .

Table 4.4 (Continued)

N	-	-	Small chains of cocci found mainly within floc.	<0.05 mm	-	-	+	0.5 µm	0.5 µm	Cocci	-	+	-	Cell details difficult to see under phase contrast microscopy.	Staphylococcal chains.
O	-	-	Short coiled chains	<0.1 mm	-	?	+	0.6 µm	1.2 µm	Rectangular	-	+	-		Undesignated
P	T	-	Short branched filaments	<0.05 mm	-	-	+	0.5 µm	0.5 - 1.0 µm	"	-	+	-	Generally found outside the floc.	*Nocardia* spp.

All strains gave a negative response to the S-test. No sulphur granules were found "*in vivo*".

± - Sometimes

? - Test not possible

F - False branching

T - True branching

Table 4.5a Filamentous microorganisms observed in return activated sludge from Runcorn plant 1 (first period of study).
x = organism dominant; / = organism secondary; 0 = organism incidental.

Organism	DAY																		
	1	2	3	4	7	8	9	10	11	14	15	16	17	18	21	22	23	24	25
A	x			0		0	/	/	/			/	0	x	0				/
B					0														
C						0	0	0	0				0	0					
D																			
E	0	0				0	0	0											
F	/				0		0												
G	/	0				0													
H								0											
I			0					0	0				0				0		0
J					0				0										
K	/	x	x	0	x	x	x	x	x	x		/	x	x	x	x	x		x
L			0		0							/		0	0		0		0
M		0	0		0	/	0	/	0	/		/	0	0	0	x	/		x
N																	0		
O																			
P																			0

Table 4.5b Filamentous microorganisms observed in return activated sludge from Runcorn plant 2 (first period of study).
x = organism dominant; / = organism secondary; 0 = organism incidental.

Organism	DAY																		
	1	2	3	4	7	8	9	10	11	14	15	16	17	18	21	22	23	24	25
A	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
B							0												
C		0			0	0	0							0					
D																			
E																			
F	0	0				0													
G				0															
H																			
I		0	0		0	0		0				0	0	0	0	0			0
J	0			0															
K		0	0	0	0		0	/	0	/				0	0	0			0
L														0		0			
M	0	0	0		/	0	0	/	0	/		/	0	0	/	0			0
N	0			0															
O			0		0		0												
P		0	/	0	0	/	0	0	0			/	/	0					

Table 4.5c Filamentous microorganisms observed in return activated sludge from Warrington South (first period of study).
x = organism dominant; / = organism secondary; 0 = organism incidental.

Organism	DAY																		
	1	2	3	4	7	8	9	10	11	14	15	16	17	18	21	22	23	24	25
A	/	0	0	/	/	/	/	/	/	/		/	/	0	/	0		0	x
B					0														
C		0	0	0	0	0	0	0	0						0	0			
D		0		0	0	0						/				0			
E		0	0	0		0		0											
F	0							0	0										
G	0			0										0	0			0	
H																			
I	0	0		0				0					0	0	/	0	0		
J								0											
K	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
L							0						0	0	0	0	0		0
M		0			0				0				0		0	0			0
N																			
O									0										
P																	0		

design or operational characteristics were involved in filament selection.

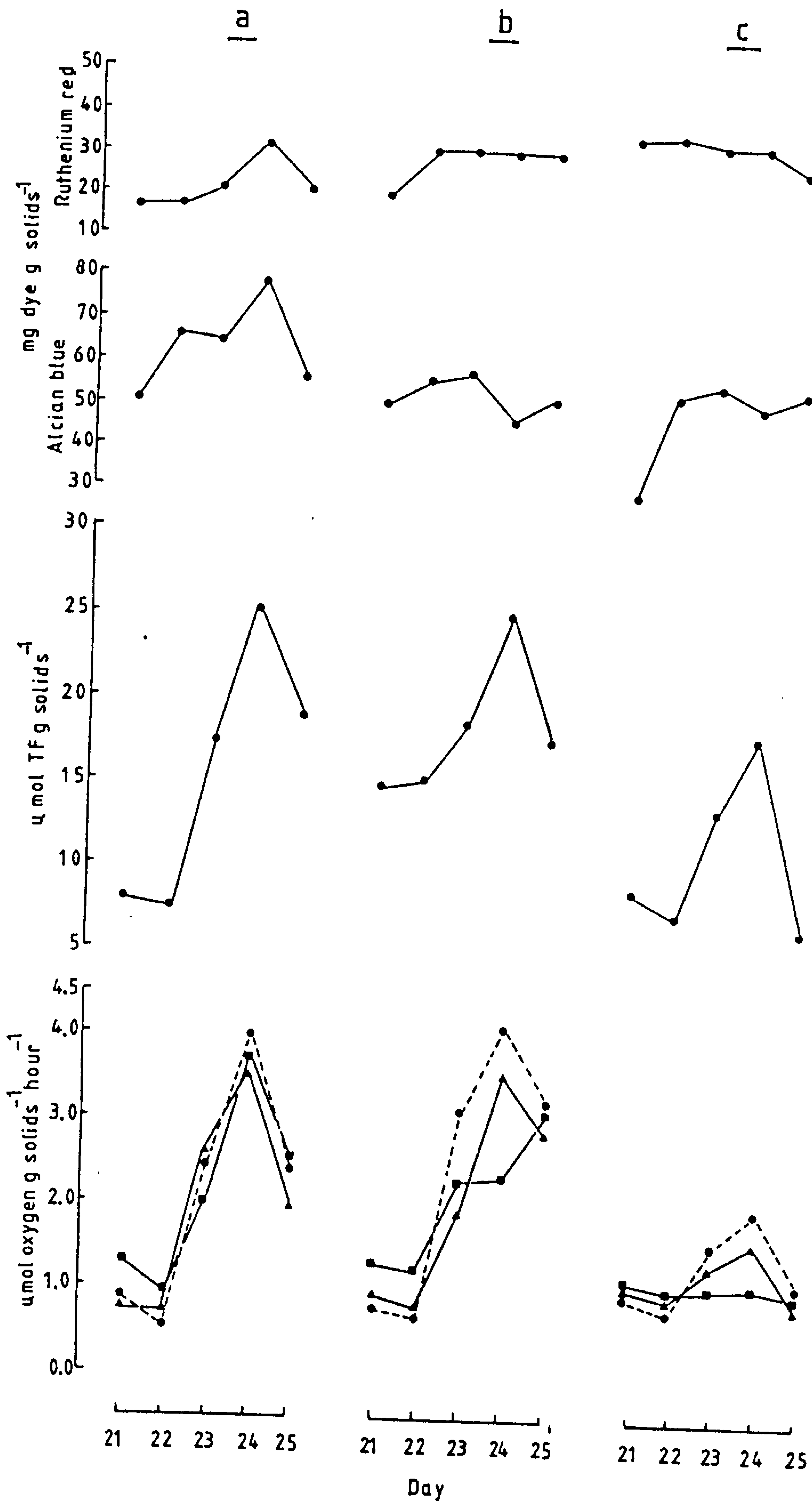
A number of other filamentous microorganisms were incidentally found in the activated sludge samples (Table 4.4). There was no apparent relationship between the appearance of specific microorganisms and plant parameters.

During the final five days of this study (28th September 1981 to 2nd October 1981), sludge activity as triphenyl tetrazolium chloride (TTC) reduction and dissolved oxygen uptake rate were measured. These results are shown in Fig. 4.4. Clearly for the period studied, trends in dissolved oxygen uptake rate closely followed those of TTC reduction. Addition of external substrates glucose and glycerol had no effect on dissolved oxygen uptake rate, probably as the sludge was not acclimated to these substrates. Mean activity for Runcorn plant 1, Runcorn 2 and Warrington South sludge respectively was 17.4, 18.9 and 11.6 μmol. TF. g $solids^{-1}$ as TTC reduction and 2.1, 2.4 and 1.3 μmol O_2 g $solids^{-1}$ as dissolved oxygen uptake rate. For both activity measurements sludge from Warrington South was least active. This probably reflects the low loading of this plant.

A second study was carried out at a later date (26th September 1982 to 7th October 1982) using dye adsorption tests on 50% activated sludge. Only the two plants at Runcorn were analysed. Plant operational data for this period are shown in Figs. 4.5 and 4.6. Mean values of this data are shown in Table 4.6.

Figure 4.4

Dye adsorption, activity and oxygen uptake rate in return activated sludge from Runcorn plant 1 (a), Runcorn plant 2 (b) and Warrington South (c). Oxygen uptake was measured with no added substrate (▲), 5 mM glucose (●) or 5 g l^{-1} glycerol (■).



a
b
c
Ruthenium red
mg dye g solids^{-1}
Alcian blue
μ mol TF g solids^{-1}
μmol oxygen g solids^{-1} hour^{-1}
Day

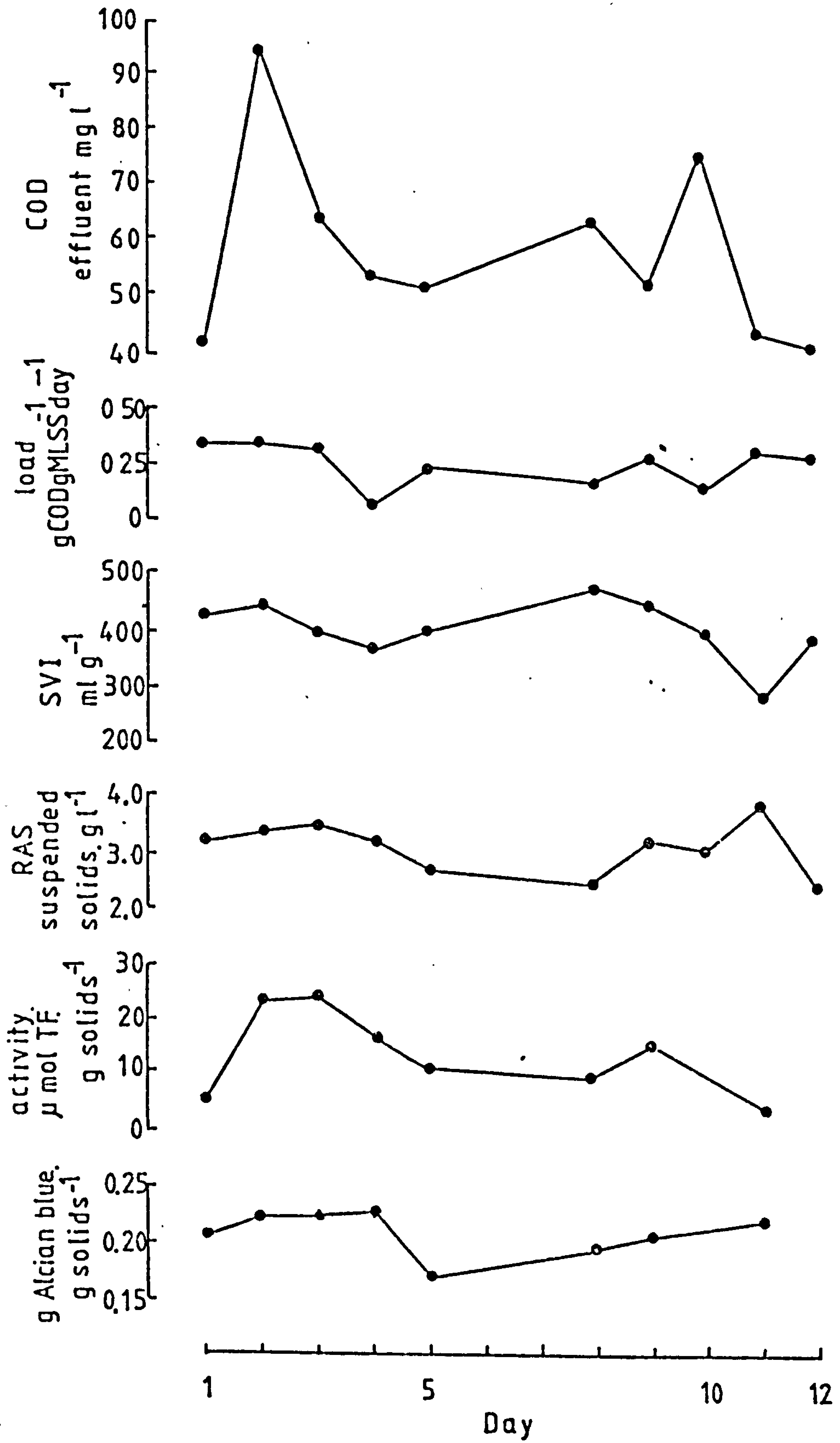
Figure 4.5

Plant characteristics throughout the second period of study at Runcorn plant 1 (26th September 1982 to 7th October 1982).

COD effluent mg l-1
80
70
60
50
40
30
load. gCOD.gMLSS-1.day-1
1.5
1.0
0.5
0
SVI ml g-1
800
700
600
500
400
300
200
RAS suspended solids g l-1
5.0
4.0
3.0
2.0
1.0
activity µmol TF. g solids-1
40
30
20
10
0
g Alcian blue. g solids-1
0.35
0.30
0.25
0.20
0.15
1
5
10
12
Day

Figure 4.6

Plant characteristics throughout the second period of study at Runcorn plant 2 (26th September 1982 to 7th October 1982).



COD effluent mg l-1
100
90
80
70
60
50
40
load gCODgMLSS-1 day-1
0.50
0.25
0
SVI ml g-1
500
400
300
200
RAS suspended solids, g l-1
4.0
3.0
2.0
activity μ mol TF. g solids-1
30
20
10
0
g Alcian blue. g solids-1
0.25
0.20
0.15
1
5
10
12
Day

Table 4.6 Means and range of data over the second study period

	Runcorn plant 1		Runcorn plant 2	
	Mean	Range	Mean	Range
Settled sewage				
BOD mg l^{-1}	143.5	98-192	143.5	98-192
COD mg l^{-1}	320.0	146-477	320.0	146-477
Ammonia nitrogen mg l^{-1}	22.5	8.1-30.2	22.5	8.1-30.2
Suspended solids mg l^{-1}	91.0	50-164	91.0	50-164
Aeration				
Load g COD g $MLSS^{-1}$ day^{-1}	0.767	0.41-1.59	0.252	0.08-3.54
SV1 ml g^{-1}	429	258-750	409.2	282-485
SV ml l^{-1}	90.3	70-100	90.5	80-97
MLSS g l^{-1}	2.13	1.24-3.15	2.24	1.96-3.08
RAS suspended solids g l^{-1}	3.19	1.86-4.22	3.17	1.52-3.54
Alcian blue adsorption mg g $solids^{-1}$	261	156-329	178	172-232
RAS activity µmol. TF. g^{-1}	12.7	5.3-37.6	11.5	3.9-25.1
Effluent				
BOD mg l^{-1}	8.1	5-13	7.4	3-17
COD mg l^{-1}	57.7	31.76	58.7	42-94
Ammonia nitrogen mg l^{-1}	10.9	0.6-21.0	9.13	0.3-21.0
Nitrate nitrogen mg l^{-1}	3.79	0.9-17.35	3.66	0.43-12.43
Nitrite nitrogen mg l^{-1}	1.02	0.23-3.42	0.724	0.25-1.41
Suspended solids mg l^{-1}	13.9	4-24	13.8	8-33

Floc size and filament category were recorded according to the method of Eikelboom (1981). These results are presented in Table 4.7. Filament category was of a similar order to that of the first study despite a change in microbial population, with filaments again more numerous in plant 2. Floc size measurements were again subject to large daily variation.

The correlation coefficient was again calculated for Alcian blue binding and plant operational parameters (Table 4.8). There is a highly significant inverse relationship between return sludge solids concentration and Alcian blue adsorption. This supports the view that sludges of low suspended solids concentration have higher dye binding capacity. In Runcorn plant 1 there is a highly significant inverse correlation between sludge loading and Alcian blue adsorption. This can be explained by the positive relationship between loading and return sludge solids concentration ($r = 0.746$ with 7 degrees of freedom, $P<0.02$). A significant inverse relationship was also found between Alcian blue adsorption and nitrite in the effluent of Runcorn plant 2. There is no relationship between return sludge solids concentration and nitrite thus it is possible that nitrite ions are combining with Alcian blue to prevent sludge binding. However, this did not occur at other works although similar levels of nitrite were found in the effluent.

Sludge activity as measured by TTC reduction increased with loading in plant 1 but not in plant 2. This may be due to the much lower loading in plant 2. Sludge activity was also related

Table 4.7 Floc size and filament category of sludges during the second period of study

Day	Runcorn plant 1				Runcorn plant 2			
	Filament category	Floc size (%)			Filament category	Floc size (%)		
		<150 μm	150-500 μm	>500 μm		<150 μm	150-500 μm	>500 μm
1	0	25	50	25	3	25	25	50
2	1	25	25	50	3		50	50
3	1		50	50	2	50	25	25
4	2	25	50	25	3	33	33	33
5	1		50	50	3		50	50
8	0	5	90	5	2	33	33	33
9	1		50	50	1	25	50	25
10	1	25	50	25	3		50	50
11	0	25	50	25	3	25	50	25

Table 4.8 Correlation between Alcian blue adsorption by 50% return activated sludge, sludge activity and plant characteristics (second period of study).

Plant operation parameter	Runcorn plant 1						Runcorn plant 2						Warrington South					
	Alcian blue adsorption			Sludge activity			Alcian blue adsorption			Sludge activity			Alcian blue adsorption			Sludge activity		
	r	n	P	r	n	P	r	n	P	r	n	P	r	n	P	r	n	P
Load (BOD)	-0.53	8	NS	0.66	8	<0.05	0.28	8	NS	0.41	8	NS	-	-	-	-	-	-
Load (COD)	-0.69	8	<0.05	0.64	8	<0.05	0.16	8	NS	0.10	8	NS	-	-	-	-	-	-
BOD influent	0.62	8	NS	0.29	8	NS	-0.07	8	NS	0.43	8	NS	0.28	7	NS	0.48	7	NS
COD influent	0.14	8	NS	0.61	8	<0.1	0.34	8	NS	0.09	8	NS	0.10	7	NS	0.51	7	NS
Nitrogen (NH_4) influent	0.12	8	NS	0.51	8	NS	0.55	8	NS	0.16	8	NS	0.16	7	NS	0.20	7	NS
Suspended solids influent	-0.43	8	NS	0.36	8	NS	0.04	8	NS	-0.07	8	NS	0.51	7	NS	-0.07	7	NS
RAS suspended solids	-0.73	8	<0.02	0.63	8	<0.1	0.67	8	<0.05	0.07	8	NS	-0.48	7	NS	0.36	7	NS
Sludge activity	-0.47	8	NS	-	-	-	0.39	8	NS	-	-	-	0.72	7	<0.05	-	-	-
BOD effluent	0.06	8	NS	0.41	8	NS	0.39	8	NS	0.60	8	<0.1	0.27	7	NS	0.29	7	NS
COD effluent	0.28	8	NS	0.39	8	NS	0.25	8	NS	0.75	8	0.02	0.38	7	NS	0.78	7	<0.05
Nitrogen (NH_4) effluent	0.53	8	NS	0.78	8	<0.02	0.12	8	NS	0.35	8	NS	0.37	7	NS	0.78	7	<0.05
Nitrogen (NO_3) effluent	0.56	8	NS	-0.32	8	NS	0.05	8	NS	-0.59	8	NS	0.61	7	NS	-0.30	7	NS
Nitrogen (NO_2) effluent	0.37	8	NS	-0.39	8	NS	-0.76	8	<0.02	-0.21	8	NS	0.05	7	NS	-0.22	7	NS

to ammonia nitrogen in the effluent of plant 1.

Results of microscopic analyses can be seen in Table 4.9. During the time between the two studies the flow patterns of both Runcorn plants were changed (See Chapter 3). Subsequently the loading to both plants was reduced. This reduction in loading was accompanied by a change in the microbial population. The dominant filamentous microorganism in Runcorn plant 1 sludge changed from *Microthrix parvicella* to Type 0961. There was also an increase in the occurrence of *Nostocoida limicola* types I, II and III in both sludges at the time of the second study.

Further studies were carried out to assess the change in sludge characteristics along the flow path of Runcorn 1 and Runcorn 2 activated sludge plants. These results are presented in Figs. 4.7a and 4.7b. For each plant, samples were taken on three separate days over a weekly period (between 28th September 1982 and 7th October 1982). Clearly there is a large variation in many of the factors studied. In plant 1 sludge reaeration was carried out in the first pocket thus mixed liquor suspended solids were high until dilution with settled sewage in pocket 2. No reaeration was carried out at plant 2 so sludge dilution occurred in pocket 1.

Sludge activity shows a marked increase on addition of sewage to plant 2. Activity then gradually decreases as organic material is utilized and eventually exhausted by the floc. The situation is less clear in plant 1. Activity is stimulated by the addition of fresh sewage, but the pattern of activity throughout

Table 4.9 Filamentous organisms observed in return activated sludge(second period of study). Description of organisms can be seen in Table 4.4.
x - organism dominant; / - organism secondary; 0 - organism incidental.

Day / Filaments	Runcorn plant 1									Runcorn plant 2								
	1	2	3	4	5	8	9	10	11	1	2	3	4	5	8	9	10	11
A	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x		x
B			0		0					0								
C										0		0	0			0		
D																		
E																		
F		0				0	0			0	0				0			0
G	/	0	0		0		0		0		0	/		0		/		0
H												0			0			
I	0					0				0	0		0	0		0		0
J			0						0	0								
K	0	0	/	0	0	0	0		0	0		0	0		0			0
L		0		0	0				0		0	0	0		0	0		
M																		
N	0	0	0		0	0	0		0	/	/	0	0	0	/	0		0
O		0																
P		0		0	0	0				0	0	0	/	0	0	/		0

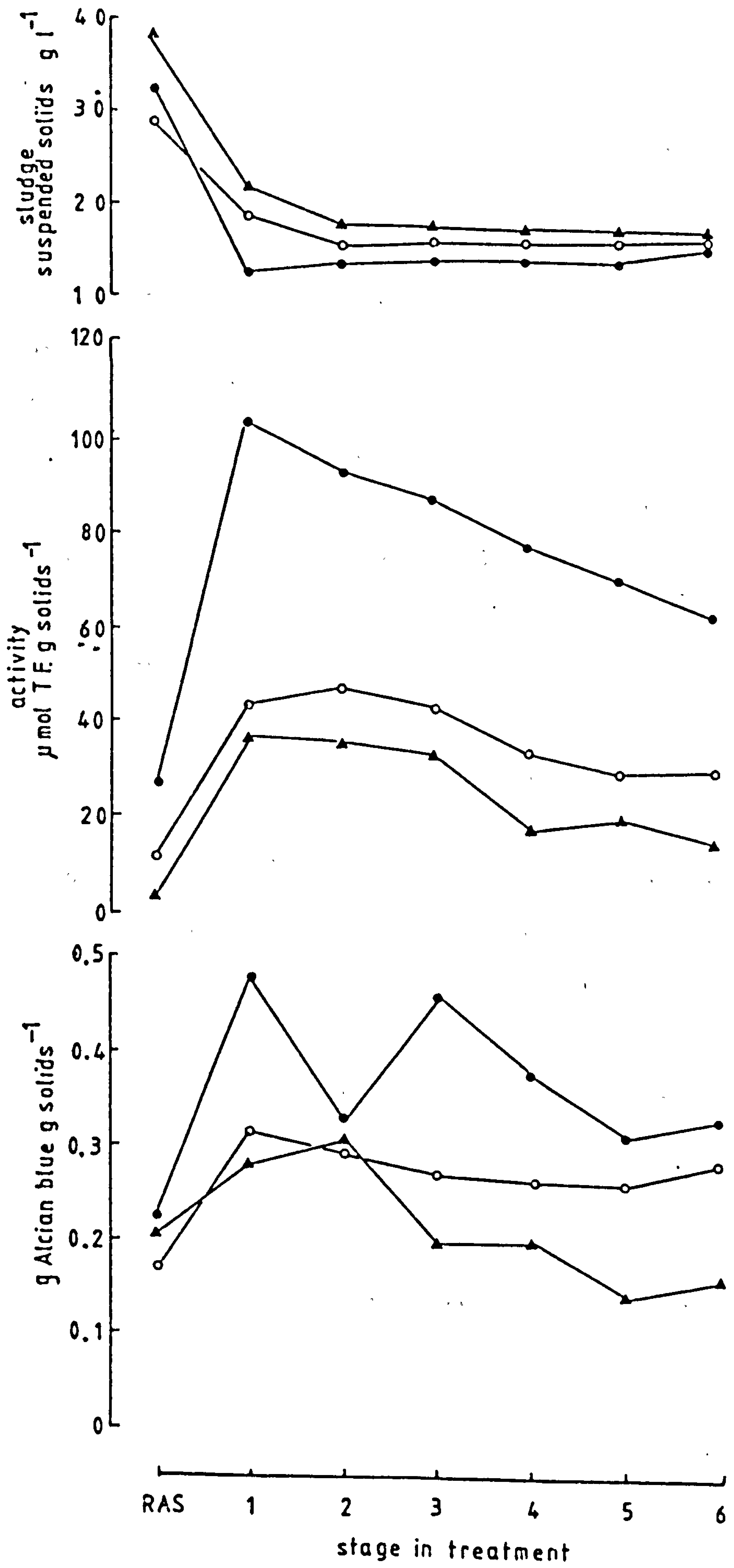
Figure 4.7

Alcian blue adsorption, sludge activity and suspended solids concentration throughout each stage of Runcorn plant 1 (a) and Runcorn plant 2 (b). Studies were performed three times for each plant over a period of 2 weeks (between 28th September 1982 to 7th October 1982).

Results for total extracellular polymer measured as ethanol insoluble material (e.i.m.) are also presented for plant 1.

The arrangement of aeration lanes at the time of study are shown in Figs 3.1b and 3.1d for plants 1 and 2 respectively.

a
mg eim.g sludge^{-1}
80
70
60
50
3.0
2.0
1.0
0
sludge
suspended solids - g l^{-1}
90
70
50
30
1.0
activity
μ mol TF g solids^{-1}
0.6
0.5
0.4
0.3
0.2
0.1
g Alcian blue g solids^{-1}
RAS
1
2
3
4
5
6
7
8
stage in treatment


b
sludge suspended solids g l^{-1}
4 0
3 0
2 0
1 0
activity µ mol T.F. g solids^{-1}
120
100
80
60
40
20
0
g Alcian blue g solids^{-1}
0.5
0.4
0.3
0.2
0.1
0
RAS
1
2
3
4
5
6
stage in treatment

aeration is variable. This is probably a result of the step loading in plant 1, where the point of sewage addition varies with flow rate. In both plants, return activated sludge gave rise to less TTC reduction than the mixed liquor of the final aeration pocket. Mean values for these respective stages were 14.81 and 36.5 μmol TF g solids^{-1} for plant 1 and 13.37 and 34.14 μmol TF g solid^{-1} for plant 2. This is in disagreement with the results of Coackley and O'Niell (1975) who recorded a two to three fold increase in activity in return activated sludge. This they suggested was due to a release of hydrogenase enzymes on cell lysis. A mean increase in activity was found on sludge reaeration in pocket 1 of plant 1, with activity increasing from 13.37 to 25.65 μmol TF g solids^{-1}. This may have been due to cell lysis or stimulation of bacterial dehydrogenases by aeration.

Alcian blue binding was not affected by sludge reaeration. However, binding increased at the sludge/sewage mixing stage at both plants (i.e. pocket 2 of plant 1 and pocket 1 of plant 2). This was followed by a decrease in dye binding as the sludge progressed along the aeration lanes. This may have been due to initial deflocculation causing an increase in surface area, followed by later reflocculation. Alternatively the changes in dye binding throughout the plant may reflect the chemical nature of the sludge surface. This was further investigated in plant 1 by the measurement of total sludge extracellular polymer, as ethanol insoluble material (Forster, 1976). Total polymer is highest at the stage of highest Alcian blue binding. The nature

of sludge extracellular polymer at various stages throughout activated sludge plants is described further in Chapter 6.

4.2 Bulking activated sludge

During the Summer of 1982 an incident of sludge bulking occurred at Runcorn E.T.W. Both plants 1 and 2 were affected. At the onset of the problem the flow patterns of both works were changed (see Chapter 3). Poor settleability was ascribed by the works staff as being due to shock loadings of brewery effluent. A sample of bulking return activated sludge was taken from Runcorn plant 1 (15th July 1982) and compared with sludge from the same plant taken several weeks prior to (4th May 1982), and several weeks following (9th August 1982) the bulking incident. Results of analysis of heterotrophic, aerobic bacteria isolated on CGY agar are given in Chapter 5. Plant operational characteristics at the time of each sample are shown in Table 4.10. It is noticeable that although SV and SVI in bulking sludge were high, effluent BOD and suspended solids were low. However, the 30:50 standard was exceeded on several occasions.

Adsorption isotherms of the three samples are shown in Fig. 4.8. These were fitted to Langmuir and mass action equations and these results can be seen in Table 4.11. Isotherms for all three sludges fitted both equations with a high degree of significance. There is some evidence that the bulking sludge has a high adsorption capacity than the non-bulking sludges. The constants K_1 of the Langmuir equation and θ for the mass action equation are

Table 4.10 Plant data at time of removal of return activated sludge. A - sludge not bulking. B - sludge bulking. C - sludge not bulking.

	A	B	C
Settled sewage			
BOD mg l^{-1}	94	140	-
COD mg l^{-1}	230	349	-
Ammonia nitrogen mg l^{-1}	-	21	-
Suspended solids mg l^{-1}	42	61	62
Aeration			
Load g COD g $MLSS^{-1}$ day^{-1}	0.93	1.05	-
SV1 ml g^{-1}	320	578	402
SV ml	40.0	100.0	80.0
MLSS g l^{-1}	1.25	1.73	2.09
RAS suspended solids g l^{-1}	3.09	2.584	2.310
RAS activity μ mol TF g^{-1}	41.0	29.7	42.3
Viability C.F.U. g^{-1}	1.99×10^{11}	3.4×10^{11}	3.5×10^{11}
Mean floc size μm	200	232	181
Effluent			
BOD mg l^{-1}	8	12	9
COD mg l^{-1}	54	56	76
Ammonia nitrogen mg l^{-1}	4.3	13	12
Nitrate nitrogen mg l^{-1}	2.8	0.8	9
Nitrite nitrogen mg l^{-1}	3.9	0.5	3
Suspended solids mg l^{-1}	10	13	9

Figure 4.8

Alcian blue adsorption by return activated sludge.

Samples were taken from Runcorn plant 1 approximately one month prior to a bulking incident (■), at the time of bulking (●) and one month after bulking was resolved (▲). Adsorption tests were carried out on 50% activated sludge. Initial suspended solids concentrations were 2.204 g l^{-1}, 2.584 g l^{-1} and 2.31 g l^{-1} for the three sludges respectively.

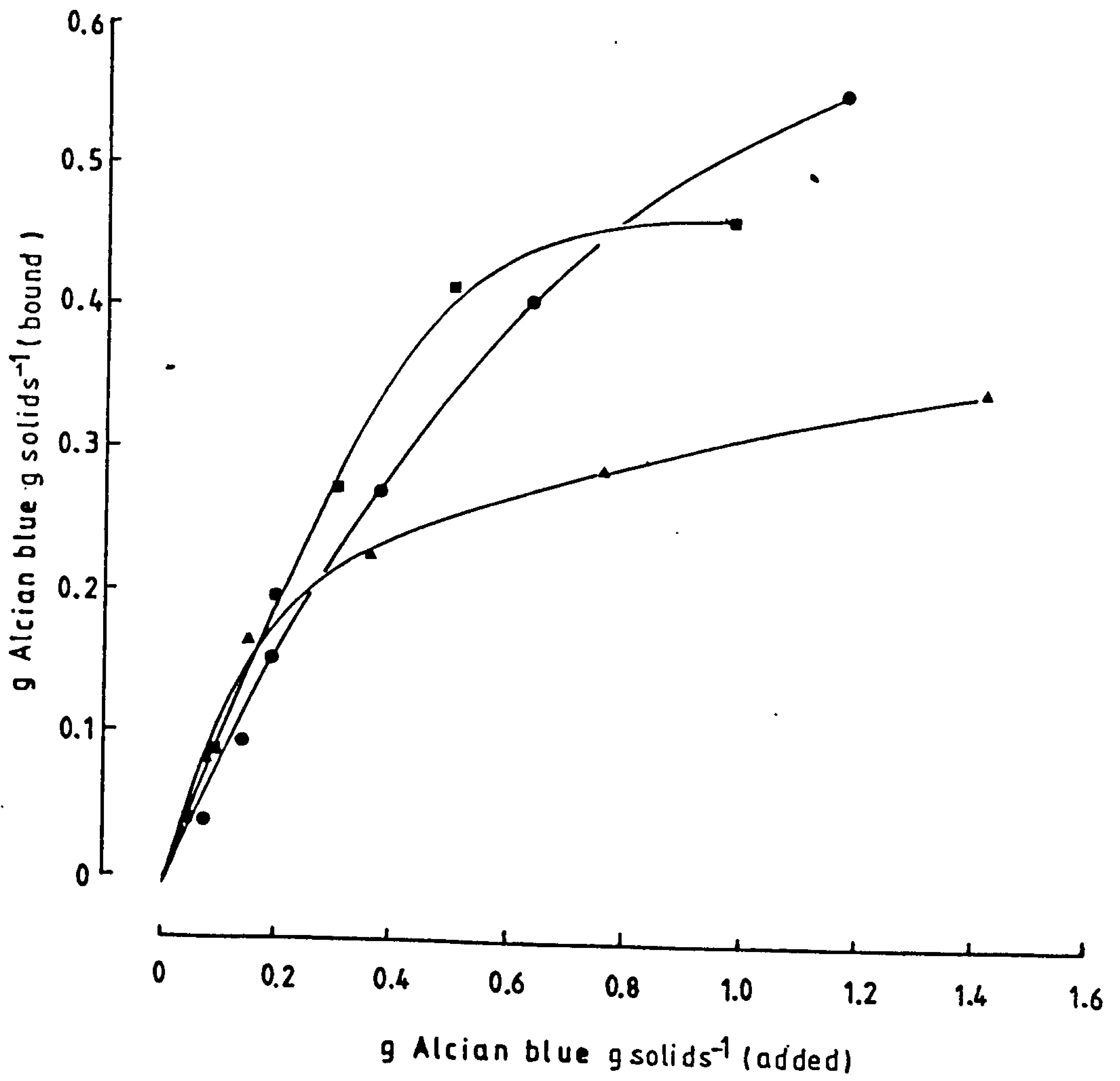

0.6
0.5
0.4
0.3
0.2
0.1
0
g Alcian blue g solids^{-1} (bound)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
g Alcian blue g solids^{-1} (added)

Table 4.11 Adsorption isotherms of return activated sludge from Runcorn plant 1.

	Langmuir equation $x = c$, $y = c/m$					Mass Action equation $x = 1/m$, $y = 1/D$						
	regression	r (df)	P	Constants		regression	r (df)	P	Constants			
				K_1	K_2				β	σ'	K_B ($\times 10^5$)	σ ($\times 10^{-6}$)
Sludge A	y = 2.08x + 25	0.998(2)	<0.01	0.481	0.083	y = 0.319x + 2.7	0.969(2)	<0.05	0.319	0.370	31.285	1.002
Sludge B	y = 1.62x + 0.21	0.96 (4)	<0.005	0.617	7.717	y = 117.62x + 2.69	0.995(4)	<0.001	117.62	0.372	0.084	1.008
Sludge C	y = 3.13x - 0.12	0.976(3)	<0.005	0.319	26.12	y = 5.4x - 4.02	0.960(3)	<0.01	5.396	0.249	2.74	0.675

Sludge A - taken several weeks prior to a bulking incident.
Sludge B - bulking sludge.
Sludge C - taken after normal working was resumed.

measures of sludge adsorption capacity. These are highest in the bulking sludge. However, it is apparent that in this case differences between dye adsorption in bulking and non-bulking sludges are not large enough to enable adsorption tests to be used as a useful guide to sludge settleability.

Photographs of bulking and non-bulking sludges are shown in Figs 4.9a and b, taken using dark ground microscopy. A bright field photograph of bulking sludge stained with Alcian blue can be seen in Fig. 4.10. Adsorption of dye to both floc and filaments can be clearly seen.

DISCUSSION

Fifteen types of filamentous microorganism were observed in return activated sludge from the three works studied. These organisms were classified according to the descriptions of Eikelboom (1981). A number of problems were encountered in identification. Three filamentous strains (I, J and M) all fell within Eikelbooms description of Type 0041. According to this worker, cells of these filaments vary in length from 0.7 to 2.3 µm and in width from 1.0 to 1.4 µm. Branching occurs incidentally and the cells may give a positive result in the S-test of Fanquhar and Boyle (1971). With such large diversity in filament morphology with regard to Type 0041 there is a possibility that more than one strain of this organism may coexist.

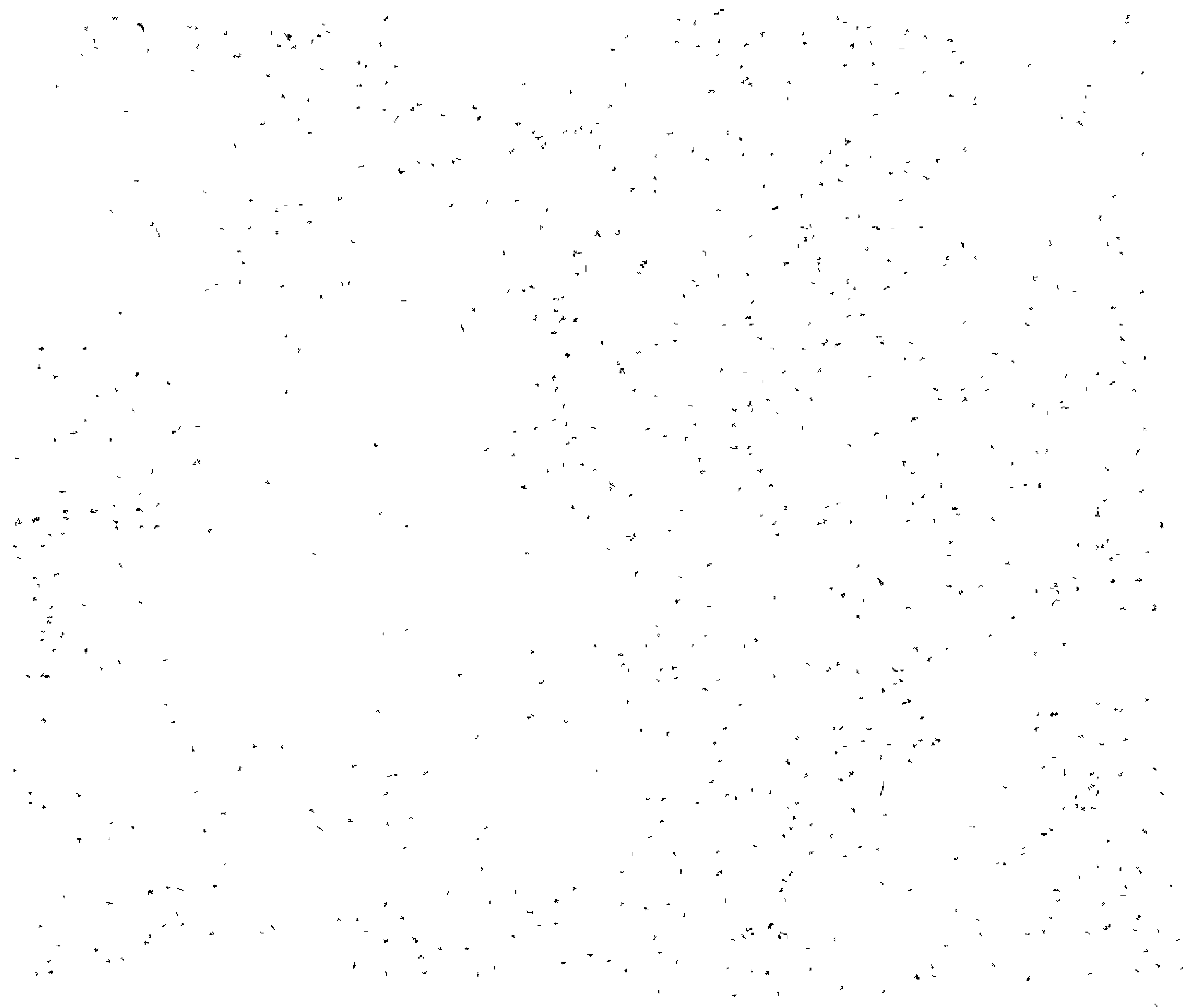
Identification of filaments using light microscopy proved difficult in several respects. In particular, sheath detection

Figure 4.9

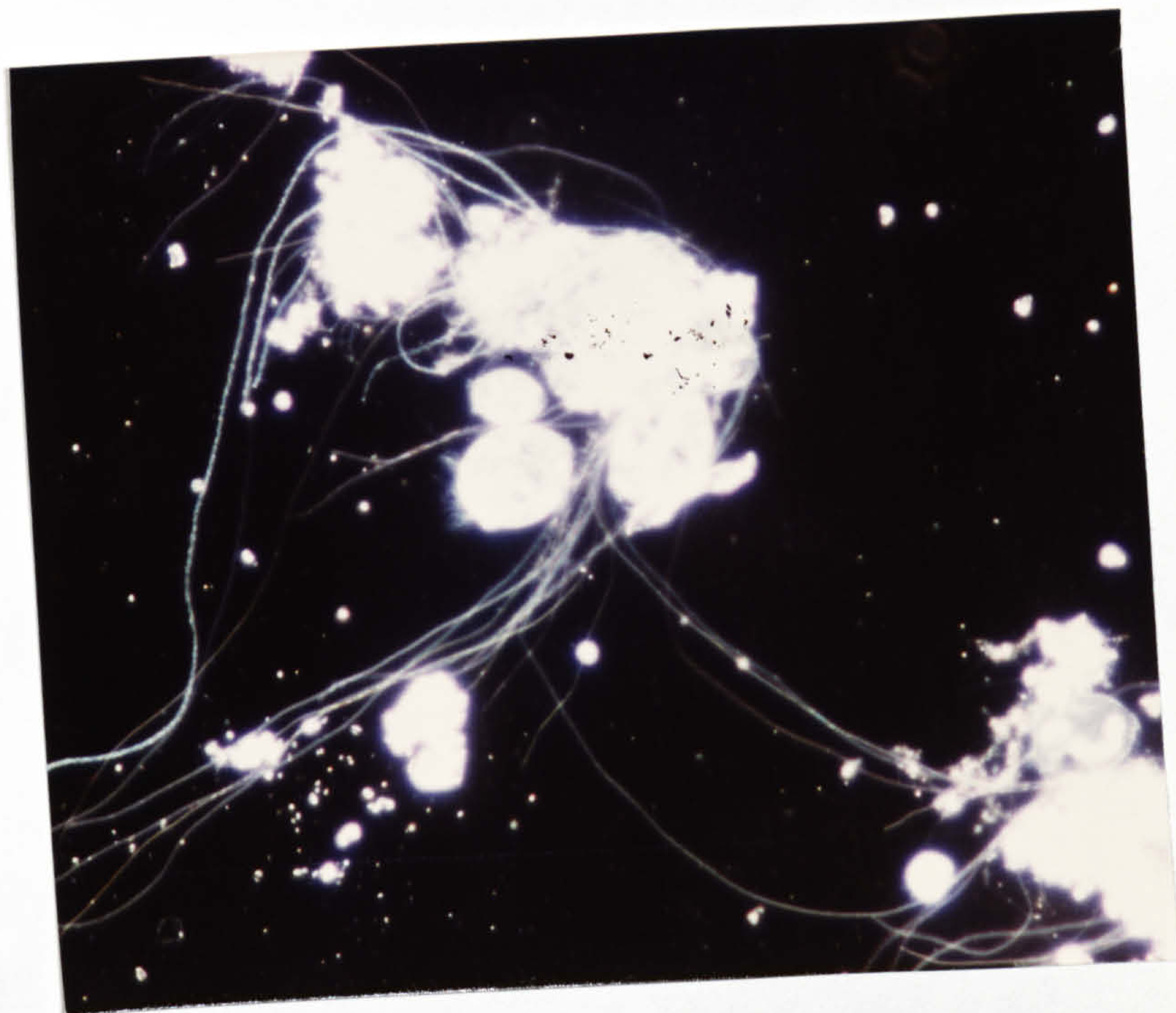
Return activated sludge from Runcorn plant 1 as seen using dark ground microscopy. Total magnification approximately x250.

(a) bulking sludge.

(b) non-bulking sludge.



a



b

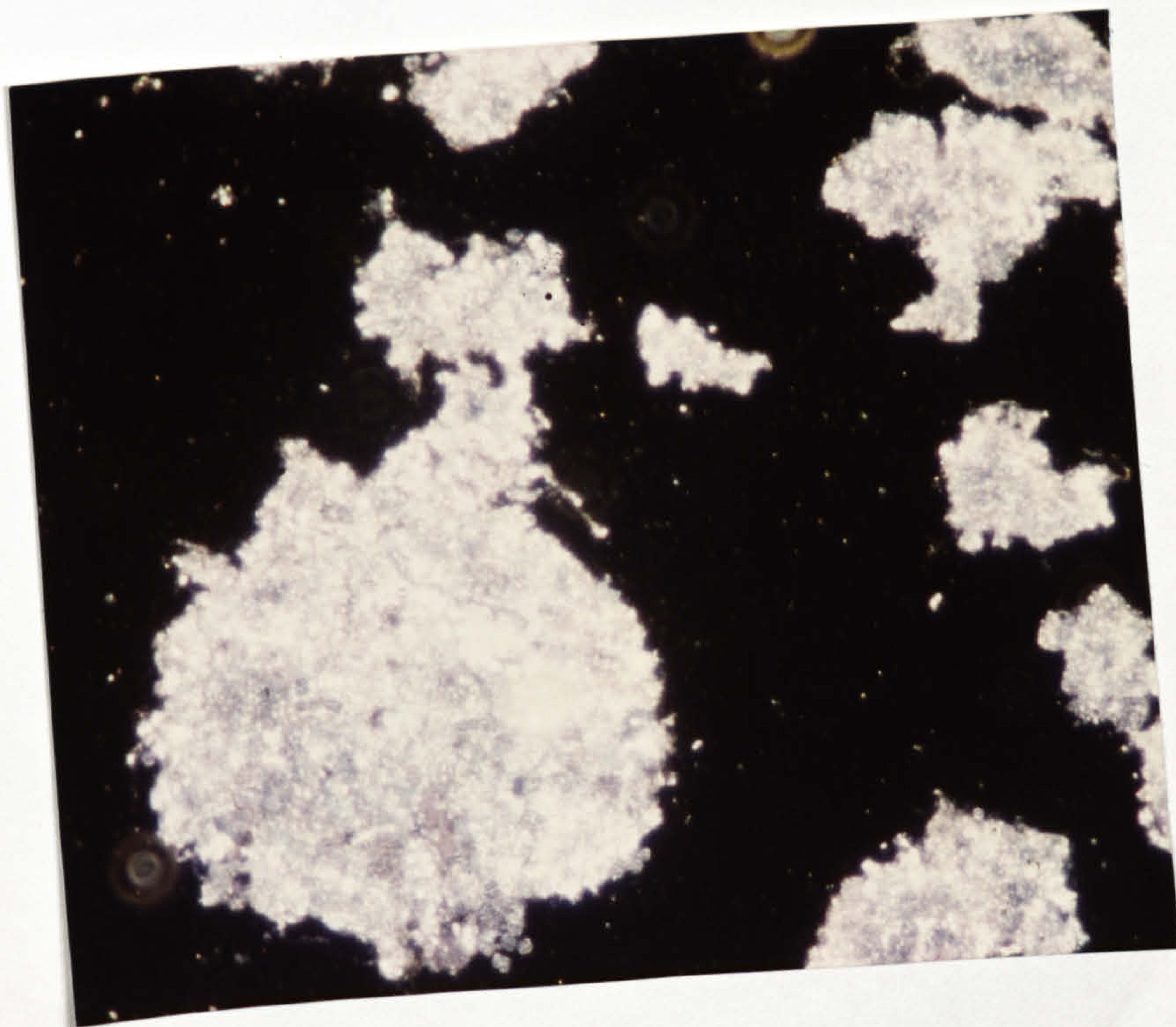


Figure 4.10

Bulking return activated sludge from Runcorn plant 1 stained with Alcian blue. Total magnification approximately x500.

using a phase contrast microscope, or the methylene blue staining technique was unsatisfactory. Sheaths were in any case obscured where there was significant attached microbial growth. The situation was further confused by the presence of "pseudo-sheaths". These were noticed by Eikelboom (1975) and Strom and Jenkins (1984) and occur as "ghost" cells, i.e blank spaces within the bacterial trichrome.

Sheath detection and other morphological features were often difficult to detect due to the small size of some filaments, especially Haliscomenobacter hydrossis. Internal granules or features were often obscured by attached growth and measurement of filament length was often impossible due to their penetration of the floc.

Finally, all reported descriptions of filamentous microorganisms in activated sludge have their origin in America (Strom and Jenkins, 1984) the Netherlands (Eikelboom, 1975, 1977) or West Germany (Wagner, 1982). No literature is available on the situation in the U.K. and it is possible that differences exist in filament morphology and diversity.

Filamentous microbial populations were clearly affected by activated sludge plant operation and design. The plants at Runcorn E.T.W. received sewage of identical chemical composition yet produced sludges of distinctly different filamentous characteristics. Plant 1 always produced a sludge with less filaments than plant 2. At the time of the initial studies the most prevalent organism in plant 1 sludge was Microthrix

parvicella, whilst in plant 2 sludge Type 0961 was most numerous. At this time loading to the two plants was 0.79 and 0.84 g COD g $MLSS^{-1}$ day^{-1} respectively. At a later date, when plant operation was changed (see Chapter 3) this resulted in a decrease in loading to plant 2 to around 0.25 g COD g $MLSS^{-1}$ day^{-1} yet this was followed by no apparent change in filamentous bacteria population. Conversely, a minor change in loading to plant 1 resulted in a change in the dominant filamentous organism from Microthrix parvicella to Type 0961.

Thus factors other than plant loading are important in filament selection. Aeration tank sizes at the two plants were 343.0 m^3 at plant 1 and 468.7 m^3 at plant 2. Coupled with the fact that plant 1 in any case received a higher proportion of the sewage inflow to the works (about 60%) this means that there was a higher COD concentration at the sludge/sewage mixing stage in plant 1. This may explain the differences in filamentous bacterial populations between the two plants at the time of the first study.

At the time of the second study, the sewage/sludge mixing stage in plant 2 was unchanged. However, at plant 1 step loading was introduced, effectively reducing the initial COD concentration. This could explain the increase in numbers of Type 0961 filaments and the reduction of Microthrix parvicella in the sludge. It is likely that the higher initial nutrient concentration was also responsible for the superior sludge settlement properties in plant 1 at the time of the initial study.

It may be significant that during the second study where step loading was introduced, sludge quality had deteriorated. The relationship between initial COD concentration and settlement has been noted by a number of workers (Chudoba *et al*., 1974; Rensink *et al*., 1982; Chambers, 1982).

There is only limited information in the literature with regard to Type 0961. In activated sludge plants this organism has been ranked as the 9th (Wagner, 1982), 10th (Eikelboom, 1977) and 15th (Strom and Jenkins, 1984) most common filament found in activated sludge. Strom and Jenkins (1984) found Type 0961 in only 2 out of 78 activated sludge plants in the U.S.A., and of these the filaments were most numerous in only one plant. Wagner (1982) found the organism to be responsible for 2.8% of all bulking incidents in West Germany. Type 0961 has been associated with low loading levels (Lau *et al*., 1980) and also nitrogen deficiency (Wu *et al*., 1984b). The bulking incident at Runcorn was thought to be a result of shock loads to the works as a result of an increased output by a brewery. Brewery effluents are characterized by a high carbon to nitrogen ratio, and thus nitrogen deficiency may have been a contributory factor in the bulking problem.

Microthrix parvicella was commonly found in sludges from all three plants studied. The physiology of this organism has been extensively studied in pure culture (Slijkhuis and Deinema, 1982; Slijkhuis *et al*., 1984) and is unusual in that it has an essential requirement for oleic acid. In the surveys of Eikelboom (1977),

Wagner (1982) and Strom and Jenkins (1984) this organism was ranked 1st, 2nd and 8th respectively in terms of appearance in activated sludge. It is the most common organism in bulking sludge in the Netherlands (Eikelboom, 1977). It has been associated with bulking problems where organic loading is low, and also where plants treat chemical waste.

A major difference between the filamentous populations at Runcorn plants 1 and 2 was the presence of large amounts of Nocardia spp. in sludge from the latter. These organisms have often been reported in activated sludge but have not been associated with bulking. However, Nocardia spp. are often deemed responsible for a viscous brown nuisance-foam. Foaming was indeed a minor problem at Runcorn plant 2. Although not common in European sludges (Eikelboom, 1977) Nocardia spp. are more prevalent in sludges in the U.S.A. Strom and Jenkins (1984) point to the higher sludge loads generally received by American works than those in Europe as the reason for this discrepancy. It is likely that the situation in the U.K. closely resembles that of the United States as these two countries utilize mainly activated sludge plants, compared with the Netherlands where oxidation ditches remain an important method of wastewater treatment.

In addition to microbial studies, sludges were also tested for dye adsorption capacity. The objective was to develop a simple test for adsorption and to evaluate its use as a guide to settlement problems or characteristics. Two studies were carried out, one on Ruthenium red and Alcian blue adsorption by 5% return

activated sludge and a second to evaluate Alcian blue adsorption by 50% return activated sludge. In all cases an inverse relationship was found between dye adsorption and return sludge solids concentration. This was thought to be due to dilution of the floc at lower solids concentration providing a larger surface area for adsorption. Eikelboom (1982) found that the COD adsorption capacity of a sludge was directly related to the floc loading, this being a measure of the loading at the sewage/sludge mixing stage. It is likely that Eikelboom's measured increase in biosorption with an increase in floc loading was also due to an increase in floc surface area available for the adsorption of nutrients. It is possible that as the floc surface area increases, comparatively more nutrients become available to floc forming microorganisms than to filaments, thus reducing the probability of filament growth.

Eikelboom also found that sludges from different sources varied in their adsorption capacity where floc loadings were similar. Similarly, the effect of dilution on dye adsorption was found to be a property of the sludge rather than purely a function of suspended solids concentration (see Chapter 3). The extent to which sludge surface area is increased when diluted with sewage is likely to be a function of the surface chemistry of the floc. Thus sludge extracellular polymers may have two roles in nutrient uptake; firstly, their adsorption properties, and secondly, their function in floc formation and integrity.

Measurement of Alcian blue adsorption by activated sludge from individual aeration compartments throughout Runcorn plants 1 and 2 revealed a high adsorption capacity in the sludge/sewage mixing phase. Again, this was thought to be due to floc disruption due to sewage addition. Reduction in dye binding along the aeration lanes may have been due to reflocculation or possibly a saturation of dye binding sites with nutrients, though if this was the case it would be expected that adsorption would increase as organic material was utilized.

Sludge activity measured as triphenyl tetrazolium chloride (TTC) reduction was also assessed throughout aeration at Runcorn. In all but one case highest activity was found in the sewage/sludge mixing stage with a subsequent decrease in later stages. Thus the general trends in sludge activity are similar to those of Alcian blue adsorption.

Since this work was completed Miksch (1983, 1985) has criticized TTC reduction as a measure of sludge activity on the basis that the test is affected by the oxygen concentration within the test solution. This is because oxygen acts as a competitive hydrogen acceptor to TTC. The method is improved by the addition of sodium sulphite to samples to provide anaerobic conditions. However if this was a major factor in activity measurements by this method, it would be expected that return sludge reaeration would give rise to a large decrease in TTC reduction. This was not the case.

Summary

1. Fifteen types of filamentous microorganism were observed in return sludge from three activated sludge plants. The organisms were identified and their relationship with plant performance discussed.

2. Two studies were carried out to evaluate dye adsorption by return activated sludge and this was correlated with plant performance data. An inverse relationship was found between dye binding and suspended solids concentration. However, no clear correlation was found between adsorption and sludge settlement.

3. Adsorption isotherms were constructed for bulking and non-bulking sludges. Some evidence was found for higher binding properties in bulking sludge. However, this increase was not significant enough to be used as an indicator of bulking problems.

4. Sludge activity as triphenyl tetrazolium chloride reduction and dye adsorption were measured throughout the aeration lanes at two activated sludge plants. Both showed a peak at the sludge/sewage mixing stage.

CHAPTER 5

Heterotrophic Aerobic Bacterial Populations of Activated Sludge

A detailed study was carried out to evaluate the heterotrophic aerobic bacterial populations in three return activated sludge samples from Runcorn plant 1. The prime objective of the study was to investigate the changes in the floc-forming activated sludge populations during an incident of filamentous bulking. Sludge samples were taken several weeks prior to a bulking incident (4th May 1982) - A, at the time of bulking (15th July 1982) - B, and several weeks after normal working had been restored (9th August 1982) - C.

Plant performance data and sludge characteristics at the time of sampling are shown in Table 4.10. At the onset of bulking, slight changes were made in the feed pattern to the plant (see Chapter 3).

Sludge samples were taken from the return activated sludge channel prior to reaeration. These were then diluted by 50% in 1/100 CGY medium and sonicated for 20 seconds prior to serial dilution and inoculation onto CGY agar. Viability was calculated as colony forming units (c.f.u.) gram sludge^{-1}. Values for the three samples were 1.99×10^{11}, 3.4×10^{11} and 3.5×10^{11} c.f.u. gram^{-1} for samples A, B and C respectively. In order to identify isolated microorganisms, agar plates containing approximately 100 colonies were selected for further study. Every colony on each of these plates was subcultured onto fresh CGY agar. After incubation at 20°C for 1 week, survival of isolates was found to be 59.3, 68.7 and 82.4% for samples A, B and C respectively.

This variation in survival rate may have been due to a change in bacterial population or to increased experience in handling.

Isolated bacterial cultures were maintained on CGY agar plates at 20°C by subculture at six weekly intervals. The cultures were subjected to a variety of biochemical and physiological tests and these results are presented in Tables 5.1, 5.2 and 5.3. This data is summarized in Table 5.4. Statistical significance between samples was calculated using Chi squared (χ^2) analysis of frequencies (Parker, 1979). This test is not valid for values of less than 5.

Clear differences can be seen between the three microbial populations. The two non-bulking samples A and C differ significantly only in growth in thioglycollate medium. There were 59.6% of strains from sample C capable of some growth in this medium compared with 17.8% of strains from sample A. No significant difference was found in the number of strains capable of anaerobic growth between these two samples. However, the number of strains capable of aerobic growth only increased from 8.2% in A to 45% in C. Thus there was apparently a development of a microbial population of aerobic bacteria capable of growth on thioglycollate medium between the taking of these two samples. This population was also present at the time of sludge bulking.

Bulking sludge was unique amongst the three samples studied in that it contained a large number of strains capable of anaerobic growth in thioglycollate medium. Moreover, a higher proportion of strains from sample B were found to give a

Tables 5.1, 5.2 and 5.3

Biochemical and pysiological characteristics of herterotrophic, aerobic bacteria isolated from Runcorn plant 1 activated sludge. Sludge samples were taken several weeks prior to a bulking incident (Table 5.1), at the time of sludge bulking (Table 5.2) and several weeks after normal working was restored (Table 5.3).

Key:

- \- negative result;
- \+ positive result.

O/F Test:

- O - Acid in aerobic tube only;
- F - Acid produced in both tubes;
- a - Growth but no acid produced;
- \- - No growth.

Thioglycollate broth:

- Ma - Microaerophillic;
- A - Aerobic growth;
- An - Anaerobic growth.

Table 5.1 (A) Runcorn plant 1 activated sludge (not bulking)

Isolate number	Gram	Cell shape	Morphology on CGY			Spore	Motility	Catalase	Oxidase	O/F	Thioglycolate broth	Designation
			Pigment	Colony size (mm)	Slime production							
1a	+	C				-	-	-	-	F	-	5
2a	-	R	Cream	4	2	-	+	-	+	-	-	0
3a	-	R	Yellow	4	2	-	-	-	-	-	-	0
7a	-	R			2	-	-	-	-	F	-	0
8a	-	C	Light brown	6	2	-	-	-	-	O	-	11
10a	-	R	"	3	2	-	-	+	+	-	-	13
11a	-	R			-	-	-	+	+	-	-	13
14a	+	R			-	+	-	-	-	-	-	1
15a	-	R	Yellow	6	1	-	-	+	+	-	-	12
17a	-	R	White	9	0	-	-	+	+	O	-	8
18a	-	R	Cream	4	-	-	-	-	+	-	-	0
19a	-	C			-	-	-	+	-	-	-	11
20a	-	R	Cream	5	3	-	-	+	+	O	-	8
21a	-	C			-	-	-	+	-	-	-	11
22a	-	C			-	-	-	+	-	-	-	11
23a	-	R	Light brown	3	-	-	-	+	+	-	-	13
24a	-	R			-	-	-	+	+	F	AAn	9
25a	-	R			-	-	-	+	+	F	AAn	9
26a	-	R	White	-	3	-	-	+	+	O	-	8
28a	-	R			-	-	-	+	+	-	-	13
29a	-	R			-	-	+	+	+	-	-	13
30a	-	R			-	-	-	+	+	-	-	13
33a	-	R			-	-	-	+	+	-	-	13
34a	-	R	White	1	-	-	-	-	-	-	-	0
36a	-	R			-	-	-	+	-	-	-	7
37a	-	R			-	-	-	-	+	-	-	0
43a	-	R			-	-	-	+	+	-	-	13
48a[+]	-	R	White	0.5	-	-	-	+	-	-	Ma	0
50a	+	R	White	-	1	-	-	-	+	F	Ma	4
53a	-	R			-	-	-	+	+	-	-	13
54a	-	R	Yellow	6	2	-	-	+	+	-	-	12
57a	-	R	Light brown	3	2	-	-	+	+	-	Ma	13
60a	-	R	Cream	7	-	-	-	+	+	-	-	13
61a	-	R			-	-	-	+	+	-	-	13
62a	-	R			0	-	-	+	+	-	-	13
66a	-	R			-	-	-	+	+	-	-	13
69a	-	R			-	-	-	-	+	-	-	0
70a		Yeast			-	-	-	+	-	F	-	14

Continued over/. . .

Isolate number	Gram	Cell shape	Morphology on CGY			Spore	Motility	Catalase	Oxidase	O/F	Thioglycolate broth	Designation
			Pigment	Colony size (mm)	Slime production							
2b	-	R	Yellow	2	0	-	-	+	+	-	-	12
4b	-	R	Cream	10	2	-	-	+	+	-	-	13
*5b	-	R			-	-	-	+	-	F	-	7
6b	-	R	White	1	1	-	-	-	+	-	-	0
9b	-	R	White	3	0	-	-	+	+	F	-	9
*10b	-	R	Colourless	8	-	-	-	+	-	-	AAn	7
+11b	-	R	Yellow	15	-	-	-	+	-	-	-	0
16b	-	R			-	-	-	+	+	-	-	13
17b	-	R	Cream	3	2	-	+	+	+	-	-	13
18b	-	R	Light brown	4	3	-	+	+	+	-	-	13
20b	-	R	Green	2	1	-	-	+	+	-	-	13
21b	+	C	Colourless	3	0	-	-	-	-	F	-	5
22b	+	C	"	3	0	-	-	-	-	F	AAn	5
23b	-	R	Cream	4	2	-	-	+	+	-	-	13
25b	-	R	Light brown	5	1	-	-	+	+	-	-	13
26b	-	R	Cream	2	1	-	-	+	+	-	-	13
28b	-	R	White	5	2	-	-	+	+	O	AAn	8
29b	-	R	Cream	6	4	-	-	+	+	-	-	13
30b	-	R	Green	3	2	-	+	+	+	-	Ma	13
33b	-	R	Cream	4	2	-	-	+	+	F	Ma	9
34b	-	R	White	1	1	-	+	+	+	-	-	13
35b	-	R	Cream	0.5	1	-	-	-	+	-	-	0
37b	-	R	Yellow	5	2	-	-	+	+	-	-	12
38b	-	C			-	-	-	+	-	-	-	11
42b	-	R	Cream	5	2	-	-	+	+	-	-	13
43b	-	R	Cream	3	2	-	+	+	+	-	-	13
44b	+	R	Grey	1	2	-	-	+	-	-	-	2
45b	-	R	Cream	8	2	-	-	+	+	-	-	13
46b	-	R	Cream	10	1	-	-	+	+	F	-	9
48b	-	R	White	4	1	-	-	+	+	O	-	8
49b	-	R	Light brown	4	2	-	+	+	+	-	Ma	13
50b	-	R	Cream	6	1	-	-	+	+	F	-	9
51b	-	R	Yellow	5	1	-	-	+	+	-	-	12
52b	-	R	Yellow	5	0	-	-	+	+	-	-	12
53b	-	C			1	-	+	+	-	O	MaAn	11

Table 5.2 (B) Runcorn plant 1 activated sludge (bulking)

Isolate number	Gram	Cell shape	Morphology on CGY			Spore	Motility	Catalase	Oxidase	O/F	Thioglycolate broth	Designation
			Pigment	Colony size (mm)	Slime production							
2a	-	R	Yellow	25	3	-	-	+	+	F	Ma	9
3a	-	R	Yellow	20	3	-	-	+	+	F	Ma	9
4a	-	C	Colourless	0.5	0	-	-	+	-	-	A	11
5a	-	R	Cream	10	2	-	-	+	+	-	Ma	13
6a	+	C	White	1	0	-	-	-	-	F	Ma	5
7a	+	R	Yellow	2	1	-	-	-	+	-	-	4
9a	-	R	White	1	0	-	-	-	-	F	-	0
11a	-	R	Yellow	10	2	-	-	+	+	-	-	12
14a	-	R	Yellow	25	2	-	+	+	+	F	-	9
*15a	-	R	Dark brown	15	-	-	+	-	-	F	-	7
17a	-	R	Yellow	3	-	-	-	+	+	-	A	12
18a	-	R	Cream	20	0	-	-	+	+	0	-	8
19a	-	R	"	10	1	-	-	-	+	F	A	0
20a	-	R	Yellow	10	2	-	-	+	+	-	Ma	12
22a	-	R	Cream	20	1	-	-	+	+	0	-	8
23a	-	R	"	5	2	-	-	-	-	-	Ma	0
26a	-	R	"	5	2	-	-	+	+	-	A	13
27a	+	R	White	20	0	+	-	+	-	F	Ma	1
28a	-	R	Yellow	10	1	-	-	+	+	-	Ma	12
29a	-	R	Yellow	20	3	-	+	+	+	F	AnMa	9
31a	-	R	Cream	5	1	-	-	+	+	-	-	13
32a	-	C	"	10	0	-	-	-	+	-	Ma	10
34a	-	R	Brown	12	1	-	-	+	+	-	Ma	13
35a	+	R	Yellow	12	4	-	-	+	-	F	MaAn	2
36a	-	C	Cream	10	1	-	-	+	-	-	Ma	11
40a	+	R	Yellow	12	4	-	-	+	-	F	AAn	2
42a	-	R	Yellow	-	2	-	-	+	+	-	Ma	12
43a	-	R	Yellow	20	2	-	+	+	+	F	MaAn	9
44a	-	R	Cream	20	-	-	-	+	-	F	MaAn	7
45a	-	R	Colourless	3	2	-	-	+	+	-	-	13
46a	-	R	Yellow	15	-	-	+	+	+	F	MaAn	9
48a	-	R	White	10	1	-	-	-	+	-	-	0
50a	+	C	"	0.5	0	-	-	-	-	-	MaAn	5
51a	-	R	"	5	2	-	+	-	-	-	-	0
52a	-	C	"	4	2	-	-	+	+	F	Ma	0
57a	-	R	Brown	swarmer	2	-	+	+	+	F	MaAn	9
60a	-	R	White	10	1	-	-	-	+	-	-	0
*61a	-	R	Cream (metallic sheen)	5	-	-	+	+	-	-	MaAn	7
62a	-	R	Yellow	25	2	-	+	+	+	-	MaAn	12
*63a	-	R	Colourless	2	-	-	-	+	-	-	AAn	7
64a	-	R	Brown	swarm	2	-	+	+	+	F	MaAn	9

Continued over/. . .

Isolate number	Gram	Cell shape	Morphology on CGY			Spore	Motility	Catalase	Oxidase	O/F	Thioglycolate broth	Designation
			Pigment	Colony size (mm)	Slime production							
68a	-	R	White	mucoid mass	2	-	-	+	+	F	AAn	9
69a	-	R	Light brown	20	2	-	+	+	+	0	AAn	8
70a	-	R	"	20	2	-	+	+	+	0	MaAn	8
73a	-	R	Dark brown	20	2	-	+	-	+	-	MaAn	0
75a	-	R	Yellow	3	2	-	-	+	+	0	Ma	12
76a	-	R	"	15	2	-	-	+	+	0	MaAn	12
77a	-	R			-	-	-	+	+	-	Ma	13
*78a	-	R	Cream	2	-	-	-	+	-	F	Ma	7
79a	-	R	"	5	2	-	-	+	+	-	-	13
1b	+	R	Yellow	10	2	-	-	+	-	F	Ma	2
2b	-	R			-	-	-	+	+	F	Ma	9
3b	-	R	Dark brown	15	1	-	-	-	+	-	-	0
*4b	-	R	Yellow	2	-	-	-	+	-	-	Ma	7
7b	-	R	Light brown	5	0	-	-	+	+	-	Ma	13
8b	-	R	Yellow	2	1	-	-	+	+	-	-	12
9b	-	R	Cream	6	1	-	-	-	+	-	Ma	0
10b	-	R	"	20	2	-	-	-	-	-	MaAn	0
11b	-	R	Colourless	20	3	-	+	+	+	F	MaAn	9
12b	-	R	Yellow	12	2	-	+	-	+	-	MaAn	0
15b	-	R	Light brown	15	2	-	-	+	+	-	Ma	13
16b	-	R	Cream (metallic sheen)	10	2	-	-	-	+	-	MaAn	0
17b	-	R	Light brown	10	2	-	-	+	+	-	Ma	13
18b	-	R	Brown	8	3	-	-	+	+	-	-	13
19b	+	C	White	4	0	-	-	-	-	-	MaAn	5
20b	-	R	Light brown	10	-	-	+	+	+	F	MaAn	9
21b	-	R	"	20	2	-	+	+	+	F	MaAn	9
22b	-	R	"	20	2	-	+	+	+	F	MaAn	9
26b	+	C	Cream	20	4	-	-	+	+	-	Ma	6
27b	-	R	Brown	8	2	-	-	+	+	-	-	13
28b	-	R	Light transparent	20	2	-	+	+	+	F	MaAn	9
30b	-	R			-	-	-	-	-	-	MaAn	0
31b	-	R	Light transparent	20	2	-	+	+	+	F	MaAn	9
32b	-	R			-	-	-	-	-	-	Ma	0

Continued over/. . .

Isolate number	Gram	Cell shape	Morphology on CGY			Spore	Motility	Catalase	Oxidase	O/F	Thioglycolate broth	Designation
			Pigment	Colony size (mm)	Slime production							
33b	+	R	White	12	-	+	+	+	-	F	Ma	2
35b	-	R	"	10	0	-	+	-	+	-	Ma	0
36b	+	R	"	12	0	-	-	+	-	F	A	2
37b	+	C	Orange	3	0	-	-	+	-	-	Ma	6
38b	-	R	Light brown	20	2	-	-	+	+	F	MaAn	9
*39b	-	R	Yellow	5	1	-	-	+	-	-	-	7
42b	-	R	Cream	10	2	-	-	-	+	-	-	0
*44b	-	R	"	4	2	-	-	+	-	-	-	7
45b	-	R	(metallic sheen Cream	3	2	-	-	-	+	-	-	0
46b	-	R	Colourless	20	2	-	-	+	+	F	AAn	9
47b	-	R	"	20	2	-	+	+	+	F	AAn	9
48b	-	R	"	20	3	-	+	+	+	-	AAn	13
49b	-	C	White	5	2	-	+	+	+	F	Ma	0
50b	-	R	Yellow	5	3	-	+	+	+	-	-	12
51b	-	R	White	11	0	-	+	+	+	-	-	13
52b	-	R	"	1	-	-	+	-	-	-	Ma	0
54b	+	R	Yellow	25	4	-	+	-	-	-	-	2
55b	-	R	White	10	0	-	+	+	+	-	-	13
56b	-	R	Yellow	-	4	-	+	+	+	-	Ma	2
57b	-	R	Light brown	4	2	-	+	-	-	-	-	0
58b	-	R	"	4	2	-	+	-	-	-	-	0
59b	-	R	White	8	0	-	+	+	+	-	Ma	13
60b	-	R	Light brown	10	3	-	+	+	+	O	MaAn	8
61b	-	R	White	10	1	-	+	+	+	-	Ma	13
64b	-	C	Cream	2	2	-	+	+	+	F	Ma	0
66b	-	R	"	8	2	-	+	+	+	-	AAn	13
67b	-	R	Colourless	20	2	-	+	+	+	F	AAn	9
68b	+	R	Cream	20	2	-	-	+	-	F	Ma	2
69b	-	R	Colourless	20	2	-	-	+	+	F	MaAn	9
70b	-	R	Light brown	5	0	-	-	+	+	-	-	13

Continued over/. . .

Table 5.3 (C) Runcorn plant 1 activated sludge (not bulking)

Isolate number	Gram	Cell shape	Morphology on CGY			Spore	Motility	Catalase	Oxidase	O/F	Thioglycolate broth	Designation
			Pigment	Colony size (mm)	Slime production							
1a	+	C	Orange	10	4	-	-	+	-	-	A	6
2a	+	R	White	4	1	-	-	-	-	-	Ma	4
3a	-	R	Dark brown	3	2	-	-	+	+	-	-	13
4a	-	R	Light brown	4	2	-	+	+	+	-	Ma	13
5a	-	R	Grey	12	0	-	-	+	+	-	Ma	13
6a	-	R	White	2	1	-	+	+	+	-	A	13
7a	-	R	Cream	8	2	-	+	+	+	-	A	13
8a	-	R	Grey	8	1	-	-	+	+	0	Ma	8
9a	-	R	White	2	4	-	-	+	+	-	Ma	13
10a	-	R	White	3	2	-	-	+	+	-	-	13
12a	-	R	Yellow	6	1	-	-	+	+	-	-	12
14a	+	C	White	10	3	-	-	+	-	0	-	11
15a	-	R	"	3	1	-	-	+	+	-	Ma	13
16a	-	R	"	3	1	-	-	+	-	-	-	0
17a	+	C			-	-	-	+	-	-	Ma	6
18a	-	R	Colourless	5	3	-	-	+	-	-	Ma	0
19a	-	R	White	4	1	-	-	-	+	-	Ma	0
20a	-	R	"	3	1	-	-	-	+	-	Ma	0
+21a	-	R	"	2	-	-	-	+	-	-	Ma	0
22a	-	R	Colourless	10	1	-	-	-	+	-	Ma	0
23a	-	R	Yellow	6	2	-	-	+	+	-	Ma	12
24a	-	R	"	4	2	-	-	+	+	-	-	12
25a	-	R	Cream	2	2	-	-	+	+	-	-	13
26a	-	R	"	2	2	-	-	+	+	-	-	13
27a	-	R	Yellow	4	-	-	-	+	+	-	A	12
29a	+	R	"	4	3	-	-	+	-	F	MaAn	2
31a	-	R	"	4	2	-	-	+	+	-	A	12
32a	-	R	White	4	2	-	-	+	+	-	A	13
33a	-	R	Cream	12	1	-	-	+	+	0	A	9
34a	-	R	"	12	1	-	-	+	+	-	Ma	13
35a	+	R	Yellow	4	0	-	-	+	-	-	Ma	2
36a	-	R	Cream	5	0	-	-	+	+	-	A	13
37a	-	R	White	3	1	-	-	+	+	-	A	13
38a	-	R	Cream	4	1	-	-	+	+	-	Ma	13
39a	-	R	"	1	1	-	+	+	+	-	Ma	13
41a	+	R	Cream	1	1	-	-	+	+	-	Ma	2
43a	+	R	White	2	1	-	-	+	+	-	MaAn	9
44a	+	R	"	2	1	-	-	-	+	-	Ma	4
*45a	-	R	"	0.5	1	-	-	+	-	-	-	7
46a	-	R	"	4	1	-	-	+	+	-	Ma	13
47a	-	R	Yellow	2	1	-	-	+	+	-	A	12
48a	-	R	"	3	1	-	-	+	+	-	-	12

Continued over/. . .

Isolate number	Gram	Cell shape	Morphology on CGY			Spore	Motility	Catalase	Oxidase	O/F	Thioglycolate broth	Designation
			Pigment	Colony size (mm)	Slime production							
49a	-	R	Yellow	3	1	-	-	+	+	-	-	12
50a	-	R	Colourless	5	3	-	-	-	-	-	Ma	0
53a	-	R	Light brown	20	2	-	+	+	+	F	MaAn	9
55a	-	R	Colourless	1	2	-	-	-	-	-	-	0
56a	-	R	Yellow	20	2	-	-	+	+	-	-	12
57a	-	R	Colourless	20	2	-	+	+	+	-	MaAn	9
58a	-	R	Cream	-	3	-	+	+	+	-	MaAn	9
59a	-	R	Colourless	20	2	-	+	+	+	F	MaAn	9
60a	-	R	"	20	2	-	+	+	+	F	MaAn	9
61a	-	R	Yellow	10	2	-	-	+	+	O	Ma	12
62a	+	C	Orange	10	3	-	-	+	-	F	A	6
63a	-	R	Light brown	7	2	-	+	+	+	-	-	13
65a	-	R	White	2	1	-	-	-	+	-	Ma	0
66a	-	R	Light brown	1	1	-	-	-	+	-	Ma	0
68a	-	R	Cream	3	2	-	-	-	+	-	-	0
69a	-	R	Grey	10	3	-	-	-	-	-	Ma	0
71a	-	R	Brown	5	2	-	-	+	+	-	-	13
72a	-	R	Green	4	2	-	-	+	+	-	Ma	13
73a	-	R	Yellow	-	2	-	-	+	+	-	-	12
74a	-	R	"	0.5	1	-	-	+	+	-	Ma	13
75a	+	R	"	-	4	-	-	+	-	O	A	2
1b	-	R	Cream	1	1	-	-	-	+	-	Ma	0
2b	-	C	Yellow	swarm	1	-	-	+	-	-	Ma	11
3b	-	R	Cream	5	2	-	-	+	+	-	-	13
4b	-	R			-	-	-	+	+	-	-	13
6b	-	R	Cream	15	2	-	-	-	+	-	-	0
7b	-	R	Light brown	5	2	-	+	+	+	-	Ma	13
9b	-	R	Brown	3	2	-	+	+	+	-	-	13
10b	-	R	White	20	4	-	-	-	-	-	Ma	0
11b	-	R	Light brown	20	2	-	-	+	+	-	-	13
12b	-	R	"	6	2	-	-	+	+	-	-	13
13b	-	R	Cream	5	2	-	-	+	+	-	-	13
14b	-	R	"	5	2	-	+	+	+	-	-	13
16b	-	R	"	1	1	-	+	-	+	-	-	0
18b	-	R	Light brown	1	1	-	-	-	+	-	-	0
+19b	-	R	"	20	-	-	-	+	-	-	-	0
20b	-	R	"	8	2	-	-	+	+	-	-	13
21b	-	R	Cream	0.5	2	-	+	+	+	-	-	13
22b	-	R	"	0.5	1	-	-	-	+	-	-	0

Continued over/. . .

Isolate number	Gram	Cell shape	Morphology on CGY			Spore	Motility	Catalase	Oxidase	O/F	Thioglycolate broth	Designation
			Pigment	Colony size (mm)	Slime production							
24b	-	R	White	2	1	-	-	+	+	-	-	13
26b	-	R	Colourless	10	1	-	-	+	+	-	-	13
27b	-	R	Light brown	4	2	-	-	+	+	-	-	13
28b	-	R	"	2	1	-	+	+	+	-	Ma	13
31b	-	R	Cream (metallic	10	0	-	-	+	+	-	-	13
+32b	-	R	sheen) Cream	8	-	-	-	+	-	-	-	0
34b	-	R	Colourless	3	2	-	-	+	+	-	-	13
36b	+	R	Cream	0.5	1	-	+	+	+	-	Ma	2
37b	-	R	"	5	2	-	-	+	+	-	-	13
38b	-	R	Colourless	10	2	-	+	-	+	F	Ma	9
39b	-	R	Light brown	3	2	-	-	+	+	-	-	13
40b	-	R	"	4	2	-	-	+	+	-	Ma	13
41b	+	R	Yellow	4	0	-	-	+	-	-	Ma	2
42b	-	R	Cream	4	1	-	+	+	+	-	Ma	13
43b	-	R	"	3	1	-	+	+	+	-	A	13
44b	+	C	"	4	1	-	+	+	+	-	Ma	6
45b	-	R	"	2	0	-	+	+	+	F	Ma	9
47b	-	R	Light brown	4	1	-	+	+	+	O	Ma	8
48b	-	R	Cream	2	1	-	+	+	+	-	Ma	13
49b	-	R	"	3	0	-	+	+	+	-	-	13
50b	-	R	"	2	1	-	+	+	+	-	Ma	13
51b	-	R	"	3	1	-	+	+	+	-	A	13
52b	-	R	"	3	1	-	+	-	-	O	A	8

Continued over/. . .

Isolate number	Gram	Cell shape	Morphology on CGY			Spore	Motility	Catalase	Oxidase	O/F	Thioglycolate broth	Designation
			Pigment	Colony size (mm)	Slime production							
53b	-	R	White	1	1	-	-	+	+	-	-	13
54b	-	R	Light brown	6	2	-	+	+	+	0	-	8
55b	-	R	Colourless	10	2	-	+	+	+	-	-	13
56b	-	R	White	2	2	-	+	+	+	-	A	13
57b	-	R	"	4	2	-	+	+	+	-	-	13
58b	-	R	"	4	1	-	+	+	+	-	AAn	13
59b	-	R	Yellow	2	0	-	+	+	+	-	-	12
60b+	-	R	Light brown	3	2	-	+	-	+	-	-	0
61b	-	R	Colourless	10	2	-	-	+	+	F	A	9
62b	-	R	Light brown	10	1	-	+	+	-	-	A	0
+63b	-	R	White	0.5	-	-	+	+	-	-	Ma	0
+65b	-	R	Light brown	4	-	-	-	+	-	-	-	0
66b	-	R	"	3	2	-	-	+	+	-	Ma	13
67b	-	R	White	1	2	-	-	+	+	-	Ma	13
69b	-	R	Colourless	10	2	-	+	-	+	-	A	0
70b	-	R	Cream	2	1	-	+	+	+	-	-	13
71b	-	R	Colourless	1	2	-	+	+	+	F	MaAn	9
72b	-	R	Dark brown	4	1	-	-	+	+	-	-	13
74b	-	R	Light brown	12	2	-	-	+	+	-	A	13
75b	-	R	White	3	1	-	-	+	+	-	-	13
76b	-	R	Cream	8	2	-	-	+	+	-	-	13
78b	-	R	"	20	1	-	+	+	+	-	-	13
+79b	-	R	Dark brown	4	-	-	-	+	-	-	-	0
81b	-	R	Colourless	swarm	2	-	+	+	+	F	MaAn	9
82b	-	R	"	"	2	-	+	+	+	F	MaAn	9
83b	-	R	"	"	2	-	+	+	+	F	MaAn	9
84b	-	R	"	10	2	-	+	+	+	F	MaAn	9

Table 5.4 Biochemical and physiological characteristics of heterotrophic, aerobic bacteria isolated from activated sludge.
A - sludge several weeks prior to bulking.
B - bulking sludge.
C - sludge several weeks after normal working was restored.

Chi squared analysis of frequencies (Parker, 1979) used except for:
* mean colony size; students t test.
+ mean slime production; chi squared test for homogeneity (Parker, 1979).

	Summary of microbiological tests						Statistical probability					
	A		B		C		AB		BC		AC	
	No.	%	No.	%	No.	%	χ^2	P	χ^2	P	χ^2	P
Total strains in sample	73	100	121	100	131	100	-	-	-	-	-	-
Gram-positive cocci	3	4.1	5	4.1	5	3.8	-	-	-	-	-	-
Gram-positive rods	3	4.1	11	9.1	5	3.8	-	-	-	-	-	-
Gram-positive bacteria	6	8.2	16	13.2	10	7.6	-	-	-	-	-	-
Gram-negative cocci	5	11.0	2	1.6	1	0.8	-	-	-	-	-	-
Gram-negative rods	61	83.6	103	85.1	116	88.5	-	-	-	-	-	-
Gram-negative bacteria	66	94.6	105	86.7	117	89.3	0.28	NS	0.55	NS	0	NS
Catalase positive	57	78.1	93	76.8	109	83.2	0	NS	1.22	NS	0.5	NS
Oxidase positive	51	69.9	84	69.4	106	80.9	0.01	NS	3.9	<0.05	0.5	NS
Oxidative) Hugh and	7	9.6	9	7.4	8	6.1	0.07	NS	0.03	NS	0.4	<0.05
Fermentative) Leifsons	12	16.4	41	33.9	15	11.4	6.12	<0.05	17.0	<0.001	0.63	NS
No result) O/F test	54	73.7	71	58.7	108	82.4	4.0	<0.05	17.4	<0.001	1.56	NS
Aerobic) Growth on	6	8.2	42	40.5	59	45.0	15.77	<0.001	4.27	<0.05	33.55	<0.001
Facultatively anaerobic) thioglycollate	7	9.6	42	34.7	13	9.9	13.86	<0.001	21.28	<0.001	0.03	NS
No growth) medium	60	82.2	37	30.6	53	40.4	46.44	<0.001	2.26	NS	31.37	<0.001
Motility	9	12.3	42	34.7	45	34.3	10.65	<0.01	0	NS	10.59	<0.01
Mean colony size on CGY agar (mm)	4.4		10.6		5.5		*7.38	<0.001	*5.86	<0.001	*1.66	NS
Mean slime production index on CGY agar	1.5		1.7		1.5		+5.2	NS	+19.9	<0.01	+3.85	NS

fermentative reaction in the Hugh and Leifsons Oxidative/Fermentative (O/F) test than in either sample A or C. In addition a significantly smaller number of strains from sample B failed to give a result in this test, i.e. they either failed to produce acid in either tube or failed to grow.

Thus bulking sludge appears to be accompanied by a large bacterial population capable of facultatively anaerobic respiration.

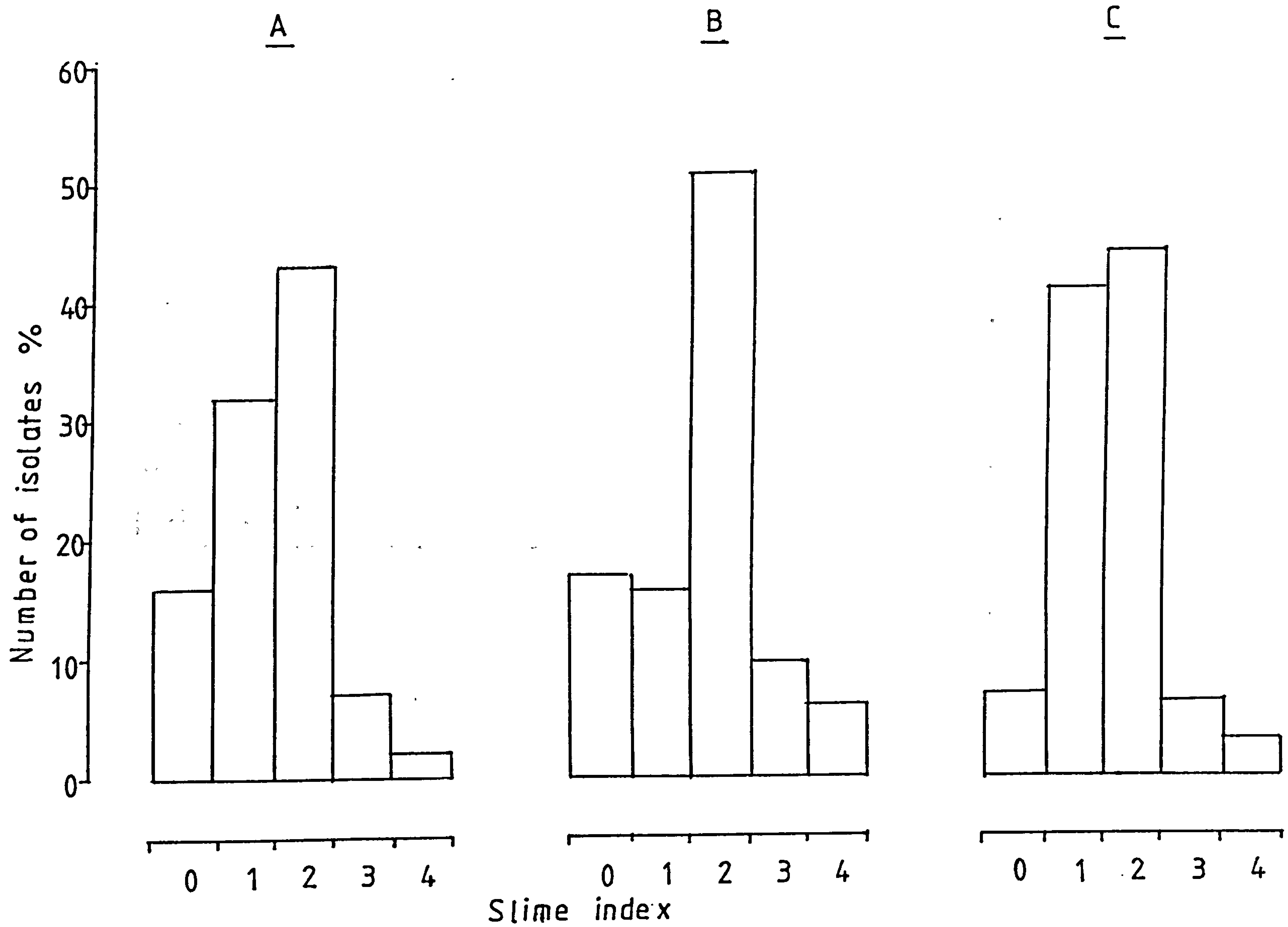
Mean colony size of isolates on CGY agar was also measured. Isolates obtained from bulking activated sludge produced significantly larger colonies than those from non-bulking sludges. This may have been due to either a greater production of extracellular polysaccharide by the bacteria or a larger number of cells. It is an indication, however, that strains from sample B are better adapted to growth on CGY agar. This may be due to acclimation to a high carbohydrate content in the sewage influent at the time of sludge bulking.

Slime production on CGY agar was also recorded. Colonies were designated 0 for zero slime production and 5 for maximum production. The results are presented in histogram form in Fig. 5.1. These results were analysed statistically using the Chi squared test for homogeniety (Parker, 1979). No significant variation was found between samples.

Isolated strains of microorganisms were tentatively classified as shown in Table 5.5. Criteria for identification are shown in Table 5.6. As identification was tentative, no

Figure 5.1

Distribution of slime production by isolates from activated sludge on CGY media. Sludge samples were taken several weeks prior to a bulking incident (A), at the time of sludge bulking (B) and several weeks after normal working was restored (C).



A
B
C
Number of isolates %
0
10
20
30
40
50
60
0
1
2
3
4
Slime index

Table 5.5 Classification of bacteria isolated from activated sludge into taxonomic groupings.

A - Sludge sample taken several weeks prior to a bulking incident.

B - Bulking sludge.

C - Sludge sample taken several weeks after normal working was restored.

Group	A		B		C	
	No.	%	No.	%	No.	%
0	11	15.1	25	20.7	26	19.8
1	1	1.4	1	0.8	0	0
2	1	1.4	9	7.4	6	4.6
3	0	0	0	0	0	0
4	1	1.4	1	0.8	2	1.5
5	3	4.1	3	2.4	0	0
6	0	0	2	1.6	4	3.0
7	3	4.1	10	8.3	1	0.8
8	5	6.8	6	5.0	4	3.0
9	6	8.2	24	19.8	15	11.4
10	0	0	1	0.8	0	0
11	6	8.2	2	1.6	2	1.5
12	6	8.2	10	8.3	12	9.2
13	29	39.7	27	22.3	59	45.0
14	1	1.4	0	0	0	0
TOTAL	73	100	121	100	131	100

Table 5.6 Criteria for classification of heterotrophic, aerobic microorganisms.

Group Number	Criteria for classification
1	Endospore forming, Gram-positive, catalase positive rod shaped bacteria. Genus Bacillus.
2	Asporogenous, Gram-positive, catalase positive bacteria. Rod shaped or pleiomorphic. Aerobic. Members of "the coryneform group of bacteria".
3	Bacteria tending to form filaments. Members of the Order Actinomycetales.
4	Gram-positive, non-motile, facultatively anaerobic rod shaped bacteria. Catalase negative. Produce acid in both tubes of Hugh and Leifsons test. These organisms belong to the family Lactobacillaceae.
5	Gram-positive, spherical bacteria. Catalase negative, oxidase negative. Metabolism fermentative, and produce acid in both tubes of Hugh and Liefsons test. Family Streptococcaceae.
6	Gram-positive, spherical bacteria. Catalase positive, oxidase positive. Metabolism fermentative (Staphylococcus spp.) or oxidative (Micrococcus spp.). Both of family Micrococcaceae.
7	Gram-negative, rod shaped bacteria. Produce acid in both tubes of Hugh and Liefsons test. Catalase positive, oxidase negative. Facultative anaerobes. Members of family Enterobacteriaceae.
8	Gram-negative, rod shaped bacteria. Catalase positive, oxidase positive. Production of acid in the presence of air in Hugh and Liefsons test. Motile. Includes Pseudomonadaceae, Azotobacteraceae, Rhizobiaceae and Acetobacteriaceae.
9	Gram-negative, rod shaped bacteria. Produce acid in both tubes of Hugh and Liefsons test. Fermentative, catalase positive, oxidase positive. Includes all members of Vibrionaceae.

Table 5.6 continued over:

Table 5.6 (continued)

10	Gram-negative, spherical bacteria. Catalase positive, oxidase positive. Non-motile. Belonging to family Neisseriaceae or genus *Paracoccus*.
11	Gram-negative, spherical bacteria. Catalase positive, oxidase negative. Of the genus *Acinetobacter*.
12	Gram-negative, rod shaped bacteria producing a yellow pigment on CGY agar. *Flavobacterium* spp.
13	Gram-negative, rod shaped bacteria. Catalase positive, oxidase positive. Give a neutral reaction in Hugh and Liefsons test. Includes genera *Alcaligenes*, *Moraxella* and *Brucella*. Motile or non-motile.
14	Yeasts.

statistical analysis was carried out. In each sample the largest number of isolates fell into group 13, containing the *Alcaligenes/Moraxella* group of bacteria. This group have previously been reported to dominate sludges (see Table 1.1). However, clear differences in bacterial populations can be seen. Bulking sludge has a high number of strains belonging to groups 2, 7 and 9. These represent the coryneform group of bacteria, the Vibrionaceae and the Enterobacteriaceae respectively. An increase in numbers of the latter two families would be expected to accompany increases in the number of facultatively anaerobic microorganisms. Other workers (Takii, 1977, Adamse, 1968) have reported an increase in coryneform bacteria where conditions of high carbohydrate are prevalent in the influent sewage. It is reasonable to assume that such conditions accompanied the shock loadings responsible for bulking at Runcorn.

The Enterobacteriaceae isolated in this study were further investigated using the API 20E identification system. Organisms subjected to this study were those found to be Gram-negative, catalase positive, oxidase negative rods. The results are presented in Table 5.7. Two isolates from sample A were lost during storage. Three species of organism were identified in activated sludge; *Serratia rubideae*, *Citrobacter freundii* and *Yersinia enterolitica*. One colony was identified as either *C.freundii* or *Klebsiella ozaenae*.

Table 5.7 Enterobacteriaceae isolated from activated sludge.

Sample	Strain No.	Designation	API comment
A	36a	-	-
	5b	-	-
	10b	*Citrobacter freundii*	very good
B	15b	*Serratia rubideae*	acceptable
	44a	*Citrobacter freundii*	excellent
	61a	*Citrobacter freundii* or *Klebsiella ozaenae*	good likelihood, low selectivity
	63a	*Citrobacter freundii*	excellent
	78a	*Serratia rubideae*	acceptable
	4b	*Yersinia enterolitica*	very good
	39b	*Serratia rubideae*	acceptable
	44b	*Citrobacter freundii*	very good
	80b	*Serratia rubideae*	acceptable
	93b	*Citrobacter freundii*	very good
C	45a	*Citrobacter freundii*	very good

A - sludge taken several weeks prior to a bulking incident.

B - bulking sludge.

C - sludge taken several weeks after normal working was restored.

DISCUSSION

Comparison of these results with those of other workers is difficult due to the variety of media used for isolation and the different criteria used for microbial identification. However, virtually all the major groupings found here have been previously reported in large numbers in sludge samples (see Table 1.1).

Bulking sludge showed a clear increase in the number of facultatively anaerobic bacteria. This was reflected by an increase in the number of isolates which fell into groups 7 and 9. The latter includes all members of the Vibrionaceae and Pasteurellaceae and comprised about 20% of the isolates from bulking sludge. _Aeromonas_ spp. have been found in activated sludge by several workers (Austin and Forster, 1969, Benedict and Carlson, 1971; Ueda and Earle, 1972; Hart and Melmed, 1980) and others have found _Vibrio_ spp. (Adamse, 1968; Bisz-Konarzewska, 1978). However, no large groups of the above families have previously been reported in sludge samples.

Members of group 7 (the Enterobacteriaceae) were also highest in bulking sludge. This clearly has implications with regard to the removal of pathogens in water treatment. The efficiency of sewage treatment processes in this respect has been reviewed (Kabler, 1959; Pike, 1975). Conventional activated sludge plants are very effective at removing coli-aerogenes bacteria, and removals of 90 to 99% are generally reported. However, with a typical sewage coliform count of 3.7×10^5 ml^{-1} this still leaves a large number of potential pathogens in the

effluent. No studies were found in the literature where the number of coliforms were monitored in effluents of bulking activated sludge plants, although an increase within sludge flocs does not necessarily mean a similar increase in the effluent. McKinney and Weichlein (1953) found that E.coli and Citrobacter (Escherichia) freundii were both capable of forming flocs within 48 hours of aeration.

This is the first time that a change in the non-filamentous bacterial population has been found to accompany a case of filamentous bulking. The increase in facultative anaerobes suggests a reduction of oxygen in the aeration tanks. This is consistent with the possibility of shock loadings at Runcorn, although oxygen deficiency has not been previously reported as a cause of Type 0961 bulking. It is possible that the facultative anaerobes are directly involved in settlement, although more likely, that conditions favouring their growth also favour bulking organisms.

Changes in floc forming bacterial populations have been reported by several workers. Generally, the change in species is the result of some external factor introduced with the influent such as heavy metals (Barth et al., 1965; Kunz et al., 1976; Singleton and Guthrie, 1977), aluminium sulphate (Unz and Davis, 1975), derivatives of cyclohexane (Bisz-Konarzewska, 1978) and phenol (Kunicka-Goldfinger and Wlotowski, 1980). There is also some evidence that changes in operating parameters lead to floc population changes. Jasewicz and Porges (1956) found that

populations differed between sludges in "assimilation phase", where the culture was fed normally and in "endogenous phase"when the culture was aerated without nutrition for several days. Brodisch and Joyner (1983) found that increased duration in an anoxic zone of a pilot plant led to an increase in the percentage of Gram-positive organisms, accompanied by a decrease in the Acinetobacter/Moraxella group. However Toerien et al. (1979) in their study of laboratory activated sludge plants with or without anoxic zones found no large scale, selective population changes. Annual variations in bacterial populations have also been reported (Seiler et al., 1984) though Seiler and Blaim (1982) found little change in the dominant microbial population over a period of several months.

As in this study the majority of workers have found that Gram-negative organisms dominate in activated sludge. Where Gram-positive bacteria have been most numerous, the influent has usually been of a high carbohydrate content (Takii, 1977; Seiler et al., 1980). At Runcorn the proportion of Gram-positive isolates was never higher than 13.2%. However, this population was highest during bulking and may possibly have reflected an increased carbohydrate content in the influent sewage.

Of the Gram-negative families, virtually all types of activated sludge have been found to include large numbers of the Pseudomonadaceae. Members of this family isolated from Runcorn sludge were placed in group 8 along with the Azotobacteriaceae, Rhizobiaceae and Acetobacteriaceae. This group made up only a

minor fraction of isolates from all three sludge samples studied. Of this group, Zoogloea ramigera has frequently been implemented as of major importance in sludge settlement. Seiler and Blaim (1982) proposed that the efficiency of water treatment correlated directly with the presence of Z.ramigera in activated sludge and that seeding problem sludge with this organism could improve performance. Their findings are in disagreement with those of Williams and Unz (1983) who found Z.ramigera to represent only 0.01% of activated sludge microorganisms. These workers identified Z.ramigera by its ability to form flocs whereas Seiler and Blaim relied on biochemical characteristics. Clearly the two methods are not compatible.

At Runcorn, organisms of group 8 made up only 6.8, 5.0 and 3.0 percent of sludges A, B and C respectively. This supports the view that Z.ramigera is not directly involved in effluent quality.

Of the remaining large groups of bacteria isolated from Runcorn sludge, there was little variation between the three samples. Flavobacteria have frequently been found to be most numerous in isolates from activated sludge (see Table 1.1). However with the major criterion for classification as production of a yellow pigment there is bound to be confusion with other genera. Seiler et al. (1984) reported large fluctuations in Flavobacterium spp. over a long period, though not related to plant performance. Although populations remained stable at Runcorn the evidence again suggests that they do not have a role

in settlement. Acinetobacter populations also remain stable in the three sludge samples. These may have a role in phosphorus removal (Hart and Melmed, 1982).

The dominant group of isolated microorganisms in both bulking and non-bulking sludges at Runcorn fell into category 13. This group includes all members of the genera Alcaligenes, Moraxella and Brucella. The primary factor in classification was a negative result in the Hugh and Leifsons medium. Clearly this is not an ideal method of identification and other genera may fall into this grouping. Possibly due to their unreactivity, little research has been carried out on the sludge ecology of Alcaligenes spp. although they have frequently been found to dominate activated sludge (see Table 1.1). Clearly they may have an important role and perhaps warrant further investigation.

From the large number of studies on the activated sludge microflora it is clear that each plant has its own characteristic bacterial population. This is influenced by the nature of the sewage and the plant characteristics. The stability of sludge populations appears to remain static over long periods of time unless influenced by some external factor. Here, a visible increase in filamentous organisms has been shown to be accompanied by a change in floc-forming microorganisms. It is possible that this population change may also play a role in sludge settlement.

Summary

1. The populations of heterotrophic aerobic bacteria in three return activated sludge samples were investigated. Samples were taken approximately one month before a bulking incident, at the time of bulking and about one month after bulking was resolved. Isolates were divided into 15 groups depending upon their physiological characteristics.
2. The dominant bacteria found in each sample fell into group 13. This group included the genera *Alcaligenes*, *Moraxella* and *Brucella*.
3. Bulking activated sludge showed a marked increase in the number of facultatively anaerobic bacteria and several species of Enterobacteriaceae were identified.
4. The significance of these changes was discussed.

CHAPTER 6

Effects of Sewage Addition on Activated Sludge Extracellular Polymers

It is likely that activated sludge extracellular polymers not only play a key role in sludge settlement but also in the removal of some toxic sewage constituents such as heavy metals. Although there has been a number of reports with regard to the influence of sewage composition on extracellular polymer production (Kiff, 1978; Forster and Clarke, 1983) less has been written about the effect of plant design. In this study, sludge polymer production was investigated at Runcorn E.T.W. Two plants were in operation which received sewage of identical chemical composition and differed only in design. At the time of the study plant 1 was operating as described in Fig 3.1b, whilst a description of plant 2 can be found in Fig 3.1d.

Samples were taken at each stage of both treatment works. This included settled sewage, activated sludge from each aeration pocket, return activated sludge and plant effluent. Extracellular polymer was measured as ethanol insoluble material (e.i.m.) by the heat extraction and ethanol precipitation method of Forster (1976). After extraction e.i.m. was assayed for COD, hexose sugars, protein, hexuronic acids and DNA. Samples from the two plants at Runcorn were taken on different days, 1 week apart. Plant performance data and details of polymer in settled sewage and plant effluents on the days of study are shown in Table 6.1.

Sewage polymer accounted for only 3.8% and 6.3% of the total COD of settled sewage on the days of sampling at plant 1 and plant 2 respectively. This disagrees with the results of

Table 6.1 Plant performance data and details of sewage and effluent polymer.

	Plant 1	Plant 2
Settled sewage		
BOD mg l^{-1}	186	98
COD mg l^{-1}	371	229
Ammonia nitrogen mg l^{-1}	27.2	13.1
Suspended solids mg l^{-1}	96	84
Polymer mg l^{-1}	151.0	172.6
Sewage polymer analysis		
COD mg g^{-1}	94	87
Hexoses mg g^{-1}	14.0	0
*(Theoretical COD mg g^{-1})	14.9	0
Protein mg g^{-1}	65.4	46.4
*(Theoretical COD mg g^{-1})	55.8	39.6
Hexuronic acids mg g^{-1}	41.2	21.0
DNA mg g^{-1}	0	3.48
Aeration		
Load g COD g $MLSS^{-1}$ day^{-1}	0.56	0.32
SVI ml g^{-1}	495	571
SV ml l^{-1}	91	80
MLSS g l^{-1}	1.70	1.43
RAS suspended solids g l^{-1}	3.31	2.05
Effluent		
BOD mg l^{-1}	7	8
COD mg l^{-1}	46	51
Ammonia nitrogen mg l^{-1}	11.3	0.3
Nitrate mg l^{-1}	0.94	11.11
Nitrite mg l^{-1}	0.63	0.31
polymer mg l^{-1}	132.8	88.2
Effluent polymer analysis		
COD mg g^{-1}	44.1	20.1
Hexoses mg g^{-1}	23.1	22.6
*(Theoretical COD mg g^{-1})	24.6	24.11
Proteins mg g^{-1}	46.8	68.2
*(Theoretical COD mg g^{-1})	59.8	58.2
Hexuronic acid mg g^{-1}	12.8	17.1
DNA mg g^{-1}	6.1	4.6

* Theoretical COD for carbohydrate was calculated using the value for glucose = 1.067 mg mg^{-1} (APHA-AWWA-WPCF, 1975). The conversion factor for protein was then obtained by multiplying this value by the average Respiratory Quotient for protein (0.80) = 0.854 mg mg^{-1}.

Kiff (1978) who found that acetone precipitated extracts from sewage accounted for up to 83% of the COD and 70% of the BOD values. Acetone precipitation has been shown to give greater quantitites of polymers than ethanol (Novak and Haugan, 1981) though Kiff obtained lower values (100 - 150 mg l^{-1}) for the extraction of sewage material than the ethanol technique used in this study (163-376 mg l^{-1}). Assuming theoretical COD values of 1.067 mg mg^{-1} for hexoses and 0.854 mg mg^{-1} for protein, the contribution to polymer COD from these fractions can be calculated (see Table 6.1). These values suggest that results of COD analysis (particularly of effluent) may be slightly low and this may in part explain the discrepancy between values here and those of Kiff.

In order to further investigate the composition of sewage polymers, samples of settled sewage were taken from a number of effluent treatment works. These results are presented in Table 6.2. There is clearly a large range in polymer content with no apparent relationship with sewage source. In no case did the polymer COD contribute more than 37.5% of the total COD. The major biopolymer found in the sewage extracts was protein with only small amounts of DNA and hexose sugars. However, the maximum value for protein content (39.4 mg g $polymer^{-1}$) represented only 3.94% of the total polymer extracted although from Table 6.2 it can be seen that protein makes up a major part of the e.i.m. COD. This clearly suggests that sewage polymers are made up largely of some other constituent. This may of

Table 6.2 Properties of e.i.m, from different sewages

SOURCE	TYPE	COD filtered sewage mg l^{-1}	e.i.m.											
						Protein				Hexoses				DNA	
			mg l^{-1} sewage	COD mg g^{-1}	% total COD	mg g^{-1}	% e.i.m.	Theoretical COD mg g^{-1}	% e.i.m. COD	mg g^{-1}	% e.i.m.	Theoretical COD mg g^{-1}	% e.i.m. COD	mg g^{-1}	% e.i.m.
Lymm	Domestic	1922	376	247	4.8	39.4	3.94	33.6	13.6	0.4	0.04	0.4	0.16	8.7	0.87
Appleton	Domestic	38	195	73	37.4	30.2	3.02	25.8	35.3	5.4	0.54	5.8	7.94	7.1	0.71
Warrington South	Domestic	225	194	33	4.6	23.6	2.36	20.1	60.9	0	0	0	0	3.6	0.36
Gateworth	Industrial	914	265	41	1.2	12.4	1.24	10.6	25.8	0	0	0	0	2.1	0.21
Runcorn*	Brewery waste	315	160.1	96	4.9	38.7	3.87	33.0	34.4	3.8	0.38	4.27	2.56	2.6+	0.26

* Mean of 4 samples

\+ Mean of 3 samples

course be lipid material although Carr and Ganczarczyk (1974) have pointed out that inorganic salts may be precipitated by ethanol and mistaken for bacterial polysaccharides.

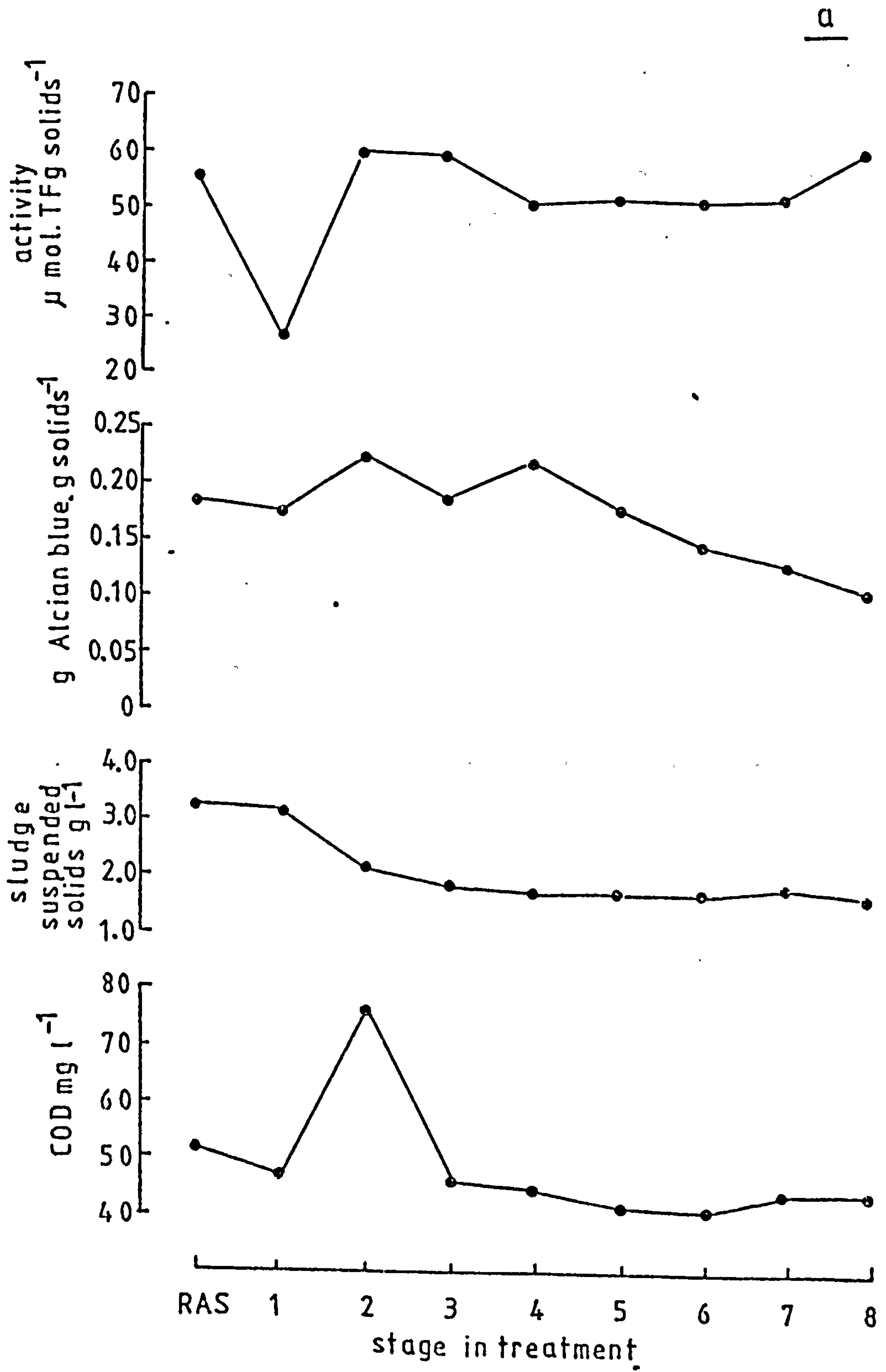
Large amounts of ethanol insoluble material were also found in the effluent from both plants at Runcorn. Treatment by both plants, however, resulted in an overall decrease in polymer content with a reduction of 87.9% at plant 1 and 51.1% at plant 2. Polymer COD as a percentage of total COD was low; 12.6% and 3.5% for plants 1 and 2 respectively. Changes can be seen in polymer isolated from plant effluent when compared to that of the sewage inflow, the former containing higher levels of hexoses and DNA. This is presumably due to production of these polymers by activated sludge bacteria.

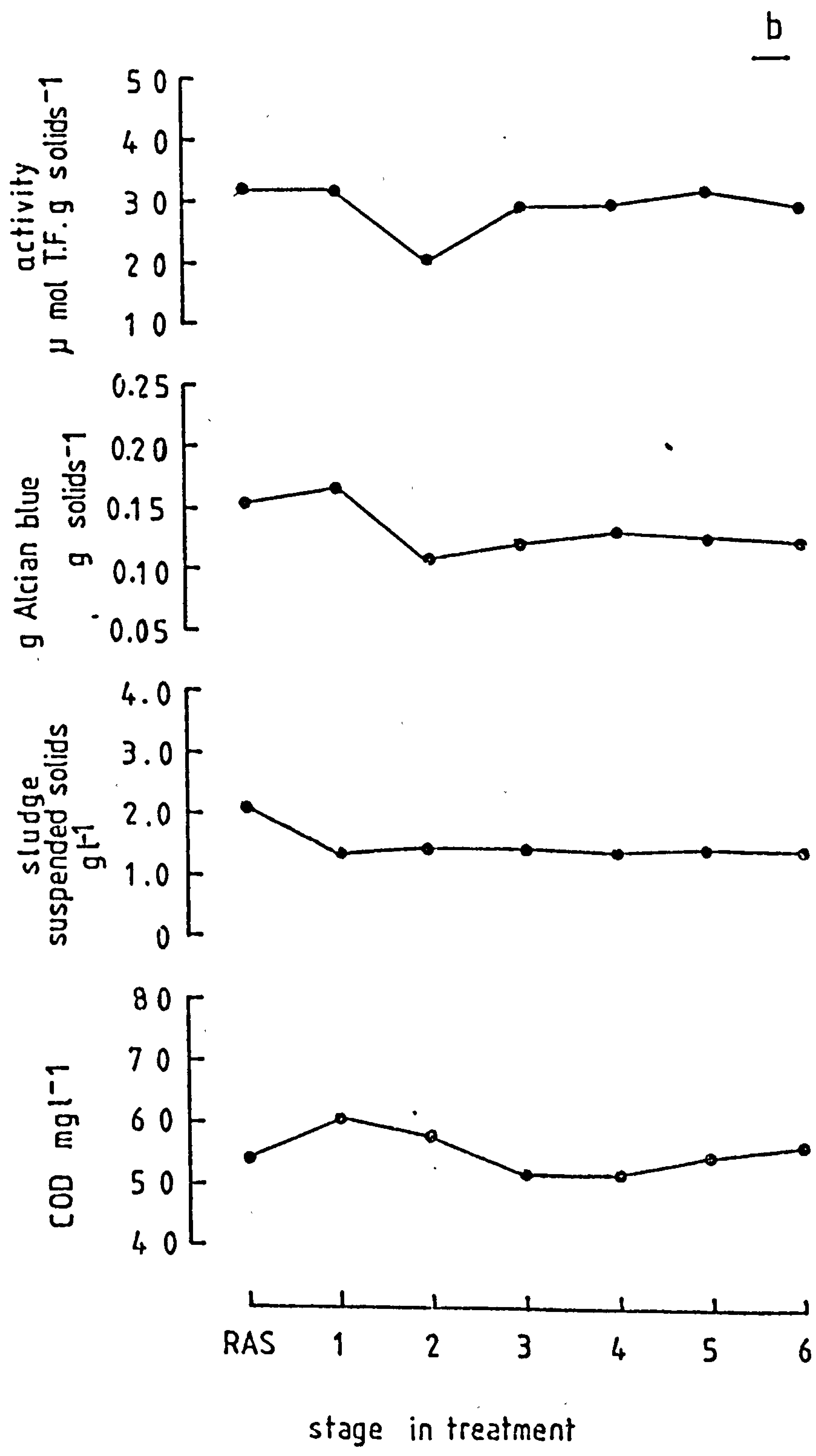
Sludge suspended solids concentration, Alcian blue binding, activity as TTC reduction and supernatant COD concentrations for each stage in treatment at both plants are shown in Fig. 6.1. These results are similar to those found in earlier studies at the same works (see Chapter 4). COD was removed very rapidly in both plants with supernatant COD levels equal to that of the plant effluent in the aeration pocket following sludge/sewage mixing. In the case of plant 1 a step feeding system was in operation with sewage entering at pockets 1, 2, 3, 4 and 5. By calculation from the sludge suspended solids concentration in the aeration pockets, the sludge : sewage mixing ratio's in pockets 1, 2 and 3 were 2.12:1, 7.3:1 and 10.1:1 respectively. With a settled sewage COD of 371 mg l^{-1} this means that the available

Figue 6.1

Supernatant COD, sludge suspended solids, Alcian blue adsorption and sludge activity at different stages of treatment in Runcorn plant 1 (a) and plant 2 (b).

a




b
activity
μ mol T.F. g solids-1
50
40
30
20
10
g Alcian blue
g solids-1
0.25
0.20
0.15
0.10
0.05
sludge
suspended solids
gl-1
4.0
3.0
2.0
1.0
0
COD mgl-1
80
70
60
50
40
RAS
1
2
3
4
5
6
stage in treatment

COD in pocket 1 can be calculated as 150.7 mg l^{-1} of which 74.7 mg l^{-1} or 49.6% was taken up by the activated sludge. This represents an uptake of 29.4 mg COD g $sludge^{-1}$ and it can be assumed that sludge is saturated with regard to COD at this point.

In plant 2, all of the sewage COD is adsorbed in the first aeration pocket, representing a COD uptake of 48.1 mg g sludge $^{-1}$ suspended $solids^{-1}$. Thus the COD removal capacity appears to be higher in plant 2 than in plant 1. Furthermore, the sludge does not become saturated with COD in plant 2.

Differences in COD adsorption capacity of the sludges from the two plants were not reflected in Alcian blue adsorption values. Dye binding by plant 2 return activated sludge was lower than in plant 1 with a binding of 0.151 and 0.156 g g $solids^{-1}$ respectively.

Table 6.3 shows the mean quantity and composition of extracellular polymers from activated sludge at Runcorn plants 1 and 2. Results are presented for both the soluble polymer fraction and polymer bound to the sludge biomass. Yields of extracted material (5.12% of total suspended solids (TSS) for plant 1 and 8.26% for plant 2) compare well with those of other workers using a steaming or boiling method of extraction. Published values have ranged from 1.13 - 6.47% TSS (Kiff, 1978; Kiff and Thompson, 1979), 0.9 - 9.1% (Forster and Clarke, 1983), 11.5 - 19.4% (Beccari _et al._, 1980) and 1.7 - 4.25% (Water Pollution Research Laboratory, 1971). Brown and Lester (1982a)

Table 6.3 Mean values of sludge e.i.m. composition from plants 1 and 2.

	Plant 1		Plant 2	
	Bound	Soluble	Bound	Soluble
Yield (% TSS)	5.12	7.49	8.26	5.43
mg protein g sludge^{-1}	5.82	3.37	7.50	3.56
mg protein g e.i.m.$^{-1}$	113.55	49.8	90.7	60.0
mg hexose sugars g sludge^{-1}	2.56	1.93	5.69	1.27
mg hexose sugars g e.i.m.$^{-1}$	49.9	25.8	68.9	21.4
mg DNA g sludge^{-1}	0.68	0.28	1.65	0.30
mg DNA g e.i.m.$^{-1}$	13.22	3.8	20.01	5.1
mg hexuronic acids g sludge^{-1}	0.74	1.35	2.33	0.83
mg hexuronic acids g e.i.m.$^{-1}$	14.5	18.1	28.2	14.0
Protein:hexose sugars ratio	2.27:1	1.93:1	1.32:1	2.80:1
hexose sugars:hexuronic acid ratio	3.44:1	1.42:1	2.44:1	1.52:1
Protein:hexose sugar:DNA ratio	8.6:3.8:1	13.1:6.8:1	4.5:3.4:1	11.8:4.2:1

reported a yield of 4.7% TSS from sludge of a laboratory activated sludge plant.

Similarly, protein content of extracellular polymer from Runcorn sludge is of a similar magnitude to that found by other workers. Forster and Clarke (1983) found that their extracted material contained 157 mg protein g polymer^{-1} which compares well with values of 113.5 and 90.7 mg g polymer^{-1} found at Runcorn. Some workers have expressed their results only as mg polymer protein g TSS^{-1}. Brown and Lester (1982b) reported a range of values between 10.5 and 15.1 mg polymer protein g TSS^{-1} for sludge from a laboratory activated sludge plant and a variety of sludge ages, although in earlier work (Brown and Lester, 1980) they found higher values (47.6 - 75.0 mg g TSS^{-1}) in full scale activated sludge plants. Ranges of 11.6 - 18.9 mg g TSS^{-1} and 1.9 - 4.8 mg g TSS^{-1} were reported by Rudd _et al._ (1983) and Carr and Ganczarcyk (1974) respectively. Polymer protein content of Runcorn sludge was equal to 5.82 mg g TSS^{-1} for plant 1 and 7.5 mg g TSS^{-1} for plant 2.

Concentrations of hexose sugars in extracellular polymers from Runcorn sludge were lower than those found by most other workers. Mean values at Runcorn plant 1 were 49.9 mg hexoses g polymer^{-1} or 2.56 mg g TSS^{-1} whereas at plant 2 the respective figures were 68.9 and 5.69 mg g^{-1}. Forster and Clarke (1983) reported a concentration of 280 mg hexoses g extracted material^{-1}. Brown and Lester (1982b), Rudd _et al._ (1983) and Carr and Ganczarczyk (1974) found the carbohydrate concentration

in their extracts to fall within the ranges 24.9 to 28.4, 1.9 to 52.4 and 1.5 to 38.0 mg g TSS^{-1} respectively. Kiff (1978) found that the concentration of carbohydrate in activated sludge extracts ranged from 5.0 to 16.4 mg g TSS^{-1} depending upon the quantity of carbohydrate in the influent.

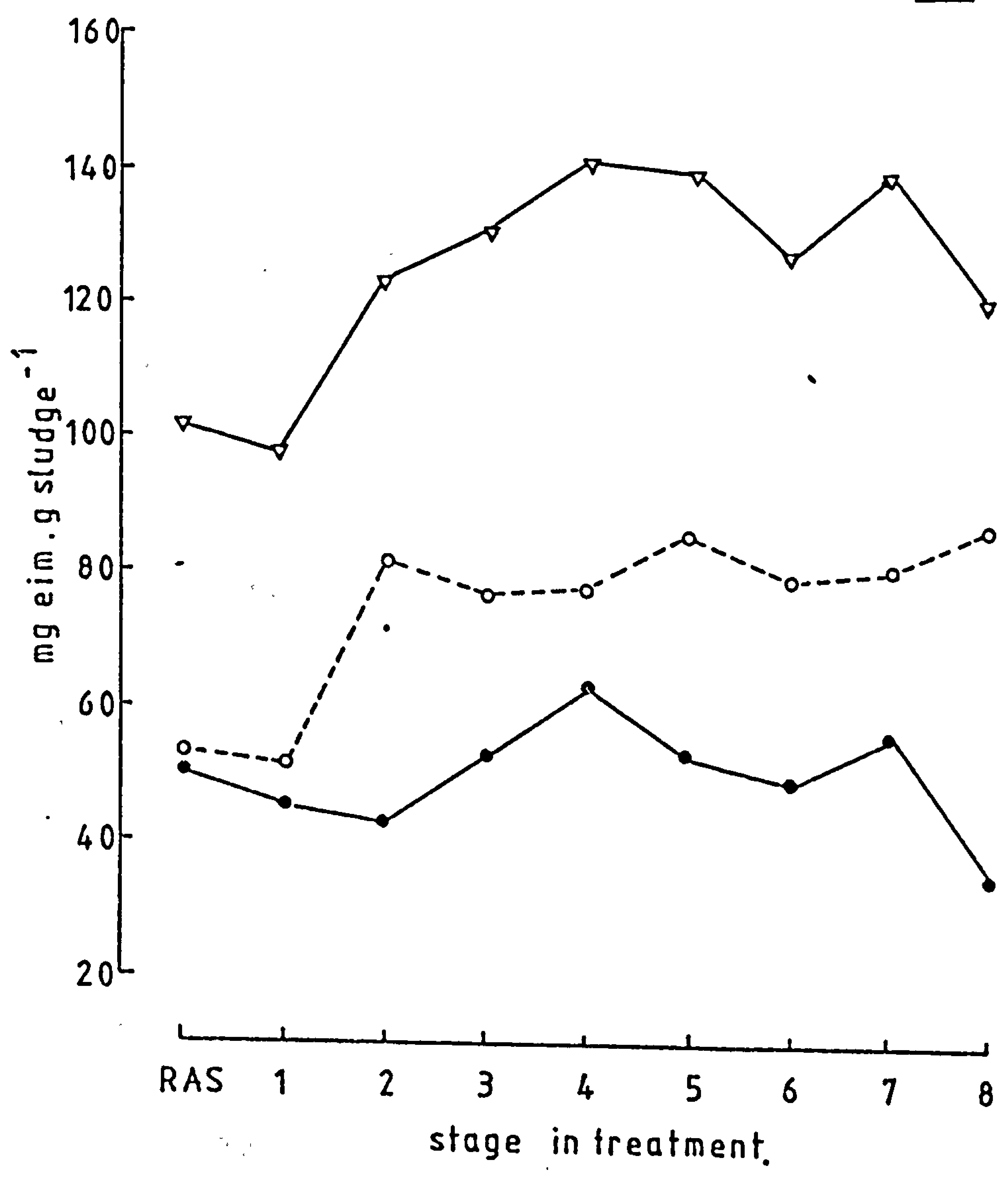
Mean values of polymer yields reveal differences between plants 1 and 2. Plant 1 sludge had a lower bound polymer content although more soluble polymer was found in the sludge supernatant. Differences were also found in polymer composition, plant 1 being lower in hexoses, DNA and hexuronic acids than plant 2, yet higher in protein content.

Fig. 6.2 shows the quantity of extracellular polymer extracted at various stages throughout Runcorn plants 1 and 2. Total extracellular polymer showed a similar pattern in both plants with a gradual increase along the aeration lanes followed by a decrease in the final pocket of aeration. Bound polymer was higher than soluble polymer in plant 2 yet lower than soluble polymer in plant 1. Soluble polymer in plant 1 ranged from 48.6 to 181.2 mg l^{-1} with the highest level found in pocket 2. The corresponding range in plant 2 was 85 to 96.6 mg l^{-1}. With only small differences in the quantity of polymer in the settled sewage inflow, this suggests that soluble polymer throughout an activated sludge plant is a function of the sludge and plant operating conditions.

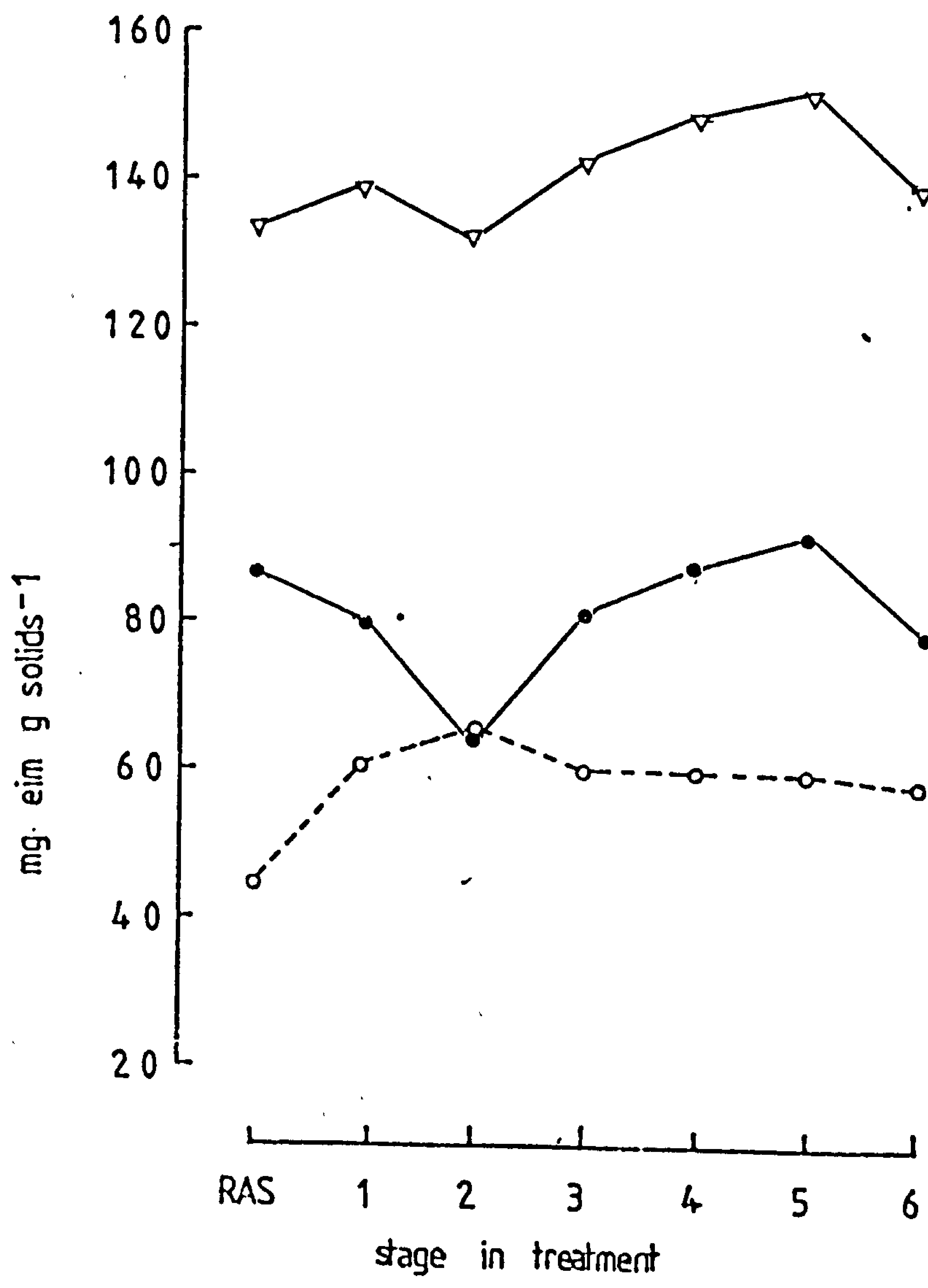
When expressed as mg polymer g $solids^{-1}$ soluble polymers showed a marked increase in the sludge/sewage mixing stage at

Figure 6.2

Total (▽), bound (●) and soluble (○) polymer (as e.i.m.) throughout Runcorn plant 1 (a) and plant 2(b).


a
160
140
120
100
80
60
40
20
mg eim . g sludge -1
RAS
1
2
3
4
5
6
7
8
stage in treatment

b

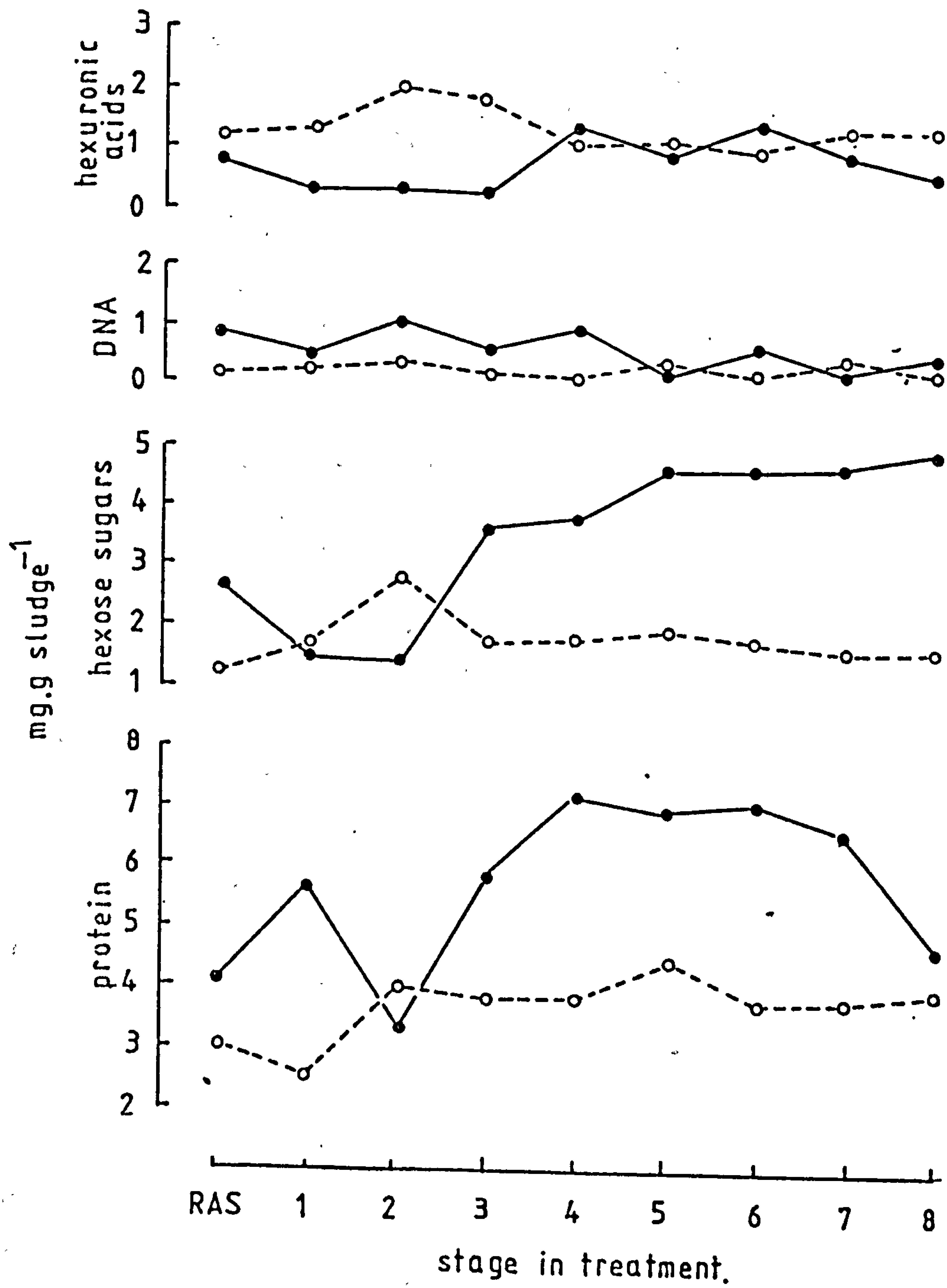


both plants, i.e. pocket 1 of plant 2 and in pocket 2 of plant 1. This reflected the dilution of sludge with sewage inflow. As material was passed out at the effluent a corresponding decrease was found in soluble polymers in return activated sludge. However, there was no evidence for an increase in bound sludge extracellular polymers at the time of sewage and sludge mixing. Bound material throughout plant 2 was characterized by a decrease in pocket 2. This corresponded with a drop in both sludge activity and Alcian blue binding although the reason for this is not clear.

Results of chemical analyses of sludge extracellular polymers at each stage throughout both plants at Runcorn are expressed as mg.g sludge^{-1} in Fig. 6.3 and mg g extracellular polymer^{-1} in Fig. 6.4. Composition of soluble polymers remained constant throughout each stage of treatment in both plants. Some changes in composition were, however, apparent in bound polymers. This was particularly evident at the sewage/sludge mixing phase. In plant 2 sludge polymer there was a fall in the levels of protein, hexuronic acid, hexoses and DNA at this point. A corresponding drop was found in protein and hexoses at the similar stage of plant 1. This was presumably due to adsorption of polymeric material from sewage by activated sludge. The nature of this material is not known. Following sludge/sewage mixing, components of bound polymer from plant 2 gradually returned to the levels found in return activated

Figure 6.3

Protein, hexose sugar, DNA and hexuronic acid content of bound (●) and soluble (○) polymer (as e.i.m.) as a proportion of sludge solids concentration in Runcorn plant 1 (a) and plant 2 (b).


a
hexuronic acids
DNA
hexose sugars
protein
mg.g sludge^{-1}
3
2
1
0
2
1
0
5
4
3
2
1
8
7
6
5
4
3
2
RAS
1
2
3
4
5
6
7
8
stage in treatment.

b

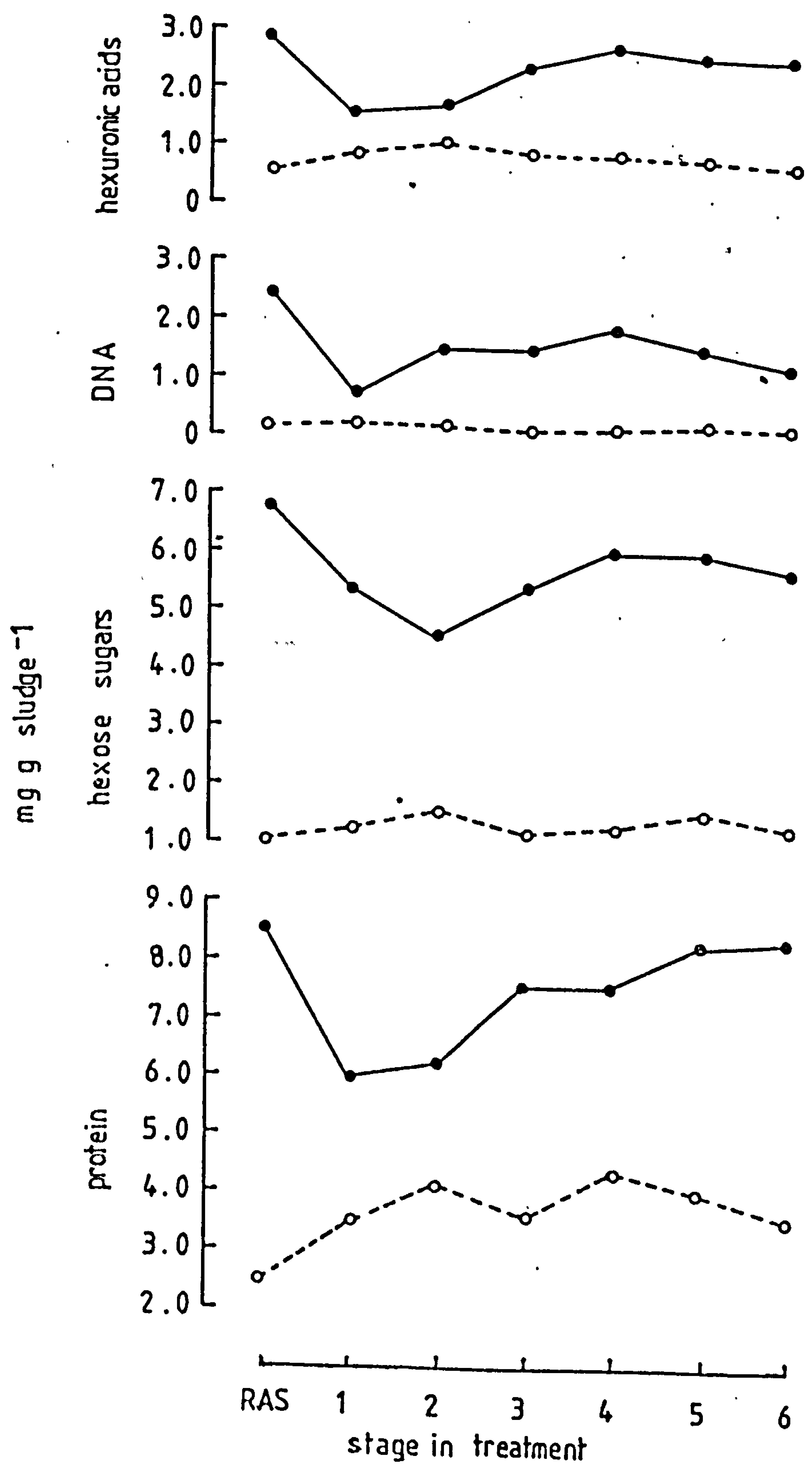
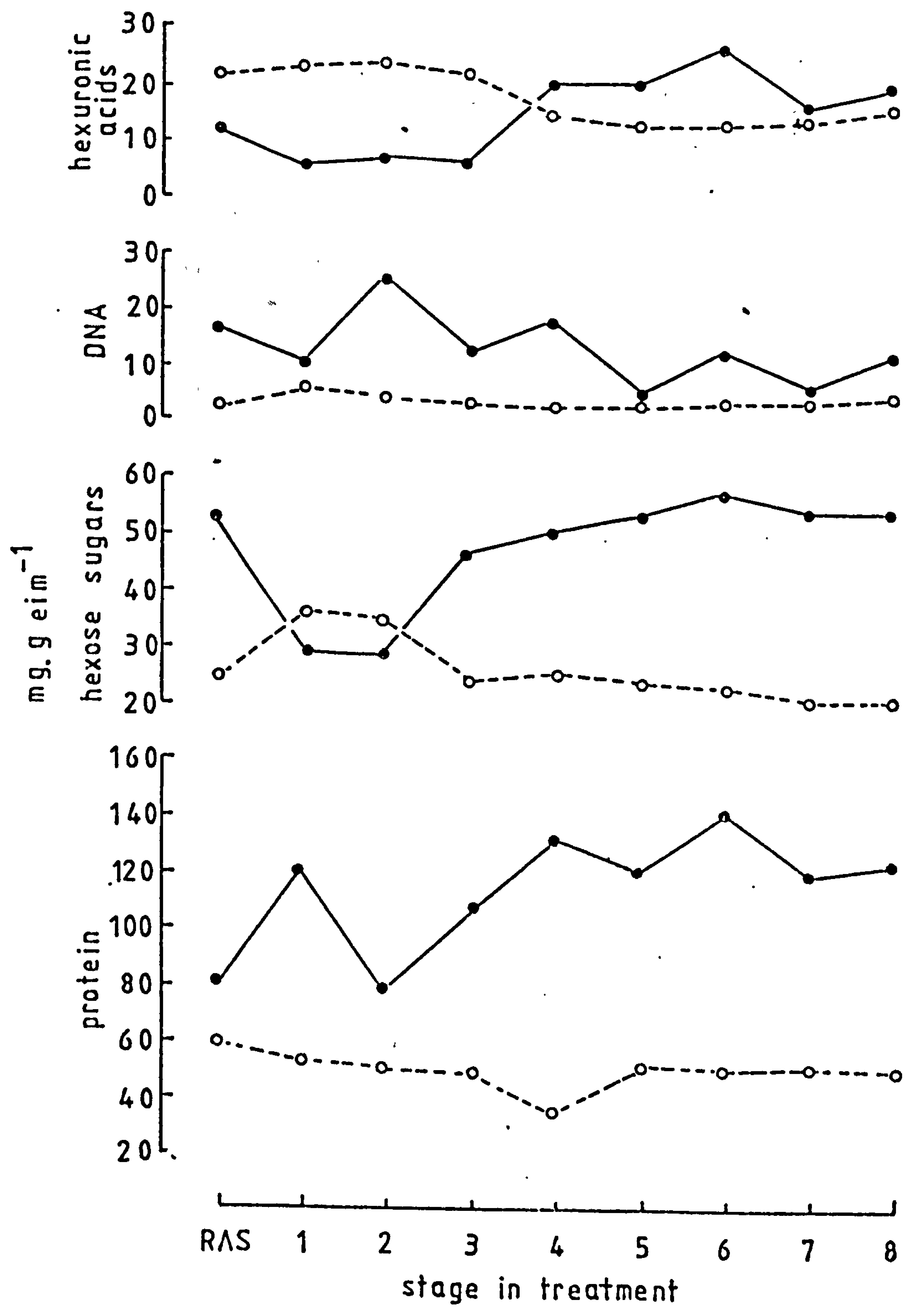
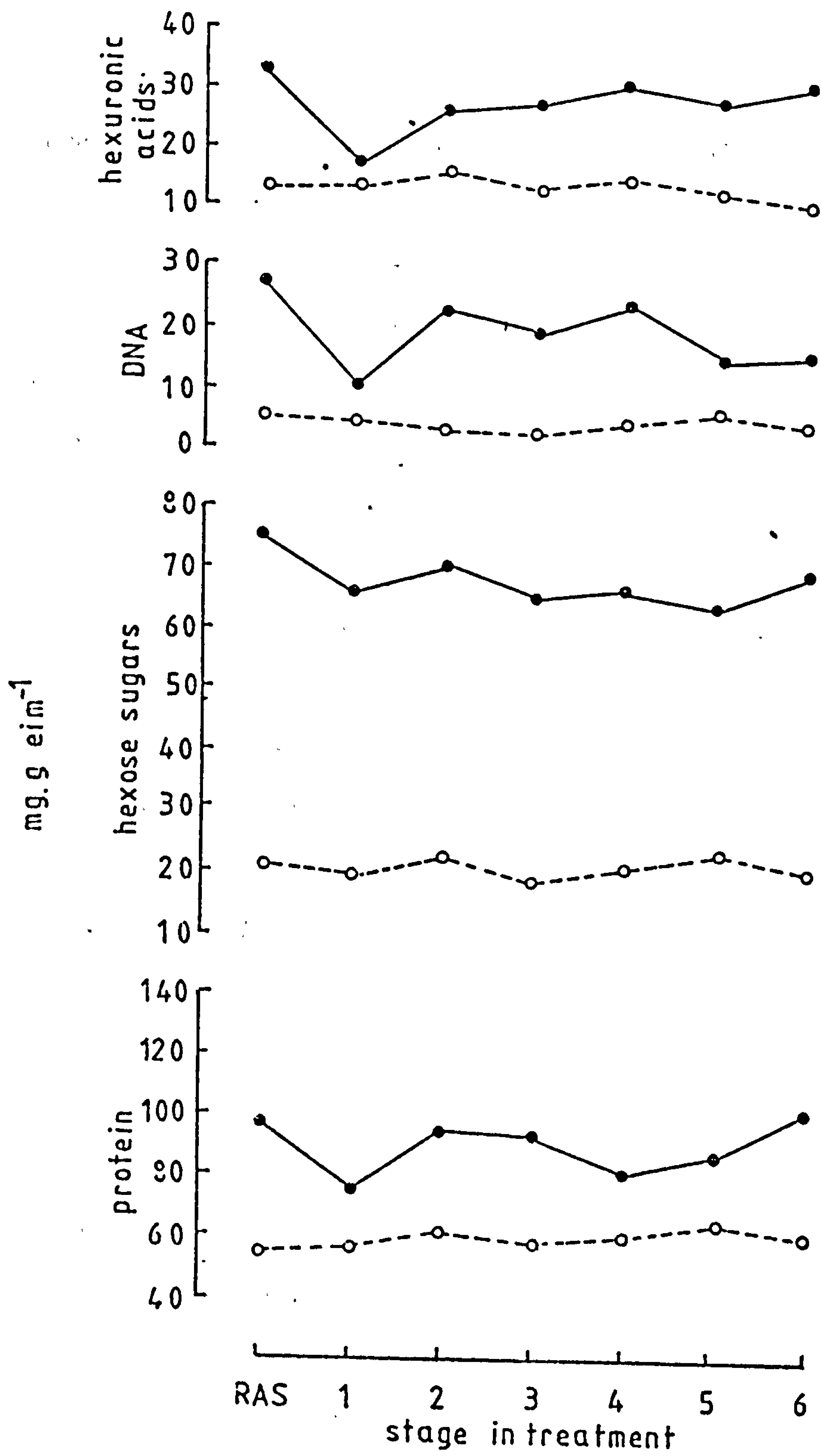


Figure 6.4

Protein, hexose sugar, DNA and hexuronic acid content of bound (●) and soluble (○) polymer (as e.i.m.) in Runcorn plant 1 (a) and plant 2 (b).



a
hexuronic acids
30
20
10
0
DNA
30
20
10
0
hexose sugars
60
50
40
30
20
mg. g eim^{-1}
protein
160
140
120
100
80
60
40
20
RAS
1
2
3
4
5
6
7
8
stage in treatment


b
hexuronic acids
40
30
20
10
DNA
30
20
10
0
hexose sugars
80
70
60
50
40
30
20
10
protein
140
120
100
80
60
40
mg.g eim-1
RAS
1
2
3
4
5
6
stage in treatment

sludge. The situation in plant 1 was more erratic probably due to the step loading employed.

The results suggest that the most pertinent changes in sludge polymer production and composition are found in the sewage/sludge mixing stage. This was further investigated in the laboratory using a 10 l fermenter (L-H Engineering) to which was added 3 l of freshly collected return activated sludge. This was then aerated for 40 minutes by stirring at 250 r.p.m., followed by the addition of an equal volume of settled sewage. The point of sewage addition was taken as t = 0. Samples of 250 ml were removed from the fermenter at t = -40 minutes, t = 0 immediately prior to sewage addition, t = 10 minutes, and at t = 1, 2.5, 4, 5.5, 7, and 24 hours. Samples were analysed for polymer content and composition, Alcian blue adsorption, suspended solids concentration, SVI, sludge activity and COD. Three combinations of sludge and sewage were investigated:

Experiment A : Runcorn plant 1 RAS and Runcorn sewage.
Experiment B : Runcorn plant 2 RAS and Runcorn sewage.
Experiment C : Runcorn plant 1 RAS and Warrington South sewage.

The characteristics of each plant and sewage on the days of sampling are shown in Table 6.4.

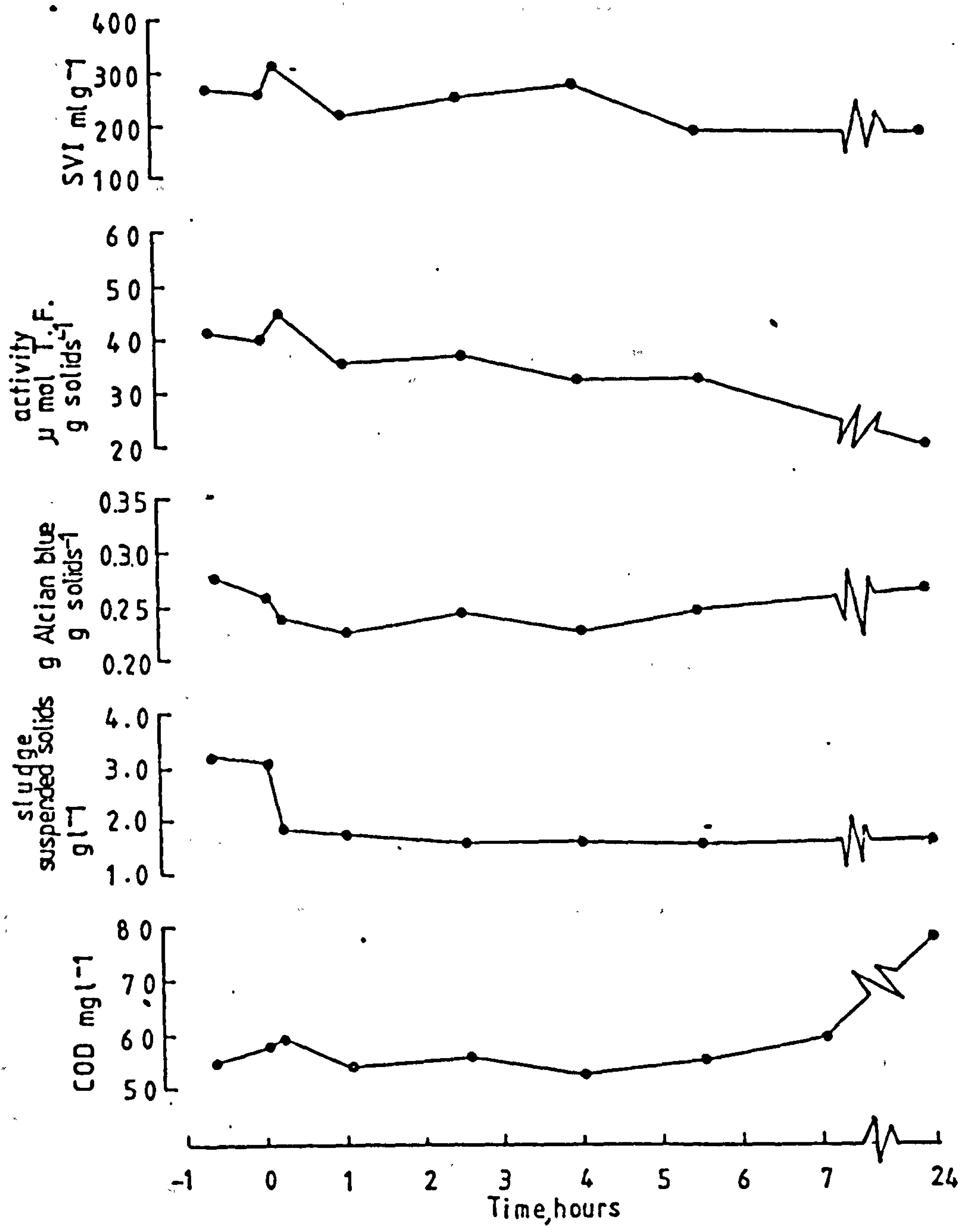
Fig. 6.5 shows the supernatant COD concentration, suspended solids concentration, Alcian blue adsorption, SVI and sludge activity (as TTC reduction) which occurred during aeration of

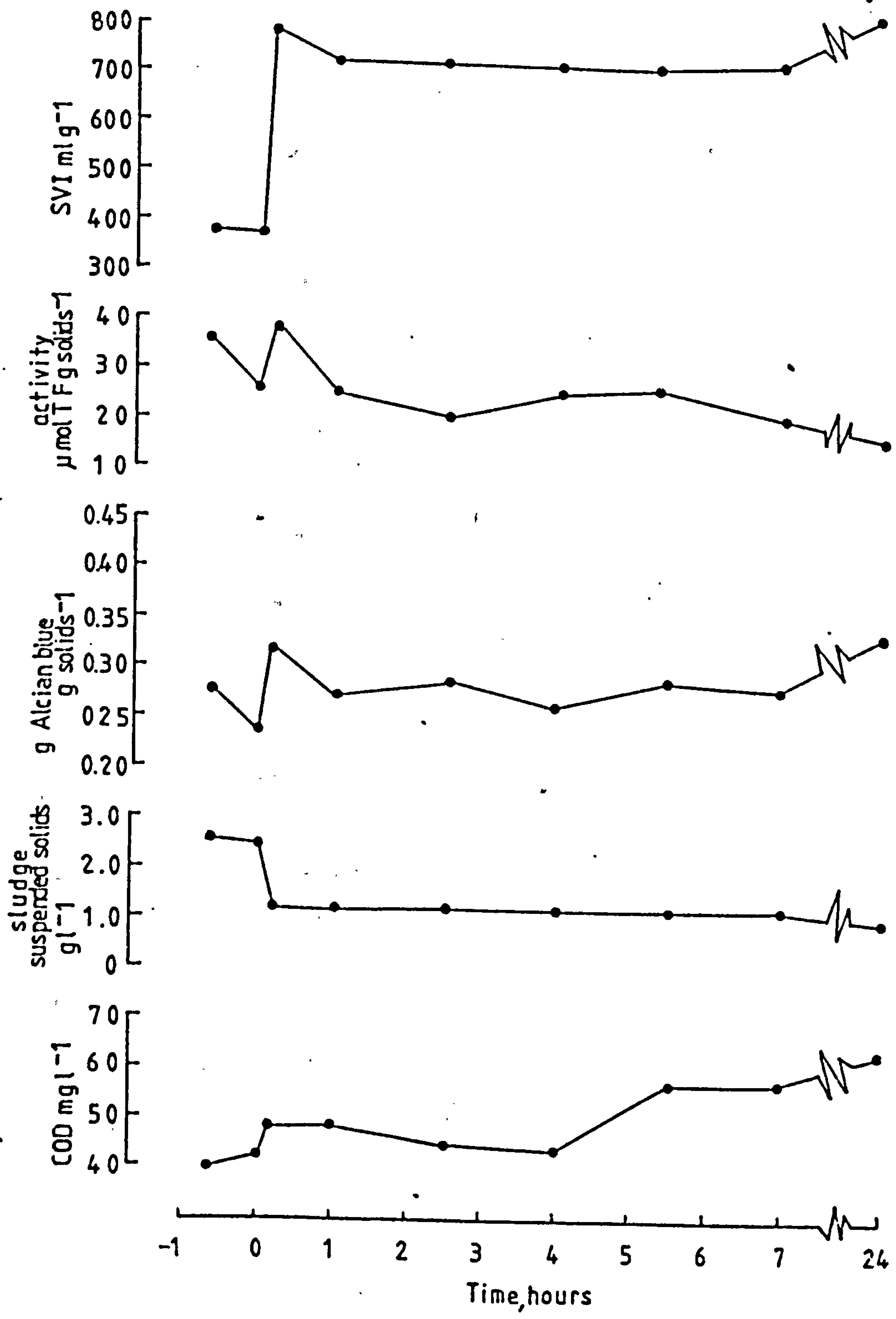
Table 6.4 Characteristics of plants and sewage on the days of sampling. For sample C, characteristics of both Runcorn plant 1 and Warrington South are shown so that comparisons can be made between acclimated and non-acclimated sewage.

	A	B	C	
			Runcorn plant 1	Warrington South
Settled sewage				
BOD (mg l^{-1})	126	164	192	84
COD (mg l^{-1})	307	353	477	225
Ammonia nitrogen (mg l^{-1})	28.0	22.0	30.2	23.0
e.i.m. (mg l^{-1})	160.3	156.6	143.8	194.0
COD e.i.m. % total COD (mg g total COD^{-1})	4.3 (82)	11.5 (121)	ND	17.4 (33)
Hexose sugars (mg g^{-1})	0	1.2	ND	0
Protein (mg g^{-1})	32	11.1	ND	23.6
DNA (mg g^{-1})	1.8	2.4	ND	3.6
Effluent				
BOD (mg l^{-1})	10	9	13	5
COD (mg l^{-1})	31	28	76	31
Ammonia nitrogen (mg l^{-1})	0.6	8.2	21	12.0
Nitrate (mg l^{-1})	0.72	2.52	1.11	4.11
Nitrite (mg l^{-1})	17.35	9.02	0.23	0.67
e.i.m. (mg l^{-1})	140.4	101.3	131.2	129.0
COD e.i.m. % total COD (mg g total COD^{-1})	27.7 (612)	13.1 (401)	ND	ND
Hexose sugars (mg g^{-1})	23.9	19.2	ND	ND
Protein (mg g^{-1})	38.2	41.4	ND	ND
Hexuronic acids (mg g^{-1})	4.3	17.2	ND	ND
DNA (mg g^{-1})	3.8	11.1	ND	ND
MLSS (final aeration tank) (g l^{-1})	1.86	1.96	1.24	1.36
RAS suspended solids (g l^{-1})	3.21	2.62	3.414	1.51
Plant loading Kg BOD Kg^{-1} day^{-1}	0.165	0.177	0.38	0.164
Kg COD Kg^{-1} day^{-1}	0.402	0.599	0.99	0.44

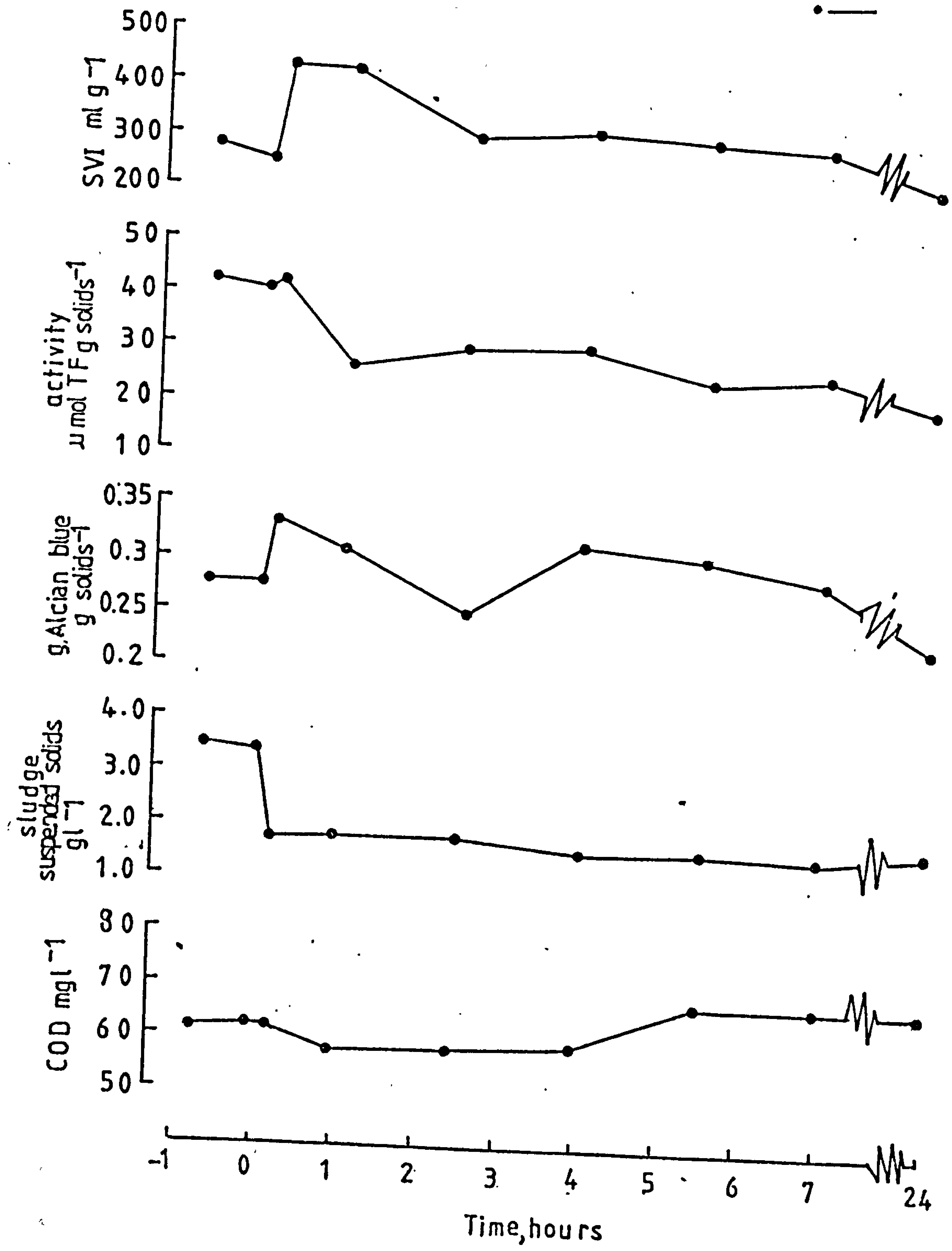
Figure 6.5

Supernatant COD, suspended solids, Alcian blue binding, sludge activity and SVI for (a) Runcorn 1 RAS mixed with Runcorn sewage (b) Runcorn 2 RAS mixed with Runcorn sewage and (c) Runcorn 1 RAS mixed with Warrington South sewage.


a
SVI ml g⁻¹
400
300
200
100
activity
μ mol T.F.
g solids⁻¹
60
50
40
30
20
g Alcian blue
g solids⁻¹
0.35
0.30
0.25
0.20
sludge
suspended solids
g l⁻¹
4.0
3.0
2.0
1.0
COD mg l⁻¹
80
70
60
50
-1
0
1
2
3
4
5
6
7
24
Time, hours



b
SVI ml g⁻1
800
700
600
500
400
300
activity
μ mol T F g solids⁻1
4 0
3 0
2 0
1 0
g Alcian blue
g solids⁻1
0.45
0.40
0.35
0.30
0.25
0.20
sludge
suspended solids
gl⁻1
3.0
2.0
1.0
0
COD mg l⁻1
7 0
6 0
5 0
4 0
-1
0
1
2
3
4
5
6
7
24
Time, hours



c
500
400
300
200
SVI ml g⁻¹
50
40
30
20
10
activity
µ mol TF g solids⁻¹
0.35
0.3
0.25
0.2
g Alcian blue
g solids⁻¹
4.0
3.0
2.0
1.0
sludge
suspended solids
gl⁻¹
80
70
60
50
COD mgl⁻¹
-1
0
1
2
3
4
5
6
7
24
Time, hours

each combination of sludge and sewage over a 24 hour period. In each case most of the settled sewage COD was taken up by the sludge within the first 10 minutes. With an influent sewage COD of 307, 253 and 255 mg l^{-1} and a return activated sludge supernatant COD of 63.58 and 43 mg l^{-1}, this means that the available COD at the point of sludge/sewage mixing was 185, 155.5 and 149 mg l^{-1} for experiments A, B and C respectively. Thus the COD uptake by each sludge was 95.8, 131.8 and 85.6 mg COD g $sludge^{-1}$ for the three respective experiments. This means that the amounts of COD adsorbed by sludge from both plants 1 and 2 in the laboratory exceeded the uptake capacity calculated at the full scale plants.

Eikelboom (1982) pointed out that biosorption increased with an increase in floc loading. Loading to the fermenter vessel was higher than that in the first pocket of either plant 1 or plant 2 on the days studied and this may explain the higher adsorption capacity.

Sludge supernatant COD remained constant up to a period of 7 hours. At 24 hours there was a large increase in the COD presumably due to cell lysis and subsequent release of cell constituents into the medium. SVI and Alcian blue binding both increased at the point of sludge and sewage mixing in experiments B and C. This was consistent with the observations found in full scale plants, probably due to deflocculation of sludge on dilution with sewage. Also consistent with full-scale observations was the reduction of sludge activity (as TTC

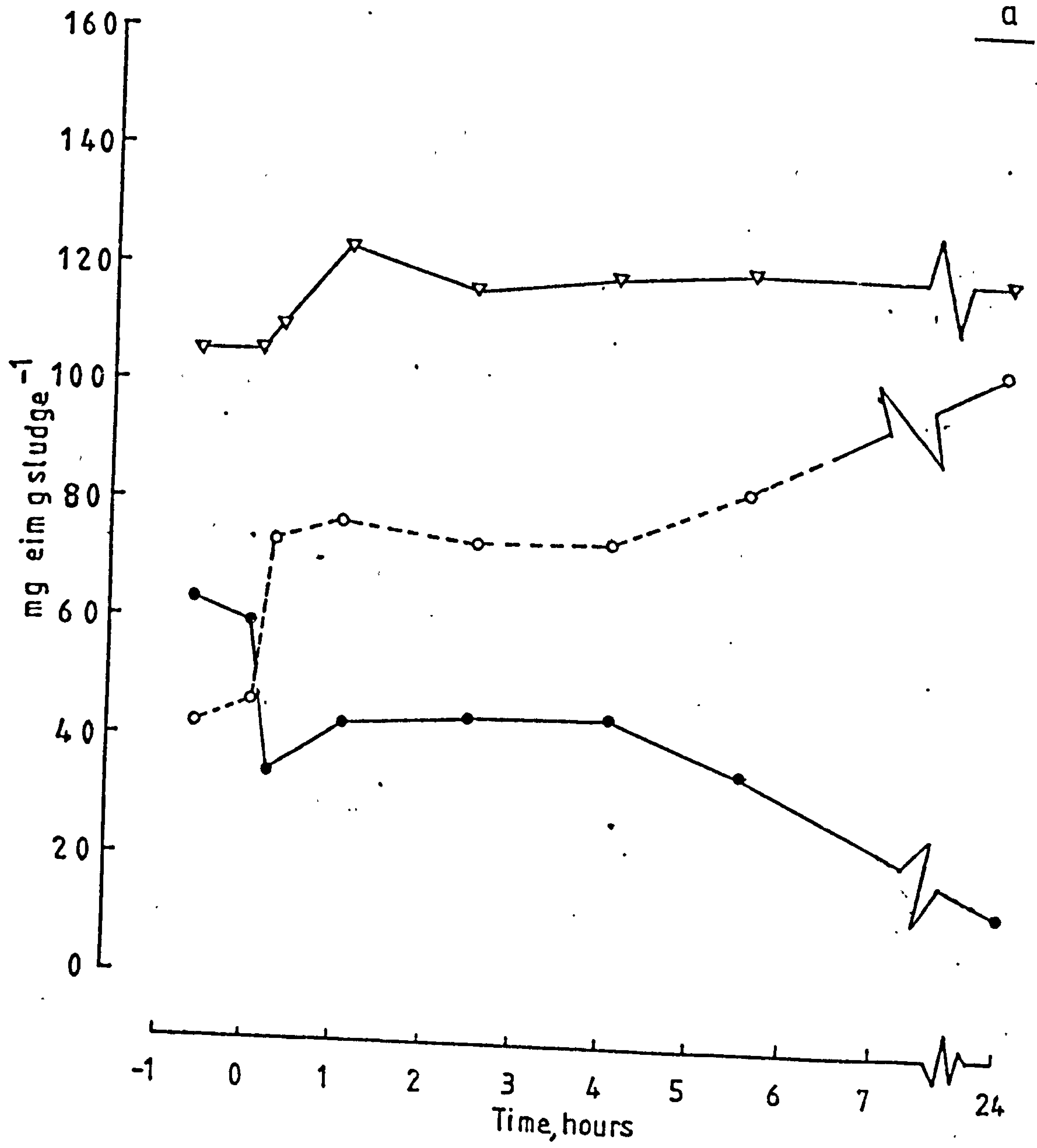
reduction) brought about by reaeration. Surprisingly, only small increases in activity were measured after sewage addition.

The quantities of extracellular polymer present in sludge samples is expressed as mg g sludge suspended solids^{-1} in Fig. 6.6. The concentration of polymers in sewage were similar to that of the sludge supernatant, thus there were no marked changes in total polymer concentration on sewage addition. After 7 hours the supernatant polymer concentrations for each experiment were 141.6, 116 and 147.1 mg l^{-1} for A, B and C respectively. These values are of a similar magnitude to that of effluent from full-scale plants. On addition of sewage to sludge there was a large increase in the soluble fraction of polymer. In all cases this was accompanied by a decrease in the amount of bound polymer extracted, indicating a release of polymers from the activated sludge surface. There was no indication that continued aeration resulted in readsorption of released material. These results were not consistent with those found in full-scale plants where there was found an increase in soluble polymers on sewage addition but no significant decrease in bound material. In plant 1 such an effect would be masked by the step loading. Loading to Runcorn plant 2 was somewhat lower than that of the laboratory experiments and this may account for the differences in bound polymer.

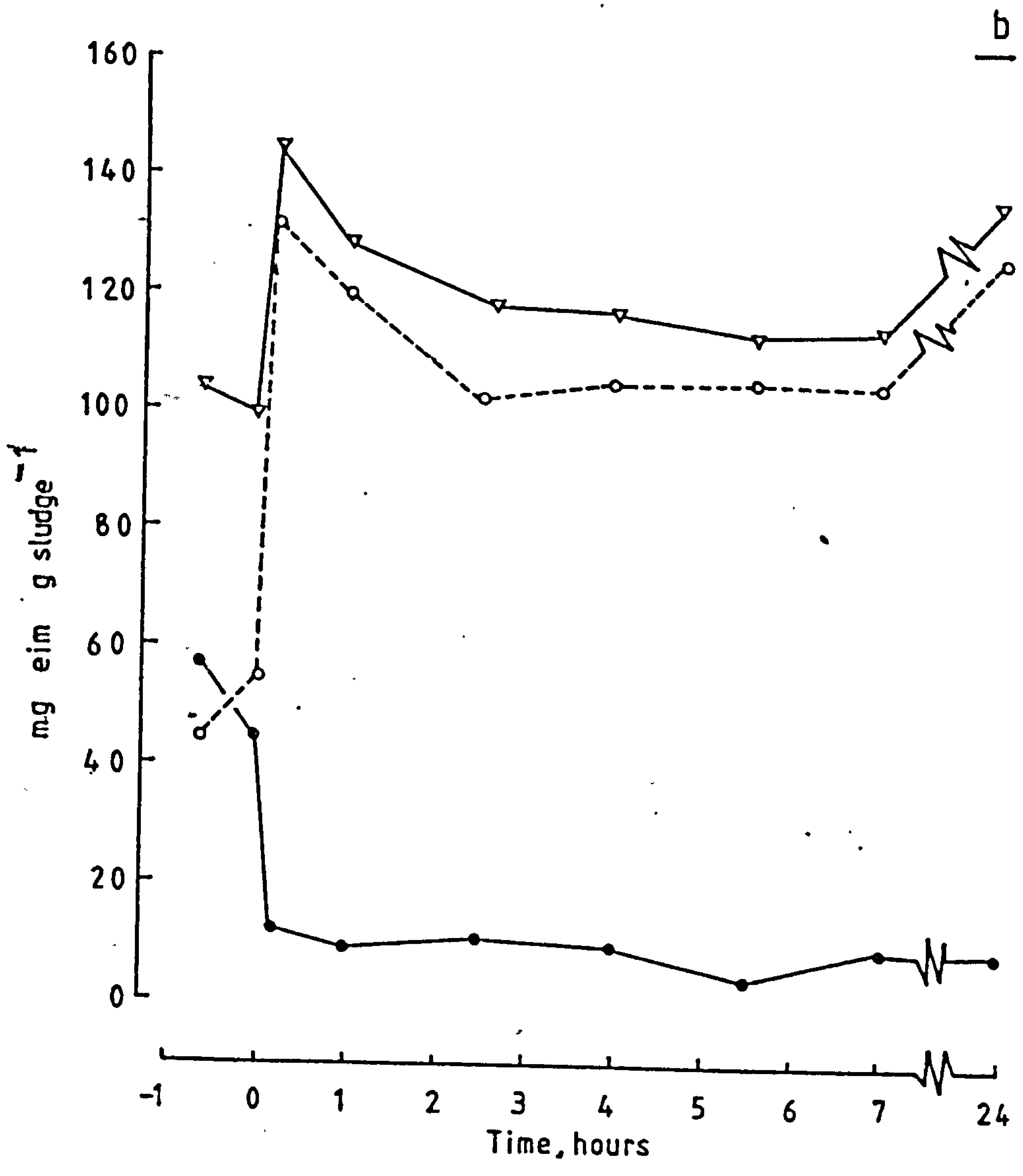
The composition of polymers extracted from sludges during the three experiments can be seen in Fig. 6.7. The composition of soluble polymers remained fairly constant under all

Figure 6.6

Total (▽), bound (●) and soluble (○) polymer (as e.i.m.) for (a), Runcorn 1 RAS mixed with Runcorn sewage, (b) Runcorn 2 RAS mixed with Runcorn sewage and (c) Runcorn 1 RAS mixed with Warrington South sewage.



a
mg eim g sludge⁻¹
160
140
120
100
80
60
40
20
0
-1
0
1
2
3
4
5
6
7
24
Time, hours


b
160
140
120
100
80
60
40
20
0
mg eim g sludge⁻¹
-1
0
1
2
3
4
5
6
7
24
Time, hours

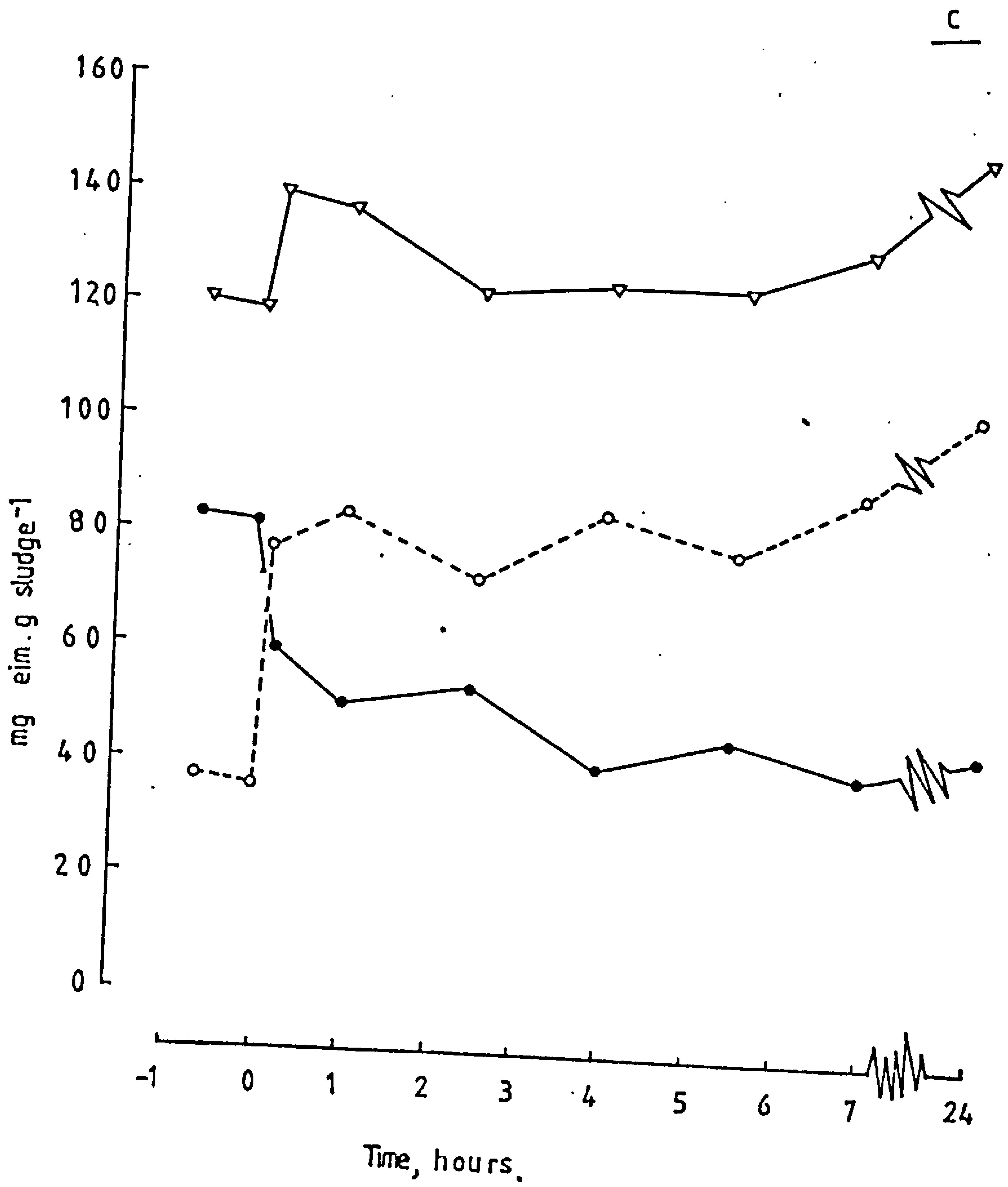
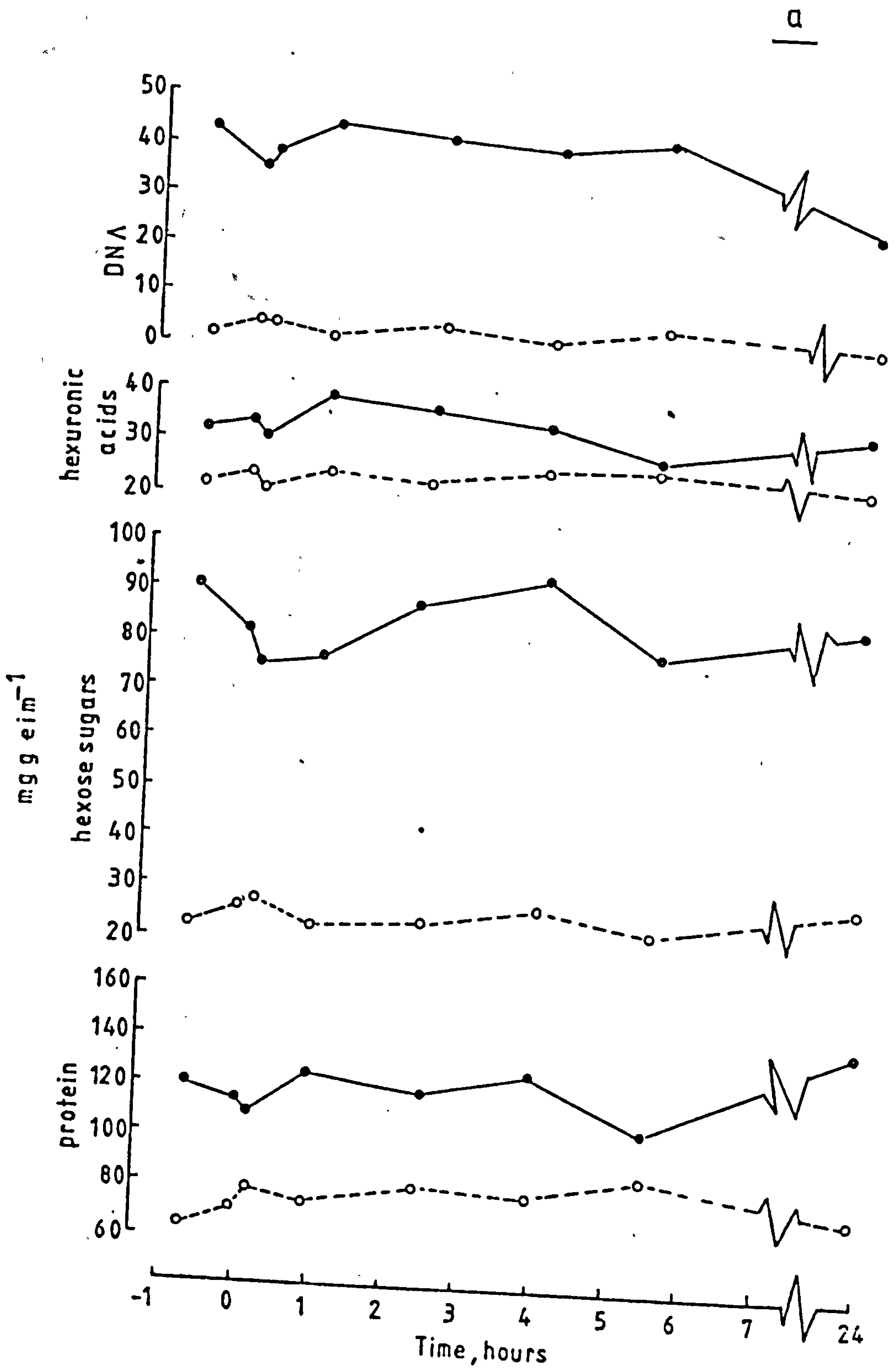
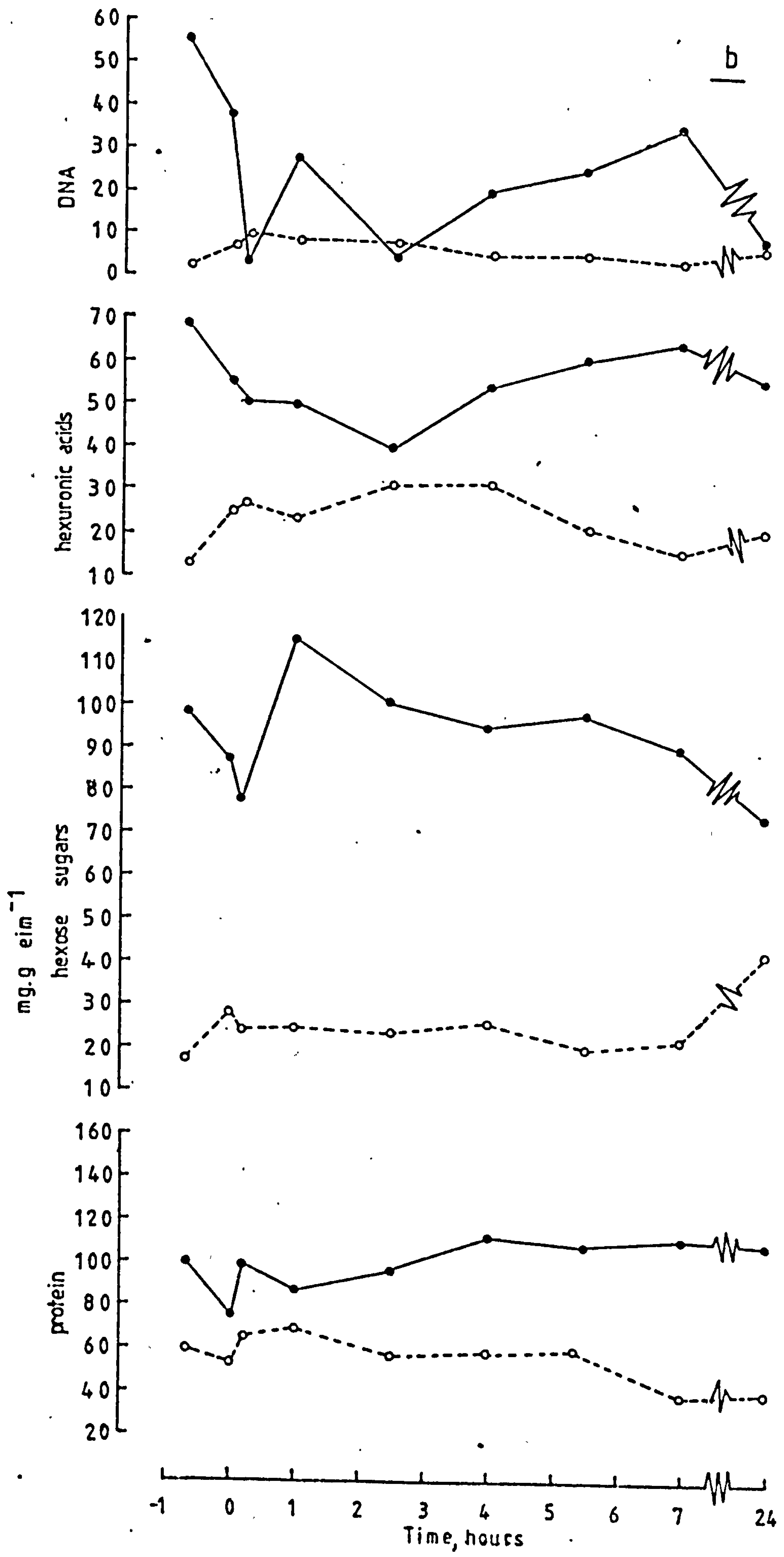

c
160
140
120
100
80
60
40
20
0
mg elm. g sludge-1
-1
0
1
2
3
4
5
6
7
24
Time, hours.

Figure 6.7

Composition of (O) soluble and (●) bound polymer (as e.i.m.) from (a) Runcorn 1 RAS mixed with Runcorn sewage, (b) Runcorn 2 RAS mixed with Runcorn sewage and (c) Runcorn 1 mixed with Warrington South sewage.


a
DNA
hexuronic acids
hexose sugars
protein
mg g eim^{-1}
Time, hours
50
40
30
20
10
0
40
30
20
100
90
80
70
60
50
40
30
20
160
140
120
100
80
60
-1
0
1
2
3
4
5
6
7
24



b
DNA
hexuronic acids
mg.g eim^{-1}
hexose sugars
protein
Time, hours

C
DNA
hexuronic acids
hexose sugars
protein
mg.g eim^{-1}
Time, hours

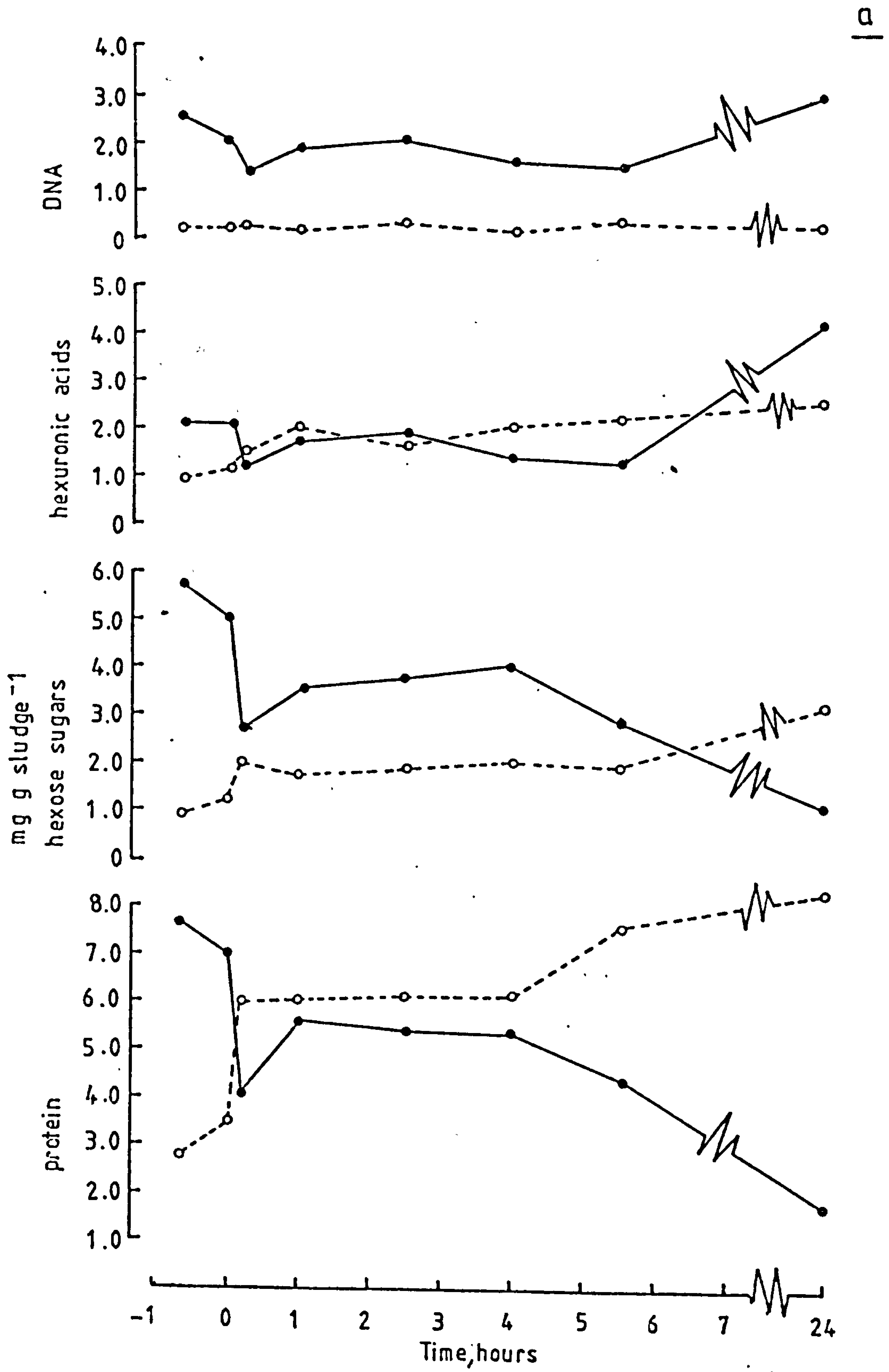
conditions, yet differed significantly from the composition of polymers in settled sewage. This suggests that sewage polymers were rapdily adsorbed to the activated sludge surface. If this was so, the composition of bound extracellular polymers would be expected to change. This was particularly evident in experiment C where mixing of sewage and sludge resulted in large increases in bound polymer protein, hexose sugar and hexuronic acids. If this increase was due purely to adsorption of sewage, only an increase in protein content would be expected. Thus some physiological change in sludge appears to occur after sewage addition. Large fluctuations did not occur in experiments A and B where sludge was acclimated to the added sewage. Thus the addition of a new sewage type to activated sludge may result in an increased release of biopolymers. In full-scale activated sludge plants, polymer and carbohydrate content were found to decrease in the sewage/sludge mixing phase. These results were not borne out in the laboratory experiments. However, when results were expressed as mg g sludge suspended solids^{-1} (Fig. 6.8) it can be seen that there was a large decrease in protein, hexoses, DNA and hexuronic acid when the sewage and sludge were mixed in experiments B and C.

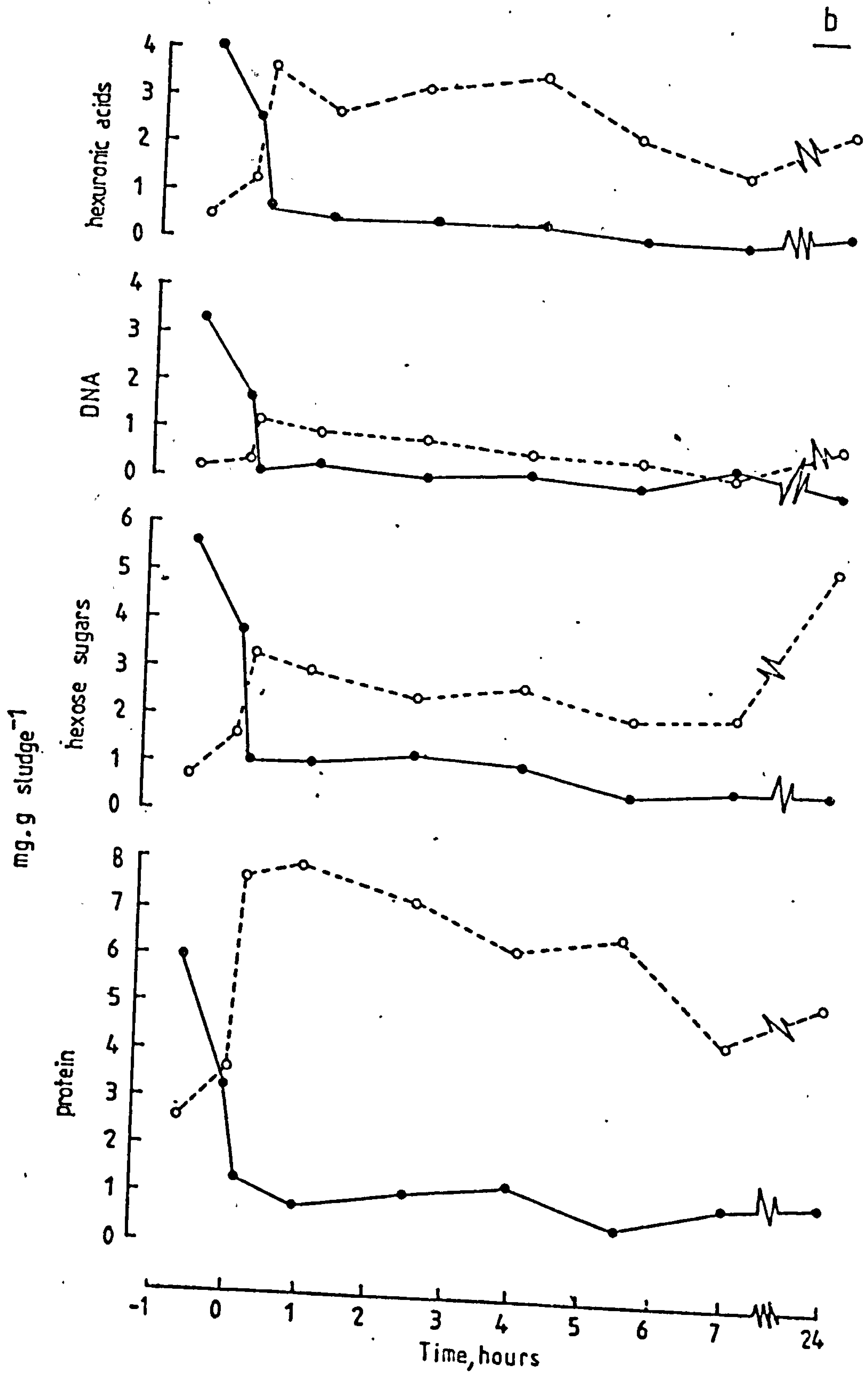
DISCUSSION

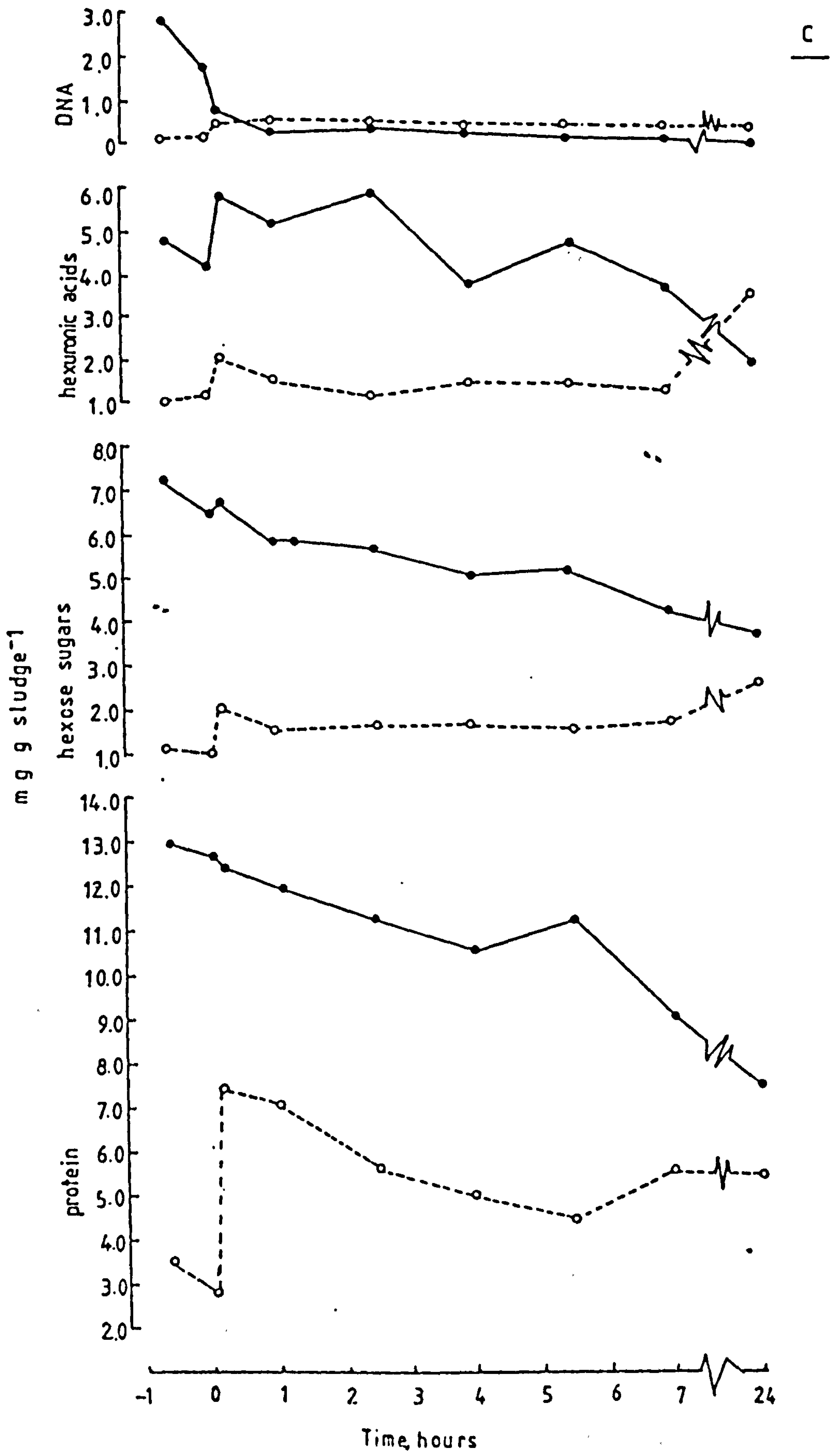
A number of workers have reported that an adequate period of sludge aeration in the absence of oxidizable substrates is required to ensure maximum COD uptake in the sewage/sludge mixing

Figure 6.8

Composition of polymers (as e.i.m.) of (○) soluble and (●) bound material expressed as mg g TSS^{-1} of (a) Runcorn 1 RAS mixed with Runcorn sewage (b) Runcorn 2 RAS mixed with Runcorn sewage and (c) Runcorn 1 RAS mixed with Warrington South sewage.


a
DNA
4.0
3.0
2.0
1.0
0
hexuronic acids
5.0
4.0
3.0
2.0
1.0
0
mg g sludge⁻¹
hexose sugars
6.0
5.0
4.0
3.0
2.0
1.0
0
protein
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
-1
0
1
2
3
4
5
6
7
24
Time, hours


b
hexuronic acids
DNA
hexose sugars
protein
mg.g sludge^{-1}
Time, hours
-1
0
1
2
3
4
5
6
7
24



C
DNA
3.0
2.0
1.0
0
hexuronic acids
6.0
5.0
4.0
3.0
2.0
1.0
hexose sugars
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
m g g sludge⁻¹
protein
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
-1
0
1
2
3
4
5
6
7
24
Time, hours

phase of activated sludge treatment. Chudoba et al. (1982) referred to a period of regeneration in the absence of substrate in order to return the accumulation capacity of sludge to its full value. Similarly, a sufficiently long endogenous phase was deemed necessary to increase biosorption capacity by Eikelboom (1982) and substrate uptake rate by Houtmeyers (1978).

Results obtained at the two plants at Runcorn suggest that activated sludge from plant 2 possessed a higher COD adsorption capacity than that of plant 1. It is likely that the step loading system operating in plant 1 was responsible for reducing the length of the endogenous phase and thus reducing the adsorption capacity. Filamentous microoganisms were consistently found in higher quantities in plant 1 and the loading pattern may have been a contributing factor in their development. When comparing adsorption capacities in plants 1 and 2 it should be remembered that samples were taken on different days. It is possible that daily variation in sewage composition may also influence COD adsorption.

The observation by Kiff (1978) that sewage polymer extracted with acetone accounted for up to 83% of the COD and 71% of the sewage BOD values disagrees with the results presented here, although COD values measured here may have been slightly low. Ethanolic extraction of sewage yielded precipitates containing less than 37.5% and in one case only 1.2% of the sewage COD. Assuming that the method of extraction precipitates all large polymers from sewage, this suggests that the sewage

organic component in these sewage samples consisted mainly of small molecular weight compounds such as amino acids, monosaccharides and fatty acids. It would be expected that such compounds vary in their proportion in various sewages, and that some sewages would contain a higher proportion of their COD in a polymeric form. The nature of sewage COD could be an important factor in substrate uptake and microorganism selection. Indeed Houtmeyers (1978) found that in laboratory activated sludge plants, bulking was more likely to occur where the polymers starch or protein were used as substrates.

The precise composition of the polymers extracted from the sewage samples was not certain. For a range of sewages, DNA, protein and hexoses accounted for less than 5% of the extracted material. Forster and Clarke (1983) reported that using an ethanolic extraction technique, 83% of the extracellular polymer from activated sludge was lipid. It is possible that large quantities of lipid were present in sewage polymer, though if this was the case it would be expected that higher COD values would have been recorded. Indeed, theoretical calculations indicated that the protein fraction made up a significant proportion (13.6 to 60.9%) of the sewage polymer COD. Carr and Ganczarczyk (1974) reported that high quantities of metal salts were present in their polymer extracts and these could be mistaken for bacterial polysaccharides.

As expected there was a significant variation in the nature of polymers extracted from settled sewage and activated sludge

supernatant. The latter was characterized by an increase in all biopolymers assayed and in particular hexose sugars. Thus some component of sewage polymer is apparently removed and replaced by polymers from the sludge.

On addition of sewage to sludge there is bound to be competition between constituents of the sewage and those already bound to the sludge surface. Earlier adsorption experiments using Alcian blue in competition with amino acids have clearly demonstrated that one substance can be displaced by a second from the floc surface. Thus it is likely that sludge biopolymers are displaced by compounds within the influent sewage. The situation is further complicated by the effect of dilution on activated sludge flocs, causing subsequent dispersal of bacteria. This is in itself likely to cause a release of sludge biopolymers into the medium.

After the sludge/sewage mixing stage only small changes were recorded in the water soluble fraction of polymer. The ethanol insoluble material in the final effluent was lower in plant 2 than in plant 1 despite similar levels in the influent sewage. Clearly the plant design pattern has some effect on the amount of polymer in solution.

The yield of polymer bound to the sludge surface remained fairly constant throughout the aeration lanes of both Runcorn plants, with the exception of the second pocket of plant 2. Kiff (1978) reported that activated sludge extracellular polymer remained at a constant level throughout each stage of a

laboratory plug-flow plant. He suggested that changes in the composition of sludge extracellular polymers were responsible for changes in sludge settlement properties. The results of the study at Runcorn indicate that the extracellular polymer composition remains unchanged throughout both plants with major changes occurring only at the sewage/sludge mixing stage. These take the form of a drop in the proportion of protein and hexose sugars. If these are subsequently displaced by certain sewage components it is likely that this will affect flocculation and settlement. It seems unlikely that material adsorbed by the sludge at Runcorn is in a polymeric form. Disturbances in floc structure are themselves likely to influence microorganism selection.

Hexose sugars, protein, DNA and hexuronic acids in bound sludge polymers were found to constitute up to only 25% of the extracted material. Thus as with sewage polymer, sludge polymer contained large quantities of unidentified material.

The anthrone method of hexose sugar determination is sensitive to free glucose, its disaccharides and polysaccharides. Galactose, mannose and fructose also give a similar reaction to glucose, whilst other sugars give a lower value. Thus the total sugar concentration in the polymers is likely to be higher than that assessed by the anthrone method. Changes in the proportion of particular sugar residues may thus lead to apparent changes in total hexose concentration. Conversely, measurement of protein by the Folin-Ciocalteau method is likely to over estimate due to

interference. The reagent is subject to modification by a wide variety of substances such as glucose, aromatic amines, urea, uric acid and sulphides. It is not possible to say to what extent these were present in extracellular polymer samples. Similarly, the hexuronic acid assay is subject to interference by pentoses.

The degree to which cell lysis occurs in the extraction process can to some extent be assessed by the quantity of biological polymers which are released. Rudd et al. (1983) found that the ratio of protein to carbohydrate in extracted material remained at approximately 3:1 unless drastic methods of sludge disruption were used. They suggested that flocs are conglomerates of whole, viable cells, dead and disintegrated cells bound together by a gel matrix composed of extracellular metabolites, and intracellular products of lysis. Thus the matrix would be of a similar composition to whole cells. Protein to carbohydrate ratio's in polymers extracted from activated sludge in this study were lower than the majority of methods used by Rudd et al., suggesting a low degree of cell lysis. Brown and Lester (1982b) found that the ratio of carbohydrate:protein:DNA remained constant over a range of sludge ages at approximately 5:2:1. This represents a higher carbohydrate quantity than in polymer extracted from Runcorn sludge, but a similar protein to DNA ratio, suggesting a similar degree of cell breakage.

The adsorption of sewage polymers by activated sludge was further investigated in the laboratory. Clearly the mixing of

sewage and sludge in batch systems does not ideally replicate continuous addition as in the full scale process, and this probably accounts for some differences in the results recorded between full-scale and laboratory systems. In all three experiments a large increase was found in soluble polymers at the sludge/sewage mixing phase. This was also found in full scale plants. The composition of polymer in the sludge supernatant at the mixing stage differed significantly in composition from polymer in the sewage. As with full scale plants there was an increase in hexoses, although there were similar levels of protein and DNA. This provides further evidence that some sewage substance exchanges for hexoses at the sludge surface.

The increase in concentration of soluble polymers in the sludge supernatant at the point of sewage and sludge mixing was particularly marked where sludge was not acclimated to the influent sewage. Where sewage of polymer concentration 194.0 mg l^{-1} from Warrington South was added to Runcorn 1 sludge, the supernatant polymer concentration increased to 329.8 mg l^{-1}. It is likely that one aspect of sewage acclimation is the retention of bacteria capable of flocculation in the sewage influent and that addition of a new sewage increases deflocculation and polymer release.

On addition of sewage there was a decrease in the amount of bound polymers extracted from activated sludge in all three experiments. There was only minor evidence for this occurring in full-scale plants. Where sludge was acclimated there was no

evidence of changes in bound polymer composition. Where Warrington South sewage was added to Runcorn 1 sludge, however, there were large increases in protein and hexose content. This was not accompanied by changes in soluble polymer composition and high levels of hexoses and protein were not found in the added sewage. Possibly the addition of a foreign sewage gave rise to changes in the extent to which protein and carbohydrate were extracted.

DNA was found at similar levels in sludge/sewage mixing experiments in the laboratory and in full scale plants. On no occasion was an increase in DNA measured when sewage was added, indicating that there was no cell lysis at this stage. Hexuronic acid levels were erratic and not related to hexose concentration nor Alcian blue binding. Possibly there may be some compound in polymer extracts which interferes with the hexuronic acid assay (Brown and Lester, 1982b) though this was not obvious during its performance.

Summary

1. Polymers extracted from a variety of sewages accounted for between 1.2 to 37.5% of the total sewage COD.
2. DNA, protein and hexoses together accounted for less than 5% of polymer extracted from sewage and less than 25% of polymers extracted from the sludge surface.
3. Substantial differences were found between the composition of sewage polymers and those present in the sludge

supernatant at the sludge/sewage mixing stage. This was the case for both scale treatment plants and in laboratory experiments. Sludge supernatant polymer was characterized by an increase in the proportion of biopolymers.

4. Major changes in polymer composition and content of both soluble and sludge surface polymers occurred only at the sewage sludge mixing phase. In both full scale plants and laboratory experiments, polymer remained comparatively constant during aeration.

CHAPTER 7

General Discussion

It might be concluded from a survey of the literature that there is to be no universal cure for activated sludge filamentous bulking. Too many variables contribute towards its manifestation and with no two plants subject to identical conditions each bulking incident must be treated on its own merits. Nevertheless advances have been made. A large number of publications have revealed that plug-flow plants are less likely to bulk than those which are completely mixed (Chudoba, 1973a, b; 1974; Tomlinson and Chambers, 1979a; Chambers 1981; Wu et al., 1984a). Filamentous bacteria have been identified in bulking sludges and factors leading to their presence determined (Strom and Jenkins, 1984; Eikelboom, 1977; 1981), offering at least some aid to the suffering plant operator. However, there is still a need for the development of any technique which can predict or cure bulking.

In this project a method was devised by which adsorption of polycationic dyes to activated sludge could be measured. Evidence that dye adsorption may be related to sludge settlement was provided by Andreadakis (1978) who found a positive correlation between SVI and adsorption of the anionic dye Lissamine Scarlet 4R. Other workers (Hall, 1982b; Wu, 1978; Banks et al., 1976; Devloo et al., 1983; Smith and Coackley, 1983) have also speculated upon the use of dye adsorption as a guide to sludge condition. Several factors known to affect dye adsorption have been shown to influence sludge settlement. Sludge surface area is clearly a factor in dye adsorption and is a function of both floc size and filament length. The latter

increases with SVI (Finstein and Heukelekian, 1965; Pipes, 1979; Sezgin *et al.*, 1978; 1980; Sezgin, 1982), whereas SVI may increase (Barahona and Eckenfelder, 1984; Magara *et al.*, 1976) or decrease with floc size (Forster and Choudhry, 1972; Forster, 1983). The nature of the floc surface is also likely to be a factor in dye adsorption. Surface charge certainly varies between sludges (Forster, 1985a; 1968; Steiner *et al.*, 1976) although any relationship with settlement is uncertain. Both composition and production of activated sludge extracellular polymer are subject to large variation and there is evidence that both may influence settlement (Kiff, 1978; Forster and Dallas-Newton, 1980; Forster, 1985).

Activated sludge adsorption isotherms were obtained in this study for two polycationic dyes, Alcian blue and Ruthenium red. With the plant operator in mind a simple test was developed for Alcian blue adsorption. The procedure is simple and requires the minimum use of reagents and equipment. The following were found to influence dye adsorption.

i. Sludge concentration : In the laboratory, binding of both Alcian blue and Ruthenium red was unaffected by solids concentrations above 1.0 g l^{-1}. On site studies revealed an inverse relationship between dye adsorption and solids concentration of return activated sludge. According to Eikelboom (1982) filamentous growth may be prevented if the initial biosorption capacity of the sludge is high.

Assuming that substrate uptake is at least partly dependent upon adsorption, these results suggest that biosorption can be increased by assuring that the solids concentration in the sewage/sludge mixing stage is low, and certainly less than 1.0 g l^{-1}. However, the precise effect of sludge dilution may be dependent upon the nautre of the substrate.

ii. Dye concentration : Adsorption increased with dye concentration up to a critical level after which binding was not influenced by the amount of dye. This value for Alcian blue was approximately four fold that of Ruthenium red. If dye adsorption is analogous to substrate uptake then clearly the nature of the substrate is critical in the adsorption kinetics during the sewage/sludge mixing stage.

iii. Nature of the sludge : There was a large variation in dye adsorption capacity for a range of return activated sludges. Some evidence was found that a bulking sludge possessed a higher dye binding capacity than non-bulking sludges, but this variation was not enough to be used as an indicator of bulking incidents.

iv. pH : Adsorption of both cationic dyes was not affected by changes in pH within the range 6.0 to 8.0. Outside this range changes in adsorption were dependent upon the dye used.

v. Sludge activity and viability : Dye adsorption was unaffected by sludge activity as measured by triphenyl tetrazolium chloride reduction. This was to be expected as earlier experiments showed that dye adsorption was instantaneous. Sonication, however, had a marked affect on adsorption due to deflocculation and a subsequent increase in surface area. This was accompanied by a decrease in viability.

vi. Temperature : Dye adsorption was unaffected by temperature between 10°C and 30°C. At higher temperatures adsorption rapidly decreased probably due to cell lysis and the larger surface area offered by released polymers. This and further experiments with metabolic inhibitors and biocides suggested that bacterial membrane integrity was an important factor in dye adsorption at normal operating, temperature.

vii. Solutes : Alcian blue adsorption was inhibited by the presence of amino acids in the medium, though unaffected by a number of other solutes at similar concentration. This was thought to be due to competition between amino-acid and Alcian blue molecules for binding sites on the sludge surface. Similar competition must exist between substrates in sewage. This is likely to be an important factor in substrate adsorption by different microorganisms in the

sewage/sludge mixing phase. The surviving bacteria in an acclimated sludge will be those best at adsorption of the sewage substrate.

viii. Nature of microorganisms : Pure cultures of the filamentous bacterium *Sphaerotilus natans* were found to have a higher adsorption of Alcian blue than the floc-forming *Zoogloea ramigera*. This was thought to be due to the fact that filaments have a larger surface area than flocs.

Despite the large number of factors which influence both dye adsorption and sludge settleability, it seems unlikely that the plant operator can use dye adsorption to predict bulking problems. Although different sludges were shown to possess different dye binding capacities, there was no obvious relationship between this and SVI. In addition the higher COD adsorption capacity shown by Runcorn plant 2 when compared to plant 1 was not reflected in Alcian blue adsorption. Clearly the adsorption of substrates by sludge is influenced by many factors not applicable to dye adsorption. Nevertheless an important contribution has been made towards the knowledge of the adsorption properties of activated sludges.

In addition to adsorption studies, a large proportion of this project has been devoted to the bacterial populations of activated sludge. Microscopic observation revealed fifteen types of filamentous microorganisms present in sludges from the three

works studied. These organisms were classified according to the scheme of Eikelboom (1981). Problems were encountered in identification and one factor in this may be that this is the first report of activated sludge microorganisms in the U.K., all previous studies being performed in the Netherlands (Eikelboom, 1977; 1980), West Germany (Wagner, 1982) and the United States (Strom and Jenkins, 1984). There was a clear effect of feed pattern on the microbial population. Runcorn plants 1 and 2 received sewage of identical composition, yet the sludge of plant 1 was dominated initially by *Microthrix parvicella*, whereas the dominant organism in plant 2 sludge was Type 0961. When the feed to plant 1 was changed to a step loading pattern, this too became dominated by Type 0961. A possible explanation for the different microbial population was the higher COD concentration in the sludge/sewage mixing tank of plant 1. When this was reduced due to step loading, there was an accompanying change in bacterial population.

Heterotrophic, aerobic bacterial populations were also examined, representing the floc-forming bacteria of activated sludge. Clear differences were found between filamentous bulking and non-bulking sludge samples. This is the first time that changes in floc-forming bacteria have been found to accompany change in filamentous populations. In particular, bulking sludge showed an increase in the number of organisms capable of anaerobic respiration. Organisms increasing in numbers in bulking sludge included members of the Virbionaceae,

Pasturellaceae and Enterobacteriaceae. This clearly has implications with regard to the removal of pathogens by activated sludge. It was concluded that these organisms increase in bulking sludge as conditions favouring their growth also favour bulking organisms. However, the results underline the possibility that bulking may be not just a consequence of the growth of filamentous organisms but may be a function of changes in the floc-forming population. This may be accompanied by changes in factors such as floc size, surface charge and extracellular polymer concentration.

Sludge extracellular polymers were also studied in some detail, with particular emphasis on their role in adsorption of polymers from sewage. Surprisingly, sewage polymer was found to constitute only 1.2 to 37.5% of the total sewage COD for a range of sewages. The precise nature of the polymers in sewage and those on the sludge surface is unclear. DNA, protein and hexoses accounted for less than 5% of sewage polymer and less than 25% of sludge polymer. A substantial proportion of remaining "polymers" may be lipid (Goodwin and Forster, 1985) or ash (Carr and Ganczarczyk, 1974). Polymers were also found in plant effluents, though this is not surprising since effluent composition is similar to that of the supernatant of the final aeration pocket.

In both on site and laboratory experiments, the most pertinent changes in polymer composition and concentration were found in the sewage/sludge mixing stage. In all cases, COD removal occurred very rapidly, within ten minutes in the

laboratory, and in the first or second aeration pocket of full-scale plants. Sewage/sludge mixing was always accompanied by a large increase in the quantity of soluble polymers in the activated sludge supernatant, often in concentrations as high as in the sewage itself. The composition of these polymers, however, was significantly different from those of the sewage, characterized by an increase in all biopolymers assayed, and in particular the hexoses. In full-scale plants, increases in soluble polymer were not accompanied by a decrease in bound polymer, suggesting an exchange between sludge polymer and some sewage constituent, possibly soluble organic material.

Both composition and concentration of all polymers remained comparatively constant after the initial sewage and sludge mixing. The results agree with the observation by Kiff (1978) that activated sludge extracellular polymer remains constant throughout each stage of a plug-flow treatment plant. However, his suggestions that changes in the composition of polymers were responsible for changes in sludge settlement properties throughout the plant are not confirmed here.

To summarise, this project has made a contribution towards improving our knowledge of three areas in the activated sludge wastewater treatment process. Firstly, the nature of, and factors affecting the adsorption of polycationic dyes by activated sludge has been studied in detail. The possibility that dye adsorption may be used as an indicator of bulking problems has been rejected. Secondly, both the filamentous and

floc-forming bacterial populations of activated sludge have been examined and the composition of both has been shown to change during an incident of bulking. Thirdly, the dynamics of activated sludge and sewage polymers have been investigated. It has been established that major changes in sludge extracellular polymer composition occur only at the sludge and sewage mixing stage, with only minor changes occurring during further aeration.

REFERENCES

ADAMSE, A.D. (1968) Formation and final composition of the bacterial flora of a dairy waste activated sludge. Water Res. 2, 665.

AIBA, S., WATANABE, T. AND HIRATA, M. (1970) Flocculation and deflocculation of aqueous suspensions of Chlorella. J. Ferm. Tech. 48, 125.

ALLEN, L.A. (1944) The bacteriology of activated sludge. J. Hyg. (Camb.) 43, 424.

ANDERSON, R. AND McCOY, E. (1963) Floc forming bacteria from activated sludge. Bact. Proc. 8, 162.

ANDREADAKIS, A. (1978) Physico-chemical aspects of activated sludge. Ph.D. Thesis, University of Strathclyde, Glasgow.

APHA-AWWA-WPCF (1975) Standard methods for the examination of water and waste water. American Public Health Association, Washington.

AUSTIN, B.L. AND FORSTER, C.F. (1969) The microbial ecology of a Lubeck Activated Sludge plant. Water Waste Treat. 12, 208.

BAKER, C.A., CLAUS, G.W. AND TAYLOR, P.A. (1983) Predominant bacteria in an activated sludge reactor for the degradation of cutting fluids. Appl. Env. Microbiol. 46, 1214.

BANERJI, S.K., EWING, B.B., ENGLEBRECHT, R.S. AND SPEECE, R.E. (1968a) Mechanism of starch removal in the activated sludge process. J. Water Pollut. Control Fed. 40, 16.

BANERJI, S.J., EWING, B.B., ENGLEBRECHT, R.S. AND SPEECE, R.E. (1968b) Kinetics of removal of starch in activated sludge systems. J. Water Pollut. Control Fed. 40, 161.

BANKS, C.J., DAVIES, M., WALKER, I., AND WARD, R.D. (1976) Biological and physical characterization of activated sludge; a comparative experimental study at ten treatment plants. Water Pollut. Contol. 75, 492.

BANKS, C.J. AND WALKER, I. (1977) Sonication of activated sludge flocs and the recovery of their bacteria on solid media. J. Gen. Micro. 98, 363.

BARAHONA, L., AND ECKENFELDER, W.W. Jr., (1984) Relationships between organic loading and zone settling velocity in the activated sludge process. Water Res. 18, 91.

BARTH, E.F., ETTINGER, M.B., SALOTTO, B.V. AND McDERMOTT, G.N. (1965) Summary report on the effects of heavy metals on the biological treatment processes. J. Water. Pollut. Control Fed. 37, 86.

BECCARI, M., MAPPELLI, P., AND TANDOI, V., (1980) Relationship between bulking and physiochemical-biological properties of activated sludges. Biotechnol. Bioeng. 22, 969.

BENEDICT, R.G. AND CARLSON, D.A. (1971) Aerobic, heterotrophic bacteria in activated sludge. Water Res. 5 1023.

BENEFIELD, L.D., RANDALL, C.W. AND KING, R.H. (1975) The stimulation of filamentous microorganisms in activated sludge by high oxygen concentrations. Wat. Air Soil Poll. 5, 113.

BEVERIDGE, T.J. (1981) Ultrastructure, chemistry and function of the bacterial wall. Int. Rev. Cytol. 72, 229.

BHATLA, M.N. (1967) Relationship of activated sludge bulking to oxygen tension. J.Water Pollut. Control Fed. 39, 1978.

BIOSOGNI, J.J. AND LAWRENCE, A.W. (1971) Relation between biological solids retention time and settling characteristics of activated sludge. Water Res. 5, 753.

BISZ-KONARZEWSKA, A. (1978) The effect of cyclohexane derivatives on selection of bacterial groups forming activated sludge microflora. Acta. Microbiologica Polonica, 27, 155.

BITTON, G. AND KOOPMAN, B. (1982) Tetrazolium reduction - malachite green method for assessing the viability of filamentous bacteria in activated sludge. Appl. Environ. Microbiol. 43 964.

BRODISCH, K.E.U. AND JOYNER, S.J. (1983) The role of microorganisms other than Acinetobacter in biological phosphate removal in activated sludge processes. Water Sci. Technol. 15, 117.

BROWN, M.J. AND LESTER, J.M. (1979) Metal removal in activated sludge. The role of extracellular polymers. Water Res. 13, 817.

BROWN, M.J. AND LESTER, J.M. (1980) Comparison of bacterial extracellular polymer extraction methods. Appl. Environ. Microbiol. 40, 179.

BROWN, M.J. AND LESTER, J.M. (1982a) Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge I; Effect of metal concentration. Water Res. 16, 139.

BROWN, M.J. AND LESTER, J.M. (1982b) Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge II; Effects of mean cell retention time. Water Res., 16, 1549.

BROWN, M.R.W. AND MELLING, J. (1971) Inhibition and destruction of microorganisms by heat. In; Hugo W.B. (ed). Inhibition and destruction of the microbial cell; Academic Press, p.1.

BUCKSTEEG, W. (1966) Determination of sludge activity; A possibility of controlling activated sludge plants. Proceedings 3rd International Conference on Water Pollution Research, Munich.

BURTON, K. (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62, 315.

BUSCH, P.L. AND STUMM, W. (1968) Chemical interactions in the aggregation of bacteria. Environ. Sci. Technol. 2, 49.

CARLSON, D.A. (1965) Utilization of free and combined amino acids by activated sludge. Ph.D. Thesis, University of Washington, College of Engineering.

CARR, D.F. AND GANCZARCZYK, J. (1974) Activated sludge exocellular material extraction methods and problems. In Zaijic, J.E., and Kosaric, N. (Eds.), Proceedings of the 9th Canadian Symposium on Water Pollution Research in Canada, Institute for Environmental Studies, University of Toronto, Toronto. Ont. pp. 250-261.

CECH, J.S. AND CHUDOBA, J. (1983) Influence of accumulation capacity of activated sludge microorganisms on kinetics of glucose removal. Water Res. 17. 659.

CHAMBERS, B. (1982) Effect of longitudinal mixing and anoxic zones on settleability of activated sludge. In Chambers, B. and Tomlinson, E.J. (Eds.), Bulking of activated sludge. Preventative and remedial methods. Ellis Horwood, Chichester, pp. 166-186.

CHAO, A.C. AND KEINATH, T.M. (1979) Influence of process loading intensity on sludge clarification and thickening characteristics. Water Res. 13, 1213.

CHIESA, S.C. AND IRVINE, R.L. (1985) Growth and control of filamentous microbes in activated sludge; an integrated hypothesis. Water Res. 19, 471.

CHUDOBA, J., BLAHA, J. AND MADERA, V. (1974) Control of activated sludge filamentous bulking III. Effect of sludge loading. Water Res. 8, 231.

CHUDOBA, J., CECH, J.S., FARKAC, J. AND GRAU, P. (1985) Control of activated sludge filamentous bulking. Experimental verification of a kinetic selection theory. Water Res. 19, 191.

CHUDOBA, J., DOHANYOS, M. AND GRAU, P. (1982) Control of activated sludge filamentous bulking IV. Effect of sludge regeneration. Water Sci. Technol. 14, 73.

CHUDOBA, J., OTTOVA, V. AND MADERA, V. (1973a) Control of activated sludge filamentous bulking I. Effect of hydraulic regime or degree of mixing in an aeration tank. Water. Res. 7, 1163.

CHUDOBA, J., GRAU, P. AND OTTOVA, V. (1973b) Control of activated sludge filamentous bulking II. Selection of microorganisms by means of a selector. Water Res. 7, 1389.

CLARKE, A.R. AND FORSTER, C.F. (1983) The significance of ATP in the settlement of activated sludge. J. Chem. Technol Biotechnol. 338, 127.

COACKLEY, P. (1969) Some aspects of activated sludge. Process Biochem. 4, 27.

COACKLEY, P. AND O'NEILL, J. (1975) Sludge activity and full-scale plant control. J. Water Pollut. Control Fed. 74, 404.

COLE, C.A., STAMBERG, J.B. AND BISHOP, E.D. (1973) Hydrogen peroxide cures filamentous growth in activated sludge. J. Water Pollut. Control. Fed. 45, 829.

COOK, W.B. AND PIPES, W.O. (1970) Occurrence of fungi in activated sludge. Mycopathol. Mycol. Appl. 40, 249.

COWAN, S.T. (1974) Manual for the identification of medical bacteria. Second edition. Cambridge University Press, Cambridge.

CRABTREE, K., BOYLE, W., McCOY, E. AND ROHLICH, G.A. (1966) A mechanism of floc formation by Zoogloea ramigera. J. Water Pollut. Control Fed. 38, 1968.

CROMBIE-QUILTY, M.B. AND McLOUGHLIN, A.J. (1983) The adsorption of bovine serum albumen by activated sludge. Water Res. 17, 39.

CURDS, C.R. (1975) Protozoa. In Curds, C.R. and Hawkes H.A. (Eds.), Ecological aspects of used-water treatment. I. The organisms and their ecology. p. 203.

CURDS, C.R. (1982) The ecology and role of protozoa in aerobic sewage treatment processes. Ann. Rev. Microbiol. 36, 27.

CURDS, C.R. AND COCKBURN, A. (1970) Protozoa in biological sewage-treatment processes. 2. Protozoa as indicators in the activated sludge process. Water Res. 4, 237.

CURDS, C.R., COCKBURN, A. AND VANDYKE, J.M. (1968) An experimental study of the role of the ciliated protozoa in the activated sludge process. Water Pollut. Control. 67, 312.

CYRUS, Z. AND SLADKA, A. (1970) Several interesting organisms present in activated sludge. Hydrobiologia 35, 383.

DALAS-NEWTON, J. AND FORSTER, C.F. (1980) Filamentous microbes in activated sludge. Environ. Technol. Lett. 1, 131.

DANIELS, S.L. (1980) Mechanisms involved in sorption of microorganisms to solid surfaces. In Bitton, G. and Manshall, K.C. (Eds.) Adsorption of microorganisms to surfaces. Wiley-Interscience, N.York. p. 7.

DAWES, E.A., McGILL, D.J. AND MIDGLEY, M. (1971) Analysis of fermentation products. In Norris, J.R. and Ribbons, D.W. (Eds.), Methods in Microbiology, vol. 6A, Academic Press Inc., London/N.York. p. 53.

DEINEMA, M.H. AND ZEVENHUIZEN, L.P.T.M. (1971) Formation of cellulose fibrils by Gram-negative bacteria and their role in bacterial flocculation. Arch. Microbiol. 78, 42.

DEPARTMENT OF THE ENVIRONMENT (1971) Water pollution Research 1970. London H.M.S.O.

DEPARTMENT OF THE ENVIRONMENT (1977a) Chemical oxygen demand (dichromate value) of polluted and waste waters (1977 version). H.M.S.O. London.

DEPARTMENT OF THE ENVIRONMENT (1977b) Continuous flow methods for the determination of total oxidized nitrogen or nitrate. Tentative methods. H.M.S.O. London.

DEVLOO, M.O., DRAKIDES, C. AND BENAIM, R. (1983) Activated sludge flocs; characteristics and evolution. Review Francaise des Sciences de l'eau 2, 41.

DIAS, F.F. AND BHAT, J.V. (1964) Microbial ecology of activated sludge. I. Dominant bacteria. Appl. Microb. 12, 412.

DICK, R.I. AND VESILIND, P.A. (1969) The sludge volume index - What is it? J. Water Pollut. Control Fed. 41, 1285.

DISCHE, Z. (1947) A specific colour reaction for glucuronic acid. J. Biol. Chem. 171, 725.

DROSTE, R.L. AND SANCHEZ, W.A. (1983) Microbial activity in aerobic sludge digestion. Water Res. 17, 975.

EDWARDS, G.L. AND SHERRARD, J.H. (1982) Measurement and validity of oxygen uptake as an activated sludge process control parameter. J. Water Pollut. Control Fed. 54, 1546.

EIKELBOOM, D.H. (1975) Filamentous organisms observed in activated sludge. Water Res. 9, 365.

EIKELBOOM, D.H. (1977) Identification of filamentous organisms in bulking activated sludge. Prog. Water Technol. 8, 153.

EIKELBOOM, D.H. (1981) Microscopic sludge investigation manual. IMG-TNO Report A94, Delft, The Netherlands.

EIKELBOOM, D.H. (1982) Biosorption and prevention of bulking sludge by means of a high floc loading. In Chambers, B. and Tomlinson, E.J. (Eds.), Bulking of activated sludge. Preventative and remedial methods. Ellis Horwood and WRC. pp. 90-110.

ENDO, T., NAKAMURA, K. AND TAKAHASHI, H. (1976) Pronase-susceptible floc forming bacteria. Relationship between flocculation and calcium ions. Agric. Biol. Chem. 40, 2289.

FARRAH, S.R. AND UNZ, R.F. (1976) Isolation of exocellular polymer from Zoogloea strains MP6 and 106 from an activated sludge. Appl. Environ. Microbiol. 32, 33.

FARQHAR, G.J. AND BOYLE, W.C. (1971) Identification of filamentous microrganisms in activated sludge. J. Water Pollut. Control Fed. 43, 604.

FARQUHAR, G.J. AND BOYLE, W.C. (1972) Control of Thiothrix in activated sludge. J. Water Pollut. Control Fed. 44, 14.

FINSTEIN, M.S. AND HEUKELEKIAN, H. (1965) Filamentous microorganisms and the bulking of activated sludge. Proc. 20th ind. waste. conf. Purdue Univ. W. Lafayette incl. ext. ser. 118, 175.

FORD, D.L., YANG, J.T. AND ECKENFELDER, W.W. (1966) Dehydrogenase enzyme as a parameter of activated sludge activities. Proc. 21st Ind. Waste. Conf. Purdue Univ. p. 538.

FORSTER, C.F. (1968) The surface of activated sludge particles in relation to their settling characteristics. Water. Res. 2, 767.

FORSTER, C.F. (1971) Activated sludge surfaces in relation to SV1. Water Res. 5, 861.

FORSTER, C.F. (1976) Bioflocculation in the activated sludge process. Water (S.A.) 2, 119.

FORSTER, C.F. (1983) The size of activated sludge flocs in relation to their surface characteristics. Environ. Technol. Lett. 4, 329

FORSTER, C.F. (1985) Factors involved in the settlement of activated sludge I. Nutrients and surface polymers. Water Res. 19, 1259.

FORSTER, C.F. AND CHOUDHRY, N.M. (1972) Physio-chemical studies on activated sludge and bioflocculation. Effl. Water Treat. J. 12, 127.

FORSTER, C.F. AND CLARKE, A.R. (1983) The production of polymer from activated sludge by ethanolic extraction and its relation to treatment plant operation. Water Pollut. Control 82, 430.

FORSTER, C.F. AND DALLAS-NEWTON, J. (1980) Activated sludge settlement, some suppositions and suggestions. Water Pollut. Control. 79, 338.

FORSTER, C.F. AND LEWIN, D.C. (1972) Polymer interactions at activated sludge surfaces. Effl. Water Treat. J. 12, 520.

FRIEDMAN, B.A. AND DUGAN, P.R. (1968) Identification of Zoogloea species and the relationship to zoogloeal matrix and floc formation. J. Bact. 95, 1903.

FRIEDMAN, B.A., PFISTER, R.M. AND REMSEN, C.C. (1968) Fine structure and composition of the zoogloeal matrix surrounding Zoogloea ramigera. J. Bact. 96, 2144.

FUHS, G.W. AND CHEN, M. (1975) Microbiological basis of phosphate removal in the activated sludge process for the treatment of waste water. Microb. Ecol. 2, 119.

FUNG, D.Y.C. AND KRAFT, A.A. (1968) Microtitre method for evaluation of viable cells in bacterial cultures. Appl. Microb. 16, 1036.

GABBITA, K.V. AND HWANG, J.Y.C. (1984a) A modified method for determination of dehydrogenase activity of activated sludge. Toxicol. Env. Chem. 8, 119.

GABBITA, K.V. AND HWANG, J.Y.C. (1984b) Dehydrogenase activity of activated sludge. Toxicol. Env. Chem. 8, 151.

GANCZARCZYK, J. (1970) Variation in the sludge volume index. Water Res. 4, 69.

GAUDY, E. AND WOLFE, R.S. (1962) Composition of an extracellular polysaccharide produced by Sphaerotilus natans. Appl. Microb. 10, 200.

GAYFORD, C.G. AND RICHARDS, J.P. (1970) Isolation and enumeration of aerobic heterotrophic bacteria in activated sludge. J. Appl. Bact. 33, 342.

GEHR, R. AND HENRY, J.G. (1983) Removal of extracellular material. Techniques and pitfalls. Water Res. 17, 1743.

GENETELLI, E.J. (1967) DNA and nitrogen relationships in bulking activated sludge. J. Water Pollut. Control. Fed. 39, (Part 2), R32.

GENETELLI, E.J. AND HEUKELEKIAN, H. (1964) The influence of load and chemical composition of substrate on the performance of activated sludge. J. Water Pollut. Control Fed. 36, 643.

GILES, C.H., MacEWAN, T.H., NAKHWA, S.N. AND SMITH, D. (1960) Studies on adsorption Part XI. A system of classification of solution adsorbing isotherms and its use in diagnosis of adsorption mechamisms and in measurement of specific area of solids. J. Chem. Soc. 3972.

GOODWIN, J.A.S. AND FORSTER, C.F. (1985) A further examination into the composition of activated sludge surfaces in relation to their settlement characteristics. Water Res. 19, 527.

GREEN, G.P. (1982) An attempt to predict bulking in activated sludge by the development of a method to measure filamentous organisms. In Chambers, B. and Tomlinson E.J. (Eds.) Bulking of activated sludge. Preventative and remedial methods. Ellis-Horwood, Chichester. pp. 63-74.

GURR, E. (1965) The rational use of dyes in biology. Hill, London.

HAAS, C.N. (1979) Oxygen uptake rate as an activated sludge control parameter. J. Water Pollut. Control. Fed. 51, 938.

HALASZ, A. (1972) Zusammenhang zwischen dehydrogenaseakivitat und wachstumstrate des belebtschlammes. Hydrologie, 34, 94.

HALL, T. (1981) Sonication for the study of floc strength and reflocculation of activated sludge. Environ. Technol. Lett. 2, 579.

HALL, T. (1982a) Sonication induced changes of particle size and their effects on activated sludge de-waterability. Environ. Technol. Lett. 3, 79.

HALL, T. (1982b) Particle size and physico-chemical influences on activated sludge solids - liquid separation. D.Phil. Thesis, University of York.

HAMMERTON, C. AND SHERRATT, J.G. (1972) Analysis of raw, potable and waste waters. H.M.S.O., London.

HAMMOND, S.M., LAMBERT, P.A. AND RYCROFT, A.N. (1984) The bacterial cell surface. Croom Helm, London and Sydney, and Kapitan Szabo Washington D.C.

HART, M.A. AND MELMED, L.N. (1982) Microbiology of nutrient removing activated sludge. Water Sci. Technol. 14, 1501.

HAWKES, H.A. (1963) The ecology of waste water treatment. Pergamon Press, Oxford.

HEIDE, B.A., PASVEER, A. (1974) Oxidation ditch; prevention and control of filamentous sludge. H_2O 7, 373.

HERBERT, D., PHIPPS, P.J. AND STRANGE, R.E. (1971) Chemical analysis of microbial cells. In Norris J.R. and Ribbons, D.W. (Eds.), Methods in Microbiology, vol. 5B. Academic Press Inc., pp. 209-344.

HEUKELEKIAN, H. AND GURBAXANI, M. (1949) Effect of certain physical and chemical agents on the bacteria and protozoa of activated sludge. Sewage Works J. 21, 811.

HITZ, H.R., HUBER, W. AND REED, R.H. (1978) The adsorption of dyes on activated sludge. J. Soc. Dyeists and Colourists. 94, 71.

HOUTMEYERS, J. (1978) Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. Agricultura. 26, 1978.

HOUTMEYERS, J. (1980) Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. Eur. J. Appl. Microbiol. Biotechnol. 9, 63.

HOUTMEYERS, J., POFFÉ, R. AND VERACHTERT, H. (1977) Hydrogen peroxide as a supplement oxygen source for activated sludge. Microbiological investigations. Eur. J. Appl. Microbiol. Biotechnol. 4, 295.

HOUTMEYERS, J., VAN DEN EYNDE, E., POFFÉ, R. AND VERACHTERT, H. (1980) Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processses. Part 1. Influence of process parameters. European J. Appl. Microbiol. Biotechnol. 9, 63.

JASEWICZ, L. AND PORGES, N. (1956) Biochemical oxidation of dairy wastes. III. Isolation and study of sludge microorganisms. Sewage Ind. Wastes 28, 1130.

JENKINS, D., NEETHLING, J., BODE, H. AND RICHARD, M. (1982) The use of chlorination for control of activated sludge bulking. In Chambers, B. and Tomlinson, E.J. (Eds.). Bulking of activated sludge. Preventative and remedial methods. Ellis Horwood, Chichester. pp. 187-210.

JONES, P.H. (1964) Studies on the ecology of the filamentous sewage fungus Geotrichum candidum. Ph.D. Thesis, Northwestern University.

JONES, P.H. AND PRASHAD, D. (1969) The use of tetrazolium salts as a measure of sludge activity. J. Wat. Pollut. Control. Fed. 41, R441.

JORGENSEN, K.P. (1984) Determination of enzyme activity of activated sludge by methylene blue reaction. J. Water Pollut Control. Fed. 56, 89.

JUNI, E. AND HEYM, G.A. (1964) Pathways for biosynthesis of a bacterial capsular polysaccharide. IV. Capsule resynthesis by decapsulated resting cell suspensions. J. Bact. 87, 461.

KABLER, P.W. (1959) Removal of pathogenic microorganisms by sewage treatment processes. Sewage Ind. Wastes 31, 1373.

KAKII, K., SHIRAKASH, T. AND KURIYAMA, M. (1984) Some properties of mucilage polysaccharides extracted from sewage activated sludge. J. Ferm. Tech. 62, 429.

KATO, A., IZAKI, K. AND TAKAHASHI, H. (1971) Floc forming bacteria isolated from activated sludge. J. Gen. Appl. Microbiol. 17, 439.

KIFF, R.J. (1978) Study of the factors affecting bioflocculation in the activated sludge process. Water Pollut. Control 77, 464.

KIFF, R.J. AND THOMPSON, R. (1979) Physical methods for the extraction of bacterial exopolymers from activated sludge biomass. Biotechnol. Lett. 1, 183.

KIPLING, J.J. (1965) Adsorption from solutions of non-electrolytes. Academic Press Inc., London.

KLAPWIJK, A., DREUT, J. AND STEENVOORDEN, I.H.A. (1974) A modified procedure for the TTC dehydrogenase test on activated sludge. Water Res. 8, 121.

KRIEG, N.R. (Ed.) (1984) Bergey's Manual of Systematic Bacteriology, vol. 1, Williams and Wilkins, Baltimore/London.

KRUL, J.M. (1977) Experiments with Haliscomenobacter hydrossis in continuous culture with and without Zoogloea ramigera. Water Res. 11, 197.

KUNICKA-GOLDFINGER, W. AND WLOSTOWSKI, T. (1980) Application of semi-continuous culture on membrane filters for the study of activated sludge bacteria. Act. Micro. Polonica. 29, 407.

KUNZ, R.G., GIANELLI, J.F. AND STENSEL, H.D. (1976) Vanadium removal from industrial waste waters. J. Water Pollut. Control Fed. 48, 762.

LAU, A., STROM, P. AND JENKINS, D. (1980) The competitive growth of Pseudomonas sp. and Sphaerotilus natans. A model for activated sludge bulking. 53rd Ann. Conf. Water Pollut. Control Fed.

LAU, A.O., STROM, P.F. AND JENKINS, D. (1984a) Growth kinetics of Sphaerotilus natans and a floc former in pure and dual continuous culture. J. Water Pollut. Control Fed. 56, 41.

LAU, A.O., STROM, P.F. AND JENKINS, D. (1984b) The competitive growth of floc-forming plus filamentous bacteria. A model for activated sludge bulking. J. Water Pollut. Control Fed. 56, 52.

LEE, S.E., KOOPMAN, B., BODE, H., JENKINS, D. (1982) Evaluation of alternative sludge settleability indices. Water Res. 17, 1421.

LENHARD, G., NOURSE, L.D. AND SCHWARTZ, H.M. (1964) The measurement of dehydrogenase activity in activated sludge. Adv. Water Pollut. Res. 2, 105.

LIGHTHART, B. AND OGELSBY, R.T. (1969) Bacteriology of an activated sludge wastewater treatment plant. A guide to methodology. J. Water Pollut. Control. Fed. 41, R267.

LILLIE, R.D. (1928) The Gram stain 1. A quick method for staining Gram-positive organisms in the tissues. Arch. Path. 5, 828.

LOGUE, C., KOOPMAN, B. AND BITTON, G. (1983) INT-Reduction assays and control of sludge bulking. J. Envir. Eng. ASCE. 109, 915.

LOVETT, D.A., KAVANAGH, B.V. AND HERBERT, L.S. (1983) Effect of sludge age and substrate composition on the settling and dewatering characteristics of activated sludge. Water Res. 17, 1511.

LOVETT, D.A., TRAVERS, S.M. AND DAVEY, K.R. (1984) Activated sludge treatment of abattoir wastewater. I. Influence of sludge age and feeding pattern. Water Res. 18, 429.

LOWRY, O.H., ROSEBROUGH, N.J. FARR, A.L. AND RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265.

LUFT, J.H. (1971) Ruthenium red and violet. I. The chemistry, purification, methods of use for electron microscopy and mechanism of action. Anat. Rec. 171, 347.

LUI, D. (1983) Resazurin reduction model for activated sludge control. Envir. Sci. Tech. 17, 407.

MAGARA, Y., NAMBU, S. AND UTOSAWA, K. (1976) Biochemical and physical properties of an activated sludge on settling characteristics. Water Res. 10, 71.

MARSH, W.H., FINGERHUT, B. AND MILLER, H. (1965) Automated and manual direct methods for the determination of blood urea. Clin. Chem. 11, 624.

McKINNEY, R.E. AND WEICHLEIN, R.G. (1953) Isolation of floc-producing bacteria from activated sludge. Appl. Microb. 1, 259.

McLOUGHLIN, A.J. AND CROMBIE-QUILTY, M.B. (1983) The kinetics of protein removal by activated sludge. Water Res. 17, 161.

MERKEL, G.J. (1975) Observations on the attachment of Thiothrix to biological surfaces in activated sludge. Water Res. 9, 881.

METCALFE AND EDDY Inc. (1972) Wastewater engineering. Collection, treatment and disposal. McGraw-Hill. New York (Maidenhead) (McGraw-Hill Series in water resources and environmental engineering).

MIKSCH, K. (1983) The influence of the concentration of dissolved oxygen on the determination of the dehydrogenase activity of activated sludge using the method of the TTC test. Acta. hydrochim. et hydrobiol. 11, 17.

MIKSCH, K. (1985) The influence of the TTC concentration on the determination of activated sludge activity. Acta hydrochim. hydrobiol. 13, 67.

MINISTRY OF TECHNOLOGY (1969) Water Pollution Research 1968. London H.M.S.O.

MORAND, J.M. (1964) Physical factors affecting the size of bacterial flocs. Ph.D. Thesis, University of Wisconsin, USA.

MULDER, E.G. (1964) Iron bacteria, particularly those of the sphaerotilus-leptothrix group. J. Appl. Bact. 27, 151.

NASH, N., KRASNOFF, P.J., PRESSMAN, W.B. AND BRENNER, R.C. (1977) Oxygen aeration at Newton Creek. J. Water Pollut. Control Fed. 49, 388.

NISHIKAWA AND KURIYAMA, M. (1968) Nucleic acid as a component of mucilage in activated sludge. Water Res. 2, 811.

NORBERG, A.B. AND ENFORS, S. (1982) Production of extracellular polysaccharide by Zoolgoea ramigera. Appl. Environ. Microbiol. 44, 1231.

NOVAK, J.T. AND HAUGAN, B.E. (1981) Polymer extraction from activated sludge. J. Water Pollut. Control. Fed. 53, 1420.

NOVAK, J.T., BECKER, H. AND ZURROW, A. (1977) Factors influencing activated sludge properties. J. Envir. Eng. A.S.C.E. 103, 815.

ORFORD, H.E., HEUKELEKIAN, H. AND ISENBERG, E. (1961) Effect of sludge loading and dissolved oxygen on the performance of the activated sludge process. Int. J. Air. Wat. Pollut. 5, 251.

PALM, J.C., JENKINS, D. AND PARKER, D.S. (1978) The relationship between organic loading, dissolved oxygen concentration, and sludge settleability in the completely mixed activated sludge process. Presented at the Fifty first ann. Conf. Water Pollut. Control Fed. Anaheim CA.

PALM, J.C., JENKINS, D. AND PARKER, D.S. (1981) The relationship between organic loading, dissolved oxygen concentration and sludge settleability in the completely mixed activated sludge process. J. Water Pollut. Control Fed. 52, 2484.

PARKER, R.E. (1979) Introductory statistics for biology (2nd Ed.). Edward Arnold, London.

PARSONS, A.B. AND DUGAN, P.R. (1971) Production of extracellular polysaccharide matrix by Zoogloea ramigera. Appl. Microb. 21, 657.

PASVEER, A. (1969) A case of filamentous activated sludge. J. Water Pollut. Control Fed. 41, 1341.

PAVONI, J.L., TENNEY, M.V. AND ECHELBERGER, W.F. (1972) Bacterial exocellular polymers and biological flocculation. J. Water Pollut. Control Fed. 44, 414.

PIKE, E.B. (1975) Aerobic bacteria. In Curds C.R. (Ed.). Ecological aspects of used water treatment. Academic Press Inc., London, p. 1.

PIKE, E.B. AND CARRINGTON, E.G. (1972) Recent developments in the study of bacteria in the activated sludge process. Water Pollut. Control 71, 583.

PIKE, E.B., CARRINGTON, E.G. AND ASHBURNER, P.A. (1972) An evaluation of procedures for enumerating bacteria in activated sludge. J. Appl. Bact. 35, 309.

PIPES, W.O. (1967) Bulking of activated sludge. Adv. Appl. Microbiol. 9, 185.

PIPES, W.O. (1978) Microbiology of activated sludge bulking. Adv. Appl. Microbiol. 24, 85.

PIPES, W.O. (1979) Bulking, deflocculation and pinpoint floc. J. Water Pollut. Control Fed. 51, 1.

PITMAN, A.R. (1980) Settling properties of extended aeration sludges. J. Water Pollut. Control Fed. 52, 524.

POWELL, K.R., HENDLEY, J.O., POHL, A.F. AND VOLK, W.A. (1982) Quantitation of acidic capsular polysaccharides by alcian blue binding. Anal. Biochem. 119, 31.

QUINTARELLI, G.J.E., SCOTT, J.E. AND DELLOVO, M.C. (1964) The chemical and histochemical properties of alcian blue. II Dye binding of tissue polyanions. Histochemie 4, 86.

RACHWAL, A.J., JOHNSTONE, B.W.M., HANBURY, M.J. AND CRITCHARD, T.J. (1982) The application of settleability tests for the control of activated sludge plants. In Chambers, B. and Tomlinson, E.J. (Eds.), Bulking of activated sludge. Preventative and remedial methods. Ellis Horwood. Chichester, pp. 224-244.

RAWLINGS, D.E. AND WOODS, D.R. (1978) Bacteriology and enzymology of fellmongery activated sludge systems. J. Appl. Bact. 44, 131.

RENSINK, J.H. (1974) New approach to preventing bulking sludge. J. Water. Pollut. Control. Fed. 46, 1888.

RENSINK, J.H. (1979) Cure and prevention of bulking sludge in practice. La Tribune du Cabadeau 432, 445.

RENSINK, J.H., DONKER, H.J.G.W. AND IJWEMA, T.S.J. (1982) The influence of feed pattern on sludge bulking. In Chambers, B. and Tomlinson, E.J. (Eds.), Bulking of activated sludge. Preventative and remedial methods. Ellis Horwood, Chichester, pp. 147-165.

RENSINK, J.H., JELLEMA, K. AND YWEMA T. (1977) The influence of the substrate gradient on the development of bulking sludge. H_2O 10, 338.

RENSINK, J.H., LEENTVAAR, J. AND DONKER, H.J. (1979) Control of bulking combines with phosphate removal by ferrous sulphate dosing. H_2O 12, 150.

RIDEAU, J.B. AND MORFAUX, J.N. (1976) Etude des polyosides du mucilage des boues activees. Water Res. 10, 999.

ROE, P.C. (1982) ATP as a control parameter for activated sludge processes. J. Water Pollut. Control. Fed. 54, 244.

ROGOVSKAYA, Z.I. AND LAZAREVA, M.F. (1959) Intensification of biochemical purification of industrial sewage I. A microbiological specification of activated sludge purification of various industrial sewages. Mikrobiologia 28, 565.

ROY, D., LE DUY, A. AND ROY, P.H. (1983) One year survey of ATP and dynamic behaviour of an activated sludge treatment plant. J. Water Pollut. Control Fed. 55, 1348.

RUDD, T., STERRITT, R.M. AND LESTER, J.N. (1983) Extraction of extracellular polymers from activated sludge. Biotechnol. Lett. 5, 327.

RYSSOV-NIELSEN, H. (1975) Measurement of the inhibition of respiration in activated sludge by a modified determination of the TTC-dehydrogenase activity. Water Res. 9, 1179.

SATO, T. AND OSE, Y. (1980) Floc-forming substances extracted from activated sludge by sodium hydroxide solution. Water Res. 14, 333.

SAUNDERS, F.M. (1975) Effect of cell residence time on the extracellular organic compounds in activated sludge suspensions. Ph.D. Thesis, University of Illinois at Urbana-Campaign.

SCHEFF, G., SALCHER, O. AND LINGENS, F. (1984) *Trichococcus flocculiformis* gen. nov. sp. nov. A new Gram-positive filamentous bacterium isolated from bulking sludge. Appl. Microbiol. Biotechnol. 19, 114.

SCHENK, E. (1981) Note from the biological stain commission. A newly certified dye Alcian blue 8GX. Stain Technol. 56, 129.

SCHWARTZ, H.G., POPOWCHA, T. AND BECKER, K. (1980) Control of sludge bulking in the brewing industry. J. Water Pollut. Control Fed. 52, 2977.

SCOTT, J.E. (1972a) Histochemistry of Alcian blue. II. The structure of Alcian blue. Histochemie 30, 215.

SCOTT, J.E. (1972b) Histochemistry of Alcian blue. III. The molecular biological basis of staining by Alcian blue and analogous phtalocyanins. Histochemie 32, 191.

SCOTT, J.E. (1973a) Affinity, competition and specific interactions in the histochemistry and biochemistry of polyelectrolytes. Biochem. Soc. Trans. 1, 787.

SCOTT, J.E. (1973b) Short communication. Alcian Dyes; ICI cease manufacture and release details of composition. Histochemie 37, 379.

SCOTT, J.E. AND DORLING, J. (1965) Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian blue in salt solutions. Histochemie 5, 221.

SCOTT, J., QUINTARELLI, M.C. AND DELLOVO, M.C. (1964) The chemical and histochemical properties of Alcian Blue. I. The mechanism of Alcian blue staining. Histochemie 4, 73.

SCOTT, J.E., TIGWELL, M.J. AND GUILDORD, H. (1972) The composition and structure of Alcian blue 8GX. Proc. Roy. Micr. Soc. 7, 45.

SEILER, H. AND BLAIM, H. (1982) Population shifts in activated sludge from sewage treatment plants of the chemical industry. A numerical cluster analysis. European J. Appl. Micro. Biotech. 14, 97.

SEILER, H., BLAIM, H. AND BUSSE, M. (1984) Ecological studies on bacterial populations in activated sludge aerated with pure oxygen. Zeitschrift fur Wasser und Abwasser Forschung 17, 82.

SEILER, H., BRAATZ, R. AND OHMAYER, G. (1980) Numerical cluster analysis of the coryneform bacteria from activated sludge. Zbl. Bakt. Hyg. I. Abt. Orig. C 1, 353.

SEZGIN, M. (1980) The role of filamentous microorganisms in activated sludge settling. Prog. Water Technol. 12, 97.

SEZGIN, M. (1982) Variation of sludge volume index with activated sludge characteristics. Water Res. 16, 83.

SEZGIN, M., JENKINS, D. AND PALM, J.C. (1980) Floc size, filament length and settling properties of prototype activated sludge plants. Prog. Water Technol. 12, 171.

SEZGIN, M., JENKINS, D. AND PARKER, D.S. (1978) A unified theory of filamentous sludge bulking. J. Water Pollut. Control Fed. 50, 362.

SIGMA CHEMICAL COMPANY (1973) Colorimetric enzymatic procedure for serum or plasma at 520 nm. Sigma Technical Bulletin, No. 115.

SIGMA CHEMICAL COMPANY (1980) The colorimetric determination of urea nitrogen in blood, plasma or serum at 515-540 nm. Sigma Technical Bulletin, No. 535.

SINGLETON, F.L. AND GUTHRIE, P.K. (1977) Aquatic bacterial populations and heavy metals. I. Composition of aquatic bacteria in the presence of copper and mercury salts. Water Res. 11, 639.

SLIJKHUIS, H. (1983) Microthrix parvicella; a filamentous bacteria isolated from activated sludge. Cultivation in a chemically defined media. Appl. Environ. Microbiol. 46, 832.

SLIJKHUIS, H. AND DEINEMA, M.H. (1982) The physiology of Microthrix parvicella, a filamentous bacterium from activated sludge. In Chambers, B. and Tomlinson, E.J. (Eds.) Bulking of activated sludge. Preventative and remedial methods. Ellis Horwood, Chichester. pp. 75-85.

SLIJKHUIS, H., VAN GROENESTIJN, J.W. AND KYLSTRA, D.J. (1984) Microthrix parvicella, a filamentous bacteria from activated sludge. Growth on Tween 80 as carbon and energy source. J. Gen. Micro. 130, 2035.

SMITH, P.G. AND COACKLEY, P. (1983) A method for determining specific surface area of activated sludge by dye adsorption. Water Res. 17, 595.

STEINER, A.E., McLAREN, D.A. AND FORSTER, C.F. (1976) The nature of activated sludge flocs. Water Res. 10, 25.

STEPHENSON, T., LESTER, J.N. AND PERRY, R. (1983) Adsorption of nitrilotriacetic acid by mixed liquor suspended solids and its effect on heavy metal solubility in the activated sludge process. Chemosphere 12, 1131.

STOBBE, G. (1964) Aber das verhalten von belebtem schlamm in aufsteigender wasserbewegung. Veroffentilichungen, des Institutes fur Sidelungswasserwirtschaft der Technischen Hochschule Hannove Heft 18.

STOKES, J.L. (1954) Studies on the filamentous sheathed iron bacterium Sphaerotilus natans. J. Bact. 67, 278.

STROM, P.F. AND JENKINS, D. (1984) Identification and significance of filamentous microorganisms in activated sludge. J. Water Pollut. Control Fed. 56, 449.

TAGO, Y. AND AIDA, K. (1977) Exocellular mucopolysaccharide closely related to bacterial floc formation. Appl. Environ. Microbiol. 34, 308.

TAKIGUCHI, Y. (1968) Studies on the mucilage in activated sludge. I. On characteristics of the mucilage and its production. J. Ferm. Tech. 46, 488.

TAKIGUCHI, Y. (1972) Purification of the mucilage in activated sludge and its physiochemical properties. J. Ferm. Tech. 50, 331.

TAKII, S. (1977) Accumulation of reserve polysaccharide in activated sludges treating carbohydrate wastes. Water Res. 11, 79.

TEBBUTT, T.H.Y. AND PARASKEVOPOULOS, A.G. (1981) Viability parameters for activated sludge. Environ. Technol. Lett. 2, 293.

TENNEY, M.W. AND VERHOFF, F.H. (1973) Chemical and autoflocculation of microorganisms in biological wastewater treatment. Biotechnol. Bioeng. 15, 1045.

TEZUKA, Y. (1969) Cation dependent flocculation in a Flavobacterium sp. predominant in activated sludge. Appl. Microb. 17, 222.

TEZUKA, Y. (1973) A Zoogloea bacterium with gelatinous mucopolyaccharide matrix. J. Water Pollut. Control Fed. 45, 531.

THOMPSON, I.M. AND FORSTER, C.F. (1983) Nutrient conditions in relation to bacterial flocculation, extracellular polymer production and sludge settlement. Biotechnol. Lett. 5, 761.

TOERIEN, D.F., VANVUURE, H.J., SADIE, D.N. AND TRACE, R.P. (1979) Influence of an anaerobic zone in activated sludge systems on the bacterial population structure. Water S.A. 5, 109.

TOMLINSON, E.J. (1976) Bulking - a survey of activated sludge plants. Water Research Centre, Technical Report TR35.

TOMLINSON, E.J. AND CHAMBERS, B.C. (1979a) Effect of longitudinal mixing on settleability of activated sludge. Water Research Centre, Technical Report TR122.

TOMLINSON, E.J. AND CHAMBERS, B. (1979b) The use of anoxic mixing zones to control the settleability of activated sludge. Water Research Centre, Technical Report TR116.

TOMLINSON, E.J. AND CHAMBERS, B. (1979c) Methods for prevention of bulking in activated sludge. Water Pollut. Control 78, 524.

TRICK, I. AND LINGENS, F. (1984) Characterization of Heterosiphon sp., a gliding filamentous bacterium from bulking sludge. Appl. Microbiol. Biotechnol. 19, 191.

TRICK, I., SALCHER, O. AND LINGENS, F. (1984) Characterization of filament forming Bacillus strains isolated from bulking sludge. Appl. Microbiol. Biotechnol. 19, 120.

TROY, F.A. (1979) Chemistry and biosynthesis of selected bacterial capsular polymers. Ann. Rev. Microb. 33, 519.

UEDA, S. AND EARLE, R.I. (1972) Microflora of activated sludge. J. Gen. Appl. Microbiol. 18, 239.

UNZ, R.F. AND DAVIS, J.A. (1975) Microbiology of combined chemical biological treatment. J. Water Pollut Control Fed. 47, 185.

VAN DEN EYNDE, E., GEERTS, J., MAES, B. AND VERACHTERT, H. (1983) Influence of the feeding pattern on the glucose metabolism of Arthrobacter sp. and Sphaerotilus natans growing in chemostat culture, simulating activated sludge bulking. Eur. J. Appl. Microbiol. Biotechnol. 17, 35.

VAN DEN EYNDE, E., VRIENS, L. AND VERACHTERT, H. (1982) Relation between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. Part III. Applications with industrial waste waters. Eur. Appl. Microbiol. Biotechnol. 15, 246.

VAN GILS, H. (1964) Bacteriology of activated sludge. IG-TNO report No. 32. Res. Inst. for Public Health Engng. The Hague. Holland. p. 101.

VAN VEEN, W.L., KRUL, J.M. AND BULDER, C.J.E.A. (1982) Some growth parameters of Haliscomenobacter hydrossis (Syn. Streptothrix hyalina) a bacterium occurring in bulking activated sludge. Water Res. 16, 531.

VAN VEEN, W.L., VAN DER KOOY, E.E.W., GEUZE, A. AND VAN DER VLIES, A.W. (1971) The classification of a multicellular bacterium isolated from activated sludge. J. Gen. Micro. 69 X-XI.

VAN VEEN, W.L., VAN DER KOOY, D., GEUZE, E.C.W.A. AND VAN DER VLIES, A.W. (1973) Investigations on the sheathed bacterium Haliscomenobacter hydrossis. gen. n. sp. n. isolated from activated sludge. Antonie van Leeuwenhoek 39, 207.

VERACHTERT, H., RAMASAY, K., MEYERS, M. AND BEVERS, J. (1982) Investigations on cellulose biodegradation in activated sludge plants. J. Appl. Bact. 52, 185.

VERACHTERT, H., VAN DEN EYNDE, E., POFFÉ, R. AND HOUTMEYERS, J. (1980) Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. Eur. J. Appl. Microbiol. Biotechnol. 9, 137.

VOELKEL, K.G., MARTIN, D.W., AND DEERING, R.W. (1974) Joint treatment of municipal and pulp mill effluents. J. Water Pollut. Control Fed. 46, 634.

WAGNER, F. (1982) Study of the causes and prevention of the bulking of sludge in Germany. In Chambers, B. and Tomlinson, E.J. (Eds.) Bulking of activated sludge. Preventative and remedial methods. Ellis Horwood, Chichester, pp. 29-46.

WALKER, A.P. (1982) Quantitative filament counting - a quick, simple method for prediction and monitoring of filamentous bulking. In Chambers, B. and Tomlinson E.J. (Eds.) Bulking of activated sludge. Preventative and remedial methods. Ellis Horwood, Chichester. pp. 245-250.

WALKER, E. AND WHEALE, G. (1981) Controlling activated sludge bulking with a cationic polymer. Env. Technol. Lett. 2, 129.

WALKER, I. AND DAVIES, M. (1977) The relationship between viability and respiration rate in the activated sludge process. Water Res. 11, 575.

WALLEN, L.L. AND DAVIS, G.N. (1972) Biopolymers of activated sludge. J. Envir. Sci. Technol. 6, 161.

WARREN, G.H. AND GRAY, J. (1955) Studies on the properties of a polysaccharide constituent produced by Pseudomonas aeruginosa. J. Bact. 70, 152.

WASE, D.A.J. and BALASIND, V. (1980) Separation of polymer from activated sludge. Biotechnol Lett. 2, 537.

WATER POLLUTION RESEARCH LABORATORY (1971) Report of the Director, H.M. Stationery Office, pp. 72-74.

WEDDLE, C.L. AND JENKINS, D. (1971) The viability and activity of activated sludge. Water Res. 5, 621.

WHITE, M.J.D. (1975a) Instruction manual for WRC settling apparatus for activated sludge. Water Research Centre, Technical Memorandum TM103.

WHITE, M.J.D. (1975b) Settling of activated sludge. Water Research Centre, Technical Report TR11.

WILLIAMS, T.M. AND UNZ, R.F. (1985) Filamentous sulphur bacteria of activated sludge. Characterisation of Thiothrix, Beggiatoa and Eilkelboom Type 021N. Appl. Environ. Microbiol. 49, 887.

WILLIAMS, T.M. AND UNZ, R.F. (1983) Environmental distribution of Zoogloea strains. Water Res. 17, 779.

WOOD, D.K. AND TCHOBANOGLOUS, G. (1974) Trace elements in biological waste treatment with specific reference to the activated sludge process. Presented to the 29th Ind. Waste Conf. Purdue University, West La Fayette, Indiana.

WOOD, D.K. AND TCHOBANOGLOUS, G. (1975) Trace elements in biological waste treatment. J. Water Pollut. Control Fed. 47, 1933.

WU, Y.C. (1978) Chemical flocculability of sludge organisms in response to growth conditions. Biotechnol. Bioeng. 20, 677.

WU, Y.C., HSIEH, H.N., CAREY, D.E. AND OU, K.C. (1984a) Control of activated sludge bulking. J. Envir. Eng. ASCE 110, 472.

WU, Y.C., ARNOLD, S.L. AND OU, K.C. (1984b) Ecological study of activated sludge settling property in the completely mixed system. Water Res. 18, 1535.

ZEVENHUIZEN, L.P.T.M. AND EBBINK, A.G. (1974) Exocellular pyruvate containing galactoglucan of Achromobacter sp. Arch. Microbiol. 96, 75.