THE EFFECT OF POLYELECTROLYTES USED AS FLOCCULANTS ON MICROORGANISMS PRESENT IN RECEIVING STREAMS.

by

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RESUME

Des polyélectrolytes utilisés comme floculants dans les procedés de traitement des eaux, eaux usées et boues activées, ont été expérimentés pour leur effet toxique sur des cultures mixtes de bactéries aquatiques isolées du fleuve St Laurent. Les polyélectrolytes testés ne demontrèrent pas d'effet inhibiteur sur la densité optique de la population microbiènne ainsi que sur l'activité metabolique de ceux-ci. Ces polyélectrolytes ne purent pas pour autant servir de source nutritive pour les cultures microbiènnes mixtes inoculées.

Cependant, il fut possible d'isoler du fleuve St Laurent une espèce de Pseudomonas ayant la faculté de biodegrader les 6 polyélectrolytes testés. La biodegradation aérobe de ces polyélectrolytes fut associée à la respiration des bactéries et donc controlée par la consommation d'oxygène de ces microbes. La consommation d'oxygène fut mesurée par un respiremètre éléctrolytique. Les efforts furent concentrés sûr la biodegradation du polymère de Chlorure d'Ammoniaque de Diallyl Dymethylique (DMDAAC). Les expériences ont en effet démontré que le Pseudomonas isolé de la rivière avait la capacité d'utiliser le polymère comme seule source de carbone et d'azote. Pendant le procedé de

biodégradation, il fût possible d'isoler une enzyme extracellulaire de la solution inoculée de polymère de DMDAAC. Cette enzyme démontra la capacité de dégrader le polymère dans une solution stérile. Basé sur des resultats experimentaux ainsi que sur une revue litteraire, un mechanisme hypothétique de biodegradation du polymère par le pseudomonad aquatique est presenté dans cette étude.

Des tests de toxicité et biodegradation conduits sur les boues activées demontrent que les polymères dûment ajoutés n'ont pas d'effet toxique sur les bactèries contenues dans les boues activées. Ces tests ont aussi permis de confirmer qu'une concentration résiduelle de polymère demeure en solution apres la sédimentation du lit bactérien. Quand le Pseudomonas isolé, fût ajouté à cette solution enrichie de polymère non sedimenté, il fût possible de démontrer la biodegradation du polymère par le contrôle de consommation d'oxygène ainsi que par la titration du polymère restant en solution.

ABSTRACT

The effect of polyelectrolytes used as flocculants in water, wastewater and sludge treatment was tested on mixed aquatic microbial populations isolated from the St Lawrence River. The tested polyelectrolytes did not exhibit toxicity on the overall microbial population density or on the microbial metabolic activity. However, these polyelectrolytes could not serve as nutrient sources for the mixed cultures and were therefore not readily biodegradable.

However, it was possible to isolate from the St Lawrence River a Pseudomonas species which had the ability to biodegrade all 6 polyelectrolytes tested. Experiments were concentrated on the biodegradation of Dimethyl Diallyl Ammonium Chloride (DMDAAC) polymer, which the isolated pseudomonad could use as source of both carbon and nitrogen. An exoenzyme was also extracted from the inoculated polymer solution, which showed the ability to lyse DMDAAC polymer in a cell-free solution. Based on experimental results and literature review, it was possible to propose a hypothetical biodegradation pathway for DMDAAC polymer.

Sludge-Supernatant toxicity/biodegradation tests showed that when polymers were added to activated sludge mixed

liquor, the polymers adsorbed on the settled sludge did not show toxic effects to the microbes present in the sludge. It also was demonstrated that some added polymer remained in the supernatant after the sludge had settled. When the isolated Pseudomonas was inoculated into these supernatants, polymer degradation, as monitored by oxygen uptake and polymer titration, occurred.

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TABLE OF CONTENTS

Table of contents contd.

Table of contents contd

Table of contents contd

Table of contents contd

Table of contents contd

LIST OF TABLES

LIST OF FIGURES

List of Figures, contd

List of Figures, contd

List of Figures, contd

10.0 APPENDIX

10-1 APPENDIX 1

LIST OF CANADIAN WATER AND WASTEWATER TREATMENT PLANTS

This survey covers water and waste treatment plants using polyelectrolytes as coagulants in four Canadian provinces. The survey uses information obtained from two major manufacturers selling polyelectrolytes (Allied Colloids and Calgon Corp. of Canada).

Municipality	Plant Name	Average Flow cu.m/d	Application	Flocculant Type
<u>Province of Quebec</u>				
Candiac	Candiac WTP	13619	Potable Water	Non ionic polyacrylamide
Contrecoeur	Centrale de Trait D'Eau	2687	Potable Water	Non ionic polyacrylamide
Dolbeau	Usine de Filtration	9987	Potable Water	Non ionic polyacrylamide
Donnacona	Usine de Traitement	5447	Potable Water	High MW Slightly Cationic
Hull City	Usine de Traitement D'Eau de Parc Mouse	68099	Potable Water	High MW nonionic
Lachute	Usine de Filtration	10895	Potable Water	Nonionic Polyacrylamide
Lauzon	Usine de Filtration	6174	Potable Water	Nonionic polyacrylamide
Lavaltrie	Usine de Filtration	522	Potable Water	Nonionic polyacrylamide
L'Assomption	Usine de Filtration	6809	Potable Water	Nonionic polyacrylamide
L'Islet	Usine de Filtration	-	Potable Water	High MW Slightly Cationic
Mirabel	Usine Depuration de St. Janvier	1452	Sludge Dewatering	High MW Slightly Cationic
Nicolet	Usine de Traitement D'Eau	5302	Potable Water	High MW Slightly Cationic

Municipality	Plant Name	Average Flow cu.m/d	Application	Flocculant Type
Plessisville	Usine de Filtration de Plessisville	5674	Potable Water	Nonionic polyacrylamide
Quebec	Usine de Traitement	181599	Potable Water	High MW Slightly Cationic
Quebec	Imperial Oil	-	Sludge Dewatering	High MW Slightly Cationic
Repentigny	Centrale de Filtration	8217	Potable Water	Nonionic Polyacrylamide
Riveriere du Loup	Usine de Traitement	8170	Potable Water	Nonionic Polyacrylamide
Roberval	Usine de Filtration	10895	Potable Water	Nonionic Polyacrylamide
St. Agathe	Usine de Filtration	3631	Potable Water	Nonionic Polyacrylamide
St.Francois du Lac	Filtration Conventionelle	-	Potable Water	Nonionic Polyacrylamide
St. Georges (Beauce)	Usine de Filtration	2905	Potable Water	High MW Slightly Cationic
St. Lambert	Usine de Filtration	49939	Potable Water	Nonionic Polyacrylamide
Sorel	Usine de Traitement D'Eau	24520	Potable Water	Nonionic Polyacrylamide
Terrebonne	Usine de Filtration	17705	Potable Water	Nonionic Polyacrylamide
Varennes	Usine de Filtration de Varennes	6968	Potable Water	Nonionic Polyacrylamide
Vaudreuil	Usine Epuration de Vaudreuil	4085	Sludge Dewatering	High MW Slightly Cationic

Municipality	Plant Name	Average Flow cu.m/d	Application	Flocculant Type
Province of Ontario				
Aylmer Town	Aylmer Municipal Plant	7590	Potable Water	High MW Nonionic
Buckingham Town	-	-	Potable Water	High MW Nonionic
Casselman Village	Casselman WTP	404	Potable Water	High MW Nonionic
Chapleau Township	Chapleau Municipal Plant	3314	Potable Water	High MW Nonionic
Chatam City	Chatam WPCP	15889	Sludge Dewatering	High MW Cationic
Durham Town	Durham Sewage Works	1098	Sludge Dewatering	High MW Cationic
Hamilton City	Woodward Avenue Sewage Treatment	239257	Sludge Dewatering	High MW Cationic
Kingston City	Kingston Water Pollution Control	54479	Sludge Dewatering	High MW Cationic
London City	-	-	Sludge Dewatering	High MW Cationic
Marmora Village	Marmora Municipal	499	Potable Water	High MW Nonionic
Midland Town	Midland WPCP	8171	Sludge Dewatering	High MW Cationic
Moosonee	Moosonee WTP	635	Potable Water	High MW Nonionic
North Bay City	North Bay WPCP	31325	Sludge Dewatering	High MW Cationic
Ottawa City	Green Creek Pollution Control Center	267859	Phosphorous Removal	High MW Nonionic
Prescott	Prescott Municipal Plant	31325	Clarification	High MW Anionic
Sarnia City	Sarnia Sewage City	39634	Phosphorous Removal	High MW Anionic
Sault Ste. Marie City	Highland Creek Plant	105781	Phosphorous Removal	High MW Anionic
St. Mary	St. Mary's Municipal Supply	3191	Potable Water	High MW Slightly Cationic

Municipality	Plant Name	Average Flow cu.m/d	Application	Flocculant Type
St. Thomas City	St. Thomas Municipal WPCP	17592	Sludge Dewatering	High MW Cationic
Sudbury City	Sudbury WPCP	49939	Sludge Thickening	High MW Cationic
Toronto	Toronto Main Plant	776339	Sludge Dewatering	High MW Cationic
Toronto	Toronto Main Plant	776339	Sludge Thickening	High MW Cationic
Toronto City	Metropolitan Toronto	-	Potable Water	Poly DADMAC
	Molson's Brewery	-	Sludge Dewatering	High MW Cationic
	Molson's Brewery	-	Sludge Thickening	High MW Cationic
	Ontario Hydro	-	Water Clarification	High MW Anionic
	Chrysler Canada	-	Flotation	High MW Anionic
	Collis Leather	-	Sludge Dewatering	High MW Cationic
	Interlake Steel	-	Wastewater Clarificaicn	High MW Anionic
	Green Gaint of Canada	-	Sludge Dewatering	High MW Cationic
	Metal Koting	-	Wastewater Clarification	High MW Anionic
Verner	-	-	Potable Water	High MW Nonionic
Windsor City	Little River Pollution Control Plant	100333	Sludge Dewatering	High MW Cationic
			Sludge Thickening	High MW Cationic
			Phosphorous Remcval	High MW Anionic
Windsor City	Windsor City Main Plant	127555	Potable Water	High MW Slightly Cationic

Municipality	Plant Name	Average Flow cu.m/d	Application	Flocculant Type
Province of Saskatchewan				
	Buffalo Pound	-	Potable Water	High MW Anionic
Regina City	Regina Wastewater Treatment Plant	61516	Sludge Dewatering	High MW Cationic
Regina City	Regina Wastewater Treatment Plant	61516	Phosphorous Removal	High MW Anionic
Estevan City	Estevan WTP	3722	Potable Water	High MW Anionic
Province of Alberta				
Calgary City	Bonnybrook Sewage Treatment Plant	282097	Sludge Thickening (Flotation)	High MW Cationic
	Indian Affairs		Potable Water	High MW Anionic

10.2 APPENDIX 2

The following appendix presents the Entner-Doudoroff pathway (Figure A.2.1) present in the pseudomonads, which converts sugars into pyruvic acid, glyceraldehyde or ribulose. Pyruvic acid is thereafter introduced into the TCA cycle after being converted to acetyl-COA (Figure A.2.2).

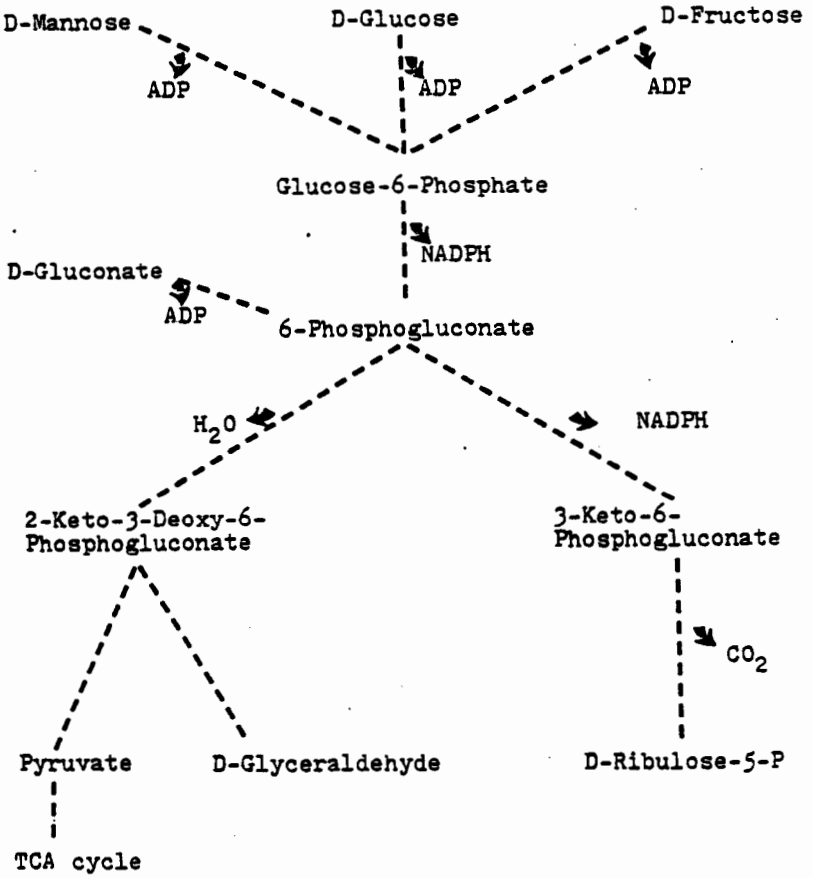


Figure A.2.1 The Entner-Doudoroff Pathway of the Pseudomonas spp. (Clarke, 1975)

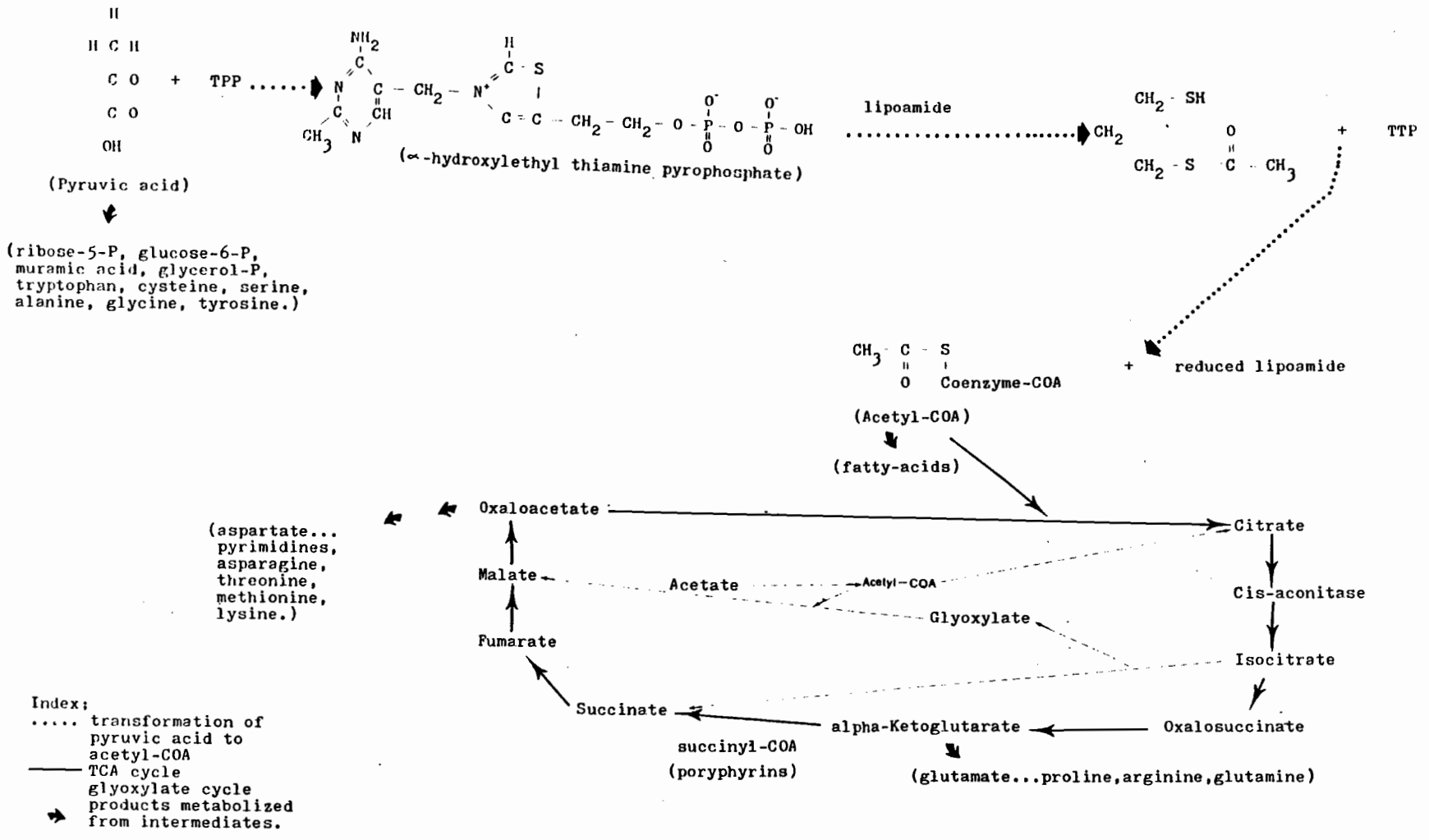


Figure A.2.2 Formation of Acetyl-COA from Pyruvic acid, the Glyoxylate cycle in the TCA cycle and Intermediates formed from the cycles.

10.3 APPENDIX-3

EXAMPLE OF TRANSFORMATION OF RAW DATA

Transformation of raw data had to be done to normalize the difference in oxygen consumption exerted by the Pseudomonas spp. for similar nutrient solutions throughout this study. Various factors could influence the oxygen uptake of a strain when inoculated into a solution. In this case, the differences in oxygen uptake observed in the raw data were principally related to variations in barometric pressures, differences in inoculum cell concentration and variations in cell vitality between the various tests. To compare the different results obtained in this study, normalization of the raw data was necessary.

The steps taken to normalize the data have been detailed in section 5.3. However, to achieve a better understanding of the steps executed, an example of data manipulation is presented here. For convenience, the results used in this example are not real results and the values given as examples are not actually used in the generation of the sample curves.

As mentioned previously, each experiment performed consisted of 5 reaction vessels; a distilled water vessel (which compensates for barometric effects), a minimal salts vessel (which compensates for endogenous respiration), a glucose control vessel, and 2 sample vessels. The raw oxygen consumption curves generated from the print-out of the respirometer are therefore similar to Figure A.3.1

Note:

Distilled water curve: DW
Minimal salts curve: MS
Glucose curve: G
Sample 1 curve: S1
Sample 2 curve: S2

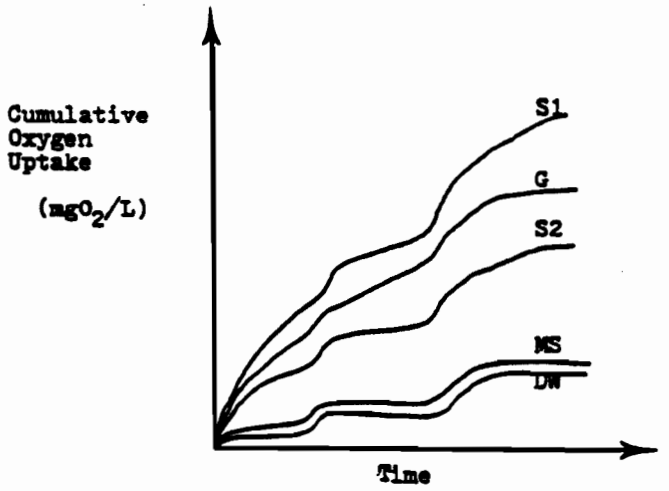


Figure A.3.1 Raw cumulative oxygen uptake as printed by the electrolytic respirometer and plotted.

Normalization steps:

1) The first normalization step consists of the subtraction of the barometric effects from the control curve, the minimal salts curve and the sample curves. The corrected curves are presented in Figure A.3.2.

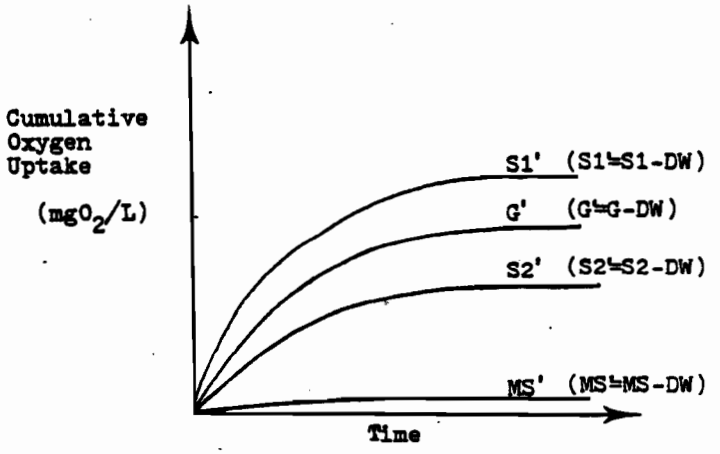


Figure A.3.2 Cumulative oxygen uptake corrected for barometric effects.

2) The second step is the subtraction of the endogenous respiration exhibited by the minimal salts vessel. The endogenous respiration is subtracted from both glucose and sample curves. The curves corrected for barometric effects and endogenous respiration are presented in Figure A.3.3.

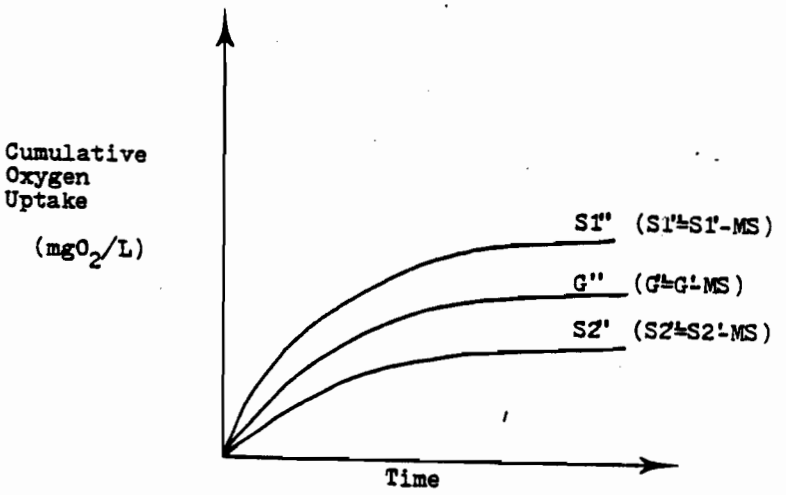


Figure A.3.3 Cumulative oxygen uptake corrected for both barometric effects and endogenous respiration.

3) The oxygen consumption exhibited by the Pseudomonas inoculated on glucose was assumed to have to be constant from experiment to experiment, since the same glucose solution was used in every test. The glucose control curves were therefore taken as a basis for normalization of the sample curves. Hence, the normalization of the results was conducted by comparing the sample curves to the corrected glucose curve. An example of numerical comparisons is presented in Table A.3.1.

Table A.3.1. Normalization Tables: Difference Between Glucose Curve and Sample Curves.

Time (hrs)	Cumulative Oxygen Uptake by Glucose Vessel. (mgC_2/L)	Cumulative Oxygen Uptake by Sample 1 Vessel (mgO_2/L)	Difference (mgO_2/L)
0	0	0	0
5	50	60	+10
10	75	90	+15
15	100	110	+10
20	105	120	+15
25	105	130	+25

4) Since the samples are now numerically related to their glucose control, one has to normalize the glucose curves obtained in all experiments. To achieve this objective, the data points of all corrected glucose curves were plotted in the same graph. A normalized glucose curve was then generated by passing a best-fit curve through the data points. This normalized curve has now been corrected for differences in inoculum cell concentration and vitality. An example of a normalized glucose control curve is presented in Figures 12 and A.3.4.

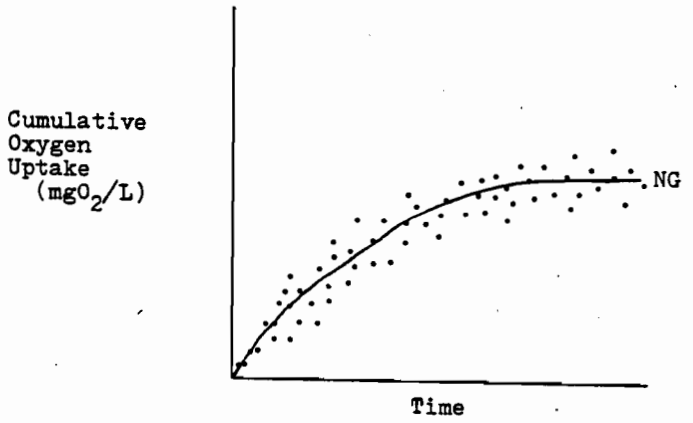


Figure A.3.4 Normalized glucose control curve.

5) The differences in oxygen uptake presented in Table A.3.1 are then used to generate oxygen consumption curves for the samples by algebraically adding them to the normalized glucose curve, in Figure A.3.4 Since the glucose curve had been corrected for some factors causing oxygen uptake differences, the sample curves now related to this normalized glucose curve will also be corrected. Before setting up the new curves, a second table is generated as follows:

Table A.3.2. Normalization Table, Example of Calculation of Normalized Sample Curve Data Points

Time	Cumulative Oxygen consumed by glucose normalized curve. (from Fig. A.3.4)	Difference in Oxygen Uptake between Glucose and Sample 1 (from Table A.3.1)	Calculated Oxygen Consumed by Sample
(hrs)	(mgO_2/L)	(mgO_2/L)	(mgO_2/L)
0	0	0	0
5	60	+10	70
10	85	+15	100
15	100	+10	110
20	120	+15	135

6) The normalized sample data points calculated from Table A.3.2 are then plotted on a graph and a best-fit curve is passed through the scattered data points. An example of this normalized final cumulative oxygen uptake curve can be seen in Figure 14 for Polymer C.

1.0 INTRODUCTION

1.0 INTRODUCTION

Polyelectrolytes, or charged water soluble synthetic polymers, are high molecular weight compounds, consisting of long-chained ionizable monomers. In the environmental engineering field, these polymers are used as flocculants or coagulant-aids in the processes of flocculation, sludge dewatering, phosphorus removal, air flotation and chemical precipitation (Benefield and Randall, 1980; Metcalf and Eddy, 1979; Weber, 1972).

Nowadays, a large number of treatment plants are using these polyelectrolytes, taking advantage of their bridging and electrolytic activities. In Quebec, a survey of 2 major suppliers (Allied Colloids and Calgon), demonstrated that as many as 30 treatment plants, including water and wastewater plants, use polyelectrolytes in their processes (refer to Appendix 1).

In wastewater treatment plants and particularly in air-flotation processes, polyelectrolytes are added to the sludge to enhance flocculation. It used to be believed that all the polyelectrolyte added to the mixed liquor would adsorb on the settling sludge, and therefore would be recycled or wasted with the sludge. But studies have shown

that some of the added polymer did not adsorb completely on the sludge. The unadsorbed polyelectrolyte remained in the supernatant, ie. in the treated effluent (Gehr and Henry, 1982, 1983; Goppers and Straub, 1976).

Only a few studies on the effects of these polyelectrolytes on aquatic life have been conducted. Biesinger et al (1976), concluded that most of the 7 polymers tested (Superfloc 330, Calgon M-500, Gendriv 162, Magnifloc 570C, Magnifloc 521C (cationic polymers), Dow AP-30 (anionic polymer) and Magnifloc 905N (a non-ionic polymer)) were toxic to trout, Mysis relecta, Limnocalanus macrurus and Daphnia magna. In general, the 5 tested cationic polyelectrolytes exhibited a higher toxicity to the tested organisms than the anionic and non-ionic polymers. Among the 5 tested species, Daphnia magna exhibited the highest sensitivity the polyelectrolyte. Spraggs et al (1982), by means of LC50 tests concluded that polymers were toxic to rainbow trout at concentrations as low as 1 ppm for a polyacrylamide polymer and 0.5 ppm for Dimethyl Diallyl Ammonium Chloride polymer (DMDAAC polymer).

The effect of polyelectrolytes on macro-aquatic life leads to the question of their effect on the microorganisms present naturally in the streams. If toxic, the presence of these polyelectrolytes could inhibit the purifying action of

aquatic microorganisms, and slowly destroy the stream. If biodegradable, the polymers could serve as nutrients to the microbial population. Alternatively, the degradation products could be even more harmful to the macro and micro biota or to subsequent users of the water.

The objective of this study was therefore to examine the effect of various polymers used in the field of sanitary engineering (with emphasis on DMDAAC polymer) on the microbial population present in a polluted stream. Toxicity and biodegradation studies were conducted in order to gain a general understanding of the fate of the discharged polyelectrolytes in the streams and the microbial activity associated with these polyelectrolytes.

2.0 LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 DEFINITION OF THE WORD POLYELECTROLYTE

Packham (1967), defined the word "polyelectrolyte" as a chemical, combining the properties of both polymers and electrolytes. These long chain molecules could be either natural (eg. proteins) or synthetic, and classified as both organic or inorganic.

Metcalf and Eddy (1979), described synthetic polyelectrolytes as being chains of simple monomers, polymerized into high molecular weight molecules. These polymers were then classified as cationic, anionic or non-ionic, depending on whether their charge, once dissolved in water, was positive, negative or neutral respectively. Examples of the 3 types of polyelectrolytes are given by Weber (1972): non-ionic (polyacrylamide), anionic (hydrolyzed polyacrylamide) and cationic (poly diallyl dimethylammonium).

2.2 HISTORY AND APPLICATIONS OF POLYELECTROLYTES IN THE SANITARY ENGINEERING FIELD

Polyelectrolytes have been used significantly for at least 20 years in the field of water and wastewater treatment. In water treatment, polyelectrolytes have been employed in the processes of water clarification, effective in the removal of organic and inorganic particles with particle sizes varying from 0.001 to 100 microns (Herner, 1976; Packham, 1967; Schwoyer, 1981).

In wastewater treatment, polyelectrolytes have a broader range of applications. As early as 1966, 14 U.S. treatment plants were using anionic polymers to improve primary settling (Garwood, 1967). Separation of biological flocs from the treated liquid in activated sludge processes has been improved by the addition of polyelectrolytes (Herner, 1975). Phosphorus removal from sewage has also been enhanced by the usage of polymers (Herner, 1975). Air flotation increases in efficiency with the addition of polyelectrolyes (Gehr and Henry, 1976; Garwood, 1967).

Polyelectrolytes are also utilized in sludge treatment processes such as sludge elutriation, conditioning of inorganic sludges and dewatering of biological sludges.

(Herner, 1975)

2.3 THEORY OF THE ACTIVITY OF POLYELECTROLYTES ON COLLOIDS

Knowledge of chemical and physical interactions occurring between polyelectrolytes and colloids is important when choosing the type and dosage of polymer in the coagulation process; a brief description of these phenomena will therefore be discussed here.

The role of the coagulant is to destabilize suspended particles. To destabilize the colloid, the polyelectrolyte has to overcome natural repulsive forces and water-colloid interactions (solvation). Natural repulsive forces have been associated with phenomena such as electrical double layer repulsion (zeta-potential), short range hydration repulsive forces, and protective colloid repulsion (Burke and Dajani, 1966; Schwoyer, 1981; Weber, 1972). Solvation as described by Weber (1972) is a poorly understood phenomenon and its effect on the rate of colloidal aggregation has not yet been clarified.

Polyelectrolytes used as coagulants or coagulant-aids have the ability, when added prior to rapid-mixing

processes, to overcome the repulsive force on the particle and thus aggregate the destabilized particles (Weber, 1972). These flocs, now heavier and denser than individual colloids can settle more easily and be removed from the liquid phase.

Burke and Dajani (1966), Metcalf and Eddy (1979), Schwoyer (1981) and Weber (1972) have classified the action of polyelectrolytes on the unstable colloid into 3 categories;

1- Double-layer compression (accomplished by polymers of opposite charge compared to the colloid).

2- Charge neutralization by polymer adsorption on the particle's surface.

3- Interparticle bridging, where the polymer, attached to a particle at one or more adsorption sites, also adsorbs on other particles, creating a bridge-type bond between various colloids.

2.4 EFFECTS OF POLYELECTROLYTES ON BIOLOGICAL SYSTEMS

A limited number of studies have dealt with the effect of polyelectrolytes on microorganisms when used as coagulants in water and wastewater treatment. This lack of information emphasizes the importance of the present study.

In order to obtain information on the subject, investigations were pursued into similar chemical compounds or sub-components of the tested polyelectrolytes.

DMDAAC polymer (dimethyl diallyl-ammonium chloride), a quaternary ammonium compound, hereafter called Polymer C, appeared to be a widely used, cationic coagulant. Its chemical structure is detailed in Figure 26, page 138. Since no information was available on the biological effects of this specific polymer, research was conducted on the general microbial effects of quaternary ammonium compounds.

In general, the smaller quaternary ammonium molecules such as CETAVLON, AJATIN and SEPTONEX, are recommended as disinfectants and exhibit great bactericidal activity, even with resistant organisms such as the Pseudomonas spp. (Taborsky et al, 1967).

Dean-Raymond and Alexander (1977) investigated the biodegradability of 10 quaternary ammonium compounds by means of the standardized BOD technique, inoculated with sewage and soil microorganisms. Eight of the tested compounds were not biodegraded by the inoculated organisms, but Hexadecyltrimethylammonium bromide and Decyltrimethylammonium (DTM), were biodegraded at concentrations down to 25 and 50 microgram per Liter respectively. It is interesting to note that the two organisms isolated from the biodegraded quaternary ammonium compounds could only develop on such substrates when grown simultaneously. This example of mutualism (Pelczar, et al, 1977) has been exhibited by two cooperative bacteria, identified as members of the Pseudomonas and Xanthomonas genera. Examples of mutualism between microorganisms are not rare, but in this case, the two bacteria work together to transform an unavailable ammonium compound into a necessary nutrient.

The second main polyelectrolyte tested during the research described herein was a polyacrylamide based co-polymer (Polymer A), composed of 63% by weight of dimethyl amino ethyl acrylate quaternised with methyl chloride and 37% of acrylamide. Suzuki, et al (1978) investigated the biodegradability of various water-soluble polyelectrolytes, including a polyacrylamide polymer. After

recognizing in a previous study (Suzuki et al, 1976) that those water-soluble polymers were not biodegraded by a strain of Pseudomonas aeroginosa, the authors partially degraded the long chains by ozonation. After this procedure, Suzuki and co-workers concluded that ozonation did not improve biodegradation of the shorter acrylamide-based polymer, when inoculated with microorganisms present in river mud. They therefore suggested that resistance of polyacrylamide polymers was not related uniquely to their high molecular weight, but also to the amide group present in acrylamide.

However, Brown et al (1979) showed that acrylamide concentrations of 0.5mg/L to 5.0mg/L, when introduced into non-sterile stream and sea samples, were biodegraded (as shown by liquid chromatography) by the mixed microbial population present in those waters. In 1982, the same authors reported on the ability of microorganisms present in wastewater, artificially spiked with acrylamide, to biodegrade the acrylamide. The authors then compared the results obtained from wastewater samples to in-situ tests performed directly on the river and concluded that in-vitro and in-situ tests could be related. Brown et al (1982) demonstrated by adding toxic substances to a solution that the disappearance of acrylamide was directly related to the number of viable microbial cells.

Croll, et al (1974) and Lande, et al (1979) also studied the biodegradation of acrylamide in mixed populations, but all mention a lag period of a few days, where no biodegradation could be observed. Thus, acrylamide (Monomer A) biodegradation could be expected in the present study, but a long lag phase will most probably be observed.

The toxicity of acrylamide to animals with a central nervous system, including humans, has been extensively investigated. For example Davenport, et al (1976) and Keeson, et al (1977), concluded that acrylamide is a potent neurotoxin, causing neurological disorders in humans and animals by affecting the mitochondrial function of neuro-filaments in distal neural axons. Croll, et al (1974), studied the effect of polyacrylamides on rats and concluded that the polymers themselves were not toxic to the test organisms. However the monomers resulting from partial degradation were highly toxic to the same organisms. No toxicity studies of acrylamide or polyacrylamide to bacteria have been reported.

Biesinger et al (1976) compared the toxicity of 7 polyelectrolytes used as coagulant-aids on 5 aquatic animal species (rainbow trout, lake trout, Mysis relicta, Limnocalanus macrurus and Daphnia magna) by acute and chronic tests. Their results showed that most

polyelectrolytes assayed were toxic to one or more species, particularly to Daphnia magna, which among those 5 animals showed the highest sensitivity to polyelectrolytes.

Spraggs, et al (1982) demonstrated by avoidance tests that rainbow trout (Salmo gairdneri) avoid Polymer A (polyacrylamide polymer) and Polymer C (DMDAAC polymer) at concentrations of 1.0mg/L and 0.5mg/L respectively, whereas at similar concentrations, the same fish were attracted to the respective monomers. The authors related the avoidance by trout to polymer toxicity, by performing 3 static tests: An algae inhibition test, performed with the species Selanastrum capricornutum, a Microtox LC50 test using the fluorescent bacterium Photobacterium phosphoreum as an indicator and, finally LC50 toxicity tests conducted with rainbow trout. Based on the results obtained from these tests, the authors concluded that both Monomers A and C were less toxic than their respective polymers to all species tested. Therefore the results supported the conclusions derived from the avoidance tests. In contrast, Goppers and Straub (1976), stated that the toxicity of polymers was mainly related to the monomers liberated by partial degradation, and not to the polymer itself.

2.5 A STUDY ON THE EFFECTS AND BIODEGRADATION OF DIFFERENT POLYMERS ON BIOLOGICAL SYSTEMS

As mentioned previously, literature was not available on the effects of the tested polyelectrolytes to aquatic bacteria, nor was it possible to find information on the possible biodegradation of the tested polymers. Information was therefore collected for a variety of other polymers, used for purposes other than water and wastewater treatment.

Surfactants have been studied extensively since their introduction into detergents. These long polymeric chains retain their foaming properties even at high dilutions, causing foaming problems in aerated activated sludge basins treating domestic wastewaters. Swisher (1970), described in his book the effects and biodegradation of these surfactants. He mentioned that surfactants can exhibit a bactericidal effect, especially to Gram positive bacteria. Swisher also studied the degradation of the surfactants by microorganisms, and concluded that the longer the molecule, the higher its biodegradation rate. This was because in larger molecules, the sulphonate groups are located farther apart on the molecule and hence the sulphonates seem to have a less negative effect on the ability of the microorganisms to use the surfactants as nutrient sources.

Various authors have studied the biodegradation of a broad variety of surfactants. Aryl-sulphonates and N-alkane-1-sulphonates were shown to be biodegraded by a Pseudomonas strain (Johnston, 1975; Thysse and Wanders, 1972, 1974). Pseudomonas testosteroni was inoculated into various arylsulfonates and could utilize individual detergents as a sole carbon source (Ripin, et al 1971). Focht and Williams (1970), adapted a Pseudomonas strain to use P-Toluenesulphonate as sulfur and carbon sources.

Guy, et al (1976), investigated the effect of Arquad T50 (an alkyl quaternary ammonium chloride surfactant) on bacteriophages and enteroviruses. Although the surfactant was found to be toxic to the bacteriophages, the tested poliovirus was resistant to the action of the surfactant. Hales et al (1982), studied the biodegradation of sodium dodecyltriethoxy sulfate surfactant by a strain of Pseudomonas. The surfactant was used as a sole carbon source and the strain was able to grow on it. Hales and co-workers also investigated different biodegradation pathways, through the analysis of metabolic intermediates by the method of thin layer chromatography. The authors described the initial steps of surfactant biodegradation, after presenting evidence that these steps involved the breakage of ether bonds.

These examples are only a small sample of the extensive research conducted on the subject of surfactant biodegradation. These detergents, originally considered as non-biodegradable are now being slowly reclassified as biodegradable.

Since the early 1950's, polyethylene glycols (PEG's) have been massively employed in pharmaceutical, cosmetics, tobacco and textile industries. As a consequence, enormous quantities of these chemicals were discharged into streams and lakes, and the possible biodegradation of polyethylene glycols was thoroughly investigated. In many cases bacteria of the Pseudomonadaceae family were recognized as being responsible for the biodegradation of PEG's attaining 20,000 molecular weight (MW) (Haines and Alexander, 1975; Kawai et al, 1978; Watson and Jones, 1977). Flavobacterium species have also demonstrated the ability to biodegrade such high molecular weight PEG's (Kawai et al, 1978). Haines and Alexander (1975) also reported that the biodegradation rate of these polyethylene glycols decrease with increasing chain length. Suzuki et al (1976), on the other hand concluded that even Pseudomonas aeroginosa could not grow on large polyethylene glycols, and that growth was only possible on 8000 MW PEG's after ozonation. In the paper cited, Suzuki and co-workers were probably unfortunate with their research, isolating a strain of Pseudomonas

aeroginosa with a limited facility of adaptation.

2.6 REVIEW OF PSEUDOMONAS BACTERIA

Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) classifies the Pseudomonas bacteria as a unicellular Gram negative rod, of length between 1.5 and 4.0 microns. Most species are motile by means of one or more polar flagella, do not produce endospores, and are strict aerobes (except for the denitrifying species). Some species have been identified as facultative chemolithotrophs, using hydrogen gas or carbon dioxide as energy sources (Holt, 1977). In total, 149 species of pseudomonads have been classified in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) but many intermediate strains also exist. Table 1 presents the 29 most common species, but the other 120 species can be seen in the appendix of Bergey's Manual of Determinative Bacteriology.

The pseudomonads are ubiquitous but have been isolated mainly from water and soil habitats. These Gram negative rods are also of special interest to environmentalists, since members of the family have been isolated which have

Table 1 Principal species of Pseudomonas

P. aeroginosa	P. marginata
P. putida	P. lemoignei
P. fluorescens	P. testosteroni
P. chlororaphis	P. acidovorans
P. aureofaciens	P. delafieldii
P. syringae	P. solanacearum
P. cichorii	P. faecalis
P. stutzeri	P. saccharophila
P. mendocina	P. ruhlandii
P. alcaligenes	P. flava
P. pseudoalcaligenes	P. palleronii
P. pseudomallei	P. maltophilia
P. mallei	P. vesicularis
P. caryophylli	P. diminuta
	P. cepacia

From: Holt (1977).

the ability to degrade a wide variety of natural and synthetic materials (Palleroni, 1964). These versatile organisms have been associated with the biodegradation of compounds previously classified as both biodegradable and non-biodegradable. As an example, Klages (1981), showed that a Pseudomonas strain was able to use 4-Chlorophenylacetic acid as a source of carbon. Swisher (1970), associated Pseudomonas spp. with the degradation of a broad number of surfactants, as did authors such as Clarke and Richmond (1975), Focht and Williams (1969), Johnston, et al (1975), Ripin, et al (1971), and Thysse and Wanders (1974).

Other "non-biodegradable" molecules such as polyethylenes (Haines and Alexander, 1975; Kawai et al, 1978; Watson and Jones, 1977), petroleum hydrocarbons (Van der Linden and Thijsse, 1965) and pesticides (1,4,5-Trichlorophenoxyacetic acid) (Chatterjee, et al, 1982) have also been demonstrated to be biodegraded by bacteria of the Pseudomonas group.

The pseudomonads are not only recognized as bacteria capable of degrading very diversified substrates, but they are also known to possess a highly adaptive metabolic system. This enables the species to use a wide variety of compounds as carbon sources (Clarke and Richmond, 1975).

As a consequence of such enormous metabolic variety, the pseudomonads have been extensively studied by scientists searching for new biosynthetic and catabolic pathways. Results of this research are discussed in the following section.

2.7 BASIC METABOLIC PATHWAYS COMMON TO THE *PSEUDOMONAS* GENUS

Catabolic and anabolic pathways common to the *Pseudomonas* bacteria have been extensively studied and detailed. A detailed description of these pathways would be out of the scope of this review, but the common mechanisms shared by most pseudomonads are described in this chapter. Detailed pathways are presented in Appendix 2.

Bacteria of the *Pseudomonas* genus are classified as microorganisms whose metabolism is always respiratory. Most strains are aerobic, with oxygen as the final electron acceptor. Some organisms have the ability to denitrify, using an anaerobic respiration pathway with nitrate as the terminal electron acceptor of the electron transport system. (Clarke and Richmond, 1975).

In 1952, Entner and Doudoroff established that *P. saccharophila* metabolized its sugar through a catabolic pathway now known as the Entner-Doudoroff pathway, an oxidative catabolic pathway. Since then, further research has revealed that the Embden-Meyeroff Pathway is effectively absent in all pseudomonads (see Clarke and Richmond, 1975). In some species however, the absent Emden-Meyeroff Pathway

is replaced by the Entner-Doudoroff Pathway. Members of the pseudomonads, possessing the oxidative Entner-Doudoroff pathway, can therefore transform ingested sugars such as glucose into pyruvate and glyceraldehyde. Pyruvate can then enter the classical tricarboxylic acid cycle (Kreb's cycle, detailed in Appendix 2) which has been shown to be present among the pseudomonads (Campbell and Stokes, 1951; Clarke and Richmond, 1975; Kogut and Podoski, 1953). The Kreb's cycle isolated in the Pseudomonas bacteria appears to have the same function and organization as in most biological systems: Converting pyruvate into building blocks and/or energy. From the TCA cycle, compounds such as amino-acids, pyrimidines, and fatty-acids are formed. The cycle also generates phosphorylated ATP's which can either be stored or converted into energy by the electron transport system (see Clarke and Richmond, 1975). Researchers have isolated cytochromes from the Pseudomonas cell membrane, confirming that an electron transport system is effectively present in the cell (Horio and Kamen, 1970; Stanier, et al, 1966). In these pseudomonads, when sugars such as glucose are not oxidized through the Entner-Doudoroff pathway, these sugars can be directly oxidized and decarboxylated to the form of Ribulose-5-Phosphate which can thereafter enter the Pentose-phosphate pathway (Clarke and Richmond, 1975).

Chapman and Dugglely (1967), demonstrated the existence

in most Pseudomonas spp. of a Glyoxylate cycle which complements the TCA cycle, allowing isocitrate and citrate to be transformed into malate and hence insuring the continuity of the cycle.

It has been mentioned that most Pseudomonas strains can utilize a variety of simple or complex substrates as carbon sources, thus explaining the presence of this Gram-negative rod in most habitats. But in order to adapt to such different nutrient sources, the species requires a complex, adaptable metabolic system, which can transform the uncommon and complex substrates into simple compounds, able to be incorporated into the cell central metabolic pathways.

This unique metabolism has been associated with a number of factors; these include the following:

1) The large number of substrates catabolized by a single pseudomonad cell implies that as large a number of enzymes should be present in the cell, since enzymes are generally known to be substrate-specific (Bernhard, 1968; Malcolm, 1971). Palleroni (1964) demonstrated that most of the enzymes involved in the catabolism of complex substrates were inducible. This regulation of enzyme products is a common physical trait in

bacteria. Clarke and Richmond (1975) and Hegeman (1966, see Clarke and Richmond, 1975) isolated a series of enzymes which could be induced by a number of different chemical analogues in a Pseudomonas species.

2) Kay and Grunland (1969), Kundig, et al (1964), Phibbs and Hagen (1970) and Romano et al (1970) concluded that inducible transport systems were present in the pseudomonad cell membrane. Inducible transport systems are induced by specific substrates which are thereby actively transported through the membrane of the bacteria. Generally, this type of transport mechanism is induced by substrates required by the cell, and to which the cell has the enzymatic complex to catabolize (Neat, 1979). Higgins and Mandelstan (1972) for example, discovered a transport system for mandelate, which was induced by the presence of low concentrations of mandelate in the medium.

3) The presence of exoenzymes or extracellular enzymes in the pseudomonads has been widely documented (Inove, et al, 1963; Maeda and Taga, 1976; Markovitz, et al, 1956; Morihara, 1963; Robyt and Ackerman, 1971; Zucker and Hankin,

1970). These enzymes enable the bacterium to cleave molecules, which are too large (> 600MW) to be transported through the cellular membrane. These smaller molecules could thereafter pass through the membrane and serve as nutrients.

4) The presence of multifunctional genes have also been proposed for the Pseudomonas bacteria (Gryder and Adams, 1969; Gunsalus et al, 1968). These genes have effectively been shown to regulate the synthesis of more than one enzyme when induced by specific substrates.

5) Bayly and Dagley (1969), Dagley (1971), and Sala-Trepat and Evans (1971), confirmed the existence of divergent enzymatic pathways (metabolic pathways present simultaneously in the cell, all induced by the same substrate, but providing different products). This adaptation enables the cell to produce various end-products from the same substrate.

6) Finally, it has been shown that most Pseudomonas strains have the ability to acquire the required catabolic enzymes, necessary to degrade a new substrate, by recombination of its

genes through transduction, conjugation (Clarke and Richmond, 1975; Kemp and Hegeman, 1968) and transformation (Carlson et al, 1983). Such an activity allows the cell to acquire a new fragment of genetic information, present in its sister cell, and consequently adapt more easily to new situations.

2.8 REVIEW OF LABORATORY METHODS

2.8.1. MEASUREMENT OF BACTERIAL GROWTH

A large number of methods have been developed to measure the growth of microorganisms. Most methods are based on the principle that microbial growth is related to replication.

Gaudy and Gaudy (1980), defined and commented on various methods developed to measure bacterial growth.

1) Measurement of biomass by dry weight after filtration or centrifugation
2) Measurement of volume of centrifuged cells.
3) Measurement of turbidity or optical density (restricted to low population density).
4) Measurement of the number of visible particles with a light microscope
5) Measurement of viable cells, using
   - culture count by pour method
   - culture count by spread method
   - culture count by spot plating
   - culture count by membrane filtration
   - null point dilution (eg. MPN Technique).
6) Measurement of cell constituents (DNA, proteins).
7) Measurement of oxygen consumption over time period.
8) Measurement of oxygen uptake rate.
9) Measurement of decrease in nutrient.

For further information on each method, the reader is referred to Gaudy and Gaudy (1980) or any microbiology textbook such as Pelczar, et al (1977). For the present

research, growth was followed firstly by optical density measurements and later by oxygen uptake measurements.

2.8.2 MEASUREMENT OF POLYMER BIODEGRADATION

Swisher (1970), described biodegradation methods applicable for the measurement of surfactant biodegradation. The methods mentioned are generally not specific for a particular surfactant and apply to a large number of microorganisms. As an example, the author described in his book different die-away tests, where the rate of biodegradation is measured by analyzing the concentration of the compound at regular time intervals.

Brown et al (1979, 1982), Degens et al (1950), and Hammerton (1955), used river-water die-away tests for their research, where the monomer was added to a river sample and analyzed at time intervals.

Borstlap and Kooijman (1963), and Heinz and Fischer (1967) developed modifications of the standard BOD test to follow surfactant biodegradation. Borstlap and Kooijman (1963), achieved surfactant biodegradation by performing

typical BOD tests with a highly concentrated inoculum of activated sludge. Once diluted, the inoculum reached 500mg/L of suspended solids. In contrast Heinz and Fischer (1967) developed an open flask test where the samples, once diluted in BOD dilution water and seeded, were shaken for 10 days.

Huddleston and Allred (1963), Renn (1964) and Tomiyama et al (1968), converted the standard microbiolcgical method of shaked culture to study surfactant biodegradation. In all cases, the cultures were continuously shaken in an incubator, and the residual surfactant concentration measured at regular time intervals.

In 1966, a test called the British STCSD Test (Standard Technical Committee on Synthetic Detergents) was developed for measuring surfactant biodegradation. The test consisted of BOD dilution water inoculated with air-dried activated sludge, and completed by the addition of surfactant in different concentrations. Surfactant biodegradation was then measured over 21 days by a methylene-blue reduction method (see Swisher, 1970).

Other biodegradation tests involving pilot plant trickling filters, activated sludge and anaerobic processes have also been used world-wide (Barnhart 1963).

Respirometric biodegradation tests have been utilized for many years, by adapting the Warburg respirometer to this function (Barden and Isaac (1957), Bogan and Sawyer(1955)). Factors such as endogenous respiration have been reported by Dietrich and Burris (1967) to cause interferences, and must therefore be taken into consideration by running controls. Busch and Myrick (1961) concluded from their experience with respirometric biodegradation tests, that degradation cannot be monitored by expecting the calculated theoretical oxygen consumption to be reached. According to the authors, the theoretical oxygen demand is only related to complete degradation of a component when the compound is completely oxidized to carbon dioxide and water. But when microbial growth takes place, complete degradation does not always occur, since biodegradation intermediates are often incorporated into the cell protoplasm, and are therefore not further biodegraded.

Biodegradation of polymers has also been studied more recently by labelling the polymers and following the incorporation of the labelled Carbon^{14} atoms into the cell protoplasm (Mulders and Gilain, 1977). In fact, the authors mention that following biodegradation by oxygen consumption may lead to false conclusions, such as misinterpreting co-oxidation phenomena (chemical oxidation, where the oxidant is a pollutant itself). These authors state that

the method of following biodegradation by die-away tests can only lead to the conclusion that degradation occurred to a point where the compound can no longer be detected by analytical methods. The authors also mention that pilot plant activated sludge processes cannot be solely used for biodegradation tests, since one cannot distinguish between the adsorbed and the biodegraded product. In the case of the present study, co-oxidation could not interfere with the results since the polymers were dissolved in a synthetic solution and not in a polluted river water. The effect of oxidation by a second pollutant was therefore not a problem.

2.8.3 MEASUREMENT OF TOXICITY TO MICROORGANISMS

Toxicity tests were developed in the early 1800's, with the objective of testing the potential hazardous effects induced by new chemicals on humans and other animals (Zapp, 1980). Since the late 1940's, aquatic animals, especially fish, have been tested for their response to chemicals discharged into streams and lakes (Buikema et al, 1982). Fish have been selected as the best test organism in these waters, since their biology, physiology and behaviour are

well understood. A number of recommendations have been proposed by various international organizations concerning the monitoring and interpretation of toxicity tests. Buikema et al (1982), discussed the following recommendations:

1) Chronic and acute tests should be performed.

2) Acute tests should be performed over a minimum of 2 days.

3) Toxicity tests are only valid when 4 or more different test organisms are involved. The organisms should represent different classes and each organism should be considered as important as the others.

4) Average toxicity should not be calculated. Instead, the results should be presented in detail, and the most sensitive organism specified as such.

Since measurement of behavioural response is not commonly performed in microbiology studies, the measurement of toxicity to microorganisms has been generally related to growth inhibition. Thus the methods developed to measure

microbial toxicity are closely related to the methods developed to measure microbial growth. These methods were mentioned previously and will not be repeated here.

Lamikanra and Allwood (1976) studied the effect of non-ionic surfactants on Staphylococcus aureus, a Gram positive coccus. The toxicity of the polymers was measured by the classical methods of plate counts, at different surfactant concentrations and contact times. The same enumeration method was utilized by Hallas, et al (1982), to measure the toxicity of tin to estuarine microorganisms. Malka-Mor and Stark (1982) correlated the toxicity of carcinogens to Salmonella typhimurium with the toxicity of the same carcinogens to animals. They measured microbial toxicity by the spot method, a modification of the standard plate count.

Although most researchers prefer to use classical methods such as plate counting to measure microbial toxicity, others use different techniques, involving analytical instruments. Mowat (1976) for example, measured the toxicity of heavy-metals to microorganisms by means of the standardized Biochemical Oxygen Demand (BOD) test.

Green et al (1975), studied the toxic effect of various pollutants with laboratory-scale activated sludge units.

The performance of the treatment plants, measured by respiration rates, was then directly related to the toxicity of the chemical tested.

2.8.4 MEASUREMENT OF RESIDUAL POLYELECTROLYTE CONCENTRATIONS

In 1955, Michaels and Morelos related the concentration of polyacrylamide to the optical density of the supernatant remaining after settling of a kaolinite suspension to which polymer had been added. This method was later modified by Crummet and Hummel (1963) and Wimberley and Jordan (1971) to accomodate anionic polyelectrolytes, and more recently by Gehr and Kallari (1983) by using mono-dispersed latex suspensions.

Thin layer chromatography has been utilized by Goppers and Straub (1976), to analyze water samples containing various polyelectrolytes. According to the authors, the method chosen exerted high sensitivity for the analysis of polymers. The authors recommended thin layer chromatography for the analysis of polymers, based on its sensitivity, small sample size requirement, short testing time and high resolution of mixed samples.

Packham (1967), described and commented on the following methods to analyze polyelectrolytes:

1) Suppression of oxygen's polarographic maximum in a potassium chloride solution
   ... poor reproducibility
2) Viscosity measurement
   ... poor reproducibilty due to wall adsorption
3) Kaolin suspension settling rate
   ... subject to interferences
4) Kaolin suspension filtration rate
   ... subject to interferences
5) Silver bromide flocculation
   ... poor for low concentrations
6) Quaternary ammonium compound precipitatcn
   ... unsuitable for high MW polymers
7) Light scattering
   ...good for concentrations down to 0.2mg/L in pure solutions
8) U.V. absorption (230nm)
   ... only accurate if no interferences
9) Carbon^{14} labelling, measured by liquid scintillation
   ...reliable but costly and complex

More recently, analytical methods such as liquid chromatography have been described as being efficient for

the analysis of polyelectrolytes. Levy and Dubin (1982) applied aqueous gel permeation chromatography (GPC) to analyze industrially significant cationic polymers. Gel permeation chromatography was also employed by Furusawa et al (1978) to measure anionic polyacrylamides.

Polyelectrolyte concentration has also been analyzed by simpler and more standardized methods such as Chemical Oxygen Demand (COD) (Tenney et al, 1969) and Total Kjeldahl Nitrogen method (TKN) (Black, et al, 1965).

Wang, et al (1974), developed a titration method applicable for both anionic and cationic polyelectrolytes. The method consists of a charge titration, utilizing an indicator which changes color when all the charge of the polymer has been complexed. This method measures polyelectrolytes in concentrations above 1.0mg/L. In 1979, Wang and co-workers improved the method so that it was applicable to concentrations as low as 0.2mg/L.

2.8.5 ISOLATION AND MEASUREMENT OF EXTRACELLULAR ENZYME ACTIVITY

Exoenzymes are known to cleave macromolecules into smaller molecules that can then be transported across the cellular membrane (Moat, 1979). Their existence enables bacteria to benefit from large substrates such as proteins, polysaccharides and other polymers.

Extraction of exoenzymes has been carried out for many years. The fact that these enzymes are present outside the cell gives an advantage to scientists aiming to isolate this type of protein. Since simple centrifugation was efficient for extraction of those proteins, early researchers could isolate exoenzymes and study their activity (Morihara, 1963; Ueda and Nanri, 1967). More recently, more sophisticated techniques have been developed to extract enzymes; these include continuous-flow centrifugation (Maeda and Taga, 1976); and high speed centrifugation (Kobori and Taga, 1980).

Studies of the specific activity of enzymes, and the kinetics of enzyme-catalyzed reactions, started mostly with the work of Michaelis and Menten (see Lehninger, 1975), which provided a mathematical basis for the analysis of

enzymatic reactions. From their extensive work resulted the Michaelis-Menten equation and constant, and of course, a better understanding of enzyme kinetics.

At present, the measurement of enzymatic activity can be done by a variety of methods. The methods employed by various authors to measure the specific activity of the enzyme are usually based on the decrease in concentration of the substrate catalyzed. Many authors developed colorimetric methods which allowed them to measure the concentration of the substrate.

Maeda and Taga (1976) related the decrease in fluorescence intensity to the activity of an extracellular nuclease. Kobori and Taga (1980) assayed the activity of phosphatase (isolated from a marine Pseudomonas bacteria) by measuring the difference of absorbance between the sample and the control. Ueda and Nanri (1967) measured the activity of an isoamylase enzyme by means of a spectrophotometer set at 620nm. Walsh et al (1981) studied the activity of 1-Aminocyclopropane-1-carboxylate deaminase extracted by centrifugation from a Pseudomonas strain. The enzyme activity was assayed by monitoring the disappearance of NADH (Nicotinamide adenine dinucleotide hydrogenase, a coenzyme involved in oxido-reduction reactions) by absorption at 340nm. Morihara (1964) studied the activity

of two enzymes, proteinase and elastase, both isolated from a Pseudomonas bacteria. Proteinase activity was measured by the conventional absorbance technique, but elastase was monitored by the plate assay method. This method, developed by Sbarra in 1960 (see Morihara 1964) relates the enzyme activity to the dimension of clear zones on elastin agar.

3.0 SEQUENCE OF EXPERIMENTS

3.0 SEQUENCE OF EXPERIMENTS

The experiments presented in this thesis have been designed to provide an indication of the effects of various polyelectrolytes on microorganisms isolated from a river.

The first series of experiments consisted of preliminary experiments, in which effects such as toxicity or biodegradation were considered. Microbial growth monitored by optical density measurements and by oxygen uptake were established as parameters to measure the effect of the polymers on the microorganisms. These microorganisms consisted of an undefined mixed culture obtained from St Lawrence River water.

Since the polymers appeared to exert no toxic effects on the overall number and activity of the aquatic microorganisms, biodegradation studies were then considered. Since biodegradation was now considered, it was decided to monitor microbial activity uniquely by oxygen uptake. All experiments were thereafter conducted with the electrolytic respirometer.

Oxygen uptake measurements confirmed that the polymers were not toxic to most or all microorganisms present in the

inoculum . But these experiments also demonstrated that the polymers could not serve as unique carbon sources. They could, however, actually enhance growth in the presence of an available carbon source.

Based on the results obtained to that date, and since the polymers did not appear to exert toxic effects on the microorganisms tested, it was decided that biodegradation tests should be conducted. However, previous experiments demonstrated that the river mixed bacterial population was unable to utilize the polymers tested as sole carbon sources. So it was decided to test individual species for their ability to biodegrade Polymer C. These trial and error experiments proved to be successful, since a microbial species which was able to metabolize Polymer C, was indeed isolated from the St Lawrence River, near Montreal.

Biodegradation tests were then conducted with the isolated strain, testing the possible biodegradation of various polymers and monomers. In addition, additional experiments, such as exoenzymatic activity tests and sludge-supernatant toxicity/biodegradation tests were conducted.

4.0 APPARATUS AND METHODS

4.1 THE EFFECT OF POLYELECTROLYTES ON FRESHWATER MICROBIAL MIXED CULTURES

4.1.A GROWTH MEASURED BY OPTICAL DENSITY

4.1.A.1 APPARATUS

-Bausch and Lomb spectrophotometer (Model 20)
set at 690nm.
-Lab-Line Junior orbit shaker set at 100rpm
-Fisher BOD incubator (model B2)
set at 20°C
-Sterilized glassware
-Side-arm growth flasks (250mL)
(29mL liquid capacity
in the side-arm).

These flasks were used as growth vessels with the advantage that optical density measurements could be taken via the side arm, without having to open the flask and risk contamination.

4.1.A.2 REAGENTS

-Minimal Salts: 20g Ammonium chloride
4g Ammonium nitrate
8g anhydrous Sodium sulfate
12g anhydrous Potassium phosphate monobasic
4g Potassium phosphate dibasic
0.4g Magnesium sulfate
diluted to 1L, filtered and sterilized

-Saline solution: 8.5g Sodium chloride diluted to 1L

-Nutrient solutions:

-0.2% Glucose solution: 25mL Minimal salts
1.0mL 20% Glucose (200,000mg/L)
diluted to 100mL

-1.0% Polymer A solution (10,000mg/L) (a cationic polyacrylamide-based polymer):
1g Polymer A

1ml Ethanol to help dissolve

dilute to 100ml

-0.2% Polymer A solution: 25mL Minimal salts

20mL 1% Polymer A solution

diluted to 100mL

-0.2% Monomer A solution: 25mL Minimal salts

1.0mL 20% Acrylamide

diluted to 100mL

-0.2% Polymer C solution: 25mL Minimal salts

1.33mL 15% Dimethyl Diallyl

Ammonium Chloride (DMDAAC) Polymer

diluted to 100mL

-Inoculum: Cultures isolated from the St Lawrence river, at Nun's Island. The cultures were grown on nutrient agar slants (Difco agar #0001-01) and incubated at 20°C

4.1.A.3 PROCEDURE

Side-arm flasks were filled with 25ml nutrient solution. The flasks were then inoculated with a 4% (4 mL inoculum in 100mL solution) inoculum of a saline suspension composed of mixed cultures isolated from the river. Microbial replication was enhanced by a rotary movement (100rpm) and incubation was at 20°C. At regular time intervals, the absorbance of the inoculated solutions was measured by transferring (without opening the bottle) the liquid into the side-arm by tilting the flask. Curves of absorbance versus time were plotted in order to obtain the mixed population growth curve, specific for each nutrient solution. A blank consisting of inoculated minimal salts diluted into water was run, together with a control (sterile glucose solution).

4.1.B GROWTH MEASURED BY OXYGEN UPTAKE

4.1.B.1 APPARATUS

-Exidyne Electrolytic Respirometer
(models # ER-101,# ER-102)
-Leedal Automatic Temperature Control unit
-Sterilized glassware

4.1.B.2 REAGENTS

-Minimal salts (recipes described in section 4.1.A.2)
-Saline solution
-0.2% Polymer A solution
-0.2% Monomer A solution
-0.2% Polymer C solution
-0.2% Monomer C solution : 25mL Minimal salts
0.32mL 62.23% DMDAAC
diluted to 100mL
-Inoculum: See section 4.1.A.2

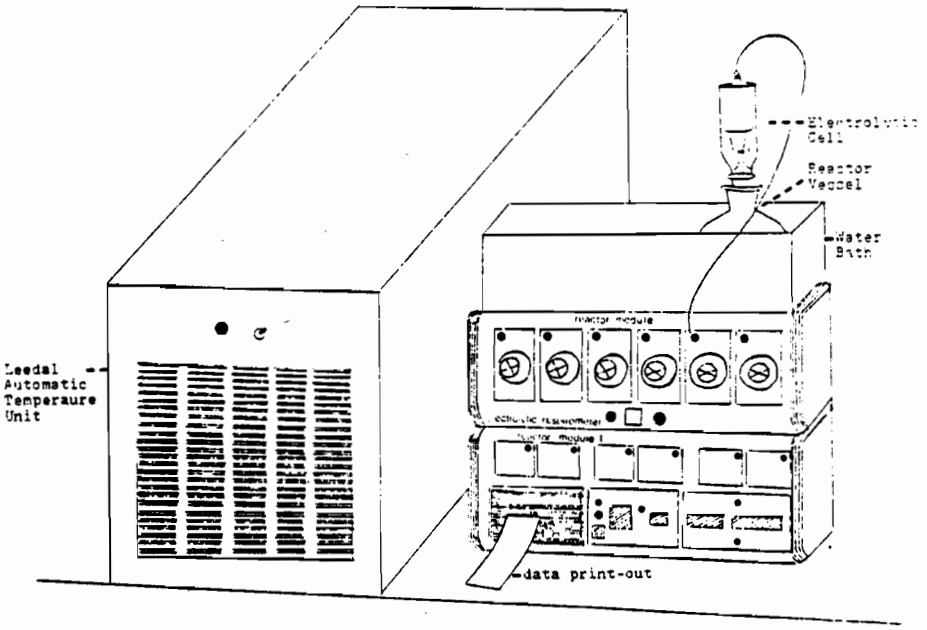


Figure 1. Electrolytic Respirometer

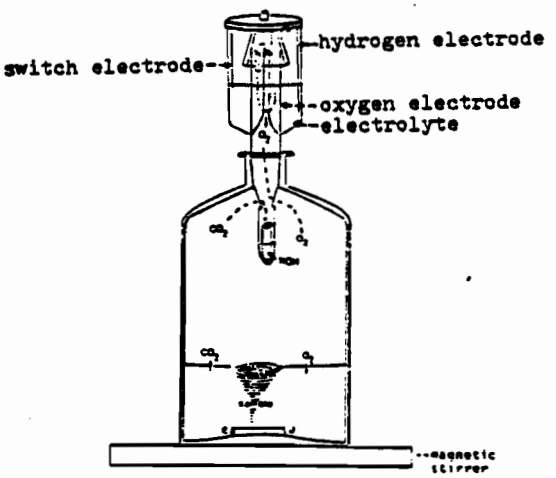


Figure 2. Electrolytic Cell

Principle of operation of the electrolytic respirometer:

The electrolytic respirometer consists of three major components (Figure 1): A constant temperature bath, a reactor (including the electrolytic cell) and a control module which records the amount of oxygen produced for each reactor.

The reaction vessel (Figure 2), is again divided into three components: A reaction vessel (bottle) where the sample is stirred continuously; an adaptor containing a glass fiber filter paper immersed into potassium hydroxide; and an electrolytic cell.

The electrolytic cell is a modified manometer, containing an electrolyte (sulfuric acid) compensating for any change in the vessel's manometric pressure. When the microorganisms respirate, they consume oxygen and produce carbon dioxide. The liberated carbon dioxide is fixed by potassium hydroxide, present in the adaptor, and the loss of oxygen consumed decreases the vessel internal pressure. To compensate for this decrease in pressure, the electrolyte rises in the cell's internal tube and the external electrolyte level decreases, activating the switch electrodes present in the cell. As a consequence, oxygen is

produced, recorded in the control module and printed at regular time intervals.

4.1.B.3 PROCEDURE

Sterilized glass bottles (1L) were partially filled with 100mL nutrient solution and inoculated with 4mL of inoculum. Boiled magnetic stirrers were introduced into the vessels to insure complete mixing of the solution. The reaction vessels were placed in a constant temperature bath set at 14°C. The bottles were then connected to the electrolytic respirometer and oxygen consumption data were printed at regular intervals. The cumulative oxygen consumed by each bottle was then plotted versus time. A blank containing inoculated minimal salts and a control consisting of inoculated glucose solution were carried in each experiment.

4.2 ISOLATION OF AN AQUATIC BACTERIA BIODEGRADING DEDAAC POLYMER

4.2.1 APPARATUS

See section 4.1.B.1

4.2.2 REAGENTS

-Minimal salts (recipes described in Section 4.1.A.2)

-Saline solution " " " "

-0.2% Polymer C solution " " " "

-Inoculum: Cultures isclated from the St Lawrence River.
Pure cultures were obtained by subsequently inoculating nutrient agar petri dishes.
Pure cultures were suspended into saline solution.

4.2.3 PROCEDURE

See section 4.1.B.1

4.3 BIODEGRADATION AND ASSIMILATION OF POLYELECTROLYTES BY AN AQUATIC PSEUDOMONAS STRAIN

4.3.1 APPARATUS

See section 4.1.B.1

4.3.2 REAGENTS

-Minimal salts (recipes in section 4.1.A.2)

-Saline solution " " " "

-0.2% Glucose solution " " " "

-0.2% Monomer A solution " " " "

-0.2% Polymer A solution " " " "

-0.2% Monomer C solution (recipe in section 4.1.B.2)

-0.2% Polymer C solution- 25mL Minimal salts
16.67mL dialyzed DMDAAC Polymer
diluted to 100mL.

-Polymer D solution- 25mL Minimal salts
20mL Polymer D (a cationic polyacrylamide-based polymer)
diluted to 100mL.

-Polymers 2318-2319/1-2319/4 solutions (cationic acrylamide-based polymers)
25mL Minimal salts
20mL 1% respective polymer
diluted to 100mL.

-Galactose solution- 25mL Minimal salts
10mL 2% Galactose
diluted to 100mL

-Inoculum:

The culture which showed an ability to degrade Polymer C in the previous experiment was maintained as a pure culture in the laboratory by subsequently transferring isolated colonies to fresh nutrient agar plates every 4 days. This culture was later identified as a Pseudomonas species, but further species identification could not be made.

4.3.3 PROCEDURE

See section 4.1.B.3, however incubation temperature was maintained at 20°C. Chemical Oxygen Demand (COD) tests, were perfcrmed on some samples by the Reflux Dichromate Method, as per Standard Methods (AWWA, APHA, WPCF, 1980).

4.4 POLYMER C AS A SOURCE OF CARBON AND NITROGEN FOR A *PSEUDOMONAS* STRAIN

4.4.1 APPARATUS

See section 4.1.B.1

4.4.2 REAGENTS

-Minimal salts 8g anhydrous Sodium sulfate
12g anhydrous Potassium phosphate monobasic
4g Potassium phosphate dibasic
0.4g Magnesium sulfate diluted to 1L,
filtered and sterilized.

-Saline solution (recipe as in section 4.1.B.1).

-0.2% Polymer C solution (dialyzed) (recipe in section 4.3.2).
The polymer was dialyzed with Fisher dialysis tubes, #8-667E

-0.2% Glucose solution including Nitrogen in minimal salts
(recipe in section 4.1.B.1)

-0.2% Glucose solution excluding Nitrogen in minimal salts

-Inoculum: see section 4.3.2

4.4.3 PROCEDURE

See section 4.1.B.3

4.5 EXTRACELLULAR ENZYME ASSAY

4.5.1 APPARATUS

-Lab-Line Junior Orbit Shaker set at 100rpm

-Superspeed Refrigerated Centrifuge, Dupont Instruments (model RC-5)

-Leedal Automatic Control Temperature Unit

-10mL titration buret

-Sterilized glassware

4.5.2 REAGENTS

-Minimal salts (See section 4.1.A.2)

-Saline solution " " "

-Polymer C solution (dialyzed) (see section 4.3.2)

-Toluidine blue O (TBO) - 1g TBO (Eastman #C1756) dissolved in 1L water

-Polyvinylsulfuric acid potassium salt stock solution: 1.6221g PVSAK (eastman #8587) dissolved in 1L water

-Polyvinylsulfuric acid potassium salt titrant 0.3216g/L 20mL Stock Solution in 1L water

ASSAY

Phase 2 film

-Lab-Li 00rpm

-Superspeed [illegible] Dupont Instruments

(model RC-5)

-Leedal Automatic Contrcl Temperature Unit

-10mL titration buret

-Sterilized glassware

4.5.2 REAGENTS

-Minimal salts (See section 4.1.A.2)

-Saline solution " " "

-Polymer C solution (dialyzed) (see section 4.3.2)

-Toluidine blue O (TBO) - 1g TBO (Eastman #C1756)
dissolved in 1L water

-Polyvinylsulfuric acid potassium salt stock solution:
1.6221g PVSAK (Eastman #8587)
dissolved in 1L water

-Polyvinylsulfuric acid potassium salt titrant 0.3216g/L
20mL Stock Solution in 1L water

4.5.3 PROCEDURE

The *Pseudomonas* strain isolated from the river and maintained as a pure culture in the laboratory, was inoculated into 200mL of Polymer C solution and incubated at 20°C for 12 hours. The turbid suspension was centrifuged at 12,000rpm for 20min at constant temperature. The supernatant, now free of cells, was enriched with a specific concentration of dialyzed DMDAAC polymer. This enriched sterile sample was shaken at 50rpm and concentrations of Polymer C were determined by titration at regular time intervals. The sterility of the supernatant was insured by plate counts. Concentrations of polymer measured by PVSAK titration were then plotted versus time.

4.6 SLUDGE-SUPERNATANT TOXICITY/BIODEGRADATION TESTS

4.6.1 APPARATUS

- Exidyne Electrolytic Respirometer
- Leedal Automatic Temperature Control Unit
- Jar Test Apparatus

  (6-paddle mixing unit manufactured by Phipps and Bird Inc. and 6-2L square jars)
- Titration apparatus
- Autoclave and sterilized glassware

4.6.2 REAGENTS

-Minimal salts (recipe described in section 4.1.B.1)

-0.2% Glucose solution " " " " "

-Saline solution " " " " "

-15% DMDAAC Polymer (Polymer C)

-1% Polacrylamide co-polymer (Polymer A)

-Mixed liquor from the Ste Rose Wastewater Treatment Plant (Laval, Quebec).

Inoculum: -Microorganisms present in the mixed liquor

-Pseudomonad bacteria isolated from the river

4.6.3 PROCEDURE

A mixed liquor sample, collected from the aeration tank of a conventional activated sludge wastewater treatment plant, was poured intc 2L standard square jars. 10mg of polymer per gram cf dry solids was added to the sample and complete mixing was insured by 30s rapid mixing at 100rpm. The process of flocculation was enhanced by mixing the samples at 30rpm fcr 20min. The solids were then allowed to settle for 1h and the supernatant and sludge separated. The sludge samples recovered from the jars were enriched with glucose solution and oxygen uptake measurements were conducted with the respirometer. The clear supernatant, which should ideally be polymer free, was inoculated with the Pseudomonas culture, and enriched with minimal salts. No additional source cf carbon was added to the sample. Oxygen uptake was recorded and plotted versus time.

5.0 RESULTS

5.0 RESULTS

5.1 THE EFFECT OF POLYELECTROLYTES ON FRESHWATER MICROBIAL MIXED CULTURES

The experiments presented here are preliminary tests, necessary to acquire a minimal notion on the effect of the tested polyelectrolytes on freshwater microbial mixed cultures. As mentioned previously, these cultures were isolated from the St Lawrence river, off Nun's Island, near Montreal, Quebec.

In this series of tests, two sets of similar experiments were performed on the same microorganisms and experimental conditions. The first set of growth curves presented (see Figures 3 to 5) were generated by conducting optical density measurements on the inoculated solutions. The second set of curves were generated from data printed by the electrolytic respirometer and are therefore cumulative oxygen uptake curves. The reasons for this change in methods are as follows;

1) Optical density measurements are restricted by turbidity or color. Biodegradation can involve lengthy experiments, hence being restricted by turbidity could limit the studies.

2) Biodegradation is a metabolic phenomenon, which is not always related to bacterial growth. Therefore, monitoring polymer biodegradation by optical density measurements can lead to false results. On the other hand, aerobic biodegradation and assimilation is always related to oxygen uptake. Therefore, a method permitting one to measure oxygen uptake and relate it to biodegradation would represent a technical improvement.

3) Since biodegradation can be a lengthy phenomenon, a method which could automatically generate data for long as well as short time intervals, would be useful and would allow generation of longer and more accurate metabolic curves.

The instrument chosen to monitor oxygen consumption was the electrolytic respirometer. This instrument allows measurement of oxygen uptake in an inoculated vessel for an

extensive period of time. Neither oxygen uptake, nor carbon dioxide produced, become inhibitory factors, since oxygen is produced by electrolysis at the same rate as it is consumed, and carbon dioxide is fixed by potasssium hydroxide (see Figure 2). Although the electrolytic respirometer was developed to measure Biochemical Oxygen Demand (BOD) of water and wastewaters, it was used in this study to measure microbial metabolism, which is closely related to biodegradation. The advantages of this method, in comparison to the optical density method, are as follows:

1) Oxygen uptake is a more sensitive and direct method to measure bacterial respiration, and thus indirectly biodegradation and assimilation.

2) The respirometer supplies oxygen at the same rate it is consumed and it also fixes the carbon dioxide generated as a result of respiration. In other words the initial oxygen and carbon dioxide levels are maintained throughout the experiment.

3) Microbial metabolism can be followed without time limit since turbidity created by growing cultures is not a limiting factor.

To insure that the results obtained with the

respirometer would be relevant, parallel experiments were conducted between the methods (optical density and oxygen uptake). Based on the similarity obtained between these parallel experiments, the respirometer was considered suitable as a means to measure microbial metabolism. In general, the results obtained from the optical density measurements were related to the cumulative oxygen consumption results, emphasizing on the validity of both methods to monitor microbial activity.

Figure 3 represents the condition where the tested polymer or monomer is the sole carbon source present in the media. As in Figure 7, Polymer A solution appeared to support some growth. However, 2000mg/L of Polymer C (no glucose added) did not support more growth or induce more oxygen uptake than the control curve (compare Figures 3 and 9). This anomaly will be further discussed later. It is also interesting to note in Figure 3 that growth with glucose as a substrate, occurred only after a lag phase of 14hr. This extensive lag phase for glucose is however not expressed in the related cumulative oxygen uptake curve (see Figure 6).

The questions generated from these first experiments were related to the possible toxicity of both Polymer C and Monomer A to the aquatic microbes. Experiments were therefore conducted with the same nutrient solutions, but in

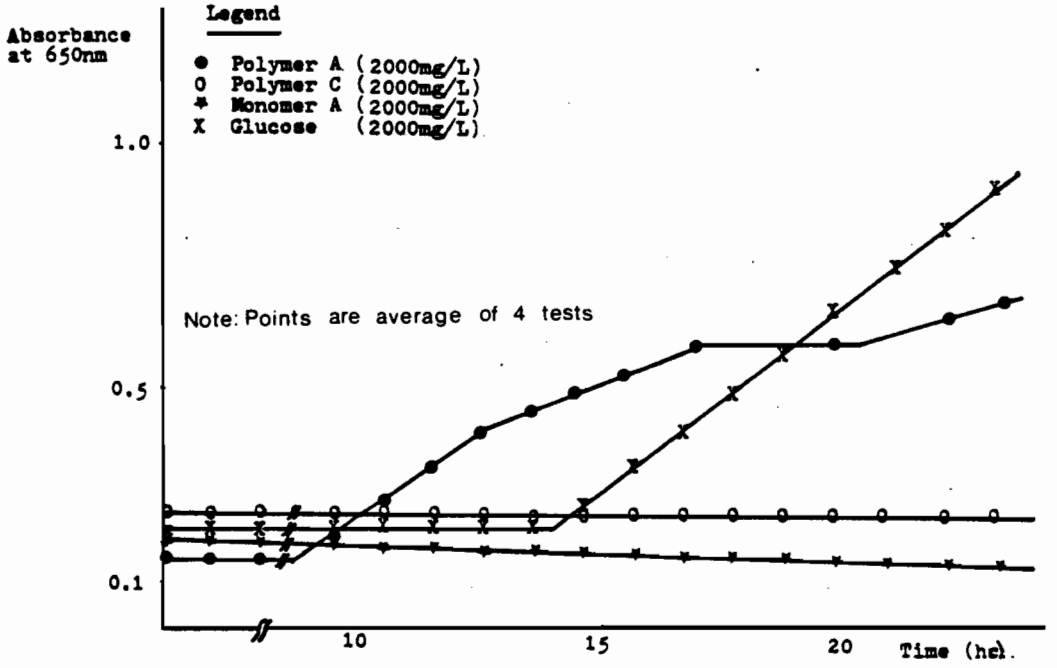


Figure 3. Growth curves of mixed aquatic microorganisms inoculated on nutrient solutions and incubated at 14°C.

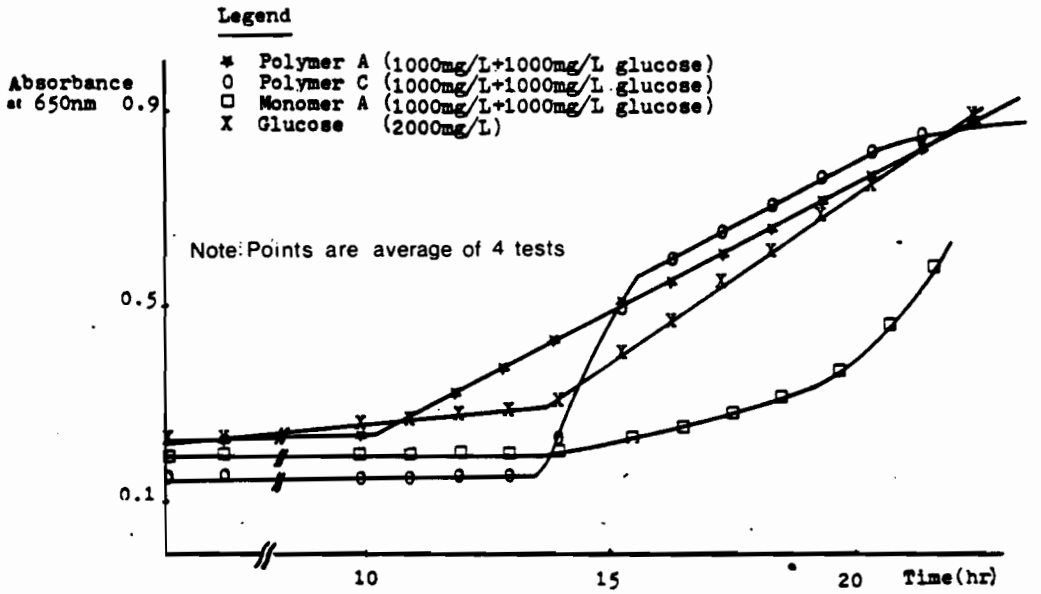


Figure 4. Growth curves of mixed aquatic microorganisms at 14°C.

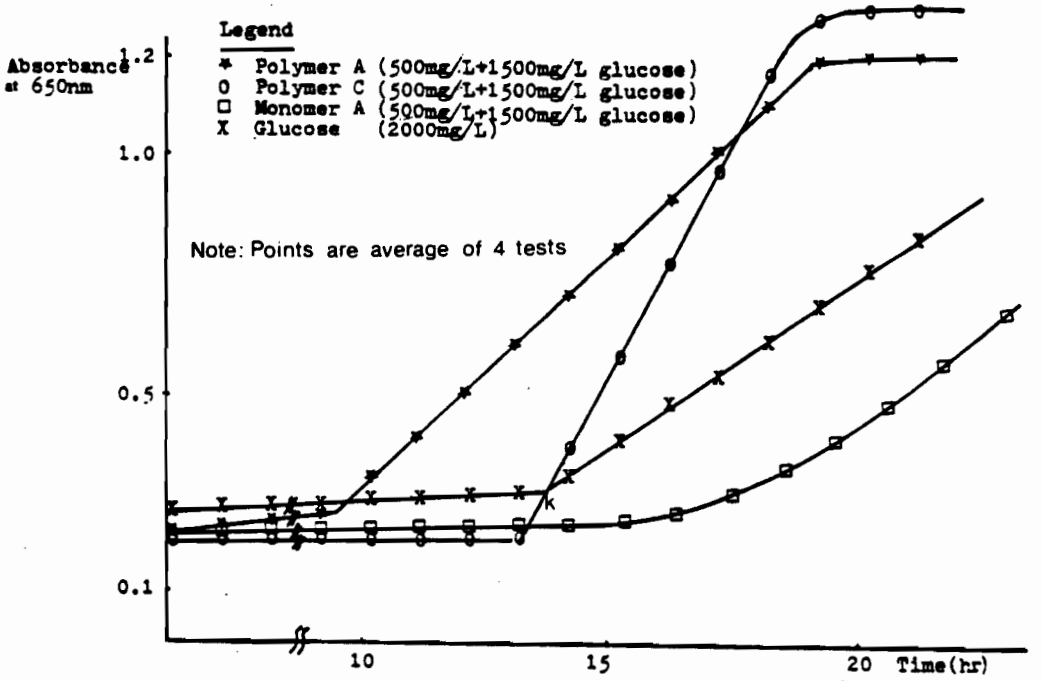


Figure 5. Growth curves of mixed aquatic microorganisms at 14°C.

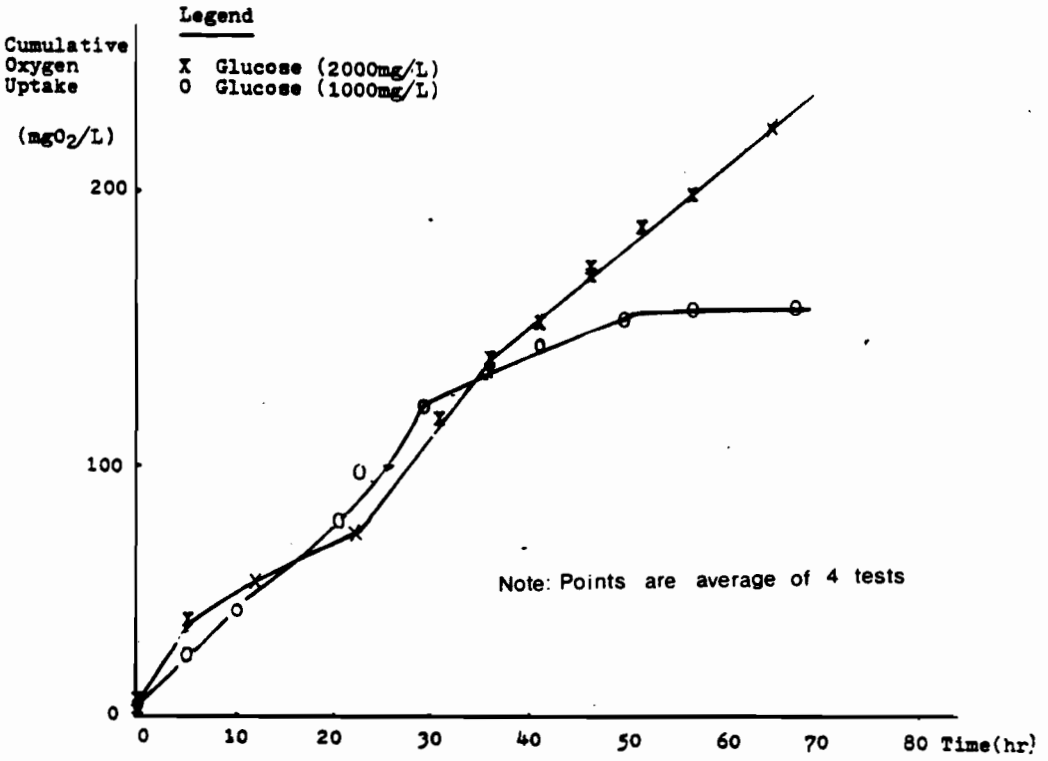


Figure 6. Cumulative oxygen uptake curves of mixed aquatic microorganisms inoculated into nutrient solutions and incubated at 14°C.

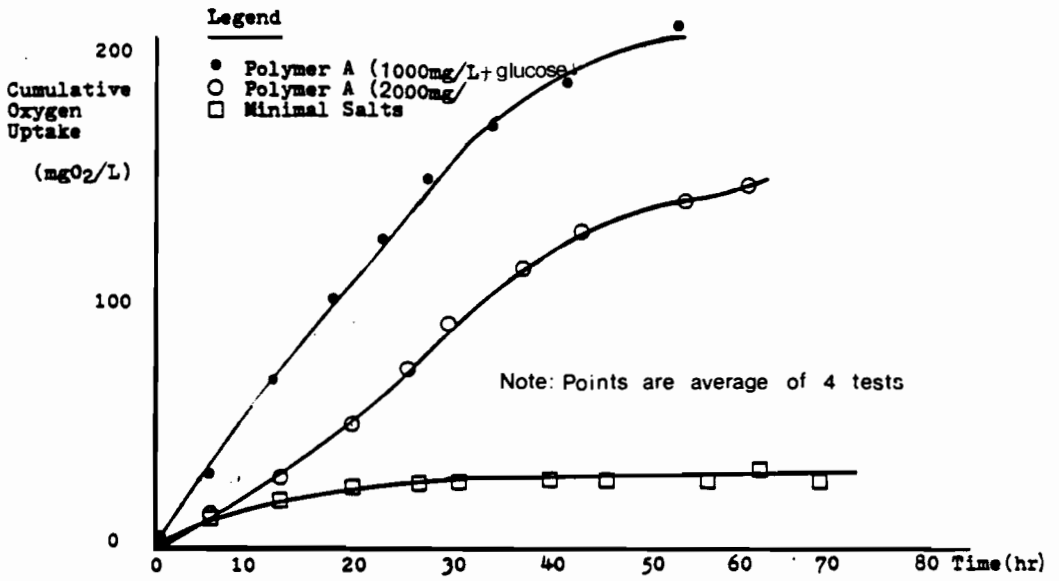


Figure 7. Cumulative oxygen uptake curves of mixed aquatic microorganisms inoculated into Polymer A solutions and incubated at 14°C.

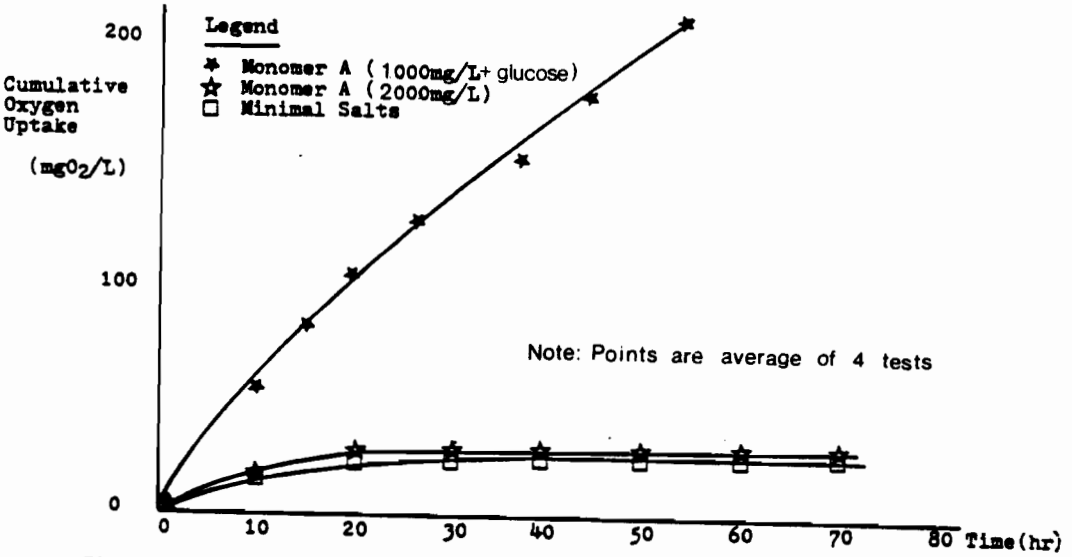


Figure 8. Cumulative oxygen uptake curves of mixed aquatic microorganisms inoculated into Monomer A solutions and incubated at 14°C.

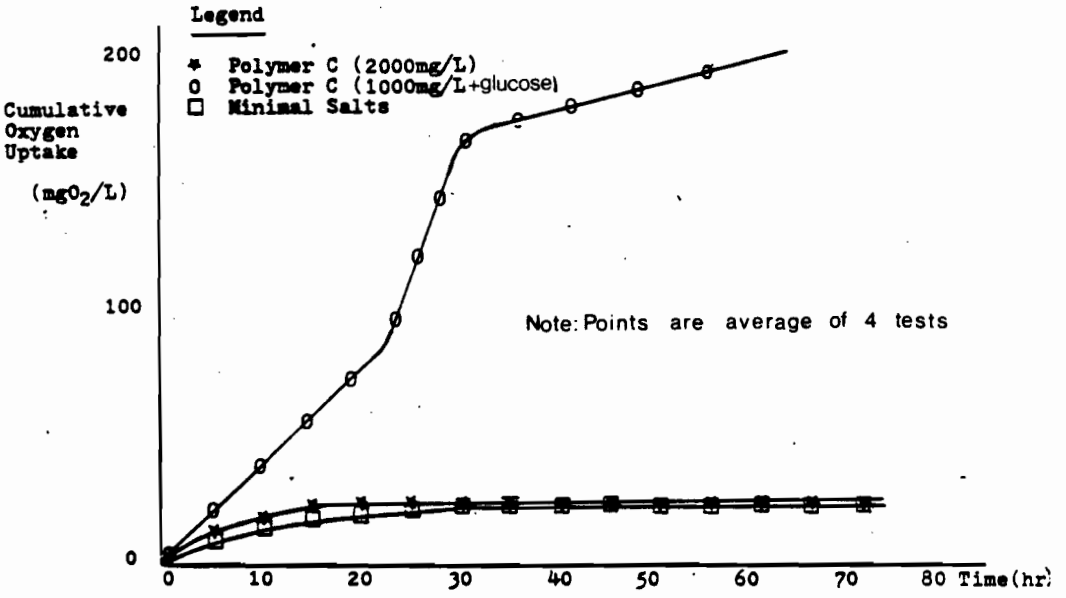


Figure 9. Cumulative oxygen uptake curves of mixed aquatic microorganisms inoculated into Polymer C solutions and incubated at 14°C.

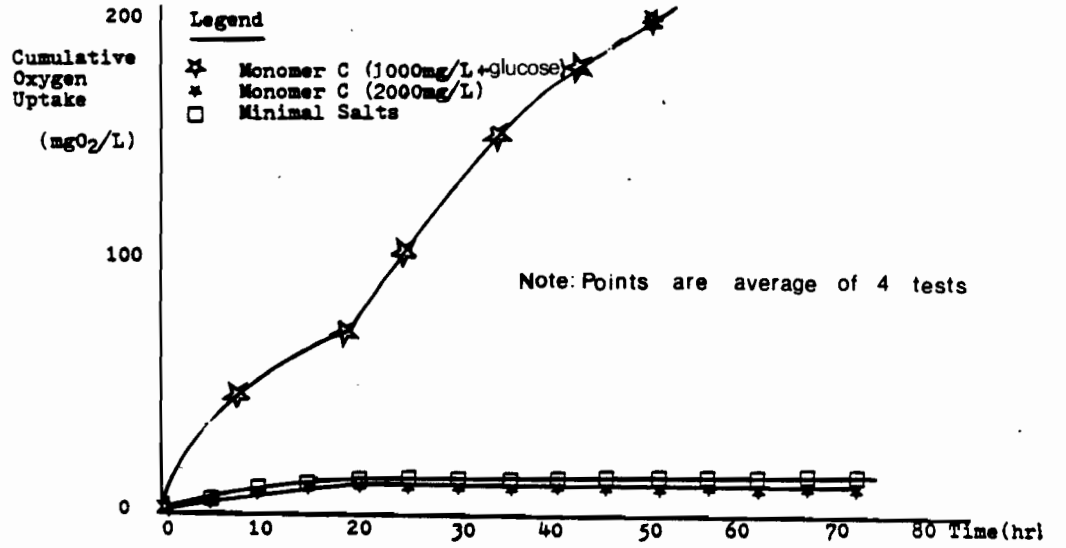


Figure 10. Cumulative oxygen uptake curves of mixed aquatic microorganisms inoculated into Monomer C and incubated at 14°C.

addition glucose in different concentrations was added as an available carbon source. Growth inhibition when compared to the growth level induced by the glucose control could be interpreted as a sign of toxicity.

Figures 4 and 5 were therefore generated from experiments in which the polymer or monomer solution was enriched with glucose solution. From both curves, it can be concluded that Polymer C did not exhibit toxicity, based on the overall number of microorganisms growing. In fact, instead of growth inhibition, it was possible to observe growth enhancement when compared to the control growth curve. The same conclusion can be derived from Figure 9 for the enriched polymer oxygen uptake curve (1000mg/L curve).

Although growth on Polymer A and Polymer C exhibited similar results when tested by absorbance and oxygen uptake, this was not the case for Monomer A (acrylamide) enriched with glucose: Figures 4 and 5 show growth inhibition for the acrylamide enriched solution (1000mg/L Mon. A), when compared to the glucose control; in contrast the curves in Figure 8 show that the oxygen consumption for the acrylamide enriched solution is at least as high as the glucose control solution (Figure 6).

Polymer C, as previously mentioned, is a homo-polymer,

or a polymer in which chains are composed of a single monomer. This monomer (DMDAAC), hereafter called Monomer C, was also tested for its effect on the aquatic mixed bacterial population (Figure 10). As expected, Monomer C exhibited the same effect on the test microorganisms as its corresponding polymer (Figure 9). In other words, DMDAAC monomer did not exhibit toxic effects, neither was it biodegraded by the test microorganisms.

Although it was mentioned that the curves generated from both methods were similar (Figures 3 to 10), it is possible to observe some differences in the shape of the generated curves. In fact, the oxygen consumption curves do not exhibit the traditional S-shape growth curve, with its lag, exponential and stationary phases. Instead the cumulative oxygen consumption curves lack an apparent lag phase step. In fact, oxygen consumption during this initial time period indicates that metabolic activity is taking place, but not necessarily growth in terms of mass or numbers. It is likely that the bacteria require oxygen to adapt to the new environmental conditions, also called endogenous growth.

Several conclusions may be derived from these first experiments (Figures 3 to 10):

1) Polymer A, Polymer C, Monomer A and Monomer C do not exhibit toxic effects to a number of aquatic microorganisms inoculated.

2) Polymer C, Monomer A and Monomer C, do not appear to support growth as sole carbon sources.

3) Polymer A solution seems to support some growth among the inoculated mixed population.

4) In the presence of an available carbon source; Polymer A, Polymer C, Monomer A and Monomer C seem to enhance growth.

The question then arises: Are there any aquatic bacteria capable of biodegrading such polyelectrolytes by using them as nutrients? To answer this question, biodegradation experiments with a pure aquatic culture were conducted with Polymer C. The reasons for this choice of polymer are as follows:

1) The molecular structure of Polymer C (DMDAAC) is known.

2) Polymer C solution appeared to be more

resistant tc biodegradation than Polymer A solutiсn.

3) Polymer C was available in liquid form; solvents required to assist dissclving the polymers in agueous solution might have interfered with the results.

5.2 ISOLATION OF AN AQUATIC BACTERIA BIODEGRADING DMDAAC POLYMER

The objective of this series of experiments was to isolate from the St Lawrence river a bacterial strain which could biodegrade Polymer C and utilize the polymer as a sole carbon source.

Based on the main objective of this study, it is obvious that it was important to isolate from the river a bacterial strain which would be indigenous to this river. Isolation of an enteric species, recently discharged into the water via sewage discharge, was undesirable since it would not represent the natural condition of the river. Hence, to maximize the chances of isolating an aquatic strain, the sampling site chosen is located on the south shore of Nun's Island (south of Montreal in the St Lawrence River), upstream from the island sewage discharge. Although sampling was conducted to avoid the possible isolation of enteric microorganisms, the success of this objective could not be totally insured. Monitoring of fecal coliform pollution by the MF-C membrane filtration technique (APHA, 1980), showed that an average of 45 fecal coliform cultures per 100ml filtered could be counted after 45hr of incubation at 35°C. This number was low when compared to the number of

microbial cultures present on the agar dishes after spreading 1mL of river sample and incubating them for 24-48hr, approximatly 300 colonies were counted.

A variety of pure colonies; originally isolated from the St Lawrence River water sample, were inoculated into Polymer C solution (DMDAAC polymer + Minimal salts) and oxygen consumption was monitored. Biodegradation was suspected when the inoculated reaction vessel consumed more oxygen than the minimal salts vessel. The pure cultures inoculated on Polymer C sclution were obtained by subsequent transfers of initial isclates.

The choice of strains to be incubated, and therefore chosen as test organisms, had to be made from a large number of isolated colonies, which grew on nutrient agar plates inoculated with river water and incubated overnight. The selection was made randomly, but preference was given to dominant colonies, determined by the number of individual but similar cultures counted on the agar plates. It is however clearly understood that dominance on nutrient agar does not necessarily relate to dominance in the stream.

The cultures selected to be tested were morphologically and microscopically studied. The cultural and morphological characteristics of the 5 cultures are described in Table 2.

Table 2. CHARACTERISTICS OF THE ISOLATED CULTURES

Culture number	Characteristics
1	Gram+ cocci in pairs. Large, mucoid, white sharp cultures with blue reflects
2	Gram+ large cocci. Small, whitish, nucleated cultures
3	Gram- small rods. Large, white, irregular shaped cultures
4	Gram- cocci in chains. Very small, transparent cultures
5	Gram- rods. Medium, irregular shaped beige colonies, with characteristic odor.

Figure 11 presents the oxygen uptake curves of the 5 isolated pure cultures, when incculated on Polymer C solution and incubated at 20°C. It can be noticed that among all five colonies, only culture #5 exhibited a significant oxygen consumption, with 130mg/L oxygen consumed after 30hr of incubation at 14°C.

Colonies #2 and #4 also exhibited some oxygen consumption. In both cases, the maximum oxygen consumed was reached after 20hr of incubation. Culture #2 consumed 30mg/L of oxygen and #4 consumed 50mg/L after 20hr. The oxygen uptake of both cultures were small when compared to culture #5 uptake. Therefore the oxygen consumed by cultures #2 and #4 was assumed to be related to the biodegradation of impurities present in the solution. All further experiments were therefore conducted with dialyzed DMDAAC polymer.

Culture #5 thus showed some ability to use Polymer C solution as a nutrient and was consequently chosen to be the strain with which all further experiments would be performed.

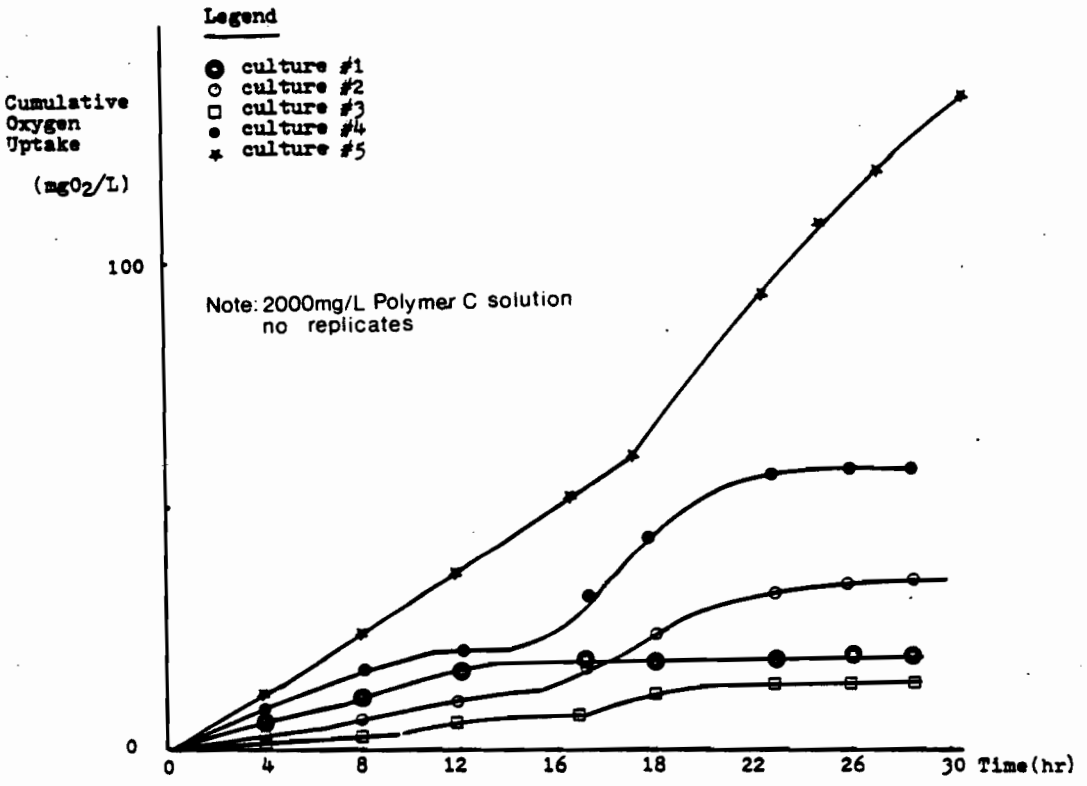


Figure 11. Cumulative oxygen uptake curves of 5 pure aquatic bacteria cultures inoculated into Polymer C solution and incubated at 14°C.

5.3 BIODEGRADATION AND ASSIMILATION OF POLYELECTROLYTES BY AN AQUATIC PSEUDOMONAS STRAIN

Since the polymers tested in the previous tests did not exhibit toxicity to the mixed population of microorganisms inoculated, biodegradation tests were considered as a subject for further research. All tests performed in this series of experiments were conducted with the Gram negative rod isolated in Series C (culture #5). The strain was maintained in the laboratory for an extensive period of time by subsequently transferring the cultures every 4 days to a fresh nutrient agar plate. Pure culture was insured by inoculating petri-dishes by the streak method.

The isolated Gram negative rod was tentatively identified by 3 microbiology laboratories (The Microbiology Faculty of Mc Gill University, the Royal Victoria Hospital Clinical Microbiology Laboratory and the Microbiology Laboratory of the Quebec Ministry of Public Health) without further possible identification. All 3 laboratories identified the strain to be a member of the Pseudomonas genus. But no species identification could be accomplished for reasons mentioned later in section 6.3.

Each set of experiments (5 bottles running

simultaneously) consisted of a control (glucose solution), a blank (thermobarometer) (distilled water)), a second control consisting of inoculated minimal salts, and 2 sample vessels. The glucose control enabled normalization of the sample oxygen consumption curves. It also made it possible to compare the oxygen consumed by the bacteria when inoculated on the tested polymer, to the oxygen consumed when the same organism was inoculated on glucose (assumed to be a readily available source of carbon). A distilled water blank was run to correct the oxygen consumption curves for any barometric effect, caused by temperature or barometric pressure variations. Finally, the minimal salts control permitted one to estimate the oxygen consumed by endogenous respiration, exerted during the initial adaptation phase.

Each oxygen consumption curve generated in this series (including glucose and sample curves) was treated as follows: (see Appendix 3 for a more detailed example)

Oxygen consumed by sample 1 (S1) = NG − $\triangle$S1

where: $\triangle$S1 = (G − MS − DW) − (S1 − MS − DW)

NG = Normalized glucose curve

(this curve has been normalized by plotting all corrected data points obtained from all experiments run and passing a best-fit line through the points (see for example, Figure 12)

(G − MS − DW) = corrected glucose curve

(S − MS − DW) = corrected sample curve

5.3.1 EXPERIMENTS CONDUCTED WITH GLUCOSE

The corrected oxygen curve for glucose is presented in Figure 12, where all experimental data points were plotted. An average curve was then passed through the scattered data points. Although all points were corrected for barometric effects and endogenous respiration, it is still possible to notice a large standard deviation among the various data points. The normalized curve reaches a cumulative oxygen consumption of 100mg/L after 14hr of incubation at 20°C, with a visible decrease in oxygen uptake rate after 4.5hr.

Based on the normalized glucose curve (Figure 12) an oxygen uptake rate curve was calculated. This new curve can be seen in Figure 13. The oxygen uptake rate curve exhibits a high uptake rate of 16.5mg/hr-L during the first 4hr of incubation, followed by a rapid decrease, tc finally reach a steady state value at 2.5mg/hr-L. This state extended to the 33rd hour of incubation. Although Figure 13 shows the oxygen uptake rate for the first 15hr of incubation only, it can be noticed in Figure 12 that there is a second period of oxygen consumption after this 33rd hour.

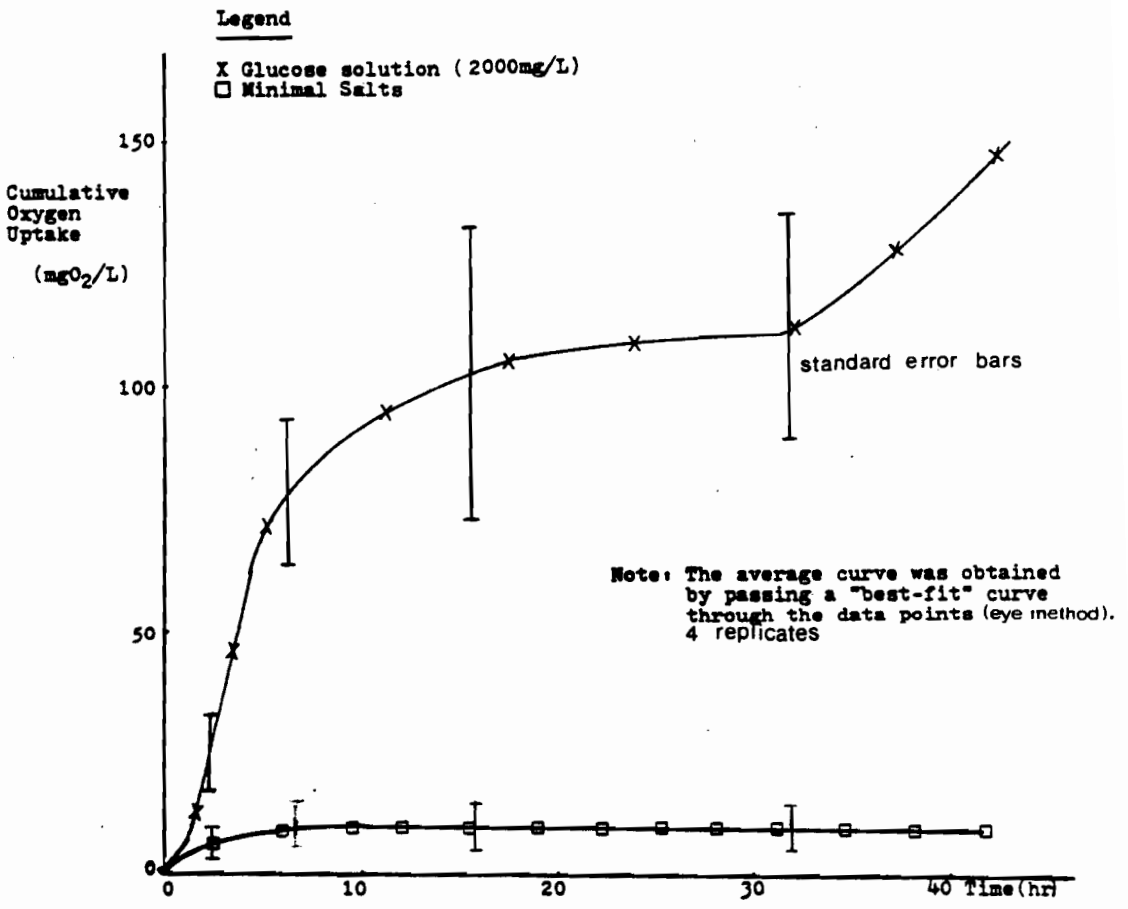


Figure 12. Cumulative oxygen uptake curves of a Pseudomonas species inoculated on glucose solution and incubated at 20°C.

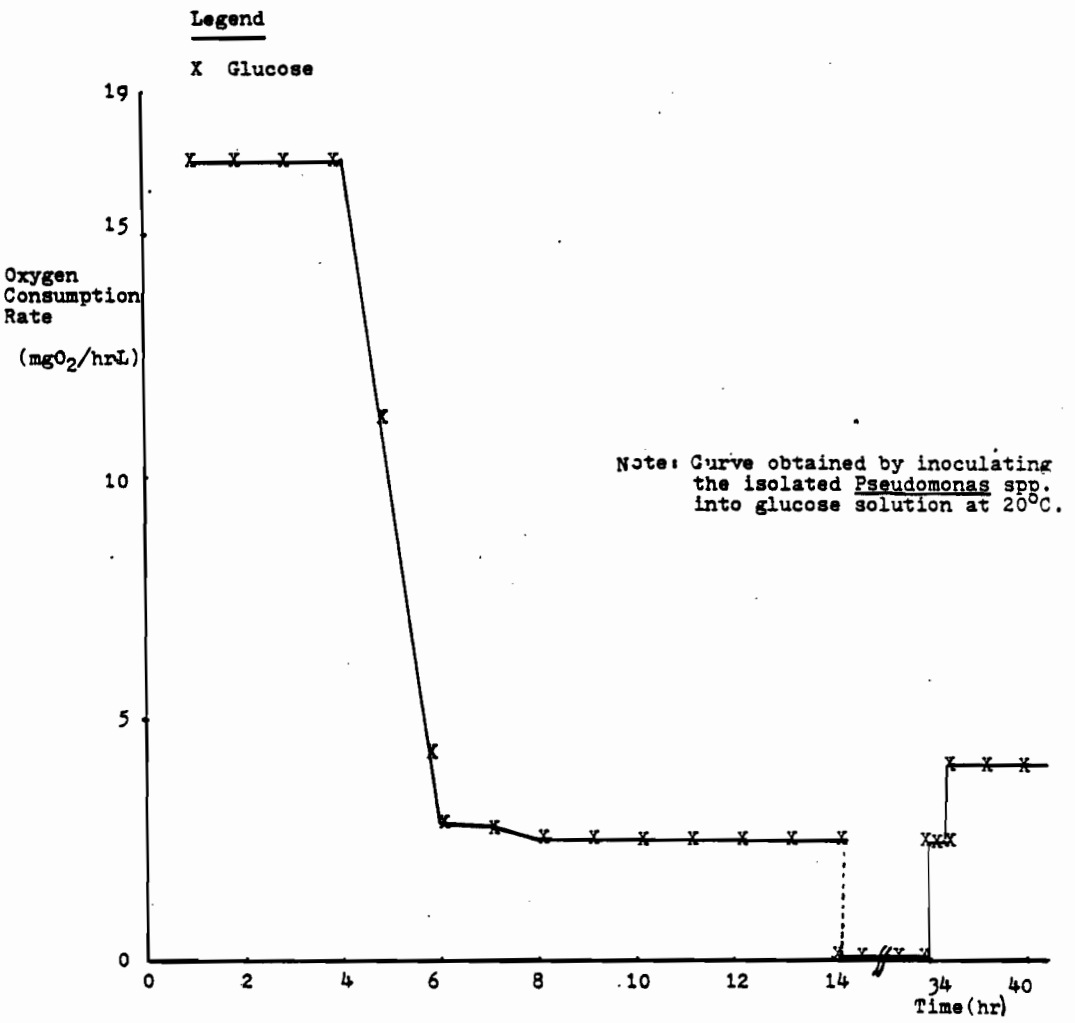


Figure 13. Oxygen consumption rate curve, from slope of Figure 12.

5.3.2 EXPERIMENTS CONDUCTED WITH POLYMER C AS A SOURCE OF CARBON

The cumulative oxygen uptake curves generated from the experiments conducted with Polymer C solution as a nutrient can be seen in Figure 14. The average curve has been normalized as described previously. Once normalization was performed, the data points showed a relatively small deviation from the mean curve.

The generated mean oxygen uptake curve for Polymer C reaches a cumulative oxygen level of 110mg/L after 15hr of incubation at 20°C, which is higher than the glucose curve (compare Figures 12 and 14). Note that the sample curves were normalized using the glucose curve (Figure 12), hence a higher oxygen uptake represents a significant increase in oxygen consumption.

Figure 15 shows that, as with the glucose curve (Fig. 12), a high oxygen uptake can be seen at the beginning of the experiment and this slowly decreases in the next few hours. It can also be noticed here that the oxygen consumption rate for Polymer C solution is similar to the glucose consumption rate curve. Both curves show an initial

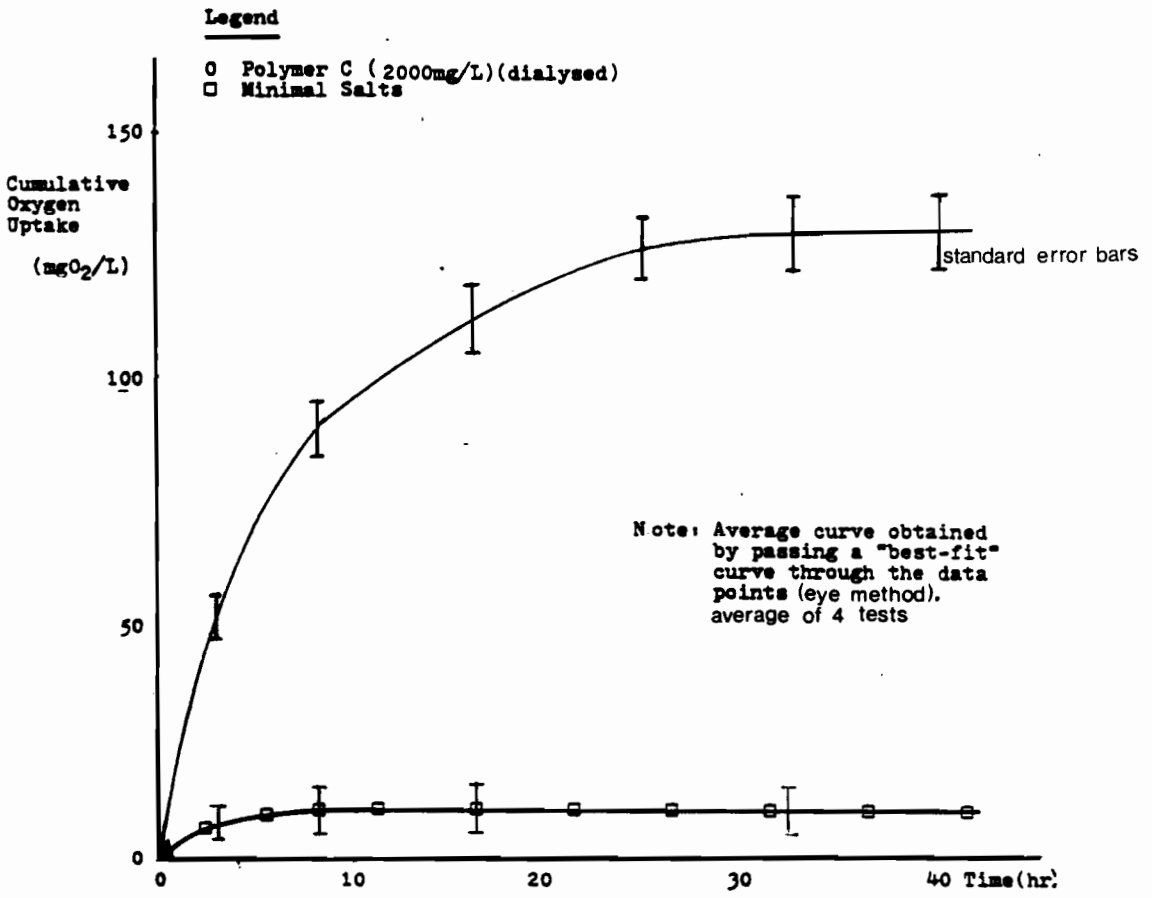


Figure 14. Cumulative oxygen uptake curves of a Pseudomonas species inoculated on Polymer C solution and incubated at 20°C.

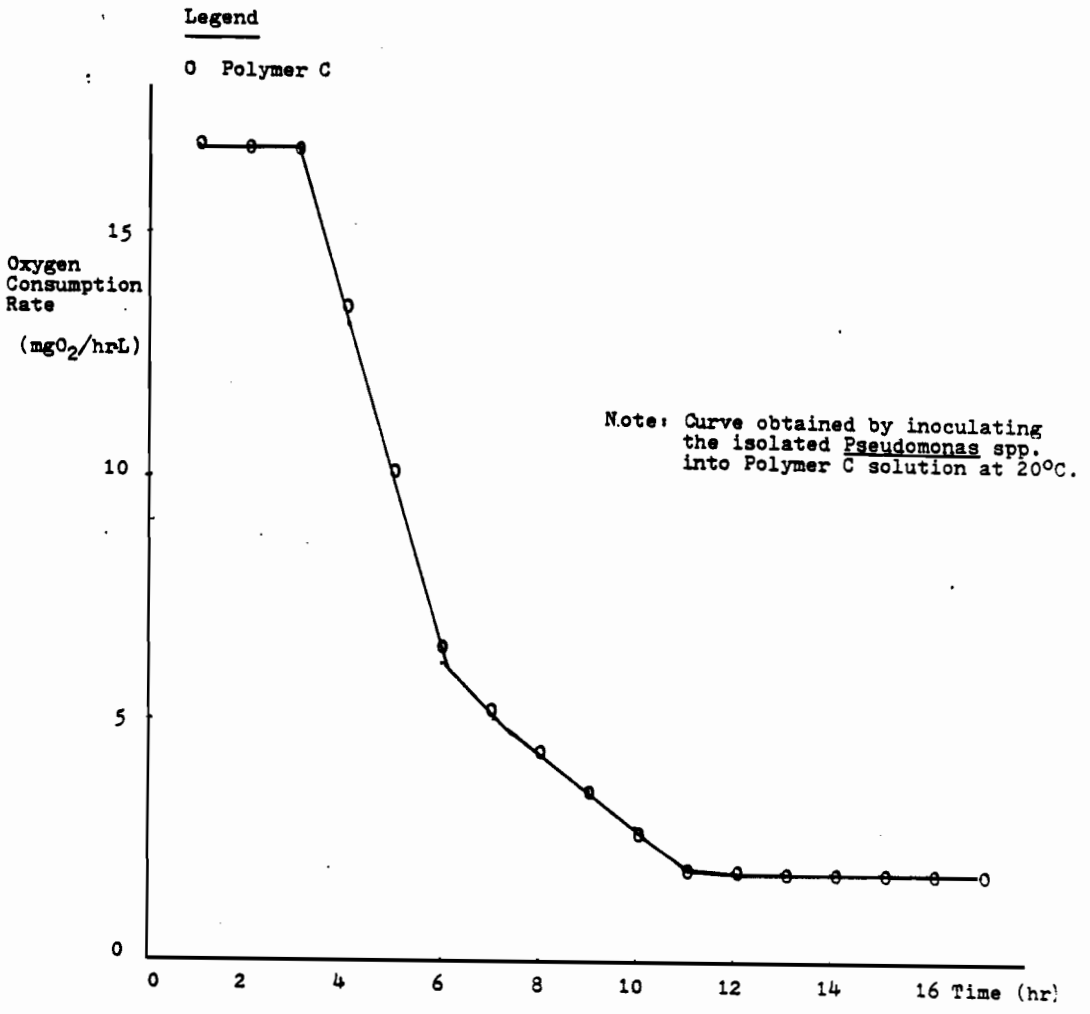


Figure 15. Oxygen consumption rate curve, from slope of Figure 14.

oxygen consumption of 16.6mg/hr-L, but in the case of Polymer C, the high consumption rate lasts only for 3hr. However, the decrease in oxygen consumption rate for Polymer C is more gradual, with a final nadir of 1.8mg/hr-L reached after 11hr of incubation, compared to 6hr in the case of glucose.

5.3.3 EXPERIMENTS CONDUCTED WITH MONOMER C

Monomer C cumulative oxygen consumption curves are presented in Figure 16. Contrary to Polymer C, but similarly to glucose, the experimental data points are scattered with a noticeable high deviation from the mean curve. The "fitted" mean curve exhibits a rapid oxygen consumption for the first 4hr of incubation, followed by a gradual decline. After 13hr of incubation, a cumulative oxygen consumption level of 110mg/L could be observed. It is interesting to note that some data points have a tendency to reach this same 110mg/L faster than the mean curve. In general, the oxygen consumption appears to be more rapid for Monomer C, than for glucose or Polymer C. This faster consumption rate, however, could not be noticed by studying the mean cumulative oxygen curve for Monomer C (Figure 16), since some lower data points have a negative effect on the normalized curve.

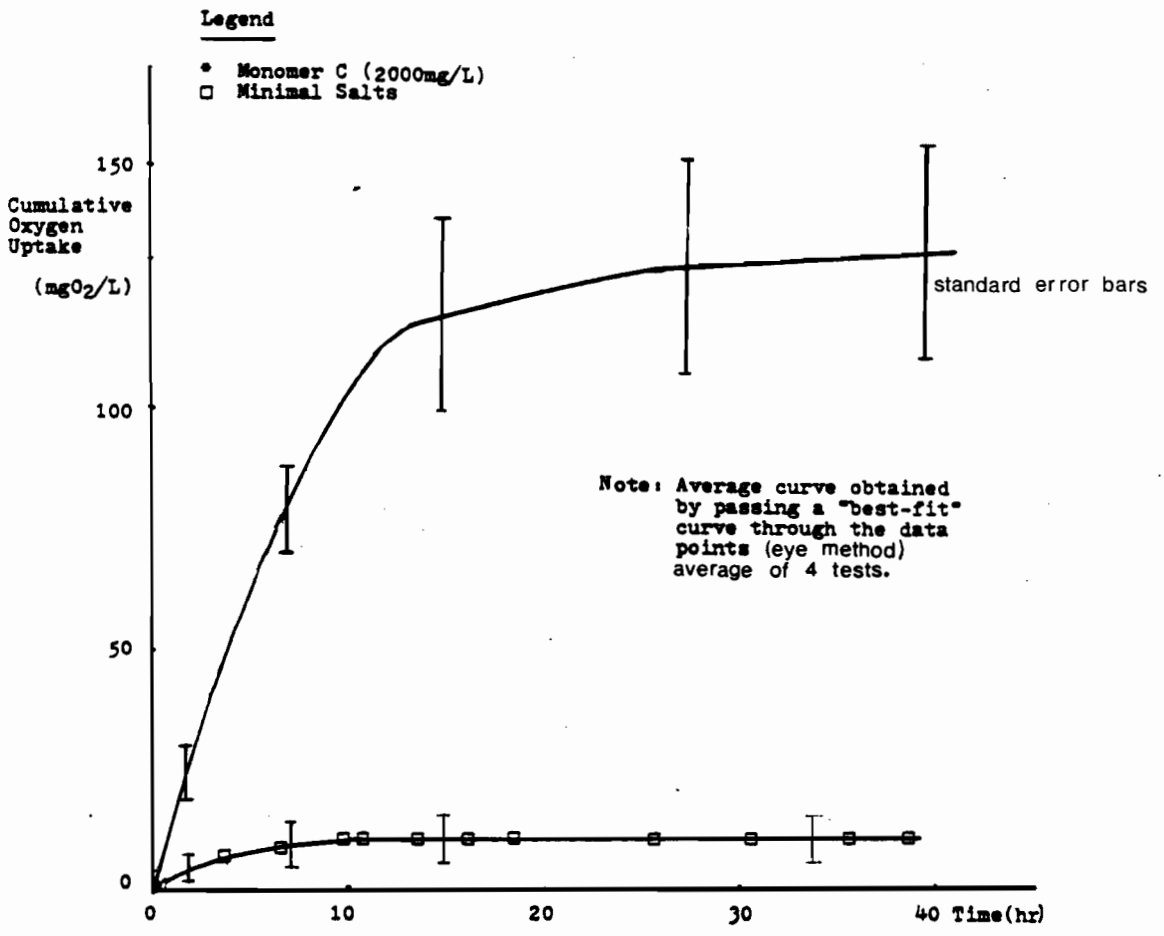


Figure 16. Cumulative oxygen uptake curves of a Pseudomonas species inoculated on Monomer C solution and incubated at 20°C.

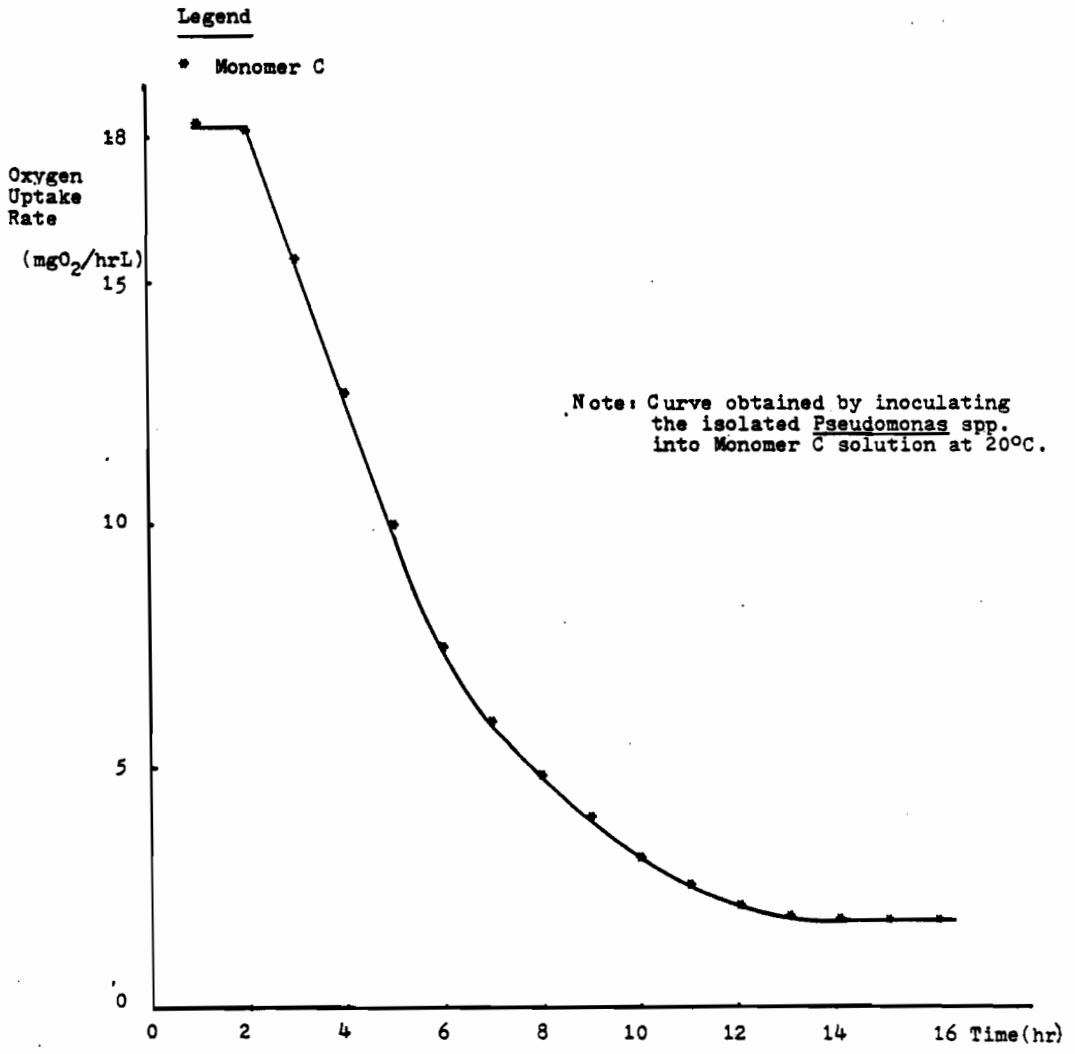


Figure 17. Oxygen consumption rate curve, from slope of Figure 16.

When the oxygen uptake rate is calculated from the mean curve, an initial oxygen uptake of 18.2mg/hr-L is established (Figure 17). However, this high rate could only be observed during the first 2hr of incubation. As with Polymer C and glucose, the high oxygen uptake is followed by a progressive decline, reaching a nadir of 1.8mg/hr-L after 14hr.

Among the 3 analyzed oxygen uptake rate curves (Figures 13, 15 and 17) the one generated by inoculating Monomer C as a sole carbon source exhibited the highest rate of oxygen uptake, for the shortest time period. Monomer C curve also attained its nadir at 1.8mg/hr-L, after the longest time of incubation. It is interesting to note that this final nadir is equal to 1.8mg/hr-L for both Monomer C and Polymer C, whereas it reaches 2.5mg/hr-L for glucose.

5.3.4 LONG-TERM EXPERIMENTS

A number of complementary experiments was conducted in connection with the Polymer-Monomer C series of experiments.

The first question arising from the results expressed so far, is related to the nadir reached in all generated curves after a certain period of incubation. Is this decrease in metabolic activity a final step, or an intermediary stage? In order to answer this question, 4 days incubation experiments were conducted with various substrates, but particularly with dialyzed Polymer C as a sole carbon source. The cumulative oxygen uptake curve obtained for Polymer C after 4 days is presented in Figure 18 and more detailed in Figure 19. It can be noticed that the same nadir as expressed in Figure 14 was reached after 15hrs of incubation. In addition, a second rise in metabolic activity appeared after 65hr. This second activity period raised the cumulative oxygen consumed from 125mg/L to 210mg/L in 35hr of incubation. Such a phenomenon suggests that the biodegradation of Polymer C is a stepwise process. The first metabolic activity period (Figure 18) which extends to 20hr of incubation, correlates in shape and level with the mean oxygen uptake curve for Polymer C, presented in Figure 14.

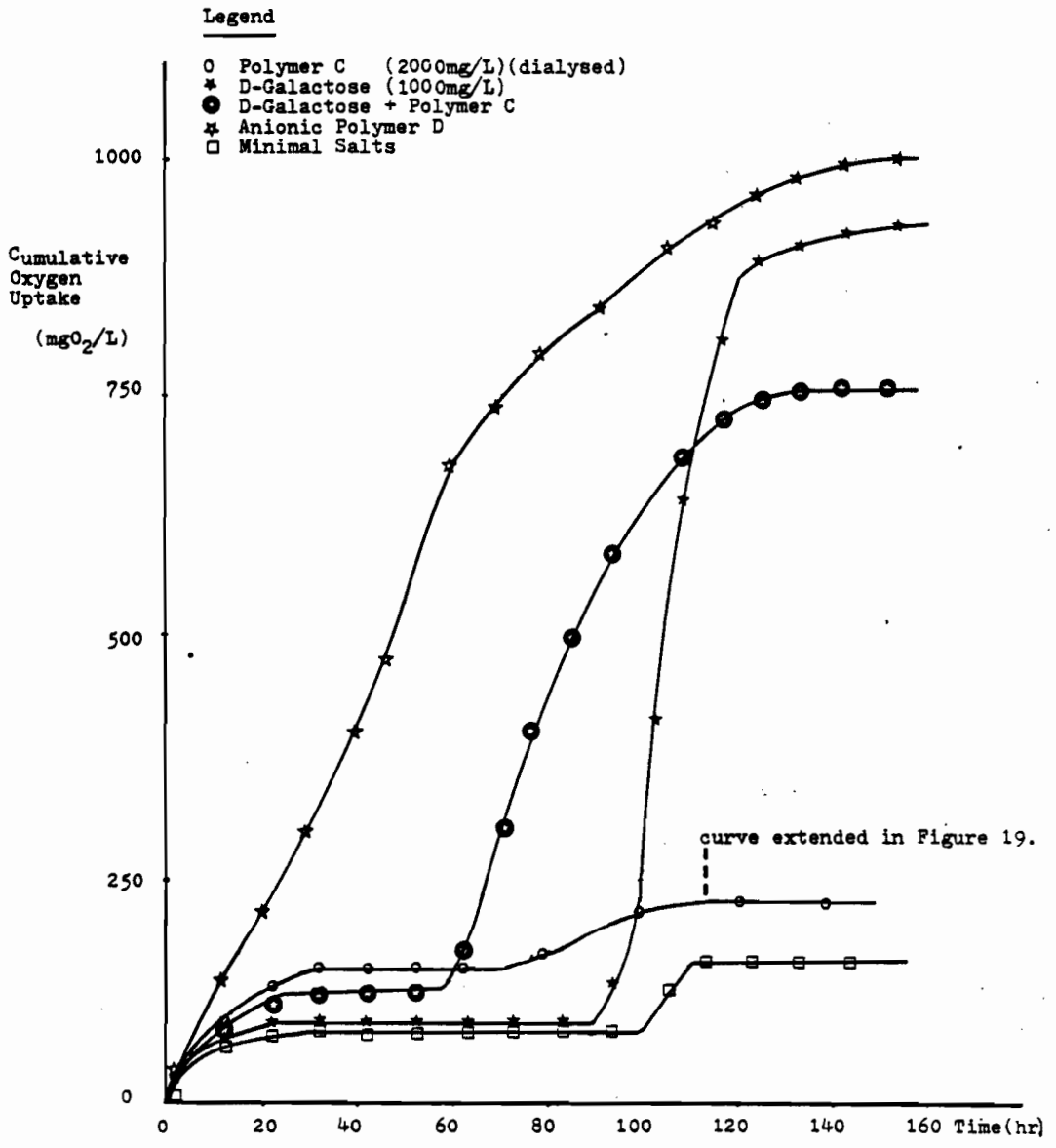


Figure 18. Cumulative oxygen consumption curves of a Pseudomonas species inoculated on a variety of nutrient solutions and incubated at 20°C.

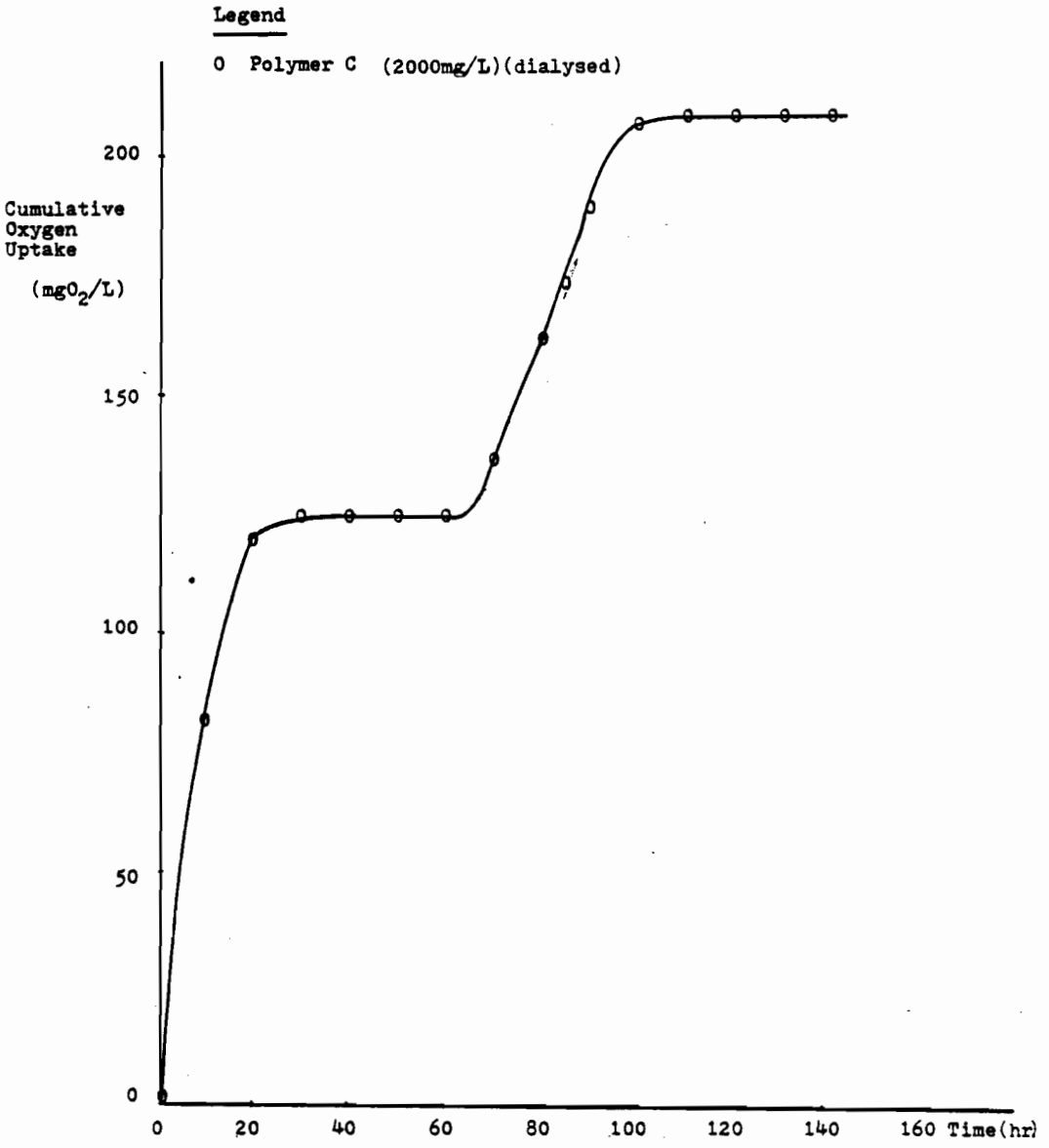


Figure 19. Cumulative oxygen uptake curve of a Pseudomonas species inoculated on dialyzed Polymer C and incubated at 20°C.

5.3.5 NUTRIENT RESIDUAL EXPERIMENTS

The second question resulting from the experiments conducted so far, relates to the real nutritional availability of the polymer and its monomer. Although inoculation in minimal salt demonstrated that the strain used was unable to grow on salts alone (as autotrophs), a nutrient residual experiment was conducted. The objective was to prove by chemical analysis that the substrate did effectively decrease in concentration, as a result of assimilation. The initial and final substrate concentrations were therefore analysed by Chemical Oxygen Demand (COD) tests after 20hr of incubation, after separation of the cells from the liquid by centrifugation at 10,000rpm.

The results of this analytical test are presented in Table 3. They demonstrate that the concentration of Polymer C, Monomer C and glucose did decrease with time. These results can be interpreted as bacterial nutrient consumption since polymer adsorption on the cells became negligible after addition of salts prior to COD analysis. The addition of salts reverses ionic charges, liberating the adsorbed polymer from the cell. Hence, in theory, the concentration of nutrient recovered and analyzed can be used to calculate microbial assimilation.

Table 3. NUTRIENT RESIDUAL EXPERIMENTS CONDUCTED WITH THE ISOLATED PSEUDOMONAD.

Sample	Concentration (mg/L)	Initial conc. (mg/L)	Final conc. (mg/L)	Average uptake (%)
Pol.C	2000	650	400	38.5
Mon.C	2000	800	544	32.0
Glucose	2000	675	435	32.9

In all tests, optical density (O.D.) measurements were conducted on the inoculated sample, prior to and after incubation. The O.D. of seeded glucose, Polymer C and Monomer C solutions were always higher at the end of the experiment than initially. The numerical results of such measurements are not presented in this study, since the purpose of these measurements was simply to confirm that growth had effectively occurred, and therefore that oxygen uptake could be correlated to growth.

5.3.6 EXPERIMENTS CONDUCTED WITH POLYMER C AS A SOURCE OF CARBON AND NITROGEN

Based on the previous results, and the fact that DMDAAC polymer includes nitrogen, it was decided to test the polymer as a source of both carbon and nitrogen for the Gram negative rod. Oxygen uptake measurements were therefore performed on Polymer C solution with nitrogen-free minimal salts. The normalized cumulative oxygen uptake curves for both Polymer C and glucose in the absence of an additional nitrogen source are presented in Figure 20. It can be noticed that the glucose medium (nitrogen free) supported solely endogenous respiration and its oxygen consumption is comparable to the minimal salts control. The polymer was able to induce some metabolism and cause an average of 60mg/L of oxygen to be consumed in 15hr. This oxygen consumption is lower than with the presence of inorganic nitrogen in the salts (Figure 14) but it remains significant. It can also be noticed that a plateau was reached very soon after initiation of incubation (about 6hr).

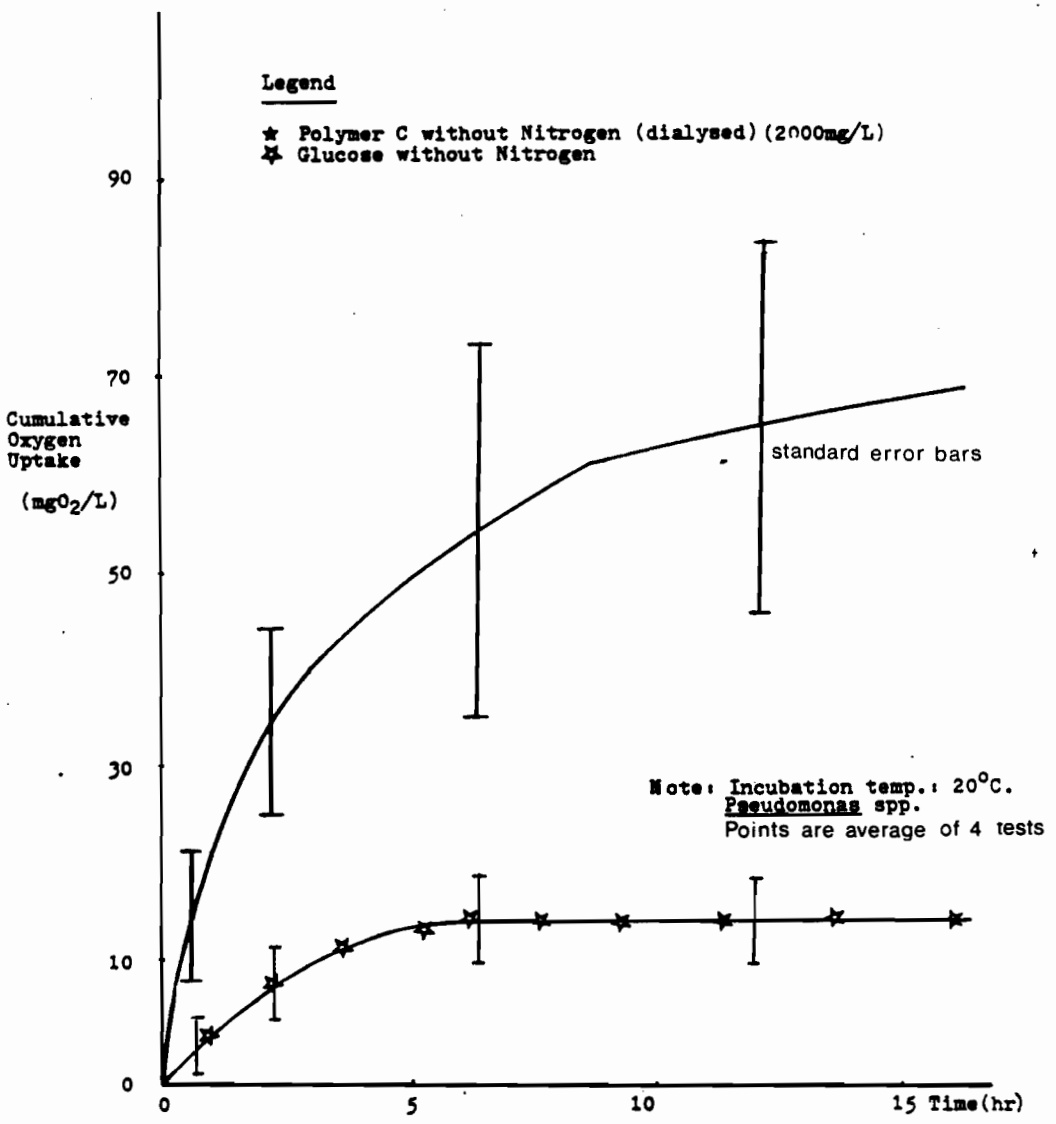


Figure 20. Cumulative oxygen uptake curves for Polymer C and Glucose without Nitrogen.

5.3.7 EXPERIMENTS CONDUCTED WITH GALACTOSE

The controls had been set as glucose solutions for all experiments so far, glucose being the classical carbon source used for similar types of assays. But most Pseudomonas (contrary to most microorganisms), cannot catabolise glucose, since the Embden-Meyeroff (sugar catabolic pathway) is generally absent in the pseudomonads catabolic functions. Some strains however, possess the Entner-Doudoroff pathway, which oxidises glucose to various end-products (pathway described in Appendix 2).

Although the isolated strain showed the ability to metabolize glucose and was therefore likely to possess the Entner-Doudoroff pathway among its various metabolic pathways, experiments were conducted with a different carbon source, in this case galactose. The objective was to confirm the validity of the experiments where glucose had been used as a control.

The various galactose experiments run (Figures 18 and 21) demonstrated that within incubation periods of 60hr, the oxygen uptake for this sugar was similar to glucose or DMDAAC polymer.

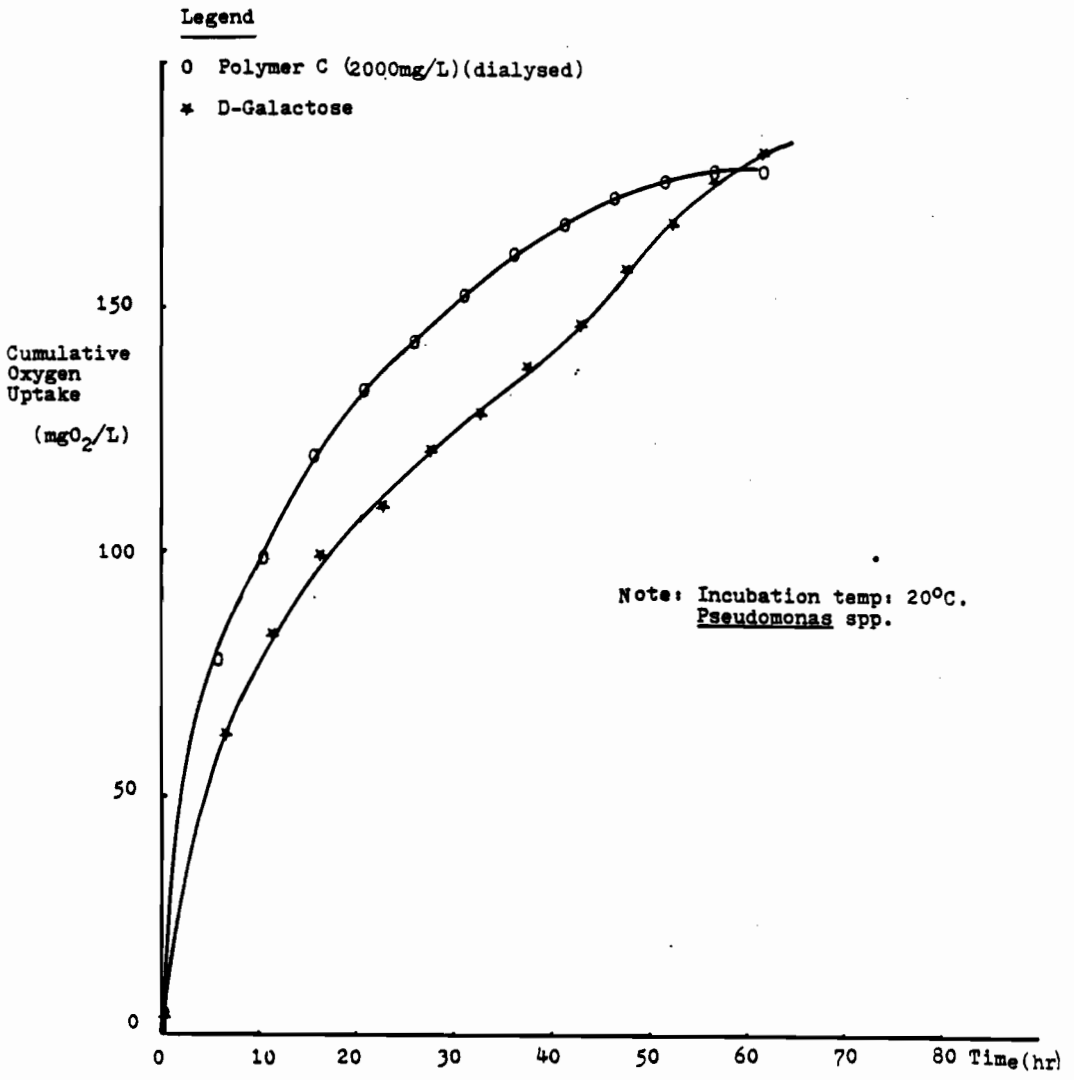


Figure 21. Cumulative oxygen uptake curves for Polymer C and D-Galactose.

It was concluded that for the purpose of this study, glucose could remain as a control, since in most cases short experiments were conducted (less than 48hr).

5.3.8 EXPERIMENTS CONDUCTED WITH POLYMER C AND AN AVAILABLE SUGAR

The results obtained so far suggested that the isolated Pseudomonas strain was able to biodegrade Polymer C and utilize its carbon and nitrogen as nutrients. But would aquatic bacteria, growing in the presence of other sources of carbon such as sugars, still metabolize the polymer? Or would they rather give preference to the available sugar? Such questions are of major importance for this study, since polluted streams normally contain a large variety of available carbon sources. If the microorganisms have preference for other sugars in-situ, the results of this study would be of minimum value. To answer these questions, a solution containing both galactose and Polymer C was inoculated with the pseudomonad strain. The concentration of the polymer was analyzed initially and after 160hr of incubation. In both cases, any adsorbed polymer was removed from the cells by addition of sodium chloride. Analysis was

conducted by titrating the polymer with 0.3216g/L PVSAK. Figure 18 presents among various curves the cumulative oxygen uptake curves, obtained for both substrates. It can be seen that the metabolic activity is enhanced in the mixed solution when compared to pure Polymer C, but is lower than with 100% galactose. The plateau existing in all curves is shortened by 30hr when the strain is inoculated in both nutrients. The increase in metabolic activity occurs at approximately the same time period as the 100% Polymer C curve.

Titration results showed that after 160hr of incubation, the concentration of polymer in solution had indeed decreased. It was therefore concluded that polymer biodegradation did occur. A control flask consisting of Polymer C solution without inoculum, was also carried for 160hr to confirm that spontaneous polymer degradation would not interfere with the results. Titration of the control sample, confirmed that in fact no significant autodegradation had occurred in 4 days at 20°C.

5.3.9 ENZYME ACTIVITY EXPERIMENTS

The large molecular size of Polymer C suggested that extracellular cleaving was necessary, prior to any possible catabolic activity. The existence of one or more exoenzymes was therefore suspected. Enzymatic experiments were conducted to demonstrate the presence of this active extracellular enzyme(s), which could cleave the polymer into smaller fragments. These would be able to penetrate the cell and serve as nutrients. Besides, exoenzyme synthesis has been recognized as being part of the great adaptation ability of the pseudomonads (Inove, et al, 1963; Kobori and Taga, 1980; Maeda and Taga, 1976; Markovitz, et al, 1956; Morihara, 1963; Robyt and Ackerman, 1971; Zucker and Hankin, 1970).

This experiment was designed to encourage considerable cell growth on Polymer C; exoenzyme(s) synthesis would then be induced. After 12hr of incubation, the cells were centrifuged at 12,000rpm for 20min in a refrigerated centrifuge. The supernatant fraction containing the exoenzyme(s) was then enriched with DMDAAC polymer, shaken at room temperature (28°C), and the concentration of polymer was analysed by the PVSAK titration method at regular time intervals. Polymer C had to be added to the supernatant

fraction, since most of the polymer present before centrifugation was adsorbed on the cells and therefore eliminated from the liquid interphase with the cells. As mentioned, centrifugation separated the cells from the liquid interphase together with the nutrient polymer, but some adsorbed exoenzyme may also have settled with the cells. However, the loss of extracellular enzyme did not affect the experimental results since this exoenzyme assay has been set as a qualitative test and measurement of the exact concentration of enzyme synthesized is beyond the scope of this study.

Measurement of polymer concentration was done by a method which could distinguish between the polymer and its smaller fragments. The PVSAK titration method, developed by Wang and Shuster (1974) was chosen since it consists of the measurement of charge by titration. As the polymer deteriorates, it loses its charge, requiring therefore less titrant. An analytical technique such as COD or Total Organic Carbon (TOC) was not suitable, since there was no carbon consumption, only a cleavage in the molecular structure.

The various experimental curves presented in Figure 22 show that the concentration of Polymer C decreases with time, until an asymptote is reached. It can be seen that

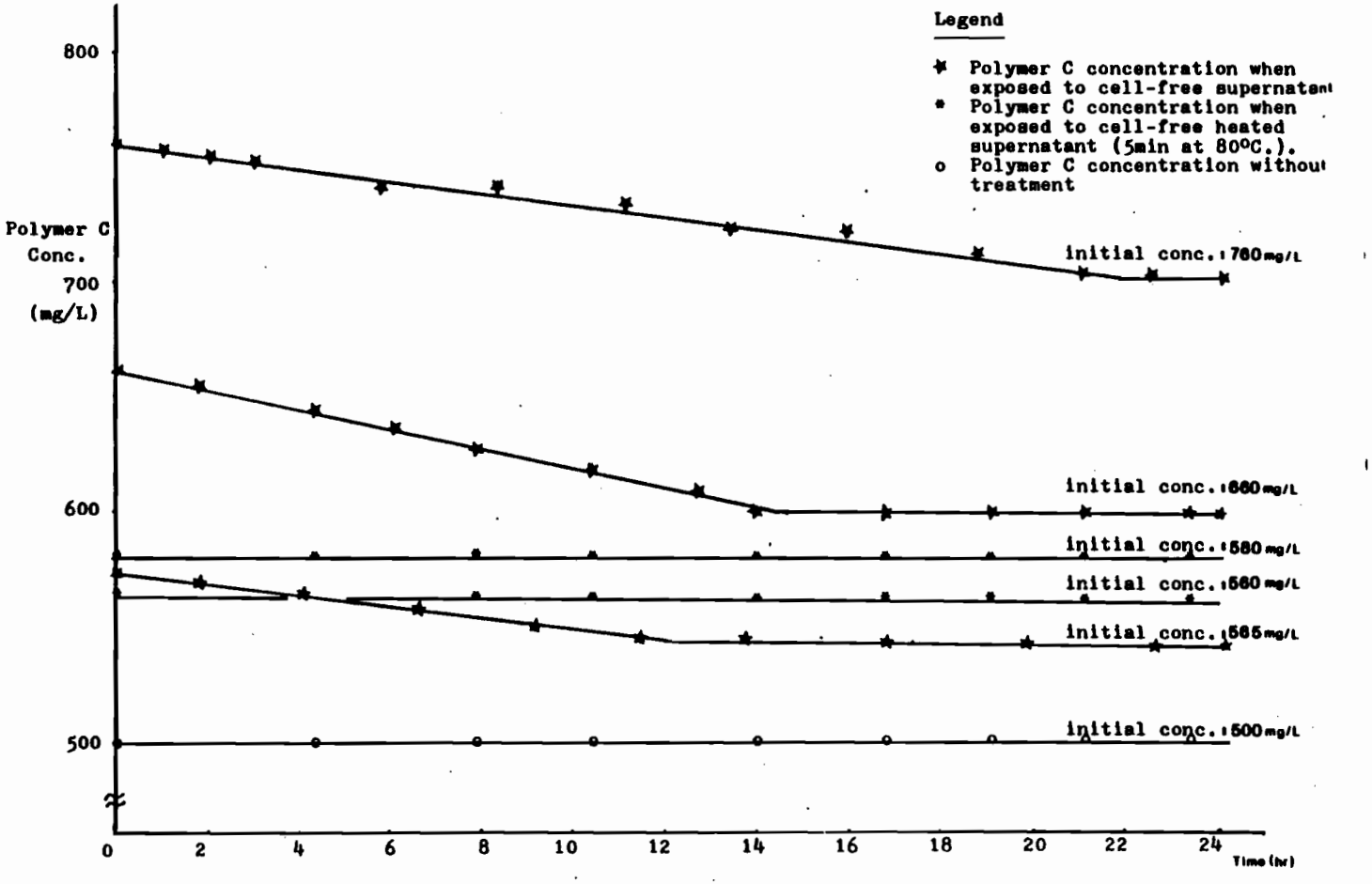


Figure 22. Extracellular Enzyme Activity Curves

the slope of polymer degradation is virtually independent of the initial concentration of polymer, but as the polymer concentration increases, the asymptote is reached later. It can also be noticed that the active excenzyme is heat-sensitive, since a short heating for 5min at 80°C inhibited its hydrolytic activity. Finally, the control (polymer without enzyme) shows that at the polymer concentrations used, Polymer C did not autodegrade.

5.3.10 BIODEGRADATION OF OTHER POLYELECTROLYTES

The biodegradation of other polyelectrolytes, also used as coagulants, was tested by inoculating the pseudomonad into solutions containing these different polymers. Among these polymers, 4 cationic and one anionic polyelectrolyte were assayed. Figure 23 presents the various oxygen uptake curves generated for each specific polymer. All curves show a similar trend, with a high uptake rate for the first 10hr, followed by a plateau. Polymer D, an anionic high molecular weight acrylamide-based polyelectrolyte exhibited the highest overall oxygen uptake rate over the first 30hr; 525mg/L oxygen was consumed after 50hr of incubation. Polymer 2319/4 (a cationic acrylamide-based polymer), also enhanced microbial activity, with an oxygen uptake curve

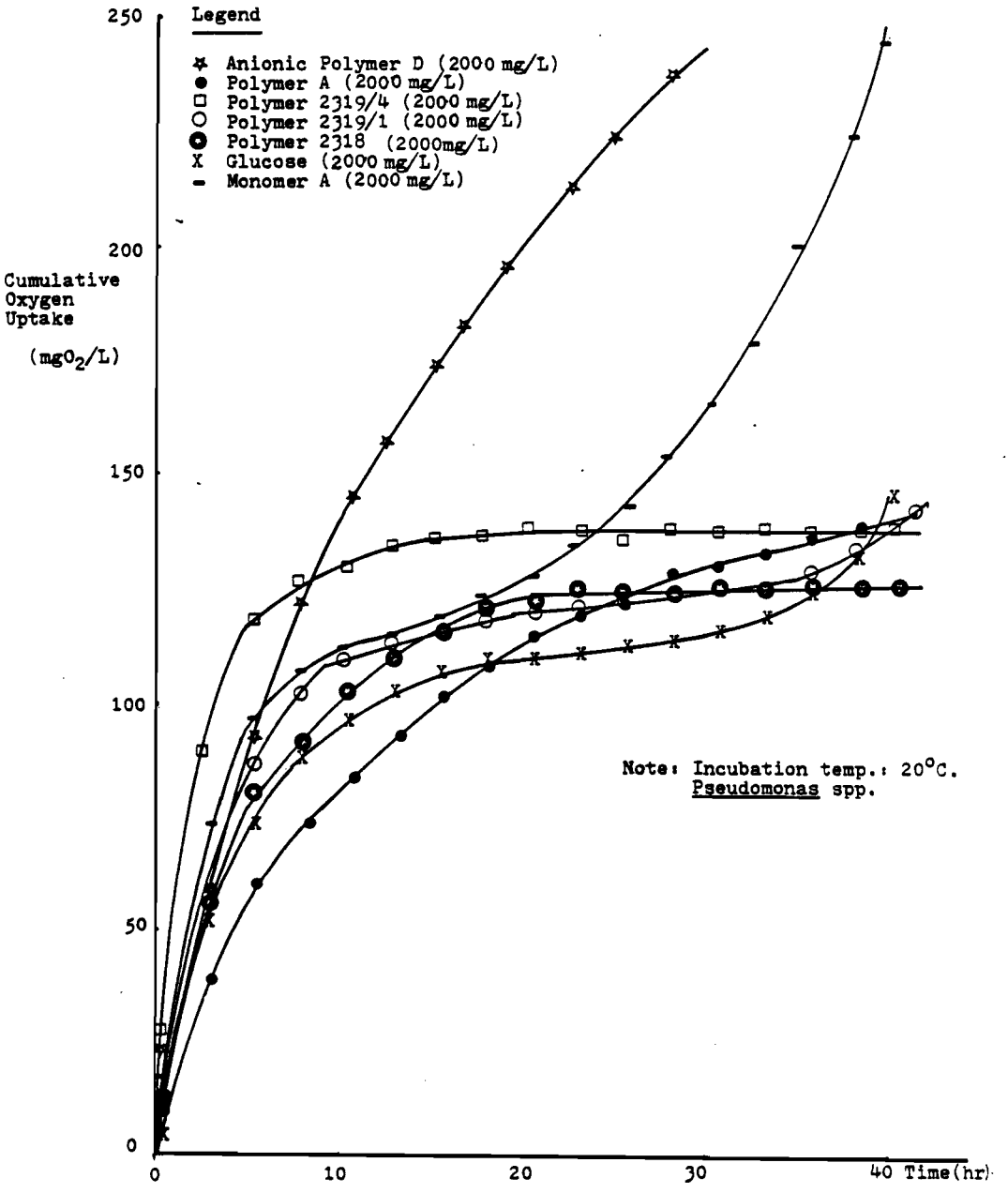


Figure 23. Cumulative oxygen consumption curves for a variety of polymers.

similar to glucose. Polymers A and 2319/1 (both cationic), supported microbial growth at a moderate rate, with almost 200mg/L of oxygen consumed after 50hr. The oxygen consumption induced by Polymers A and 2319/1, although representing signs of metabolism, is lower than the other tested polymers. A more detailed description of the polymers tested in this section is presented in section 6.3.8.

Figure 23 also presents the cumulative oxygen uptake curve for the pseudomonad when inoculated on Acrylamide (Monomer A). The curve exhibits a high metabolic activity induced by the Monomer after 20hr of incubation. Before 20hr, the cumulative oxygen curve is similar to other curves presented in Figure 23, but the inoculated bacterium starts consuming oxygen at a faster rate after the first day of incubation, reaching 250mg/L after 40hr. This result is unexpected when compared to the activity induced by Polymer A, but similar oxygen uptake curves can be observed in the case of glucose and galactose (Figures 23 and 18 respectively). Monomer A seems therefore to be a readily available source of carbon for the pseudomonad.

The Pseudomonas strain isolated from the river has therefore exhibited the capability to adapt to many types of polymers and utilize them as nutrient sources. It is

interesting to note that Polymer D (an anionic polymer), was soon colonized when stored at 10°C by various microorganisms, and in particular fungi, as evidence by fungal mycilia and turbidity in the polymer stock solution.

5.3.11 SLUDGE - SUPERNATANT TESTS

Flocculants in the form of polyelectrolytes are added to the sludge in water and wastewater treatment processes to enhance solid-liquid separation. It is important to investigate the effect of the added polyelectrolyte on the microorganisms present in the sludge.

To approach real conditions, both polymers A and C were added to a sample of 2l of activated sludge mixed liquor. Complete mixing was insured by rapid mixing at 100rpm for 30s followed by slow mixing at 30rpm for 20min. The sludge was allowed to settle and the liquid phase separated. The settled sludge was then enriched with glucose and minimal salts, and metabolic activity was monitored by oxygen uptake measurements.

High levels of oxygen uptake was exerted by the sludge (Figure 24) when a concentration of 10mg/g of Polymer C was

added to the mixed liquor. The oxygen consumed by the sludge was as high as 1300mg/L after 30hr of incubation. Based on the concentration of polymer added (when compared to 2000mg/L in previous tests), it is evident that most of the oxygen consumed is due to the biodegradation of nutrients present in the supernatant fraction and not to the polymer itself. However, the sludge with Polymer C showed a tendency to take up more oxygen than the blank (no coagulant-aid added) after 35hr of incubation. It can also be noticed that the blank sludge stops consuming oxygen after 70hr of incubation, whereas the polymerized sludge continues consuming oxygen.

The clear supernatant fraction recovered from the settled mixed liquor was inoculated with minimal salts and the isolated Pseudomonas strain, but no additional source of nutrients was added. Titration of this same supernatant fraction with 0.3216g/l PVSAK indicated that not all the polymer had settled with the sludge, since a concentration of 3.5mg/L of polymer remained in solution (see Table 4).

Oxygen taken up by microorganisms present in the blank reached 620mg/L after 45hr of incubation (Figure 25), but the supernatant enriched with polymers A and C exhibited a lower oxygen uptake. This result was unexpected and its significance will be discussed in the following section.

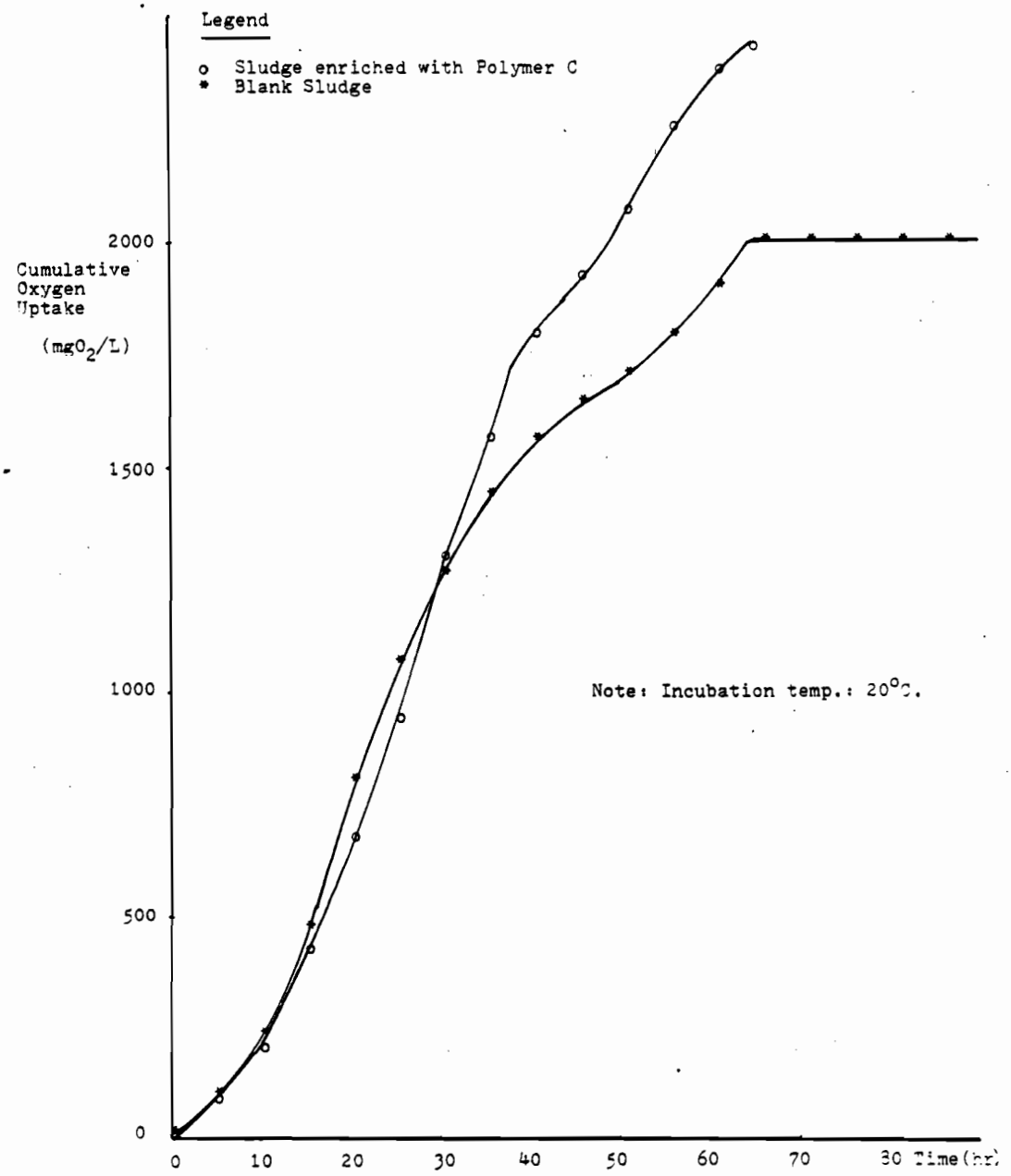


Figure 24. Cumulative Oxygen Uptake Curves Generated from Sludge-Toxicity Tests.

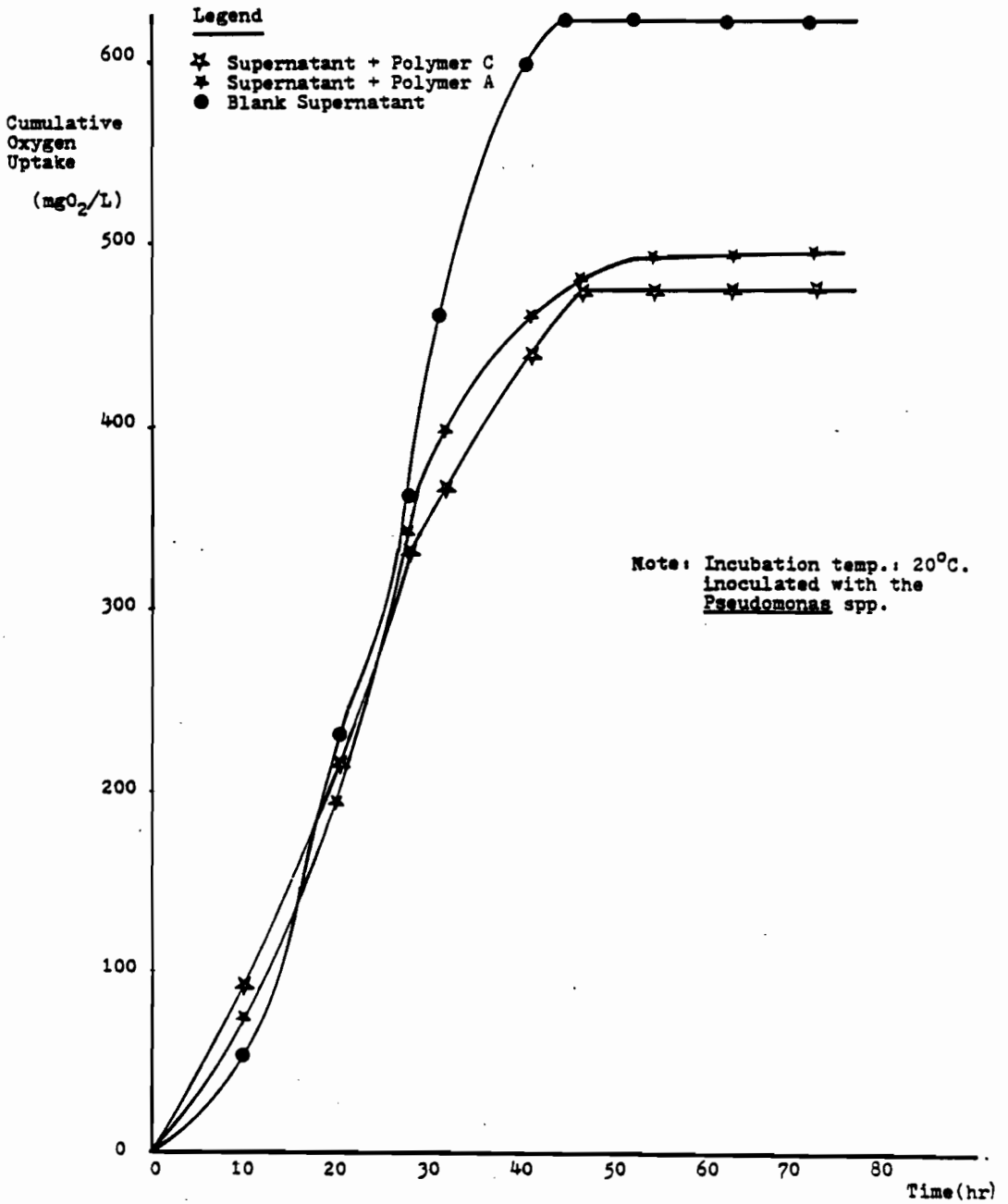


Figure 25. Cumulative Oxygen Uptake Curves (Sludge Supernatant Tests)

The availability of other sources of nutrients in the supernatant fractions enriched with polymers, and the unusually high oxygen consumption exerted by the pseudomonad in the presence of such a low concentration of polymer (only 3.5mg/L of Polymer C and 3.2mg/L of Polymer A), leads to the question of whether the polymer really was being used as a carbon source. To answer this, the supernatant fractions were tested for their concentration of polymer after 85hr of incubation (as with previous titrations, Sodium chloride was added prior to centrifugation). The results given in Table 4 show that both polymers did effectively decrease in concentration.

Table 4. CONCENTRATION OF POLYMERS IN SUPERNATANT FRACTION BEFORE AND AFTER INCUBATION.

Polymer A		Polymer C	
before incub. (mg/L)	after incub. (mg/L)	before incub. (mg/L)	after incub. (mg/L)
3.2	2.3	3.5	2.5

6.0 DISCUSSION OF RESULTS

6.0 DISCUSSION OF RESULTS

6.1 THE EFFECT OF POLYELECTROLYTES ON FRESHWATER MICROBIAL MIXED CULTURES

The results obtained in this series of experiments are important because they represent the first positive results achieved of the effect of polyelectrolytes used as coagulant-aids on aquatic bacteria. Although these preliminary results are rudimentary, they permitted one to gain a general understanding of the effect of the tested polymers on the mixed microbial population and therefore to establish the remaining series of experiments. Since little literature on the subject has been published, any possible "effect" could be expected, ranging from toxicity to the aquatic bacteria, to microbial biodegradation of the polymers. The results obtained are of major importance to the water quality of receiving streams. If the tested polymers would show toxicity to the inoculated aquatic microorganisms, the natural phenomenon of water self-purification might be inhibited with disastrous consequences. If, on the other hand, biodegradation of the polymers would occur, toxicity to aquatic animals, such as presented by various authors (Biesinger et al, 1976;

Spraggs, et al, 1982) could be inhibited or enhanced. However, inhibition of toxic effects to aquatic organisms could only be useful if the degradation intermediate and end-products would be shown to have no effect on the aquatic biota. Such toxicity tests are however beyond the scope of this study.

It is interesting to observe from Figures 3, 4, 5, 8 and 10 that neither Polymer A nor Polymer C inhibited microbial growth nor oxygen uptake when compared to the glucose control curves (Figures 3, 4, 5, and 6). Since the inoculum consisted of a mixed culture of freshwater microorganisms, it is only possible to state that the polymers tested did not inhibit overall cell growth and activity. It is in fact impossible to comment on growth inhibition of a particular species.

Polymer A solution (2000mg/l) supported some growth (Figures 3 and 7), although not as high as the glucose control. This result contradicts the literature, which states that generally, polyacrylamides are non-biodegradable, unless prior chemical degradation is initiated (Suzuki et al, 1976). In fact, the microbial activity observed in Figures 3 and 7, could be attributed to both the presence of ethanol added to dissolve the solid polymer (1.0mL of 95% ethanol in 200mg/L Polymer A

solution), and impurities present in the solid polymer. But, when complemented with a carbon source such as glucose (Figures 4, 5, 7 and 9), Polymer A, as well as Polymer C significantly enhanced microbial growth. This growth induction could be explained by the fact that although the polymers cannot be utilized as sole carbon sources by most species, they may be biodegraded to a certain extent and serve as growth factors, supplying carbon and nitrogen. It is also possible that impurities present in the stock polymer solution may have contributed as growth factors. The combination of either one or both sources would then enhance microbial growth in the presence of glucose.

When examining Figure 5, where the polymers are present in a smaller fraction, it can be seen that the growth curves are enhanced by 64% and 86% for polymers A and C respectively. Such an increase in growth could not be caused solely by impurities. In fact, further research (see section 6.3) demonstrated that all monomers and polymers assayed in the first two series of experiments were biodegraded and assimilated by at least one microbial species present in the inoculum. Plate counts demonstrated that this species was present in significant quantities in the inoculum, but was not in the majority. Based on this knowledge, it is possible to assume that this Pseudomonas, present in all experiments, would perform polymer

biodegradation, therefore liberating in the solution additional growth factors such as nitrogen and carbon. These liberated growth factors could then enhance growth of other microorganisms also present in the sample. However, in order for these growth factors to be effective, an available source of carbon such as glucose has to be present in significant concentration, to serve as a primary nutrient source. It is indeed possible to relate the amount of sugar available and the growth enhancement caused by the above mentioned factors. Figures 4 and 9, which present curves where 1000mg/L of glucose and 1000mg/L of Polymer C are present, show that growth enhancement is lower than in Figure 5, where glucose solution represents 75% of the solution and Polymer C only 25%. In both curves, the polymer enriched curves are higher than the control curve (2000mg/L glucose), but the effect of the liberated growth factors is more significant in Figure 5, for both Polymer C and Polymer A.

In Figure 6, it is possible to notice a difference between the oxygen uptake curves of the solutions containing 2000mg/L glucose and 1000mg/L glucose. In fact, the less concentrated samples induce oxygen uptake until a plateau is reached; the richer samples induce the same initial oxygen uptake, but the curves do not display this plateau. It can therefore be concluded that 1000mg/L of glucose in such

solutions is not sufficient to sustain growth after 50hr of incubation. Glucose is thus a limiting factor in the two curves presented in Figure 6 (it is important to remember that the only difference between the 1000mg/L and the 2000mg/L glucose solution is the concentration of glucose; all other elements remain constant). Therefore, in the case where the low concentration of glucose limits growth, there are fewer microorganisms to benefit from the liberated growth factors. However, when glucose is not a limiting factor, other compounds such as nitrogen or phosphorus can limit microbial growth. In such a situation, the possible breakdown of Polymer C by a Pseudomonadacae known to be present in the inoculum, would liberate carbon and nitrogen atoms which could act as growth enhancers. The higher concentration of glucose has also been shown to induce microbial growth, and therefore there would be more microorganisms present in the sample to benefit from the liberated growth factors.

The optical density curves and oxygen uptake curves for 2000mg/L Polymer C solution (Figure 3 and 9 respectively) showed an absence of microbial activity, indicating that DMDAAC could not support microbial activity among most aquatic bacterial species. The organic carbon present in the polymer molecule is therefore unavailable to most or all microorganisms present in the inoculum. As with Polymer A,

Polymer C solution enhanced growth (Figures 4, 5 and 9) when complemented with an available carbon source such as glucose. The explanation of such a phenomenon would be similar to that of growth enhancement due to Polymer A.

Monomer A (or acrylamide), as with Polymer C could not serve as a sole nutrient source (Figure 5) and therefore did not support microbial growth. Unlike the two polymers mentioned, the monomer did not enhance growth in the presence of glucose (Figures 4 and 5), but rather inhibited growth as much as 37% for 21hr of incubation in a 1/3 mixture (Figure 5). Figures 4 and 5 may also be interpreted as a toxicity effect of Monomer A on most or some inoculated bacteria, since the growth curves obtained are in all cases lower than the control. However, oxygen uptake curves (Figure 8) contradicted this hypothesis, and proved that Monomer A did not inhibit oxygen uptake in the mixed microbial population. In other words, Monomer A did not show any toxic effect based on the overall oxygen consumption exhibited by the mixed aquatic culture. In Figure 10 where 1000mg/L of acrylamide was incculated with aquatic microorganisms, the microbial oxygen consumption was generally as high as the control vessel (glucose). Thus it can be concluded that acrylamide is not toxic to the majority of aquatic microorganisms inoculated. The fact that the tests conducted with Monomer A as a carbon source

did not exhibit microbial growth, is not necessarily related to the unavailablitity of the monomer as a nutrient to all microbial species. The absence of oxygen consumption by the microorganisms in the presence of acrylamide could be related to the presence of a long lag phase, where the microorganisms slowly adapt to the new medium. The existence of a long initial adaptation phase for acrylamide is supported by the work of Croll, et al (1974) and Lande, et al (1979). Both studies mention that acrylamide monomer spiked in a stream is effectively biodegraded, but generally a lag phase of a few days is observed. The existence of such a long lag phase is most likely related to the number of microbial species adapting to biodegrade the acrylamide. It is also important to notice that the above mentioned studies were conducted on polluted rivers, where the presence of other sources of carbon, more easily biodegraded, could be detected. Even under such conditions, the acrylamide decreased in concentration (as monitored by chromatography).

In addition to the tested polymers and monomers, Monomer C was also assayed for its effect on the microbial population. The results obtained were as predicted: Monomer C could not serve as a carbon source to the inoculated microorganisms, but as Polymer C, it enhanced growth when mixed with glucose.

In Figures 3 to 5, the control or glucose solution showed an initial lag phase as long as 14hr, where no microbial growth could be observed. Although the existence of this long lag phase cannot be discussed with precision since the microbial populations present in the media have not been identified, it is possible to assume that a longer lag phase was required since the microorganisms were maintained in the laboratory on nutrient agar slants incubated at 20°C. The medium used for the test was poorer in nutritional value than nutrient agar and the experiment was only conducted at 14°C. It is interesting to note that the Polymer A curves in Figures 3, 4 and 5 had a shorter lag phase than glucose. Once again, this phenomenon could be explained by the presence of both ethanol and impurities in Polymer A solution. Ethanol is a readily available source of carbon for a large number of microbes, whereas the nutrient value of the impurities cannot be discussed.

Although the final results obtained from both series of experiments are similar, it is interesting to note the major differences existing between the curves generated from the two different analytical methods. Firstly, it would be a mistake to interpret the oxygen consumption curves generated in Series B as growth curves. They cannot be considered as growth curves because actual population multiplication was not monitored. The oxygen consumption curves can however be

considered as metabolic activity curves, directly related to microbial aerobic respiration. Metabolic curves do not display the traditional growth curve shape, with its lag, exponential and stationary phases. This fact is related to the principle that cell respiration is not necessarily related to cell growth. As an example, it has been established that during the so-called "lag phase", an intensive metabolic activity does take place, where the cell, without multiplying, increases in size and adapts to new environmental conditions (Moat, 1979). This adaptation period involves the transformation and induction of a large number of metabolic pathways and their specific enzymes. As mentioned previously, bacteria induce the synthesis of some enzymes when required. But generally, before enzyme synthesis can be initiated, the specific substrate to be catalyzed must be present in the proximity of the cell. When microorganisms are inoculated into these substrates, a high rate of metabolic activity starts, necessary to synthesize all enzymes required for survival. This initial adaptation phase has also often been called "endogenous respiration" (Busch and Myrick, 1961; Dietrich and Burris, 1967; Fincher and Payne, 1962). The author does not agree with the term, since by definition endogenous respiration takes place when the organism has transformed its environment in such a way that the environment is no longer suitable for replication of this organism. Transformation of the environment (ie.

the medium) occurs as a result of metabolic activity, where intermediate and end-products are excreted into the environment. In this case, the term "adaptive respiration" would be more suitable, since the microorganisms have not yet had a chance to transform their environment. Independently from its given name, this initial adaptive respiration has to be taken in account. In this study, "adaptive respiration" was measured by inoculating minimal salts solution and carrying such a control in every experiment. The oxygen uptaken by the minimal salts control was then subtracted from every sample curve. The adaptive respiration would also permit one to correct for oxygen uptake by microorganisms biodegrading impurities present in the nutrient solutions or in the salts. It can be noticed that in all cases, the curve for adaptive respiration reaches a final plateau after few hours of incubation. (See for example Figure 16), which proves that the Pseudomonas isolated does not have the capability to grow in pure water, as do some members of the family.

In most cases, the O.D. growth curves exhibit a plateau after a few hours of incubation. This plateau could be interpreted as a stationary phase where growth ceases. When comparing these curves to the corresponding oxygen uptake curves, a similar decrease in microbial activity is not observed (compare for example Figures 5 and 7). This

difference can be explained by the turbidity attained when incubating inoculated solutions for long periods of time (for example, for active microbes an incubation time of 20hr can bring the sample absorbance to 1.3, at which the O.D. reading is no longer precise (see Figure 5)). In such a case, the growth curve would display a plateau, suggesting a false inhibition of growth. In such a case, the sample would have to be diluted for further measurements to be possible.

The results obtained for Polymers A and C, and Monomers A and C suggest that the barrier for polymer biodegradation by most tested microorganisms is not the absence of a hydrolytic exoenzyme, which could lyse the large molecules into smaller oligomers or monomers. Since most inoculated microorganisms did not exhibit the ability to biodegrade both the polymers and the monomers, it can be assumed that the reason for such a lack in biodegradation is related to the absence of required catabolic pathways to degrade the basic molecule expressed in the monomer.

Enhancement of microbial activity and growth, observed when both polymers were complemented with an available source of carbon (glucose) gives the impression that some minor polymer biodegradation is indeed occurring in the vessel. This metabolic enhancement could be noticed in the

case of both growth curves and oxygen consumption curves (Figures 3 to 10). Biodegradation of the polymer would therefore liberate entrapped growth factors which could then act as additional nutrients for other microbes. The presence of carbon and nitrogen atoms in both Polymers A and C, supports this last assumption. It is also possible that a few microorganisms, adapted to degrade the polymers, would liberate as intermediate or end-products, molecules that could enhance growth of other microorganisms. The results obtained in Series A and B suggest that there may exist at least one species in the inoculum that has the ability to degrade to some extent Polymer A, Polymer C, Monomer A and Monomer C (further results confirmed this hypothesis).

Further research was therefore oriented towards the isolation of this particular microorganism.

6.2 ISOLATION OF AN AQUATIC BACTERIA BIODEGRADING DMDAAC POLYMER

The method utilized to isolate from the river the microorganism(s) which had the ability to biodegrade Polymer C, was a trial and error method. The cultures chosen to be tested were present in large numbers in the nutrient agar plates inoculated with river water. However no culture could be considered as dominant. It is understood that the number of cultures growing on an inoculated agar plate is not necessarily related to dominance in the river, but some type of arbitration had to be made. It is also understood that only non-fastidious organisms, able to replicate on nutrient agar at room temperature, were represented on the inoculated plates.

As mentioned in Section 5.2, the sampling site was chosen to avoid, if possible, the isolation of enteric coliforms recently discharged into the stream. The results of fecal coliform counts showed that the number of fecal bacteria is negligible when compared to the total counts.

The five inoculated vessels (Figure 11) showed that only culture #5, a Gram negative rod, consumed enough oxygen for biodegradation of Polymer C to be considered as effective. The other cultures consumed different quantities

of oxygen, but none comparable to culture #5.

The results obtained by this series of experiments suggested that it was worthwhile to investigate further the possible biodegradation of Polymer C and other polymers by aquatic microorganisms present naturally in a river. The sampling site also insured that no prior adaptation to the tested polymer could have taken place in the river. Thus, culture #5, or similar bacteria could likely be isolated from any river with similar environmental conditions as the St Lawrence River.

6.3 BIODEGRADATION AND ASSIMILATION OF DMDAAC BY A PSEUDOMONAS STRAIN

Most polymers and monomers tested in this study have been observed to be toxic to Salmo gairdneri (rainbow trout) (Spraggs, et al, 1982). Consequently, this series of experiments was conducted in an effort to determine whether these toxic substrates can be biodegraded into potentially less toxic intermediate and end-products.

The studies indicated that these polymers and monomers are indeed readily biodegraded by a Pseudomonas bacterium, isolated from the St Lawrence river. The effect of the polymer and monomer degradation products on Salmo gairdneri, however, was not determined in this study.

The test pseudomonad was tentatively identified by 3 microbiology laboratories, but identification of the specific species was not possible. Such a failure of specific identification is not uncommon when dealing with members of the Pseudomonas genus. Due to their high adaptability and genetic mutation capabilities, bacteria of this genus are difficult to identify.

It is also understood that genetic transformation of

the strain could have occurred by maintaining the microorganism for one year in the laboratory. The original strain was isolated from a polluted river, and in this river the strain was constantly exposed to various external pressures. In a laboratory, these adaptation pressures are generally absent. In order to control any biochemical adaptation of major interest for this study, basic glucose and Polymer C experiments were regularly conducted. The results obtained were then compared to the initial oxygen uptake curves obtained at the beginning of this study. The similarity in results confirmed that no major transformation involving the metabolism of glucose or Polymer C occurred.

6.3.1 EXPERIMENTS CONDUCTED WITH GLUCOSE

As mentioned, glucose was chosen as a control nutrient. This decision was made while ignoring the genus of the working microorganism. Identification of the strain as being a member of the Pseudomonas species generated a problem, since most pseudomonads are recognized as glucose "hating" microorganisms (Clarke and Richmond, 1975). The inability of these species to consume glucose has been related to the absence in most pseudomonads of the glycolytic Embden-Meyerhoff pathway (Clarke and Richmond, 1975; Moat, 1979).

The observed catabolism of glucose suggests that the isolated Pseudsomonas strain does pcssess the Entner-Doudoroff pathway (detailed in the Appendix). The Entner-Doudoroff pathway illustrates oxidation of sugars such as glucose, fructose and mannose to pyruvic acid and D-Glyceraldehyde-3-P or D-ribulose (see Clarke and Richmond, 1975). Pyruvic acid would most likely be incorporated into the Kreb's cycle (also known as the TCA cycle, detailed in Appendix 2) where both energy and building blocks would be generated. It has indeed been recognized that pseudomonads possessing the Entner-Doudoroff pathway, are able to utilize glucose as a source of carbon as easily as any other available carbon source (Clarke and Richmond, 1975).

The metabolism observed in these experiments suggests that the Pseudomonas strain demonstrated the ability to catabolize glucose as a sole carbon source, but it was observed that a large deviation existed among the data points generated after conducting various similar experiments. This high deviation was mainly related to differences in the inoculum, since the glucose solutions and the experimental conditions were constant in all experiments. Variations in the inoculum could be related to differences in cell concentration and cell age. The cell age of the inoculated cultures could affect the vitality of

the cells and therefore their ability to adapt to new conditions. To compensate for these sources of variations, all oxygen uptake curves obtained from the experiments conducted with glucose were normalized. Normalization was achieved by plotting all data obtained after correction (see section 5.3 and Appendix 3) and passing a "best fit" curve through the points (Figure 12).

When analyzing the data obtained for experiments conducted with glucose solutions, it could be noticed that even if some vessels consumed oxygen for a longer time at a lower rate than others, all vessels attained a similar final cumulative oxygen consumption. All vessels also exhibited a similar cumulative oxygen uptake curve, suggesting that independently of the time required for metabolizing, the same available glucose concentration was catabolized. This indicates that the same mechanism for glucose catabolism is present in all inoculated cells, since the same quantity of oxygen is required for the catabolism of a similar concentration of available nutrient. It can also be noticed that independently of the number and vitality of cells in the inoculum, an identical stepwise degradation pattern occurs (see Figures 12 and 18), suggesting that this stepwise activity is part of the normal biodegradation ritual of glucose by the isolated Pseudomonas strain.

Similar oxygen uptake curves could be noticed for Polymer C, Monomer C, D-Galactose and other polymers tested (Figures 14, 16, 18, 21 and 23 respectively). It was interesting to observe that some authors alsc reported a similar stepwise metabolic pattern, when studying biodegradation of various substrates by oxygen uptake measurements (Kawai et al, 1978; Ripin, et al, 1971). The metabolic steps observed can be tentatively explained by the fact that when a ccmpound is biodegraded, various mechanisms are involved. Each catabolic pathway produces intermediate-products, which cannot always be readily further biodegraded. Currently, the formed intermediates have to first induce the enzymes responsible for their further biodegradation. However, the time required for such adaptation is normally shorter than the extensive hours observed in the curves generated.

If the cells have already accumulated enough energy from the first biodegradation step, synthesis of these induced enzymes is not necessarily coupled to oxygen uptake. This statement may not be applicable to the initial lag phase, where the cells are also adapting to new nutrients, since it has been previously demonstrated that cells in their lag phase consume oxygen and increase significantly in size (Moat, 1979). In the case of induction of pathways which will degrade these intermediates, it would be

reasonable to assume that the cells do not increase in size as with the lag phase, and hence, less oxygen should be consumed. It is important to mention that this statement is purely tentative and no special experiment was conducted to measure cell enlargement.

6.3.2 EXPERIMENTS CONDUCTED WITH POLYMER C

As predicted, the pseudomonad had the ability to use dialyzed DMDAAC polymer as a sole carbon source (Figure 14) and as sole source of both carbon and nitrogen (Figure 20). In other words, Polymer C represented a rich nutrient source for the isolated Pseudomonas strain.

Contrary to glucose oxygen uptake curves, Polymer C data points, after normalization, exhibited a low deviation from the mean curve (Figure 14). Normalization for differences in cell concentration and vitality could not be the only factor affecting the deviation observed among the various data points, since oxygen curves for Monomer C (DMDAAC Monomer) (Figure 16) exhibited a large variance from the normalized curve.

The small deviation observed for Polymer C data

suggests that there may exist a controlling step in the biodegradation of the polymer, which affects microbial oxygen consumption. This controlling step occurs in every experiment conducted with Polymer C, since the various data points were obtained from a large number of experiments conducted over a period of 8 months. Based on the large size of Polymer C, it could be hypothesized that the controlling step could be the need for synthesis of an extracellular enzyme, which would cleave the molecule into smaller available monomers or oligomers.

6.3.3 EXPERIMENTS CONDUCTED WITH MONOMER C

It may be noticed that the oxygen uptake rate curve for Monomer C (Figure 17) exhibits a higher oxygen consumption rate for a shorter time than the Polymer C rate curve (Figure 15). Thus microbial nutrient uptake occurs more quickly when the microorganism is inoculated into DMDAAC monomer.

The faster oxygen uptake rate exhibited for Monomer C, combined with the above mentioned hypothetical controlling step related to Polymer C biodegradation, support the assumption that this controlling step may be related to the synthesis of an exoenzyme. In fact, Polymer C being a

homopolymer composed of chains of Monomer C, should normally be catabolized in the same fashion as the monomer. The oxygen consumption curves (Figures 14 to 16) show that in reality, Monomer C is biodegraded faster than its related polymer and with no common controlled pattern. Based on the fact that the major difference existing between Polymer C and Monomer C is the molecular size, it is reasonable to conclude that an exoenzyme controls the degradation of Polymer C.

6.3.4 EXPERIMENTS CONDUCTED WITH GALACTOSE

Galactose tests were conducted with the same Pseudomonas strain and the generated oxygen consumption curves were compared to the control curves (Figures 18 and 21). After the first 40hr of incubation, the oxygen consumption rate is comparable for the glucose, Polymer C and galactose solutions. However, after 90hr of incubation, the oxygen consumption rate for galactose test surpassed the oxygen consumption of both glucose and Polymer C solutions. Galactose, in similar fashion to the other tested compounds, was degraded stepwise with a long stationary phase of 70hr between each activity period (Figure 18).

Based on the results obtained with galactose for the first 40hr of incubation, and that the working strain

demonstrated the ability to catabolize glucose, it was concluded, for the purpose of this experiment, that glucose could be accepted as a control sugar.

6.3.5 EXTRACELLULAR ENZYME ACTIVITY TESTS

As mentioned in sections 6.3.2 and 6.3.3, the controlling step for Polymer C and Monomer C catabolism could be the synthesis of an exoenzyme. This exoenzyme would cleave the large polymer molecule into smaller oligomers and monomers, which would then be of lower MW and thus could permeate or be transported through the cell membrane. The time required for the synthesis of the exoenzyme, and the time required for its catalytic activity could control the metabolic activity of most cells and consequently synchronize the activity of the microbial population. The existence of extracellular enzymes among the pseudomonads has been reported by many authors, among which are: Haines and Alexander (1975), Inove et al (1963), Maeda and Taga (1976), Markovitz, et al (1956), Morihara (1963), Robyt and Ackerman (1971) and Zucker and Hankin (1970).

To confirm the above hypothesis, extracellular enzyme activity experiments were conducted. The enzyme activity

was related to the decrease in concentration of the added polymer in a cell-free solution. Measurement of polymer concentration by the PVSAK charge titration method developed by Wang and Shuster (1974), demonstrated that the polymer concentration had decreased with time (Figure 22). Interference of spontaneous degradation of the polymer due to temperature effects was compensated for by running controls, in which pure polymer solution was assayed at regular time intervals. This control experiment demonstrated that at room temperature and over 24hr, no significant degradation of the polymer occurred (Figure 22). Hence, it was concluded that any decrease in concentration in the test vessels would be caused by enzymatic degradation.

A study of the curves generated for the enzyme activity experiment (Figure 22) leads to some interesting conclusions, regarding the nature and activity of the extracted exoenzyme. It was previously mentioned that the slope of Polymer C degradation by the exoenzyme in solution was similar for all generated curves presented in Figure 22, and was independent of the polymer or enzyme initial concentrations. The concentration of Polymer C decreased with time (in a linear fashion) until an asymptote was reached. After this stage, the concentration of polymer remained constant in all cases for the rest of the

experiment. The enzyme activity was thus limited after a certain time by some factor which was present in all tests. It can also be noticed that the slope of the curve is independent of the initial polymer concentration. However, the time at which the final concentration level was reached depended on the initial concentration of the polymer. Higher initial concentrations took longer to reach the steady value.

From the facts cited, it seems that the enzyme was catalyzing the polymer in a zero-order fashion, since the concentration of polymer does not influence the rate of reaction. However, enzymes do not follow zero-order reaction pathways. This false impression given by the shape of curves in Figure 22, is related to the fact that in all tests, the concentration of substrate was in great excess (from 500mg/L to 800mg/L). Based on the Michaelis and Menton enzyme rate curve (see Lenhinger, 1975), the enzyme had reached its maximum reaction rate due to the excess of polymer.

A conventional analytical method such as COD or TOC could not be used to measure the concentration of Polymer C in this particular experiment, since in the absence of cells, the monomers or oligomers liberated by the hydrolytic action of the enzyme are not consumed and are released in the solution. As a consequence, no carbon consumption occurs

and the results of TOC or COD would not reflect the breakdown of Polymer C. A method was therefore required which could differentiate between the polymer and its degradation products. The charge titration method developed by Wang and Shuster (1974) was therefore applicable.

The absence of cells to consume the smaller molecules in the cell-free extract, also leads to a tentative explanation of the presence of the asymptotes observed in the polymer degradation curves (Figure 22). It appears that the exoenzyme ceases its catalytic activity, even if the concentration of polymers remain high in the solution. Two tentative explanations can be offered for this result. Firstly, the exoenzyme may be a repressible type of enzyme. These enzymes cease their catalytic activity in the presence of a specific compound. In most cases, the repressor compound is the enzyme's end-product (Lehninger, 1975). In this case, the cell is not present to catabolize the smaller molecule (enzyme end-product), and as a consequence, a build-up in degradation products occurs. These products may inhibit the enzyme's activity.

A second tentative explanation to the asymptotes observed could be that the enzyme might lyse the polymer into charged monomers and/or oligomers. As mentioned, the PVSAK titration method titrates the charge on the polymer.

The polymer may not lose its charge completely when transformed to the state of smaller products. These oligomers may thus be responsible for the observed asymptotes.

6.3.6 HYPOTHETICAL BIODEGRADATION PATHWAY FOR POLYMER C

The various biodegradation experiments conducted with Polymer C and its respective monomer, enabled a possible biodegradation pathway of Polymer C by the isolated pseudomonad to be advanced. In summary, the experiments conducted established the following;

1) Polymer C could serve as sole sources of both carbon and nitrogen to the pseudomonad strain. (see Figures 14 and 20)

2) Nutrient residual experiments showed that the concentration of Polymer C and Monomer C decreased in concentration after the incubation period (see Table 3).

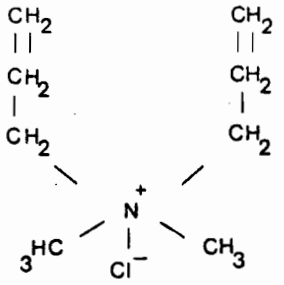
3) An extracellular enzyme was extracted from the inoculated polymer solution. This enzyme could

degrade the polymer over a period of time in a cell-free extract (see Figure 22).

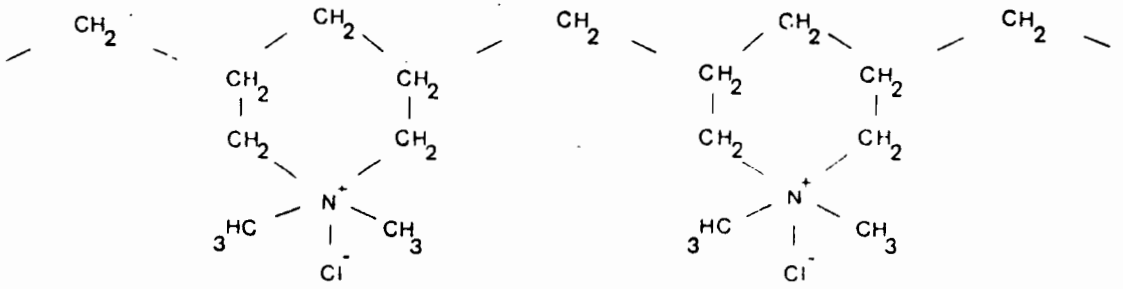
Since polymer biodegradation was established, a biodegradation pathway is proposed below. In general, microorganisms catabolize a nutrient to utilize its available atoms, thus generating energy and building cellular material. There are multiple pathways by which organisms induce biodegradation, and these vary with each specific microbial species. Hence, a general pathway cannot be proposed for all microorganisms degrading Polymer C.

The molecular structure of Polymer C and its corresponding Monomer are detailed in Figure 26. From this figure, it can be noticed that the only difference between Polymer C and Monomer C is that Polymer C is a cyclic structure whereas Monomer C is an aliphatic molecule.

It was confirmed above that at least one extracellular enzyme was involved in the biodegradation of the polymer, thus the first degradation step likely involves the cleavage of the large polymer molecular structure into smaller fragments such as monomers or oligomers. The subsequent biodegradation steps, which would most likely occur inside the cell, could not be studied experimentally, so their proposal is based mainly on consulted literature. Such



Monomer C



Polymer C (DMDAAC Polymer)

Figure 26. Molecular structure of DMDAAC Polymer and Monomer.

experiments could however be conducted by C^{14} labelling. Figure 27 presents the hypothetical steps proposed in this study for the biodegradation of Polymer C. The first step is likely to be accomplished by the exoenzyme which was previously isolated. This extracellular enzyme would hydrolyze the polymer into smaller fragments, which could thereafter penetrate the cell membrane. Based on the common mechanisms of synthesis of polymers (see Schwoyer, 1981) the weakest methyl group would most likely be the linking methyl group. So the exoenzyme could firstly hydrolyze this weaker methyl group. The enzyme assays have demonstrated that effectively it was possible to follow the degradation of the polymer by charge titration, proposing that the degradation product may be a non-charged molecule. Two possible hydrolytic sites would be on the right or left of the intermediate methyl group, indicated by number 1. Subsequently, the molecule may be small enough to diffuse or be transported across the membrane (< 600MW)

Step 2 could be the separation of the various methyl groups from the nitrogen group, with formation of a branched hydrocarbon and a nitrogen-chloride molecule. Various microorganisms have been reported to use hydrocarbons as a source of carbon, among these Pseudomonas have been cited often (Gaudy and Gaudy, 1980; Moat, 1979). The most common catabolic pathway to assimilate hydrocarbons involves the

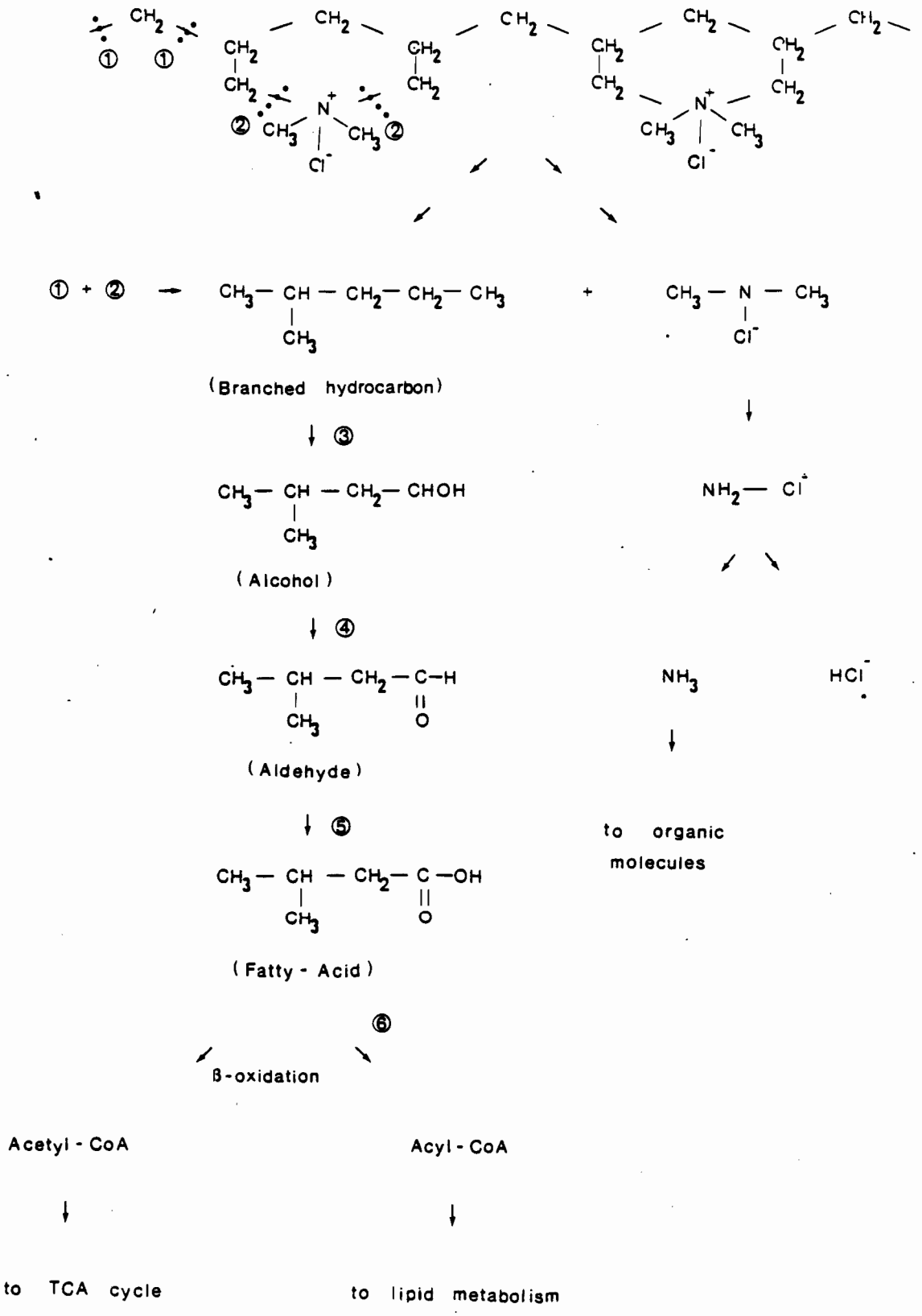


Figure 27 Hypothetical Biodegradation Pathway for Polymer C.

oxidation of the terminal methyl group, resulting in the formation of an alcohol. This step requires oxygen and is therefore specific to aerobic organisms (step 3). The newly formed alcohol is then reoxidized through the action of dehydrogenases to form successively an aldehyde and a fatty-acid (steps 4 and 5 respectively) (Clarke and Richmond, 1975; Gaudy and Gaudy, 1980; Moat, 1979). Many bacteria do not have the mechanism to biodegrade fatty-acids, but Escherichia coli and the pseudomonads, have demonstrated the ability to metabolize fatty-acids through their beta-oxidation pathway (detailed in Appendix 2). Acyl-CoA and Acetyl-CoA are formed as end-products of this beta-oxidation pathway.

Acyl-CoA has been reported to play an important role in the metabolism of lipids, involved most particularly in the generation of energy and the synthesis of membrane phospholipids (Moat, 1979). Acetyl CoA is a major substrate in the TCA cycle (see Appendix 2) and is therefore utilized to generate energy and building blocks.

Alternative pathways for hydrocarbon metabolism have also been suggested by Klug and Markovetz (1971) in their study of the metabolism of aliphatic hydrocarbons. In this paper, Klug and Markovetz demonstrated that some pseudomonads metabolize hydrocarbons by oxidizing the

subterminal methyl group. A second study by Finnik, et al (1974) stated that some strains of the Pseudomonas genus oxidize both terminal methyl groups simultaneously in straight chains and branched hydrocarbons.

The experiment conducted with the isolated Pseudomonas strain (Figure 20) demonstrated that the polymer is a source of both carbon and nitrogen, suggesting that the nitrogen-chloride molecule formed after the second step is also further biodegraded. It is known that inorganic forms of nitrogen are degraded in some microorganisms, until ammonia is liberated and thereafter incorporated into cellular molecules. It would therefore be acceptable to predict that the nitrogen-chloride compound will be dimethylyzed with subsequent removal of the chloride atom to form a molecule of ammonia nitrogen.

6.3.7 LONG-TERM EXPERIMENTS

In all oxygen consumption curves presented in this study, (Figures 6 to 20), the oxygen uptake invariably attains a plateau, whereafter very little oxygen is consumed. To interpret such a decrease in metabolism, 4 days experiments were conducted with a variety of substrates

(Figure 18). It may be concluded from the short experiments conducted previously that biodegradation generally stops before 40hr, since in most curves, the oxygen consumption attains a plateau before this time (see for example Figures 14, 16, 21 and 23). However, some long-term experiments were conducted to confirm the validity of the shorter experiments and the significance of the plateaux observed. These longer experiments demonstrated that biodegradation is in fact not completed after 40hr. It was, however, decided to stop most experiments after 40hr of incubation, since 40hr in a stream represents many miles in distance and prediction of any fate becomes impossible. In most curves presented in Figure 18, it can be seen that a second period of metabolic activity follows the abovementioned plateau. This second metabolic activity period occurs generally after 60 to 90hr of incubation (Figure 18), suggesting that there would be little difference in stopping the experiments after 40hr or 60hr. Finally, in the case of polymers which exhibit toxicity to aquatic biota, the study of immediate biodegradation of polyelectrolytes is more important than their long-term fate.

All oxygen uptake curves generated from this long run test exhibited the same plateau after an average incubation time of 20hr. This decrease (or even cessation) in metabolism could be observed for a few hours or even 2 days.

In all cases though, a metabolic activity enhancement takes place after this plateau. This fact could be due to the formation of intermediate products during the first activity period, to which the cell has to adapt and induce pathways to further biodegrade these new substrates. For example, in the case of DMDAAC polymer, the hypothetical degradation described proposed that fatty-acids resulted as an intermediate product of alkane oxidation. The cells may transform the generated fatty-acids to Acetyl-Coa and Acyl-Coa. This transformation would occur through the beta-oxidation pathway, but some enzymes of this pathway may first have to be induced. Induction of specific enzymes is likely to require some adaptation in which the cellular metabolism decreases and a second lag phase may be induced. This type of adaptation period is likely to be required for the activation of pathways such as the beta-oxidation pathway, since it is not constantly being used by the cell.

6.3.8 EXPERIMENTS CONDUCTED WITH OTHER POLYMERS

In addition to the biodegradation of Polymer C and its corresponding monomer, other polymers employed in the sanitary engineering field have also been tested. These polyelectrolytes were inoculated with the previously

isolated pseudomonad strain. Among these was Polymer A, a polyacrylamide co-polymer. Experiments with Polymer A had previously been conducted in the first two series of experiments (Figures 3 and 9) and it was possible to observe some growth among the mixed population. This growth and small oxygen uptake was tentatively related to the presence of ethanol in the solution, required to obtain complete dissolution of the polymer. Impurities present in the solid polymer were also proposed to cause the high microbial metabolism observed. In section 5.3.10, the polymer was dissolved without the help of ethanol as a solvent; as a result, complete dissolution of the polymer could not be achieved. Hence, it was impossible to state the true concentration of the polymer in the solution. However, these tests were principally qualitative tests, and the real concentrations were not of importance. The results given in Figure 23 show that the Pseudomonas strain could metabolize the polymer. As a consequence, it was concluded that Polymer A can be biodegraded by the isolated pseudomonad. Monomer A (acrylamide), one of the components of Polymer A, was also tested for its effect on the pseudomonad bacteria. This time, the monomer yielded a higher oxygen uptake than its corresponding polymer (Figure 23). In fact, Monomer A showed a faster oxygen consumption than glucose or galactose. This result contradicts the conclusions published by Suzuki and co-workers (1976), which mentioned

that polyacrylamide polymers could not be biodegraded by Pseudomonas aeroginosa, even after cleaving the polymer by ozonation. Suzuki et al (1976) added that the reason for the absence of biodegradation of polyacrylamides was the presence of amide groups. One explanation for this difference in results, could be based on the difference of working pseudomonad strains. It is possible that Suzuki and co-workers conducted their experiments with a Pseudomonas strain poorly adapted to biodegrade polymers and specifically amide groups. As a matter of fact, they also mentioned that their pseudomonad strain was unable to biodegrade polyethylene glycols of large to intermediate molecular weights, whereas other authors, (Haines and Alexander, 1975; Kawai et al, 1978; Watson and Jones, 1977) reported that some species of Pseudomonas can biodegrade polyethylene glycols attaining 20,000 MW.

Other cationic polymers, of less interest, were also inoculated with the strain isolated from the river, to test the adaptation capacity of the bacterium to biodegrade a variety of polymers (Polymer A (a polyacrylamide), Polymer D (an anionic acrylamide-based polymer), Polymers 2319/1 and 2319/4 (cationic acrylamide-based polymers with 20% dimethyl amino ethyl acrylate, quaternised with methyl chloride) and 2318 (a cationic acrylamide-based polymer with 50% dimethyl amino ethyl acrylate quaternised with methyl chloride). As

expected, the strain consumed oxygen when inoculated into all tested cationic polymers (Figure 23). It could therefore be concluded, that the isolated strain had a great capacity to biodegrade large molecules, and its catabolic steps did not seem to be specific for a single compound. This conclusion is in agreement with Hegeman (1966, see Clarke and Richmond, 1975) who isolated a number of enzymes from a pseudomonad strain, inducible by a number of different chemical analogues.

A high molecular weight, low anionic charge, acrylamide based polymer (Polymer D) was tested for its biodegradability. The results obtained with this last polymer were surprising, since the oxygen consumed by the Pseudomonas strain when inoculated on the anionic polymer is higher than with any other compound, attaining levels of 250mg/L after 30hr of incubation (Figure 23). The oxygen consumed when inoculated on the anionic polymer was not higher in the first 10hr of incubation, but it did not display a plateau, such as observed for all other compounds tested. As a consequence, after 30hr of constant oxygen uptake, the cumulative uptake was higher that any other polymer tested. The reason why this polymer induces a higher oxygen consumption than glucose and galactose could unfortunately not be answered with the results available, but this higher

oxygen uptake may not necessarily be asscciated with the fact that the polymer is anionic.

It is also of interest to mention that this same anionic polymer was in a short period of time contaminated with fungi and bacteria when stored at 10°C. It seems that this anionic polymer could serve as an available source of carbon to a number of different microorganisms. It can also be assumed that the microorganisms which invaded the stored polymer possessed active exoenzymes. The presence of exoenzymes in yeasts have indeed been reported by Spencer-Martin (1982). Since the anionic polymer seemed to be so desirable as a nutrient, it can be concluded that the negative charge of the polymer did not interfere with that of the cell. The extracellular enzyme, responsible for the initial cleavage, may even neutralize the polymer charge, and possibly transform the polymer into its monomeric state. Hence, after this first hydrolysis, the polymer becomes suitable for the cell in both size and charge.

6.3.9 SLUDGE-SUPERNATANT TOXICITY/BIODEGRADATION TESTS

In sludge thickening and dewatering processes, or in other biological sludge coagulation processes, the polymer

is added directly to the sludge. What are the effects of these high concentrations of polyelectrolytes on the microorganisms present in the sludge? Ideally, the polymer should adsorb completely on the bacterial cells and increase the flocculation efficiency, enhancing the solid-liquid separation. But how much of this added polymer does not adsorb to the sludge and hence remains free in the supernatant?

To answer these two questions, a sludge-supernatant toxicity/biodegradation test was conducted with mixed liquor from an activated sludge treatment plant.

The results obtained with the settled sludge to which glucose had been added as a nutrient source, shown in Figure 24, permitted one to conclude that the polymer did not exhibit toxic effects, based on oxygen consumption curves. In fact, the vessel containing the sludge settled with Polymer C, exhibited a higher oxygen uptake than the blank which was polymer free. Hence, the polymer is not only non-toxic to some or most of the microbial species present in the sludge, but it also seems to be biodegraded to some extent by some microorganisms present in the sludge.

A second important conclusion derived from the sludge-toxicity test correlates with the results published

by Gehr and Henry (1982, 1983) and Goppers and Straub (1976). Both studies concluded that the polymer added in a sludge treatment process does not adsorb completely on the sludge. As a consequence, some of the polymer remains in the supernantant. In fact, PVSAK titration of the supernatant remaining after the sludge had settled, showed that a concentration of 3.5mg/L of Polymer C and 3.2mg/L of Polymer A remained in the supernatant.

This tested supernatant fraction known to contain polymer was thereafter inoculated with the Pseudomonas strain, this test being called the supernatant/biodegradation test. When the pseudomonad strain was inoculated on this supernatant fraction, it could be observed (Figure 25) that the fraction containing polymer exhibited less oxygen consumption than the blank (supernatant fraction from the polymer-free jar). This difference in oxygen consumption, observed for both Polymer A and Polymer C, can tentatively be explained by the fact that both mixed liquors settled with polymers exhibited a better settling. More suspended solids were therefore settled in the sample jars than in the control jar. Hence, although the supernatant fractions were complemented with polymers, there were fewer available nutrients in the media to be consumed by the inoculated pseudomonad. To insure that polymer degradation had effectively taken place in such

a rich medium, titration analyses were conducted on the final solutions after 8Chr of incubation. In all cases, the final concentration of polymer was lower than the initial concentration detected in the supernatant fraction (see Table 4).

It may also be noticed that the addition of polymers to the sludge enhanced settling of the microorganisms themselves. Hence, the supernatant fractions separated by polymer addition may have had a lower concentration of mixed liquor microorganisms. Although the Pseudomonas strain was added in similar concentrations to all 3 vessels, the unsettled cells originating from the mixed liquor may have also consumed high quantities of oxygen. It is then obvious that the blank, containing higher concentrations of these unsettled cells, would consume more oxygen, based on its population size. To support this hypothesis of higher population density in the blank vessel, a few petri dishes were inoculated and the number of cultures estimated. As mentioned, the number of microorganisms in the blank was higher than in any other reactor vessel. It was also noticed that the number of cultures was in fact much higher than any experiment run to that date, when conducting experiments with the inoculated pseudomonad strain.

When comparing the oxygen consumption curves obtained

with the inoculated supernatant with those presented previously for polymer degradation, the oxygen consumed was 5 times higher in the case of the supernatant tests (for example 600mg/L (Figure 25) vs. 150mg/L (Figure 21)). This difference in oxygen uptake is likely related to the fact that the supernatant is rich in nutrients, of many types and quantities. However, note that the first 10hr of incubation are similar for all curves generated, displaying biodegradation (Figure 23). The difference in oxygen consumption only appears after these 10 hours, when the usual plateau is reached in the case of polymers and glucose. This plateau, as in the case of the tested anionic polymer, does not exist here for the supernatant samples. This plateau would not occur yet in such a rich source of available nutrients. There would be no need to stop metabolism to adapt for further degradation of formed intermediate products. As mentioned previously, some cells from the unsettled sludge remained in solution. Hence, even the original cell population represented by the supernatant test curves is actually much higher than any other oxygen uptake curve generated, where virtually the only microorganisms present originated from the inoculum.

7.0 CONCLUSIONS

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In general, all objectives stated in the introduction were achieved, with an increase in understanding about the fate and effect of polyelectolytes on aquatic microorganisms. The importance of these results and conclusions is heightened by the lack of information available on the subject selected. It is also important to understand the relation of the experimental results obtained *in-situ* to the *in-vivo* situation. Although the tests conducted in this study were performed in bottles, they should be constantly related to the true environmental problem of polyelectrolyte discharge.

The major conclusions derived from the experiments conducted in the study are as follows:

1) Polymers discharged into streams as a result of inefficient settling and adsorption did not show toxic effects to the inoculated non-fastidious aquatic mixed populations at concentrations as high as 2000mg/L, based on overall population density and metabolic activity. In other words, the polymers introduced into streams in residual concentrations will most probably not affect the overall microbial activity of the

stream. As a consequence, the stream's natural self-purification process is likely not to be inhibited by the discharged polymers.

2) Polymer A (a polyacrylamide-based co-polymer) and Polymer C (DMDAAC polymer) could not support microbial metabolic activity of the mixed population present in the inoculum. Neither growth nor oxygen uptake could be detected when the mixed cultures were inoculated on Polymer C. Some growth and oxygen uptake could be noticed in the Polymer A vessels. This growth was however related to the presence of both ethanol (0.02 mL of 95% ethanol in 100mL 2000mg/L Polymer solution) and impurities in the same solution.

3) All polyelectrolytes tested were biodegraded to some extent by a Pseudomonas strain indigenous to the St Lawrence River. This biodegradation occurred even in the presence of other available sources of carbon. Hence, the discharged polymers would be consumed to a certain extent by some adaptable bacteria present in the stream.

4) Although one strain was isolated from the river, with the ability to biodegrade a series of polymers, it is not stated that it will be the only species indigenous to the river with this ability. In fact, the facility by which the strain was isolated, suggests that there might be a number of bacterial species in the same stream with the ability to biodegrade polymers of this type. In such a case, the biodegradation of the residual polymer discharged in the streams could be even faster than as predicted by this study.

5) The isolated Pseudomonas strain has been shown to cleave the long polymeric chains by means of extracellular enzyme(s), easily extracted from the solution.

6) A biodegradation pathway was tentatively proposed for the biodegradation of DMDAAC polymer by the isolated Pseudomonas strain. This catabolic pathway was not based on experimental results, but on a Literature review.

7) Experiments conducted with mixed-liquor sludge demonstrated that the polymer that is added to the mixed-liquor does not inhibit the microbial metabolism of the sludge microorganisms. Experiments conducted using the supernatant of mixed-liquor settled with polymers, demonstrated that some residual concentrations of polymer remained in the supernatant.

8) The electrolytic respirometer has been demonstrated to be an effective instrument for the measurement of toxicity and biodegradation effects.

The area of research conducted in this study is a new field, where very little experimental work has been done. This study may therefore be considered a preliminary study, which gave satisfactory results, but which opens the doors to further research.

8.0 FUTURE RESEARCH

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A number of research subjects could be developed from this study. These include the following:

1) It has been shown in previous papers that the polyelectrolytes used as coagulants were toxic to fish (Spraggs et al, 1982). It would be of prime interest to measure the toxic effect of the intermediate and end-products of biodegradation on the same fish. Toxicity tests should then be conducted with extracted intermediate and end-products.

2) In the area of microbiology, it would be interesting to study experimentally, the actual biodegradation steps of the polyelectrolytes by the pseudomonad strain. A more detailed knowledge of such a pathway would permit one to choose the polyelectrolytes to be used as flocculants, based on their ability to be biodegraded.

3) Since it has been demonstrated that there is at least one aquatic species able to biodegrade the tested polymers, it would be interesting to make a survey of other possible strains sharing the same

abilities. Such information could give insight into the actual biodegradation of the polymers in-vivo.

4) In-situ tests should be conducted on the biodegradation of residual polyelectrolytes discharged by wastewater treatment plants into streams or lakes. These tests could involve sampling and analysis of water samples at various distances from the discharge site. It would permit one to correlate the results obtained in the laboratory with in-vivo situations. The results obtained from such an experiment could also lead to the generation of a mathematical model representing the stream with its decreasing concentration of polymer as a function of time and distance. Such a model, combined with LC50 fish tests of the intermediate products of polymer biodegradation, would permit one to predict at which distance from the discharge site the polymer or its products would cease to be toxic to fish.

5) In-situ experiments could also include the microbiological analysis of waters receiving polyelectrolytes in residual concentrations. Adapted microbes would have a tendency to grow on

such sites. Information on the microorganisms effectively developed at contaminated sites would permit one to visualize and understand the biodegradation of the polymers in-situ.

6) It would also be interesting to effectively analyze all polyelectrolytes actually used by treatment plants, for their ability to be biodegraded. Information on the biodegradability of such polymers would enable a list to be generated of recommended polymeric flocculants which could be used in the wastewater treatment field.

7) Finally, a closer collaboration between the companies producing the polyelectrolytes and the above-mentioned area of research would guide these manufacturing companies to produce polymers which could be more easily biodegraded by aquatic microorganisms.

9.0 REFERENCES

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