



Durham E-Theses

The biodegradation of organic chemical waste using a constructed horizontal reedbed treatment system

Stubbs, K. T.

How to cite:

Stubbs, K. T. (2007) *The biodegradation of organic chemical waste using a constructed horizontal reedbed treatment system*, Durham theses, Durham University. Available at Durham E-Theses Online:
<http://etheses.dur.ac.uk/2480/>

Use policy

The full-text may be used and/or reproduced, and given to third parties in any format or medium, without prior permission or charge, for personal research or study, educational, or not-for-profit purposes provided that:

- a full bibliographic reference is made to the original source
- a [link](#) is made to the metadata record in Durham E-Theses
- the full-text is not changed in any way

The full-text must not be sold in any format or medium without the formal permission of the copyright holders.

Please consult the [full Durham E-Theses policy](#) for further details.

Academic Support Office, Durham University, University Office, Old Elvet, Durham DH1 3HP
e-mail: e-theses.admin@dur.ac.uk Tel: +44 0191 334 6107
<http://etheses.dur.ac.uk>

The copyright of this thesis rests with the author or the university to which it was submitted. No quotation from it, or information derived from it may be published without the prior written consent of the author or university, and any information derived from it should be acknowledged.

**The biodegradation of organic chemical waste using a
constructed horizontal reedbed treatment system**

Submitted to the University of Durham
for the degree of Doctor of Philosophy

by

K. T. Stubbs, B.Sc. (Durham)

School of Biological and Biomedical Sciences

University of Durham

May, 2007



- 2 JAN 2008

ABSTRACT

The aim of the project was to evaluate the potential for a constructed reedbed (planted with *Phragmites australis*) to aid in the biodegradation of triethylene glycol (TEG) under normal climatic conditions experienced at the site. The approach taken was to design and construct a series of test beds which could be used in replicated experiments to measure the rate at which TEG solutions of different concentrations were degraded. The effect of the TEG on reeds and the interactions between TEG and physical conditions within the test beds were monitored and in addition, the potential use of two methods which might be used instead of the BOD₅ method were assessed.

Although the temperature range experienced during the series ranged from 2.5°C to 18.5°C, TEG was degraded within four days at both high and low temperatures. The initial decline in TEG concentrations was rapid and this was thought to be partly due to some dilution by water but mainly by the action of bacteria within the reedbed.

Subsequently, TEG concentration declined more slowly. The TEG concentrations used in the tests were between 0.1%v/v and 5.0%v/v with the higher concentrations degrading faster initially, but the overall degradation rates being similar. The physical conditions within the treatment tanks compared to the controls showed that temperature was not affected, pH was unaffected in the early trials but showed slight changes to acidity towards the final trials. Conductivity in both control and treatment beds showed similar unexplainable variances until the later treatments when the treatment beds displayed

higher values than those found in the control beds. Dissolved oxygen in the treatment beds was lower than in the untreated beds as was expected due to bacterial action.

The effect of dosing with TEG on the reeds was to increase the number of shoots per area and Total Kjeldhal Nitrogen (TKN) content after one season with biomass production of the treated beds increasing after two years. The stomatal count on second year plants showed a significant increase in the treated reeds against the untreated ones.

A brief investigation of the impact of TEG on micro-organisms within the test beds indicated that some species of bacteria were probably 'tegophilic' i.e. bacteria that flourish in a medium containing TEG and that ciliate protozoa were not adversely affected.

Of the two methods examined to replace the BOD₅ test, the EZBOD[®] meter was found unsuitable due, it was thought, to the bactericidal properties of the TEG. Trials using a Total Organic Carbon analyser indicated that this rapid method would be a successful supplement and/or replacement to the BOD₅ method currently used to monitor whether waste water quality meets disposal requirements imposed by the Environment Agency.

It was found that the TEG did biodegrade without having any adverse effects on the reeds and that the degradation was a first order reaction. A reaction rate was determined that will enable anybody to determine the requirements of a treatment system to deal with effluent containing TEG.

Acknowledgements

I would like to express my gratitude to several people for their assistance during this project. My supervisors from the University of Durham, Dr. Bob Baxter for his advice and guidance and Dr. Val Standen for her unstinting enthusiasm, support and advice throughout.

Dr. D .Rowe, Queens Campus, University of Durham for the crash course in practical bacteriology and Mr. Richard Daniels, Queens Campus for providing laboratory facilities.

Finally, but not least, I must thank BP CATS Terminal for their generous sponsorship of the project and in particular Alan Tapster, Rachel McKenna, Tony Little and Dave Stobbs for their help and support for the project

Declaration

I hereby declare that this thesis and material presented herein, has not previously been submitted to this or any other institution and is solely the product of the present author.

Unless otherwise stated all photographs, diagrams and analysis are by the author.

Signed

Kenneth Thomas Stubbs

Date

The copyright of this thesis rests with the author. No quotation from it should be published without their written consent and information derived from it should be acknowledged.

Contents

	Page no.	
Title page	i	
Abstract	ii	
Acknowledgements	iv	
Declaration	v	
Contents	vi	
List of plates	xi	
List of figures	xii	
List of Tables	xiv	
1.0	General introduction	1
1.1	The disposal of organic chemical waste	1
1.2	The use of constructed reedbeds in waste water treatment	4
1.3	Background to the BP CATS terminal and the constructed reed beds	6
1.4	Background to the project	12
1.5	Questions raised by the proposal to pass TEG through the constructed reedbeds at CATS	13

Page no.

1.6	Aims and objectives of the project	17
2.0	The test beds used in the experiments with TEG	19
2.1	Introduction	19
2.2	Construction of the test beds	20
2.3	Performance of the test beds and problems encountered	26
3.0	The degradation of TEG in test beds	27
3.1	Introduction	27
3.2	Methods	27
3.2.1	Detection of triethylene glycol.	27
3.2.2	Experimental Design and Procedure	37
3.2.2.1	Use of the experimental beds	37
3.2.2.2	Concentrations of test solutions	38
3.2.2.3	Trial procedure and sampling	39
3.2.3.	The addition of a TEG solution using water from the retention pond after the Klargestester had been brought into use.	41
3.2.4	Statistical Analysis	42
3.3	Results	43

Page no.

3.3.1	The GC method used to measure TEG	43
3.3.2	Rate of degradation of TEG during the trials	45
3.3.3	Results – Klargestær / retention pond trials	58
3.4	Discussion - Factors affecting the rate of TEG degradation	62
3.4.1	Start concentration	62
3.5	Interactions between TEG solution and physical chemistry of the test beds.	73
3.5.1	Temperature	88
3.5.2	Conductivity	88
3.5.3	pH	89
3.5.4	Dissolved oxygen	89
3.5.5	Discussion	91
3.5.5.1	Temperature	91

Page no.

3.5.5.2	Conductivity	91
3.5.5.3	pH	92
3.5.5.4	Dissolved oxygen	93
4.	The effect of TEG on the reeds	94
4.1	Introduction	94
4.2	Methods	96
4.2.1	Shoot density	96
4.2.2	Tallest shoot	97
4.2.3	Shoot Biomass	97
4.2.4	Stomatal count	97
4.2.5	Total Kjeldhal Nitrogen of whole shoots	97

	Page no.
4.3	Results 98
4.3.1	Shoot Density and Height 98
4.3.2	Shoot biomass 103
4.3.3	Stomatal Number 104
4.3.4	Total Kjeldhal Nitrogen 107
4.4	Discussion 108
5.	To find a quick and easy method of estimating the BOD₅ of the effluent that could be used by treatment plant personnel. 110
6.	The micro-organisms of the reedbed rhizosphere 111
6.1	Bacterial investigation 111
6.1.1	Introduction 111
6.1.2	Method 112
6.1.3	Results 114
6.1.4	Discussion 116
6.2	Effects of TEG on microscopic organisms 118
6.2.1	Introduction 118
6.2.2	Method 118
6.2.3	Results 119
6.2.4	Discussion 121
7.	General Discussion 122
8.	Conclusion 127

	Page no.
References	128
Appendix	138
Method for Biological Demand	139
Method for Chemical Oxygen Demand	139
Statistical Analysis	144
Micro-Kjeldhal Method	170
EZBOD manual	171
Biotector	175
Chapter 5 expanded version	176

List of Plates

Plate no.	Description	Page no.
1.1.	Map of Teesmouth, Middlesbrough with the location of the CATS Terminal	8
1.2	Aerial photograph of the CATS terminal, Seal Sands, Middlesbrough	9
2.1.	A test tank prior to addition of gravel and reeds	24
2.2.	The scaffolding structure before the test beds were put in place	24
2.3.	Photograph of a spare test tank complete with baffles and gravel	24
2.4.	Side view of one complete test bed with header tank and receiver in place	25
2.5.	View of the back of all the test beds on their scaffold support	25
4.1.	Photographs of the test and control beds	106
6.1.	The conical flasks with samples and nutrient after 6 days in the constant temperature room.	113
6.2.	Examples of resultant culture growths on Agar plates after 4 days at 20°C	114

List of Figures

Fig. no.	Description	Page no.
1.1	Diagram of constructed reed beds and the surrounding grassland at the CATS Terminal, Seal Sands, Middlesbrough.	10
1.2	Diagrammatic representation of the site drainage system.	11
2.1	Diagrammatic representation of the constructed reedbed, showing the approximate position of the test beds.	20
2.2	A schematic plan of one portable test bed.	23
3.1	A GC trace of 0.1%v/v DEG / 0.025v/v Pentane diol / 0.1%v/v TEG solution.	33
3.2	A GC trace of 0.01%v/v solution of DEG / 0.025% v/v Pentane diol / 0.01% v/v TEG.	34
3.3	A typical GC trace of the 0.1%v/v MEG/DEG/TEG standard	36
3.4	A typical GC trace of a trial sample	44
3.5	Mean concentrations of TEG %v/v retention pond trial 1	59
3.6	Mean concentrations of TEG %v/v retention pond trial 2	60
3.7	Comparison of concentration of TEG%v/v against COD over time , retention pond trial.	61
3.8	The change in TEG concentration of period of trial 1	63
3.9	The change in TEG concentration of period of trial 2	63
3.10	The change in TEG concentration of period of trial 3	64
3.11	The change in TEG concentration of period of trial 4	64

List of Figures (continued)

Plate no.	Description	Page no.
3.12	The change in TEG concentration of period of trial 5	65
3.13	The change in TEG concentration of period of trial 6	65
3.14	The change in TEG concentration of period of trial 7	66
3.15	The change in TEG concentration of period of trial 8	66
3.16	The change in TEG concentration of period of trial 9	67
3.17	The change in TEG concentration of period of trial 10	67
3.18	The change in TEG concentration of period of trial 11	68
3.19	The change in TEG concentration of period of trial 12	69

List of Tables

Table no.	Description	Page no.
3.1.	Results of internal standard trial.	32
3.2.	Details of trials showing start dates, duration and concentrations used in the trials.	39
3.3.	Trial 1. Mean concentrations of DEG %v/v in the test beds determined by GC analysis	46
3.4.	Trial 1. Mean concentrations of TEG %v/v in the test beds	46
3.5.	Trial 2. Mean concentrations of DEG %v/v in the test beds	47
3.6.	Trial 2. Mean concentrations of TEG %v/v in the test beds	47
3.7.	Trial 3. Mean concentrations of DEG %v/v in the test beds	48
3.8.	Trial 3. Mean concentrations of TEG %v/v in the test beds	48
3.9.	Trial 4. Mean concentrations of DEG %v/v in the test beds	49
3.10	Trial 4. Mean concentrations of TEG %v/v in the test beds	49
3.11	Trial 5. Mean concentrations of DEG %v/v in the test beds	50
3.12	Trial 5. Mean concentrations of TEG %v/v in the test beds	50
3.13	Trial 6. Mean concentrations of DEG %v/v in the test beds	51
3.14	Trial 6. Mean concentrations of TEG %v/v in the test beds	51
3.15	Trial 7. Mean concentrations of DEG %v/v in the test beds	52
3.16	Trial 7. Mean concentrations of TEG %v/v in the test beds	52
3.17	Trial 8. Mean concentrations of DEG %v/v in the test bed	53

Table no.	Description	Page no.
3.18	Trial 8. Mean concentrations of TEG %v/v in the test beds	53
3.19	Trial 9. Mean concentrations of DEG %v/v in the test beds	54
3.20	Trial 9. Mean concentrations of TEG %v/v in the test beds	54
3.21	Trial 10. Mean concentrations of DEG %v/v in the test beds	55
3.22	Trial 10. Mean concentrations of TEG %v/v in the test beds	55
3.23	Trial 11. Mean concentrations of DEG %v/v in the test beds	56
3.24	Trial 11. Mean concentrations of TEG %v/v in the test beds	56
3.25	Trial 12. Mean concentrations of DEG %v/v in the test beds	57
3.26	Trial 12. Mean concentrations of TEG %v/v in the test beds	57
3.27	Mean concentrations of TEG %v/v in the test beds, retention pond trial 1.	59
3.28	Mean concentrations of TEG %v/v in the test beds, retention pond trial 2.	60
3.29	Mean COD levels mg/l retention pond trials	61
3.30	Summary of times taken for the breakdown of TEG to 10% of original concentration.	62
3.31	The concentrations of TEG, in and out, time taken, the area of the test bed and the calculated removal rate	71
3.32	Mean physical measurements taken at points in time after the addition of TEG, Trial 1	74

Table no.	Description	Page no.
3.33	Mean physical measurements taken at points in time after the addition of TEG, Trial 2	75
3.34	Mean physical measurements taken at points in time after the addition of TEG, Trial 3	76
3.35	Mean physical measurements taken at points in time after the addition of TEG, Trial 4	77
3.36	Mean physical measurements taken at points in time after the addition of TEG, Trial 5	78
3.37	Mean physical measurements taken at points in time after the addition of TEG, Trial 6	79
3.38	Mean physical measurements taken at points in time after the addition of TEG, Trial 7	80
3.39	Mean physical measurements taken at points in time after the addition of TEG, Trial 8	81
3.40	Mean physical measurements taken at points in time after the addition of TEG, Trial 9	82
3.41	Mean physical measurements taken at points in time after the addition of TEG, Trial 10	83
3.42	Mean physical measurements taken at points in time after the addition of TEG, Trial 11	84
Table no.	Description	Page no.

3.43	Mean physical measurements taken at points in time after the addition of TEG, Trial 12	86
3.44	Summary of results of one way ANOVAs with <i>post hoc</i> Tukey tests on the physical aspects.	90
4.1	The number of shoots per transect for all beds 2001	98
4.2	The number of shoots per transect for all beds 2002	99
4.3	The number of shoots per transect for all beds 2003	99
4.4	The final number of shoots per transect 2001, 2002, 2003	100
4.5	The tallest shoots for all beds 2001	101
4.6	The tallest shoots for all beds 2002	101
4.7	The tallest shoots for all beds 2003	102
4.8	The final tallest shoots for all beds 2001, 2002, 2003	102
4.9	The biomass harvested 2002, 2003	103
4.10	The stomatal count of random leaves 2002	104
4.11	The stomatal count of random leaves 2003	105
4.12	The TKN by analysis on harvested reeds 2002, 2003	107
4.13	Summary of results of t-Tests	107
4.14	Summary of analysis of reed bed attributes using two factor ANOVA with replication	108
6.1.	Observations of slides after gram staining	115
6.2.	Results of Protozoa search of test bed water	120

1. General Introduction

1.1 The disposal of organic chemical waste

The problem of waste, its production and disposal is a matter of concern in both the commercial and private sector, with the Environment Agency and the Department for Environment, Farming and Rural Affairs, being deeply involved in trying to both reduce waste and control the treatment received by waste.

Over the past 200 years, some commercial interests in Britain have produced excessive amounts of organic chemical waste and have disposed of it without regard for the environmental consequences. Many of the end products, such as chlorofluorocarbons (CFCs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), plastics and leaded petrol have had a severe detrimental effect on the environment. These effects were probably not fully recognized until the middle of the 20th Century. Once the damage was recognized and culpability defined then ways of limiting and in some cases treating the waste, in a hope of damage limitation, were initiated.

In the last years of the 20th Century international conferences, such as the Earth Summit, 1992 and the Kyoto Summit, 1997, were held in the hope of achieving worldwide agreement on limiting the production of damaging materials and the control of waste production and disposal. There have been some international successes, such as the banning of CFCs and PCBs and a host of pesticides, but, because of political dissent, much of the pollution control has fallen to organisations in different countries.



The spectre of disposed waste returning to cause untold damage has resulted in tighter controls being exerted by the authorities responsible for the environment. In the UK the Environment Agency (EA), is charged with limiting pollution and trying to rectify existing problems. The EA monitors the state of the waterways, atmosphere and waste disposal sites in England and Wales. With the introduction of legislation to monitor waste, companies producing waste had to impose stringent controls on themselves in an effort to meet targets set by the EA and also to regenerate consumer confidence. Oil companies have received a lot of bad 'press' over the impact of their industry on the environment and are, currently, investing heavily in trying to produce environmentally friendly ways of providing sources of renewable energy (BP Solar are the largest producers of solar panels, www.BP.com) and increasing the efficient use of the ever decreasing supplies of fossil fuels.

Although all waste causes problems of disposal, organic waste appears to be the most difficult to deal with. Organic material is defined as 'material of or relating to animal or plant constituents or belonging to a class of chemicals having a carbon base'(Collins English Dictionary,1989) Most organic material derived from animal or plant source is biodegradable, which is to say that the material is acted upon by bacteria in the environment, that break down the material into simpler forms.

Biodegradation can occur in two ways, one, using oxygen to assist the bacteria termed aerobic degradation and the other, which occurs without the presence of oxygen which is termed anaerobic degradation. Aerobic degradation usually produces carbon dioxide gas and water whilst any other elements are utilised by the bacteria for their own growth. Anaerobic degradation tends to produce methane and carbon dioxide. Some hydrocarbons, as naturally occurring compounds, will eventually decompose to

harmless components but many man-made compounds are either long lasting or unpredictable in their reaction once released.

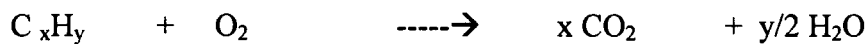
Aquatic life depends upon the oxygen that is naturally dissolved in the water. This dissolved oxygen, measured in milligrams of oxygen per litre of water, can be as high as 15 mg/l. The colder the water is, the higher the levels achievable. However, when water is polluted with organic material (material derived from a carbon source) the bacteria involved in degradation use up the oxygen present thus causing a shortage of oxygen for other aquatic life forms present. Once all the oxygen is used up anaerobic degradation is carried out by other species of bacteria and the water is described as being anoxic.

When waste organic material is to be disposed of, the impact of biodegradation on oxygen levels must be considered. In the disposal of large amounts of waste such as into landfill sites the production of methane can be dangerous unless the site is designed to collect and use the gas. Many sites have been designed to harness methane and use it to create alternative energy, either as electricity or heat or both.

In waterways the concentration of dissolved oxygen is the most important consideration. Without dissolved oxygen in the water many aquatic organisms would be unable to survive. To ensure that waterways are not polluted to anoxia the Biochemical Oxygen Demand (BOD_5) test is used as a method of testing the loading of waste organic material. (See Appendix, p 154)

The BOD_5 test determines the amount of oxygen that will be used by bacteria in a sample of water over a period of 5 days to degrade any organic material it contains. A simplistic version of the process of the biodegradation of an organic chemical is

Organic Chemical + Oxygen $\xrightarrow{\text{bacterial action}}$ Carbon Dioxide + Water



Another method used is the Chemical Oxygen Demand (COD) which determines, by a chemical titration test, the amount of oxidisable material present in a given sample of water (Appendix p 155). This figure is usually higher than the BOD₅ as it includes material that will not naturally biodegrade but can be chemically oxidised.

If an industrial site produces water effluent that is contaminated with an organic chemical, then the Environment Agency will place a limit on the BOD₅ level of that water. One such site is the BP Central Area Transmission (CATS) terminal situated on reclaimed land at Seal Sands, Middlesbrough, (Grid Ref. NZ 518 247) in the Tees Estuary.

1.2 The use of constructed reedbeds in wastewater treatment

Constructed reedbeds / wetlands have been in use since the 1960's, and their design and performance have been widely documented (e.g. Mitsch 1989; Lawson 1989; Horan 1991; Cooper 1996; Griffin 1998; Jiang 1998; Vymazal 1999; Masi 1999; Verhoeven 1999; Greenway 1999; Cerezo 2001). Although they can be expensive to install, they are low on running costs so many wetlands have been constructed to replace some small sewage treatment plants in the Severn Trent Water Authority area (Griffin and Pamplin, 1998; Vymazal, 2005). Community sewage, cess pit contents, farm effluents, mine drainage and landfill leachate are all common problems that have utilised the constructed reedbed / wetland treatment system (Lawson 1989; Wood 1994; Griffin 1998; Sun 1998; Maehlum 1999; Vymazal 1999; Masi 1999; Verhoeven

1999; Newman 1999; Greenway 1999; Kern 1999; Decamp 1999,2000; Nguyen 2000; Cossu 2001; Cerezo 2001)

Johnson (1999) found that although there was a lot of information and literature about constructed wetlands and their uses, few dealt with the treatment of pollution caused by hydrocarbons i.e. organic chemicals. The basic concept of reedbed treatment systems is that micro-organisms/ bacteria residing in the dense rhizosphere of the reeds biodegrade the waste material passing through the beds. They are aided by the ability of the reeds to 'pump' oxygen down to the rhizosphere (Cizakova 1998; van der Nat 1998; Rolletschek 1999; Vymazal 1999; Grunfeld 1999; Armstrong 2000) and so replete the oxygen used up by the bacteria. When discussing reedbed treatment systems it is important to remember that these systems are **constructed** wetlands not natural ones. In a constructed wetland, facilities such as baffles are included in design and manufacture, in an attempt to ensure that the flow of the effluent follows a pre-determined course that gives maximum exposure of the effluent to the rhizosphere to ensure maximum oxygenation and therefore maximum exposure to microbial communities. This design is not always successful as pathways may become blocked with decomposing biomass or solid waste. In horizontal flow systems water flow and residence time i.e. the time that the effluent is kept in the reed bed is controlled by the use of swivelling level pipes.

The biodegradation of organic waste is a process where the compounds are broken down into elemental carbon, carbon dioxide, water and any other elements that were part of the waste compounds. The elemental carbon is utilised by the bacteria in cell growth (Todar, K., 2005) and some of any other elements made available are often absorbed by the macrophytes themselves. The term organic waste is usually taken to be waste that has originated from a 'natural' organic source such as domestic sewage,

farm waste e.g dairy washings, silage seepages or landfill leachate from domestic waste. It does not normally refer to organic chemicals.

The basic design concepts and calculations for constructed wetlands are now accepted and for routine waste e.g. sewage or natural organic waste or heavy metals; test beds are rarely used. There are accepted formulae for the calculation of capacities and BOD₅ levels and these are used in the design of new systems. (Mitsch 1989; Horan 1991; Merritt 1994; Hawke 1995; Cooper 1996) If the load requiring treatment is known then the size of wetland required to treat the waste produced can be calculated.

The calculation widely used in Europe for horizontal treatment systems is

$$A_h = (Q_d (\ln C_o - \ln C_t)) / k_{BOD}$$

Where

A_h = surface area of bed

Q_d = average daily flow rate sewage, m³/d

C_o = daily average BOD₅ of effluent, mg/l

C_t = required daily average of effluent, mg/l

k_{BOD} = rate constant, m/d

The factor, k_{BOD} , has been recommended to be 0.1 for normal strength sewage with a BOD₅ of between 150 and 300 mg/l. (Cooper, P. F., *et al*, 1996)

1.3 Background to the BP CATS terminal and the constructed reed beds

The BP CATS terminal processes natural gas from fields in the North Sea and was built in 1996. During construction there were about 300 personnel on the site but there was no mains sewage system in place. To cope with the waste water generated and

surface run off from the site, a reed bed was constructed adjacent to the site that was designed to treat the waste produced. Prior to the construction of the reedbeds the grassland had been surveyed so that on completion the disturbed land was reseeded with a matching mix of grasses etc.(Rushall,1998) The land surrounding the beds has not been disturbed since then and is now a well established grassland is species rich and of importance to the biodiversity of the area.(Stubbs,2002) The land on which the industrial complexes are built is actually reclaimed from the Tees estuary and consists of limestone slag from the steelworks and dredgings from the estuary. This has resulted in the creation of a calcareous grassland with some plants not usually found in the area. BP are aware of the importance of the site and are making every effort to protect it. The University of Durham has been involved with the development since its inception and have used the site for some undergraduate projects and one MSc project. During a recent plant survey it was found that the site supported four species of orchid (*Dactylorhiza fuchsia* , Common Spotted Orchid; *Dactylorhiza purpurella*, Northern Marsh Orchid; *Gymnadenia conopsea*, Fragrant Orchid; *Ophrys apifera*, Bee Orchid) with over 40 other species of plants (Bell,N.,2002) , 32 species of birds (Stubbs, K.T., 2003) and in excess of 60 species of insects (Rushall, C., 1998). The ponds and reed beds support frogs, toads, common newts and voles, whilst the grassland supports rabbits, hares and foxes. Amongst the birds supported are skylarks, *Alauda arvensis* and Grey Partridge, *Perdix perdix*, both of which have bred on the site and are listed as ‘target species’ in the Tees Valley Biodiversity Action Plan, 1999.



Plate 1.1. Map of Teesmouth, Middlesbrough with the location of the CATS Terminal reed beds highlighted in yellow (map courtesy of Ordnance Survey).



Plate. 1.2. Aerial photograph of the CATS terminal, Seal Sands, Middlesbrough taken during the construction period. (Photo courtesy of BP CATS Terminal)

(Adapted from CATS Terminal Project drawing no.7983-15/00/00/40/252/0024)

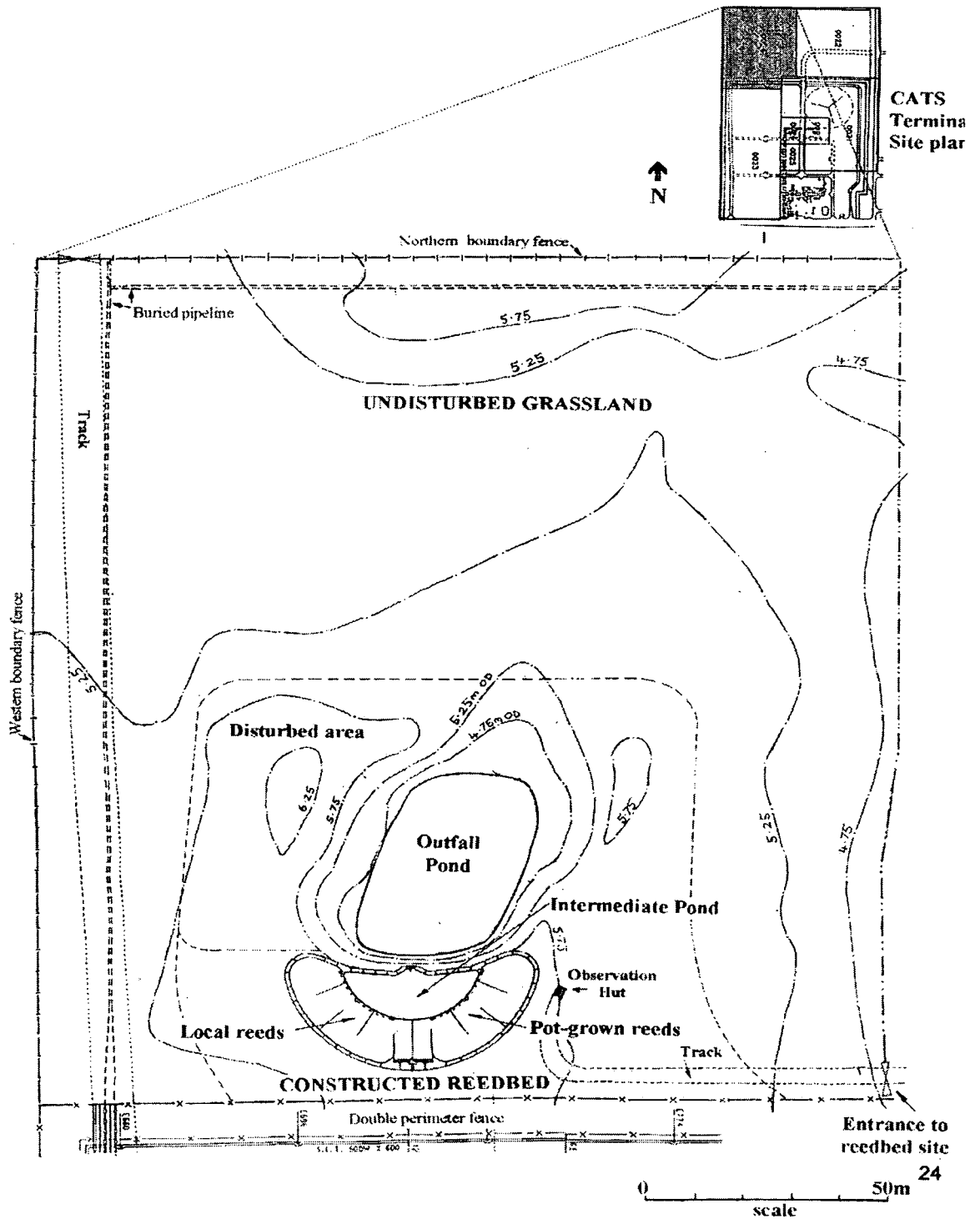
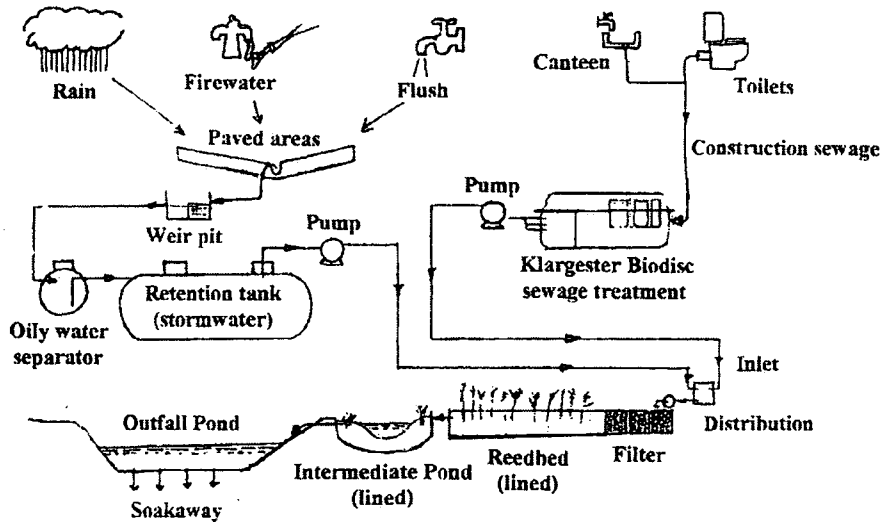


Figure 1.1 Diagram of constructed reed beds and the surrounding grassland at the CATS Terminal, Seal Sands, Middlesbrough (Courtesy of BP CATS Terminal)



(P. Weiner, 1997, Parsons Group International Ltd., personal communication)

Figure 1.2 Diagrammatic representation of the site drainage system

Figure 1.2. shows a flow diagram of the site drainage system. All site waste water was passed through a Klargester bio disc contactor primary treatment unit before passing to the reed beds.

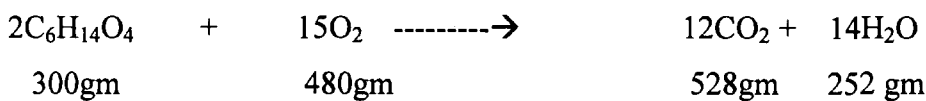
The beds are situated in an area of grassland that is species rich and in order to reduce the environmental impact the installation and commissioning of the beds in 1996-7 were monitored as part of an MSc project, (Rushall, 1998). Rushall's study showed the young reed bed was capable of assisting in the biodegradation of the primary treated effluent, produced by 300 to 400 staff, so that the water leaving the beds met the standards set by the Environment Agency. Water from the reed beds passed into an intermediate (polishing) pond and then to a larger soakaway pond. Water from the soakaway then percolated through the ground to the nearby Greatham Creek, an inlet of the River Tees. Once the site was constructed and the plant on-line, the number of staff fell and the Klargester was no longer used. A system of septic tanks was employed for amenity effluent and a system of contained drains for removing site water.

The septic tanks were emptied and the contents removed by contractors on a regular basis. Samples of drain water were analysed by an external laboratory. If the BOD₅ of the sample was over the limits set by the EA, these were also emptied by contractors and treated before being disposed of. If they were within specification they were pumped through to the reed beds. The cost of disposal by the contractors was approximately £26,000 per annum.

1.4 Background to the project

The CATS terminal uses triethylene glycol (TEG) as a drying agent for the natural gas it handles. Some of this TEG ends up in the contained drain system of the plant, some from spillages during routine maintenance and some from the reclamation process of the TEG. Because of the high molecular weight of TEG (150), small amounts of TEG give rise to high BOD₅ levels.

A simplified equation of the reaction involved can be shown as :-



There may be many intermediate steps in the process and it was hoped that these could be identified during the project.

BP are an ISO 14001 registered company with a commitment to cause as little damage to the environment as possible and to cut down on emissions, including fluid waste, as much as possible. In order to comply with this commitment, and to reduce costs of disposal, the idea of ‘treating’ the drain water by passing it through the reed beds was proposed by management, but the effect of TEG on reeds was unknown.

The amenity waste from the CATS terminal was collected in a cess pit and the management wanted to treat this material on site, once more cutting liquid waste production and costs. It was therefore proposed that the Klargestor system of treating site amenity waste prior to reed bed treatment should be reinstated.

An undergraduate project, using reeds growing in buckets, was carried out in 1999. This indicated that TEG would biodegrade with the help of reeds but some doubts were expressed about the effect of exposing the reeds to TEG in the long term.

Following presentation of the undergraduate work, BP management agreed to fund a 3yr studentship to investigate the possibility of using the reedbeds to treat waste hydrocarbons. After preliminary discussions between BP and the University team, it was decided to design and construct a series of small scale 'test beds' that replicated the established beds as far as possible but were self contained so that should there be any adverse reactions to the TEG, the products could be contained and the surrounding environment would not be contaminated.

1.5 Questions raised by the proposal to pass TEG through the constructed reed beds at CATS .

Can the reed beds help the biodegradation of TEG effluent and lower the BOD₅ without damaging the reeds?

There seems to have been little or no work carried out on treating Tri Ethylene Glycol waste using reed bed treatment systems. Johnson (1999) constructed a system to deal with the oily effluent generated from a natural gas pumping station in Musom, Florida, using *Phragmites australis* (Cav.) Trin. Ex Steud , the common reed and *Zizaniopsis miliacea* (Michx.) Doell & Aschers, Southern Wild Rice, as the

macrophytes. The target water quality standards have been met since it became operational. Although TEG was not present there were no detrimental side effects reported from the oily waste.

Chong (1999) and Revitt (1997) studied the development of constructed wetlands around Heathrow airport that were installed to deal with the runoff from the runways. This runoff was heavily contaminated with ethylene and diethylene glycol used as de-icers and 1,2-propylene glycol, used as an anti-icer on the aircraft. They reported no adverse effects on either reeds or bacteria even after 'shock' dosing of the beds of initial concentrations of 1180 mg/l and 632 mg/l to simulate large spillages. Removal efficiencies of 78% for a sub surface system and 54% for a surface flow reed bed were recorded.

If the reeds can aid the biodegradation of the TEG effluent, can they also treat the site sewage from the Klargester mixed with the TEG effluent?

The constructed reedbeds at the CATS terminal were commissioned in 1996 and their performance when used in the treatment of waste water from the Terminal construction site was monitored by Carole Rushall (unpublished MSc dissertation, 1998, University of Durham) and her results showed that the beds were capable of cleaning the site sewage to a level that was within the limits set by the Environment Agency i.e. a BOD of 50mg/l. The undergraduate work carried out in 1999, on reeds growing in buckets of gravel, indicated that TEG would be readily biodegraded but a mix of the two effluents was not used and there was no information on the effect of the two together.

Will the addition of TEG or TEG and sewage effluent affect the growth of the reeds or the bacterial populations associated with reeds?

Chong *et al* (1999) indicated that although the levels of bacteria fluctuated seasonally, population densities, after the addition of glycols, were considered to be higher than would normally be found, had glycol not been introduced.

The addition of sewage should provide a source of nutrition for the reeds and the bacteria so the theoretical outcome is optimistic provided that the plants can actually co-exist with TEG.

If the biodegradation of the TEG effluent is successful, there will be a large increase in CO₂ ventilation around the stems of the reeds - will this affect the responses of the reeds?

The elevation of CO₂ in the atmosphere is an effect of the 'global warming' phenomenon. If the biodegradation of TEG is successful, will the expected elevation of CO₂ in the atmosphere surrounding the reeds cause any reaction to the plants growth? Melkonian (1997) showed that corn grown under conditions of higher CO₂ was more productive than corn grown under usual conditions. Graves and Reavy (1996) cite research that indicates the increase in CO₂ levels results in the increase in crop production. They also point out that some work has also found this response to be limited and that the plants do sometimes revert to pre increase levels.

Will the efficiency of the system change with seasonal meteorological variances?

Temperature does not seem to have any dramatic effect on the efficiency of the reed bed treatment system (rbts) to clean the waters passing through them. Maehlum and Stalnacke (1999) found that efficiency was only reduced by about 10% in cold conditions and implied that macrophytes were not actually needed for biodegradation to occur, particularly in a horizontal subsurface flow reedbed treatment system. Similar findings were recorded by van der Nat and Middleburg (1998) but they also demonstrated that increased availability of oxygen in the rhizosphere was instrumental in achieving the levels of biodegradation recorded. This emphasises the importance of the macrophytes in maintaining oxygen supplies to the rhizosphere. Yin and Shen (1995) found that, with some engineering alterations to the beds in question, that even under frozen conditions the reed beds were more efficient than a nearby secondary treatment plant. Kern and Idler (1999) demonstrated similarities between seasonal results in the reduction of BOD₅ but pointed out that nitrification was adversely affected by lower temperatures.

The efficiency of an rbts has been shown to depend on the availability of oxygen (van der Nat 1998; Cizakova 1998; Grunfeld 1999; Armstrong 2000) and the ability of *P' australis* to provide that supply with results showing aeration to be similar in established stands and new ones. Bart (2000), however, found that rhizosphere oxidation increased as the reeds matured thus both the maturity of the system and the season may affect the efficiency of the reedbed.

1.6 Aims and objectives of the project

The main aim of the project was to determine whether waste TEG produced on the BP site could be passed through the constructed reedbeds and be biodegraded to a level that was acceptable to the Environment Agency, without having any detrimental effect on the established reeds. If a method was found, it had to utilise the existing facilities and practices. The normal procedure of designing a system for a particular problem did not apply here. The reed beds were already in place and could not be modified to any extent (dimensions were unalterable, planting was established). There was no system of constant flow to the beds. Rainwater that fell on the columns and towers of the treatment plant was collected, along with any spillages, in the retention pond/pit. This pit was kept at approximately 60% full and when the level went over this point the plant operators would discharge about 30m³ to the reed beds (A. Tapster, Engineer, pers. comm.) Instead of trying to design a treatment system for a particular effluent of known content and flow, this project was aimed at using an existing treatment system and trying to ascertain the levels of pollution that the beds would cope with, without suffering any damage.

Using the test beds as proxy to the existing constructed reed beds, the objectives were:-

- 1) To find a quick, easy and reliable means of measuring the level of TEG or the BOD in the effluent that could be used by plant personnel.
- 2) To determine whether TEG can be biodegraded when passed through an rbts.
- 3) If TEG can be biodegraded in the rbts what effect will the action have on the reeds.

4) To find the highest concentration that could be tolerated by the reeds without adverse effect.

5) To determine what effect, if any, the mixing of TEG with sewage would have on the reeds and reedbed efficiency.

6) To try and provide some indication as to the time needed to biodegrade an effluent of a particular concentration in the existing beds to levels within the parameters set by the EA.

The pathway of degradation was of interest as was the actual process and a subsidiary objective was to find how degradation was initiated and accomplished.

2.0 The test beds used in the experiments with TEG

2.1 Introduction

The current project - the attempted biodegradation of Tri Ethylene Glycol (TEG), by itself and mixed with site sewage in a reed bed system - is to the best of the authors belief, unique. Other glycols and hydrocarbons have been passed through reedbed treatment systems with great success (Strong-Gunderson 1995; Revitt *et al*, 1997; Qianxin 1998; Johnson 1999). However, TEG has a high molecular weight, 150, giving rise to a very high BOD₅ even in low concentrations, i.e. a large amount of oxygen is needed to aid biodegradation of a small amount of TEG. Because the beds at the CATS terminal had already been constructed and had proved to work with site effluent, the aim of this project was to establish whether TEG contaminated effluent produced on site could be disposed through the system that is in place. The accepted calculations for the theoretical size can only help with loadings as the bed dimensions cannot be altered. There were many unknowns to be considered about the addition of TEG effluent to reed beds and because of these unknowns it was decided to carry out the experiments in scaled down test beds that would be completely self-contained. This would protect the established reed beds from any detrimental side effects which may occur and would enable sufficient replicates to be used to give statistically valid results.

2.2. Construction of the test beds

The test beds were designed to replicate, as far as possible, conditions in the existing constructed reed bed that was already on site and which would be used for effluent disposal in the future. It was decided that the test beds should be built alongside the established beds so that any variance due to geographical or meteorological conditions could be discounted from any discussion concerning the results and their relevance to the future 'real life' disposal of the waste hydrocarbons.

Figure 2.1 shows the plans used in the construction of the wetlands at the CATS Terminal and the approximate position of the test bed assemblage in relation to the constructed beds.

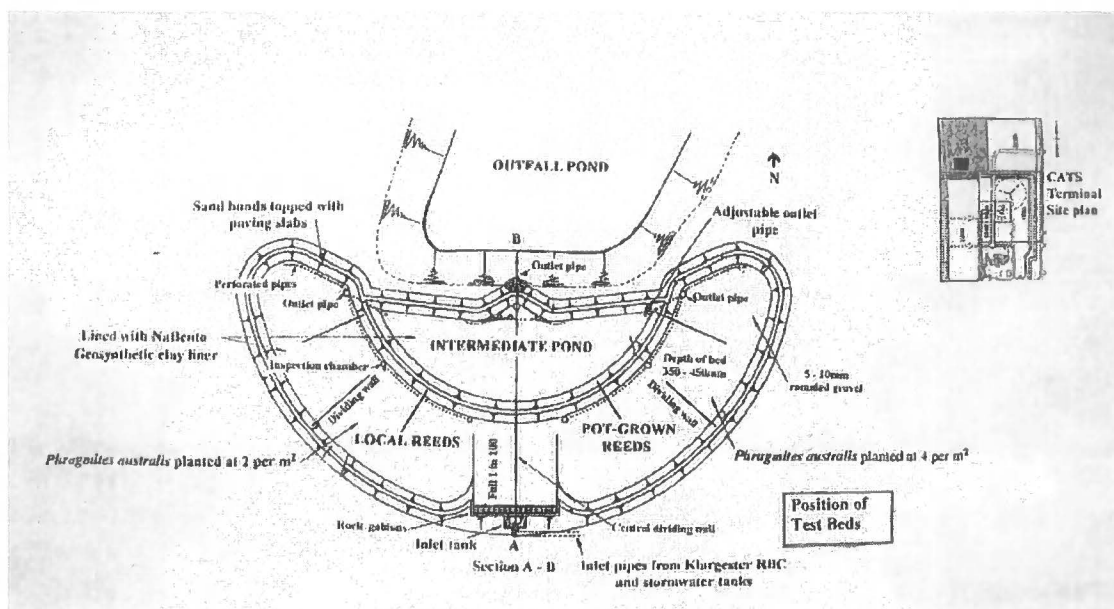


Figure 2.1 CATS Terminal horizontal subsurface flow reedbed system (Adapted from CATS Terminal project drawing no. 7893-15/00/00/40/252/0040)

It can be seen from Figure 2.1 that the constructed reed beds are in fact two separate beds. After the inlet, each bed has four baffles that direct the flow of water through the reeds. This arrangement attempts to ensure that the water will travel the maximum

possible distance from the start of the beds to the outlet rather than allowing the water to take the shortest route from inlet to outlet. The baffle arrangement thus directs the water to the maximum surface area of reed rhizosphere and gravel. The constructed beds have a 1° slope, from inlet to outlet, built into the base of the bed itself thus encouraging a gentle gravity induced flow through the system without the need for any pumping of effluent. It was decided that the test tanks should, if possible, be made to be portable. If the system proved to be successful it was hoped that the tanks could possibly be used in other venues in the future.

Black polypropylene 450 l cold water storage tanks (1.15m long x 0.6 m wide and 0.65 m deep) were used as test beds.

So as to be able to achieve replication of results, it was decided to use 3 test beds to test a solution of a given concentration and so as to increase the number of trials possible in the time allotted, it was decided to use another set of 3 units to test a solution of a different concentration. Natural occurrences could have an effect on the test beds so it was decided to have 3 beds acting as 'controls' i.e. they would only receive water but could be used to counter any claims of the results being influenced by natural occurrences. A further 3 beds were constructed to be used as either controls or spares, should anything untoward happen to a test bed. By having the control beds alongside the test beds and by ensuring that both test and control beds were treated identically until the start of the trials it was possible to say that any difference between the controls and the test beds was due to the addition of solutions of TEG. The test beds were numbered east to west from 1 to 12 inclusive. The choice of test beds was made by drawing the numbers of the test beds out of a hat. Tanks 3,5 and 10 were selected as the first group and 9,11 and 12 as the second. An area of land alongside the established reed beds was covered with a geotextile membrane and a layer of

hardcore to act as a base. The membrane was used so that when the project was finished the land could easily be cleared of the hardcore.

Twelve test units were therefore constructed, each unit consisting of a 225 l header tank, a 450 l test tank and a 225 l receiver.

The test beds were designed to try and replicate conditions in the existing constructed reed beds (Fig 2.1.) and had 4 baffles fixed within each tank using waterproof tape (Plate 2.3.). At the point where the test solutions were to be added, a further small baffle was added lengthwise to contain some larger stones that would replicate the gabion area of the beds. This baffle did not reach the bottom of the tank and was designed to assist in the dispersal of the test solutions. Marks were placed around the inside of each test bed to indicate a depth of 0.45m. Washed pea gravel, 5 – 10 mm, was added to each tank and 50, 1 year old pot grown *P' australis* were planted in the gravel and the surface level adjusted until it reached the 0.45 m line. 10 litres of reed bed water was added to each tank and then each tank was filled up to the 0.45m line with site mains water. Planting took place over a two day period, 26 & 27 April, 2001 and the plants were then allowed to become established in the test beds. They were left for 14 weeks with the water level being checked and adjusted twice a week. The test beds were supported on a scaffolding structure with the base of each bed being 1 m from the ground. The beds were arranged side by side with a lengthwise 1° slope running from north to south. Test solutions were added through a slotted 28mm copper spreader bar fed from a 28mm copper pipe fitted to the bottom of the 225l header tank placed about 2.5m above the ground. The addition of test solutions through this pipe was controlled by stopcocks.

At the southern end of the test tank, on a lower level, there was a 225l receiver used to collect the contents of the test tank after the trial was over. Prior to disposal the

contents of the receiver was analysed to ensure that the surrounding grassland was not polluted by the contents.

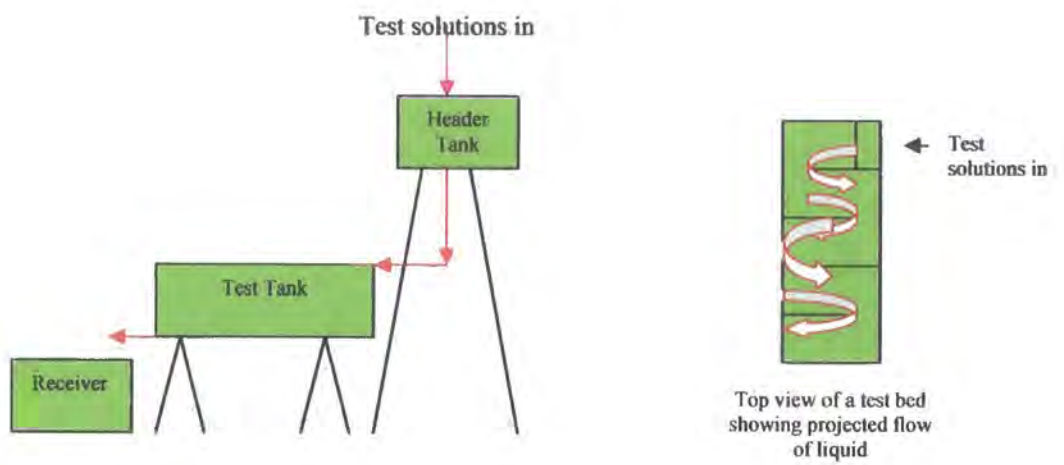


Figure 2.2. A schematic plan of one portable test bed (Author)



Plate 2.1. A test tank prior to addition of gravel and reeds



Plate 2.2. The scaffolding structure before the test beds were put in place

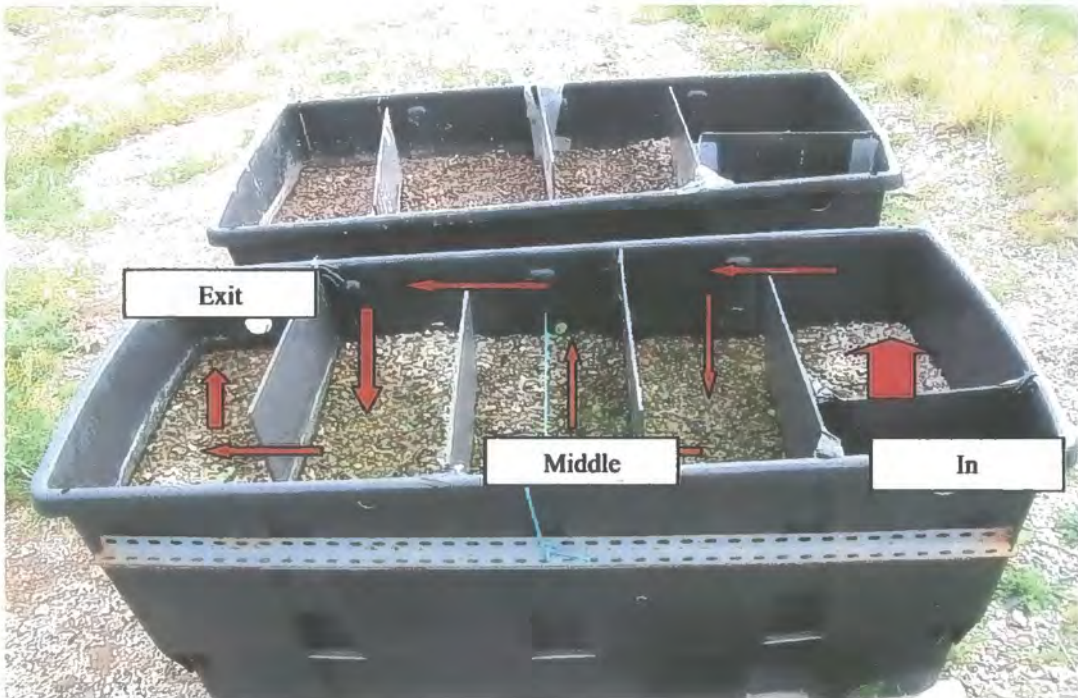


Plate 2.3. Photograph of a spare test tank complete with baffles and gravel showing sample points and direction of flow. These two beds were later used as gravel only test beds.



Plate 2.4. Side view of one complete test unit with test bed, header tank and receiver in place



Plate 2.5 View of the back of all the test beds on their scaffold support

2.3 Performance of the test beds and problems encountered

The system designed for the delivery of the test solutions proved to be unsatisfactory, in that it was difficult to maintain a regular flow of solution. As the level of solution in the header tank fell, so the rate of addition decreased. However the system used to transfer effluent from the retention pond to the reed beds worked in a similar way in that the initial flow was approximately 18000 litres /hour but as the level in the retention pond fell the flow became erratic. The water levels in the experimental beds had to be constantly monitored as the evapotranspiration effects of sun and wind caused regular level changes. If the tanks needed topping up at any time, **all** beds received the **same** volume of water. Whilst the trials were proceeding it was noticed that the water in tanks 9, 11 and 12, in particular, were turning a reddish brown colour and starting to smell. The bottom tank/receiver for these 3 systems started to develop a jelly like substance on the surface of the waste water. The water did not contain any detectable TEG and the jelly did contain some protozoan life. The reddish colour was determined to be ferric oxide whilst the 'jelly' was thought to be an iron mould (Edstrom Industries, information sheet 4230-M14146, 11/2003). The source of the ferric oxide was found to be some scaffolding clamps that were corroding above the test tanks, dripping a solution of ferrous salt into the beds where the oxygenation of the water was causing the precipitation of ferric oxide.

During Trials 11 & 12, an accidental overspray of systemic weed-killer by the site contract gardeners, coupled with the iron problem meant that trials were not conducted throughout the summer. The reeds recovered after a short period of water only additions.

3.0 The degradation of TEG in the test beds

3.1 Introduction

The 12 test beds, filled with gravel, reeds and water, were numbered in sequence, with no. 1 being the easterly bed. The beds were then used as proxy to the main constructed reed beds in a series of trials with the following specific objectives:

Although similar in appearance to a sequential batch treatment system, it must be pointed out that the test beds were NOT sequential and that they were actually individual units. Each unit consisted of a header (feed) tank, the actual test bed of gravel and reeds and a receiver, into which all material passed before being cleared for disposal into the surrounding environment. Test solutions were added to the allotted tank and remained in those tanks until the trial was finished. They were not then passed into another test tank. In a sequential batch treatment system the effluent is passed into a bed that is designed to remove a particular part/ parts of the effluent, whereas these test beds were replicates of the existing constructed reed bed at the CATS Terminal.

3.2 Methods

3.2.1 Detection of triethylene glycol.

As the test effluent was to be triethylene glycol in water with no other material added a quick reliable method of determining triethylene glycol was required. Gas chromatography is commonly used but usually to determine high concentrations of triethylene glycol (Ruifeng Li,1998).

A pilot undergraduate study (Stubbs, 2000, Unpublished dissertation, University of Durham) used a gas chromatography method but each test had taken approximately 14 minutes to complete. This, coupled with a cooling period needed by the equipment

between tests increased the time to about 20 minutes for each test which was not a workable time for the volume of analysis envisaged. Provided that there were no problems needing repeat analysis it was thought that there would be approximately 20 to 22 tests to be performed on each sampling day. So as to minimise any problems with degradation continuing in the sample vials it was hoped that all analyses could be completed on the day of sampling. As the test beds were situated approximately 8 miles from the laboratory, time was of the essence. Any samples that were not analysed on the days of sampling were stored at 4⁰C in the dark.

S.G.E.Ltd., an Australian manufacturer of chromatography columns and ancillary equipment produce a comprehensive range of GC columns for the detection of a vast range of compounds. To aid in choosing the correct column for a particular compound or range of compounds the company have a comprehensive on line library that lists all uses and shows sample traces for each usage. The column needed for the project had to be able to run at temperatures up to 350⁰C and be able to cope with aqueous samples. It had to be able to detect low boiling compounds such as alcohols and yet still provide good separation of the 3 glycols, ethylene, diethylene and triethylene glycols. After examining the properties of the columns in the range it was decided to use a BP20 column that was 30m in length with an internal diameter of 0.32mm and an internal coating of 5 microns of polyethylene glycol and was supplied by SGE (UK) in Milton Keynes. The BP20 was chosen over the BP21 as it was more suitable for aqueous samples and the temperature ranges envisaged. The column was fitted to a Unicam 600 gas chromatograph connected to a Unicam 4880 data collection unit and an Epson LQ-570+ printer.

So as to determine the optimum settings for the column conditions a aqueous solution of 0.1% v/v mono, 0.1% v/v di and 0.1% v/v tri ethylene glycol was accurately made and stored in a refrigerator at 4⁰C and measured amounts were injected into the GC using different heating programmes and gas pressure settings. The settings had to able to produce a trace that detected the presence of any low boiling compounds such as alcohols whilst still producing large well defined and separated peaks for the 3 glycols. At 0.1%, the peaks for the glycols had to be as large as possible at a low sensitivity with definite separation. The three peaks produced were in the order of mono, di and tri ethylene glycols. An isothermal setting was unsuitable as the retention time for TEG was too long, so a heating programme that could raise the oven temperature at a preset rate was used. The flow rates of the GC gases were also varied as these affect the size and separation of the peaks produced. Trials were conducted varying these flows and temperatures etc. until optimum settings were achieved.

These were:-

Start temperature	150 ⁰ C
Final temperature	350 ⁰ C
Temperature increments	15 ⁰ C /min
Injection temperature	350 ⁰ C
Detector temperature	300 ⁰ C
Carrier gas (nitrogen) flow	25 psi
Fuel gas (hydrogen) flow	12 psi
Air flow	26 psi
Injection volume	0.5µl rising to 3µl by end of project

As well as deciding on the settings for the GC, the method of GC analysis also had to be chosen. GC columns can give constantly reproducible results when used regularly but because the stationary phase i.e. the column lining, changes fractionally over time, a method of determining the columns' efficiency and its response to the target compounds at a given time is needed. There are 2 commonly used methods of quantitative GC analysis in use, one uses an internal standard and the other uses straight samples.

The first method, using an internal standard, involves adding a measured amount of a known chemical (internal standard) to a measured amount of sample. After shaking a sample of the mixture is injected into the GC. The choice of internal standard is crucial as it needs to be a compound that will elute at a point on the GC trace that will not interfere with the target compound, but will respond in a similar manner to it. The area of the target compound is proportional to its concentration and the area of the standard shows the response of the column at that moment in time. It is then possible to determine the concentration of the target compound by a simple calculation.

Concentration of target = (Area target peak x concentration of standard) ÷ Area standard peak

In theory, this method ensures that the response of the column is constantly being checked. In practice, some internal standards are not very stable and can result in incorrect analysis.

The second method is based on the area of the peak produced for a compound being directly proportional to the concentration of the compound. The response of the

column to a compound does change and because of this a 'factor' has to be calculated before each set of traces.

$$\text{Area of peak} \times \text{response factor} = \text{concentration of component}$$

Trials were conducted into both methods to determine which would be the most reliable.

For the internal standard method, a chemical was needed that would elute before the TEG peak but that would not infringe on either the TEG or the DEG peaks. After searching the physical properties of many compounds it was decided that the most suitable chemical for use as a standard would be 1,5-pentanediol. When passed through the GC it did elute between the di and tri ethylene glycol peaks, without interfering with them, so a series of trials were run. Standard solutions of 0.1%v/v DEG and TEG with 0.025%v/v 1,5-pentanediol and 0.01%v/v DEG and TEG with 0.025%v/v 1,5-pentanediol were made up and tested in the GC. Some of the results are shown in Table 3.1. These figures are the best figures obtained as the diol, itself, did not give many reproducible traces over the period of the trials.

Table 3.1. Results of internal standard trial.

Concentration of DEG/TEG	Area DEG	Area 1.5. Pentane Diol	Area TEG
0.1%v/v	475.072	194.565	427.957
	470.756	195.155	438.826
	482.044	196.383	431.675
Average factor	475.9573	195.3677	432.8193
	0.00021	0.000512	0.000231
0.01%v/v	62.368	178.253	59.602
	48.846	141.474	42.49
	53.389	176.272	42.703
	52.304	176.967	36.798
	45.103	141.753	36.202
	54.334	183.044	41.488
Average	52.724	166.2938	43.21383
Concentration by GC	0.011	0.085	0.01

When the 0.1% samples were injected the areas of the DEG and TEG were used to calculate the response factors and the areas of the diol peaks were noted. The factors were used to calculate the concentrations of the 0.01%v/v solution. The resultant traces were not very consistent but the mean result for DEG and TEG were satisfactory at 0.011% and 0.01% respectively, whereas the result for the pentane diol bore no relationship to the known concentration of the solution. Trace 3.1. is a GC trace of 0.1% DEG/ pentane diol / TEG solution and Trace 3.2 is a trace of the 0.01%v/v solution with 0.025%v/v diol. The TEG and DEG areas are proportionately less and the peak for pentane diol has reduced in area when it should have stayed the same as in the trace of the 0.1%v/v solution. This result was repeated and indicated that under the conditions suitable for the detection of the glycols, the pentane diol did not give reproducible peaks. It was therefore decided that pentane diol was unsuitable to be used as an internal standard in this case.

Figure 3.1. A GC trace of 0.1%v/v DEG/ 0.025%v/v Pentane diol/ 0.1%v/v TEG solution. Peak 4 represents DEG, peak 5 pentane diol and peak 8 TEG.

Online Channel 1
Run Type : Unknown

Run Number : 76

11:14 Thu Jun 27 2002

Batch Name : RUN

File Description

Sample Strip:

Method:

Control Method:

Raw Data:

Results:

Date

12:20 Tue Jun 11 2002

12:20 Tue Jun 11 2002

12:19 Tue Jun 11 2002

Raw Data:

Results:

Name

C:\4880\SYSTEM\TEG4.STR

C:\4880\METHODS\TEG4

C:\4880\METHODS\TEG4

C:\4880\RAWDATA\

C:\4880\RESULTS\

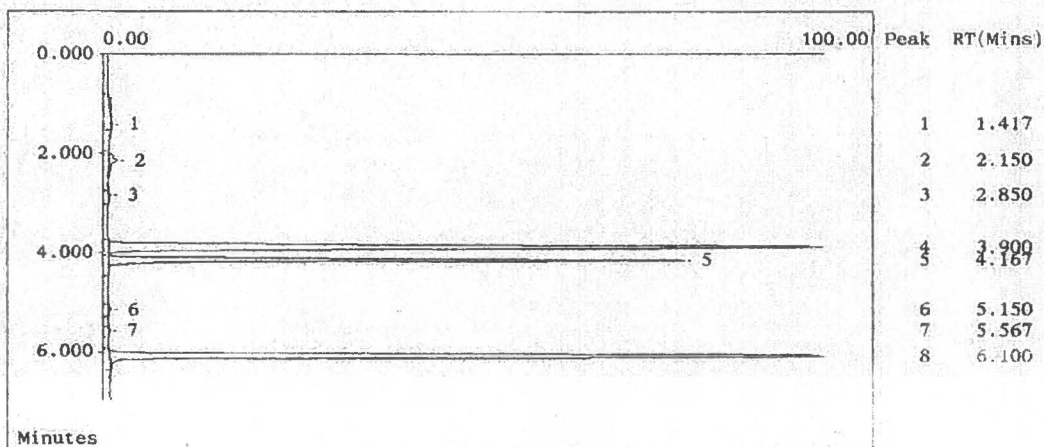
Mode : Manual

Sample Name : 2 ul 0.025% pdiol/0.1%deg/0.1% teg

Scale : 1.0000000

Sample Amount : 1.0000000

Comment:

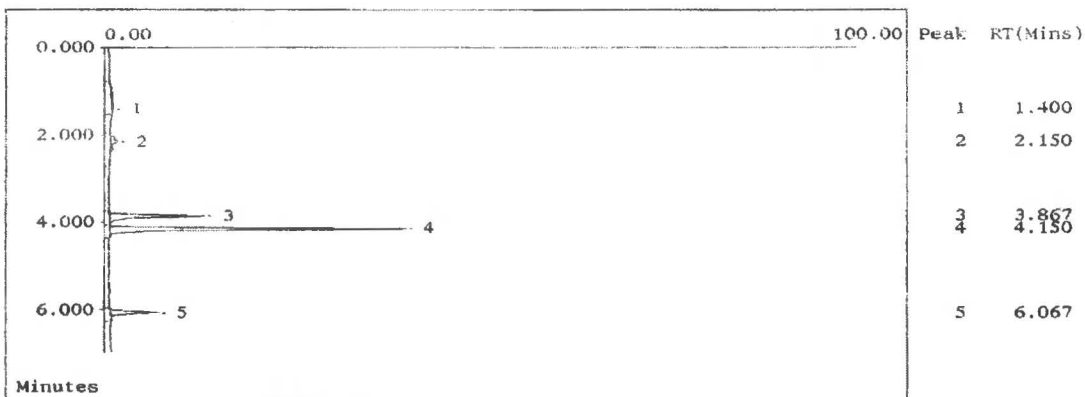


Peak Name	Type	RT(Mins)	Area	Height	Base	Conc
1	BB	1.417	9.765	0.271	1.004	0.000
2	BV	2.150	14.748	1.219	0.796	0.000
3	VB	2.850	2.112	0.376	0.699	0.000
4	BV	3.900	520.953	99.228	0.772	0.000
5	VB	4.167	278.769	79.883	0.791	0.000
6	BB	5.150	2.256	0.437	0.761	0.000
7	BB	5.567	1.185	0.360	0.789	0.000
8	BB	6.100	506.654	99.065	0.935	0.000

Figure 3.2. A GC trace of 0.01% v/v solution of DEG/ 0.025% v/v Pentane diol / 0.01%v/v TEG . Peak 3 represents DEG, peak 4 pentane diol and peak 5 TEG.

Online Channel 1 Run Number : 79 13:29 Fri Jun 28 2002
 Run Type : Unknown Batch Name : RUN

File Description Date Name
 Sample Strip: 12:20 Tue Jun 11 2002 C:\4880\SYSTEM\TEG4.STR
 Method: 12:20 Tue Jun 11 2002 C:\4880\METHODS\TEG4
 Control Method: 12:19 Tue Jun 11 2002 C:\4880\METHODS\TEG4
 Raw Data: Raw Data: C:\4880\RAWDATA\
 Results: Results: C:\4880\RESULTS\
 Mode : Manual
 Sample Name : 2ul 0.01% deg/teg/ 0.025% p-diol
 Scale : 1.0000000 Sample Amount : 1.0000000
 Comment:



Peak Name	Type	RT(Mins)	Area	Height	Base	Conc
1	BB	1.400	9.777	0.257	0.969	0.000
2	BT	2.150	14.007	1.043	0.749	0.000
3	BV	3.867	52.261	12.752	0.685	0.000
4	VB	4.150	144.655	39.400	0.722	0.000
5	BB	6.067	22.801	6.507	0.808	0.000

The second method under consideration was to use direct samples and determine the daily response factor of the column to the 3 glycols. Before a batch of samples was tested, samples of 0.1%v/v MEG / DEG / TEG aqueous solution were passed through the GC and the response of the GC to the 3 glycols was determined and a factor determined. This factor was determined by injecting the standard amount (1 μ l) of 0.1%v/v aqueous solution of mono, di and triethylene glycol.

$$\text{Response factor} = 0.1 \div \text{area of component peak}$$

The factor was determined when at least two consecutive traces produced factors within 10% of each other. This method proved to be reliable (Table 3.1) and was adopted as the method of analysis for the trials. By using diluted standard samples it was determined that it was possible to detect TEG at levels down to 0.0001%v/v.

A 'master' solution of 1.0%v/v MEG/DEG/TEG in water was prepared every month and stored at 4°C and a standard solution of 0.1%v/v was prepared from this 'master' every week during a trial. The new standard solution was tested against the 'incumbent' standard before it replaced the old one. It was found that the glycol solutions did not degrade when made with distilled water and when kept in a fridge in a sealed container at 4°C. The retention time (time from injection to elution of peak) was determined by using solutions of each chemical separately. Then, when all three were put together in the standard solution it was possible to determine which peak represented which compound. Initially 0.5 μ l was sufficient to obtain clear defined peaks but as the project progressed the volume had to be increased as the column deteriorated and small peaks were produced.

Figure 3.3. A typical GC trace of the 0.1%v/vMEG/DEG/TEG standard.

(0.3µl injection) Peak 2 represents MEG, peak 3 DEG and peak 4 TEG

Online Channel 1 Run Number : 43 10:36 Thu Jan 17 2002
 Run Type : Unknown Batch Name : RUN

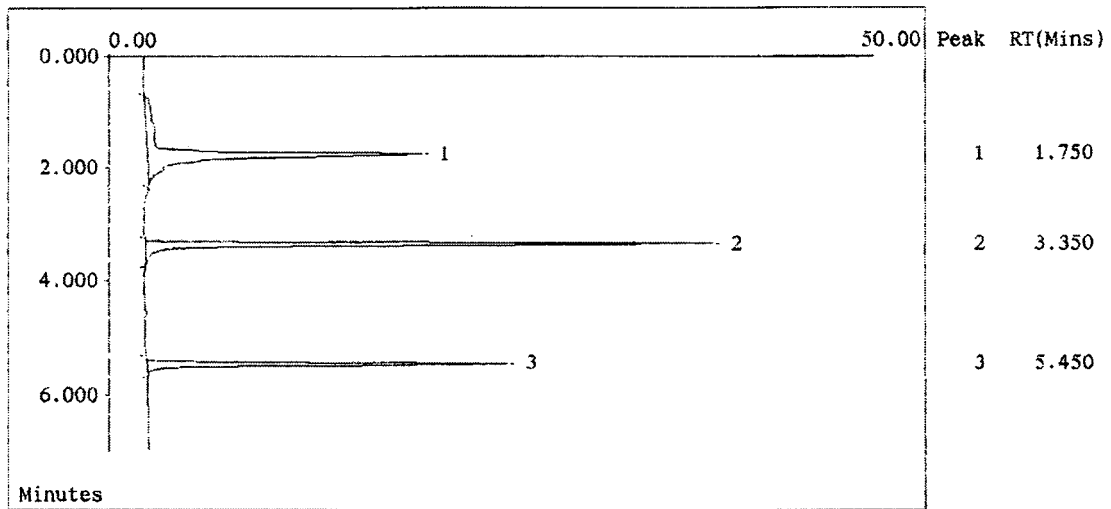
<u>File Description</u>	<u>Date</u>	<u>Name</u>
Sample Strip:	15:12 Thu Jan 03 2002	C:\4880\SYSTEM\TEG4.STR
Method:	15:11 Thu Jan 03 2002	C:\4880\METHODS\TEG4
Control Method:	15:12 Thu Jan 03 2002	C:\4880\METHODS\TEG4
Raw Data:	10:35 Thu Jan 17 2002	C:\4880\RAWDATA\RUN02.043
Results:	10:35 Thu Jan 17 2002	C:\4880\RESULTS\RRUN02.043

Mode : Manual

Sample Name : 0.1% std

Scale : 1.0000000 Sample Amount : 1.0000000

Comment:



Peak Name	Type	RT(Mins)	Area	Height	Base	Conc
1	BB	1.750	154.234	17.991	2.489	41.533
2	BB	3.350	136.697	37.225	2.366	36.811
3	BB	5.450	80.421	23.574	2.498	21.656

3.2.2 Experimental Design and Procedure

3.2.2.1 Use of the experimental beds

It was decided that 3 beds would be exposed to a solution of one concentration of TEG, another 3 would receive a solution of a different strength whilst the remaining 6 would act as controls/ spares. The beds had been numbered 1 to 12 and these numbers were drawn at random, in groups of three. Beds numbered 3, 5 and 10 were selected to receive test solutions of one concentration whilst beds 9,11 and 12 would receive solutions of another concentration. These beds were designated to be the **test beds**.

The other beds would not receive any effluent, but would receive only mains water. These beds would be designated as being the control beds. The addition of a test solution was termed a 'trial'. In each trial the groups of test beds remained the same but the concentrations of solutions varied for each trial. The solutions to be tested were made up in batches of 150l and this was divided into 3 equal lots of 50l each into the appropriate header tanks. 50l of water was drained off from each bed before addition of the test solution or in the case of the controls before the addition of water. Although the mains water contained chlorine, added by the Water Authority, it was used both in the solutions and when added to the controls because it was from the same source as that which was used on the Terminal and was used in the retention pond. The initial flow rate of the test solution was set at 18l per hour but this decreased as the volume in the header tank decreased. The solutions were passed through slotted spreader bars into the test beds.

Sampling of the contents of the tanks was at 3 points:-

- 1) The area where the solutions were added, designated 'in'
- 2) A point in the test bed that was half way between the point of addition and the end of the tank, designated 'middle'
- 3) A point at the furthest point from the area of addition, designated 'exit'

Sampling did not start until at least 24hrs after addition and was repeated as often as possible until the level of TEG was within the limits set by the EA(as BOD₅) each sample point was tested for DEG and TEG by GC analysis, temperature, pH, conductivity and dissolved oxygen.

3.2.2.2. Concentrations of test solutions

The actual concentration of the test solutions was decided after consultation with BP process personnel. It was said to be impossible to achieve concentrations greater than 5% v/v TEG as it was not thought that that amount of TEG could be lost at any one time. A major spillage or tank rupture would not go unnoticed and the material would be contained using the established containment practices. It was decided that it would be prudent to start with low levels of TEG in case there were an adverse reactions from the young reeds in the test beds and perform the trials with the highest concentrations of test solutions later. The initial concentrations of TEG used in each trial are shown in table 3.2.

Table 3.2. Details of trials showing start dates, duration and concentrations used in the trials.

	Date started	Duration of trial in days	Test beds	Initial concentration %v/v TEG
Trial 1	26/09/2001	12	3, 5 & 10	0.1
Trial 2	26/09/2001	12	9, 11 & 12	0.5
Trial 3	17/10/2001	15	3, 5 & 10	0.5
Trial 4	17/10/2001	15	9, 11 & 12	0.1
Trial 5	27/11/2001	50	3, 5 & 10	0.1
Trial 6	27/11/2001	50	9, 11 & 12	0.5
Trial 7	16/01/2002	20	3, 5 & 10	0.5
Trial 8	16/01/2002	20	9, 11 & 12	0.1
Trial 9	11/03/2002	33	3, 5 & 10	1.0
Trial 10	11/03/2002	33	9, 11 & 12	0.2
Trial 11	26/04/2002	49	3, 5 & 10	5.0
Trial 12	26/04/2002	49	9, 11 & 12	1.0

3.2.2.3. Trial procedure and sampling

Two days prior to addition, the test tanks were filled with water to the 0.45 metre level and then before the start of the trial had 50 litres drained off. Batches of the solutions, each of 50 litres, were made up, placed in the header tanks and then added to the test tanks at a rate of approximately 18 litres per hour. All beds were sampled at 3 points, in the ‘gabion’ area (in), in the middle of the tank (middle) and near the exit (exit). These points were tested for temperature, pH and conductivity initially and later for dissolved oxygen (a satisfactory D.O. meter being unavailable at the start of the trials) prior to the addition of the test solution. After the solution had been added the tanks were sampled (usually after 24 hours post addition and when possible after that) at the 3 points and the samples analysed for concentration of TEG by GC.

Sampling was by means of a bulb pipette. 20 ml of liquid was taken and placed in a glass vial which was taken to the laboratory for GC analysis. The samples were analysed as soon as possible, usually on the day of sampling, but if it was not possible they were stored at 4⁰C. They were then analysed the next day. Once the test solutions

had been added, samples were taken at interval of 2-3 days early in the trial and then at longer intervals as the trial progressed..

The test tanks were sampled until the level of TEG was such that it could not be detected by GC (<0.0001%v/v). After completion of a trial, the test beds were drained of water and were then refilled with water and then 'rested' for a short period before another trial was initiated. The trials were to be continued throughout a year so as to gather results from all weather conditions because if the project was to be a success it would have to show that the process involved would work throughout the year in the main beds. However, due to unforeseeable circumstances, only six trials, each using two concentrations, were conducted over a period of 9 months. It was not possible to conduct trials over the summer months but it was thought that if the trials were a success in the cold weather then according to Maehlum and Stalnacke (1999) they would be approximately 10% more efficient in the summer months. Newman et al found that the degradation in a constructed wetland was less efficient in the winter so by carrying out the trials in the colder months means that the system will be more efficient if the summer.

The physical measurements were carried out on the beds *in situ*.

The temperature was measured using a glass bulb thermometer that was inserted into the beds and allowed to stabilise before reading.

The pH measurements were made using a portable hand held type, Camlab KS 701.the instrument was calibrated before, during and after the tests using buffer solutions 4.0 and 6.9 as per manufacturers instructions.

Conductivity was measured with a portable model by Hanna, DIST3WP. This model detected in the range 0 to 2000 μ Siemens. Higher levels were recorded using the

Jenway, 4010 bench model. Both instruments were calibrated using the manufacturers standards before use by zeroing in distilled water on site.

Dissolved oxygen was measured by a Hanna HI 9142 that was calibrated before use as directed in the users' handbook.

3.2.3. The addition of a TEG solution using water from the retention pond after the Klargester had been brought into use.

The Klargester bio-contactors were put into operation on the site in April, 2003. All utility waste water was sent through the Klargester for primary treatment and the treated waste was then passed into the retention pond. A sample of about 500 litres was taken from the pond by plant personnel. It was contained in a large polythene tank within a wire cage and transported to the test beds using a forklift truck. The test solutions were made up using this water and TEG in the header tanks. The task was carried out with all participants wearing face, hands and body protection as the water contained sewage that had only had primary treatment and was considered to be potentially hazardous.

Two trials were carried out, in May and August 2003. The trial in May used a 2% TEG solution whilst the trial in August was 5%. In May beds 3,5 & 10 received 50 litres of water from the retention pond and beds 9,11 & 12 received 50 litres of 2% v/v TEG in retention pond water. The test solutions were added in the same manner as the earlier test solutions had been added. The test bed samples were analysed by GC for %v/v TEG as in the other trials but instead of measuring pH, conductivity, temperature and dissolved oxygen, the chemical oxygen demand (COD) was measured. The COD is a measure of the total oxidisable content of a water sample i.e.

a strong oxidising agent is added to the sample and the amount of oxidation that takes place is measured by titration. The COD is always higher than a BOD₅ as it is not just organic material that is oxidised. This test, as with the BOD₅ is not very reliable and can often give wide ranging results on the same sample. The method is described in the appendix.

3.2.4 Statistical Analysis

All the data was analysed, using Kolmogorov-Smirnov normality tests (Minitab 15[®]), for normality of distribution.

It was found that all datasets were distributed normally ($p > 0.05$) apart from those for dissolved oxygen in Trial 12 ($p < 0.01$). This particular trial was adversely affected by glyphosphate overspray and the presence of the iron mould which could account for the disparities.

As the majority of the data was normally distributed it was decided to analyse the data using the stronger parametric tests of ANOVAs and paired t-tests.

3.3. Results

3.3.1. The GC method used to measure TEG

The method that had been designed for the detection of TEG proved to be successful. The age of the GC itself caused a few problems that often required some running maintenance and it had its own peculiarities for best performance. Nevertheless, after some pilot analyses using the standard solutions, repeatable results were obtained.

The response of the column to monoethylene glycol was poor and it was decided not to count the measurements of the appropriate peak as it did not have the definition of the TEG peak and it was not possible to be sure about concentration. The diethylene glycol (DEG) peak was reliable and factors were obtained daily for both DEG and TEG when analysis was in progress. The concentrations of both DEG and TEG were calculated but only the TEG figures have been used in these analyses. The traces showed that DEG appeared as soon as the beds were sampled but the DEG also biodegraded before the TEG, so when there was no response for TEG there was also no response for DEG.

Figure 3.4. A typical GC trace of a trial sample. Peak 5 represents TEG, peaks 2 & 3 MEG and DEG respectively.

Online Channel 1 Run Number : 17 12:41 Thu Oct 18 2001
 Run Type : Unknown Batch Name : teg2

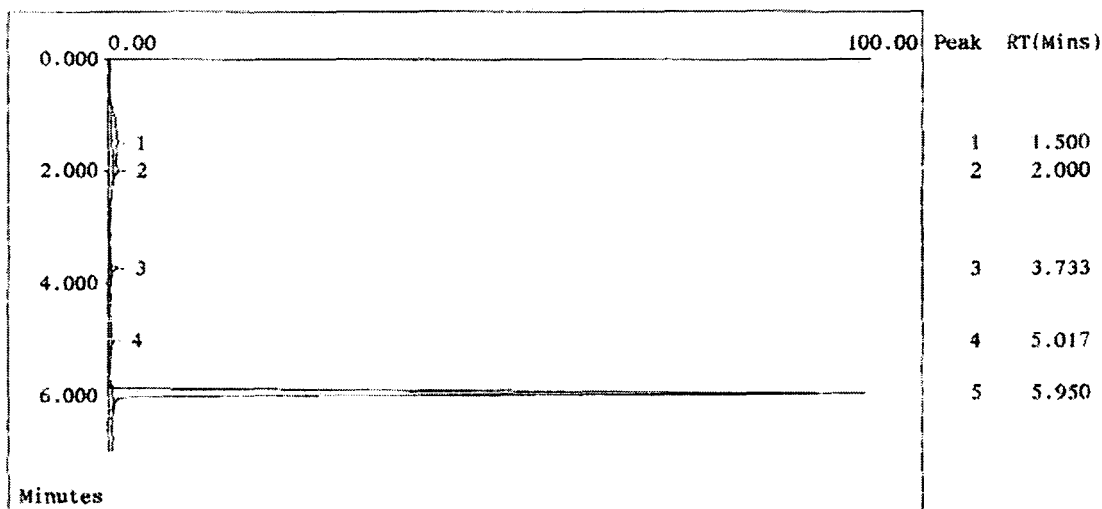
File Description	Date	Name
Sample Strip:	09:25 Thu Oct 18 2001	C:\4880\SYSTEM\TEG4.STR
Method:	09:24 Thu Oct 18 2001	C:\4880\METHODS\TEG4
Control Method:	09:24 Thu Oct 18 2001	C:\4880\METHODS\TEG4
Raw Data:	12:41 Thu Oct 18 2001	C:\4880\RAWDATA\teg201.017
Results:	12:41 Thu Oct 18 2001	C:\4880\RESULTS\Rteg201.017

Mode : Manual

Sample Name : 0.5 ul test bed 11 run 2 in/1

Scale : 1.0000000 Sample Amount : 1.0000000

Comment:



Peak Name	Type	RT(Mins)	Area	Height	Base	Conc
1	BV	1.500	40.098	0.844	0.422	8.410
2	VB	2.000	10.900	0.837	0.507	2.286
3	BB	3.733	4.250	0.875	0.325	0.891
4	BB	5.017	2.614	0.449	0.380	0.548
5	BB	5.950	418.943	98.785	0.513	87.865

3.3.2. Rate of degradation of TEG during the trials

The groups of three beds received solutions of TEG of the same concentration and each test bed was sampled at the inlet, middle and exit points. The samples were analysed by GC and the diethylene glycol (DEG) and TEG concentrations calculated for the 3 sample points. The results for 'in' for tanks in one group were averaged as were the results for middle and exit points and this was repeated for the sample points in the other group. The mean values and standard errors are shown for sampling times in **Tables 3.3. to 3.23.** There are two tables for each trial, one showing the concentration of TEG and another showing the concentration of DEG. The test solutions had been made up using TEG and water, the DEG was a product of the TEG degradation. The GC traces were unable to produce acceptable traces for monoethylene glycol (MEG) as the amounts present were so low. There was no sign of any organic acids or alcohols on the GC traces indicating that the degradation from TEG to carbon dioxide and water was a direct process with a small amount of DEG being the only detectable intermediate product.

Table 3.3. Trial 1. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 3, 5 and 10. Initial concentration of TEG 0.1%v/v.

	Sample point	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Sample 2	2	0.000		0.000		0.000	
Sample3	5	0.002	0.001	0.002	0.002	0.001	0.001
Sample4	7	0.002	0.001	0.001	0.001	0.009	0.004
Sample5	12	0.000	0.000	0.002	0.002	0.000	0.000

Table 3.4. Trial 1. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 3,5,and 10. Initial concentration of TEG 0.1%v/v.

	Sample point	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Sample 2	2	0.054	0.004	0.070	0.014	0.088	0.023
Sample3	5	0.018	0.008	0.016	0.010	0.007	0.003
Sample4	7	0.037	0.034	0.019	0.019	0.001	0.001
Sample5	12	0.002	0.002	0.010	0.010	0.001	0.001

N.B. it was not possible to take samples from test beds 3,5 and 10 after one day as the header tank had not emptied completely into the test beds of tanks 3 and 5. The problem was not discovered until the samples were due to be taken.

Table 3.5. Trial 2. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 9,11 and 12. Initial concentration of TEG 0.5%v/v.

	Sample point	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	1	0	0	0	0	0	0
Sample 2	2	0	0	0	0	0	0
Sample3	5	0.005	0.004	0.010	0.005	0.003	0.002
Sample4	7	0.000	0.000	0.004	0.004	0.002	0.001
Sample5	12	0.000	0.000	0.003	0.002	0.006	0.004

Table 3.6. Trial 2. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 9,11 and 12. Initial concentration of TEG 0.5%v/v.

	Sample point	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	1	0.053	0.014	0.146	0.073	0.072	0.024
Sample 2	2	0.049	0.011	0.127	0.057	0.060	0.011
Sample3	5	0.053	0.038	0.075	0.040	0.014	0.006
Sample4	7	0.003	0.001	0.054	0.028	0.009	0.005
Sample5	12	0.002	0.002	0.016	0.013	0.039	0.032

Table 3.7. Trial 3. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 3,5 and 10. Initial concentration of TEG 0.5%v/v.

	Sample point	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	1	0.005	0.003	0.003	0.002	0.002	0.001
Sample 2	2	0.008	0.002	0.006	0.001	0.005	0.000
Sample3	5	0.005	0.002	0.005	0.000	0.003	0.000
Sample4	7	0.007	0.005	0.003	0.002	0.001	0.001
Sample5	12	0.007	0.004	0.003	0.002	0.001	0.001

Table 3.8. Trial 3. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 3,5 and 10. Initial concentration of TEG 0.5%v/v.

	Sample point	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	1	0.363	0.013	0.186	0.045	0.188	0.080
Sample 2	2	0.160	0.078	0.079	0.030	0.075	0.027
Sample3	5	0.025	0.006	0.030	0.005	0.019	0.002
Sample4	7	0.027	0.025	0.011	0.005	0.005	0.003
Sample5	12	0.033	0.017	0.014	0.009	0.003	0.002

Table 3.9. Trial 4. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 9,11 and 12. Initial concentration of TEG 0.1%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	1	0.001	0.001	0.002	0.002	0.001	0.001
Sample 2	2	0.006	0.002	0.005	0.000	0.005	0.001
Sample3	5	0.003	0.001	0.003	0.001	0.003	0.000
Sample4	7	0.001	0.001	0.001	0.001	0.001	0.001
Sample5	12	0.005	0.003	0.000	0.000	0.000	0.000

Table 3.10. Trial 4. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 9,11 and 12. Initial concentration of TEG 0.1%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	1	0.108	0.014	0.099	0.005	0.086	0.012
Sample 2	2	0.134	0.015	0.094	0.018	0.073	0.022
Sample3	5	0.016	0.008	0.028	0.011	0.015	0.001
Sample4	7	0.008	0.005	0.006	0.001	0.005	0.002
Sample5	12	0.010	0.010	0.002	0.002	0.000	0.000

Table 3.11. Trial 5. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 3, 5 and 10. Initial concentration of TEG 0.1%v/v.

	Sample point	in		middle		exit	
	Time after addition of test solution in days	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	3	0.005	0.001	0.021	0.018	0.004	0.001
Sample 2	7	0.004	0.001	0.010	0.007	0.002	0.001
Sample3	10	0.001	0.001	0.001	0.001	0.000	0.000
Sample4	13	0.002	0.001	0.001	0.001	0.000	0.000
Sample5	16	0.002	0.001	0.000	0.000	0.000	0.000
Sample 6	20	0.001	0.001	0.000	0.000	0.000	0.000
Sample 7	50	0	0	0	0	0	0

Table 3.12. Trial 5. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 3, 5 and 10. Initial concentration of TEG 0.1%v/v.

	Sample point	in		middle		exit	
	Time after addition of test solution in days	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	3	0.091	0.009	0.044	0.002	0.039	0.011
Sample 2	7	0.032	0.006	0.013	0.001	0.010	0.006
Sample3	10	0.002	0.002	0.002	0.001	0.001	0.001
Sample4	13	0.003	0.001	0.001	0.001	0.000	0.000
Sample5	16	0.002	0.001	0.001	0.001	0.000	0.000
Sample 6	20	0.001	0.001	0.000	0.000	0.000	0.000
Sample 7	50	0.004	0.002	0.000	0.000	0.001	0.001

Table 3.13. Trial 6. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 9, 11 and 12. Initial concentration of TEG 0.5%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	3	0.008	0.001	0.007	0.000	0.007	0.001
Sample 2	7	0.012	0.004	0.018	0.006	0.006	0.001
Sample3	10	0.015	0.008	0.006	0.001	0.004	0.000
Sample4	13	0.006	0.000	0.040	0.035	0.003	0.001
Sample5	16	0.012	0.002	0.007	0.002	0.002	0.000
Sample 6	20	0.002	0.000	0.007	0.003	0.000	0.000
Sample 7	50	0.000	0.000	0.000	0.000	0.000	0.000

Table 3.14. Trial 6. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 9, 11 and 12. Initial concentration of TEG 0.5%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	3	0.251	0.015	0.162	0.048	0.081	0.015
Sample 2	7	0.173	0.020	0.106	0.038	0.029	0.005
Sample3	10	0.014	0.004	0.057	0.029	0.029	0.011
Sample4	13	0.018	0.008	0.031	0.011	0.011	0.003
Sample5	16	0.042	0.029	0.027	0.011	0.007	0.003
Sample 6	20	0.016	0.007	0.030	0.016	0.001	0.001
Sample 7	50	0.011	0.006	0.005	0.005	0.001	0.001

Table 3.15. Trial 7. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 3, 5 and 10. Initial concentration of TEG 0.5%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	2	0.010	0.002	0.008	0.001	0.006	0.001
Sample 2	5	0.013	0.003	0.011	0.003	0.006	0.001
Sample 3	7	0.020	0.003	0.010	0.003	0.004	0.001
Sample 4	9	0.012	0.002	0.009	0.002	0.004	0.000
Sample 5	12	0.009	0.003	0.002	0.001	0.000	0.000
Sample 6	16	0.013	0.006	0.002	0.001	0.000	0.000
Sample 7	20	0.002	0.002	0.001	0.001	0.000	0.000

Table 3.16. Trial 7. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 3, 5 and 10. Initial concentration of TEG 0.5%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	2	0.337	0.021	0.219	0.012	0.138	0.018
Sample 2	5	0.052	0.025	0.076	0.012	0.046	0.015
Sample 3	7	0.058	0.026	0.039	0.006	0.018	0.008
Sample 4	9	0.031	0.006	0.026	0.005	0.015	0.001
Sample 5	12	0.030	0.023	0.005	0.002	0.002	0.000
Sample 6	16	0.029	0.021	0.004	0.003	0.000	0.000
Sample 7	20	0.002	0.002	0.001	0.001	0.000	0.000

Table 3.17. Trial 8. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 9,11 and 12. Initial concentration of TEG 0.1%v/v.

	Sample point	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	2	0.004	0.000	0.004	0.000	0.005	0.001
Sample 2	5	0.010	0.005	0.006	0.002	0.005	0.001
Sample 3	7	0.003	0.001	0.003	0.001	0.002	0.000
Sample 4	9	0.002	0.000	0.001	0.001	0.000	0.000
Sample 5	12	0.009	0.003	0.002	0.001	0.000	0.000
Sample 6	16	0	0	0	0	0	0
Sample 7	20	0	0	0	0	0	0

Table 3.18. Trial 8. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 9,11 and 12. Initial concentration of TEG 0.1%v/v.

	Sample point	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	2	0.103	0.008	0.108	0.011	0.094	0.013
Sample 2	5	0.032	0.012	0.039	0.010	0.028	0.003
Sample 3	7	0.022	0.004	0.017	0.009	0.007	0.001
Sample 4	9	0.007	0.001	0.004	0.001	0.001	0.001
Sample 5	12	0.002	0.001	0.001	0.001	0.001	0.001
Sample 6	16	0	0	0	0	0	0
Sample 7	20	0	0	0	0	0	0

Table 3.19. Trial 9. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 3, 5 and 10. Initial concentration of TEG 1.0%v/v.

	Sample point	in		middle		exit	
		mean	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG
Sample 1	2	0.009	0.002	0.005	0.001	0.004	0.000
Sample 2	4	0.010	0.001	0.007	0.001	0.006	0.001
Sample 3	7	0.011	0.001	0.006	0.001	0.003	0.001
Sample 4	10	0.015	0.003	0.006	0.002	0.003	0.000
Sample 5	12	0.018	0.002	0.003	0.001	0.001	0.000
Sample 6	15	0.012	0.006	0.004	0.001	0.002	0.001
Sample 7	16	0.012	0.006	0.004	0.001	0.002	0.001
sample 8	32	0.002	0.002	0.002	0.001	0.002	0.002
sample 9	33	0.003	0.003	0.001	0.001	0.001	0.001

Table 3.20. Trial 9. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 3, 5 and 10. Initial concentration of TEG 1.0%v/v.

	Sample point	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	2	0.105	0.045	0.079	0.015	0.064	0.027
Sample 2	4	0.105	0.007	0.063	0.017	0.047	0.019
Sample 3	7	0.005	0.000	0.005	0.001	0.001	0.001
Sample 4	10	0.091	0.031	0.019	0.006	0.020	0.006
Sample 5	12	0.114	0.041	0.009	0.001	0.006	0.003
Sample 6	15	0.054	0.037	0.012	0.003	0.006	0.002
Sample 7	16	0.054	0.037	0.012	0.003	0.006	0.002
sample 8	32	0.004	0.002	0.004	0.002	0.008	0.007
sample 9	33	0.005	0.005	0.002	0.002	0.002	0.002

Table 3.21. Trial 10. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 9, 11 and 12. Initial concentration of TEG 0.2%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	2	0.004	0.001	0.004	0.001	0.003	0.000
Sample 2	4	0.006	0.001	0.006	0.001	0.001	0.001
Sample 3	7	0.005	0.000	0.005	0.001	0.001	0.001
Sample 4	10	0.011	0.002	0.005	0.002	0.000	0.000
Sample 5	12	0.007	0.003	0.005	0.005	0.001	0.001
Sample 6	15	0.000	0.000	0.000	0.000	0.000	0.000
Sample 7	16	0.000	0.000	0.000	0.000	0.000	0.000
Sample 8	32	0.000	0.000	0.000	0.000	0.000	0.000
Sample 9	33	0.000	0.000	0.000	0.000	0.000	0.000

Table 3.22. Trial 10. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 9, 11 and 12. Initial concentration of TEG 0.2%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	2	0.082	0.015	0.038	0.008	0.023	0.007
Sample 2	4	0.077	0.004	0.030	0.007	0.007	0.004
Sample 3	7	0.025	0.004	0.013	0.005	0.003	0.000
Sample 4	10	0.049	0.002	0.017	0.008	0.003	0.000
Sample 5	12	0.018	0.009	0.012	0.011	0.002	0.001
Sample 6	15	0.003	0.000	0.001	0.001	0.000	0.000
Sample 7	16	0.003	0.000	0.001	0.001	0.000	0.000
Sample 8	32	0	0	0	0	0	0
Sample 9	33	0	0	0	0	0	0

Table 3.23. Trial 11. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 3, 5 and 10. Initial concentration of TEG 5.0%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	3	0.016	0.006	0.027	0.002	0.028	0.002
Sample 2	6	0.033	0.008	0.034	0.001	0.033	0.002
Sample 3	8	0.047	0.004	0.040	0.002	0.042	0.007
Sample 4	15	0.036	0.007	0.032	0.003	0.033	0.010
Sample 5	18	0.016	0.005	0.013	0.003	0.017	0.008
Sample 6	21	0.005	0.002	0.003	0.001	0.004	0.001
Sample 7	25	0.003	0.000	0.003	0.002	0.002	0.001
Sample 8	28	0.008	0.004	0.003	0.001	0.002	0.001
Sample 9	32	0.007	0.003	0.003	0.002	0.001	0.001
Sample 10	34	0.001	0.000	0.000	0.000	0.000	0.000
Sample 11	36	0.000	0.000	0.000	0.000	0.000	0.000

Table 3.24. Trial 11. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 3, 5 and 10. Initial concentration of TEG 5.0%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	3	0.371	0.121	0.411	0.024	0.424	0.052
Sample 2	6	0.388	0.072	0.344	0.027	0.337	0.052
Sample 3	8	0.417	0.055	0.295	0.053	0.328	0.079
Sample 4	15	0.199	0.015	0.147	0.032	0.172	0.068
Sample 5	18	0.050	0.017	0.048	0.015	0.084	0.038
Sample 6	21	0.010	0.003	0.008	0.001	0.012	0.006
Sample 7	25	0.007	0.002	0.006	0.003	0.004	0.001
Sample 8	28	0.012	0.004	0.006	0.001	0.005	0.001
Sample 9	32	0.012	0.003	0.008	0.004	0.003	0.002
Sample 10	34	0.001	0.001	0.000	0.000	0.000	0.000
Sample 11	36	0.000	0.000	0.000	0.000	0.000	0.000

Table 3.25. Trial 12. Mean (n=3) concentrations of DEG %v/v and standard errors at the 3 sample points in test beds 9, 11 and 12. Initial concentration of TEG 1.0%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e	Mean %v/v DEG	± s.e
Sample 1	3	0.011	0.001	0.004	0.001	0.004	0.001
Sample 2	6	0.016	0.001	0.013	0.005	0.004	0.001
Sample 3	8	0.042	0.008	0.020	0.008	0.005	0.001
Sample 4	15	0.049	0.007	0.012	0.007	0.006	0.003
Sample 5	18	0.047	0.006	0.020	0.008	0.012	0.004
Sample 6	21	0.053	0.013	0.032	0.009	0.015	0.007
Sample 7	25	0.055	0.021	0.023	0.010	0.017	0.008
Sample 8	28	0.049	0.012	0.027	0.010	0.013	0.006
Sample 9	32	0.053	0.021	0.024	0.018	0.039	0.009
Sample 10	34	0.003	0.000	0.015	0.007	0.016	0.010
Sample 11	36	0.041	0.002	0.033	0.019	0.016	0.008
Sample 12	49	0.004	0.001	0.009	0.006	0.007	0.001

Table 3.26. Trial 12. Mean (n=3) concentrations of TEG %v/v and standard errors at the 3 sample points in test beds 9, 11 and 12. Initial concentration of TEG 1.0%v/v.

	Sample point Time after addition of test solution in days	in		middle		exit	
		Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e	Mean %v/v TEG	± s.e
Sample 1	3	0.776	0.168	0.037	0.007	0.016	0.004
Sample 2	6	0.439	0.028	0.119	0.054	0.027	0.009
Sample 3	8	0.463	0.079	0.136	0.059	0.022	0.006
Sample 4	15	0.559	0.187	0.051	0.030	0.021	0.009
Sample 5	18	0.338	0.056	0.094	0.040	0.046	0.021
Sample 6	21	0.342	0.100	0.169	0.047	0.086	0.056
Sample 7	25	0.277	0.121	0.102	0.049	0.082	0.045
Sample 8	28	0.278	0.054	0.121	0.055	0.055	0.030
Sample 9	32	0.285	0.125	0.155	0.123	0.218	0.058
Sample 10	34	0.016	0.002	0.056	0.024	0.062	0.035
Sample 11	36	0.076	0.004	0.052	0.026	0.029	0.015
Sample 12	49	0.010	0.002	0.032	0.020	0.029	0.010

Before the addition of the test solutions, 50 l of water was drained off from each tank. This left a residual water content of 22 l (Mean (n=12) volume of ALL test beds 72 l +/- 0.41 s.e.) When the test solution was added it was thought that the test solutions would be subject to a dilution with the remaining water that was calculated to be equivalent to a dilution factor of 0.69 +/- 0.004 s.e. (n=12) being operative. However, when the initial concentrations are multiplied by this factor, some of the measured concentrations AFTER addition are higher than the calculated ones in! The implication of this is that although some mixing will occur at the interface, it is not consistent throughout the system. The figures used have not been subjected to any dilution factor being applied.

3.3.3. Results – Klargester / retention pond trials

The results of the trial with Klargester material were inconclusive. The COD of the treated beds rose while the TEG concentration decreased. The most likely explanation was that because of the small size of the test beds coupled with warm drying winds causing low water levels, it was not possible to take samples of clear water with no organic debris present. The presence of organic debris could cause high COD readings even though the TEG had been degraded. Nevertheless, it appeared that the TEG was degraded in the presence of Klargester material.

Table 3.27. shows the mean concentrations of TEG in retention pond water over a period of 32 days. The concentration of TEG had fallen to a level less than 0.02 %v/v(BOD equivalent of 30mg/l) at a point between 20 and 28 days after the initial addition of test solution.

Table 3.27. Mean concentrations (n=9) of TEG %v/v in test beds 3, 5 & 10, determined by GC analysis against time in days after addition. Solution made up using water from the retention pond. May 2003

Days after addition	0	4	7	11	15	20	28	32
Concentration of TEG % v/v	2	0.107	0.073	0.056	0.053	0.079	0.005	0.003
St Error	n.a.	± 0.067	± 0.032	± 0.029	± 0.046	± 0.014	± 0.004	± 0.003

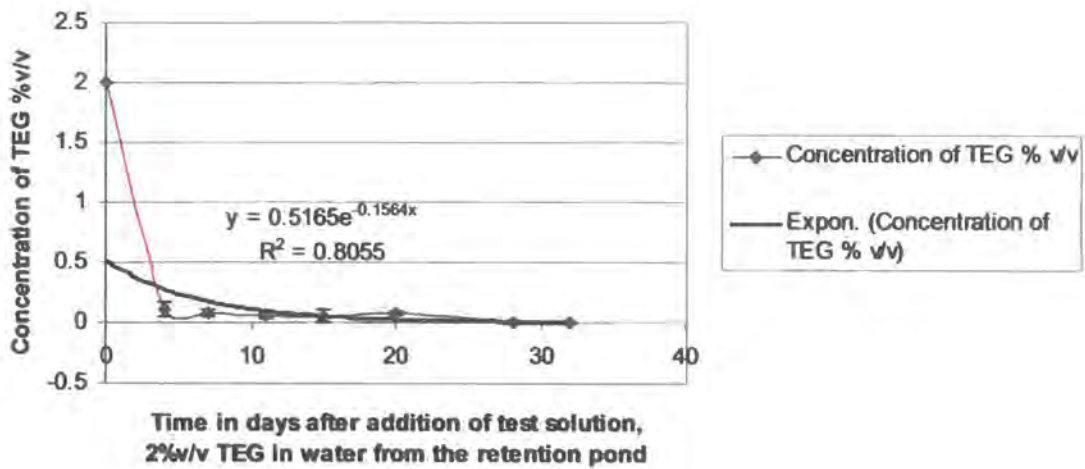


Figure 3.5. Mean concentrations (n=9) of TEG %v/v in test beds 3, 5,& 10, determined by GC analysis against time in days after addition (Retention pond trial 1)

Figure 3.5. shows the pattern of degradation almost following an exponential curve as seen earlier in the TEG only trials. As the concentration of TEG decreases so the rate of degradation decreases.

Table 3.28. Mean concentrations (n=9) of TEG %v/v in test beds 3, 5 & 10, determined by GC analysis against time in days after addition. Solution made up using water from the retention pond. August 2003

Days after addition	0	2	10	12	21
Concentration of TEG % v/v	5	0.084	0.057	0.014	0.002
St Error	n.a.	± 0.03	± 0.028	± 0.006	± 0.0003

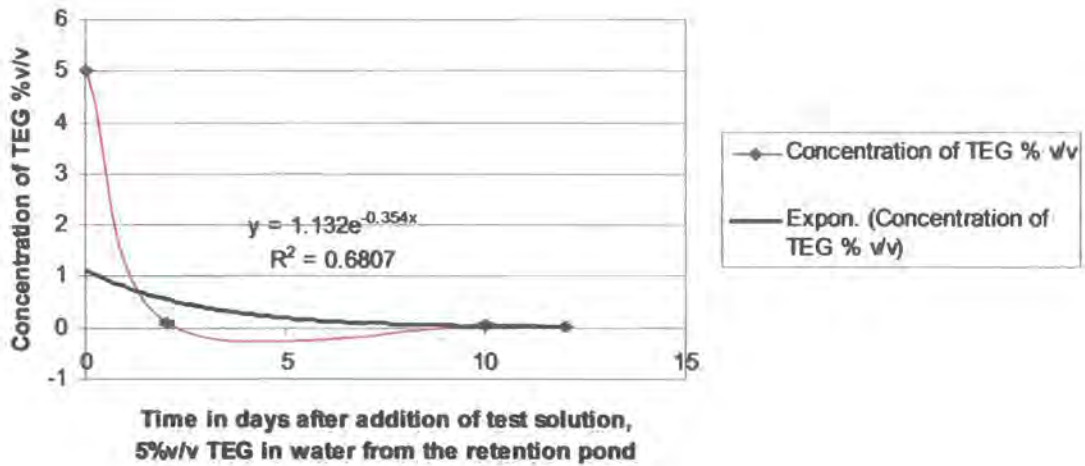


Figure 3.6. Trial 6 Mean concentrations (n=9) of TEG %v/v in test beds, 3, 5 & 10, determined by GC analysis against time in days after addition (Retention pond trial 2)

Figure 3.6. shows the degradation curve for the trial using 5%v/v TEG in water from the retention pond, which had a faster initial degradation rate than the first of these two trials. As seen earlier the higher the initial concentration the faster the initial degradation rate.

Table 3.29. Mean (n=3) COD levels mg/l measured in the test beds, retention pond trial 1 May / June 2003

Days after addition	COD mg/l retention pond+ TEG	± st. error	COD mg/l ret pond	± st. error
4	718.3	70.4	99.3	7.9
7	1731	87.5	211	15.6
11	1097	95	74	6
15	1638.7	115.9	126.3	9.5
20	1650.7	120	193	9.8

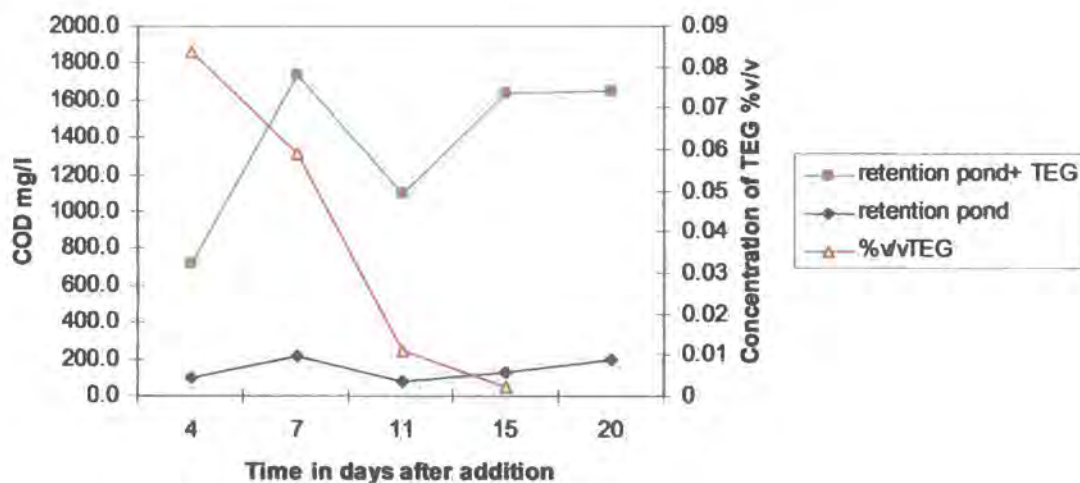


Figure 3.7. Comparison of concentration of TEG %v/v against COD over time after addition of TEG (Retention pond trial 1)

3.4. Discussion - Factors affecting the rate of TEG degradation

3.4.1. Start concentration

The breakdown of TEG in the test showed it to be a first order reaction in that the higher the original concentration the quicker the initial degradation. As the concentration of TEG decreased, so a concentration of DEG appeared but it never became the higher component and it also was degraded easily. The 3 samples taken in each test bed showed that usually the levels of TEG recorded were higher at the beginning of the test beds than at the end. The solution sampled at the exit point had passed through more of the test bed and therefore been exposed to more bacterial action. The pattern of the degradation rates would indicate that most of the rapid degradation takes place in the first half of the bed.

Table 3.30. Summary of times taken for the breakdown of TEG to 10% of original concentration and to the lowest detectable level (or suspension of trial)

	Initial concentration %v/v TEG	Time in days to 10% of original concentration	Time in days to lowest detectable level
Trial 1	0.1	5	12
Trial 2	0.5	5	12
Trial 3	0.5	4	15
Trial 4	0.1	9	15
Trial 5	0.1	9	20
Trial 6	0.5	9	20
Trial 7	0.5	6	20
Trial 8	0.1	8	16
Trial 9	1.0	2	33
Trial 10	0.2	6	32
Trial 11	5.0	3	36
Trial 12	2.0	33	49

Concentrations above 0.1%v/v were degraded within the first few days but once 0.1% was reached the rate decreased. The following Figures 3.11. to 3.23 are the TEG concentration charts for complete trials, with trendlines fitted.

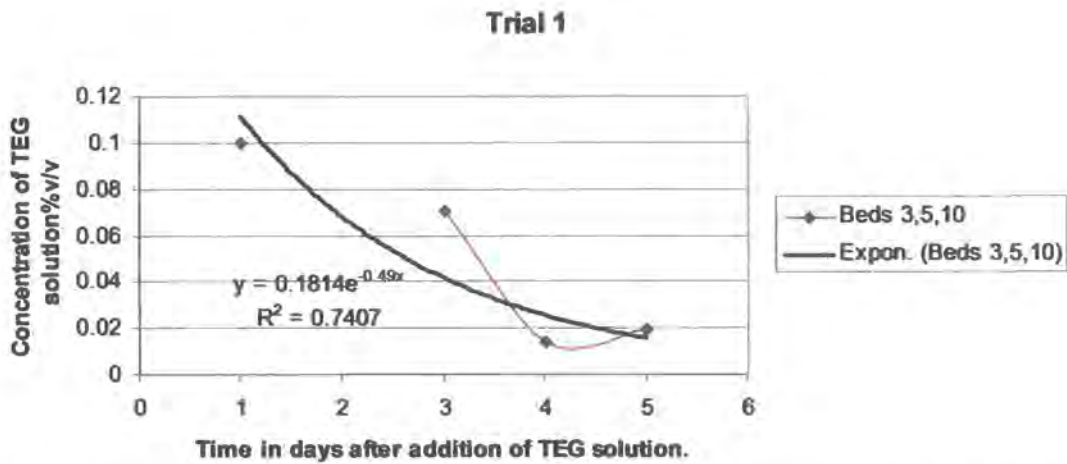


Figure 3.8. The change in TEG concentration over period of Trial 1, beds 3,5 &10 . Initial concentration 0.1%v/v TEG. (trendline fitted)

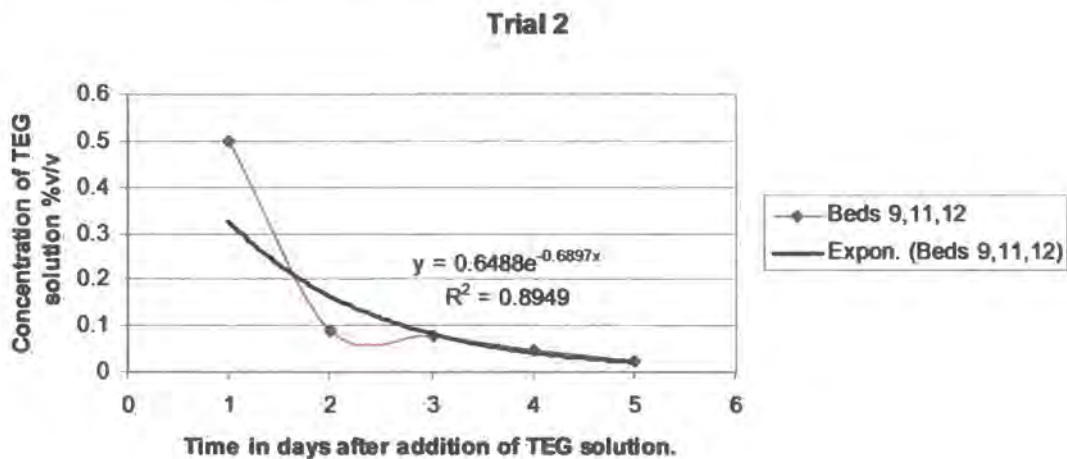


Figure 3.9. The change in TEG concentration over period of Trial 2, beds 9,11 & 12. Initial concentration 0.5%v/vTEG. (trendline fitted)

In Trial 1, beds 3,5 & 10 containing 0.1% TEG had the concentration reduced at a rate of 0.015% per day for the first two days, followed by 0.0017% per day in five days. In Trial 2, beds 9,11 & 12 with a starting concentration of 0.5% decreased at a rate

of 0.21% per day in the first two days. The five day figure was down to 0.09% per day.

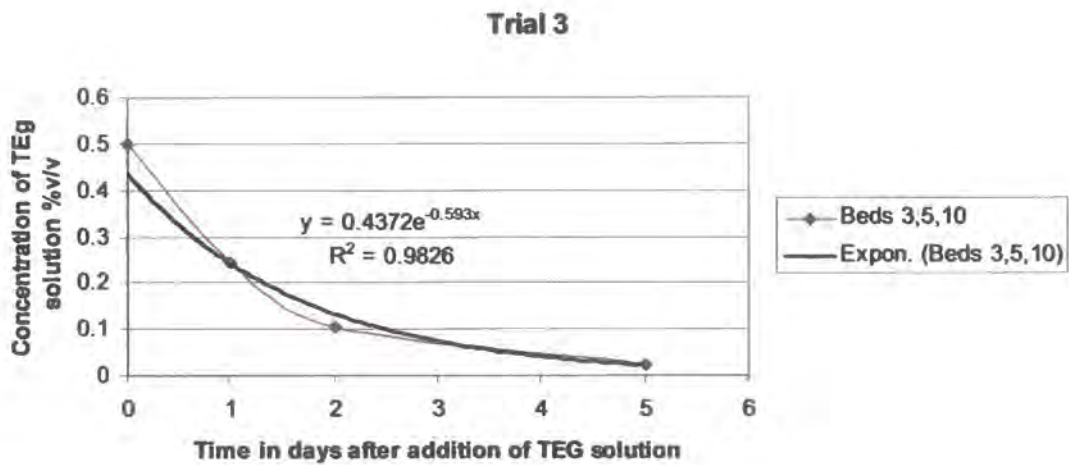


Figure 3.10. The change in TEG concentration over period of Trial 3, beds 3, 5 & 10. Initial concentration 0.5%v/vTEG. (trendline fitted)

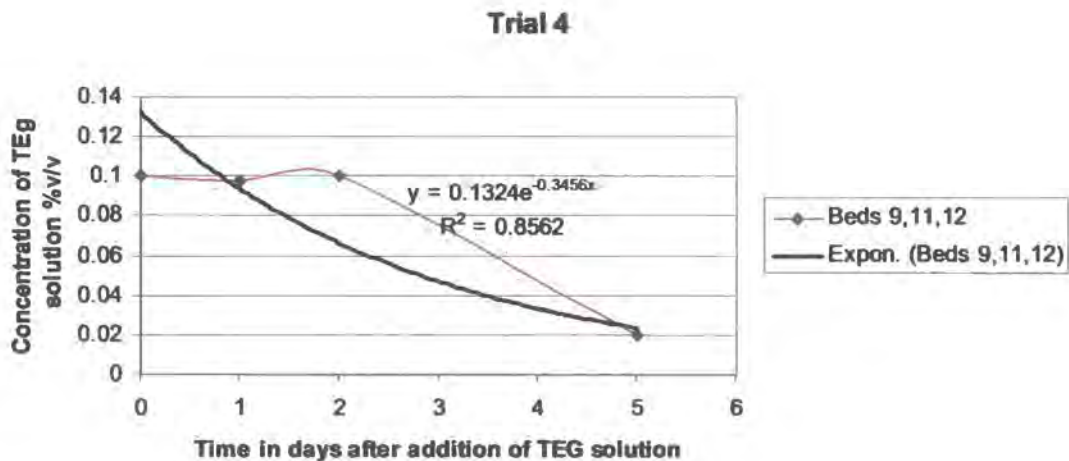


Figure 3.11. The change in TEG concentration over period of Trial 4, beds 9,11 & 12. Initial concentration 0.1%v/vTEG. (trendline fitted)

In Trial 3, beds 3, 5 & 10 started at 0.5%v/v TEG and degraded at a rate of 0.2% per day for the first two days whereas in Trial 4, the other 3 beds at a starting

concentration of 0.1% didn't start to degrade until two days had passed. After two days both sets of beds were showing mean concentrations of approximately 0.1%v/v TEG. Over the next three days the concentrations in all the test beds fell at a rate of 0.03% per day.

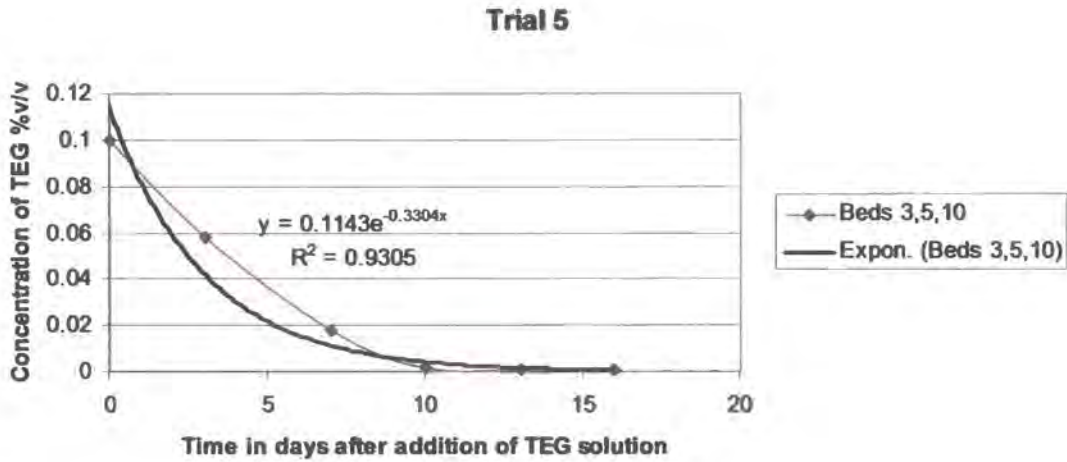


Figure 3.12. The change in TEG concentration over period of Trial 5, beds 3, 5 & 10. Initial concentration 0.1%v/vTEG. (trendline fitted)

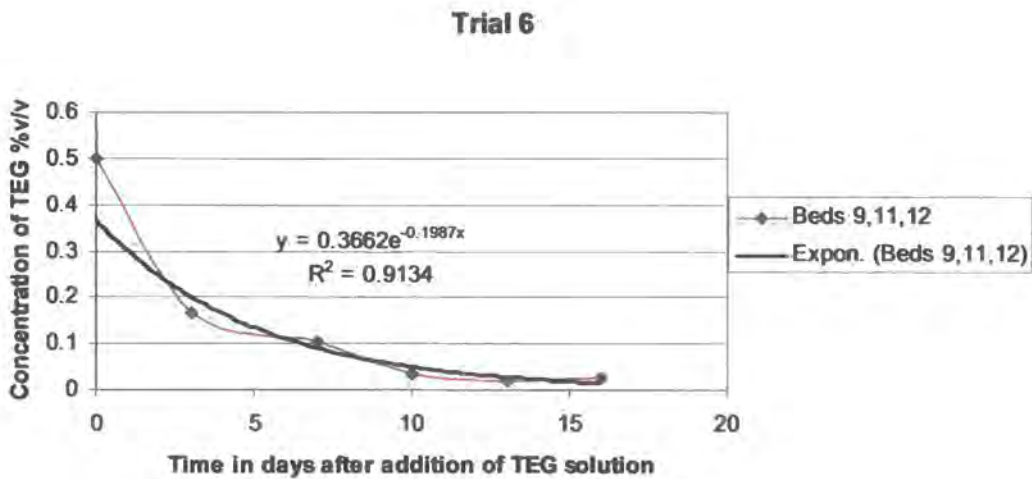


Figure 3.13. The change in TEG concentration over period of Trial 6, beds 9,11 & 12. Initial concentration 0.5%v/vTEG. (trendline fitted)

In Trial 5, beds 3, 5, & 10 with 0.1%v/v TEG initially, demonstrated a degradation rate of 0.01% per day over the period of the trial, whilst in Trial 6, with beds 9, 11 & 12 starting at 0.5%v/v TEG showed a similar pattern with the higher concentration falling at 0.11% per day to around 0.1%v/v TEG after which the rate slowed to 0.02% per day.

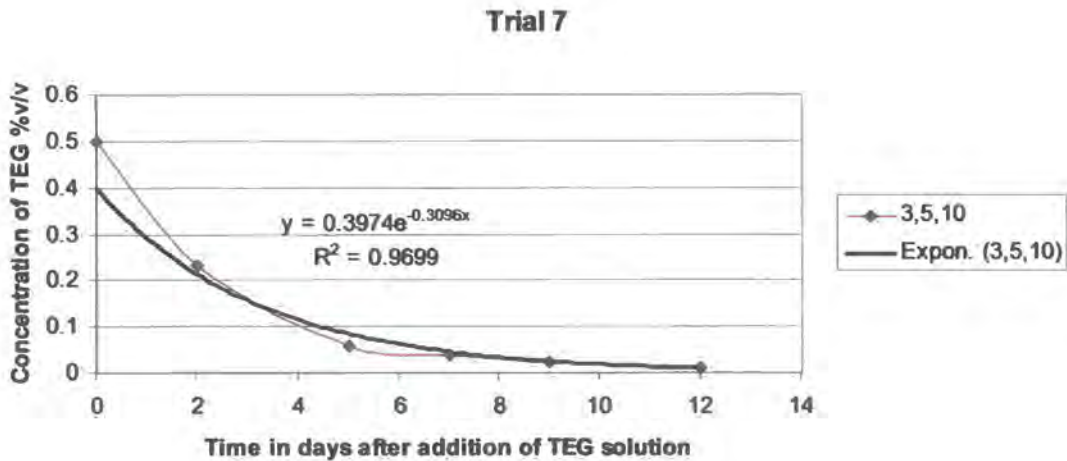


Figure 3.14. The change in TEG concentration over period of Trial 7, beds 3, 5 & 10. Initial concentration 0.5%v/vTEG. (trendline fitted)

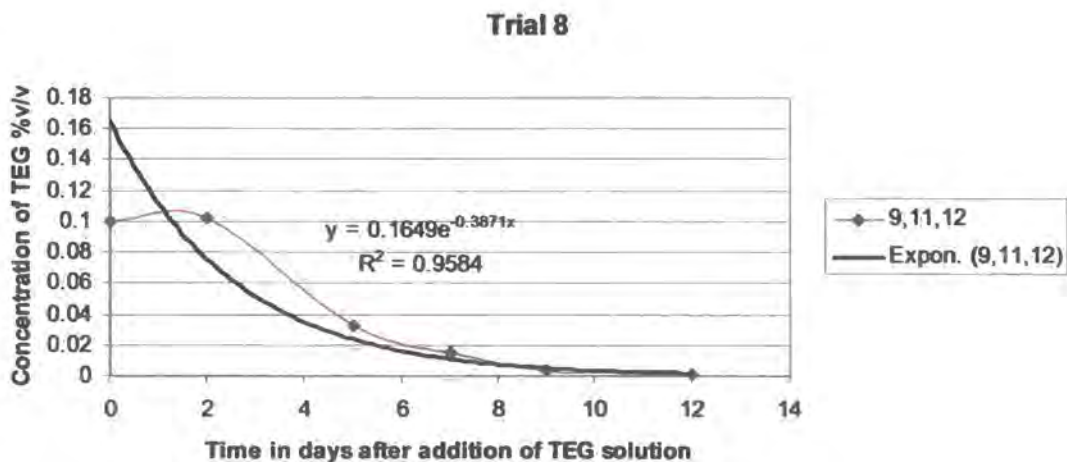


Figure 3.15. The change in TEG concentration over period of Trial 8, beds 9, 11 & 12. Initial concentration 0.1%v/vTEG. (trendline fitted)

Trial 7 had beds 3, 5 & 10 starting with 0.5% v/v TEG and demonstrated a degradation rate of 0.14% per day for the first two days, slowing down to an average of 0.03% per day over the 16 day trial period. Trial 8, beds, 9,11 & 12 with the lower concentration, once again showed no sign of degradation for the first two days but then showed a rate of 0.006% per day over the 16 day period.

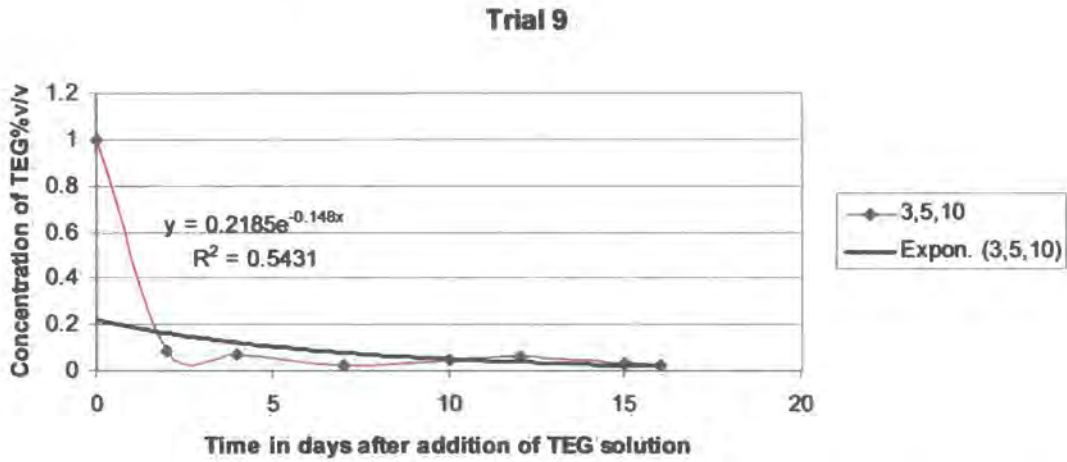


Figure 3.16. The change in TEG concentration over period of Trial 9, beds 3, 5 & 10. Initial concentration 1.0 %v/vTEG. (trendline fitted)

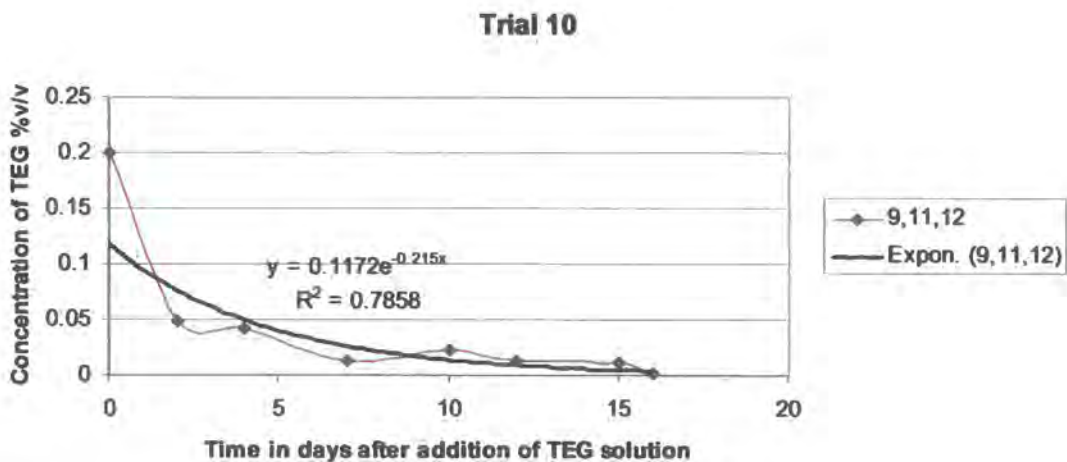


Figure 3.17. The change in TEG concentration over period of Trial 10, beds 9, 11 & 12. Initial concentration 0.2 %v/vTEG. (trendline fitted)

In Trial 9 higher concentrations were used in an attempt to determine a maximum viable concentration that could be degraded without causing distress to the reeds. Beds 3, 5 & 10 were given 1%v/v TEG solution and in the first two days the rate of degradation was 0.46% per day until a level of 0.08%v/v TEG was achieved. The rate then slowed down to 0.006% per day for two more days and then became very slow, taking a total of 32 days to reach a level of 0.003%v/v TEG. In Trial 10, beds 9, 11 & 12 received solution of 0.2%v/v TEG and demonstrated a degradation rate of 0.08% per day for the first two days slowing to 0.003% per day for the following two days. Over a period of 16 days, the rate for these beds was averaged at 0.012% per day.

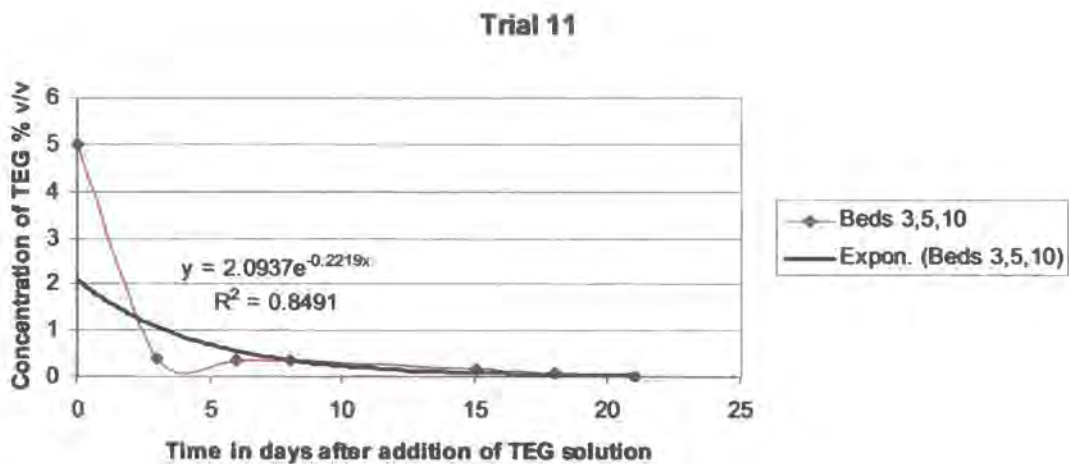


Figure 3.18. The change in TEG concentration over period of Trial 11, beds 3, 5 & 10. Initial concentration 0.5 %v/vTEG. (trendline fitted)

In Trial 11, beds 3, 5 & 10 received a solution of 5%v/v TEG whilst in Trial 12 beds 9, 11 & 12 received a solution of 1%v/v TEG. Beds 3,5 & 10 showed an initial rate of 1.57% per day whilst the other set displayed a rate of 0.24% per day. In both cases the

rates slowed down to 0.02% per day after three days and the trial continued for a total of 49 days before it was stopped.

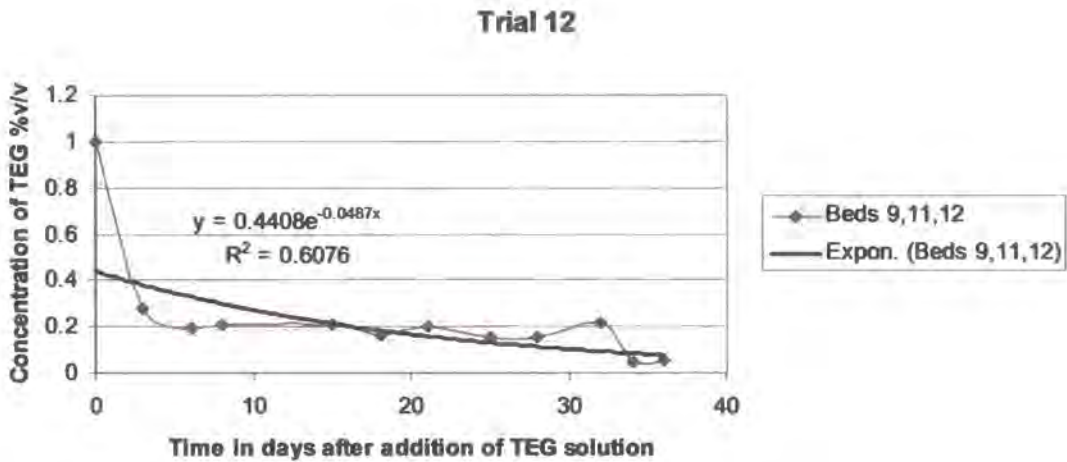


Figure 3.19. The change in TEG concentration over period of Trial 12, beds 9, 11 & 12. Initial concentration 1.0 %v/v TEG. (trendline fitted)

Beds 3, 5 & 10 degraded down to <0.0001% after 34 days but the other beds were still showing traces of TEG when the trial was stopped.

The test beds had at this point started to display signs of contamination with an iron mould which was causing a precipitation of ferric oxide which in turn appeared to be causing distress to the plants. The beds were allowed to dry out for several days before being filled up with fresh water. The reeds recovered and the mould did not return.

Thus the results show that the initial concentration of TEG has an effect on the rate of degradation in the first few days, but that once levels reach a point about 0.1%v/v TEG, this rate slows down as would be expected for a first order reaction. If the initial concentration is 0.1%v/v TEG then the rate is slow throughout the trial whereas higher concentrations show high initial rates that eventually slow down to the point

where the rates in all the beds are similar. The retention pond trials 1 & 2 displayed similar trends in degradation rates although the test solutions had been made up using water from the retention pond which had received the site sewage after primary treatment in the Klargestær. Figures 3.10. to 3.21. show that the degradation curves tend to follow the exponential trendlines closely, a strong indication of the first order reaction.

According to Tarutis et al(19991) the efficiency of a constructed wetland has been calculated using a formula first employed by Girts et al (1987) which was

$$\text{Efficiency \%} = \frac{(\text{Concentration in}-\text{Concentration out})}{\text{Concentration in}} \times 100$$

However, this equation does not include any temporal aspect so it is of little use when trying to assess how long it will take for a batch of effluent to be degraded.

A removal rate expressing the amount, either %, BOD or mass, removed in a given time would be more useful. This could be expressed thus:-

$$\text{Removal rate (final conc. time}^{-1}\text{)} = \frac{(\text{Concentration in}-\text{Concentration out})}{(\text{time})}$$

To calculate the removal rate of the test beds and to be able to transfer it to the existing rbts the areas of the beds need to be considered so it can be said

$$\text{Removal rate of test bed/area} = \frac{(\text{Concentration in}-\text{Concentration out})}{(\text{time}) \times (\text{area of bed})}$$

To enable this equation to be used for these results the concentration of TEG equivalent to the BOD target needs to be known. One method of approximating the oxygen demand of a known organic chemical is to calculate the Theoretical Oxygen

Demand (THOD) of it. THOD is the calculated amount of oxygen needed by the compound to be oxidised to its final oxidation products. Because some of the carbon will be utilised by the bacteria for growth (Todar , 2004) the THOD will always be higher than the BOD₅ so any estimations based on the THOD will err on the safe side. The THOD of DEG is 1.51 and for TEG it is 1.6 i.e. for each unit of DEG 1.51 units of Oxygen are needed and for TEG 1.6 units, to complete oxidation. To convert % TEG to mg/l oxygen demand the concentration must be multiplied by a factor of 1600 and 1510 for DEG.

The limits set by the EA for discharge at CATS was a BOD₅ of 40 mg/l but for this exercise a limit of 35 mg/l has been used. This figure equates to concentration of 0.022% TEG. From the results in Tables 3.3. to 3.26. the time, in days, taken for the combined DEG and TEG to reach 0.025% or less has been noted and used in the above equation for the removal rate. The figures are shown in table 3.31.

Table 3.31. The concentrations of TEG, in and out, time taken, the area of the test bed and the calculated removal rate expressed in %T⁻¹A⁻¹

Trial no.	Conc. In %v/v	Conc. Out %v/v	Time in days (T)	Area of bed (A) m ²	Removal rate %T ⁻¹ A ⁻¹
1	0.1	0.02	5	0.69	0.023
2	0.5	0.02	5	0.69	0.139
3	0.5	0.02	5	0.69	0.139
4	0.1	0.02	5	0.69	0.023
5	0.1	0.02	6	0.69	0.019
6	0.5	0.02	13	0.69	0.054
7	0.5	0.02	7	0.69	0.099
8	0.1	0.02	7	0.69	0.017
9	1	0.02	10	0.69	0.142
10	0.2	0.02	4	0.69	0.065
11	5	0.02	18	0.69	0.401
12	1	0.02	15	0.69	0.095
Mean					0.101
± St. Error					0.031

The concentration in and out (Conc. in, out) are in %v/v TEG and the removal rate is concentration removed day⁻¹ m⁻¹.

The equation shown in the opening chapter (Cooper,1996)

$$A_h = (Q_d (\ln C_o - \ln C_t)) / k_{BOD}$$

assumes a rate constant of 0.1 which is the mean rate arrived at in table 3.28.

From the equation

$$\text{Removal rate of test bed/area} = \frac{(\text{Concentration in} - \text{Concentration out})}{(\text{time}) \times (\text{area of bed})}$$

the time for a known concentration of TEG to degrade to a target concentration can be expressed as

$$\text{Time to degrade to target} = \frac{(\text{Concentration in} - \text{Concentration out})}{(\text{area of bed}) \times (\text{Removal rate/area})}$$

The standard practice at the Terminal is that ‘batches’ of effluent of between 30 and 60 m³ will be pumped to the reed beds. The test beds had a surface area of 0.69 m² and received batches of 50 l and the biodegradation was successful. The ratio of area over volume of effluent was 13.8. The area of the constructed beds is 547 m² and to achieve the same ratio the volume of the batch should be approximately 40 m³. The higher volumes are usually pumped out in periods of high rainfall, which would actually dilute the effluent so it would be safe to continue with the existing practices.

The ‘concentrations in and out’ can be replaced with THOD or BOD or even Total Organic Carbon figures dependant on which are available. It is hoped this equation can be used at the CATS Terminal to allow plant personnel to control the effluent flows into the reed beds.

3.5. Interactions between TEG solution and physical chemistry of the test beds.

So as to investigate interactions between physical chemistry of the beds and TEG, its breakdown products and the degradation process, the pH, conductivity, temperature and dissolved oxygen content of the water in the test beds was measured each time that a sample was taken for GC analysis. The mean results and standard errors are presented in the following tables 3.31. to 3.42.

To save space and enable the following tables to be readable the measurement units have been omitted.

The relevant units are

Temperature	(Temp.) :-	$^{\circ}\text{C}$
Conductivity	(Cond.) :-	$\mu\text{S/cm}$
Dissolved Oxygen	(D.O.) :-	mg/l

Table 3.32. Mean physical measurements taken at points in time after the addition of TEG

Trial 1, Beds 3,5,10@0.1%. Treated n=9, Untreated n=18

Days after addition of TEG	0		1		2		5		7		12	
	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error
Beds												
Control	8.6	0.2	7.7	0.3	7.4	0.2	7.7	0.2	7.8	0.1	7.9	0.2
3,5,10	8.4	0.1	7.7	0.0	7.5	0.1	7.5	0.2	7.7	0.2	7.8	0.1
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	603	41	593	33	605	47	968	68	1282	41	928	164
3,5,10	670	54	798	145	794	145	1234	29	1487	38	1155	179
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error
Control	15.1	0.6	13.5	0.1	14.7	0.4	14.2	0.3	13.1	0.5	12.3	0.1
3,5,10	14.5	0.7	13.9	0.6	15	0.4	14.7	0.5	14.3	0.7	12.5	0.1

Table 3.33. Mean physical measurements taken at points in time after the addition of TEG

Trial 2, Beds 9,11,12@0.5%. Treated n=9, Untreated n=18

Days after addition of TEG	0		1		2		5		7		12	
	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error
Beds												
Control	8.6	0.2	7.7	0.3	7.4	0.2	7.7	0.2	7.8	0.1	7.9	0.2
9,11,12	8.4	0.2	8.1	0.2	7.6	0.1	7.7	0.1	8.0	0.0	8.1	0.1
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	603	41	593	33	605	47	968	68	1282	41	928	164
9,11,12	672	110	532	175	507	108	949	74	1374	0	750	126
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error
Control	15.1	0.6	13.5	0.1	14.7	0.4	14.2	0.3	13.1	0.5	12.3	0.1
9,11,12	13.5	0.1	12.9	0.1	14.7	0.2	14.7	0.5	13.8	0.6	12.5	0.1

Table 3.34. The physical measurements taken at points in time after the addition of TEG

Trial 3. Beds 3,5,10 @0.5%;. Treated n=9, Untreated n=18.

Days after addition of TEG	0		1		2		5		12	
	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error
Beds										
Control	7.7	0.0	7.5	0.0	7.6	0.0	7.8	0.0	7.5	0.0
3,5,10	7.8	0.2	7.3	0.0	7.6	0.0	7.4	0.0	7.5	0.0
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	1408	84	946	55	990	45	914	51	instrument	
3,5,10	1511	139	1313	135	1366	89	1438	81	failure	
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error
Control	14.5	0.0	12.1	0.0	9.3	0.2	12.1	0.0	11.3	0.2
3,5,10	14.7	0.1	12.1	0.0	9.1	0.2	12.1	0.0	11.5	0.2

Table 3.35. The physical measurements taken at points in time after the addition of TEG

Trial 4, 9,11,12 @ 0.1% TEG Treated n=9, Untreated n=18.

Days after addition of TEG	0		1		2		5		12	
	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error
Beds										
Control	7.7	0.0	7.5	0.0	7.6	0.0	7.8	0.0	7.5	0.0
9,11,12	7.6	0.2	7.6	0.0	7.7	0.0	7.6	0.1	7.5	0.1
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	1408	84	946	55	990	45	914	51	Instrument	
9,11,12	1403	73	886	189	951	86	1011	190	failure	
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error
Control	14.5	0.0	12.1	0.0	9.3	0.2	12.1	0.0	11.3	0.2
9,11,12	14.8	0.0	12.1	0.2	9.1	0.2	12.1	0.0	11.4	0.1

Table 3.36. The physical measurements taken at points in time after the addition of TEG

Trial 5. Beds 3,5,10 @ 0.1% TEG. Treated n=9, Untreated n=18.

Days after addition of TEG	0		3		7		10		13		16	
	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error
Beds												
Control	7.9	0.3	8.7	0.2	7.5	0.1	7.6	0.2	8.1	0.1	8.2	0.1
3,5,10	7.8	0.1	8.4	0.1	7.2	0.1	7.4	0.1	7.8	0.1	8.0	0.1
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	1386	32	1364	42	883	75	967	13	981	9	999	10
3,5,10	876	93	1085	38	771	69	736	113	778	99	657	149
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error
Control	10.9	0.1	8.7	0.1	6.3	0.1	2.7	0.2	6.5	0.1	7.5	0.0
3,5,10	10.7	0.1	8.5	0.2	6.5	0.1	2.5	0.0	6.5	0.0	7.5	0.0
	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error
Control					6.1	1.4	8.1	1.0	8.1	0.7	7.9	1.3
3,5,10					1.1	0.2	3.7	0.3	3.5	0.4	4.3	0.8

Table 3.37. The physical measurements taken at points in time after the addition of TEG

Trial 6. Beds 9,11,12 @ 0.5% TEG. Treated n=9, Untreated n=18.

Days after addition of TEG	0		3		7		10		13		16	
	pH	\pm St. error	pH	\pm St. error	pH	\pm St. error	pH	\pm St. error	pH	\pm St. error	pH	\pm St. error
Beds												
Control	7.9	0.3	8.7	0.2	7.5	0.1	7.6	0.2	8.1	0.1	8.2	0.1
9,11,12	7.9	0.3	8.7	0.2	7.5	0.1	7.6	0.2	8.1	0.1	8.2	0.1
	Cond.	\pm St. error	Cond.	\pm St. error	Cond.	\pm St. error	Cond.	\pm St. error	Cond.	\pm St. error	Cond.	\pm St. error
Control	1386	32	1364	42	883	75	967	13	981	9	999	10
9,11,12	645	127	960	133	675	67	784	121	892	106	828	203
	Temp.	\pm St. error	Temp.	\pm St. error	Temp.	\pm St. error	Temp.	\pm St. error	Temp.	\pm St. error	Temp.	\pm St. error
Control	10.9	0.1	8.7	0.1	6.3	0.1	2.7	0.2	6.5	0.1	7.5	0.0
9,11,12	10.8	0.0	9.0	0.1	6.4	0.0	2.5	0.0	6.5	0.0	7.4	0.0
	D. O.	\pm St. error	D. O.	\pm St. error	D. O.	\pm St. error	D. O.	\pm St. error	D. O.	\pm St. error	D. O.	\pm St. error
Control					6.1	1.4	8.1	1.0	8.1	0.7	7.9	1.3
9,11,12					1.4	0.4	3.5	0.4	3.9	0.3	4.7	0.7

Table 3.38. The physical measurements taken at points in time after the addition of TEG

Trial 7. Beds 3,5,10 @0.5% TEG. Treated n=9, Untreated n=18.

Days after addition of TEG	0		2		5		7		9		12		16		20		
	Beds	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error
	Control	7.9	0.2	7.8	0.1	7.7	0.2	7.9	0.2	7.9	0.2	8.0	0.1	8.1	0.1	7.5	0.2
	3,5,10	7.8	0.1	7.6	0.1	7.4	0.1	7.4	0.1	7.4	0.1	7.3	0.1	7.3	0.1	7.4	0.2
		Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error
	Control	579	41	630	41	750	34	831	24	765	41	748	74	1011	40	966	48
	3,5,10	556	58	483	51	658	79	824	81	731	75	917	79	1160	49	1061	116
		Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error
	Control	7.8	0.0	4.6	0.1	12.8	0.1	8.8	0.1	2.1	0.1	8.3	0.1	10.1	0.0	7.8	0.3
	3,5,10	8.1	0.0	4.7	0.0	12.4	0.3	8.8	0.2	2.2	0.1	8.0	0.3	10.2	0.0	7.4	0.1
		D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error
	Control	8.0	0.8	7.7	0.6	7.0	0.5	7.0	0.5	8.9	0.6	8.8	0.3	8.5	0.5		
	3,5,10	3.3	0.5	3.3	0.3	3.4	0.2	3.5	0.2	3.8	0.2	4.2	0.2	3.8	0.3		

Table 3.39. The physical measurements taken at points in time after the addition of TEG

Trial 8. Beds 9,11,12 @ 0.1% TEG. Treated n=9, Untreated n=18.

Days after addition of TEG	0		2		5		7		9		12		16		20	
Beds	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error
Control	7.9	0.2	7.8	0.1	7.7	0.2	7.9	0.2	7.9	0.2	8.0	0.1	8.1	0.1	7.5	0.2
9,11,12	7.5	0.1	7.4	0.1	7.2	0.1	7.1	0.1	7.2	0.1	7.3	0.1	7.4	0.1	7.6	0.1
	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error	Conductivity	±St. error
Control	579	41	630	41	750	34	831	24	765	41	748	74	1011	40	966	48
9,11,12	801	47	553	40	794	69	1005	71	867	74	1011	58	1202	36	814	110
	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error	Temperature	±St. error
Control	7.8	0.0	4.6	0.1	12.8	0.1	8.8	0.1	2.1	0.1	8.3	0.1	10.1	0.0	7.8	0.3
9,11,12	8.1	0.1	4.7	0.0	11.8	0.1	8.7	0.1	2.2	0.1	7.7	0.1	10.2	0.1	6.5	0.4
	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error
Control	8.0	0.8	7.7	0.6	7.0	0.5	7.0	0.5	8.9	0.6	8.8	0.3	8.5	0.5		
9,11,12	3.6	0.5	3.6	0.4	3.5	0.3	3.4	0.4	3.3	0.1	4.1	0.3	3.2	0.4		

Table 3.40. The physical measurements taken at points in time after the addition of TEG
Trial 9. Beds 3,5,10 @ 1% TEG: Treated n=9, Untreated n=18.

Days after addition of TEG	0		2		4		7		10		12		15		16		
	Beds	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error		
	Control	7.9	0.2	7.8	0.2	7.7	0.2	7.7	0.1	7.9	0.1	7.7	0.1	7.7	0.1	7.7	0.1
	3,5,10	7.8	0.0	7.7	0.1	7.4	0.1	7.3	0.1	7.2	0.1	7.2	0.1	7.1	0.1	7.0	0.1
		Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
	Control	460	11	507	16	540	16	613	19	631	14	655	15	706	19	692	24
	3,5,10	465	25	656	15	726	30	888	59	966	67	963	89	1257	64	1175	124
		Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temperature	±St. error
	Control	7.5	0.3	7.3	0.3	4.1	0.0	11.7	0.3	8.5	0.1	11.6	0.1	11.7	0.1	12.5	0.2
	3,5,10	7.4	0.2	7.4	0.4	4.1	0.0	11.6	0.2	8.4	0.0	11.8	0.1	11.9	0.1	12.6	0.2
		D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error
	Control	9.2	0.1	7.4	0.4	7.3	0.6	7.3	0.4	7.4	0.5	7.5	0.5	6.8	0.6	5.7	0.2
	3,5,10	6.6	0.2	3.8	0.3	3.1	0.3	3.6	0.2	2.9	0.2	3.3	0.2	3.4	0.2	3.6	0.5

Table 3.41. The physical measurements taken at points in time after the addition of TEG

Trial 10. Beds 9,11,12 @ 0.2% TEG. Treated n=9, Untreated n=18.

Days after addition of TEG	0		2		4		7		10		12		15		16	
Beds	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error	ph	±St. error
Control	7.9	0.2	7.8	0.2	7.7	0.2	7.7	0.1	7.9	0.1	7.7	0.1	7.7	0.1	7.7	0.1
9,11,12	7.8	0.0	7.6	0.0	7.6	0.1	7.4	0.1	7.3	0.1	7.4	0.1	7.3	0.1	7.2	0.1
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	460	11	507	16	540	16	613	19	631	14	655	15	706	19	692	24
9,11,12	439	20	505	23	580	32	687	54	748	39	717	59	935	42	863	100
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temperature	±St. error
Control	7.5	0.3	7.3	0.3	4.1	0.0	11.7	0.3	8.5	0.1	11.6	0.1	11.7	0.1	12.5	0.2
9,11,12	7.3	0.3	7.5	0.5	4.2	0.0	11.6	0.2	8.3	0.1	12.4	0.1	12.9	0.1	13.0	0.2
	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error
Control	9.2	0.1	7.4	0.4	7.3	0.6	7.3	0.4	7.4	0.5	7.5	0.5	6.8	0.6	5.7	0.2
9,11,12	6.6	0.2	3.3	0.3	2.7	0.2	3.1	0.2	2.8	0.3	2.9	0.2	2.9	0.2	2.8	0.1

Table 3.42. The physical measurements taken at points in time after the addition of TEG

Trial 11. Beds 3,5,10 @ 5% TEG. Treated n=9, Untreated n=18.

Days after addition of TEG	0		3		6		8		15		18	
	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error
Beds												
Control	8.0	0.2	7.9	0.2	8.0	0.2	7.7	0.1	8.0	0.1	7.6	0.1
3,5,10	7.4	0.1	7.4	0.1	7.3	0.1	6.9	0.1	6.8	0.1	6.6	0.1
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	667	38	698	26	750	27	816	22	889	22	861	33
3,5,10	1102	122	971	169	1088	137	1416	122	1651	119	###	287
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error
Control	12.1	0.1	7.5	0.1	10.5	0.1	11.3	0.1	11.3	0.1	13.2	0.2
3,5,10	12.0	0.1	8.0	0.2	10.8	0.1	11.3	0.1	11.4	9.9	13.3	0.2
	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error
Control	7.4	0.5	8.0	0.5	8.0	0.4	7.9	0.6	8.0	0.4	5.8	0.6
3,5,10	3.1	0.2	5.3	0.6	2.7	0.2	3.2	0.2	3.3	0.2	2.8	0.1

Trial 11 continued

Days after addition of TEG	21		25		28		32		34		36	
	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error
Control	8.0	0.1	7.8	0.1	8.1	0.1	8.1	0.1	7.8	0.0	7.9	0.1
3,5,10	6.7	0.1	6.9	0.1	7.1	0.1	7.1	0.1	6.9	0.1	7.0	0.1
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	821	71	944	34	852	40	895	24	965	28	846	36
3,5,10	3720	369	3885	387	2989	340	3664	330	4002	292	2592	277
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error
Control	18.4	0.2	16.4	0.1	15.4	0.1	14.0	0.2	14.2	0.1	13.7	0.2
3,5,10	18.5	0.1	16.4	0.1	15.3	0.1	13.9	0.1	14.1	0.1	13.9	0.1
	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error
Control	6.7	0.6	6.0	0.5	7.7	0.6	8.1	0.7	5.5	0.6	7.6	0.6
3,5,10	2.8	0.2	2.1	0.2	2.5	0.2	2.4	0.2	2.3	0.2	2.4	0.2

Table 3.43. The physical measurements taken at points in time after the addition of TEG

Trial 12. Beds 9,11,12 @ 1% TEG. Treated n=9, Untreated n=18.

Days after addition of TEG	0		3		6		8		15		18	
	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error
Beds												
Control	8.0	0.2	7.9	0.2	8.0	0.2	7.7	0.1	8.0	0.1	7.6	0.1
9,11,12	7.8	0.1	7.4	0.1	7.6	0.1	7.2	0.1	7.0	0.1	6.7	0.1
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	667	38	698	26	750	27	816	22	889	22	861	33
9,11,12	697	60	970	117	955	126	1180	114	1528	131	1873	260
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error
Control	12.1	0.1	7.5	0.1	10.5	0.1	11.3	0.1	11.3	0.1	13.2	0.2
9,11,12	12.6	0.1	9.1	0.2	11.2	0.1	12.2	0.2	12.1	0.1	14.3	0.1
	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error
Control	7.4	0.5	8.0	0.5	8.0	0.4	7.9	0.6	8.0	0.4	5.8	0.6
9,11,12	2.5	0.2	4.8	0.5	3.2	0.2	3.0	0.2	2.9	0.2	3.2	0.2

Trial 12continued

Days after addition of TEG	21		25		28		32		34		36	
	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error	pH	±St. error
Control	8.0	0.1	7.8	0.1	8.1	0.1	8.1	0.1	7.8	0.0	7.9	0.1
9,11,12	6.9	0.1	6.6	0.1	6.9	0.1	6.7	0.1	6.6	0.1	6.7	0.1
	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error	Cond.	±St. error
Control	821	71	944	34	852	40	895	24	965	28	846	36
9,11,12	2405	336	3135	352	2508	335	3040	432	3931	473	2819	398
	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error	Temp.	±St. error
Control	18.4	0.2	16.4	0.1	15.4	0.1	14.0	0.2	14.2	0.1	13.7	0.2
9,11,12	18.8	0.1	16.7	0.1	15.9	0.0	14.5	0.2	14.6	0.1	14.4	0.1
	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error	D. O.	±St. error
Control	6.7	0.6	6.0	0.5	7.7	0.6	8.1	0.7	5.5	0.6	7.6	0.6
9,11,12	3.3	0.2	3.1	0.2	3.1	0.2	3.1	0.2	2.2	0.2	2.5	0.3

3.5.1. Temperature

The mean temperature in the test beds is shown for each trial in tables 3.32. to 3.43.

During trials 1, 2, 3 and 4 (September – October) the temperature in all the beds declined 2-4 degrees over the course of the trial with the lowest temperature being 8°C. The temperature in trials 5, 6, 7 and 8 (November – January) declined 8 – 10 degrees down to 2°C. In trials 9, 10, 11 and 12 (March – April) the temperature rose – from 4°C to 12°C in trial 5 and 8°C to 14°C in trial 11 and 12.

ANOVA showed there were no significant differences in the readings between the control, low and high dose beds for any of the 12 trials.

3.5.2. Conductivity

Changes in conductivity are shown in tables 3.32. to 3.43. for trials 1- 12

Variations in conductivity in the three treatments was fairly consistent except in trial 11 and 12. In trials 1 to 10, conductivity fluctuated between 400 – 1500 $\mu\text{S}/\text{cm}$ while in trial 11 & 12 with the highest dosage, the conductivity increased in the treated beds from 500 to 4000 $\mu\text{S}/\text{cm}$. The pattern of change varied considerably between trials. One way ANOVA with *post hoc* Tukey tests showed significant differences between control and test beds in trials 3 to 12.

In trial 3, conductivity in the high dose (0.5%v/v TEG) beds was significantly higher than in trial 4, with a low dose and the control beds. In trial 5 & 6, both the high (0.5%v/v TEG) and the low (0.1%v/v TEG) dose beds were significantly lower than the control beds. Trial 7 & 8 showed no differences between the beds, indicating that the effects of the TEG are not carried over from one trial to another. In trial 9 conductivity in the high dose treatment (1%v/v TEG) was significantly higher than that in the control while in trials 11 & 12, both beds were significantly higher than control.

3.5.3. pH

Changes in pH in the test beds are shown in tables 3.32. to 3.43. for trials 1-12.

The pH fluctuated in the first three trials between 8.6 and 7.3 in all of the beds. Some variation occurred but all beds followed the same levels while in the last three trials the pH of the treated beds fell to levels below those of the control beds. In the treated beds the pH fell from 8.0 to 6.8. ANOVAs showed no significant difference between the pH of the control, high and low dosage beds but in trials 7 to 12 inclusive the pH in the treated beds was significantly lower than in the control beds

3.5.4. Dissolved oxygen

Changes in dissolved oxygen in control and treated beds are shown in tables 3.32. to 3.43. for trials 1-12. TEG at both high and low dosage lowered the dissolved oxygen in all four trials and one way ANOVAs showed highly significant differences between the control and both dosed beds. Unfortunately, readings were not possible at the start of trials so any long term patterns of dissolved oxygen could not be ascertained.

Table 3.44. Summary of results of one way ANOVAs with *post hoc* Tukey tests on the physical aspects of the biodegradation trials 1 to 6.

n/s = not significant. n/a not available, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$
 Full analysis Appendix p 160 - 181

	% TEG	Temperature °C	pH	Conductivity $\mu\text{S/cm}$	Dissolved oxygen mg/l
Trial 1	0.1	n/s	n/s	n/s	n/a
Trial 2	0.5	n/s	n/s	n/s	n/a
Trial 3	0.5	n/s	n/s	**	n/a
Trial 4	0.1	n/s	n/s		
Trial 5	0.5	n/s	n/s	**	***
Trial 6	0.1	n/s	n/s	*	*
Trial 7	0.5	n/s	***	n/a	***
Trial 8	0.1	n/s	*	n/a	*
Trial 9	1.0	n/s	***	*	***
Trial 10	0.2	n/s	*		*
Trial 11	5.0	n/s	***	*	***
Trial 12	1.0	n/s	*	*	*

Thus the addition of TEG to the test beds affected physical factors in the following ways:-

- Temperature - No effect
- pH - No effect first three trials
 - Lowered pH last three trials
- Conductivity - No effect (trials 1 & 4)
 - Lowered (trial 3)
 - Raised (trials 2,5 & 6)
- Dissolved Oxygen – Levels lowered in each trial

3.5.5. Discussion

3.5.5.1. Temperature

One way ANOVAs showed there were no significant differences in the temperature readings between the control, low dose and high dose beds for any of the six trials. The very small differences in the temperature readings from different tanks can be attributed to the fact that the beds were raised off the ground, so the tanks themselves were open to prevailing winds and direct sunlight. Test beds at either end of the row could feel the effect of wind more than beds in the centre of the row. Likewise, shading from the sun affected different beds at different times of the day and year. Thus there was no evidence that variation of temperature was by means other than by meteorological influences.

3.5.5.2. Conductivity

Conductivity in the control beds varied considerably – especially in the first three trials. The test beds had higher conductivity than the control beds in all except trial 5/6. One way ANOVAs with *post hoc* Tukey tests showed significant differences between control and test beds in Trials 3 to 12. There appeared to correlation between conductivity readings and treated beds in the first five trials indicating that they were responding to some environmental factor.

In trial 4, the high dose beds showed significant differences from the low dose and control beds, whilst in trial 5/6, both the high and low dose beds were significantly different to the control beds.

In trial 4 the high dose beds were significantly different as were the high dose beds in trial 6. However, it cannot be said that the differences were caused by the high dosage as in trial 5, the low dose beds were also significantly different. Trial 7/8 showed no

differences between the beds, indicating that the effects of the TEG, if any, are not carried over from one trial to another.

In trials 9,10,11&12, both high and low dosage beds showed significant differences to the control beds with trial 12 displaying highly significant differences from the control beds.

Throughout the trials, the control beds displayed conductivity readings that varied from 600 μ S/cm to 1400 μ S/cm for no apparent reason. All the beds contained washed pea gravel from the same source received in one delivery and all the plants were from the same nursery also received in one delivery so there was no discernable difference that could account for these fluctuations. In trial 11/12, however, the control bed conductivity remained steady whilst the test beds produced readings in excess of 4000 μ S/cm.

In trial 11/12 the conductivity in the treated beds increased dramatically after day 15. Conductivity is a measurement of a liquid's ability to conduct electricity and this conductivity changes with the total ion concentration in the liquid. The high levels measured in trials 9,10,11 and 12 could be partly due to the presence of ferrous salts in solution in the water. Raised conductivity in beds treated with TEG may need monitoring in the long term and some method found to ameliorate the condition. The high levels measured did not appear to be caused by the presence of the TEG solution.

3.5.5.3. pH

There was no difference between pH of control and treated beds in the first three trials but subsequently, the pH of the treated beds was lower than the control. ANOVAs showed significant differences between the control beds and both the high and low

dose beds in trial 7 to 12. The control beds displayed variations in pH from 8.6 to 7.5 which must be attributed to atmospheric conditions and maybe the removal of nutrients by the reeds from the water which could alter the ionic balance. The test beds however slowly became more acidic than the control beds. This acidity could be due to the production of carbon dioxide from the degradation of the TEG which then produces a weak solution of carbonic acid. There is no evidence that minor pH fluctuations as experienced in these trials affects the rate of degradation of TEG. Bacteria are, on the whole, pH dependant with most having an optimum range of about 3 pH units (Todar,2004) so the changes noted here should not have any adverse effect on those present in the beds.

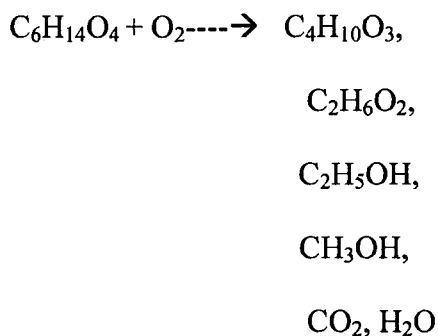
3.5.5.4. Dissolved oxygen

ANOVAs on the readings from all the beds show significant differences between control and dosed beds. Unfortunately, readings were not possible at the start of trials 5/6 and 7/8 so long term patterns of oxygen content could not be ascertained. The basic premise of the project was that TEG would be degraded by bacteria that would utilise the dissolved oxygen in the water while the reeds attempted to maintain a balance around the rhizosphere by diffusing oxygen through their roots into the water. The significant differences were expected, biodegrading bacteria use the dissolved oxygen during the process.

4. The effect of TEG on the reeds

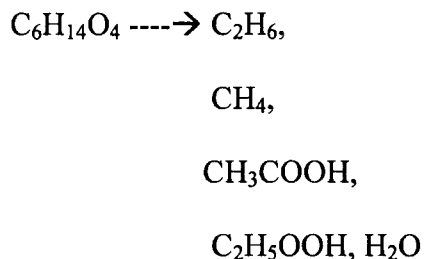
4.1 Introduction

An important aspect of the project was to determine the effects that the addition of triethylene glycol and the products of degradation may have on reeds and thus whether the reeds of the constructed reedbed treatment system would be capable of growing in a solution of TEG in the long term. Possible products of the aerobic degradation of TEG are diethylene glycol, monoethylene glycol, ethanol, methanol, carbon dioxide and water.



Earlier work by Chong(1999) found that wetland plants and micro-organism populations in the reedbed substrate were not adversely affected by 'shock-loads' of glycol based de-icers and anti-icers (mono and diethylene glycols and 1,2-propylene glycol) but alcohol and water mixes are used for killing and preserving small organisms, so if alcohols were to be produced it could cause problems with some aquatic micro-organisms present in the rhizosphere.

If the degradation is anaerobic the end products could be ethane, methane, acetic acid, proprionic acid and water.



Armstrong (1998) found that propionic acid was highly toxic to *P. australis* when found in high concentrations that gave pH readings of 4.5 and 6.0. The production of methane would not be welcome due to its 'flammability' and 'greenhouse gas' properties.

The impact of TEG on the rest of the reedbed community was also of interest as the site of the existing reedbeds is considered to be of importance in the context of local biodiversity. Any detrimental effects from the proposed biodegradation programme would have 'knock on' effects on both the flora and fauna of the site. Before the reedbeds were constructed, the surrounding grassland had been surveyed for both flora and fauna and after the construction work had been completed, the disturbed land was reseeded so as to return the land to its original state as soon as possible. Since then the land has been undisturbed apart from the small area utilised for these trials. During the trials, notes were taken of fauna seen and a survey of the flora was undertaken as an undergraduate project in the summer of 2003 to determine any changes that may have occurred since the beds were constructed. This information is not included in full in the thesis but was made available to BP, English Nature and INCA (Industry Nature Conservation Association).

The aim of the work on the physical attributes of the reeds was to establish whether the continuous application of TEG effluent would damage them and their ability to aid biodegradation. The specific objectives were to measure the impact of TEG on shoot density, height, total biomass, stomatal count and Total Kjeldhal Nitrogen content of the shoots. As reedbeds mature they are capable of aerating their rhizosphere to a greater extent (Bart,2000) but in the early stages of growth it would be advisable to monitor the degradation processes and plant well-being.

4.2 Methods

Several different methods were employed in this section, some of which evolved as the project progressed.

4.2.1 Shoot density

To determine whether shoot production by reeds was being affected by the addition of TEG the number of shoots in a predetermined area of bed was recorded at indeterminate intervals from planting until the end of the growing season 2003. As the test beds were outdoors in a windy environment it was not possible to count all shoots in each tank as those at the extremities of both the tanks and the whole structure were open to wind damage of varying degrees. So as to standardise the area counted two wires were attached to each test tank covering a strip of 10cms along the middle of the tanks giving a sample area of 0.15 m^2 (Figure 4.1) All shoots above the top of the tank lip that were in this wire corridor were to be counted.

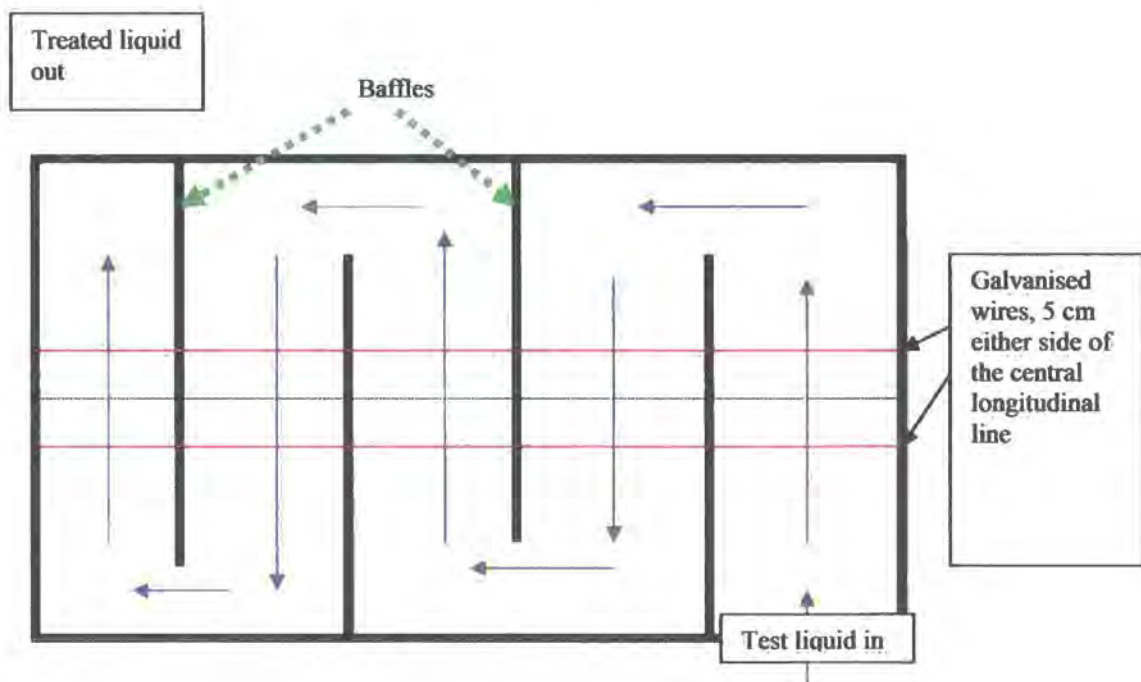


Figure 4.1. Diagram of a test bed, surface view, showing the positions of the baffles and the 'corridor' used for the density count (not to scale).

4.2.2 Tallest shoot

When the shoot density was being measured, the tallest shoot in each tank was also measured from the lip of the tank to the tip of the tallest shoot. The lip of the tank was chosen as a suitable static reference point for measurements so as to discount any surface movement of the gravel in the tanks.

4.2.3 Shoot Biomass

At the end of each growing season (2002 and 2003) all material above the lip of each test tank was cut and bagged. The lip of the tank was taken as a suitable constant point of reference. The cut material was then taken to the lab where it was weighed and dried at room temperature to constant weight.

4.2.4 Stomatal count

During the season of 2003, leaves were removed from randomly selected shoots in each test tank and examined for stomatal count. On the underside of a leaf a strip of clear nail varnish was applied. When the varnish was dry it was covered with a piece of clear 'sellotape' with the sticky side down onto the leaf. The tape was then removed, leaving the varnish adhered to the tape, along with the epithelial layer of the leaf. This sample of cells was placed on a glass microscope slide and examined under a Nikon microscope. The number of stomata per field of view was recorded, 3 fields per sample, magnification x 100.

4.2.5 Total Kjeldhal Nitrogen of whole shoots

The reeds harvested at the end of 2002 and 2003 were ground up using a Culatti grinder and the resultant material was analysed for total kjeldhal nitrogen (TKN)

TKN was determined by using the published Hach method (Appendix) with some minor alterations recommended by Mr. H. Pinnegar, senior technician Queens College, Durham University. (Appendix p 182)

4.3 Results

4.3.1 Shoot Density and Height

The number of shoots per transect for treated and untreated beds, 2002 and 2003, are shown in Table 4.1. 2-Factor ANOVA with replication shows that the reeds of the treated beds were significantly more dense than the untreated ($P=0.0002$) with significant interaction ($P=0.01$) with the two years.(d.f. 20, 23; $P = <0.001$)

Table 4.1. The number of shoots per transect for all beds, 2001

Control Beds

Date sampled	Bed 1	Bed 2	Bed 4	Bed 6	Bed 7	Bed 8
11/06/2001	38	37	35	41	40	37
27/07/2001	39	36	35	43	42	40
31/08/2001	41	37	37	43	42	41
04/10/2001	43	37	37	43	42	42
Mean	40	37	36	43	42	40
± St Error	1.1	0.3	0.6	0.5	0.5	1.1

Test Beds

Date sampled	Bed 3	Bed 5	Bed 9	Bed 10	Bed 11	Bed 12
11/06/2001	39	41	41	47	49	49
27/07/2001	39	45	34	39	43	45
31/08/2001	42	46	39	40	40	41
04/10/2001	42	47	39	43	44	45
Mean	41	45	38	42	44	45
± St Error	0.9	1.3	1.5	1.8	1.9	1.6

Table 4.2. The number of shoots per transect for all beds, 2002

Control Beds

Date sampled	Bed 1	Bed 2	Bed 4	Bed 6	Bed 7	Bed 8
18/06/2002	45	37	57	40	37	36
24/07/2002	39	36	56	45	40	35
16/08/2002	49	38	54	41	37	34
02/09/2002	49	38	59	47	38	34
20/09/2002	46	40	58	49	40	35
04/10/2002	47	39	57	48	40	36
Mean	46	38	57	45	39	35
\pm St Error	1.5	0.6	0.7	1.5	0.6	0.4

Test Beds

Date sampled	Bed 3	Bed 5	Bed 9	Bed 10	Bed 11	Bed 12
18/06/2002	32	36	35	24	31	33
24/07/2002	41	45	38	28	34	29
16/08/2002	45	55	38	34	42	32
02/09/2002	50	58	49	37	46	36
20/09/2002	53	62	53	39	50	47
04/10/2002	51	60	54	37	49	44
Mean	45	53	45	33	42	37
\pm St Error	3.2	4.1	3.5	2.4	3.2	2.9

Table 4.3. The number of shoots per transect for all beds, 2003

Control Beds

Date sampled	Bed 1	Bed 2	Bed 4	Bed 6	Bed 7	Bed 8
03/06/2003	31	32	37	32	34	32
13/06/2003	35	34	38	36	35	36
04/08/2003	35	34	39	38	34	39
04/10/2003	35	35	41	48	40	36
Mean	34	34	39	39	36	36
\pm St Error	1	0.6	0.9	3.4	1.4	1.4

Test Beds

Date sampled	Bed 3	Bed 5	Bed 9	Bed 10	Bed 11	Bed 12
03/06/2003	51	42	36	41	51	49
13/06/2003	50	46	35	38	50	42
04/08/2003	55	52	42	48	58	49
04/10/2003	60	60	51	50	63	52
Mean	54	50	41	44	56	48
\pm St Error	2.3	3.9	3.7	2.8	3.1	2.1

Table 4.4. The final number of shoots of reeds per transect in the test beds for 2001, 2002 and 2003. Beds 1,2,4,6,7 and 8 were untreated and 3,5,9,10,11 and 12 all received TEG

Bed no.	No. of shoots per transect 4/10/01	No. of shoots per transect 4/10/02	No. of shoots per transect 4/10/03	Bed no.	No. of shoots per transect 4/10/01	No. of shoots per transect 4/10/02	No. of shoots per transect 4/10/03
1	43	47	34	3	42	51	60
2	37	39	35	5	47	60	60
4	37	57	41	9	39	54	51
6	43	48	38	10	43	37	50
7	42	40	34	11	44	49	63
8	42	36	40	12	45	44	52
Mean	41	45	37	Mean	43	49	56
± St. error	1.2	3.1	1.3	± St. error	1.1	3.3	2.3

The maximum heights of shoots in the treated and untreated beds for 2002 and 2003 are shown in Table 4.5. 2-factor ANOVA showed no significant difference in the heights of the tallest shoots between the treated and untreated beds.

Measurements were taken throughout the growing season of the tallest shoots and are shown in tables 4.5 to 4.7. Table 4.8 shows the heights of the tallest shoots at the end of the growing season. In 2001, the first year in the test beds, the reeds had only experienced TEG once by October and as they appeared to be at different stages of growth they were not harvested.

Table 4.5. The tallest shoots for all beds, in mm, 2001

Control Beds

Date sampled	Bed 1	Bed 2	Bed 4	Bed 6	Bed 7	Bed 8
11/06/2001	388	703	470	414	610	523
27/07/2001	431	750	475	590	675	578
31/08/2001	460	775	476	635	735	662
04/10/2001	478	875	520	655	760	675
Mean	439	776	485	574	695	610
± St. Error	20	36	12	55	33	36

Test Beds

Date sampled	Bed 3	Bed 5	Bed 9	Bed 10	Bed 11	Bed 12
11/06/2001	612	514	575	488	478	432
27/07/2001	875	654	620	525	525	550
31/08/2001	924	800	655	580	560	625
04/10/2001	1020	995	685	650	578	675
Mean	858	741	634	561	535	571
± St. Error	87	103	24	35	22	53

Table 4.6 The tallest shoots for all beds, in mm, 2002

Control Beds

Date sampled	Bed 1	Bed 2	Bed 4	Bed 6	Bed 7	Bed 8
18/06/2002	603	722	731	615	765	677
24/07/2002	729	865	820	747	862	947
16/08/2002	648	922	852	781	921	836
02/09/2002	736	925	853	780	956	972
20/09/2002	763	962	1032	836	971	888
04/10/2002	780	920	852	828	923	996
Mean	710	886	857	765	900	886
± St. Error	28	35	40	33	31	48

Test Beds

Date sampled	Bed 3	Bed 5	Bed 9	Bed 10	Bed 11	Bed 12
18/06/2002	450	404	432	509	441	400
24/07/2002	728	632	462	762	602	398
16/08/2002	773	746	631	878	746	665
02/09/2002	918	749	707	1001	815	800
20/09/2002	926	866	794	1032	830	812
04/10/2002	923	748	785	1031	828	865
Mean	786	691	635	869	710	657
± St. Error	76	65	64	84	64	86



Table 4.7 The tallest shoots for all beds, in mm, 2003

Control Beds

Date sampled	Bed 1	Bed 2	Bed 4	Bed 6	Bed 7	Bed 8
03/06/2003	578	718	715	627	670	713
13/06/2003	648	810	810	712	696	824
04/08/2003	715	915	785	835	900	870
04/10/2003	664	915	873	1010	950	1035
Mean	651	840	796	796	804	861
± St. Error	28	47	33	83	71	67

Test Beds

Date sampled	Bed 3	Bed 5	Bed 9	Bed 10	Bed 11	Bed 12
03/06/2003	705	763	470	750	685	578
13/06/2003	792	831	490	776	688	582
04/08/2003	925	960	660	960	795	680
04/10/2003	1050	970	687	995	895	735
Mean	868	881	577	870	766	644
± St. Error	76	50	56	63	50	38

Table 4.8. The final tallest shoots, in mm, of reeds in all beds for 2001, 2002 and 2003. Beds 1,2,4,6,7 and 8 were untreated and 3,5,9,10,11 and 12 all received TEG

Bed No.	Tallest shoot 04/10/01	Tallest shoot 04/10/02	Tallest shoot 04/10/03	Bed No.	Tallest shoot 04/10/01	Tallest shoot 04/10/02	Tallest shoot 04/10/03
1	478	780	664	3	1020	923	1050
2	875	920	915	5	995	748	970
4	520	852	873	9	685	785	687
6	655	828	1010	10	650	1031	995
7	760	923	950	11	578	828	895
8	675	996	1035	12	675	865	735
Mean	661	883	908		767	863	889
± St. Error	60	31.8	54		78	42	60

As the reed were outside some shoots suffered wind damage, which meant that the tallest shoot at one sample time was not necessarily the same shoot at the next sampling. There was no significant difference between the control and test beds.

4.3.2 Shoot biomass

Table 4.9. The biomass of reeds (g) harvested from the treated and untreated beds, 2002 and 2003. Beds 1,2,4,6,7 and 8 only received water whilst beds 3,5,9,10,11 and 12 received solutions of TEG

Control bed no.	Biomass (g) 2002	Biomass (g) 2003	Test bed no.	Biomass (g) 2002	Biomass (g) 2003
1	126.7	219.3	3	212.9	499
2	203.3	301.4	5	175.7	293.2
4	197.4	284.8	9	180.3	336.6
6	148.3	258.2	10	212.5	493.8
7	176.4	244	11	152	410.7
8	194.7	303	12	182.3	344.1
Mean	174.47	268.45	Mean	185.95	396.2
\pm S Error	12.57	13.74	\pm S Error	9.55	35.2

The shoots from 2001 were not all at the same stage of growth at the end of the growing season and had only experienced the single addition of TEG so they were not harvested that October.

It can be seen from the above figures that the beds that received the solutions of TEG produced more biomass than those that only had water. It was decided that none of the beds would receive any nutrients. It can be seen from plate 4.1 that there was a visible difference between the control and the test beds. The controls had not had any nutrients added whilst the test beds had only had solutions of TEG until late into 2003 when the final solution was made up with water from the retention pond.

4.3.3. Stomatal Number

The number of stomata in each field of view at x100, 3 fields per leaf, 2 leafs per bed and averages are shown in Tables 4.9 and 4.10. As stated earlier the reeds, in 2001, appeared to be at different stages of growth by October and it was thought that any results from those leaves would not be safe.

Table 4.10. The stomatal count of random leaves from test beds and control beds. 2002.

Control bed	1	2	4	6	7	8
leaf 1-1	70	73	75	88	80	78
leaf 1-2	71	77	88	88	70	64
leaf 1-3	61	74	73	78	75	75
leaf 2-1	66	77	67	76	78	78
leaf 2-2	78	69	78	67	89	80
leaf 2-3	82	62	79	77	88	71
Mean	71	72	77	79	80	74
± Standard Error	3	2	3	3	3	2

Test bed	3	5	9	10	11	12
leaf 1-1	79	83	101	98	92	88
leaf 1-2	90	89	106	96	94	86
leaf 1-3	87	100	102	95	96	93
leaf 2-1	100	127	109	110	90	98
leaf 2-2	90	129	104	99	90	99
leaf 2-3	79	120	99	100	91	102
Mean	88	108	104	100	92	94
± Standard Error	3	8	1	2	1	3

Table 4.11. The stomatal count of random leaves from test beds and control beds, 2003

Control bed	1	2	4	6	7	8
leaf 1-1	64	85	91	96	71	68
leaf 1-2	79	71	68	92	65	61
leaf 1-3	59	77	93	86	66	65
leaf 2-1	82	71	78	70	88	67
leaf 2-2	67	60	72	81	97	79
leaf 2-3	80	59	86	72	86	68
Mean (n=6)	72	71	81	83	79	68
\pm Standard Error	4	4	4	4	5	2

Test bed	3	5	9	10	11	12
leaf 1-1	83	89	114	105	96	95
leaf 1-2	112	101	114	104	100	81
leaf 1-3	107	109	110	102	103	96
leaf 2-1	92	137	120	120	99	103
leaf 2-2	80	138	109	109	85	106
leaf 2-3	84	135	110	110	98	108
Mean	93	118	113	108	97	98
\pm Standard Error	6	9	2	3	3	4

The stomatal count for the years 2002 and 2003 ,tables 4.10 & 4.11, show that in both years the reeds in the treated beds had a greater number of stomata than the reeds in the untreated beds.



Test bed 2

Test bed 1



Test bed 4

Test bed 3



Test bed 6

Test bed 5



Test bed 8

Test bed 7



Test bed 10

Test bed 9



Test bed 12

Test bed 11

Plate 4.1. Photographs of the test and control beds to illustrate the appearance of the plants in the beds. (30th September, 2002) Beds 3,5,9,10,11 & 12 were ‘test beds’ that received TEG solutions. The remaining beds only received water.

4.3.4 Total Kjeldhal nitrogen

Table 4.12 shows that the TKN in the harvested reeds was higher in the treated beds than the untreated beds for both years of testing.

A source of nitrogen was not added to any of the test beds.

Table 4.12. The TKN found by analysis in harvested reeds from all test beds, 2002 & 2003

Treated beds no.	2002	2003	Untreated beds no.	2002	2003
3	744.2	1831	1	528.7	507.7
5	1052.2	1768.3	2	750.5	1014.5
9	1212.7	1128.4	4	668.6	809.5
10	1633.6	3636.4	6	765	1518.7
11	1050.4	2314	7	829.3	1170.5
12	1224.9	2036.9	8	1190.7	1390.6
Average TKN, mg/kg	1153	2119.2	Average TKN, mg/kg	788.8	1068.6
± St. error	119.4	343.2	± St. error	90.8	152.9

Table 4.13. Summary of results of t-Tests carried out using the above data
Full details in the appendix
n/a not available, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

	P-Value 2002		P-Value 2003	
	Significant	Not Significant	Significant	Not Significant
Shoot Density		x	**	
Shoot height		x		x
Biomass		x	*	
Stomata		x	***	
TKN	*		**	

Table 4.14. Summary of analysis of reedbed attributes using two factor ANOVA with replication (2002 and 2003)

	P-Value	
	Significance	Interaction
Shoot Density	***	**
Shoot height	ns	not
Biomass	**	*
TKN	**	ns

Single factor ANOVA on Stomata count showed that there was a highly significant difference between the treated and untreated reeds in 2003

Full statistical analysis in Appendix p 183 - 187

4.4 Discussion

The exposure of the reeds to TEG had a definite effect on them and the effect was cumulative. In 2001 only the number of and heights of shoots were recorded. In 2002 the number of shoots, the height of shoots and shoot biomass of the reeds that were dosed with TEG were not significantly different from the untreated reeds. In 2003, however, the number of shoots and the biomass of the treated reeds were significantly higher than those that were untreated. The photographs (Plate4.1) taken in 2003, show that the reeds in the treated beds were looking healthier than those in the untreated ones. The controls were grown in gravel beds without any added nutrients

as were the reeds in the established beds. Although these established beds had originally been used for sewage treatment, they had not received anything else for about 3 years.

The test for the stomatal count was not carried out on the reeds in 2001 but in 2001 a t-test indicated that there was no significant difference between the controls and test bed reeds. In 2003, however, there was a significant difference in the count of stomata present on the treated reeds than those of the untreated.

The TKN for both years was higher in reeds from treated beds.

It would appear from this short term study that the reeds benefited from being exposed to TEG, in that they were more productive and had higher levels of nitrogen in their tissues. Over the two years of experiments, the reeds did not appear to be adversely affected in any way. However, the long term consequences are not known.

It is also of interest that the respiration patterns of the reeds as indicated by the increase in the number of stomata, were altered by the addition of TEG. This increase in the number of stomata is well established and is initiated by an increase in carbon dioxide concentration around the stems(Graves and Reavy, 1996)

Actual carbon dioxide concentrations were not monitored but it is assumed that the breakdown of TEG in the reed rhizosphere released carbon dioxide which caused the response in the reeds.

5. To find a quick and easy method of estimating the BOD₅ of the effluent that could be used by treatment plant personnel.

When research was initiated into the feasibility of passing all site wastewater, run off, sumps and sewage, through the constructed reed beds it was stated, by BP, that a system of testing that would enable plant personnel to monitor BOD₅ levels quickly, easily and accurately would be advantageous. Two on-site instrumental methods which might be used by plant personnel to monitor BOD were evaluated. They were the EZBOD portable BOD meter and an in line Total Organic Carbon analyser , the Biotector, manufactured by CSIP, Weymouth. The methods employed and the results of these evaluations are in the Appendix page 175.

It was found that the EZBOD portable meter was not suitable for use at the CATs terminal as it was designed for determining BOD₅ levels in sewage treatment works rather than in effluent containing organic chemicals. The alternative method supplied by the manufacturers was not satisfactory.

The Biotector however did show that it could provide quick accurate results and that it was suitable for use at CATs as it required minimal attention from plant personnel.

6. The micro-organisms of the reedbed rhizosphere

As use of the reedbed treatment system was to be continuous and long term, preliminary investigations were made on the impact of TEG on the micro-organisms of rhizosphere.

6.1. Bacterial investigation

6.1.1. Introduction

From the start of this project it had been assumed that the degradation of the TEG, displayed by earlier undergraduate work, had been due to the bacteria in the rhizosphere of the reed beds. Chong (1999) found an increase in the populations of aerobic micro-organisms such as bacteria, fungi and actinomycetes after exposure to glycol contaminated effluent. However, during ongoing literature research, references were found that showed that TEG was being used in bactericides (Unisuprol S-25, Chesham Chemicals Ltd, Harrow, UK). These references indicated that the basic premise of the whole project could be flawed, in that if TEG was an effective bactericide, then bacterial biodegradation may not occur. It would also imply that should material from the Klargestar be mixed with site waste water, the effect of the TEG would be to destroy the bacteria responsible for the degradation of the Klargestar material when passed into the reed beds. However, it had also been found that the TEG solutions made up in the laboratory for GC standards did not degrade with standing, implying that an external agent caused the degradation. It was therefore decided to investigate whether TEG did actually act as a bactericide by trying to culture any bacteria present in the rhizosphere after the addition of TEG.

A simple pilot project was devised that would indicate whether TEG was a bactericide or not. The project was designed to be qualitative only and there was no replication.

6.1.2. Method

5 conical flasks were prepared with solutions of 'beef extract' nutrient and they were placed, along with a selection of equipment that may be necessary for future tests, in an autoclave for sterilisation. After 24 hours sterilisation 2 flasks had nutrient solution left in them with the necks sealed. These flasks were marked as controls. A sample of gravel and some rhizome material was taken from test bed 5 (a test bed which had received TEG solution in the trials) and 10 gm. of this sample was placed in each of the remaining 3 flasks. These 3 flasks were each treated differently.

Flask 1 had 20 ml. 2%v/v TEG solution added

Flask 2 had 20 ml. 5%v/v TEG solution added

Flask 3 had 20 ml. 10%v/v TEG solution added

The five flasks were all sealed with sterile wool and aluminium foil and were placed in a constant temperature room at 20°C on a slow swirl plate, 3 rd July 2003.

Signs of growth had occurred after 1 day, but they were left in the constant temperature for 6 days in total. They were then removed to a 4°C room to inhibit further growth.

Nutrient enriched Agar plates were impregnated with samples taken from these flasks. All the plates had a coating of 5% nutrient enriched Agar. Two plates had a further 5% of 2% TEG solution added, 2 had 5% of 5% TEG and 2 had a further 5% of 10% TEG solution. The plates were placed in the 20°C room for a further 6 days.

The resultant plates are shown in the photographs. The control plates and the 'TEG added' plates were compared visually and any sites on the 'TEG' plates that appeared to differ from those on the control plates were sampled for gram staining. Random samples of cultures on the control plates were also taken. The sample of the culture was taken using a hot needle, mixed with 1 drop of water on a microscope slide. The mix was spread and then set by allowing it to air dry and then was fixed with a flame. When cool the sample was placed in crystal violet for 30 seconds before being rinsed gently with water. It was then placed in Lugols' Iodine for 30 seconds and then washed with ethanol to decolourise. The slide was then washed in water before being placed in Cabol Fuchsia for 1 minute. The resultant slides were examined under a microscope.

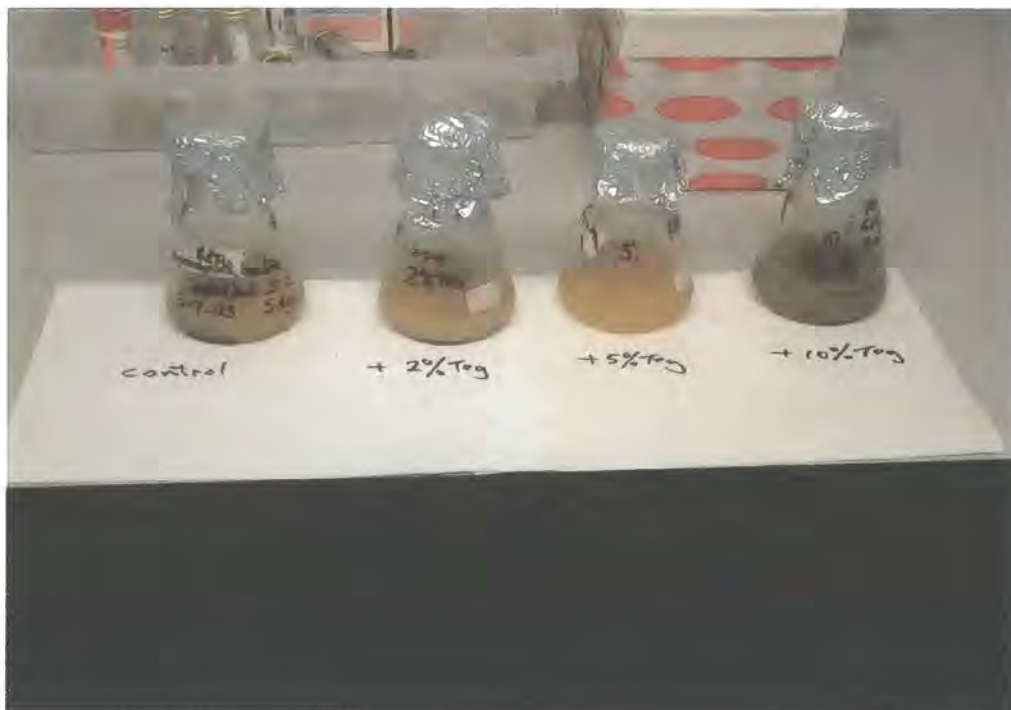


Plate 6.1. The conical flasks with samples and nutrient after 6 days in the constant temperature room.

6.1.3. Results

The results obtained from the pilot experiment and gram stained plate material are only qualitative.

Plate 6.2. shows some of the cultures that had been grown, whilst Table 6.1. describes the observations of the slides from the gram staining exercise.

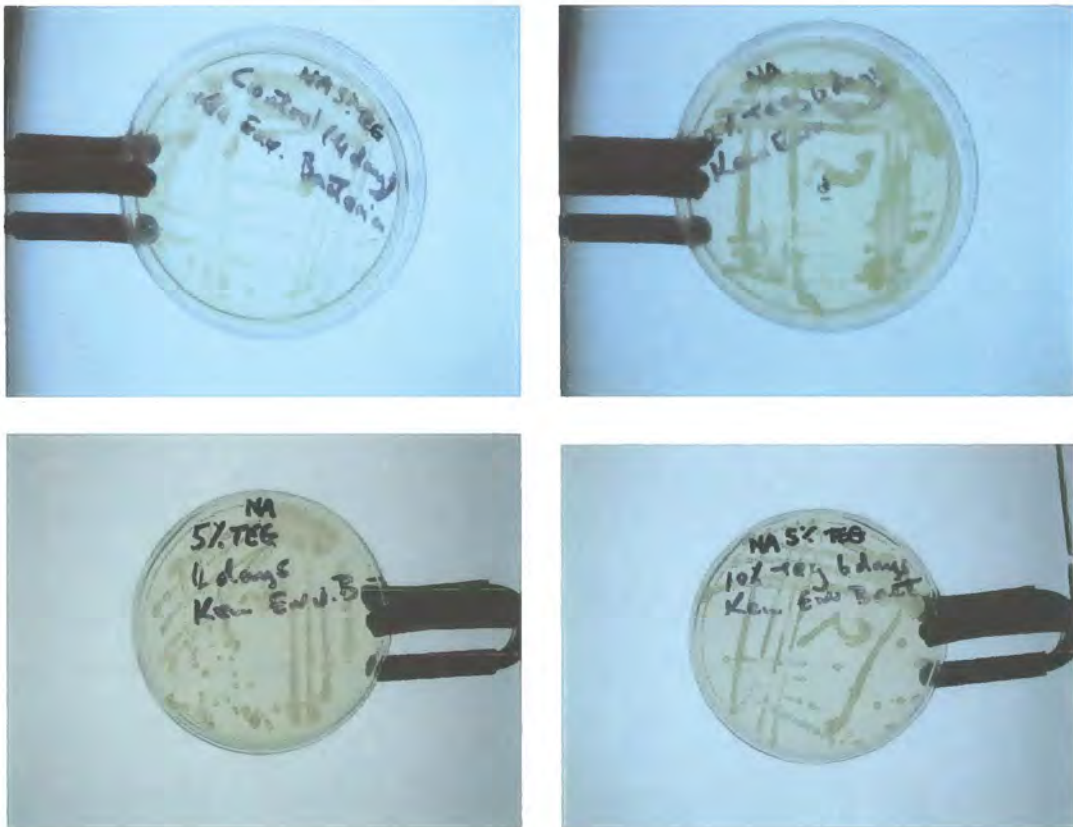


Plate 6.2. Examples of resultant culture growths on Agar plates after 4 days at 20°C

Table 6.1. Observations of slides after gram staining.

Plate Treatment	Plate observations of cultures selected	Slide observations
5% nutrient Agar control	Spreading form with rings	Gram +ve rods 1 μ , lying adjacent
5% nutrient Agar control	Round umber	Gram +ve rods 2 μ Gm +ve spheres < 1 μ
5% nutrient Agar + 5% (2% TEG)	Spherical	Gram -ve spheres <1 μ
5% nutrient Agar + 5% (2% TEG)	Thin spreading form	Gram -ve spheres <1 μ
5% nutrient Agar + 5% (5% TEG)	Spherical	Gram -ve spheres <1 μ Gram +ve spheres 1 μ Gram +ve rods 2-3 μ
5% nutrient Agar + 5% (5% TEG)	Thin spreading form	Gram -ve spheres <1 μ
5% nutrient Agar + 5% (10% TEG)	Round umber	Gram +ve rods, 0.5 -1 μ
5% nutrient Agar + 5% (10% TEG)	Thin spreading form	Gram +ve spheres 1 μ forming circles Gram +ve rods 1-2 μ lying adjacent

6.1.4. Discussion

Only a very preliminary analysis was undertaken as only one test bed had been sampled and bacteriology is a complex subject that could not be included within the remit of this project, but the investigation was considered to be a useful indication of the potential long term effects of TEG on bacterial populations. The investigation showed that there are some bacteria present in the rhizosphere of the test beds that are 'tegophilic' in that they thrive in the presence of TEG. The microscopic examination of the gram stained specimens showed that the more than one species flourished in a medium enriched with TEG.

It was suggested (Rowe, D., pers comm.) that the bacteria may be a strain of *Pseudomonas sp.*, that have a protective 'oil' layer around them that allows slow controlled assimilation of material that may otherwise be harmful. It was thought that it would be possible to isolate the tegophilic strain(s) i.e. bacteria that thrived in the presence of TEG but that it would be a time consuming exercise that could not be completed within the limits of this project.

Bacteria that have an affinity with TEG will benefit from effluent containing TEG being passed through the reed beds regularly. As they benefit from the TEG so they should increase in numbers and become more efficient in degrading the chemical. The rates of degradation of the TEG bears resemblance to the accepted growth pattern of bacterial colonies (Todar, 2002) and it is suggested that when the organic material is introduced the bacteria start to feed off it and subsequently reproduce. Bacterial reproduction is exponential so their food consumption (i.e. the degradation of TEG)

will be relative to that growth. When the food source becomes scarce the growth rate of the population of tegophilic bacteria will slow and eventually stop – coinciding with a decline in the rate of degradation of TEG until all the organic material has been degraded. The population of the bacteria grows and declines exponentially in line with the kinetics of a first order reaction.

6.2. Effects of TEG on microscopic aquatic organisms

6.2.1. Introduction

The presence of micro organisms in water can be an indicator of the 'quality' of the water. Even in highly polluted environments, it is not unusual to find some micro organism communities and in some cases, the presence of a particular species or collection of species can be indicative of the level of pollution.(Patterson and Hedley,1992) It was not known if there was any aquatic life in the test beds as, apart from the initial water from the main beds, all water had been from a mains supply or from rainfall. If microscopic organisms were present it was not known if TEG had any effect on aquatic life and certain organisms are considered to be beneficial in the breakdown of organic waste, e.g. the presence of ciliated species would be advantageous should water from the Klargestor be passed through the beds as ciliates are particularly effective at destroying *Escherichia coli*. (Decamp,1999). An investigation was therefore carried out to find the effects of TEG on microscopic aquatic organisms.

6.2.2. Method

To ensure representative sampling the samples were taken using 5 ml. disposable pipettes and flushing the water in and out of the pipette several times so as to disturb the sediment. The test bed samples taken to the lab for GC analysis were also used for micro organism examination while separate samples from the control beds were taken in the same way. The samples were allowed to settle prior to GC analysis but agitated again for microscopic examination. One drop of the agitated sample was placed on a glass slide which was covered with a glass slip. Ten separate fields were examined of

each sample and the species present, numbers of each species and relative activity of the species were noted. The species were identified using 'Free-Living Freshwater Protozoa, a colour guide', Patterson, D.J., Hedley, S., 1992.

6.2.3. Results

An example of the results of one survey is shown in table 6.2.1

When the samples were examined under a microscope the difference between the samples from the control beds and the test beds was notable. The control bed populations were limited in diversity, numbers of individuals and levels of activity, whereas the samples from the control beds, in many cases, had a greater number of species and individuals that were extremely active. However, it was not possible, in the time available, to find a method to quantify diversity, abundance and activity. The protozoa found in the samples from the test beds were so active that it was impossible to either identify all species present or count individuals. An attempt to fix and stain the samples was unsuccessful as the dead protozoa tended to congregate and obviously their activity could not be noted. When designing the experimental procedure it had been decided to view 10 fields per slide but when samples from the test beds were actually examined it was found that the numbers and rate of movement were too great for the protozoa to be accurately counted.

There are many methods set out for assessing water quality but most are based on 'ordinary pollution' not pollution by glycol so further work would be needed to provide a method that could be used as some form of quantitative 'bio indicator' of the 'health' of the micro-organism community in situations used for the disposal of waste hydrocarbons.

Table 6.2. Results of Protozoa search on water samples from the test beds, 26/04/02

Treated beds	Species found	Activity	Untreated beds	Species found	Activity
1	<i>Gymnodium, diatoms (long blunt)</i>	Low	3	<i>Gymnodium, Colpidium, Paramecium caudate, Phaslodon, Spirotomum</i>	High
2	<i>Gymnodium, Spirogyra, diatoms</i>	Low	5	<i>Gymnodium, Colpidium, Ceratiomyxa, Paramecium caudata</i>	High
4	<i>Gymnodium, Colpidium, Diatoms; navicula, long blunt</i>	Low	9	<i>Gymnodium, Colpidium, Paramecium caudate, Spirostomum</i>	Very High
6	<i>Gymnodium, Diatom navicula</i>	Low	10	<i>Gymnodium, Colpidium, Paramecium caudate, Ceratiomyxa</i>	Very High
7	<i>Colpidium, Diatom navicula</i>	Low	11	<i>Gymnodium, Colpidium, Paramecium caudate</i>	Very High
8	<i>Colpidium,, Diatom navicula</i>	Low	12	<i>Gymnodium, Colpidium, Paramecium caudate</i>	Very High

6.2.4. Discussion.

It proved impossible to put a figure on the numbers of individuals present in the samples taken from the control and treated beds and as many of the species were so active it was not possible to quantify diversity. Nevertheless, observations made during the investigation indicate that TEG had no detrimental effect on these organisms – rather the reverse.

Many of the protozoa identified are mentioned in Patterson and Hedley, 1992, as being species more often found in organically rich environments. Most species of protozoa are also bacterivores and the presence of high numbers of very active individuals indicates a ready supply of food i.e. bacteria. Many of the species that were identified were ciliates such as *spp. Paramecium, Holosticha, and Colpidium*. These are particularly effective against *Escherichia coli* (Decamp, O., 1999). A healthy ciliate population would be an added safeguard when the contents of the Klargestar are passed through the reed beds as their presence would help to ensure a more efficient *Escherichia coli* removal in the beds.

7.0 General discussion

The original aims of the project were:-

- 1) To find a quick, easy and reliable means of measuring the level of TEG or the BOD in the effluent, that could be used by plant personnel.
- 2) To determine whether TEG can be biodegraded when passed through an rbts
- 3) If TEG can be biodegraded in the rbts what effect will the action have on the reeds.
- 4) To find the highest concentration that could be tolerated by the reeds without adverse effect.
- 5) To determine what effect, if any, the mixing of TEG with sewage would have on the reeds and reedbed efficiency.
- 6) To try and provide some indication as to the time needed to biodegrade an effluent of a particular concentration in the existing beds to levels within the parameters set by the EA.

Most of the aims of the project have been achieved satisfactorily with a few minor exceptions.

The development of the laboratory based GC method for the determination of low levels of DEG and TEG produced reliable results. The chromatograph used was old and temperamental but it still managed to give accurate reproducible results. If the same procedure was transferred to a modern instrument it would be possible to reduce the time required for analysis. Unfortunately, because the CATS terminal is a gas terminal no sources of ignition are allowed on site so it would not be possible to use a GC for site analysis. This was known before the start of the project but the GC

method was the most suitable for the accurate analysis needed in the lab. The GC method could be used for the detection of TEG in other environmental samples such as contaminated soils or waterways.

The EZBOD portable BOD meter was not suitable for use on the effluent at CATS. It was designed for use on waste treatment sites that used activated sludge in the process. The suggested alternative method, suggested by Biosciences Inc., that involved the making up of an artificial activated sludge did not work. The Biotector from CSIP provided fast accurate reproducible results for the total organic content of the samples. With suitable programming the TOC figures can easily be related to the parameters set by the EA. Wilson (1997) found that the use of TOC for the determination of the efficiency of a rotating biological contactor (RBC) treatment system was an accurate and reliable system. By using the Biotector for spot samples it would be possible for the operators at CATS to monitor the performance of the Klargester system (an RBC system) as well as the input and output of the constructed reed beds.

The TEG was biodegraded when passed through the test reed beds and exhibited the exponential decay curve of a first order reaction. The higher the concentration of TEG that was put in, the faster the initial rate of degradation was. As the concentration decreased so did the reaction rate. Concentrations from 0.1%v/v TEG up to 5%v/v TEG were passed through the test beds. The lower concentrations were slow to degrade but the highest concentration, 5%, started off at a high rate but because of the appearance of an iron mould and the deposition of iron salts on the rhizosphere the trial never actually reached the low levels achieved by the others. The iron appeared to have come from some iron fittings used on the superstructure supporting the 12

test beds. Work by Batty and Younger (2003) found that iron concentrations of up to 1 mg/l didn't seem to harm the rhizosphere of reed seedlings, but any higher and the reeds suffered. The amount on the test reeds was enough to cause them some stress and they appeared to be dying. By draining the tanks, allowing the contents to dry and then refilling with fresh water, the iron deposits became dry and brittle and fell off the stems etc. The deposits could well have hampered the bacteria, making it difficult for them to act on the TEG and hampering their efforts at getting enough oxygen to enable them to perform the degradation process.

The addition of TEG to the test beds resulted in an increase in the number of shoots in the treated bed, an increase in the biomass produced with the stomatal density increasing and the total Kjeldhal nitrogen being significantly higher than the reeds from the untreated control beds. It can be argued that the control beds did not receive any form of nutrients but as the constructed beds had not received anything for several years it was decided not to add anything to the controls either. The addition of any nutrients to the controls would have meant that the same addition had to be made to the test beds which may have altered the reactions of the reeds to the TEG. The changes in the reeds in the test beds indicated that the reeds were actually benefiting from the presence of either the TEG or the results of the bacterial degradation. According to Graves and Reavey (1996) and Penuelas and Matamala (1990) and others, an increase in CO₂ levels results in a decrease in stomatal density but Royer states that the reductions recorded are in fact from long term data and that in the short term an increase in stomatal density may occur.

The degradation rate of the TEG was that of a first order reaction with the degradation rate being high at high concentrations and decreasing as the level of contamination decreases. Chong *et al* (1999) found that shock dosing of de icing glycols resulted in

higher removal rates whilst Strong-Gunderson *et al* found that ethylene glycol at 10% concentration degraded quicker than 1 – 5 % solutions. The results of this project agree. When the equation was used to calculate the removal rate over the 12 trials the mean (n=12) figure arrived at was 0.1, which is the value of the rate constant accepted in the UK.(Cooper, 1996)

By reworking the equation to

$$\text{Time to degrade to target} = \frac{(\text{Concentration in} - \text{Concentration out})}{(\text{area of bed}) \times (\text{Removal rate/area})}$$

it is possible to calculate how long it would take for an effluent 'batch' to be biodegraded to the level of the consent to discharge issued by the EA. The mixing of the Klargest material with retention pond water was not fully investigated as the plant process operators started to pass the material through the constructed beds without notification. The main beds had dealt with site effluent several years earlier and it had been shown that the mixture could be passed through the test beds without any problems so it was taken that it would work (operator decision).

The use of reed beds for water treatment has until recently been confined to the treatment of wastewater from small communities or sites producing organic waste, not particularly organic chemical waste. TEG is a fairly simple hydrocarbon consisting only of carbon, hydrogen and oxygen but the outcome was not definite until the trials had been conducted. The exponential degradation bears a similarity to the findings of Nguyen (2000) who noted that microbial respiration rates decreased with decreasing concentration as the samples became further away from the start of the bed.

The bacterial activity on the TEG produced a 'knock on' effect to the microscopic community of the beds. The protozoan communities of water are already used as bioindicators and Decamp (2000) found that even protozoan ciliates showed a greater

abundance in the first third of each bed examined. Using this information it appears that not only does the majority of fast degradation take place in the first part of the beds but also the majority of the microbial respiration and the greater number of protozoan ciliates can be found there.

The biomass of the reeds increased with the addition of TEG as did the shoot density and if this is repeated in the established beds then they will provide more shelter and food for resident wildlife. L. Batty (2005) pointed out that micro organisms in mine water and around disused mine sites are part of 'key biogeochemical cycles' and if encouraged they could start further cycles. By increasing the bacterial population in the beds the micro organism population has increased which could hopefully help to boost the food chain. The provision of more food and habitat will not necessarily increase the biodiversity of the area but it could help to increase the number in the existing populations.

It was hoped that it would be possible to detect the possible route that degradation took but from the analysis it appears that a small amount of TEG breaks down to DEG which itself rapidly breaks down to water and carbon dioxide, whilst the majority of the TEG breaks down directly to water and CO₂. There were no signs of any organic acids or alcohols on the GC traces so it can only be assumed that there are no other steps involved.

8. Conclusion

The easiest and most accurate system of monitoring the effluent in the retention pond and reed beds would be the Total Carbon Analyser and it is suggested that effluent is not sent down to the reed beds if the carbon content is over 2000 mg/l TOC or approximately 1%v/v TEG. The problems experienced in the test beds were thought to be caused by a combination of rusting clamps, iron mould and weed killer but it cannot be said that definitely was the case.

The biodegradation of Triethylene glycol when passed through a horizontal reed bed treatment system occurs as a first order reaction and follows the accepted equation (Cooper, 1996) and has a mean rate constant of 0.1. It has been possible to rework the equation to allow the process operators to calculate the time required for an effluent of known strength, either % TEG, THOD or BOD₅, to reach the parameters set by the Environment Agency.

$$\text{Time to degrade to target} = \frac{(\text{Concentration in} - \text{Concentration out})}{(\text{area of bed}) \times (\text{Removal rate/area})}$$

The adoption of this system at CATS has resulted in all the liquid waste from the site being 'cleaned' before it is discharged to the watercourse. Waste water is no longer transported by road tankers reducing carbon emissions and costs. The biodegradation of TEG in a reed bed can be said to be beneficial to both the reeds and the micro organisms in the water in the beds and it is hoped that once the system becomes established the reed bed community will flourish. By establishing that the biodegradation of TEG follows the above equation it will be possible to apply it to other sites looking for a means of disposing of TEG by environmentally friendly means.

References

- Armstrong, W., Cousins, D., Armstrong, J., Turner, D. W., Beckett, P. M., 2000, **Oxygen distribution in wetland plant roots and permeability barriers to gas exchange with the rhizosphere: a micro electrode and modelling study with *Phragmites australis***. Annals of Botany, 86: (03) 687-703, Sept. 2000, Academic Press ltd, London,
IDS 0305-7364
- Armstrong, J., Armstrong, W., 1998, ***Phragmites* die-back: toxic effects of propionic, butyric and caproic acids in relation to pH**, New Phytologist, 142,pp 201-217
- Armstrong, J., Afreen-zobayed, F., Blyth, S., Armstrong, W., 1999, ***Phragmites australis*: Effects of shoot submergence on seedling growth and survival and radial oxygen loss from roots**, Aquatic Botany, vol. 64, issue 3 - 4, pp 275 - 289, Elsevier Science B.V
- Baird, A.J., Wilby, R. L, eds., 1999, **ECO-HYDROLOGY, Plants and water in terrestrial and aquatic environments**, Routledge, London and New York pp133
- Bart, D., Hartmann, J. M., 2000, **Environmental determinants of *Phragmites australis* expansion in a New Jersey salt marsh: an experimental approach**, OIKOS, vol 89, issue 1, 2000, pp 59-69. Elsevier Science B.V.

Batty, L. C., 2005, **The Potential Importance of Mine Sites for Biodiversity**. Mine Water and the Environment (2005)24: 101 – 103. IMWA Springer-Verlag.

Batty, L. C., Younger, P. L., 2003, **Effects of External Iron Concentration upon Seedling Growth and Uptake of Fe and Phosphate by the Common Reed, *Phragmites australis* (Cav.) Trin ex. Steudel**. Annals of Botany 92: 801-806, 2003
Annals of Botany Company

Bell, N., Unpublished dissertation, 2002, University of Durham.

Brix, H., Sorrell, B.K., Lorenzen, B., 2001, **Are *Phragmites* – dominated wetlands a net source or net sink of greenhouse gases?**, Aquatic Botany, Vol. 69, issues 2-4, pp 313-324. Elsevier Science B.V.

Cerezo, R. G., Suarez, M. L., Vidal-Abaraca, M. R., 2001, **The performance of a multi-stage system of constructed wetlands for urban wastewater treatment in a semiarid region of SE Spain**. Ecological Engineering, vol.16, issue 4, pp 501-517

Chong, S., Garelick, H., Revitt, D. M., Shutes, R. B. E., Worrall, P., Brewer, D., 1999, **The microbiology associated with glycol removal in constructed wetlands**, Water Science and Technology, vol. 40, issue 3, 1999, pp 99-107, Elsevier Science B.V.

Cizakova, H., Bauer, V., 1998, **Rhizome respiration of *Phragmites australis*: effect of rhizome age, temperature and nutrient status of the habitat**. Aquatic Botany, vol. 64, issue 4, pp 239-253, Elsevier Science B.V.

Cizkova, H., Brix, H., Kopecky, J., Lukavska, J., 1999, **Organic acids in the sediments of wetlands dominated by *Phragmites australis*:evidence of phytotoxic concentrations.** Aquatic Botany, vol. 64, issue 3 - 4, pp 303 - 315, Elsevier Science B.V

Cooper, P.F., Job, G.D., Green, M.B., Shutes, R.B.E., 1996, **Reed Beds and constructed wetlands for wastewater treatment,** W.R.C. Swindon, Swindon, Wiltshire

Cossu, R., Haarstad, K., Lavagnolo, M.C., Littarru, P., 2001, **Removal of municipal solid waste COD and NH₄-N by phyto-reduction: A laboratory-scale comparison of terrestrial and aquatic species at different organic loads.** Ecological Engineering, vol. 16, issues 4, pp 459-470

Decamp, O., Warren, A., Sanchez, R., 1999, **The role of ciliated protozoa in subsurface flow wetlands and their potential as bioindicators,** Water Science and Technology, vol. 40, issue 3, 1999, pp 91-98, Elsevier Science B.V.

Decamp, O., Warren, A., 2000, **Investigation of *Escherichia coli* removal in various designs of subsurface flow wetlands used for wastewater treatment,** Ecological Engineering, vol.14, issue 3, pp 293-299

Department for the Environment, Farming and Rural Affairs,2005,

<http://www.defra.gov.uk/environment/waste/index.htm>

Drizo, A., Frost, C. A., Grace, J., Smith, K. A.,2000, **Phosphate and ammonium distribution in a pilot scale constructed wetland with horizontal subsurface flow using slate as a substrate.** Water research 34 (9) 2483-2490

Edstrom Industries, 2003, **Iron and iron bacteria in water**, Edstrom Industries, Inc., 819 Bakke Ave., Waterford, WI

Environment Agency,2005 , www.environment-agency.gov.uk/

Environment Agency, 2005,**Waste-landfill**, (http://www.environment-agency.gov.uk/yourenv/eff/1190084/resources_waste/213982/207743/?version=1&lang=_e)

Graves, J, Reavey, D, 1996, **Global Environmental Change**, Longman Group Ltd., Harlow, Uk. ISBN 0-582-21873-X. pp 28-38.

Greenway, M., Woolley, A., 1999, **Constructed wetlands in Queensland: Performance efficiency and nutrient bioaccumulation.** Ecological Engineering , vol. 12, issues 1-2, pp 39-55

Griffin, P., Pamplin, C., 1998, **The advantages of a constructed reedbed based strategy for small sewage treatment works.** Water science and technology, 1998, vol. 38, no. 3, pp 143-150. , Elsevier Science B.V.

Grunfeld, S., Brix, H., 1999, **Methanogenesis and methane emissions: effects of water table, substrate type and presence of *Phragmites australis*,** Aquatic Botany, vol. 64, issue 1, pp 63 - 75, Elsevier Science B.V

Hanks,P., Ed., **Collins Dictionary of the English Language, 2nd ed.** Reprint 1989, Collins, London and Glasgow.

Hawkes, C.J., Jose, P.V., 1995, **Reedbed management for commercial and wildlife interests.,** , Royal Society for the Protection of Birds, Sandy, Beds.
ISBN 0 9031 38 816

Horan, N. J., 1991, **Biological wastewater treatment systems, theory and operation,** John Wiley & Sons.

Johnson, K. D., Martin, C.D., Davis, T. G., 1999, **Treatment of wastewater effluent from a natural gas compressor station.** Water Science and Technology, vol. 40, issue 3, 1999, pp 51-56, Elsevier Science B.V.

Jiang, Z., Xinyuan, Z., 1998, **Treatment and utilisation of wastewater in the Beijing Zoo by an aquatic macrophyte system.**
Ecological Engineering, vol.11, issues 1-4, pp 101-110

Kern, J., Idler, C., 1999, **Treatment of domestic and agricultural wastewater by reedbed systems**, Ecological Engineering, vol.12, issues 1-2, pp 13-25.]

Kominkova, D., Kuehn, K. A., Busing, N., Steiner, D., Gessner, M. O., 2000, **Microbial biomass, growth and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake**, Aquatic Microbial Ecology, 22: (3) 271-282 , Oct 2000, IDS 374NR ISSN 0948-3055

Lawson, G.J., Cooper, P. F., 1989, **Reedbed treatment of sewage**, Acta hydrochim. Hydrobiol., vol. 17, pp190-198, Institute of terrestrial ecology, Grange-over-sands, Cumbria, water research centre, Elder Way, Stevenage, Herts.

Maehlum, T., Stalnacke, P., 1999, **Removal efficiency of three cold-climate constructed wetlands treating domestic wastewater: Effects of temperature, seasons, loading rates and input concentrations**. Water Science and Technology, vol. 40, issue 3, 1999, pp 273-281, Elsevier Science B.V.

Masi, F. Martinuzzi, N., Loiselle, S., Peruzzi, P. Bacci, M., 1999, **The tertiary treatment pilot plant of publisher SPA (Florence, Tuscany): a multistage experience**, Water science and technology, vol. 40 issue 3, pp 195 - 202, Elsevier Science B.V

Merritt, A., 1994, **Wetlands, Industry and Wildlife; a manual of principles and Practices**, The Wildfowl and wetlands Trust.

Mitsch, W. J., Jorgensen, S. E., eds, 1989, **Ecological Engineering, an introduction to ecotechnology**, Wiley-Interscience, New York, Chichester, Brisbane, Toronto, Singapore pp 235 ref to portable test unit used by Burris *et al*, Burris, J.E., Gerber, D.W.,

Newman, J. M., Clausen, J.C., Neafsey, J. A., 1999, **Seasonal performance of a wetland constructed to process dairy milkhouse wastewater in Connecticut**, Ecological Engineering, vol. 14, issues 1-2, pp 181-198

Nguyen, L., 2000, **Organic matter composition, microbial biomass and microbial activity in gravel-bed constructed wetlands treating farm dairy wastewaters**. Ecological Engineering , vol.16, issue 2, pp 199-221

Patterson,D.J., Hedley, S., 1992, **Free-living freshwater protozoa**, Wolfe publishing, London. ISBN 0-7234-1683-4

Qianxin, L., Mendelsohn, I. A.,1998, **The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands**. Ecological Engineering, vol. 10, issues 3, pp 263-274

Revitt, D.M., Shutes, R.B.E., Llewellyn, N. R., Worrall, P., 1997, **Experimental reedbed systems for the treatment of airport runoff.**

Water science and technology, , vol. 36, no. 8-9, pp 385-390. , Elsevier Science B.V.

Rolletschek, H., Hartzendorf, T., Rolletschek, A., Kohl, J, 1999,

Biometric variation in *Phragmites australis* affecting convective ventilation and amino acid metabolism, Aquatic Botany, vol. 64 issues 3-4, Sept. 1999, pp 291 – 302, Elsevier Science B.V.

Ruifeng Li, Chuanchen Miao, Yajing Wang, July 1998, **Purity analysis of Industrial Triethylene glycol by capillary gas chromatography**, Journal of chromatographic Science

Rushall, C., **Aspects of the biology of a constructed reedbed within herb-rich grassland**,1998, Unpublished M.Sc. Thesis, University of Durham.

Stolp, H., **Microbial Ecology: organisms, habitats, activities**, 1988, Cambridge University Press.

Strong-gunderson, J., Wheelis, S., Carroll, S., Waltz, D., Palumbo, A. V., (1995) **Degradation of high concentrations of glycols, antifreeze and deicing fluids**, Microbial processes for bioremediation, Papers edited by Hinchee, R.E., Wilson, J.T., Battelle Press.

Stubbs, K. T., Unpublished dissertation , 2000, University of Durham.

Stubbs, K. T., Unpublished report to BP, CATS Terminal, 2003.

Sun, G., Gray, K. R., Biddlestone, A. J., 1998, **Treatment of agricultural wastewater in downflow reedbeds: experimental trials and mathematical model.** Journal of agricultural engineering research, vol 69, pp63-71, Silsoe research Institute.

Tees Valley Wildlife Trust, **Tees Valley Biodiversity Action Plan**, 1999.

Todar, K., 2002, 2004, 2005, **On line Textbook of Bacteriology**, University of Wisconsin, U.S.A.

Van der Nat, F. W. A., Middleburg, J. J., 1998, **Seasonal variation in methane oxidation by the rhizosphere of *Phragmites australis* and *Scirpus lacustris*,** Aquatic Botany, vol. 61, issue 2, pp 95 – 110, Elsevier Science B.V.

Verhoeven, J.T.A., Meuleman, A. F. M., 1999, **Wetlands for wastewater treatment: Opportunities and limitations.** Ecological Engineering, vol. 12, issues 1-2, pp5-12

Vymazal, J., 1999, **Removal of BOD₅ in constructed wetlands with horizontal sub – surface flow; Czech experience,** Water Science and Technology, vol. 40, issue 3, 1999, pp133 - 138, Elsevier Science B.V.

Vymazal, J., 2005, **Horizontal sub surface flow and hybrid constructed wetlands systems for wastewater treatment.** Ecological Engineering, Vol. 25, Issue 5, pp 478-490. Elsevier Science B. V.

Wood, B., McAtamney, C., 1994, **The use of macrophytes in bioremediation.**

Biotechnology Advances, vol. 12, pp 653-662. Elsevier Science B.V.

Zheng, W. J., Zheng, X. P., Zhang, C. L., 2000, **A survey of photosynthetic carbon metabolism in 4 ecotypes of *Phragmites australis* in North West China. Leaf anatomy, ultrastructure and activities of ribulose 1,5-biphosphate carboxylase and glycollate oxidase.** Physiologia Plantarium 110: (2) 201-208 Oct. 2000,

Munksgaard Int. Publishing Ltd., Copenhagen

Appendix

Method for Biological (Biochemical)Oxygen Demand

Biochemical Oxygen Demand

Carefully remove the stopper from the bottle containing the sample and add, using a pipette, 2.0 cm³ of the manganese II sulphate solution followed by 2.0 cm³ of the alkaline iodide-azide solution, both just below the surface of the water. Carefully replace the stopper (avoiding the inclusion of air bubbles) and mix thoroughly by repeated vigorous inversion and swirling.

Allow the precipitate to settle to the lower third of the bottle and repeat the mixing. This allows the precipitate to settle completely. A clear upper liquid should be obtained. (With salt water the precipitate settles more slowly) Settling may be improved by rotating the bottle carefully during mixing to blend the two reagents before shaking vigorously.

Carefully remove the stopper and add 4.0 cm³ 50 % v/v sulphuric acid (CORROSIVE). Replace the stopper and thoroughly mix the contents by rotation or gentle inversion. (The precipitate should dissolve almost immediately. If it does not dissolve after standing for a few minutes repeat the mixing. If absolutely necessary add a few more drops sulphuric acid and mix again)

Pipette 100 cm³ of the solution into a conical flask and titrate with 0.0125M sodium thioisulphate solution until the solution is pale straw coloured. Add 2 cm³ starch indicator solution and continue the titration to the first disappearance of blue colour. Record the volume. Repeat titration with a second sample. (Ignore the reappearance of the blue colour in the titrated solution on standing.)

Results

Calculate the dissolved oxygen content from

$$\text{Dissolved oxygen content} = \frac{V_1 \times 0.100 \times 1000 \times F}{V_2} \text{ mg dm}^{-3}$$

V_1 = volume, in cm³, of 0.0125 M sodium thiosulphate used

V_2 = volume, in cm³, of sample titrated

F is the dilution factor caused by addition of reagents to the sample

$$F = \frac{V_3}{V_3 - V_4}$$

V_3 = volume in cm³ of sampling bottle, V_4 = 4 cm³ (reagents added in first step)

Do NOT pour your solutions down the sink, ask about disposal

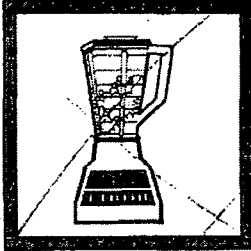
Ref:HMSO - Dissolved Oxygen in Natural and Waste Waters 1979 Version.

OXYGEN DEMAND, CHEMICAL (COD)

For water and wastewater

Dichromate Reflux Method*; USEPA approved for reporting**

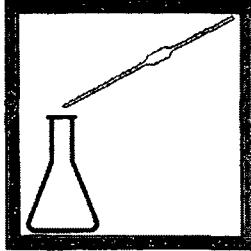
DIGESTION



1. Homogenize 500 mL of sample for two minutes in a blender.

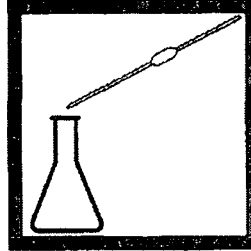
Note: Blending ensures distribution of solids and improves accuracy and reproducibility.

Note: If samples cannot be analyzed immediately, see Sampling and Storage following these steps.

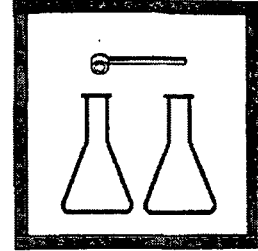


2. Pipet the appropriate sample volume and deionized water volume from Table 1 into a digestion flask to make the prepared sample.

Note: A reflux condenser and a 125-mL flask with 24/40 ground-glass joints are used for the reaction. Do not grease the glass joints, as this could contaminate the sample.



3. Pipet 10.00 mL of deionized water into a second digestion flask to make the reagent blank.



4. Add one heaping 0.2-g spoonful of COD Catalyst Powder and a few glass beads to each flask. Swirl to mix.

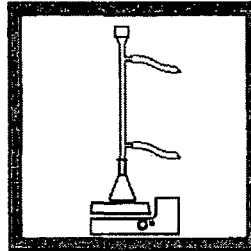
COD Range (mg/L as O ₂)	Sample Volume (mL)	Deionized Water (mL)	Multiplier
0-800	10	0	1
800-1600	5	5	2
1600-4000	2	8	5
4000-8000	1	9	10



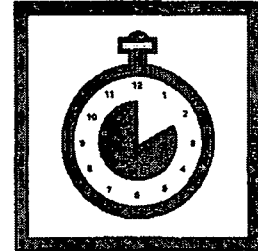
5. Pipet 5.00 mL of 0.250 N Potassium Dichromate Standard Solution into each flask



6. Measure 15.0 mL of sulfuric acid slowly into each flask. Swirl to mix thoroughly while adding



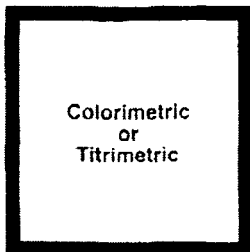
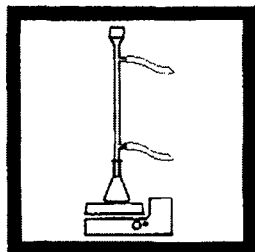
7. Attach a reflux condenser to each flask. Place each flask on a hot plate or over a flame.



8. Gently boil the solution for two hours.

Note: A pure green color in the

OXYGEN DEMAND, CHEMICAL, continued



9. Wait for the solutions to cool to room temperature with the reflux condensers attached.

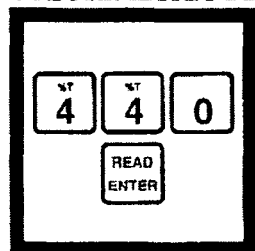
Note: Upon cooling, some of the catalyst may precipitate. When doing colorimetric determinations, wait until the particles settle before measuring the color.

10. Use one of the following analytical techniques to determine the sample concentration:

- Colorimetric determination
- Buret titration

COLORIMETRIC DETERMINATION

Method 8230



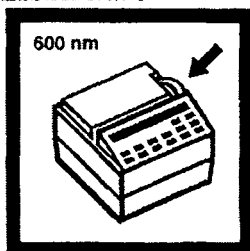
1. Enter the stored program number for chemical oxygen demand (COD)-reflux method.

Press: **4 4 0 READ/ENTER**

The display will show:
DIAL nm TO 600

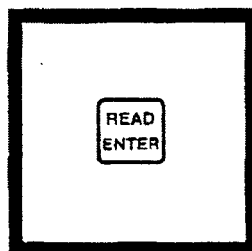
Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.



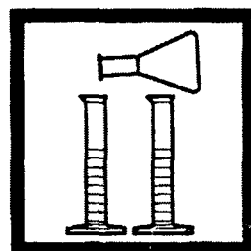
2. Rotate the wavelength dial until the small display shows:

600 nm

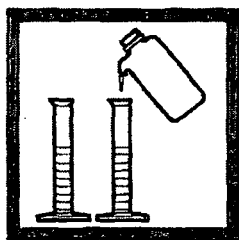


3. Press: **READ/ENTER**

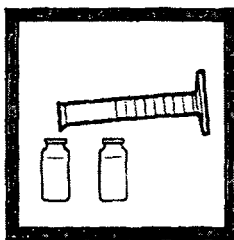
The display will show:
mg/l COD Reflux



4. Pour the contents of each flask into a dry 50-mL graduated cylinder.

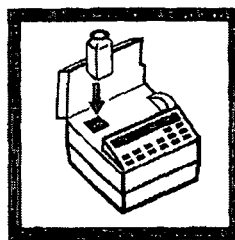


5. Adjust the volume to 29 mL, if necessary, with deionized water.

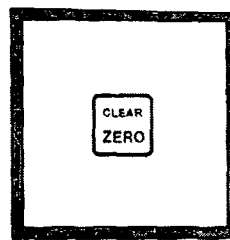


6. Pour the solutions into dry sample cells.

Note: The Pour-Thru Cell cannot be used with this procedure.



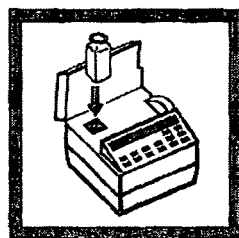
7. Place the reagent blank into the cell holder. Close the light shield.



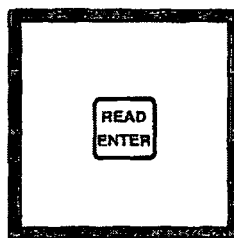
8. Press: **ZERO**

The display will show:
WAIT

then:
0. mg/l COD Reflux



9. Place the prepared sample into the cell holder. Close the light shield.



10. Press: **READ/ENTER**

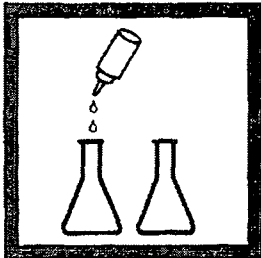
The display will show:
WAIT
then the result in mg/L
COD will be displayed.

Note: In the constant-on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

OXYGEN DEMAND, CHEMICAL, continued

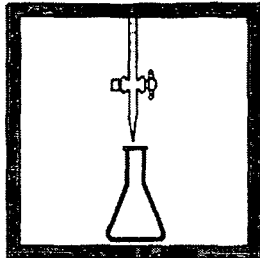
Method 8116

BURET TITRATION



1. When the flasks have cooled, rinse the inside of the condenser with a small amount of deionized water, remove the condenser from the flask. Add two drops of Ferroin Indicator to each flask.

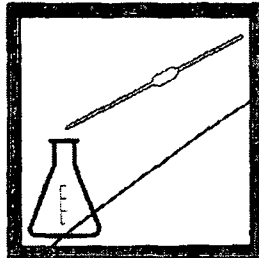
Note: If the solution color changes from blue-green to orange-brown, the COD value is out of range. Repeat the digestion with a smaller sample volume plus the deionized water specified in Table 1.



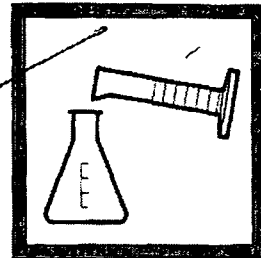
2. Titrate the solutions with 0.0625 N Ferrous Ammonium Sulfate Standard Solution (FAS) until the color changes sharply from blue-green to orange-brown. Record the number of mL of titrant required.

Note: The mL required for the prepared sample is value B. The mL required for the reagent blank is value A. Use the values in Step 8.

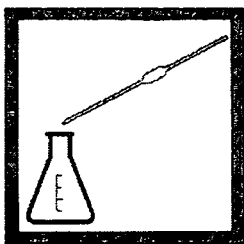
Note: Steps 3 through 7 need only be done as required because the FAS deteriorates over time.



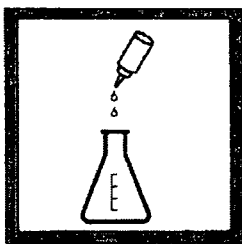
3. To make the required standard solution for titration in Step 7, pipet 5.00 mL of 0.250 N Potassium Dichromate Standard Solution into a 125-mL erlenmeyer flask.



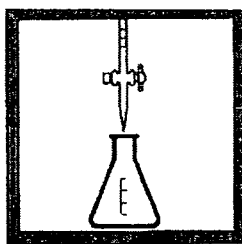
4. Add 30 to 50 mL of deionized water to the flask.



5. Add 15 mL of concentrated sulfuric acid slowly to the flask, swirling continually.



6. Add two drops of Ferroin Indicator Solution to the flask.



7. Titrate the standard solution with 0.0625 N Ferrous Ammonium Sulfate Standard Solution until the sample changes sharply from blue-green to orange-brown. Record the mL of titrant required.

Note: This is value C in the following equation.

$$(A - B) \times \frac{1000}{C} \times M = \text{mg/L COD}$$

8. Determine the mg/L COD according to the following equation:

$$(A - B) \times \frac{1000}{C} \times M = \text{mg/L COD}$$

Where:

- A = mL used in titration of reagent blank
- B = mL used in titration of prepared sample
- C = mL used in titration of standard solution in Step 7
- M = Multiplier from Table 1

For example when using a 10-mL sample volume:

- A = 19.75 mL
- B = 10.00 mL
- C = 20.00 mL
- M = 1

$$\text{COD as mg/L O}_2 = \frac{(19.75 - 10.00) \times 1000 \times 1}{20.00} = 487.5$$

SAMPLING AND STORAGE

Collect samples in glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples treated with sulfuric acid to make the pH less than 2 (at least 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days. Correct results for volume additions; see Sampling and Storage, Volume Additions, (Section I) for more information.

ACCURACY CHECK

Standard Solution Method

Check accuracy by using 10.00 mL of 300-mg/L or 5.00 mL of 1000-mg/L COD Standard Solution as sample.

Or, prepare a 500 mg/L standard solution by dissolving 425 mg of dried (120 °C overnight) potassium acid phthalate and diluting to 1 liter with deionized water.

PRECISION FOR COLORIMETRIC DETERMINATION

In a single laboratory, using a standard solution of 500 mg/L COD and one representative lot of reagent with the DR/2000, a single operator obtained a standard deviation of ±6 mg/L COD.

INTERFERENCES

The COD Catalyst Powder contains mercuric sulfate to complex up to 1000 mg/L chloride. For higher chloride concentration, dilute the sample so that the chloride concentration is less than 1000 mg/L.

One way ANOVA analysis of Temperature readings, all 6 trials

Concentration codes

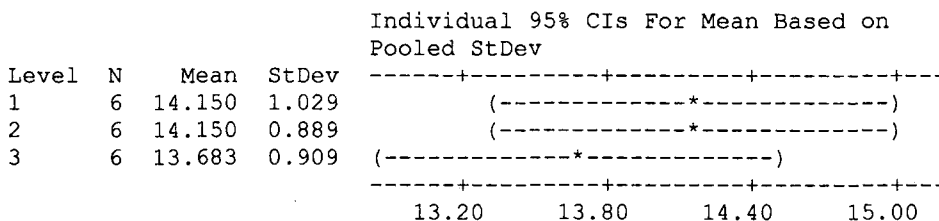
Control	1
0.1% TEG	2
0.5 % TEG	3
1.0% TEG	4
0.2% TEG	5
5.0 % TEG	6

Results for: trial 1

One-way ANOVA: Temperature versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	0.871	0.436	0.49	0.623
Error	15	13.378	0.892		
Total	17	14.249			

S = 0.9444 R-Sq = 6.11% R-Sq(adj) = 0.00%



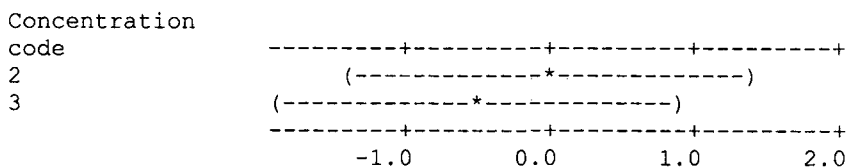
Pooled StDev = 0.944

**Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code**

Individual confidence level = 97.97%

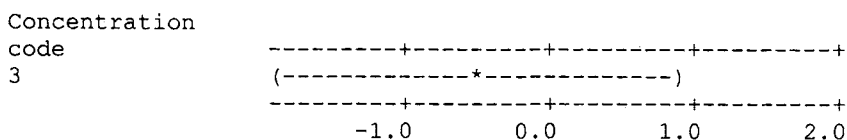
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
2	-1.4150	0.0000	1.4150
3	-1.8816	-0.4667	0.9483



Concentration code = 2 subtracted from:

Concentration code	Lower	Center	Upper
3	-1.8816	-0.4667	0.9483

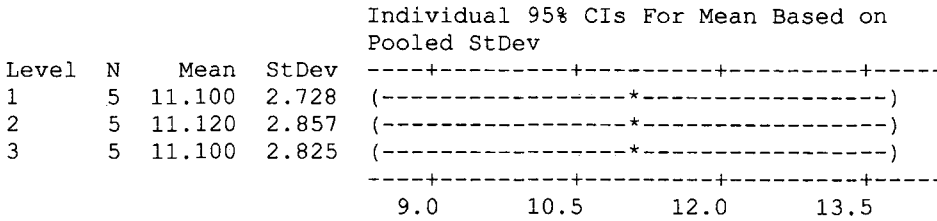


Results for: trial 2

One-way ANOVA: Temperature versus concentration code

Source	DF	SS	MS	F	P
concentration co	2	0.00	0.00	0.00	1.000
Error	12	94.33	7.86		
Total	14	94.33			

S = 2.804 R-Sq = 0.00% R-Sq(adj) = 0.00%



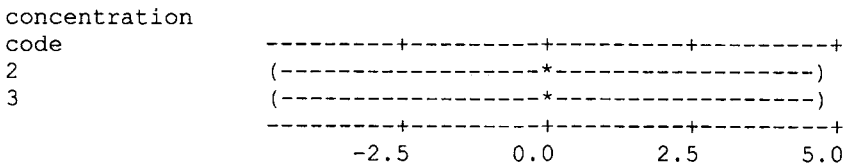
Pooled StDev = 2.804

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of concentration code

Individual confidence level = 97.94%

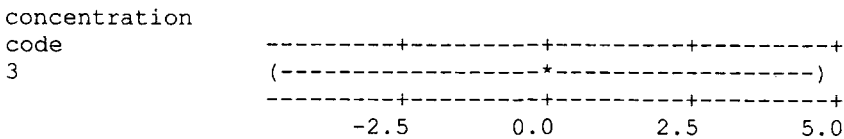
concentration code = 1 subtracted from:

concentration code	Lower	Center	Upper
2	-4.707	0.020	4.747
3	-4.727	-0.000	4.727



concentration code = 2 subtracted from:

concentration code	Lower	Center	Upper
3	-4.747	-0.020	4.707

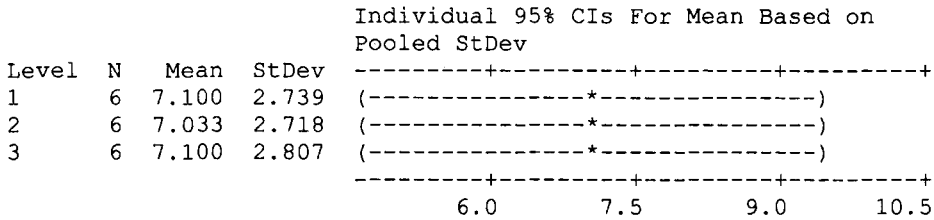


Results for: Trial 3

One-way ANOVA: temperature versus concentration code

Source	DF	SS	MS	F	P
concentration co	2	0.02	0.01	0.00	0.999
Error	15	113.85	7.59		
Total	17	113.87			

S = 2.755 R-Sq = 0.02% R-Sq(adj) = 0.00%



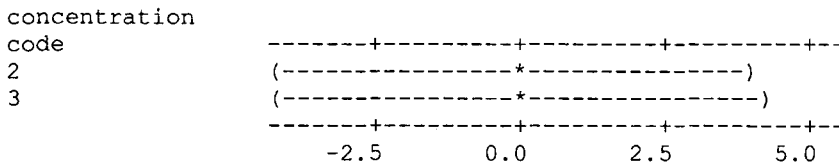
Pooled StDev = 2.755

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of concentration code

Individual confidence level = 97.97%

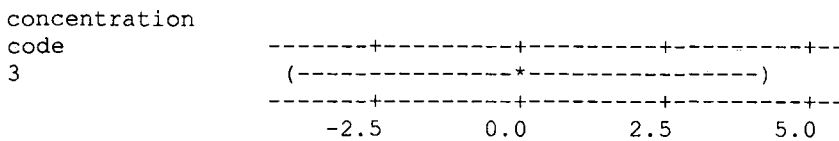
concentration code = 1 subtracted from:

concentration code	Lower	Center	Upper
2	-4.194	-0.067	4.061
3	-4.128	0.000	4.128



concentration code = 2 subtracted from:

concentration code	Lower	Center	Upper
3	-4.061	0.067	4.194

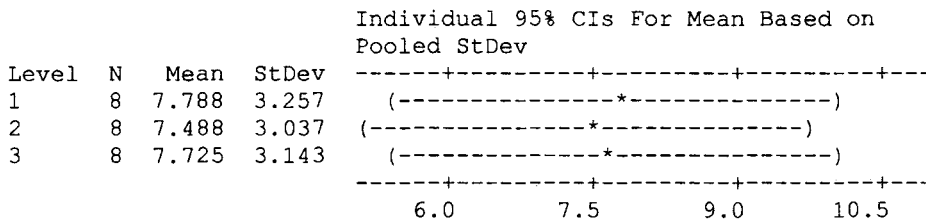


Results for: Trial 4

One-way ANOVA: Temperature versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	0.40	0.20	0.02	0.980
Error	21	207.95	9.90		
Total	23	208.35			

S = 3.147 R-Sq = 0.19% R-Sq(adj) = 0.00%



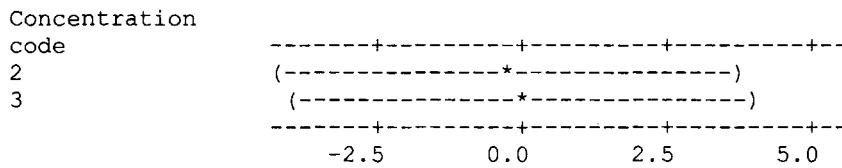
Pooled StDev = 3.147

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.00%

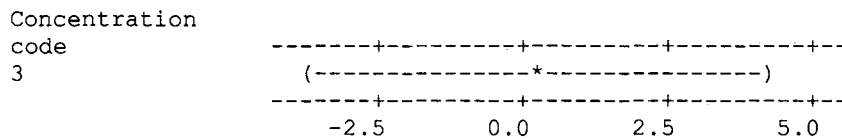
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
2	-4.261	-0.300	3.661
3	-4.023	-0.063	3.898



Concentration code = 2 subtracted from:

Concentration code	Lower	Center	Upper
3	-3.723	0.237	4.198

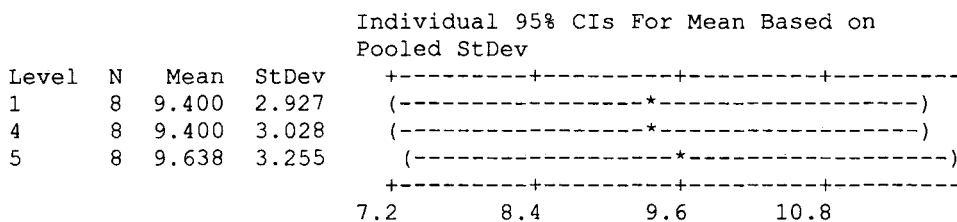


Results for: Trial 5

One-way ANOVA: Temperature versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	0.30	0.15	0.02	0.984
Error	21	198.34	9.44		
Total	23	198.64			

S = 3.073 R-Sq = 0.15% R-Sq(adj) = 0.00%



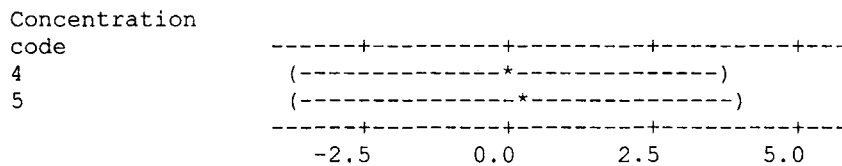
Pooled StDev = 3.073

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.00%

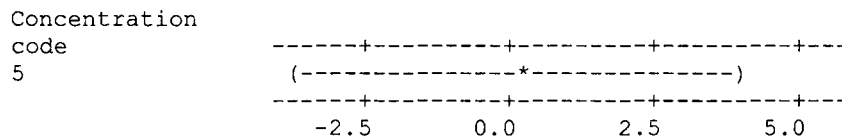
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
4	-3.868	0.000	3.868
5	-3.631	0.238	4.106



Concentration code = 4 subtracted from:

Concentration code	Lower	Center	Upper
5	-3.631	0.238	4.106

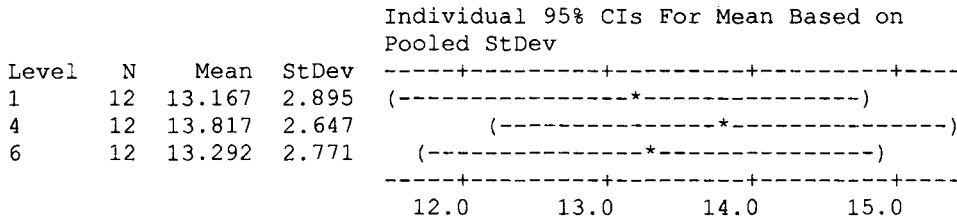


Results for: Trial 6

One-way ANOVA: Temperature versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	2.86	1.43	0.19	0.831
Error	33	253.75	7.69		
Total	35	256.61			

S = 2.773 R-Sq = 1.11% R-Sq(adj) = 0.00%



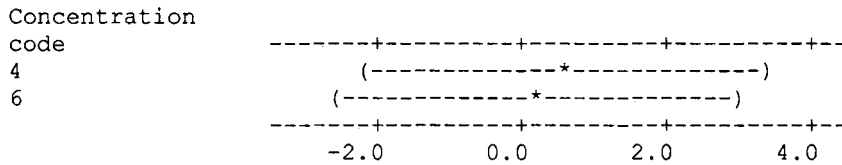
Pooled StDev = 2.773

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.04%

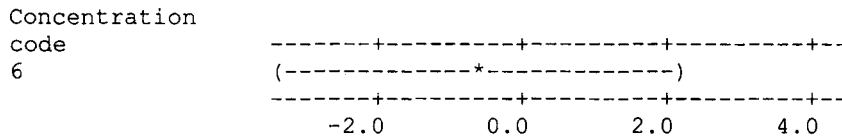
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
4	-2.128	0.650	3.428
6	-2.653	0.125	2.903



Concentration code = 4 subtracted from:

Concentration code	Lower	Center	Upper
6	-3.303	-0.525	2.253

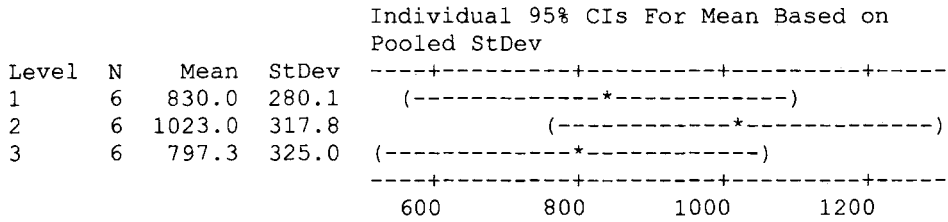


Results for: Trial 1conductivity

One-way ANOVA: conductivity versus concentration code

Source	DF	SS	MS	F	P
concentration co	2	178483	89242	0.94	0.413
Error	15	1425287	95019		
Total	17	1603770			

S = 308.3 R-Sq = 11.13% R-Sq(adj) = 0.00%



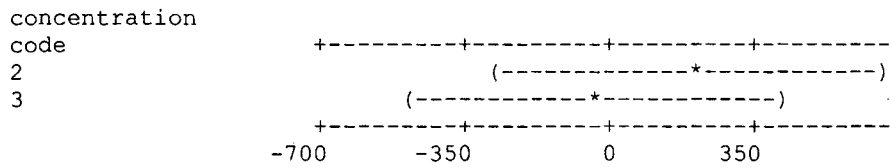
Pooled StDev = 308.3

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of concentration code

Individual confidence level = 97.97%

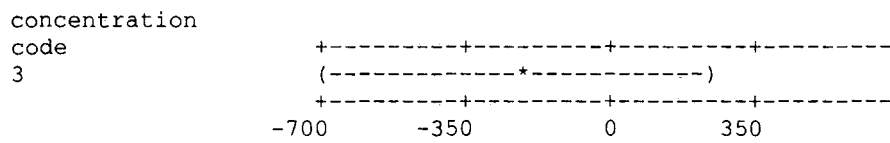
concentration code = 1 subtracted from:

concentration code	Lower	Center	Upper
2	-268.8	193.0	654.8
3	-494.5	-32.7	429.2



concentration code = 2 subtracted from:

concentration code	Lower	Center	Upper
3	-687.5	-225.7	236.2

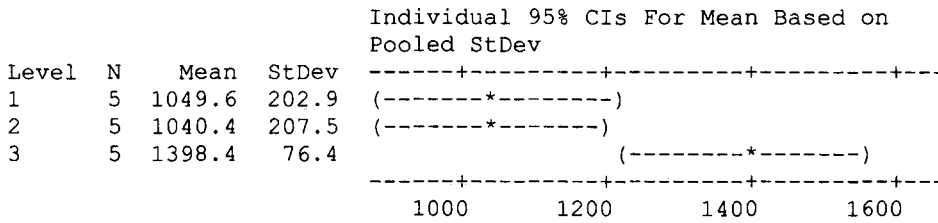


Results for: Trial 2

One-way ANOVA: Conductivity versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	416517	208258	6.94	0.010
Error	12	360180	30015		
Total	14	776696			

S = 173.2 R-Sq = 53.63% R-Sq(adj) = 45.90%



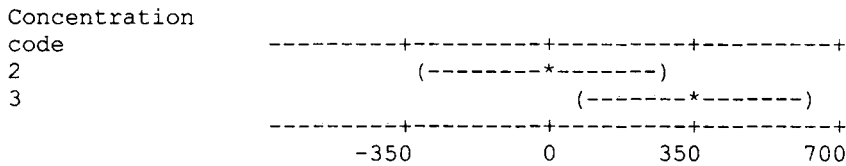
Pooled StDev = 173.2

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 97.94%

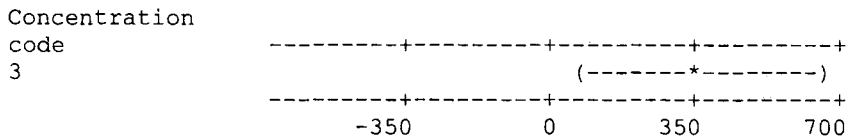
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
2	-301.3	-9.2	282.9
3	56.7	348.8	640.9



Concentration code = 2 subtracted from:

Concentration code	Lower	Center	Upper
3	65.9	358.0	650.1

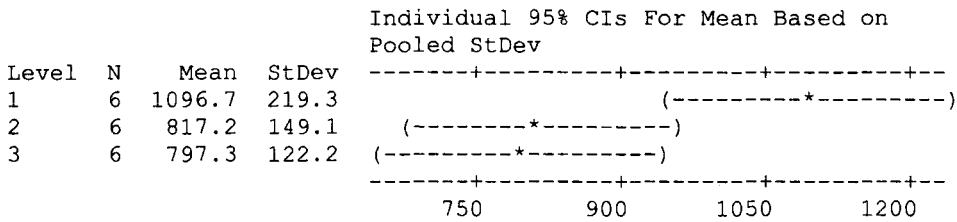


Results for: Trial 3

One-way ANOVA: Conductivity versus concentration code

Source	DF	SS	MS	F	P
concentration co	2	336228	168114	5.91	0.013
Error	15	426380	28425		
Total	17	762608			

S = 168.6 R-Sq = 44.09% R-Sq(adj) = 36.63%



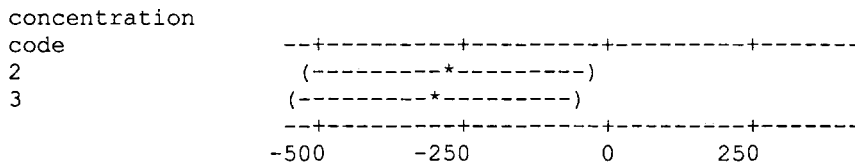
Pooled StDev = 168.6

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of concentration code

Individual confidence level = 97.97%

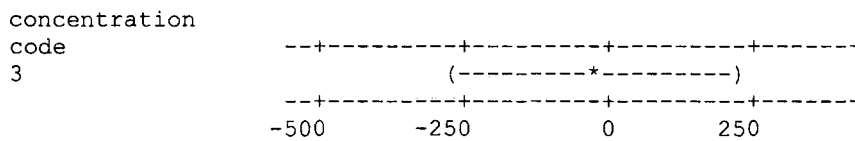
concentration code = 1 subtracted from:

concentration code	Lower	Center	Upper
2	-532.1	-279.5	-26.9
3	-551.9	-299.3	-46.7



concentration code = 2 subtracted from:

concentration code	Lower	Center	Upper
3	-272.4	-19.8	232.8

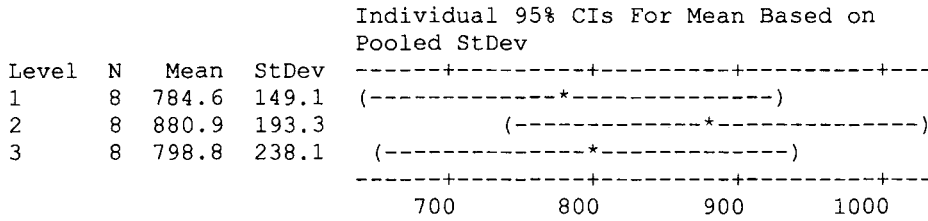


Results for: Trial 4

One-way ANOVA: Conductivity versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	43222	21611	0.56	0.581
Error	21	814116	38767		
Total	23	857338			

S = 196.9 R-Sq = 5.04% R-Sq(adj) = 0.00%



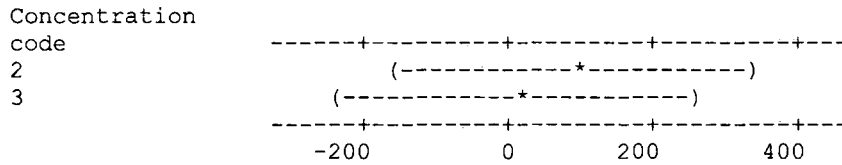
Pooled StDev = 196.9

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.00%

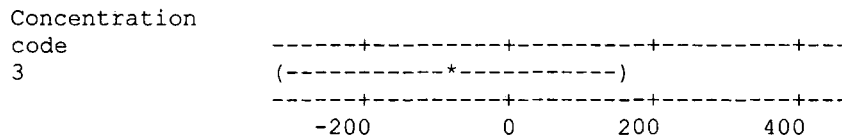
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
2	-151.6	96.3	344.1
3	-233.7	14.1	261.9



Concentration code = 2 subtracted from:

Concentration code	Lower	Center	Upper
3	-329.9	-82.1	165.7

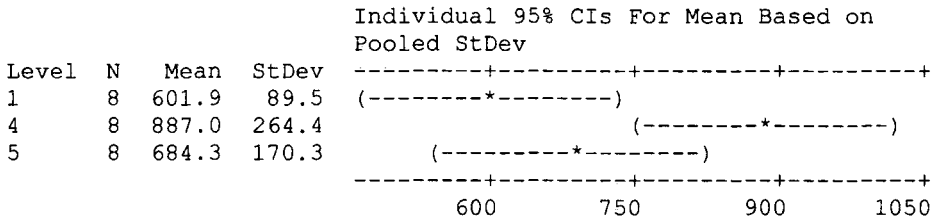


Results for: Trial 5

One-way ANOVA: Conductivity versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	344505	172253	4.83	0.019
Error	21	748428	35639		
Total	23	1092934			

S = 188.8 R-Sq = 31.52% R-Sq(adj) = 25.00%



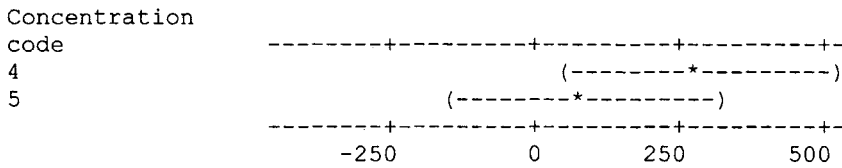
Pooled StDev = 188.8

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.00%

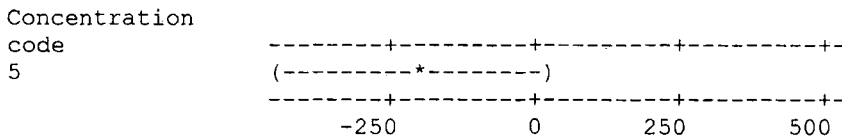
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
4	47.5	285.1	522.7
5	-155.2	82.4	320.0



Concentration code = 4 subtracted from:

Concentration code	Lower	Center	Upper
5	-440.4	-202.8	34.9

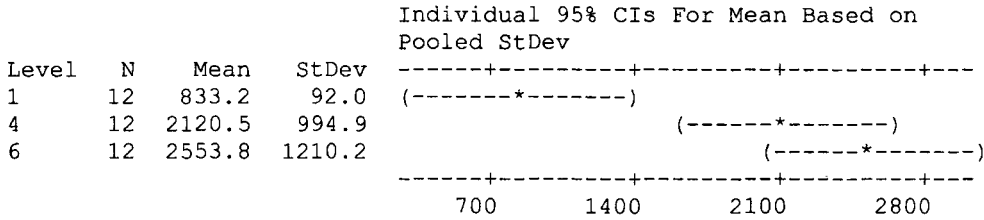


Results for: Trial 6

One-way ANOVA: Conductivity versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	19221359	9610679	11.71	0.000
Error	33	27090737	820931		
Total	35	46312096			

S = 906.1 R-Sq = 41.50% R-Sq(adj) = 37.96%



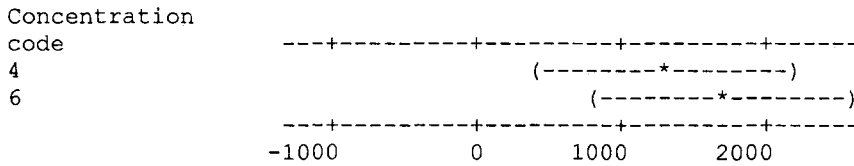
Pooled StDev = 906.1

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.04%

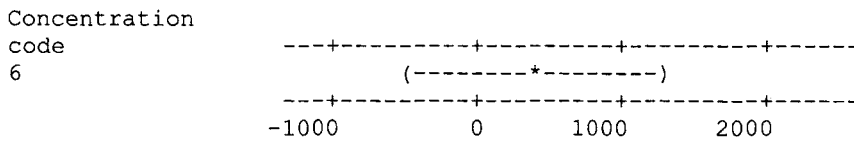
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
4	379.7	1287.3	2194.9
6	813.0	1720.6	2628.2



Concentration code = 4 subtracted from:

Concentration code	Lower	Center	Upper
6	-474.3	433.3	1340.8

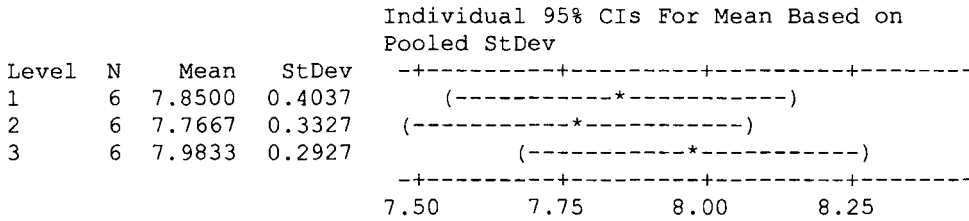


Results for: Trial 1& 2

One-way ANOVA: pH versus concentration code

Source	DF	SS	MS	F	P
concentration co	2	0.143	0.072	0.60	0.562
Error	15	1.797	0.120		
Total	17	1.940			

S = 0.3461 R-Sq = 7.39% R-Sq(adj) = 0.00%



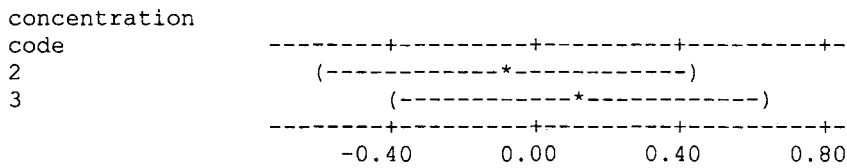
Pooled StDev = 0.3461

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of concentration code

Individual confidence level = 97.97%

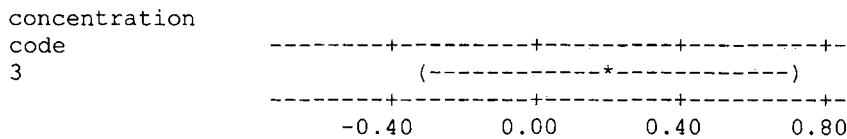
concentration code = 1 subtracted from:

concentration code	Lower	Center	Upper
2	-0.6019	-0.0833	0.4352
3	-0.3852	0.1333	0.6519



concentration code = 2 subtracted from:

concentration code	Lower	Center	Upper
3	-0.3019	0.2167	0.7352



Results for: Trial 3 & 4

One-way ANOVA: pH versus Concentration code

Source	DF	SS	MS	F	P
Concentration code	2	0.0280	0.0140	0.71	0.510
Error	12	0.2360	0.0197		
Total	14	0.2640			

S = 0.1402 R-Sq = 10.61% R-Sq(adj) = 0.00%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
1	5	7.6200	0.1304
2	5	7.6000	0.0707
3	5	7.5200	0.1924

Pooled StDev = 0.1402

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 97.94%

Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
2	-0.2564	-0.0200	0.2164
3	-0.3364	-0.1000	0.1364

Concentration code

Concentration code = 2 subtracted from:

Concentration code	Lower	Center	Upper
3	-0.3164	-0.0800	0.1564

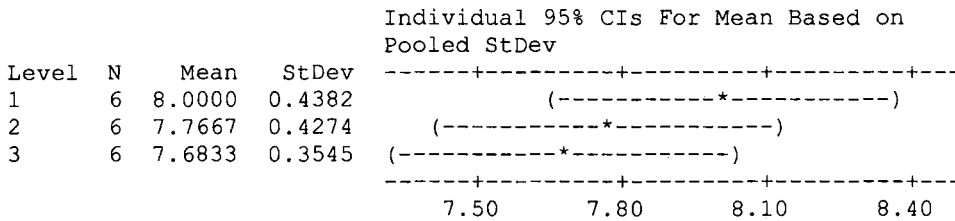
Concentration code

Results for: Trial 5 & 6

One-way ANOVA: pH versus concentration code

Source	DF	SS	MS	F	P
concentration co	2	0.323	0.162	0.97	0.402
Error	15	2.502	0.167		
Total	17	2.825			

S = 0.4084 R-Sq = 11.45% R-Sq(adj) = 0.00%



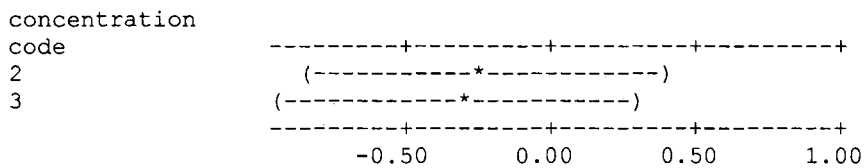
Pooled StDev = 0.4084

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of concentration code

Individual confidence level = 97.97%

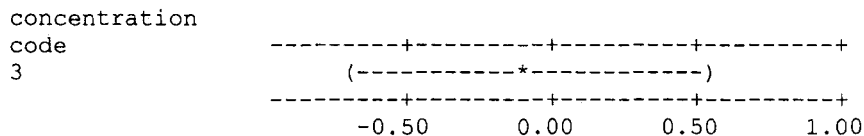
concentration code = 1 subtracted from:

concentration code	Lower	Center	Upper
2	-0.8452	-0.2333	0.3785
3	-0.9285	-0.3167	0.2952



concentration code = 2 subtracted from:

concentration code	Lower	Center	Upper
3	-0.6952	-0.0833	0.5285

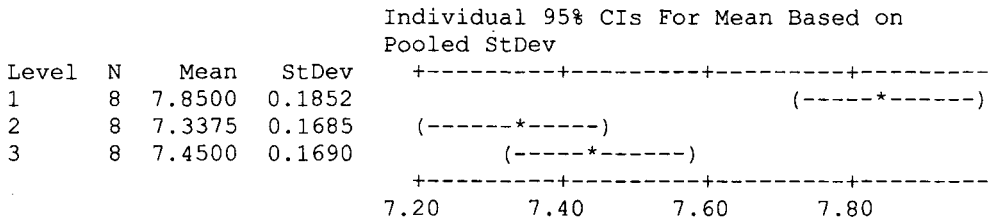


Results for: Trial 7 & 8

One-way ANOVA: pH versus Concentration code

Source	DF	SS	MS	F	P
Concentration code	2	1.1608	0.5804	19.08	0.000
Error	21	0.6387	0.0304		
Total	23	1.7996			

S = 0.1744 R-Sq = 64.51% R-Sq(adj) = 61.13%



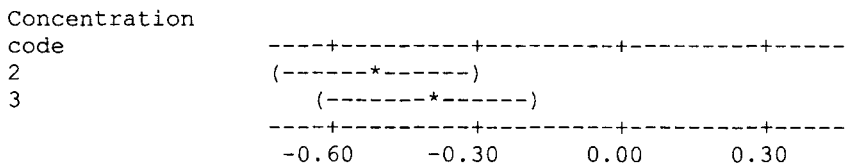
Pooled StDev = 0.1744

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.00%

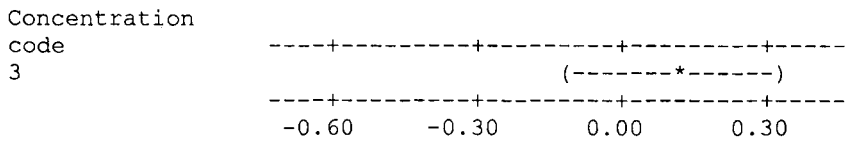
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
2	-0.7320	-0.5125	-0.2930
3	-0.6195	-0.4000	-0.1805



Concentration code = 2 subtracted from:

Concentration code	Lower	Center	Upper
3	-0.1070	0.1125	0.3320

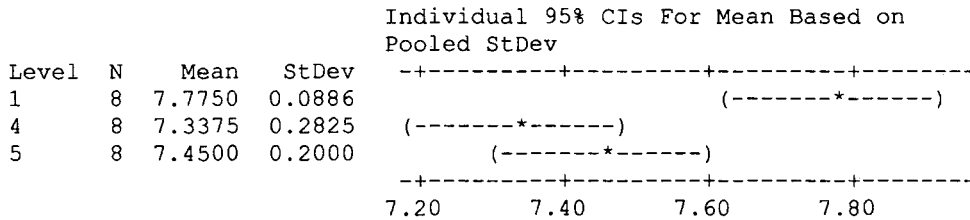


Results for: Trial 9 & 10

One-way ANOVA: pH versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	0.8258	0.4129	9.70	0.001
Error	21	0.8938	0.0426		
Total	23	1.7196			

S = 0.2063 R-Sq = 48.03% R-Sq(adj) = 43.08%



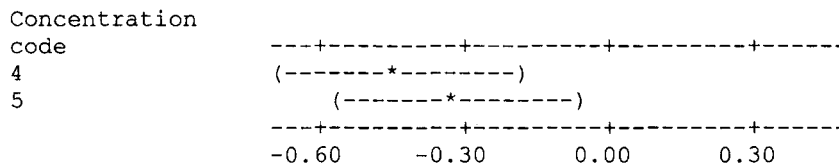
Pooled StDev = 0.2063

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.00%

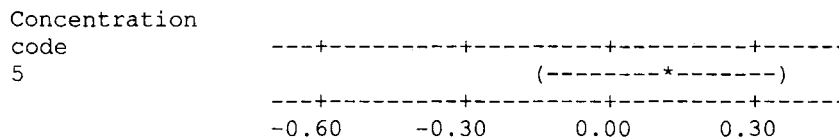
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
4	-0.6972	-0.4375	-0.1778
5	-0.5847	-0.3250	-0.0653



Concentration code = 4 subtracted from:

Concentration code	Lower	Center	Upper
5	-0.1472	0.1125	0.3722

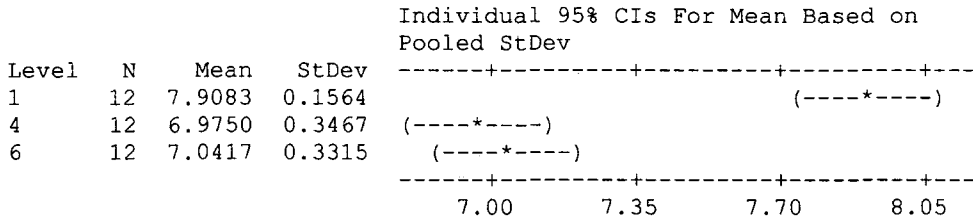


Results for: Trial 11 & 12

One-way ANOVA: pH versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	6.5067	3.2533	38.33	0.000
Error	33	2.8008	0.0849		
Total	35	9.3075			

S = 0.2913 R-Sq = 69.91% R-Sq(adj) = 68.08%



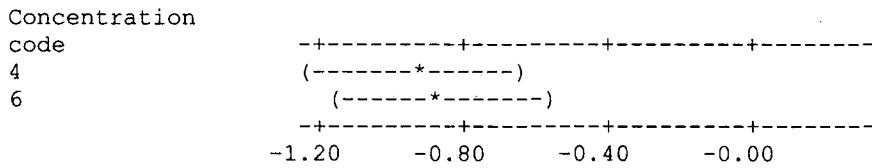
Pooled StDev = 0.2913

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.04%

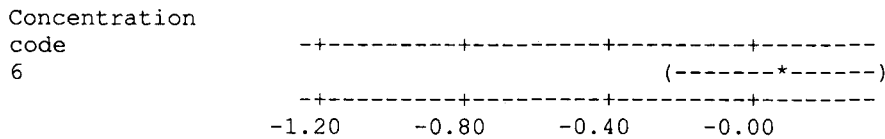
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
4	-1.2252	-0.9333	-0.6415
6	-1.1585	-0.8667	-0.5748



Concentration code = 4 subtracted from:

Concentration code	Lower	Center	Upper
6	-0.2252	0.0667	0.3585

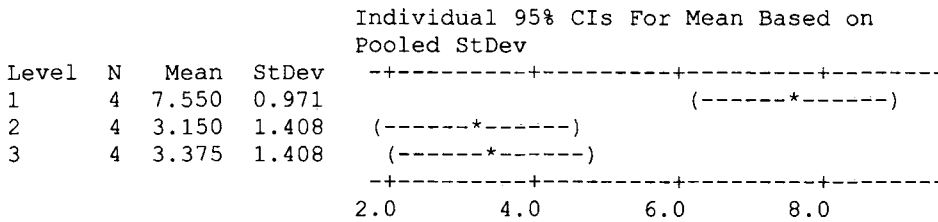


Results for: Trial 3

One-way ANOVA: Dissolved oxygen versus concentration code

Source	DF	SS	MS	F	P
concentration co	2	49.12	24.56	15.01	0.001
Error	9	14.73	1.64		
Total	11	63.85			

S = 1.279 R-Sq = 76.93% R-Sq(adj) = 71.81%



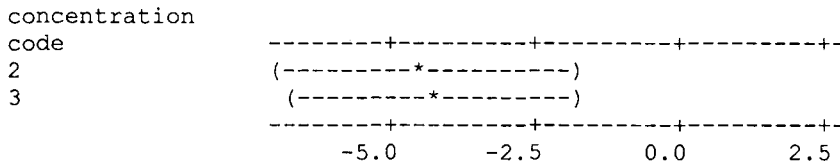
Pooled StDev = 1.279

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of concentration code

Individual confidence level = 97.91%

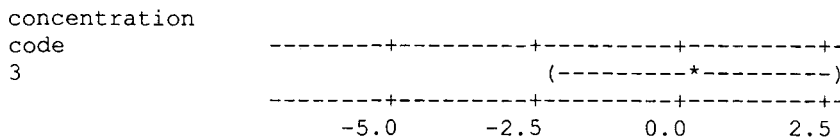
concentration code = 1 subtracted from:

concentration code	Lower	Center	Upper
2	-6.926	-4.400	-1.874
3	-6.701	-4.175	-1.649



concentration code = 2 subtracted from:

concentration code	Lower	Center	Upper
3	-2.301	0.225	2.751

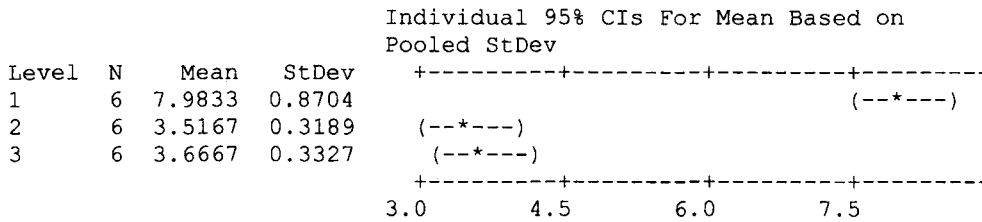


Results for: Trial 4

One-way ANOVA: Dissolved oxygen versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	77.214	38.607	119.40	0.000
Error	15	4.850	0.323		
Total	17	82.064			

S = 0.5686 R-Sq = 94.09% R-Sq(adj) = 93.30%



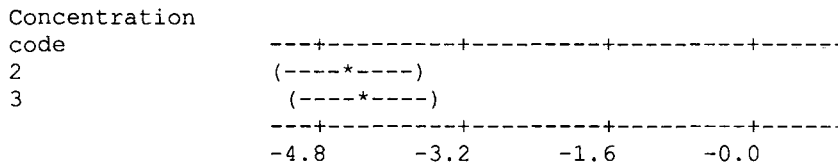
Pooled StDev = 0.5686

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 97.97%

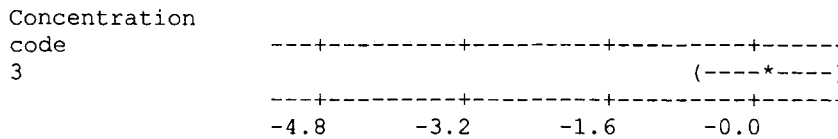
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
2	-5.3186	-4.4667	-3.6147
3	-5.1686	-4.3167	-3.4647



Concentration code = 2 subtracted from:

Concentration code	Lower	Center	Upper
3	-0.7020	0.1500	1.0020

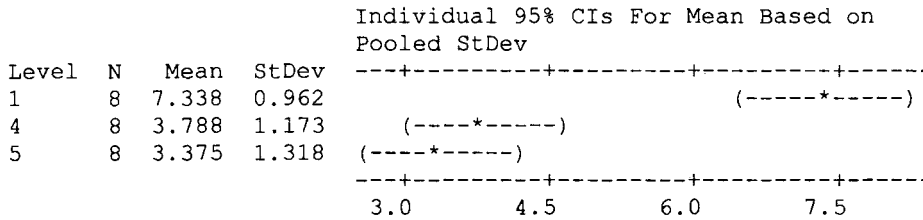


Results for: Trial 5

One-way ANOVA: Dissolved oxygen versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	75.93	37.97	28.21	0.000
Error	21	28.26	1.35		
Total	23	104.19			

S = 1.160 R-Sq = 72.87% R-Sq(adj) = 70.29%



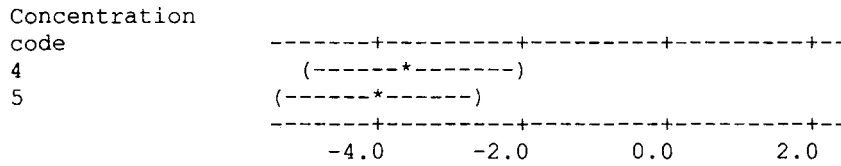
Pooled StDev = 1.160

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Concentration code

Individual confidence level = 98.00%

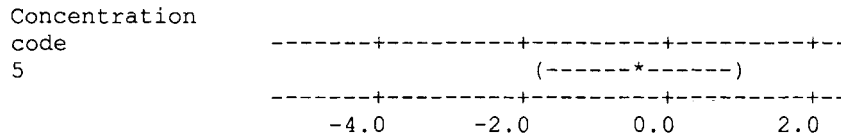
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
4	-5.010	-3.550	-2.090
5	-5.423	-3.963	-2.502



Concentration code = 4 subtracted from:

Concentration code	Lower	Center	Upper
5	-1.873	-0.413	1.048

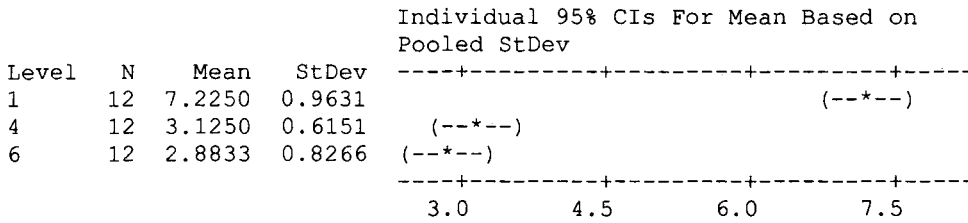


Results for: Trial 6

One-way ANOVA: Dissolved oxygen versus Concentration code

Source	DF	SS	MS	F	P
Concentration co	2	142.874	71.437	107.73	0.000
Error	33	21.882	0.663		
Total	35	164.756			

S = 0.8143 R-Sq = 86.72% R-Sq(adj) = 85.91%

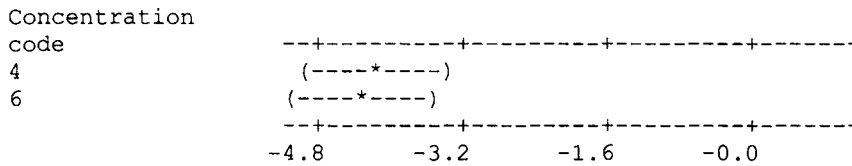


Pooled StDev = 0.8143

Tukey 95% Simultaneous Confidence Intervals
 All Pairwise Comparisons among Levels of Concentration code
 Individual confidence level = 98.04%

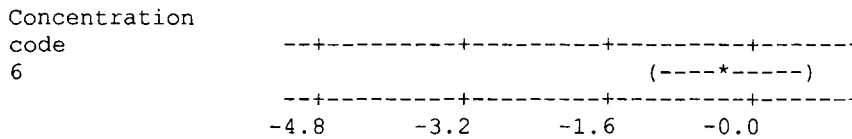
Concentration code = 1 subtracted from:

Concentration code	Lower	Center	Upper
4	-4.9157	-4.1000	-3.2843
6	-5.1574	-4.3417	-3.5260



Concentration code = 4 subtracted from:

Concentration code	Lower	Center	Upper
6	-1.0574	-0.2417	0.5740



Paired T-Test and CI: heights control 2003, heights dosed 2003

Paired T for hts control 2003 - hts dosed 2003

	N	Mean	StDev	SE Mean
hts control 2003	6	90.7167	13.4960	5.5097
hts dosed 2003	6	88.8667	14.7155	6.0076
Difference	6	1.85000	23.70306	9.67673

95% CI for mean difference: (-23.02483, 26.72483)

T-Test of mean difference = 0 (vs not = 0): T-Value = 0.19 P-Value = 0.856

Anova: Two-Factor With Replication

Shoot height

SUMMARY	2002	2003	Total
<i>control</i>			
Count	6	6	12
Sum	530	543	1073
Average	88.33333	90.5	89.41667
Variance	61.86667	179.9	111.1742
<i>Treated</i>			
Count	6	6	12
Sum	517	532	1049
Average	86.16667	88.66667	87.41667
Variance	103.7667	215.0667	146.6288
<i>Total</i>			
Count	12	12	
Sum	1047	1075	
Average	87.25	89.58333	
Variance	76.56818	180.447	

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	24	1	24	0.171245	0.683412	4.35125
Columns	32.66667	1	32.66667	0.233084	0.634489	4.35125
Interaction	0.166667	1	0.166667	0.001189	0.972832	4.35125
Within	2803	20	140.15			
Total	2859.833	23				

Paired T-Test and CI: no. of shoots control 2002, no shoots dosed 2002

Paired T for no. of shoots control 2002 - no shoots dosed 2002

	N	Mean	StDev	SE Mean
no. of shoots co	6	44.5000	7.7136	3.1491
no shoots dosed	6	49.1667	7.9854	3.2600
Difference	6	-4.66667	10.96662	4.47710

95% CI for mean difference: (-16.17542, 6.84209)

T-Test of mean difference = 0 (vs not = 0): T-Value = -1.04 P-Value = 0.345

Anova: Two-Factor With Replication

Shoot numbers

SUMMARY	2002	2003	Total
<i>Control</i>			
Count	6	6	12
Sum	267	222	489
Average	44.5	37	40.75
Variance	59.5	9.6	46.75
<i>Treated</i>			
Count	6	6	12
Sum	295	336	631
Average	49.16667	56	52.58333
Variance	63.76667	31.6	56.08333
<i>Total</i>			
Count	12	12	
Sum	562	558	
Average	46.83333	46.5	
Variance	61.9697	117.1818	

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	840.1667	1	840.1667	20.43373	0.000209	4.35125
Columns	0.666667	1	0.666667	0.016214	0.899947	4.35125
Interaction	308.1667	1	308.1667	7.494933	0.012685	4.35125
Within	822.3333	20	41.11667			
Total	1971.333	23				

Anova: Two-Factor With Replication

Biomass

SUMMARY

	2002	2003	Total
<i>control</i>			
Count	6	6	12
Sum	1046	1610	2656
Average	174.333	268.333	221.333
Variance	3	3	3
	939.866	1135.86	3353.33
	7	7	3
<i>Treated</i>			
Count	6	6	12
Sum	1115	2378	3493
Average	185.833	396.333	291.083
Variance	3	3	3
	542.566	7446.26	15715.9
	7	7	15715.9
<i>Total</i>			
Count	12	12	
Sum	2161	3988	
Average	180.083	332.333	
Variance	3	3	
	709.901	8369.33	
	5	3	

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	29190.3	8	29190.3	11.6012	0.002801922	4.35125
Columns	139080.	4	139080.	55.2752	3.54766E-07	4.35125
Interaction	20358.3	8	20358.3	8.09110	0.010018948	4.35125
Within	50322.8	3	2516.14			
		20	2			
Total	238952	23				

Anova: Two-Factor With Replication

TKN

SUMMARY	2002	2003	Total
<i>control</i>			
Count	6	6	12
Sum	4733	6411	11144
Average	788.8333	1068.5	928.6667
Variance	49460.17	140345.9	107606.4

<i>treated</i>			
Count	6	6	12
Sum	6918	12714	19632
Average	1153	2119	1636
Variance	85647.2	706852.8	614724.4

<i>Total</i>			
Count	12	12	
Sum	11651	19125	
Average	970.9167	1593.75	
Variance	97580.81	686058.6	

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	3001923	1	3001923	12.22398	0.002275	4.35125
Columns	2327528	1	2327528	9.477812	0.005925	4.35125
Interaction	706580.2	1	706580.2	2.87723	0.105351	4.35125
Within	4911530	20	245576.5			
Total	10947561	23				

Results for: stomata

Two-Sample T-Test and CI: stomata control, stomata dosed

Two-sample T for stomata control vs stomata dosed

	N	Mean	StDev	SE Mean
stomata control	36	75.6	11.0	1.8
stomata dosed	36	101.5	16.1	2.7

Difference = mu (stomata control) - mu (stomata dosed)

Estimate for difference: -25.9444

95% CI for difference: (-32.4257, -19.4632)

T-Test of difference = 0 (vs not =): T-Value = -7.98 P-Value = 0.000 DF = 70

Both use Pooled StDev = 13.7871

Micro-Kjeldhal method used to prepare solution for use in Hach method 8075

Add

20 – 100 mg. dried vegetation +

¼ selenium catalyst table (ex Merck or Fluka) +

3 ml. of concentrated sulphuric acid to micro-Kjeldhal reflux flask, with a few anti-bump granules.

With bulb stopper in the top of the neck of the flask, reflux gently as solution goes from black to orange to yellow to nearly colourless and clear. Stop refluxing, allow to cool and make up solution to 25.0 ml with distilled water.

Use this solution from step 5 in the published method.

EZ-BOD[®] Meter

Operating Manual

Model

EZ-BOD - PD

**Bioscience, Inc.
1550 Valley Center Parkway Suite 140
Bethlehem, PA 18017
(610) 974-9693
Version 2.0**

5. To find a quick and easy method of estimating the BOD₅ of the effluent that could be used by treatment plant personnel.

5.1 The EZBOD[®] Meter

Our requirements were discussed with Quintus Milieu, Holland, the European agents for Biosciences Inc., the manufacturers of the EZBOD[®] meter, and we were assured that the instrument would satisfy our needs. The meter was designed to measure the dissolved oxygen depletion rate due to the bacterial action in a given sample. This rate, measured over a short period was then used to indicate the BOD₅ of the sample. According to the product description, spot analysis of the waters using the EZBOD[®] would take 15 to 20 minutes, thus saving time (5 days for a lab BOD₅) and thus the cost of analysis. The EZBOD[®] meter was purchased in 2001. It had been planned that evaluation of the meter would take place whilst the reeds in the test beds were becoming established. However, the evaluation was assigned to an M.Sc. student from Teesside University who, unfortunately, was unable to get any readings from the instrument at all, a fact that was not made apparent until late into the year. The instrument was finally handed over at the end of 2001 and trials were started. Initially, it was impossible to get any results from the instrument. The method provided with the EZBOD[®] meter requires the use of some 'returned activated sludge' (RAS) for the test to work. RAS is bacterially-active liquor found in sewage treatment plants but due to health and safety regulations it was difficult to obtain a supply of this material. The Health and Safety Officers from both the University and BP were not willing to allow the material to be used.

QM and Biosciences Inc were contacted and they suggested that an alternative was to make up bacterially-active water using dried bacteria obtained from Biosciences. The

product suggested was Microcat[®] R , a bacterial mix that was specifically designed to deal with hydrocarbons. The COSSH handling details were obtained and the University agreed to the use of the material. The system was tested using the Microcat[®] R as and when other testing schedules allowed but with very disappointing results. It was not until October 2002 that it was possible to devote time to the evaluation.

5.1.2. Method

Although purportedly safe, certain basic precautions were taken when handling Microcat[®] R. Gloves and a facemask were worn and the material was always handled in a fume cupboard. This material was not intended to be used by site personnel, should use of the meter be adopted.

Two unused test tanks that had not been planted with reeds were filled with gravel and water. These gravel beds were used so that there would be no organic matter other than TEG present. A TEG solution was added to the beds and each sample was taken using a bulb pipette. The samples were tested for BOD₅ using the EZBOD[®] analyser and TEG was determined by GC. On each occasion, at least 2 repeat analyses were carried out for each sample.

5.1.3. Production of active liquor

5gm. of Microcat[®] R were added to 1 litre of dechlorinated water and aerated for 24 hours. Aeration was stopped and the mixture allowed to settle for about 10 minutes. Whilst allowing the mixture to settle, the dissolved oxygen probe was calibrated. (See EZBOD[®] instrument method in appendix p 187) The supernatant liquor was decanted

off to be used as the RAS. It was found, by trial and error, that this liquor gave better results if diluted in the ratio of 170 ml of liquor with 100 ml of dechlorinated water.

5.1.4. Instrument method

The aeration bottle was filled with demineralised or distilled water and placed in the instrument. The oxygen probe and aeration tube were inserted in the bottle and switched on. Simple step-by-step instructions appear on the vdu of the meter.

After a few minutes the probe was calibrated. The aeration bottle was emptied and 270 ml of the bacterially active mix added and returned to the instrument. This was the bacterially active liquor, which was then aerated to a saturated concentration of oxygen (about 7.5 mg/litre D.O.). The aeration pump and stirrer on the EZBOD[®] were adjusted to give maximum aeration to the liquid. Prior to testing, standard solutions of glucose/glutamic acid were made up and tested to provide archive data. This data enables the instrument to calculate a factor that is used in future calculations of samples, i.e. the BOD₅ of the standard solution is known, so the BOD₅ that the instrument gives will need to be 'adjusted' by a factor to arrive at the correct result. All future results are multiplied or divided by this factor. When the dissolved oxygen (D.O.) level reached 7.5 mg/l or above, aeration was stopped and the slope of the D.O. monitored, i.e. the rate at which the oxygen was being used up by bacterial activity was automatically monitored. When this slope was steady the instrument called for the addition of the test sample. The sample was added and the volumes used were entered into the instrument database. The increased oxygen uptake from the liquid when the bacteria degraded the organic material in the samples was monitored and when the D.O. slope returned to the level at which it began, the instrument calculated the oxygen uptake for the volume of sample used. Using the time taken for this to

occur and the factor calculated earlier, the instrument calculated the BOD₅ for the sample. The result was then saved on either the hard drive of the meter, a floppy disk or printed out.

5.1.5. Results

The Microcat[®] R was unreliable when attempting to create a batch of active liquor. It was not possible to determine the quality of the liquor until more than 24 hours had passed. If the liquor was not active then a new batch had to be made and a further 24 hours aeration were needed and repeat tests of the same sample. A sample of the results obtained over a 3 week period using the EZBOD[®] meter on water samples from the gravel beds is shown in Table 5.1

Table 5.1. Table of results of analysis of samples of water from the gravel test beds. 17/10/02 to 14/11/02 using the EZBOD[®] meter and GC

Sample no.	Sample date	Location	Factor	BOD ₅	%v/v TEG
1	17/10/02	Gravel A/ in	10.65164	5	0.183
		Gravel A/in duplicate	11.61991	101	0.183
2		Gravel B/in	11.16487	15	0.2
		Gravel B/in duplicate	11.30467	13	0.2
3		Gravel A, middle			
		Gravel A, middle	11.5774	87	0.17
		Gravel A, middle duplicate	6.908719	1	0.17
4	22/10/02	Unable to aerate liquor to saturation. Used 2 litres of liquor			
	23/10/02	Gravel A, in	0	0	0.137
		Gravel A, in, duplicate	11.06781	9	0.137
		Gravel A, in, trip	0	0	0.137
5		Gravel B, in	9.38399	2	0.157
		Gravel B, in, duplicate	11.51218	30	0.157
6	24/10/02	Unable to aerate liquor to saturation. Used 2 litres of liquor			
	25/10/02	20 mls of sample was too high, tried twice at this volume			
		Gravel A, middle	11.59255	171	0.153
		Gravel A, middle duplicate.	8.940957	2	0.153
7	07/11/02	Gravel bed A in 4/11/02	11.64532	1919	0.108
		Gravel bed A in 4/11/02	11.6453	1698	0.108
		Gravel bed A in 4/11/02	11.64611	1641	0.108
8		Gravel bed A middle	11.64945	2225	0.11
		Dissolved oxygen level of liquor would not stabilise			0.11
		Gravel bed A middle, 4/11/02	11.64891	2575	0.11
		Gravel bed A middle, 4/11/02	11.64931	3233	0.11
		Gravel bed A middle, 4/11/02	11.64669	2311	0.11
		Gravel bed A middle, 4/11/02	11.64552	1927	0.11
		Gravel bed A middle, 4/11/02	11.64612	2561	0.11
		Gravel bed A middle, 4/11/02	11.64579	2549	0.11
9	12/11/02	Gravel bed B in, 4/11/02	11.63956	490	0.171
		Gravel bed B in, 4/11/02	11.63843	638	0.171
		Gravel bed B in, 4/11/02	11.64608	1708	0.171
		Gravel bed B in, 4/11/02	11.63699	606	0.171
10	14/11/02	Gravel bed A, in, 13/11/02	11.64944	3441	0.064
		Gravel bed A, in, 13/11/02	11.64845	3235	0.064
		Gravel bed A, in, 13/11/02	11.64917	3419	0.064
		Gravel bed A, in, 13/11/02	11.64957	3467	0.064
		Gravel bed A, in, 13/11/02	11.64931	3264	0.064
11		Gravel bed B, in, 13/11/02	11.60984	33	0.158
		Gravel bed B, in, 13/11/02	0	0	0.158
		Gravel bed B, in, 13/11/02	11.5957	33	0.158
		Gravel bed B, in, 13/11/02	11.12651	14	0.158
		Gravel bed B, in, 13/11/02	11.1721	19	0.158

Table 5.2. Table of results of analysis of samples of water from the gravel test beds. 17/10/02 to 14/11/02 using the EZBOD[®] meter and GC

Sample no.	Average Factor used by EZBOD [®] meter	Average BOD ₅ from EZBOD [®] meter, mg/l	Average %v/v TEG by GC
1	11.136	53	0.183
2	11.235	14	0.2
3	9.243	44	0.17
4	3.689	3	0.137
5	10.448	16	0.157
6	10.267	86.5	0.153
7	11.646	1752.7	0.108
8	11.647	2417.5	0.11
9	11.64027	860.5	0.171
10	11.649188	3365.2	0.064
11	9.1008	19.8	0.158

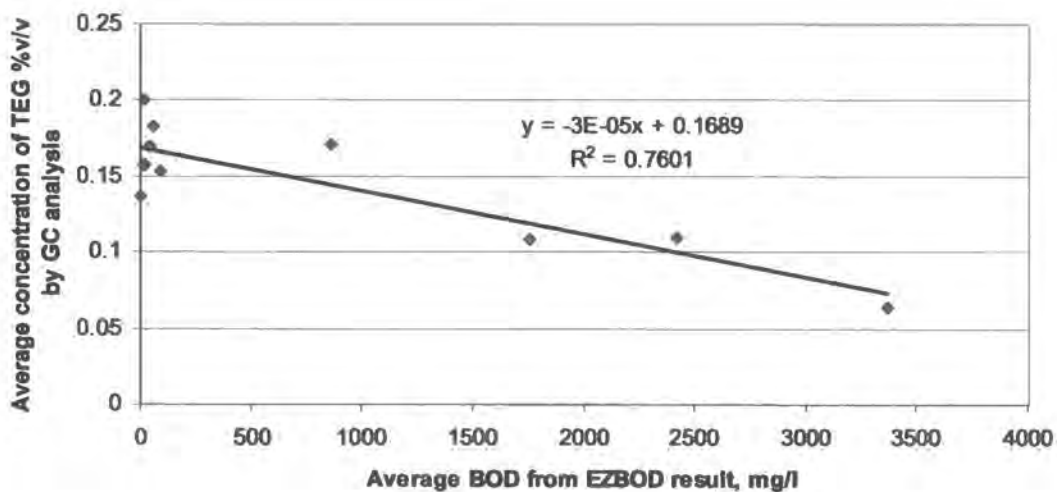


Figure 5.1. Relationship between BOD₅ mg/l from EZBOD Analyser and %TEG v/v from GC analysis figures in Table 5.2.

5.1.6. Critique of the EZBOD® method

As the liquor needed to be prepared 24 hrs in advance the EZBOD® meter could only be used from Tuesday onward, not a problem in a 24 hour plant like CATS Terminal, but still not what is thought of as 'spot testing'. At times it was not possible to aerate the liquor to saturation point. The bacteria were using oxygen faster than it could be added. Sometimes the liquor would need 48 hours aeration before it was ready for use. When the liquor was prepared, it was done in duplicate and at times it was found that one flask of liquor would be suitable and the other would not be. Attempts to make up a large flask of liquor proved to be unsuccessful. The volume of sample used was determined by trial and error. If it was to be used on effluent of unknown content it could be very time consuming to arrive at the optimum sample volume. During the trial duplicate results were not often obtained and even then they bore no relationship to the organic content of the samples. Replacement of the membrane on the oxygen probe was very time consuming and intricate. After many attempts, a technique evolved but attempts by university lab technicians were unsuccessful, an indication that untrained plant personnel would probably find the process difficult.

5.1.7. Discussion

Ease of use

The EZBOD[®] meter is easy to use and the instructions clear and to the point. However, the sample preparation is complicated and haphazard. The Microcat[®] mix is unreliable (a dried bacterial mix may work on a large scale but when reduced to 5gm., samples being representative of the whole may be hard to find.) Because of the 24 hr. preparation time, it would not be possible to deal with heavy rains or spillages, on site, as fast as would be hoped for. This meter was designed for use on an activated sludge treatment plant and would probably work under those conditions. It did not work using solutions of TEG.

Reproducibility of results

One occurrence that causes concern is that the 'factor' used by the meter, changes.

Unless other data has been entered into the archive file the factor should remain constant, in fact the whole method hinges on that point. Of the 38 results quoted the same factor was only used twice (discounting the times when the factor was zero)

On a sample that was tested 3 times (07/11/02) a factor of 11.6453 was used in two of the calculations. The results however were not within an acceptable range of experimental error and could not be used.

Table 5.3. Samples that gave results that were acceptable as being within experimental limits.

Date	Sample	Factor used	BOD mg/l	%v/vTEG
7/11/02	Gravel B/in	11.16487	15	0.2
	Gravel B/in dupl.	11.30467	13	0.2
14/11/02	Gravel bed A, in, 13/11/02	11.64944	3441	0.064
	Gravel bed A, in, 13/11/02	11.64845	3235	0.064
	Gravel bed A, in, 13/11/02	11.64917	3419	0.064
	Gravel bed A, in, 13/11/02	11.64957	3467	0.064
	Gravel bed A, in, 13/11/02	11.64931	3264	0.064

Unfortunately the higher BOD was from the sample with the lower TEG concentration.

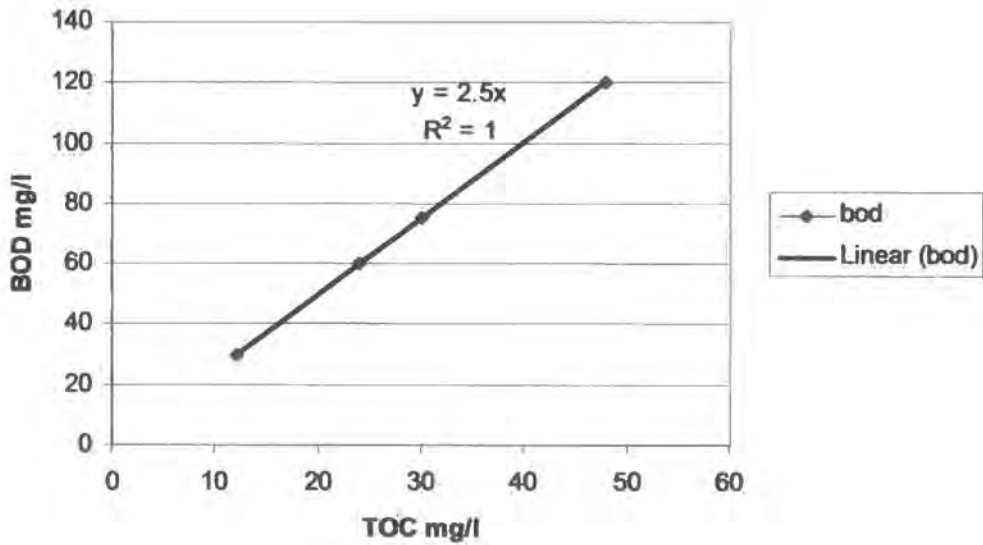


Figure 5.2. The theoretical relationship between the concentration of TEG, %v/v, and the BOD₅, mg/l

The results used in Figure 5.1 are so varied as to make the insertion of a trend line purely academic. The variance is too wide to be classed as accurate and the trendline indicates that BOD increases as TEG decreases. Figure 5.2. shows the results which had been expected theoretically i.e. a direct relationship between the amount of organic carbon present (calculated from concentration and molecular formula of TEG) and the amount of oxygen needed to convert that carbon to carbon dioxide. The expected result was a positive straight-line relationship between the TEG and the BOD.

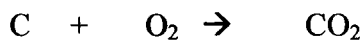
To summarise, it was felt that the EZBOD[®] method was not suitable for use in this situation, where the BOD₅ is due to the presence of TEG. This was because preparation of the Microcat[®] mix was time wasting and frustrating, but mainly because the results that were obtained bear no resemblance to either the theoretical BOD or to the organic content of the samples.

It was also decided that the use of any 'bacterially active' material by plant personnel was unacceptable from a health and safety point of view.

5.2. The Total Organic Carbon Analyser

5.2.1 Introduction

Although the BOD₅ test is still widely used and specified as the international measure of the organic contamination of water, many organisations are turning to other forms of determining the levels. The BOD₅ test takes five days so the actual result is historical. Many companies need results quicker so as to avoid any damaging discharges. An alternative method is to determine the total organic carbon (TOC) content of a sample. If the TOC is found a simple calculation can provide the maximum possible oxygen demand for that sample.



Using atomic wts. 12 32 44

Thus every 12 mg. of carbon will require 32 mg. of oxygen.

Modern instrumentation has meant that TOC analysis can be carried out in 10 to 15 minutes and the results can either be recorded or passed to a control room. They can also be programmed to safe limits and used to automatically control containment procedures in the event of 'out of spec' results. The determination of the TOC is by measuring the carbon dioxide produced when a sample is completely oxidised.

TOC analysers can be divided into two categories, stand-alone bench analysers or 'in-line' instruments that are fully automated. The bench analysers are usually used in laboratories for both control and project analysis and need trained analysts to operate them.

The in-line analysers are placed near the effluent line in question and sample automatically at preset time intervals. Results are recorded and can be transmitted to a

control room. Apart from periodic servicing and the maintenance of reagent levels, the in-line analysers need very little attention. Spot samples can be tested but the sample must be introduced by operators.

CSIP, Weymouth, have developed the Biotector[®] TOC analyser and agreed to loan one of their demo models for a trial to be run on site. The actual processes involved in the analysis are protected by various patents but are summarised in the CSIP sales brochure (Appendix p 191) All the stages are automatic. The Biotector[®] takes a representative unfiltered sample from the effluent under test. The volume of sample is determined for the optimum range of carbon expected. Acid is added and the inorganic carbon is measured by infra red detection. Ozone is then generated, the pH of the sample raised using sodium hydroxide and total oxidation of the carbon occurs. The carbon dioxide is released by lowering the pH and it is measured by the infra red detector. The total organic carbon is determined by subtracting the total inorganic carbon content from the total carbon content.

Prior to the trial CSIP arranged for a site visit to be made to BASF, Seal Sands, who already have 2 of their instruments in use. The aim of the trial was twofold. Firstly to attempt to establish a relationship between TOC and BOD₅ and/or COD which would enable the waste water quality to be monitored on a daily time scale without having to have lab analysis performed as regularly as at present. Secondly, to evaluate the performance of the instrument with a view to using one in the future, especially as the waste from the Amenity block was about to pass through a Klargester and into the retention pond.

The units at BASF are 'in-line' instruments, housed in a small metal hut near the discharge point of the BASF effluent line. Samples are taken at preset time intervals and the results recorded in the control room. The instrument is set up so that should levels rise above the allowed limits an alarm is raised and the discharge is automatically stopped to allow plant personnel to rectify the problem. BASF reported no problems with the machine and very little time had to be devoted to it other than routine checking of reagents and servicing.

5.2.2. Method

The analyser loaned for the trials was from the Billingham dept. of CSIP and was housed in a small trailer. It was placed alongside the retention pond and sampled the water at preset time intervals. The pond was also sampled every two hours and these samples analysed in the lab for COD, BOD₅ and triethylene glycol content by gas chromatography. The COD and BOD₅ methods are in the appendix. The unit was installed in February, 2003, but due to a few unforeseen events it was not available for as long as hoped. Thirty one samples were taken from the retention pond followed by a further 22 samples from the glycol sump. The instrument has to be set up to detect specified 'ranges' of carbon and was consequently set up to deal with the low levels found in the retention pond. Unfortunately Train 2, glycol sump was sampled in error and was too high in TOC for the parameters set into the instrument. This meant that only 17 of the sample results could be used as being within the calibration range.

5.2.3. Results

The results of analysis by Biotector[®] of samples from the retention pond are shown in table 6.3.1. The COD and BOD results from the retention pond gave values and ratios that were in line with accepted relationship figures for waste water but bore no relationship to the TOC figures achieved

Table 5.4. Results of analysis of samples from the retention pond. The TOC results were obtained using a CSIP Biotector[®] whilst the other analysis was from the laboratory at Durham University

Date	Time	Bod mg/l	toc mg/l	Bod/Toc	Bod/Cod	Cod mg/l	Cod/Toc	cod/bod
18/02/03	800	26	19.6	1.33	0.04	35	1.79	1.35
	1000	11	23.9	0.46	0.03	15	0.63	1.36
	1200	11	23.8	0.46	0.02	20	0.84	1.82
	1400	11	24.4	0.45	0.03	15	0.61	1.36
19/02/03	800	5	26	0.19	0.02	10	0.38	2.00
	1000	5	26.5	0.19	0.02	10	0.38	2.00
	1200	5	22.4	0.22	0.02	10	0.45	2.00
	1400	5	21.5	0.23	0.02	10	0.47	2.00
20/02/03	800	41	21.4	1.92	0.03	65	3.04	1.59
	1000	5	22.3	0.22	0.02	10	0.45	2.00
	1200	5	21.9	0.23	0.02	10	0.46	2.00
	1400	21	19.8	1.06	0.04	30	1.52	1.43
21/02/03	1000	36	22.3	1.61	0.03	60	2.69	1.67
	1200	5	21.9	0.23	0.02	10	0.46	2.00
	1400	7	19.8	0.35	0.04	10	0.51	1.43
24/02/03	800	5	20.7	0.24	0.02	10	0.48	2.00
	1000	16	20.9	0.77	0.04	20	0.96	1.25
	1200	5	20.9	0.24	0.02	10	0.48	2.00
	1400	16	20.2	0.79	0.04	20	0.99	1.25
25/02/03	800	16	23.8	0.67	0.03	25	1.05	1.56
	1000	6	26.1	0.23	0.02	10	0.38	1.67
	1200	16	25	0.64	0.03	20	0.80	1.25
	1400	21	24.6	0.85	0.03	25	1.02	1.19
26/02/03	800	41	25.6	1.60	0.03	55	2.15	1.34
	1000	16	25.1	0.64	0.03	25	1.00	1.56
	1200	21	25	0.84	0.03	30	1.20	1.43
	1400	29	26.1	1.11	0.02	45	1.72	1.55
27/02/03	800	16	26	0.62	0.02	25	0.96	1.56
	900	14	26.3	0.53	0.03	20	0.76	1.43
	1000	29	23.7	1.22	0.03	40	1.69	1.38
	1100	21	24.2	0.87	0.03	30	1.24	1.43
Average				0.68	0.03		1.02	1.61

Anomalies occurred in the comparison of the results and after looking at COD and BOD results from samples analysed by a contract laboratory it was decided to run trials using EXACTLY the same sample for analysis in the Biotector® and in the lab.

5.2.4. Alternative Method

The on-site trials with the Biotector® Total Organic Carbon analyser in February and March, 2003, proved inconclusive in that there was poor correlation between the total organic carbon (TOC) and the chemical oxygen demand (COD) and the biological oxygen demand (BOD). Both the retention pond and the glycol sump were tested with better correlation occurring from the sump samples. It was thought that this may have been due to sampling differences and possible 'layering' of impurities. The analyser had been set up to sample the retention pond 'in line' whilst the sump was manually 'spot sampled'. All samples for lab analysis were spot sampled.

The analyser was on limited loan and had to leave the site but a further 15 samples were taken at 30 minute intervals, 24/03/03 and submitted for analysis by the analyser back at its base and in the labs. Once again the correlation was poor and it was decided to remove as many variables as possible. The remains of the 15 samples used in the lab had been stored at 4⁰C in the dark and should have changed little from the first results. The samples were sorted into groups of 3 of similar COD levels (from earlier analysis) and these groups blended together in equal proportions. Some samples had contained visible particles so the blends were filtered through No. 1 papers to remove any debris. At such low levels, a particle of organic material being analysed for TOC and not COD could seriously affect the correlation.

Each one of the five blends were divided into 3 samples again to give 15 samples for analysis. Two of the blends had a small amount of triethylene glycol solution added to raise levels, purely as an exercise that would show if the tests were detecting organic material or not. This time each bottle was tested for TOC, COD and BOD removing any chance of variation. The main reason for the trial was to compare the different methods of testing not determine the precise level in each sample. The results from this method were better, with good correlation being obtained between TOC and COD, TOC and BOD and reasonable correlation between COD and BOD. Historically COD and BOD levels on samples from CATS have not had good correlation. Levels in the 'doctored' blends were proportionately higher than those found in the untouched ones.

5.2.5. Results

Table 5.5. Results of analysis of 15 samples of filtered water from the retention pond. The 15 samples consisted of 5 sets of 3 blends.

Sample no.	BOD mg/l	COD mg/l	TOC mg/l
1	3	45	10.4
2	5	24	7.5
3	3	15	6.5
4	4	24	5.8
5	2	36	6.4
6	18	67	11.4
7	2	40	6.1
8	6	14	7.1
9	4	29	5.9
10	4	29	9.1
11	22	66	13.2
12	24	65	13.1
13	16	63	12.7
14	20	67	12.8
15	11	57	12.7

Table 5.6. The constituent samples of each blend.

Blend no.	Samples in blend
1	1,5,7
2	2,4,9
3	3,8,10
4*	6,11,15
5*	12,13,14

* denotes blends that had a small amount of triethylene glycol solution added

Table 5.7. The blends and the averages of the BOD, COD and TOC for each blend

Blend 1	BOD mg/l	COD mg/l	TOC mg/l
Sample no.1	3	45	10.4
Sample no. 5	2	36	6.4
Sample no. 7	2	40	6.1
Average	2	40	7.6
Standard error	0.33	2.6	1.39
Blend 2			
Sample no. 2	5	24	7.5
Sample no. 4	4	24	5.8
Sample no. 9	4	29	5.9
Average	4	26	6.4
Standard error	0.33	1.67	0.55
Blend 3			
Sample no. 3	3	15	6.5
Sample no. 8	6	14	7.1
Sample no. 10	4	29	9.1
Average	4	19	7.6
Standard error	0.88	4.84	0.79
Blend 4			
Sample no. 6	18	67	11.4
Sample no. 11	22	66	13.2
Sample no. 15	11	57	12.7
Average	17	63	12.4
Standard error	3.21	3.18	0.54
Blend 5			
Sample no. 12	24	65	13.1
Sample no. 13	16	63	12.7
Sample no. 14	20	67	12.8
Average	20	65	12.9
Standard error	2.31	1.15	0.12

Table 5.8. The average results of BOD, COD and TOC mg/l for each of the blends .Averages obtained from 3 samples of each blend of the filtered samples. Table also shows the ratios of BOD/TOC, COD/TOC, COD/BOD and BOD/COD for these results.

Blend no.	BOD mg/l	COD mg/l	TOC mg/l	BOD/TOC	COD/TOC	COD/BOD	BOD/COD
1	2	40	7.6	0.31	5.28	17.29	0.06
2	4	26	6.4	0.68	4.01	5.92	0.17
3	4	19	7.6	0.57	2.56	4.46	0.22
4	17	63	12.4	1.37	5.09	3.73	0.27
5	20	65	12.9	1.55	5.05	3.25	0.31
Ave.				0.9	4.4	6.93	0.21

Table 5.9. Table of correlation coefficients and coefficients of determination obtained from charts 1, 2 and 3

Variables	Correlation coefficient, r	Coefficient if determination, r²
COD against TOC	0.935	0.8747
BOD against TOC	0.970	0.9413
COD against BOD	0.888	0.7892

5.6.5 Discussion

From Table 5.9, it can be seen that the three methods of analysis all performed to approximately the same degree of accuracy. Blends 4 and 5 had had some triethylene glycol solution added and this was picked up by the 3 methods. Although this trial was not actually carried out on 'real life' retention pond samples, it was supposed to be a comparative trial and as such needed to be carried out on the same samples. As mentioned earlier, at levels of <10 mg/l TOC, a small particle of organic material tested for one aspect would not be present in another aspect and would result in a discrepancy in results.

The Biotector[®] is designed to sample at predetermined time periods so the results gathered over a day would give a good overall indication of the effluent quality.

The recent analysis of the retention pond contents after the Klargestar had been commissioned also showed a wide variation in results as was found when the unfiltered samples had been tested. The correlation coefficients derived from the results (Table 5) are highly satisfactory. The ratios in Table 4 are slightly lower than some available from Klargestar Co. which were BOD/COD of 0.4 to 0.6 and BOD/TOC of 1.0 to 1.6. The BOD/COD ratio result is reinforced by a BOD/COD ratio from Caleb Brett (the independent laboratory used by BP for analysis) results of 0.27. The effluent at CATS is unique in its constituent make up which would explain this slight disparity.

To summarise, the Biotector[®] in-line analyser provided accurate results that could be strongly correlated to results obtained by conventional means. The analyser requires a minimum of attention (topping up of reagents) and the results can be recorded in the control room. Total Organic Carbon is fast becoming accepted as a reliable indicator of effluent quality, being accurate, faster and cheaper than conventional analysis and a true indicator of the actual organic content of an effluent providing that non organic material such as NH_4^+ don't contribute to the BOD / COD measurements. Although the BOD_5 is still the required level to be met, TOC is being used on a greater scale as an accurate control over effluent quality as BOD and COD can only supply the data on a historic scale whereas TOC is almost instantaneous.

