LAND DISPOSAL OF WINERY AND DISTILLERY WASTEWATERS

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by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

SUMMARY

Winemaking is traditionally divided into two periods, vintage and non-vintage. Vintage is a 12–20 week period in which the harvesting of grapes, extraction of juice, and fermentation of the juice to wine take place. The remainder of the year is the non-vintage period. During non-vintage the wine is clarified, stabilised, and matured for final bottling. Defective wine and by-products from the manufacturing operations may also be distilled for ethanol spirit, used for the production of fortified wines.

Wineries produce about 3–5 kl of wastewater per tonne of grapes crushed, which represents a few hundred Ml per major wine producing district within Australia, annually. About half the annual volume of wastewater is produced during vintage. In the past, the wastewater was disposed of by evaporation and direct discharge into water courses. Future management of the natural water resources within Australia will ultimately lead to the use of winery wastewater as a supplementary source of water for irrigation of vineyards.

This thesis establishes a model for the treatment of organic materials in wastewaters produced by the wine industry by irrigation of soils. The aim is to: (I) promote the reuse of water for crop production, and (II) the utilisation of the organic carbon in the wastewater to improve the fertility and structural stability of soils, through its conversion to humus.

Winery and distillery wastewaters were analysed to determine the origins and concentrations of the major organic and inorganic components. Winery wastewater contained 0.9–3.7 g/l total organic carbon (TOC) during vintage, which decreased to <1.5 g/l during non-vintage. Wastewater generated from the distillation of lees (residues of the fermentation operation) typically contained >11 g/l TOC. Greater than 90% of the TOC of winery wastewater was present in soluble forms, whereas soluble organic materials made up to 55% of the TOC of distillery wastewater. Simple carboxylic acids, sugars and alcohols dominated the organic composition of the wastewaters.

The salinity of winery wastewater was highly variable ranging from <1 to 3.5 dS/m. Juice and wine added both organic and inorganic salts, whereas the external supply of water, and chemicals used for processing, cleaning, and sterilisation were important sources of inorganic salts. During vintage a greater abundance of organic acids relative to caustic cleaning wastes was produced, resulting in a more acidic wastewater (pH 4–6) than during non-vintage (pH 6–8). Distillation of lees always produced a highly saline (>6 dS/m), and acidic (pH <4) wastewater.

The removal of soluble organic carbon in winery and distillery wastewaters after their application to soil was determined. Uniformly labelled ^{14}C-lactic acid and glycerol were added to typical winery and distillery wastewaters. Decreases in the solution concentrations of ^{14}C-compounds in soil extracts and the amounts of added ^{14}C evolved as $^{14}CO_2$ were determined.

Between 10–50% of the ^{14}C-compounds were adsorbed by the soils within the first hour after application of the wastewaters. Microbial uptake and metabolism was responsible for the removal of ^{14}C-compounds remaining in solution to concentrations of <5% of ^{14}C input. Removal times were dependent on the length of the lag phase, if present, and on the rate of metabolism of ^{14}C-compounds in solution. Metabolism of ^{14}C-compounds involved the formation and subsequent turnover of ^{14}C within the microbial biomass and products, as well as mineralisation to $^{14}CO_2$. A logistic model was used to quantify the adsorption and microbial metabolism of the ^{14}C-compounds.

The effects of organic loading, soil texture, and acclimatisation of microbial populations on the removal of soluble organic carbon were determined. Increases in organic loading were accompanied by higher solution concentrations of ^{14}C-compounds after initial adsorption, and often prolonged lag periods. Thus, the soils took longer to reduce the solution concentration of ^{14}C-compounds to <5% of ^{14}C input.

Acclimatisation of microbial populations was found to occur in 2 stages. Adaptation of the microbial population in soils newly-exposed to the wastewater after 3 to 17 applications involved a concurrent decrease in the length of the lag period and an increase in the rate of microbial metabolism of ^{14}C-compounds in solution. The second level of acclimatisation involved the maintenance of sufficient populations of active microbial cells through regular application of the wastewater. An interaction with acclimatisation influenced the rates by which soils of different texture metabolised the ^{14}C-compounds.

Acclimatised soils were able to reduce the solution concentration of ^{14}C-compounds added in winery wastewater to <5% of ^{14}C input within 1 day after application. This nominal level of treatment could be achieved when the lag period was <2 hours in duration, and when microbial metabolism resulted in a loss of soluble-^{14}C/day exceeding the ^{14}C input.

By contrast to microbial metabolism, adsorption of ^{14}C-compounds by the soils varied little with repeated irrigations with wastewater.

The above results were confirmed using undisturbed soils, which accounted for the effects of: (I) the movement of water during irrigation on the distribution of the organic substrates, and (II) the ability of the soils wetted to field capacity to transport oxygen to the sites where it is required for microbial metabolism. Acclimatised soils were able to reduce the solution concentration of ^{14}C-compounds by >85% of ^{14}C input within 1–2 days after the application of winery wastewater to 10% of the gravimetric moisture content of the 0–15 cm layer at - 10 kPa.

A different balance of mechanisms was involved in the removal of organic carbon from distillery wastewater containing suspended material. Removal of suspended material was due to physical filtration. Of the soils studied, the fine, non-structured sand (solod) was more effective in filtering out suspended material, than the structured sandy loam (brown

earth). Evolution of $^{14}CO_2$ was low from both soils in this treatment, which suggested that only the "readily available" soluble organic compounds were being mineralised.

The above results were used to develop a model for the management of soils irrigated with winery wastewaters. The model partitioned the soil into: (I) the 0–15 cm layer in which adsorption and metabolism by aerobic populations of micro-organisms would reduce the solution concentration of organic carbon by 95% of input levels within 1–2 days after application of the wastewater, and (II) the 15–100 cm layer to provide a buffer in which further reduction in the solution concentration of organic carbon would result from adsorption and metabolism by anaerobic populations of micro-organisms.

A number of implications for the management of the volume and composition of wastewaters produced by wineries and distilleries, of irrigation systems, and of soils arise from this work. These have been outlined, along with suggestions for further scientific inquiry.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief this thesis contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying, with acknowledgment.

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Jeanette Anne Chapman

August, 1995

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CHAPTER 1

INTRODUCTION

The Australian wine industry is characterised by its concentration within a number of major locations, primarily in South Australia (50% of total production), New South Wales (25%), and Victoria (20%). In the 1992/93 season approximately 6×10^5 tonnes of grapes were yielded for winemaking. For each tonne of grapes crushed 3–5 kl of wastewater are generated. Thus winery wastewater represents a considerable resource of water.

In the past the Australian wine industry disposed of its wastewater primarily by evaporation or direct discharge into watercourses at times of high flow. Malodours and other problems associated with anoxic conditions caused by the high organic loading of the wastewater, have led to these methods of disposal becoming environmentally and aesthetically unacceptable. In recent times management of the scarce natural water resources within Australia has caused the wine industry to seek information to develop protocol for the management of its wastewater as a supplementary source of water for irrigation of vineyards.

Tare and Bokil (1982) stressed the need to develop appropriate systems for land application of wastewaters which allowed acceptable rates of irrigation, and acceptable levels of improvement of water quality to avoid pollution of groundwater. Wastewater produced by the wine industry has a characteristically high organic carbon content, yet little is known about the fate of the organic carbon in soil. For this reason work was undertaken to quantify the removal of soluble organic carbon from wastewaters produced by the wine industry, by irrigation of soil. The term "wastewater" is used here to refer to the untreated liquid waste.

Little information exists on the identities and relative concentrations of the dominant organic and inorganic components of the wastewaters, and the proportion of the total organic carbon content contained in soluble forms. Hence the composition and origins of the major organic and inorganic components of winery and distillery wastewaters, analysed as part of the initial investigations, have been described.

Soil has been actively used for the disposal of organic-rich wastewaters and the reclamation of water for many years. In the past, quantitative information on the removal of organic carbon from wastewaters in irrigated soils has been based on measurement of the reduction of the biological oxygen demand (BOD) or chemical oxygen demand (COD) of the soil solution (eg., Gambrell and Peele 1973, Jewel 1976, Tare and Bokil 1982). This approach has provided useful information on the depth below which no significant reduction in BOD or COD would be expected, and the effect of particle-size distribution of soils on the efficacy of treatment of the wastewaters.

The inherent weakness of the above approach is that it does not provide information about the processes responsible for the reduction of the BOD or COD. For this reason work was undertaken to establish the roles of adsorption and microbial metabolism in the removal of organic compounds from solution, and to determine the optimal soil conditions needed for rapid removal of the soluble organic carbon added in wastewaters produced by the wine industry by irrigation. A description of the methodology is given, followed by a discussion of the results.

This thesis aims to examine a basis for promoting: (I) the reuse of water for irrigation of vineyards, and (II) the utilisation of the organic carbon in the wastewater to improve the fertility and structural stability of soils, through its conversion to humus. Hence recommendations on the management of irrigation of sites used for the reuse of organic-rich wastewaters will be given, and the impact of the wastewaters on the environment will be discussed.

CHAPTER 2

DISPOSAL OF ORGANIC-RICH WASTEWATERS BY IRRIGATION OF SOILS

2.0   Introduction

Food industries generate wastewater from water used for cleaning purposes, and to a lesser extent processing operations. The wastewaters have a characteristically high total organic carbon content, and a high requirement of oxygen for biological decomposition (Table 2.1), although the organic composition varies considerably between industries, and even within an industry. The total organic carbon content of a wastewater consists of dissolved materials and suspended solids (Table 2.1), which are defined by Rump and Krist (1992) as particles which have a diameter ≤0.45 μm or >0.45 μm, respectively.

Irrigation systems have been traditionally used in the management of wastewaters for the disposal of water after varying levels of pretreatment (Tcholbanoglous and Burton 1991). The soil is relied on to remove the remaining BOD and inorganic nutrients before the water enters aquifer systems. Insufficient removal of the BOD may indirectly lead to degradation of geological matrices forming the aquifers and mobilisation of colloidal materials (McCarthy and Zachara 1989). Excess leaching of nitrates and phosphates can result in the loss of potable quality of aquifers, and facilitate eutrophication in waterways that leads to algal outbreaks (Lehane 1995).

The above problems highlight the need for better management of the organic carbon and nutrients added in irrigation wastewaters particularly: (I) their transportation and distribution within the soil matrix, and (II) removal from the soil solution, which will be reviewed here.

Table 2.1: Biological oxygen demand (BOD), and suspended solids content (SS) of wastewaters generated by food industries in South Australia.

	ANIMAL PRODUCTS			PLANT PRODUCTS	
	SS (g/l)	BOD (g/l)		SS (g/l)	BOD (g/l)
Meat Processors			*Beverages*		
Abattoirs	0.3 – 1.2	2.0 – 3.5	Brewery	0.5 – 1.5	2.3 – 4.0
Poultry	0.2 – 0.5	1.2 – 1.6	Maltsers	0.1 – 0.4	1.5 – 5.0
Fish	0.1 – 0.3	0.3 – 0.9	Fruit juice	0.2 – 0.5	3.0 – 4.0
Smallgoods	0.1 – 0.8	0.8 – 2.0	Soft Drinks	0.1 – 0.2	0.5 – 3.0
Dairygoods			Winery	0.3 – 1.8	0.5 – 7.0
Ice cream	0.8 –1.3	<1.0 – 3.4	Distillery	1.0 – >30	15 – 35
Milk	0.8 – 1.0	3.0 – 4.0	*Other*		
Cheese	0.8 – 1.3	<1.0 – 2.5	Bakery	0.2 – 0.8	0.7 – 1.7
			Restaurants	0.2 –6.0	0.8 – 2.3

Source: B. Townsend, State Water Laboratories of South Australia (pers. comm.)

2.1 Water as a medium for the transport of solutes

Water is the primary medium in which organic materials in wastewaters are transported in soil. Since water is potentially able to move from soil to other environments (Figure 2.1), an important aim of management of irrigation is to optimise the removal of organic materials in the wastewater by adsorption and microbial metabolism before the water moves from soil to other environments. Surface runoff is not normally allowed from sites irrigated with wastewaters, however, water is allowed to move off-site underground, eventually reaching groundwaters (Thomas 1991). Evapotranspiration is also an important pathway for the removal of water (Figure 2.1).

2.1.1 Distribution of water and nutrients under different irrigation systems

The potential for excess leaching of dissolved organic carbon and nitrates can be strongly influenced by their distribution within the soil matrix during irrigation. The type of system used for irrigation of wastewaters can affect the uniformity by which the upper layer of soil is wetted, and the depth of penetration of the water front, and hence the uniformity and depth to which the organic carbon is distributed within the soil matrix.

Trickle systems rely on the soil matrix to distribute the water both horizontally and vertically. Scotter and Clothier (1986) found that capillary forces dominated the initial stages of irrigation, allowing the water to fan out hemispherically. With time gravitational forces became more apparent, and hence the water front began to move preferentially in the vertical rather than the horizontal direction. As a result, the upper few cm were not wetted uniformly. In contrast to trickle irrigation, sprinkler systems are designed to provide an even horizontal coverage. As a result, the soil matrix distributed the water vertically during irrigation, allowing more even wetting of the upper layer (Scotter and Clothier 1986).

INPUTS

Precipitation

Irrigation

OUTPUTS

Evapotranspiration

ATMOSPHERE

Infiltration and subsurface drainage

vertical

lateral

SURFACE WATER

GROUNDWATER
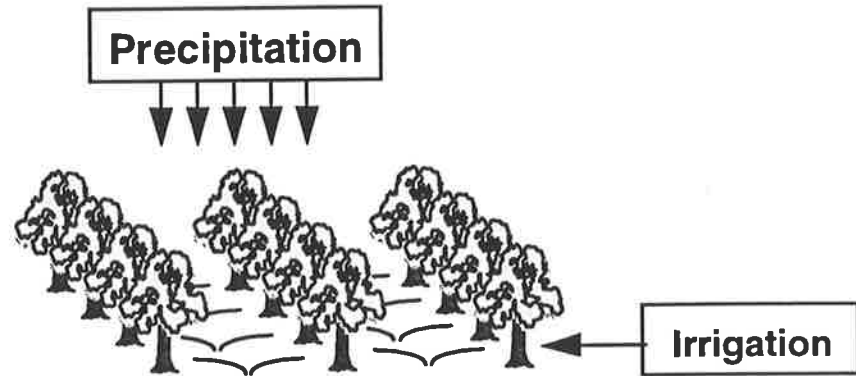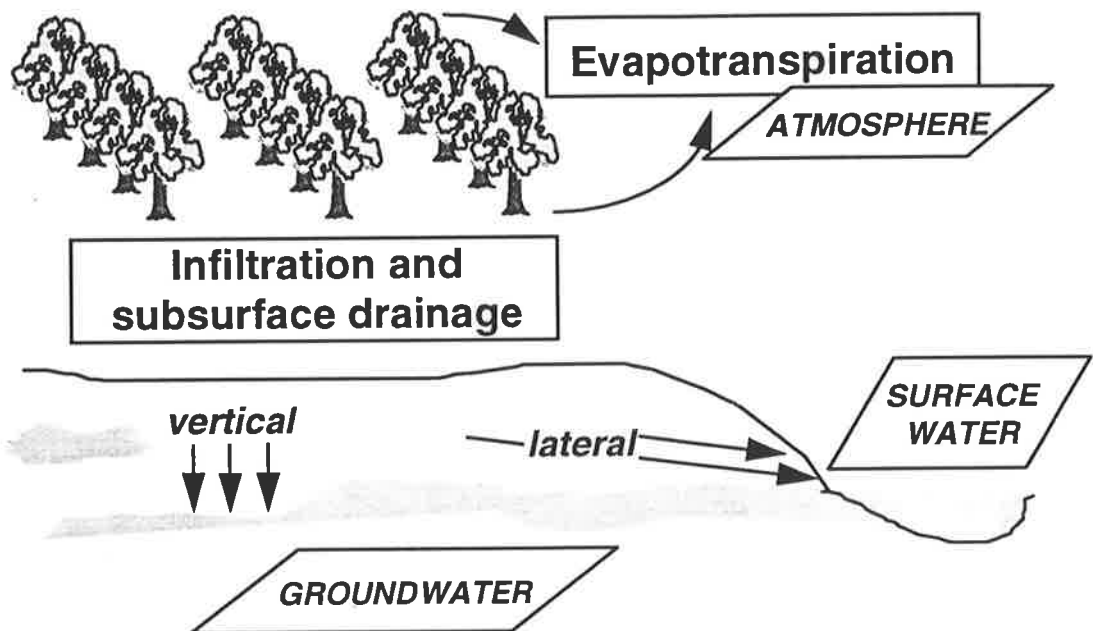
Figure 2.1: Inputs and major outputs of water from soil irrigated with wastewater.

Scotter and Clothier (1986) found that soil near a dripper tended to become saturated, or even pond during irrigation. For this treatment, the rate of infiltration of water into the soil was controlled by the infiltration capacity of the ponded soil, rather than by the rate of application. As a result, the trickle system resulted in deeper penetration of the water front during irrigation, compared with the sprinkler system, when the same volume of water was applied to the soil. Troiano et al. (1993) found that flood irrigation also resulted in deeper penetration of water, than when the same volume of water was applied through a sprinkler system. The authors attributed differences in the depth of penetration of water during irrigation to the lower efficiency of sprinkler systems in delivering water to the soil surface, as the result of evaporation of the droplets. It would have been interesting to compare distribution patterns after the same volume of water had been added to the soil surface.

2.1.2   Movement of the water and solute fronts

During irrigation, water is added to the surface layer of soil at an average rate (I, m/h). The water infiltrates into the profile behind a wetting front where the initial volumetric moisture content (θ_I, m^3/m^3) is raised to a new level (θ, m^3/m^3) as the percolating water fills part of the existing air space (Clothier and Scotter 1985). Assuming homogeneity of the soil, the depth of penetration of the water front per unit time (Z_{WF}, m/h) is given by:

$$Z_{WF} \;=\; I/(\theta - \theta_I) \qquad\qquad [2\text{--}1]$$

Movement of solutes contained in the irrigation water requires displacement of the initial soil solution ahead of the invading chemical front (Z_{CF}), which moves at the slower rate:

$$Z_{CF} \;=\; I/(\theta) \quad (m/h) \qquad\qquad [2\text{--}2]$$

Movement of the chemical front is further retarded due to interactions with clay and organic colloids (R, dimensionless). The extent of the interactions can be estimated from the adsorption distribution coefficient (K, cm^3/g), which expresses the partitioning of solutes between the bound and solution phases (Smettem 1986). Thus the rate of penetration of the chemical front will be a function of the rate of infiltration, the water

holding capacity of the soil, and the degree of retardation of the solutes due to interaction with the solid phase (Clothier and Scotter 1985, Smettem 1986), where:

$$Z'_{CF} \ = \ Z_{CF} / R \qquad\qquad\qquad [2\text{--}3]$$

$$R \ = \ 1 + (\rho K / \theta) \qquad \rho \text{ is the bulk density of the soil (g/cm}^3).$$

Figure 2.2 shows the relationship between movement of the solute front in relation to that of the wetting front. Essentially the water front is displaced ahead of the solute front by an amount equivalent to the initial volume of water present in the soil (θ_I). Further retardation of the solutes will depend on the level of interaction with soil constituents.

Over the range of irrigation rates normally used, it is assumed that the change in mean volumetric moisture content in homogeneous soils (eg., sands) is unaffected by the intensity of irrigation. When no surface runoff occurs the mean residency time of the chemical front within a given depth is linearly related to the inverse of the volume of irrigation water (Fig. 2.3A; Smettem 1986).

In structured soils lower rates of irrigation lead to more uniform wetting due to exclusion of larger pores from the infiltration process (Scotter and Clothier 1986), resulting in better exchange of water and solutes within aggregates with those of the percolating solution (Youngs and Leeds–Harrison 1990). As a result, the mean residency time of both the water front and the solute front within a given depth is increased (indicated by a higher value of θ). Since adsorption is also time dependent, the amount of adsorption will be higher in relation to the rate of flow at lower intensities of irrigation (indicated by a higher value of R), thereby extending the lag time between the movement of the solute front relative to the movement of the water front (Figure 2.3B; Smettem 1986).
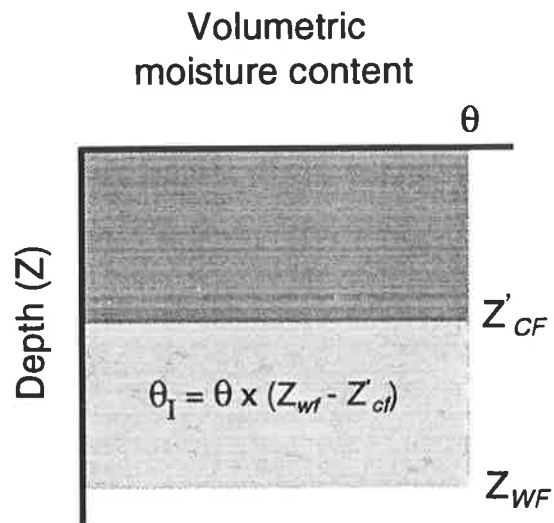
Figure 2.2: Theoretical movement of the water and chemical fronts through a homogeneous soil. θ_I and θ are the initial and final moisture contents of the soil; Z_{WF} and Z'_{CF} are the depths of the water and chemical fronts after a given time (h).

A

Mean
residency
time
within
depth
"Z"

Rate of irrigation

B

Mean
residency
time
within
depth
"Z"

———Wetting front
·····Chemical front
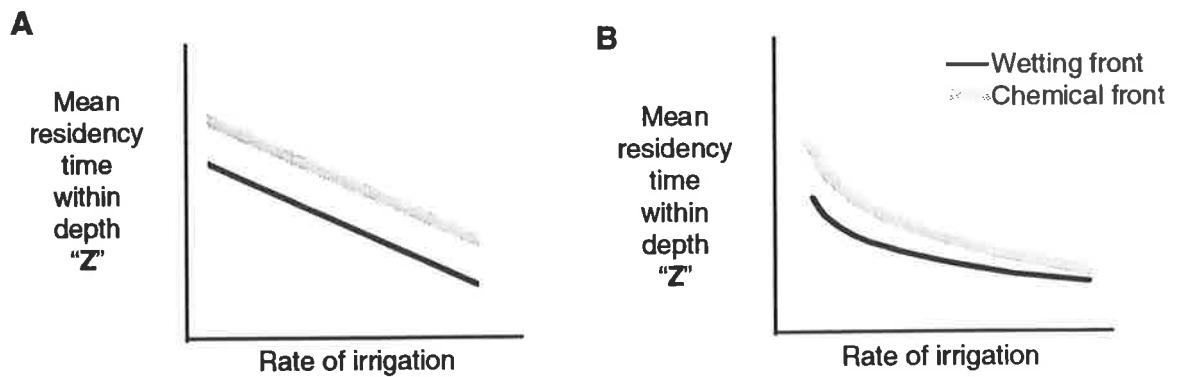
Rate of irrigation

Figure 2.3: Schematic representation of the mean residency time of the water front and chemical front as a function of the intensity of irrigation assuming: A) constant θ and K, and B) variable θ and K. It is assumed that the infiltration capacity of the site is not exceeded.

(Adapted from Smettem 1986)

2.1.3 Movement of suspended solids

Suspended solids may vary in diameter from 0.001 to greater than 1 mm. Thus, the downward movement of particulate materials can be hindered by their large size and irregular shape, and hence much of the particulate material can be removed from the infiltrating water by physical filtration (Metcalf and Eddy 1980; McCarthy and Zachara 1989). Colloidal material (<10 μm in diameter) is known to reach groundwater systems (McCarthy and Zachara 1989), its movement apparently facilitated by preferential flow though cracks and macropores (Jardine et al. 1989).

2.2 Removal of organic materials in wastewaters by adsorption and microbial metabolism in soils.

Optimal management of irrigation systems used for the disposal of organic-rich wastewaters requires an understanding of: I) the respective roles of adsorption and of microbial metabolism in the rapid removal of the organic substrates (ie., <1 week), and II) the requirements for long-term sustainability of the two processes in soils.

2.2.1 Adsorption

Adsorption is a physico-chemical process which involves the increase of solute at the soil-water interface (Sims et al. 1986). Adsorption has a dual role: I) to retain the organic materials in the surface soil following infiltration and transport of the wastewater during irrigation (as explained in Section 2.1.2), where microbial population densities and oxygen levels are high, and II) to reduce the solution concentration of the organic substrates.

Adsorbed materials exist in dynamic equilibrium with solutes in the soil water. Hence the extent of adsorption is largely determined by the sorptive capacity of the soil, and by the chemical characteristics of the organic sorbates and competing species. Environmental factors which change any of the above conditions, eg., irrigation with wastewater, will alter

the equilibria, and hence can lead to a portion of adsorbed materials re-entering the soil solution and *vice versa* (Weber et al. 1991). Table 2.2 lists some of the important factors determining sorptive behaviour in soils.

Adsorption often consists of 2 phases, a rapid initial phase of non-specific adsorption, lasting from a few minutes to a few hours, followed by a slower phase of specific adsorption, which may take from a week to a few months to reach equilibrium (Sims et al. 1986). Non-specific adsorption involves rapid attraction via weak electrostatic forces, whereas specific adsorption may involve diffusion within the micropores of aggregates, and/or the displacement of water from surfaces to make way for the formation of stronger sorbate-sorbent bonds (Pignatello 1989). Thus non-specific forces appear to be responsible for adsorption of organic substrates in wastewaters during the relatively short infiltration stage, and in the time elapsed from irrigation to the onset of rapid microbial metabolism.

Adsorption may not always guarantee immobilisation, since the adsorbents can themselves be mobile. Adsorption of small non-polar organic contaminants has been found to be strongly correlated to the organic carbon content of soils (Bailey and White 1970). Part of the total organic carbon content of soils consists of suspended colloidal materials, which can be mobile within the solution phase (McCarthy and Zachara 1989).

2.2.2 Microbial metabolism

Microbial activity accounts for the metabolism of more than 90% of the soluble organic carbon in wastewaters into other forms, mainly by oxidation to carbon dioxide, and assimilation into microbial biomass and products. Uptake by plant roots and other soil-dwelling fauna account for the removal of the remaining carbon (Scow 1982). Thus,

Table 2.2: Major factors influencing sorptive behaviour of organic materials in soils[†].

Properties of the sorbent	Properties of the sorbate	Environmental Factors
• clay content	• chemical form	• pH
• type of clay mineral	-ionic	• redox potential
• cation exchange capacity	-polar	• temperature
• presence of iron and	-non polar	• moisture content
aluminium oxides	• size and shape	• chemical form and
• organic carbon content	• concentration	concentration of
	• solubility in water	competing species

† Descriptions of the effects of the above factors can be found in Bailey and White (1970), Sims et al. (1986, 1991), Calvert (1989), McCarthy and Zachara (1989), and Weber et al. (1991).

microbial metabolism is essential to ensure the near complete removal from the soil solution of organic materials added in wastewaters by irrigation. Factors affecting the rate and the extent of microbial metabolism of organic substrates are summarised in Table 2.3.

2.2.2.1 *Properties of the substrates*

According to Scow (1982) the size, composition, and solution concentration of organic substrates can strongly influenced both the pattern and rate of microbial metabolism. Monomeric sugars, and low molecular weight alcohols and carboxylic acids, which often dominate the organic carbon content of wastewaters generated by food industries, show a phase of rapid transformation to carbon dioxide and microbial biomass, ending abruptly when the substrate is exhausted, and subsequent breakdown depends on turnover of the biomass itself (Haider and Martin 1975, Van Veen et al. 1985).

The phase of rapid transformation may not be as pronounced for polymeric, or branched substances, or be delayed as the initial structure is transformed into one which can subsequently be readily degraded. No appreciable degradation may be shown by complex substances, or by halogenated man-made substances (Alexander 1973, Scow 1982). Haider and Martin (1975) found that simple sugars, amino acids, carboxylic acids, and phenolics were rapidly and extensively mineralised, but when present in polymers, the same substances were stabilised against biodegradation. An extensive summary of the effects of branching, chain-length, oxidation, saturation, and substitution on rates of biodegradation of organic substances is provided by Scow (1982); an outline is given in Table 2.4.

The solution concentration of organic substrates is determined in part by their solubility in water, and by adsorption processes. According to Alexander (1973) organic compounds which have a low solubility in water persist longer in soils than water-soluble compounds,

Table 2.3: Factors affecting microbial metabolism of organic substrates in soils[†].

SUBSTRATE	ORGANISM	ENVIRONMENT
• size	• composition	• temperature
• physico-chemical composition	• spatial distribution	• pH
	• population density	• moisture
• concentration	• previous history	• oxygen availability
	• interactions—intra or interspecific	• salinity
		• nutrition, particularly available nitrogen
	• enzymatic make-up and history	• toxins
		• other eg., adsorption reactions

† Descriptions of the effects of the above factors can be found in Scow (1982), and Oades (1989).

Table 2.4: Effects of the composition of organic compounds on rates of biodegradation.

FACTOR	COMMENTS
Branching	• highly branched compounds are more resistant to biodegradation than unbranched compounds.
Chain Length	• short chains are not as easily degraded as long chains.
Oxidation	• highly oxidised compounds may resist further oxidation under aerobic conditions, but may be more readily degraded under anaerobic conditions.
Saturation	• unsaturated aliphatics are more readily degraded than corresponding saturated hydrocarbons.
Substitution	• increased substitution, and substitution at the meta position of ring compounds usually lowers the biodegradability of organic compounds (there are exceptions); • replacement of hydrogen in aliphatic alcohols or carboxylic acids with a methyl group, or a halogen reduces the rate of biodegradation.

Source: Scow (1982, Table 9-20)

due to the former having a reduced ability to reach reaction sites in microbial cells. Miller and Alexander (1991) found that the rate of microbial decomposition of benzylamine in a clay suspension was lower than in the same solution containing no clay. The authors attributed the differences to adsorption of benzylamine to the clay particles which lowered the solution concentration available for biodegradation. Rajbanshi et al. (1992) found that the rate of microbial metabolism of dicyandiamid in a silty loam increased when a higher concentration of the substrate compound was added to the soil. The duration of the lag period also increased with increasing solution concentration of diacyandiamid, which led to the soil taking longer to remove the substrate compound.

Simple and low molecular weight organic components of wastewaters are likely to be removed from the soil solution by microbial metabolism, whereas the removal of polymeric and/or complex components, which are commonly insoluble and present in colloidal solution and suspension, may be reliant on adsorption and on physical filtering. Jewel (1976) found that following the application of food processing wastewater, consumption of oxygen was most rapid during the first 24 hours, falling off rapidly to a low constant rate, which was similar to the pattern of degradation shown by readily biodegradable substances. It may be possible to use the times elapsed from irrigation to the end of rapid microbial metabolism of the readily available soluble organic carbon, to estimate the minimum times required between successive irrigations with wastewaters for treatment of the organic substrates. Thus, the proportion of the total organic carbon content of the wastewater present in readily degradable forms will be an important determinant of both: (I) the extent of removal of the organic carbon from the soil solution, and (II) the time taken.

2.2.2.2 *Properties of the microbial populations*
The availability of organic substrates and of water usually limits the capacity of soil to support populations of micro-organisms (Oades 1989). For sites irrigated with wastewater
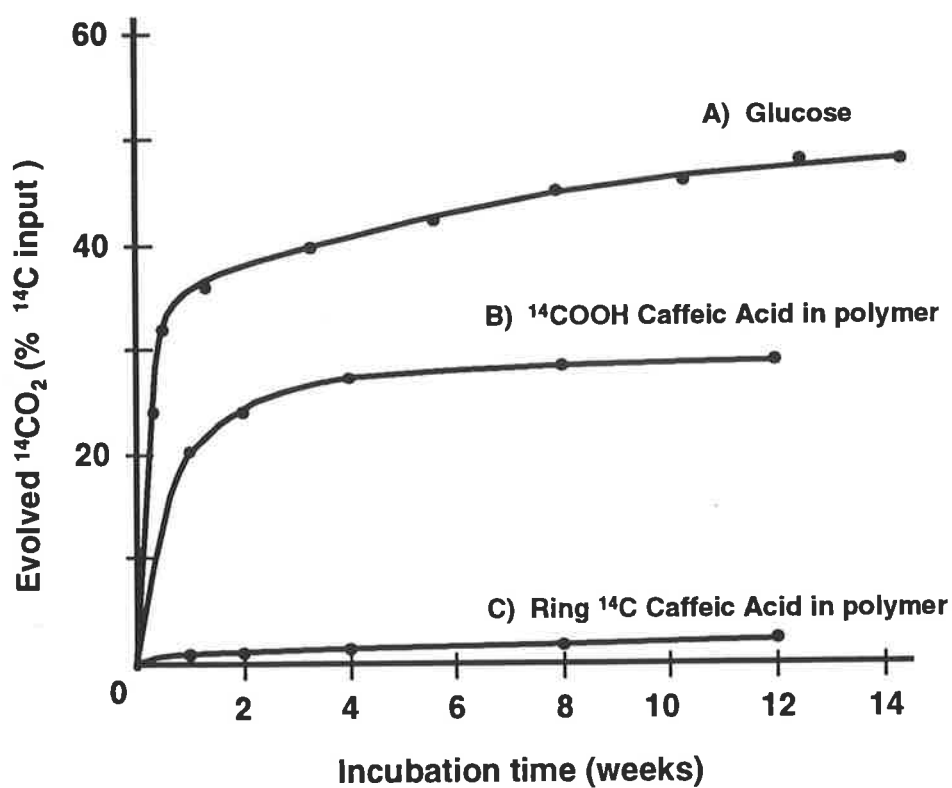
Figure 2.4: Patterns of biodegradation. Data for glucose from Van Veen et al. (1985), and for $^{14}COOH$ caffeic acid in polymer and ring caffeic acid in polymer from Haider and Martin (1975).

rich in organic carbon, the density of microbial populations is more likely to limit the capacity of soil to rapidly remove the organic substrates. Low population densities can lead to observed delays (lag phases) in the onset of microbial metabolism of organic substrates (Scow 1982). Thus, rapid microbial metabolism of the organic carbon added in wastewaters by irrigation may be largely confined to the upper 20–30 cm depth of soil, where the majority of microbial populations exist, and where nutrient and oxygen levels are high (Paul and Clark 1989). Gambrell and Peele (1973) and Tare and Bokil (1982) found that the greatest reduction in the chemical oxygen demand of leachate collected from soils irrigated with wastewaters occurred within the upper 10–15 cm of the soils.

Prior exposure of microbial populations in soils to the organic substrates can significantly increase the rate of biodegradation following a subsequent application of the organic compounds (Scow 1982). Jewel (1976) found that soils containing microbial populations adapted to the organic substrates (acclimatised) were able to degrade the organic materials added in wastewater generated by the processing of vegetables within 48 hours following irrigation, whereas soils newly-exposed to the wastewater (non-acclimatised) could not fully degrade the organic materials in one week. Consumption of oxygen was almost three times higher in the acclimatised soils than in the non-acclimatised soils after application of the wastewater. Rajbanshi et al. (1992) found that pretreatment of a silty loam with dicyandiamid eliminated lag periods, and significantly increased the rates of microbial metabolism following a further application of dicyandiamid, compared with the same soil not previously exposed to the substrate compound.

2.2.2.3 *Effects of the environment*

Environmental factors can either directly affect the reaction rate, eg., temperature, or indirectly influence microbial metabolism, through effects on the availability and chemical form of the substrate and/or concentration and activity of micro-organisms. The proportion of the added organic substrates mineralised to carbon dioxide, or assimilated into microbial biomass is also influenced by environmental factors (Scow 1982). At similar rates of

carbon loading, mineralisation is normally more rapid in sandy soils, whereas heavy-textured soils retain a larger proportion of residual carbon as biomass (Kunc and Stotzky 1974, Marshall 1975, Van Veen et al. 1985, Ladd et al. 1992). Marshall (1975) suggested that heavy-textured soils enhanced survival of micro-organisms added to soils due to their better protection from predators. Enhanced turnover of biomass carbon, following rewetting of initially dry soils, was also attributed to greater predation of the biomass (Van Veen et al. 1985).

The application of organic-rich wastewaters can have a dramatic effect on the soil environment. The single most immediate and important effect is the high requirement of oxygen for microbial metabolism of the organic substrates, which will be discussed in section (2.3). Rudd (1987) found that the continued irrigation of a red brown earth with wastewater generated by a winery led to an increase in the organic carbon content, and contents of nitrogen, phosphorus, and potassium within the 0–10 cm layer of the soil. These additional levels of energy and nutrition are likely to increase the capacity of the soil to support larger populations of micro-organisms, and hence lead to reduced times for the removal of the soluble organic substrates in the wastewater after subsequent irrigations.

Many different types of microbial populations are likely to contribute to the metabolism of organic substrates added in wastewaters by irrigation, with each population having its own set of optimal environmental condition.

2.2.3 Interactions between adsorption and microbial metabolism of organic compounds

Both sorption and microbial metabolism are optional processes providing alternative sinks for soluble forms of organic carbon (Stevenson 1976, Sims et al. 1986, Calvert 1989). Figure 2.5 shows the inter-relationships between the two processes in the removal of organic materials from wastewaters disposed of by irrigation of soils.
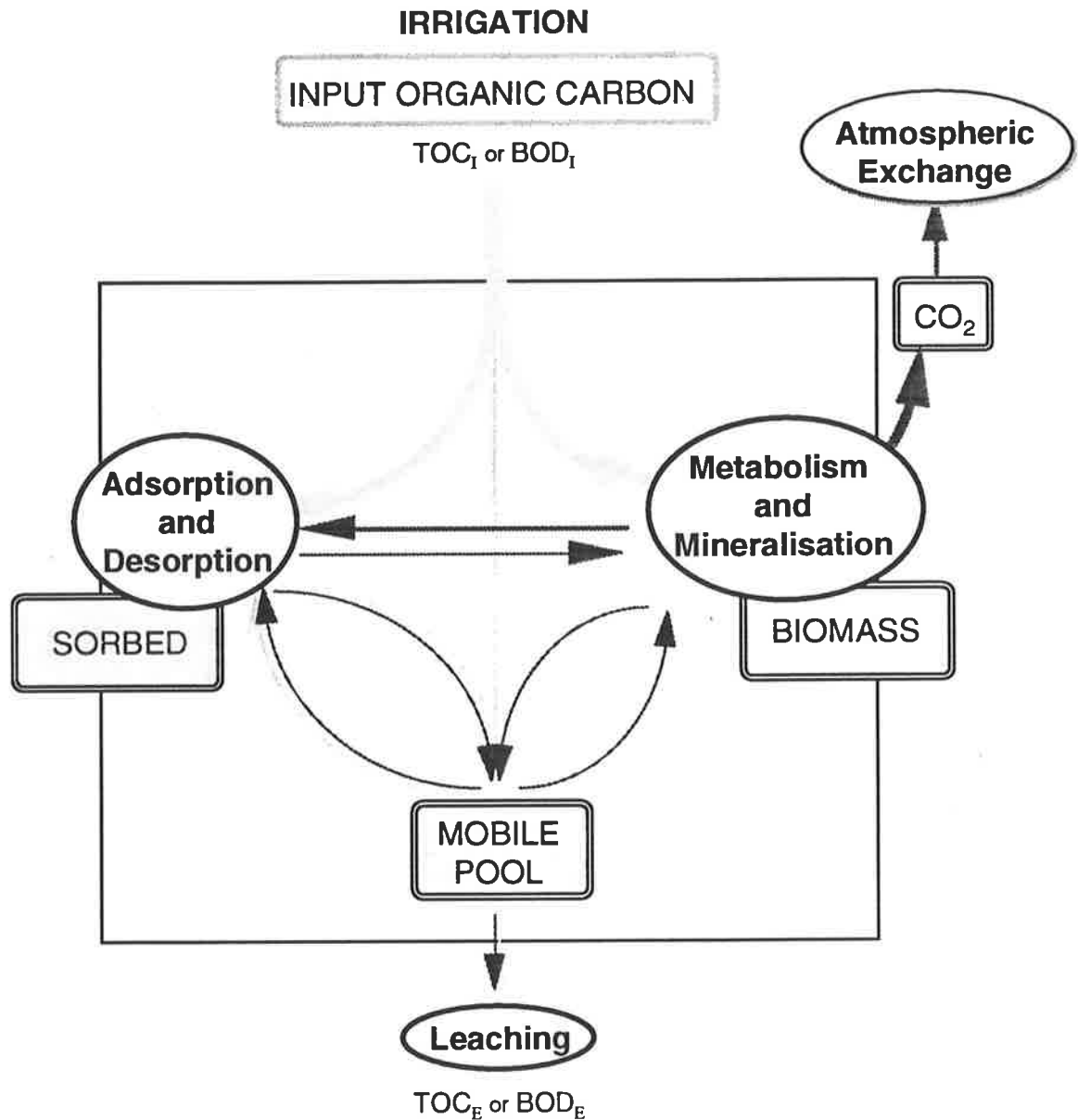
Figure 2.5: Inter-relationships between adsorption and microbial metabolism in the removal of organic materials from wastewaters by irrigation of soils. The light grey lines represent substrate organic carbon, the dark grey lines represent altered products, the shaded boxes represent major pools of the organic materials, and the circled boxes represent processes involved in the movement of the organic carbon from different pools, and from soil to other environments. The carbon loading of influent (I) and effluent (E) waters is measured as total organic carbon (TOC) and/or biological oxygen demand (BOD).

Key information on the availability to microbial populations of adsorbed substrates, and the effects of microbial metabolism of substrates from the soil solution on desorption, have been difficult to quantify under natural field conditions. Studies have indicated that adsorbed organic materials are generally not available for uptake by micro-organisms, and hence must be desorbed before they can be metabolised (Sims et al. 1991, Weber et al. 1991). Sustained availability of existing adsorption sites may, in turn, be dependent on microbial metabolism of the organic substrates in solution. Miller and Alexander (1991) found that the removal of benzylamine from the soil solution as a result of microbial decomposition promoted desorption of initially adsorbed benzylamine. Models on the persistence of pesticides in soil, have demonstrated that adsorption can provide a reservoir of pesticide, which can re-enter the solution phase in response to declining solution concentrations (Yaron et al. 1985, Jury et al. 1987).

2.2.3.1 Nitrogen and mineralisation

Total nitrogen in soils exists in a variety of chemical forms, of which nitrate is most readily absorbed by micro-organisms and plants (Scow 1982). To meet the demand for nitrate, micro-organisms will convert part of the nitrogen present in organic forms and as ammonium to nitrate during mineralisation of organic carbon. Nitrate not utilised by the micro-organisms or absorbed by plants can pose an environmental risk due to its susceptability to leaching (Lehane 1995).

According to Lehane (1995) irrigation leads to a sharp increase in the rate of mineralisation of organic nitrogen in soil. This may be advantageous for the rapid treatment of organic-rich wastewaters, since the rate of metabolism of organic carbon is enhanced by higher concentrations of nitrate (Scow 1982).

2.3 Meeting the requirement of oxygen for prevention of malodours.

Levels of oxygen contained in wastewaters generated by the food industry are insufficient to meet their biological oxygen demands (BOD), and hence the balance must be met in the soil. Oxygen is about 21% by volume of the free atmosphere. Assuming that 10% of the total pore volume remains air-filled after irrigation, a soil with a total pore volume of 50% will contain about 15g of oxygen per m^3. Given that most wastewaters generated by the food industry have a BOD in excess of 0.5 g/l (Table 2.1), microbial metabolism of the organic substrates will create an immediate deficit of oxygen in most soils. For example, if 50 litres of wastewater with a BOD of 1.5 g/l are applied to $1m^2$ of the soil and penetrates to a maximum depth of 0.2m, the atmosphere within the pore spaces would need to be renewed at least 25 times to provide the necessary oxygen for microbial metabolism of the organic substrates. Competition for the oxygen, by microbial populations located closer to the soil surface for metabolism of the organic substrates, would further limit the supply of oxygen to microbial populations at depth.

Jewel (1976), and Russell et al. (1976) highlighted the need to allow sufficient times between successive irrigations with wastewaters generated by the food industry to allow the soil to become aerobic, in order to maintain a high capacity within the soil to microbially metabolise organic substrates added by the wastewaters.

2.3.1   Oxygen exchange into soils

Oxygen exchange into soil occurs by a combination of mass flow and diffusion. Diffusion is considered to be the more important process, as mass flow is normally confined to the upper 15-20 cm of the profile, and can be substantially reduced by restrictions in the circulation of air at the soil surface (Hillel 1980). Diffusion of oxygen is about 10 000 times more rapid in air than in water. Oxygen exchange in soil will thus be largely dependent on the air-filled porosity (Hillel 1980).

Air-filled porosity is determined by the balance of air versus water in the pore spaces. Following irrigation, only macropores >30 μm in diameter (corresponding to a "field capacity" drainage suction of 10 kPa) are expected to remain air-filled (Oades 1984). The presence of interconnecting macropores is therefore essential for maintaining sufficient rates of oxygen diffusion. Oades (1984), and Tisdall and Adem (1985) concluded from research on the ideal properties of soils used for irrigation, that a minimum of 10–15% of the total porosity should be present as macropores, to allow adequate aeration and drainage.

Rates of diffusion of oxygen through even the largest macropores is still much lower than through dry, atmospheric air, since air-filled pores are often at 100% relative humidity (Hillel 1980). Stolsky et al. (1961) examined the effects of moisture content on the rate of diffusion of oxygen into soil. The rate of diffusion of oxygen into the soil was reduced by half in saturated conditions.

The optimal moisture content for adequate aeration of soils may also be dependent on the type of wastewater used for irrigation, and the carbon loading. Rice et al. (1988) and Neilson and Pepper (1990) used the evolution of carbon dioxide as an index of soil aeration, to establish for a given type of soil, and rate of application of sewerage sludge, a maximum moisture content above which diffusion of oxygen into the soil was limiting mineralisation of the organic substrates (and hence promoting denitrification processes). The moisture content at which aeration became limiting, decreased as the rate of application of the sludge was increased. Increasing the bulk density of the soils also decreased the moisture content at which aeration became limiting, probably due to the loss of macroporosity.

2.4 A model for the management of irrigation systems used for the disposal of organic-rich wastewaters

2.4.1 Aims of management

As mentioned in Section 2.1, soil can potentially allow water, and hence contaminants to move to other environments. Malodours produced during storage of the wastewaters can create a public nuisance. Management of irrigation systems used for the disposal of wastewaters rich in organic carbon will therefore need to based on the principle for minimising exposure to harmful substances:

"All exposures should be kept as low as reasonably achievable, economic and social factors being taken into account" (Shapiro 1981) .

Exposure of environments or humans to harmful substances can be kept to a minimum, but at increased cost. Successful management of sites irrigated with organic-rich wastewaters requires finding the right balance between environmental, social and economic principles of management. It is therefore proposed to:

- optimise the rapid removal of organic materials in the wastewater by adsorption and microbial decomposition, in soils, for improved quality of drainage water,
- promote the conversion of the organic substrates to humus, for improved structural stability and fertility of soils,
- maximise the reuse of water for irrigation, as determined by crop needs.

The above principles and aims of management will be further discussed in Chapter 8, in relation to disposal of wastewaters generated by the wine industry.

2.4.2 The model system

The model system (Figure 2.6) is analogous to batched-flow, fixed-film secondary treatment systems, as described by Tcholbanoglous and Burton (1991). Wastewater is added to the soil surface via the irrigation system. The movement of water within the soil matrix transports the organic materials to depths which support varying populations of potentially-active micro-organisms. Sufficient contact time is allowed for microbial growth and activity to remove the organic carbon.

The key feature of the model is a nominal depth of soil, called the zone of activity, in which an arbitrarily defined percentage of the organic materials added by the wastewater can be removed from the soil solution within a given period of time. The irrigation system can then be managed to: (I) keep the organic substrates added by the wastewater during irrigation within this depth, and (II) allow sufficient time between successive irrigations to enable the organic substrates to be removed from the soil solution by adsorption and microbial metabolism.

2.4.3 Factors influencing the efficacy of the model system

It is hypothesised that microbial metabolism will be essential for the near complete removal from the soil solution of organic materials added in wastewaters by irrigation; the main roles of adsorption are as given in section 2.2.1. The time required for removal of the soluble organic carbon from wastewaters used for irrigation will thus depend on: I) the length of the lag period before the onset of rapid microbial metabolism, and II) the rate of metabolism attained. The presence of a lag period is essentially lost time, and hence needs to be avoided.

On the basis of information provided in this review, it is further hypothesised that:

- increasing the concentration of organic carbon added to the soil will increase the rate of microbial metabolism of the organic substrates, but that the duration of the lag period

will also increase, resulting in longer times required for removal of the soluble organic carbon from the wastewaters (cf., Rajbanshi et al. 1992; Section 2.2.2.1).

- for a given level of carbon loading, the duration of the lag period will be shorter, and the rate of microbial metabolism of the organic substrates will be faster in acclimatised soils than in non-acclimatised soils (cf., Section 2.2.2.2).

- for a given level of carbon loading, the duration of the lag period will be shorter, and the rate of microbial metabolism of the organic substrates will be faster in sands than in heavy textured soils (cf., Section 2.2.2.3).

- for a given type of soil and carbon loading, the duration of the lag period will be shorter, and the rate of microbial metabolism of the organic substrates will be faster in soils containing a higher amount of native organic matter, and/or amounts of total nitrogen and phosphorus (eg., the 0–15 cm layer, cf. Section 2.2.3.1).

- for a given type of soil and carbon loading, increasing the moisture content of the soil towards field capacity may increase the duration of the lag period, and decrease the rate of microbial metabolism of the organic substrates, if the supply of oxygen through the air-filled pores becomes rate limiting (cf. Section 2.3.1).

- for a given level of organic loading, greater adsorption of the organic substrates will occur in clays than in lighter textured soils (cf., Section 2.2.1), which may, in turn, reduce the rate of microbial metabolism of the organic substrates remaining in solution (cf., Miller and Alexander 1991, Section 2.2.2.1).

Experiments have been designed to test these hypotheses for wastewaters produced by the manufacture of wine, and associated distillation operations (Chapters 4–7). The production and characteristics of wastewaters generated by the wine industry have been detailed in the next Chapter.

INPUT CARBON

TOC_I or BOD_I

DEPTH OF "ZONE OF ACTIVITY"

REMOVAL OF ORGANIC CARBON BY ADSORPTION AND MICROBIAL METABOLISM

UPTAKE OF WATER BY PLANTS

OUTPUT CARBON

TOC_E or BOD_E

Depth of root zone

H_2O
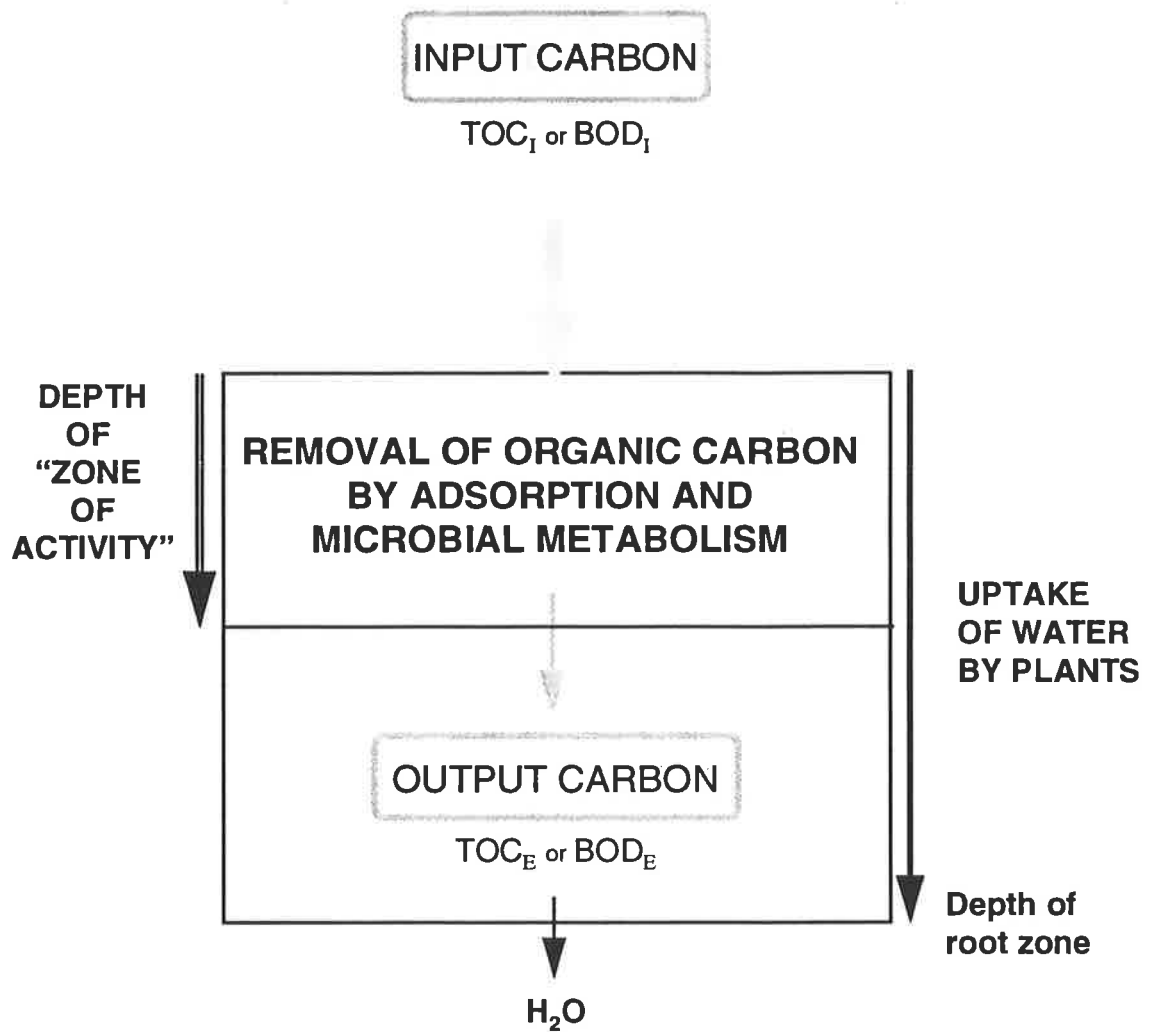
- **Figure 2.6:**   A model for the treatment of organic materials in wastewaters by irrigation of soils. The carbon loading of influent (I) and effluent (E) waters is measured as total organic carbon (TOC) and/or biological oxygen demand (BOD).

CHAPTER 3

PRODUCTION AND CHARACTERISTICS OF WINE INDUSTRY WASTEWATERS

3.0 Introduction

Winemaking is a multistep process divided into two periods, vintage and non-vintage. Vintage is a 12 to 20 week period in which the harvesting and crushing of wine grapes, and the fermentation of grape juice to wine take place. The remainder of the year is the non-vintage period. During this time the cellar operations of stabilisation, maturation and blending of wine are conducted to produce the final product for bottling. There is no clear boundary between the vintage period and non-vintage period. Processes associated with the latter usually occur year-round, and each major style of wine involves different processing operations.

Defective wine and lees may be distilled to provide ethanol spirit, which is used for the production of fortified wines such as port, sherry, and liqueur muscat or tokay. Lees are a by-product of the fermentation operation, and consists of a slurry of yeast, bacteria, and wine.

Crude wine is also distilled for the manufacture of brandy. The first distillation converts the wine to "low wine" during a 7–8 hour period. Low wine is 30% alcohol by volume (approximately 3x the original strength) and contains no suspended solids. The low wine, with an added portion of "heads" and "tails", is then distilled to the crude brandy, ready for maturation and blending. Heads is the distillate collected during the first 20–30 minutes of the second distillation. Distillate collected over the next several hours is diverted as brandy, with distillate collected over the remaining 3–4 hours, forming the tails. In more recent times the double distillation process has been replaced by a continuous column system.

3.1 Production of wastewater by wineries and distilleries.

3.1.1 Wastewater produced by the manufacture of wine

Wineries produce three main categories of wastewater:

1. Cleaning water

Winemaking involves handling several types of wine at any given time. Since the same facilities are used to make the various wines, meticulous attention must be paid to keeping equipment and surrounding areas clean to avoid contamination and spoilage. Cleaning water is subdivided into two categories as follows:

- *Alkali washwater* Grape juice and wine are rich in potassium-hydrogen bitartrate which adheres to the walls of equipment during storage and processing operations, and is deliberately precipitated during stabilisation of the wines. Alkali washwater (5% NaOH by weight) is used to dissolve the precipitates. Polyphenolic pigments are also more easily removed by alkali. The washwater is recycled whenever possible. Sodium hydroxide, sodium metasilicate, and sodium carbonate are the most commonly used reagents for alkali washing (Leske 1992).

- *Rinsewater* Filtered mains water and citric/tartaric water is used to rinse equipment following alkali washing to remove all traces of alkali. Outsides of equipment and cellar floors are also regularly washed.

2. Process water

The following processes also generate wastewater.

- *Ion exchange* Ion exchange columns are used to acidify wine through exchange of mainly potassium ions, for hydrogen. Removal of potassium ions also helps stabilise

wine against crystallisation of potassium-hydrogen bitartrate, once bottled. Once a wine is passed through the column, the ion exchange resin is regenerated with a mineral acid, usually sulfuric acid. Several washings are then needed to remove the potassium sulfate residues, and the excess sulfuric acid. Small volumes of highly acidic wastewater rich in potassium and sulfate are produced.

- *Earth filtering* Rotating drum earth filters consist of a coating of diatomaceous earth around a perforated drum through which juice being clarified is forced as the drum rotates. The outermost layer of earth is continuously sliced off to remove the filtered solid components of the juice. Repacking the earth coat to the correct consistency requires approximately 20 kl of water (the exact volume depends of the size of the filter), and may need to be done twice daily during vintage.

3. Cooling tower water

Juice and wine must be kept cool to avoid spoilage. Evaporative towers use about 20% of the total volume of mains water used within a winery. About 5% of the cooling tower water is continuously "bled-off" and replaced to prevent the buildup of salts.

Wineries produce about 5 kl of wastewater per tonne of grapes crushed (Sanders 1975). This represents a few hundred megalitres of wastewater per major wine producing district within Australia, annually. The extra processing steps operating during vintage result in a sharp increase in water usage; just under half the annual volume of wastewater is generated during the vintage period, as shown for a medium sized winery in Figure 3.1.

The relative annual contributions of wastewater from cleaning, processing, and cooling operations are shown for a non-distilling winery in Figure 3.2. Cleaning waste is by far the biggest contributor of wastewater, with alkali washing producing up to 35% of the annual
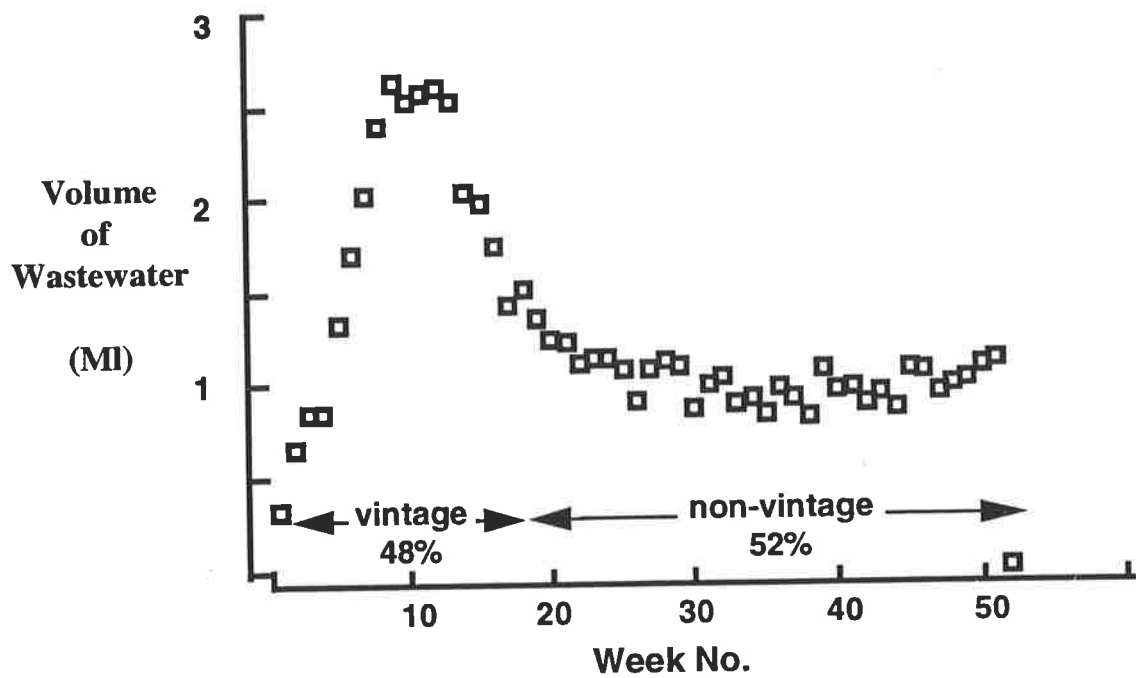
Figure 3.1: Weekly volumes of wastewater generated by a medium-sized, non-distilling Australian winery; week 1: 1st – 7th January, week 2: 8th – 14th January, etc.

Source: the supplier of the raw data is acknowledged (to remain anonymous)

cooling tower waste

alkali washwater

non-alkali rinsewater

ion exchange waste

earth filtration
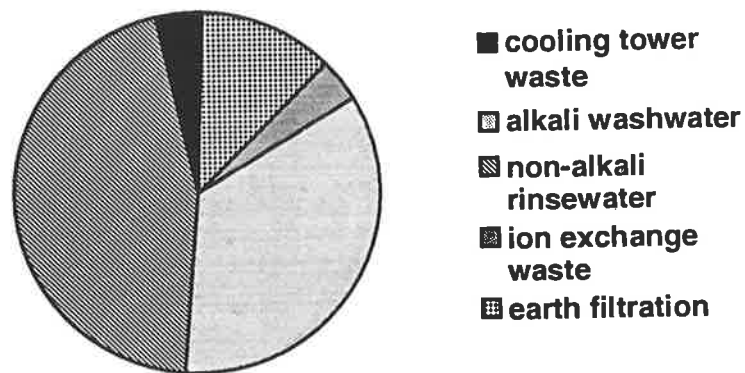
Figure 3.2: Relative annual contributions of wastewater from cleaning, processing, and cooling operations by a non-distilling winery.

Source: the supplier of the information is acknowledged (to remain anonymous)

volume of wastewater generated by a non-distilling winery. It should be noted, however, that the relative proportions of wastewater produced by cleaning, processing, and cooling operations differs for each winery within Australia, and are subject to change as wineries strive to reduce the volume of wastewater generated, annually.

3.1.2 Wastewater produced by the manufacture of alcohol spirit and brandy

Distillation operations produce three main categories of wastewater:

1. *Still wash, spent wine, and spent water*

Still wash is the residue left over after the recovery, by distillation, of alcohol from lees or defective wine. Grape marc may also be steam distilled, but does not produce a liquid waste (Sanders 1975).

Residues left over from the distillation of wine to low wine is called spent wine. Spent water is the residues left over from the second distillation of the low wine mixture to brandy. Residues left over after continuous column distillation of wine to brandy is also referred to as spent wine, but is really a mixture of both spent wine and spent water. The term "spent" infers that the residue does not contain alcohol.

2. *Cleaning waste*

Alkali washwaters and non-alkali containing rinsewaters are used for cleaning, as outlined above.

3. *Cooling tower waste*

Distillate is rapidly cooled by heat exchange with water recycled through evaporative towers. About 5% of the cooling tower water is continuously "bled-off" and replaced to prevent the buildup of salts.

About 90% of the lees or defective wine distilled for alcohol spirit remains as residual stillwash, whilst about 80% of the crude wine distilled for the production of brandy ends up as spent wine (68%) or spent water (12%)[1]. Condensed steam, washwater, and water bled-off from the cooling towers, can generate a further 300–500l of wastewater per kl of material distilled. An example of the types of flow of water through a distillery is given in Figure 3.3.

3.2 Chemical composition of juice and wine

Residues from both grape juice and wine are invariably contained in wastewaters generated by wineries and distilleries. It is therefore important to have a basic understanding of the major differences in the composition of juice and wine, and of the characteristics that juice and wine can potentially add to the overall composition of the wastewaters.

The manufacture of red and white varieties of wine is shown in Figure 3.4; major differences in the organic and inorganic composition of juice and wine are given in Table 3.1. Juice extracted by the crushing and pressing of grapes reflects the composition of the variety of grape and seasonal growing conditions. Fermentation of the juice into wine produces the most significant changes in the organic composition. Sugars are used as the chief source of carbon by the yeast and bacteria (Amerine and Ough 1980), resulting in a dramatic drop in sugar content between juice and wine. Anaerobic conditions favour production of ethanol and glycerol, which are both important components of wine. Malolactic fermentation naturally occurs in red wine varieties, and less commonly in white wines. Malolactic fermentation is a biochemical process which results in the transformation of malic acid to lactic acid.

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1   J. Conradie, KWV South Africa; pers. comm.
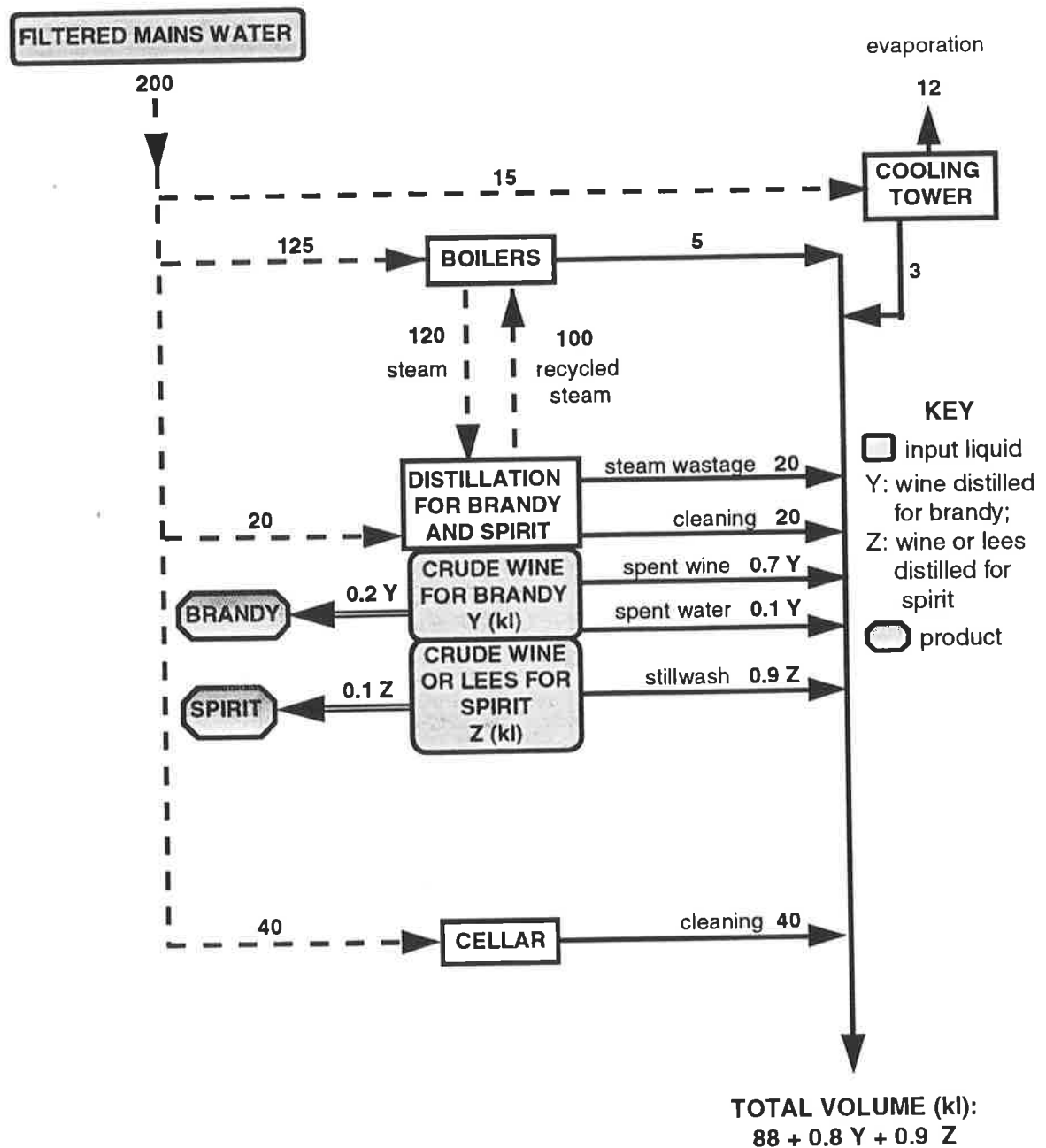
Figure 3.3: Daily inputs of liquids (dashed lines), and outputs of products (double lines) and of wastewater (solid lines) from a distillery; No. of days of operation: approximately 70.

Source: the supplier of the information is acknowledged (to remain anonymous)
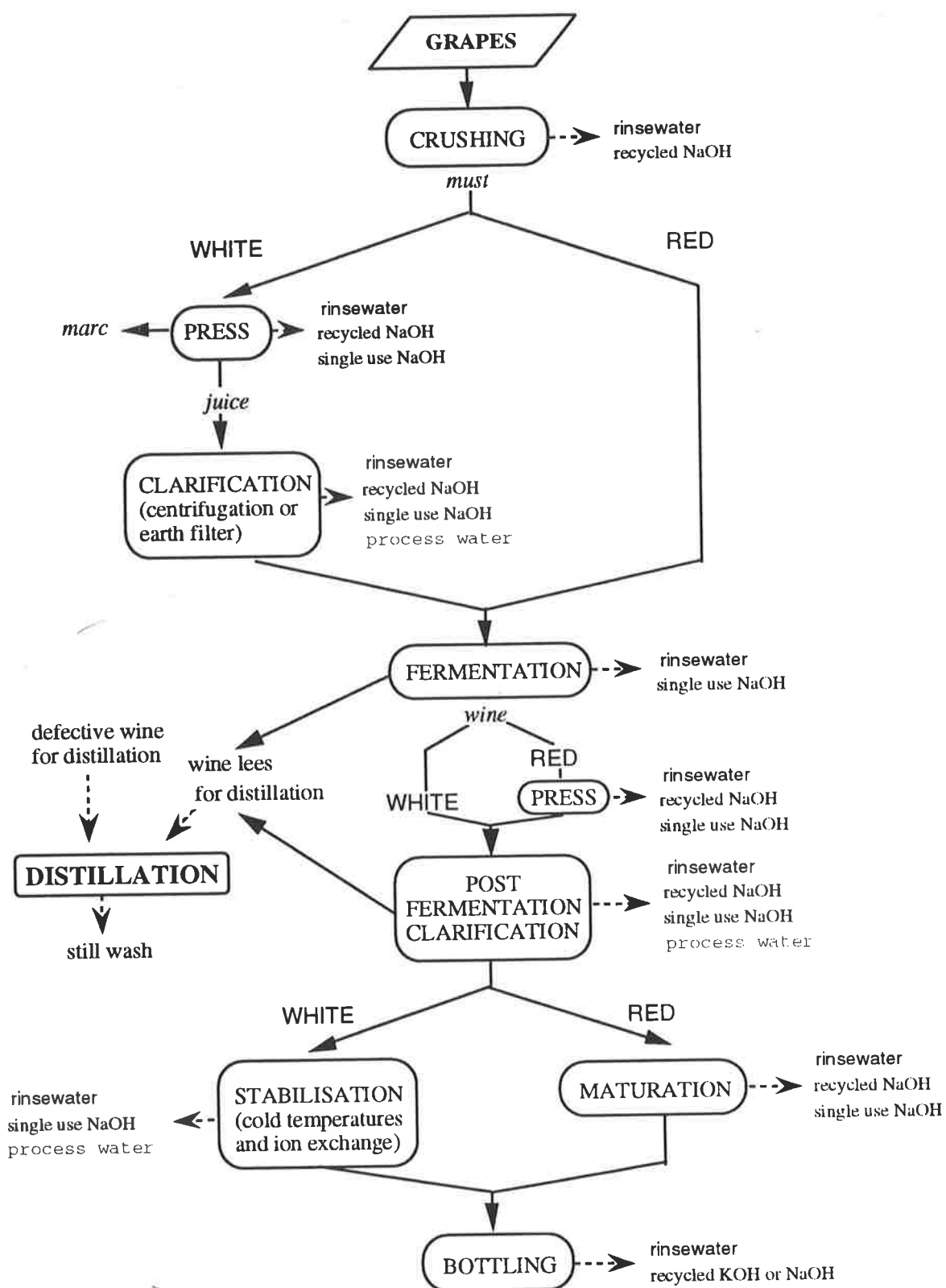
Figure 3.4: Origins of wastewater from the manufacture of wine.

(Source: B. Duncan, Southcorp Wines; pers. comm.)

Table 3.1: Typical composition of juice and wine produced in the Barossa Valley of South Australia.

	Juice	Wine	
Organic composition (g/l)			
Tartaric acid	3–5	1–2	
Citric acid	<0.1	<0.5	
Malic acid	2–6	0–6	higher in white wine
Lactic acid	0	0–6	higher in red wine
Acetic acid	0	<1	higher in red wine
Glucose	90–150	0–20	table wine
Fructose	90–150	0–150	table wine
Glycerol	0	3–10	
Ethanol (% V/V)	0	8–12	table wine
		17–18	fortified wine
Inorganic composition (g/l)			
Sodium	0.1–0.5	0.1–0.5	
Potassium	2–3	0.1–2.0	
Calcium	0.05–0.15	0.05–0.15	
Sulfur dioxide	<0.01–0.15	0.02–0.27	
pH	3.0–3.5	3.0–3.8	table wine
		3.6–4.0	fortified wine
EC (dS/m)	2.5–3.5	2.5–3.5	

Source: I. Codrington, S. Smiths and Sons Pty. Ltd.; pers. comm.

Potassium-hydrogen bitartrate can form unwanted crystals in wine after bottling, and hence is removed during post fermentation operations. The concentrations of other inorganic components changes relatively little during the manufacture of wine; small amounts of sodium may be contributed by additives. Both juice and wine have an electrical conductivity of around 3 dS/m, due to the presence of potassium salts of the organic acids (Amerine and Ough 1980).

It can be seen from Figure 3.4 that cleaning waste is generated at each stage of manufacture of red and white wines, whereas processing waste is largely produced by clarification and stabilisation operations. It is important to note that pre-fermentation operations are confined to vintage, and contribute juice-like characteristics to the wastewater. Post-fermentation operations may occur year-round, and contribute wine-like characteristics to the wastewater. The extra pre-fermentation operations during the vintage period are reflected by the higher biological oxygen demand of wastewater generated, compared with wastewater generated by post-fermentation operations during the non-vintage period (Table 3.2).

Wastewater generated by a distillery is always more concentrated than wastewater generated from the manufacture of wine (Table 3.2). Further distinctions between wastewaters generated by wineries and distilleries will be outlined in the following Sections.

Table 3.2: Averaged analyses of wastewaters originating from eight wineries located in the Barossa Valley of South Australia.

Analysis (mg/l except pH)	Distillery	Winery vintage	Winery non vintage
Biological oxygen demand	16 000	8 000	3 000
Total organic carbon	13 000	–	2 000
Suspended solids	26 000	1 000	1 000
Total Dissolved Solids	4 500	1 200	1 200
Total Kjeldahl Nitrogen	1 700	–	28
Ammonia Nitrogen	50	–	1.3
Total Phosphorus	130	–	9.8
pH units	4.2–5.0	–	4.8
Total No. Samples	24	8	22

Source: EWS (1979)

3.3 Detailed analysis of wastewaters produced by wineries and distilleries.

As part of the initial investigations, a detailed analysis has been conducted of the major organic and inorganic components of wastewaters produced from the manufacture of wine and the distillation of lees, and of wastewaters generated from selected processing and cleaning operations within a non-distilling winery. Wastewater sampled from a winery and a distillery located in the Barossa Valley of South Australia was used for the analyses. Both companies have been disposing the wastewaters by irrigation, as outlined in Section 4.2.1. It was therefore important to obtain a detailed analysis of the wastewaters to: (I) determine the proportion of the total organic carbon content of the wastewaters present as readily degradable substances (cf., Section 2.2.2.1), and (II) identify key components that will affect the management of sites irrigated with the wastewaters. Management issues will be discussed in Chapter 8.

All of the sampling, and analysis of the major organic and inorganic components of the wastewaters were conducted by the participating wineries, and by the State Water Laboratory of South Australia. Wastewaters generated from selected processing and cleaning operations were sampled by S. Smiths and Sons Pty. Ltd; analysis of the wastewaters summarised in the Tables was conducted by myself. The immense contribution of the participating organisations in the above activities is acknowledged, as the need to conduct the analyses within a few hours to a few days from sampling, and the high costs of analysis would have severely limited the range of analyses that could have been conducted by myself.

3.3.1   Sampling

Ten, weekly samples of stillwash originating from the distillation of lees were taken during the late vintage period and early non-vintage period of 1992. The stillwash was sampled after the ethanol spirit had been removed by distillation, and was taken from the base of the still via the exit valve, and immediately cooled (4°C).

Wastewater produced by a non-distilling winery was sampled during the vintage periods, and non-vintage periods of 1992–93. Both fresh wastewater, and wastewater which had been stored in a lagoon to remove solid material were analysed. The fresh wastewater was a composite of 125 ml subsamples taken every 30 minutes over 24h (a proportional flow sampler was not available). Wastewater sampled after storage to remove solids was taken from the exit line of the lagoon. The mean residency time within the lagoon varied from 2–3 days during vintage to >7 days during late non-vintage. Wastewater was not sampled during winter due to excessive dilution by stormwater. Ten composite samples of wastewater produced during the vintage period, and 14 composite samples of wastewater produced during the non-vintage period were analysed.

Wastewaters originating from ion exchange processing, and alkali and non-alkali cleaning, were sampled during 1993. Ten, 20 litre composite samples for each type of wastewater were taken by the participating winery at random from a range of the various stages of manufacture during the vintage and non-vintage periods (cf., Figure 4.4). Samples of the filtered water used by the winery were also taken.

3.3.2   Analyses

The following analyses were conducted by the participating wineries, and the State Water Laboratory of South Australia, according to the specified methods—The organic acid composition of the wastewaters was determined by High Performance Liquid Chromatography (HPLC), as outlined in Table 3.3. Samples of wastewater were passed through a 0.45 μm filter prior to injecting 20 μl into the column. Results were calibrated against standards. Distillery wastewater was analysed before and after dilution (1:4) with concentrated hydrochloric acid to provide a comparison between free and total acids, and monomeric and polymeric sugars. "Total acids" includes the conjugate bases of the acids, normally present as salts.

Table 3.3   Operating conditions for organic acid analysis by HPLC.

HPLC System	
Column:	Interaction ION–300 pn. 25342
Temperature:	$75°C \pm 1°C$
Eluents:	HNO_3 buffer pH 2.9 (allow resolution of malic acid from glucose);
	$0.015M$ HNO_3 (allows resolution of citric acid from tartaric acid)
Flow rate:	0.6 ml/min
Detector:	Erma Optical Works Ltd, Refractive Index Detector,
	model ERC–7510
Range:	1/16x attenuation

Source: I. Codrington, S. Smith and Sons Pty. Ltd.; pers. comm.

The "Standard Methods" were used to determine the biological oxygen demand (BOD), chemical oxygen demand (COD), total and suspended solids, and total nitrogen and phosphate contents of the wastewaters (Standard Methods 1989). Costs prevented the analysis of wastewaters originating from ion exchange processing, and alkali and non-alkali cleaning.

COD was determined by potassium dichromate consumption. BOD was determined by the quantity of oxygen consumed as the result of biological oxidation of the wastewater diluted in oxygen-rich solution, after 5 days incubation at 20°C. Total nitrogen was determined as total Kjeldahl nitrogen. Total phosphate was determined spectrophotometrically after all forms of phosphate had been converted to ortho-phosphate, which, in turn, was converted to molybdenum blue. Total solids were determined by drying a known volume of wastewater at 100°C. Suspended solids were defined as the proportion of the total solids greater than 0.45 μm. Suspended solids were determined by difference between total solids and the weight of a known volume of wastewater which had been passed through a 0.45 μm filter.

The following analyses were conducted by myself, according to the specified methods— Total organic carbon was determined by the dichromate wet oxidation method as described by Nelson and Sommers (1982). Inorganic composition, other than total nitrogen, and phosphorus was determined by an Inductively Coupled Plasma Optical Emission Spectroscopy (ARL 3580 model). Distillery wastewater was analysed before and after dilution (1:4) with concentrated hydrochloric acid. Samples were passed through a 160 μm filter prior to analysis.

Electrical conductivity (EC) and pH were measured 60 seconds after immersing the electrodes in entire samples of wastewater kept continuously stirred at 20° C. The hourly variation in pH and EC of fresh wastewater generated by the winery was determined by measuring the individual samples taken to form the composite sample.

3.3.3   Characteristics

3.3.3.1   Carbon

Simple dissolved compounds dominated the organic composition of winery wastewater (Table 3.4), with ethanol alone contributing about 70% of the total organic carbon content (TOC). Amounts of ethanol present in winery wastewater (1–3 g/l) were much lower than found in wine (40–100 g/l).

Separation of solids during storage, reduced the TOC of winery wastewater produced during vintage by 35%, and the TOC of wastewater produced during non-vintage by 60%. Partial degradation of the organic substrates occurred during storage, as indicated by the increases in the concentrations of acetic acid and butyric acid, particularly after the longer residency times of wastewater within the lagoon during the non-vintage period (Table 3.4).

Following the removal of ethanol spirit by distillation, the residual stillwash contained mostly carboxylic acids, including tartaric, lactic and acetic acids, and smaller concentrations of monomeric sugars. Glycerol, which is another dominant component in wine (Table 3.1), and is further produced by hydrolysis of glycerol-esters during distillation, was also present in high concentrations (Table 3.5).

Monomeric sugars and free acids, however, accounted for only one third of the total organic carbon content of the stillwash. Salts of these acids and polymeric sugars, normally present in suspension, and the remains of the yeast and bacteria, made up the remaining organic carbon. According to DePuy and Rinehart (1975) strongly acidic conditions favour: (I) the ionisation of insoluble carboxyl salts (eg., potassium-hydrogen bitartrate), and the conversion of the anions to the free acids, and (II) the breakdown of sugar-containing dimers and polymers into the individual components. Acidification of the wastewater with hydrochloric acid was therefore used to obtain an indication of the "total acid and sugar" content (Table 3.5).

Table 3.4: Organic composition of winery wastewater.

	Vintage	Non-vintage	Vintage	Non-vintage
	Fresh effluent		After separation of solids	
Organic acids (g/l)				
Tartaric	0.53 ± 0.22	0.35 ± 0.22	0.20 ± 0.13	0.02 ± 0.03
Citric	trace	trace	trace	trace
Malic	trace	trace	trace	trace
Lactic	0.35 ± 0.17	0.12 ± 0.07	0.15 ± 0.12	0.01 ± 0.01
Acetic	0.10 ± 0.03	0.05 ± 0.06	0.22 ± 0.13	0.37 ± 0.14
Sugars (g/l)				
Glucose	0.30 ± 0.51	0.28 ± 0.39	trace	trace
Fructose	0.53 ± 0.87	0.27 ± 0.33	0.08 ± 0.20	trace
Alcohols (g/l)				
Glycerol	0.19 ± 0.11	0.12 ± 0.05	0.06 ± 0.05	0.02 ± 0.02
Ethanol	3.13 ± 2.91		1.49 ± 0.42	
Ethanol + butyric acid†		3.38 ± 3.65		2.21 ± 1.45
Total Organic Carbon (g/l)	1.4 ± 0.2	0.9 ± 0.5	0.8 ± 0.2	0.3 ± 0.1
No. Samples	10	14	10	14

† Wastewater sampled after separation of solids contained a mixture of butyric acid, formed during storage, and ethanol, which co-elute.

Table 3.5: Organic composition of stillwash originating from the removal, by distillation, of ethanol from lees.

	Without acidification	After acidification with HCl
Organic Acids (g/l)		
Tartaric	1.54 ± 0.62	7.55 ± 1.04
Citric	0.48 ± 0.38	peak hidden by Cl⁻
Malic	0.11 ± 0.17	0.99 ± 1.09
Lactic	1.91 ± 1.55	3.32 ± 1.94
Acetic	1.21 ± 1.01	1.38 ± 0.71
Sugars (g/l)		
Glucose	0.09 ± 0.13	2.47 ± 0.58
Fructose	0.16 ± 0.18	2.25 ± 0.35
Alcohols (g/l)		
Glycerol	2.23 ± 1.40	3.64 ± 1.12
Ethanol	0.78 ± 1.41	0.05 ± 0.12
Organic Carbon (g/l)	**Total:** 11.3 ± 1.5	**Dissolved** (<0.45μm) 3.9 ± 1.2
No. Samples	10	10

3.3.3.2 Oxygen Requirement

Winery wastewater produced during the vintage period, and stillwash have characteristically high requirements of oxygen for biological decay (BOD), in excess of 1 g/l and 13 g/l for the respective wastewaters (Table 3.6). Santos Oliveira et al. (1975) and Mauganet (1978) found that the carboxylic acids and sugars, which dominate the organic carbon composition of the wastewaters (Tables 3.4–3.5) have BOD/COD ratios >0.5, which was concluded to be an indicator of readily biodegradable substances.

The BOD of winery wastewater appeared to be almost entirely due to decomposition of dissolved organic components, mainly ethanol. In contrast to winery wastewater, the combined BOD of the individual soluble components found in stillwash accounted for only 30–40% of the total BOD, a similar proportion to the TOC content accounted for by the soluble organic components.

3.3.3.3 Salinity

The electrical conductivity of winery wastewater averaged 2.6 dS/m during the vintage period, and 1.1 dS/m during the non-vintage period (Table 3.7). The source of water used for cleaning was a major contributor to the total salt content of winery wastewater (27–64%). Alkali washwater produced an effluent with a conductivity ranging from 19 to greater than 100 dS/m, becoming more saline with the extent of recycling. Given that alkali washwater was an important contributor to the total volume of wastewater generated by the winery, annually (cf., Figure 3.2), large amounts of salts are added by this activity. The washing and recharging of the ion exchange column always produced small volumes of extremely saline waste (>100 dS/m). Despite the differences in input salts between the vintage period and non-vintage period, the salt content of winery wastewater after separation of solids remained relatively constant (Table 3.7).

The extraction of ethanol by distillation concentrates the wine lees, and hence the residual stillwash was highly saline (>5.5 dS/m, Table 3.7).

Table 3.6: Oxygen requirements for biological decomposition (BOD) and chemical oxidation (COD) of wastewaters produced by the wine industry, and of organic components[†].

	BOD	COD	BOD/COD
Organic acid	gO_2/g	gO_2/g	
Tartaric acid	0.27	0.51	0.53
Citric acid	0.35	0.70	0.50
Lactic acid	0.57	0.97	0.59
Malic acid	0.50	0.72	0.70
Acetic acid	0.56	1.03	0.54
Sugar	gO_2/g	gO_2/g	
Glucose	0.75	1.09	0.69
Alcohol	gO_2/g	gO_2/g	
Glycerol	0.64	1.20	0.53
Ethanol	1.37	2.12	0.65
Wastewater	gO_2/l	gO_2/l	
Winery: vintage	1.5 – 6	4 – 8	0.2 – 0.8
Winery: non-vintage	0.5 – 3.5	1 – 4.5	0.2 – 0.8
Stillwash	13 – 35	30 – 45	0.4 – 0.7
No. Samples	**10**	**10**	**10**

† Information for individual components from Santos Oliveira et al. (1975) and Mauganet (1978).

Table 3.7: Electrical conductivity of wastewaters produced by the wine industry.

Source	Conductivity (dS/m)	No. Samples
Filtered mains water used by the winery	0.6 – 0.8	10
Winery wastewater: fresh effluent		
vintage	2.6 ± 0.9	10
non-vintage	1.1 ± 0.2	14
Winery wastewater: after separation of solids		
vintage	1.9 ± 0.2	10
non-vintage	1.9 ± 0.2	14
Alkali washwater	19 – >100	10
Non-alkali containing rinsewater	1.0 ± 0.1	10
Ion exchange waste	>100	10
Stillwash	6.0 ± 0.8	10

3.3.3.4 Soluble cations and sodicity

Winery wastewater had an excess of sodium relative to the concentrations of the other major soluble cations (Table 3.8). The concentrations of all the soluble cations fell as the result of separation of solids during storage. The major source of sodium was alkali washing (Table 3.8). In excess of 20 tonnes of sodium hydroxide can be used for washing by a medium-sized winery, annually. Depending on the origin, water used for cleaning can also be naturally high in sodium (Cobb 1986).

The concentrations of soluble sodium, potassium, calcium and magnesium in stillwash were similar to their concentrations in grape juice (cf., Table 3.1).

Sodicity of solutions describes the relationship between the concentration of monovalent sodium, relative to the concentrations of divalent calcium and magnesium, expressed as the sodium adsorption ratio (SAR, Rengasamy and Olsson 1991). Fresh winery wastewater produced during the vintage period and the non-vintage period, and stillwash had SAR values around 7–9. It is interesting to note that the SAR of the fresh winery wastewater was often double that of the filtered water used by the winery (Table 3.8).

3.3.3.5 Acidity

The pH of fresh winery wastewater depended on the relative concentrations of alkali and organic acids. The additional vintage operations from crushing through to fermentation produced wastewater containing larger quantities of acids than did the cellar operations during non-vintage (cf., Table 3.4), and hence a comparatively more acidic wastewater was produced during the vintage period than in the non-vintage period (Table 3.9). Winery wastewaters sampled after separation of the solids were also more acidic than the fresh winery wastewaters. This was partly due to the production of short chain fatty acids during storage, including acetic acid, butyric acid (cf., Table 3.4), and propionic acid (data not shown). A small amount of the carbon dioxide released by micro-organisms during storage was also likely to contribute towards acidity.

Table 3.8 Concentrations of soluble cations and the sodium adsorption ratio of wastewaters produced by the wine industry[†].

Source	Soluble Cations (mg/l)				SAR[‡]
	Na+	K+	Ca2+	Mg2+	
Filtered water used by the winery	124 ± 11	65 ± 7	23 ± 4	9 ± 3	4
Winery wastewater: fresh effluent					
vintage	250 ± 59	131 ± 48	26 ± 13	29 ± 18	8
non-vintage	328 ± 129	204 ± 132	30 ± 15	15 ± 5	9
Winery wastewater: after separation of solids					
vintage	148 ± 36	98 ± 17	25 ± 4	10 ± 4	4
non-vintage	219 ± 73	55 ± 15	22 ± 1	10 ± 1	7
Alkali washwater	9591 ± 11856	194 ± 174	20 ± 24	12 ± 21	1184
Non-alkali rinsewater	94 ± 12	122 ± 49	16 ± 8	11 ± 4	4
Ion exchange waste	216 ± 57	2537 ± 2684	165±155	184±150	3
Stillwash	430 ± 184	1396 ± 649	102 ±45	80 ± 33	8

[†]   Number of samples analysed as given in Table 3.7.

[‡]   Sodium adsorption ratio $(SAR) = [Na^+]/([Ca^{2+}] + [Mg^{2+}])^{1/2}$, with concentration in millimoles.

Table 3.9: pH of wastewaters produced by the wine industry, and dissociation constants (pK) of selected components.

Wastewater	pH	No. Samples
Filtered mains water used by the winery	7.0 – 7.1	10
Winery wastewater: fresh effluent		
vintage	4 – 8	10
non-vintage	6 – 10	14
Winery wastewater: after separation of solids		
vintage	4 – 6	10
non-vintage	4 – 8	14
Alkali washwater	10 – 13	10
Non-alkali rinsewater	4 – 8	10
Ion exchange waste	3 – 4	10
Stillwash	3 – 4	10
	pK[†]	
Tartaric acid	2.98	
Lactic acid	3.08	
Acetic acid	4.75	
	pH of a 0.1M solution[†]	
Sodium hydroxide	13.5	
Potassium hydroxide	13.5	

† Source: Weast and Astle (1982)

Stillwash was always acidic due to the high concentrations of organic acids in the residues (cf., Table 3.5).

3.3.3.6 Variability

Figure 3.5 shows an example of the variation in the pH, and the electrical conductivity of fresh winery wastewater obtained at 1h intervals, over 24h. The rapid variation between extremes in composition of fresh winery wastewater was expected, given the many concurrent processing and cleaning stages, each contributing effluent of widely differing properties (eg., ion exchange processing waste, alkali and non alkali washwaters shown in Tables 3.7–3.9).

There is no typical winery or distillery wastewater. Rather, they are complex mixtures with components which will vary within a range of concentrations, usually independently of each other.

3.3.3.7 Nutrition

Relative to total carbon, amounts of nitrogen and phosphorus were low (Table 3.10). Nitrogen originated from proteins removed from wine to prevent haze. Additional phosphorus may be obtained from small amounts of phosphate detergents used for cleaning laboratory glassware, etc.

The high level of sulfur found in winery wastewater during vintage, and in the stillwash, was due to the natural level of sulfate in wine grapes, some wastage of sulfur dioxide, and sulfate added from ion exchange processing waste. Sulfur dioxide is added during winemaking to preserve the wine against oxidation and spoilage (Amerine and Ough 1980).

A. pH
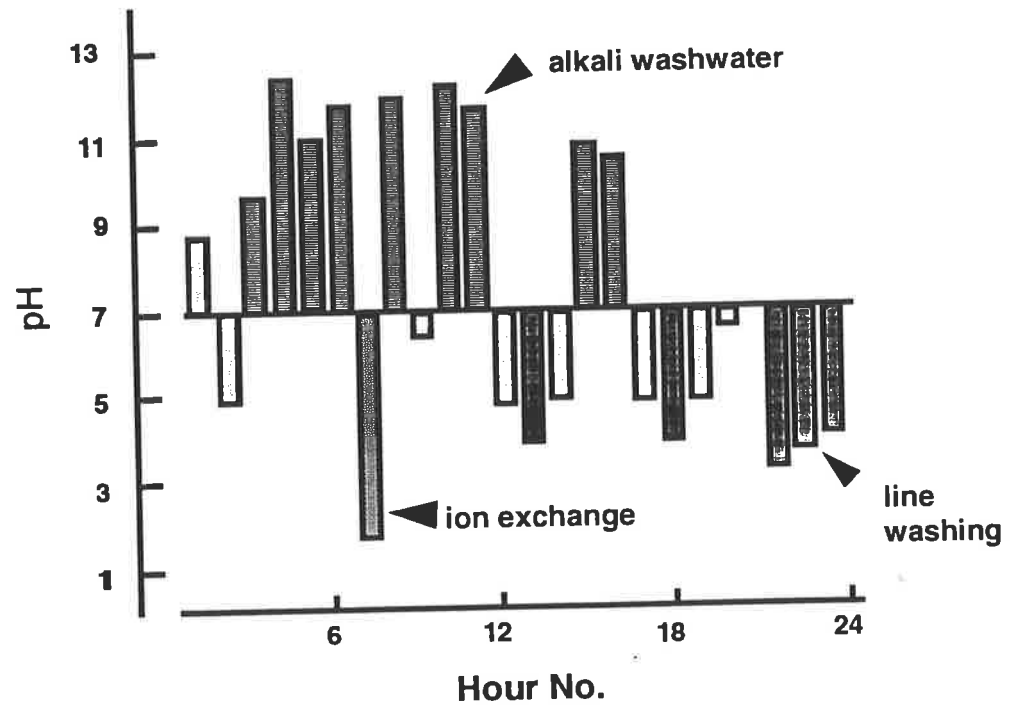
B. Electrical Conductivity
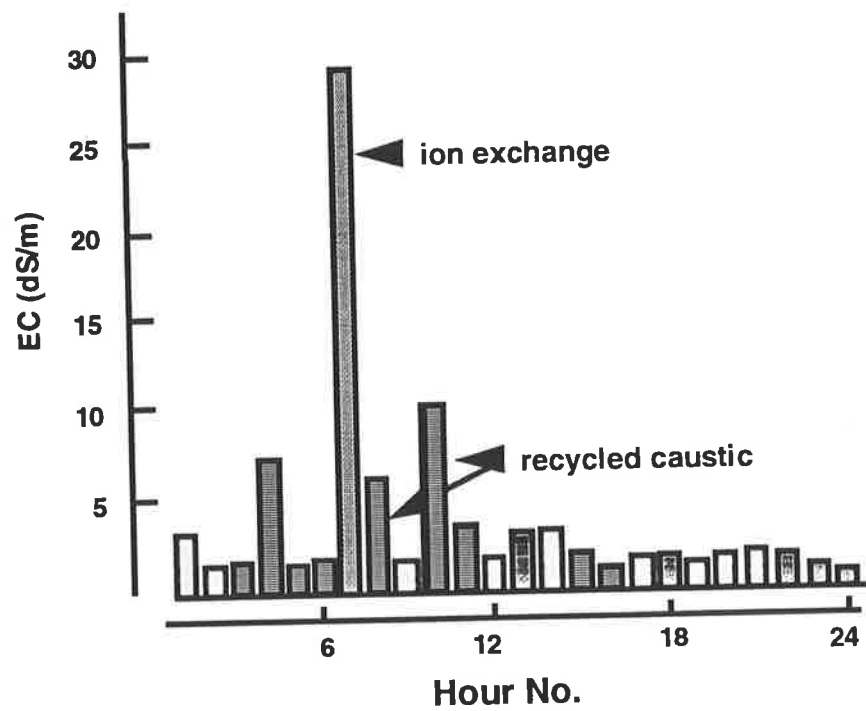
Figure 3.5: An example of the hourly variation in pH and electrical conductivity of winery wastewater.

Table 3.10: Major and secondary nutrient composition of wastewaters produced by the wine industry.

	Vintage	Non-vintage	Vintage	Non-vintage
	Fresh effluent		After separation of solids	
Major elements				
Total organic carbon (g/l)	1.4 ± 0.14	0.4 ± 0.1	0.8 ± 0.2	0.3 ± 0.1
Total Kjeldahl nitrogen (mg/l)	47 ± 13	31 ± 9	31 ± 5	19 ± 3
Nitrate + Nitrite (mg/l)	0.09 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Total phosphorus (μg/l)	19.9 ± 9.3	9.5 ± 4.0	2.2 ± 1.8	2.0 ± 0.6
C:N:P	30 : 2.8 : 0.3	30 : 6.1 : 1.3	30 : 1.4 : 0.1	30 : 5.2 : 0.5
Secondary elements[†]				
Sulfur (mg/l)	349 ± 428	209 ± 266	6.7 ± 1.7	30.6 ± 6.3
Iron (mg/l)	2.8 ± 2.1	2.2 ± 0.8	0.95 ± 0.53	1.2 ± 0.4
No. Samples	10	14	10	14

Stillwash

Major elements

Total organic carbon (g/l)	11.3 ± 1.5
Total Kjeldahl nitrogen (mg/l)	870 ± 300
Total phosphorus (μg/l)	330 ± 141
C:N:P	30 : 0.2 : 0.1

Secondary elements[†]

Sulfur (mg/l)	281 ± 267
Iron (mg/l)	not available
No. Samples	10

† Values for calcium and magnesium are given in Table 3.7.

3.3.3.8 Solids

Winery wastewater contained about 1.5 g/l of solids. The solids comprised of small amounts of pulp, skin and seeds of grapes, filter media, and precipitates. Most of the earth material, skins and seeds were separated during storage of the wastewaters in a lagoon. However the formation of microbial biomass (algae) during storage resulted in a net increase in the total solids content of the wastewaters sampled from the lagoon compared with the fresh wastewaters.

Distillation of lees produced a wastewater with the consistency of a thick soup. The residual stillwash contained about 20 g/l suspended solids, and a similar amount of dissolved solids (Table 3.11). Most of the solid material originated from yeast and bacteria, and residues of grapes (Hartley 1977).

Table 3.11: Solids content of wastewaters produced by the wine industry (g/l).

Wastewater	Total Solids	Suspended Solids (>0.45 µm)	No. Samples
Winery wastewater: fresh effluent			
vintage	1.57 ± 1.05	0.19 ± 0.11	10
non-vintage	1.64 ± 0.45	0.41 ± 0.35	14
Winery wastewater: after separation of solids[†]			
vintage	2.43 ± 0.73	0.23 ± 0.18	10
non-vintage	2.80 ± 1.14	0.41 ± 0.22	14
Stillwash	46.0 ± 20.0	20.1 ± 9.8	10

† Algal growth was evident in samples.

3.4 Discussion

The Australian wine industry has sought to develop irrigation strategies for their wastewaters involving the re-use of water, and acceptable levels of removal of the soluble organic carbon for improved water quality. The soluble organic carbon in the wastewater is further utilised to improve the fertility and structural stability of soils, through its conversion to humus. Thus, the main question of the research described in this thesis was whether the organic carbon added in the wastewaters by irrigation could be extensively and rapidly removed from the soil solution.

The organic carbon content of wastewaters produced by the wine industry was found to be dominated by simple carboxylic acids, sugars, and alcohols (cf., Tables 3.4–3.5). It was therefore hypothesised that the organic carbon in wastewaters produced by the wine industry would be extensively and rapidly removed from the soil solution, by the processes of adsorption and microbial metabolism. Furthermore it was hypothesised that rapid metabolism of the organic carbon would be facilitated by acclimatisation of the microbial populations and mineralisation of the organic nitrogen added by the wastewater and present in the soil, as outlined in Chapter 2.

Allowing for differences in the organic carbon content of winery wastewater produced during the vintage period and the non-vintage period, the application of 1 Ml of wastewater per hectare would add about 0.5 tonnes of organic carbon to the soil. According to Oades (1989) about 10% of the added organic carbon could be converted to humus in the long-term. On the basis of this assumption, it was hypothesized that the continued use of wastewaters produced by the wine industry for irrigation would result in the gradual increase in the organic carbon content and of soils.

These hypotheses formed the basis of the experimentation described in the following Chapters.

CHAPTER 4

ADSORPTION AND MICROBIAL METABOLISM OF SOLUBLE ORGANIC CARBON IN WINERY WASTEWATER

4.0   Introduction

It was hypothesised in Chapter 3 that the soluble organic carbon in wastewaters produced by the wine industry would be extensively and rapidly removed from the soil solution, by the processes of adsorption and microbial metabolism.   Adsorption and microbial metabolism provide alternative sinks for the soluble forms of organic carbon (Figure 4.1). As discussed in Chapter 2, part of the soluble organic carbon is adsorbed to the surfaces of clay and organic colloids as the wastewater moves through the topsoil during irrigation. Metabolism of the organic compounds remaining in solution, which involves the formation and subsequent turnover of microbial biomass and products, and mineralisation to carbon dioxide, further reduces the solution concentration of organic carbon.   Thus, the overall removal of soluble organic carbon after each irrigation with wastewater involves both the removal of the organic substrates added by the wastewater, and to a lesser extent, the removal of soluble organic compounds produced by microbial metabolism (Figure 4.1).

The removal of soluble organic carbon in winery wastewater by the processes of adsorption and microbial metabolism in soil will be quantitatively described.   Two assays will be used: (I) the evolution of carbon dioxide, and (II) the removal of soluble organic carbon from soil extracts.   Evolution of carbon dioxide is commonly used to determine rates of mineralisation of organic substrates (Scow 1982).   A more direct measurement of the extent of removal of the added organic carbon from the soil solution can be achieved by the application of concentrated solutions of salts to extract the remaining unbound organic materials.
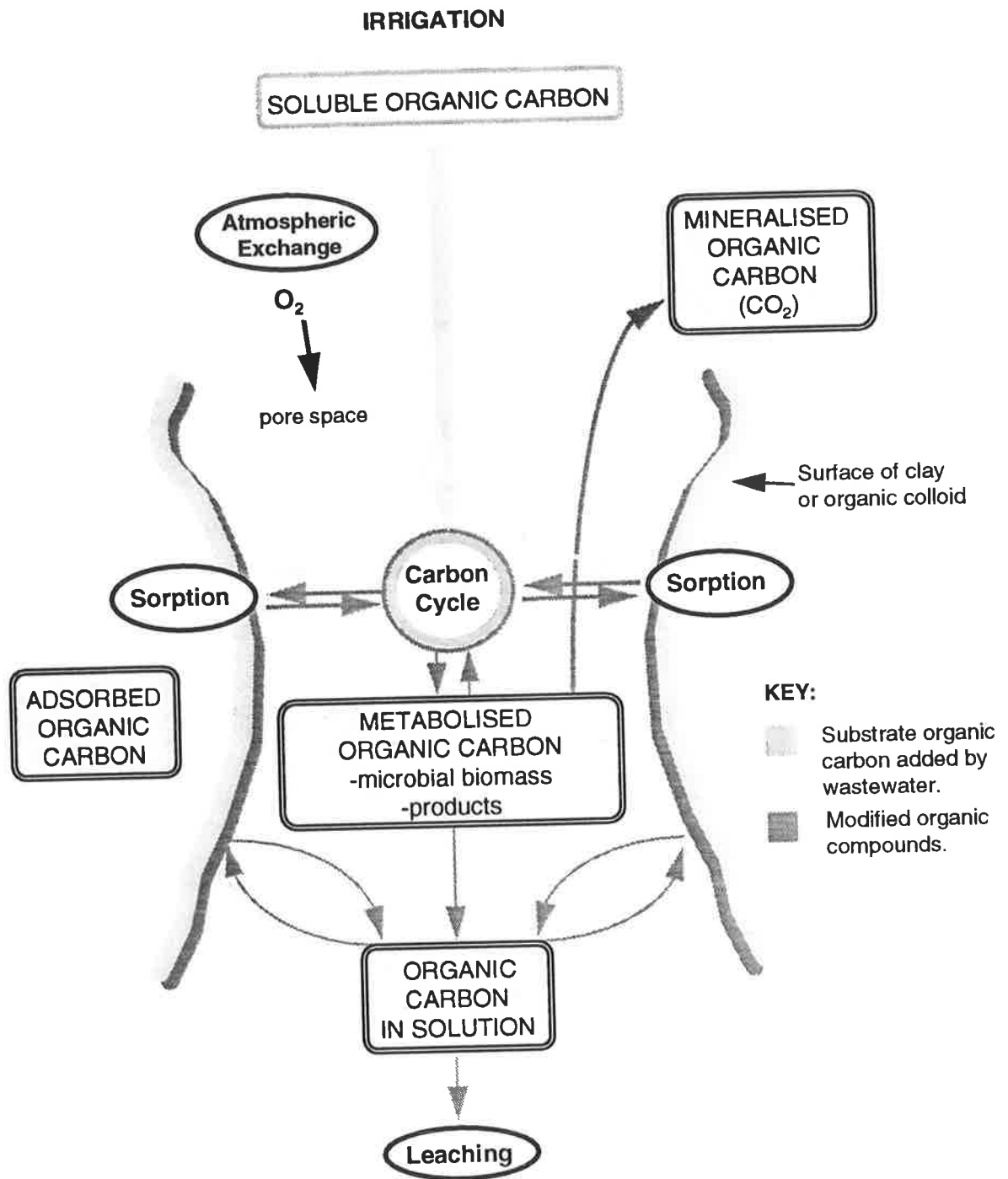
Figure 4.1:   Removal of soluble organic carbon in wastewater by the processes of adsorption and microbial metabolism in soil.

4.1 Materials and Methods

4.1.1 Soils

The surface layers (0–10 cm) of a solod (Australian Map Grid (AMG) Reference: 54H UG 133829) and a brown earth (AMG: 54H UG 200792) were sampled from the Barossa Valley, a premier wine district in South Australia. The solod consisted of a sand of 0–0.2m to 0–1.0m overlying a 0.3–0.7m massive yellow-grey clay of poor permeability (Northcote et al. 1954). The soil was sampled from within a 2 year-old woodlot irrigated with wastewater from a winery-distillery. The brown earth consisted of a sandy loam of 0–0.3m overlying a 0.5–0.7m brown clay, with both soil layers impregnated with honeycomb limestone (Northcote and deMooy 1957). The site has been irrigated with winery wastewater for 17 years, and used as a permanent pasture for grazing.

Thirty samples were taken at random from an area considered representative of each soil type. The soils were air-dried to about 25% of their gravimetric moisture contents at a potential of -10 kPa ($0.25W_{-10}$), and passed through a 2 mm sieve (solod) or a 4.75 mm sieve (brown earth), before being mixed to form a composite sample for each soil type, and stored in sealed containers at room temperature. Properties of the solod and brown earth, listed in Table 4.1, were determined as follows.

The gravimetric moisture content at -10 kPa (W_{-10}) was determined by placing replicate samples of soil (100g) on sintered funnels (porosity 4) at -1 kPa (10 cm suction) to wet the soil relatively slowly (24h), and then a positive head (10 cm) was used to wet the soil until free water was just visible at the surface. A tension of -10 kPa (100 cm suction) was subsequently applied for 7 days before determination of the gravimetric moisture content.

Particle-size analyses were determined by the hydrometer method (Day 1965). A 1:5 ratio of soil:reverse osmosis (RO) water (<5 dS/m) was used to determined pH and electrical

Table 4.1: Characteristics of the acclimatised brown earth and solod (0–10 cm).

	Brown earth	Solod
% sand, silt, clay	63/32/5	85/15/0
%W at -10 kPa	35.9	14.5
$pH_{1:5:\ water}$	5.9	6.0
$EC_{1:5:\ water}$ (dS/m)	1.2	0.9
Total Organic Carbon (%)	5.7	2.7
Total Kjeldahl N (mg/g)	59.4	19.5
Bicarbonate P (μg/g)	208	250
Exchangeable Cations (mg/100g)		
Sodium	19	7
Potassium	118	38
Calcium	344	156
Magnesium	35	10
$SAR^{†}$	0.26	0.15

† Sodium Adsorption Ratio $SAR = [Na^+] / (([Ca^{2+}] + [Mg^{2+}])/2)^{½}$; concentration of exchangeable cations in millimoles.

conductivity (Rhoades 1982). Organic carbon was determined by difference between total and carbonate carbon. Total carbon was determined by combustion in a high temperature induction furnace (Nelson and Sommers 1982). Carbonate carbon was determined by the volumetric calcimeter method described by Allison and Moodie (1965). Nitrogen was determined as total Kjeldahl nitrogen and, available phosphorus by bicarbonate extraction (Heanes 1981). Exchangeable cations were determined by difference between total cations and soluble cations (Thomas 1982).

4.1.2 Wastewater

To enable reproducibility of composition without the need for storing large volumes of wastewater, a synthetic wastewater was prepared based on the dominant organic and inorganic components of winery wastewater sampled during the vintage period after separation of solids. The organic constituents were chosen to represent the biodegradabilities of the major organic groups, and blended to obtain a total organic carbon content close to the upper range found in the natural wastewater. To avoid precipitation of potassium-hydrogen bitartrate, higher levels of acetic acid were required in the synthetic wastewater than found in the natural wastewater. Similarity in pH was obtained by using free organic acids and the hydroxides of the inorganic ions. The composition of the synthetic winery wastewater is shown in Table 4.2. The wastewater was prepared 16h before each experiment and allowed to stand at room temperature to avoid crystallisation of potassium-hydrogen bitartrate. RO water was used throughout for making up the solution and for dilutions.

Immediately before each experiment, a mixture of 99.9% pure L-[U-^{14}C]-lactic acid (la) and [U-^{14}C]-glycerol (gly; 4 kBq) was added to the synthetic wastewater in the proportion of 70:30 (W:W), a proportion commonly found in winery wastewater sampled during the vintage period after separation of solids. The isotope mixture never exceeded 3% of the total volume. Costs prevented the use of labelled forms of the other organic constituents.

Table 4.2: Composition of the synthetic winery wastewater.

Organic component (g/l)	
Tartaric acid	1.0
Lactic acid	1.5
Acetic acid	2.0
Ethanol	1.5
Glycerol	1.0
Inorganic component (mg/l)	
Sodium	148
Potassium	97
Calcium	24
Magnesium	9
SAR[†]	6.2
pH	4.3
EC (dS/m)	2.1

[†] Sodium Adsorption Ratio SAR = $[Na^+] / (([Ca^{2+}] + [Mg^{2+}])/2)^{1/2}$; concentration of exchangeable cations in millimoles.

This research is thus based on the assumption that the rate of disappearance of the ^{14}C-lactic acid and glycerol in solution in the synthetic wastewater is similar to that of the total water-soluble carbon in winery wastewater. In Chapter 3 it was found that >95% of the total water-soluble organic carbon consisted of constituents with similar biodegradabilities (Table 3.6). Polyphenolic flavour and pigment compounds, which represent <1% of the total organic content of winery wastewater, have lower indices of biodegradability (BOD/COD of approximately 0.2, Mauganet 1978). A preliminary measurement of the mineralisation of the individual ^{14}C-compounds, and the 70:30 la:gly mixture added to the acclimatised soils in RO water is shown in Figure 4.2.

4.1.3 Incubation: evolution of $^{14}CO_2$

Samples equivalent to 30g oven-dried basis, of the soils stored at $0.25W_{-10}$ were placed in a 120 ml container and equilibrated to $0.35W_{-10}$ (24h), before being amended with a volume of synthetic winery wastewater equivalent to $0.6W_{-10}$. Thus, the relative moisture content of the soils for incubation was $0.95W_{-10}$, chosen to reflect field levels immediately after irrigation. Due to the lower gravimetric moisture content at -10 kPa of the solod compared with the brown earth, the amount of organic carbon (OC) added to the solod (245 mg/kg) was about 40% of that added to the brown earth (634 mg/kg). Each amended sample was lightly tamped down within its container to an approximate bulk density of 1.3 Mg/m^3.

The 120 ml vessel containing the amended soil was placed in a 1 litre, gas-tight glass jar containing 5 ml of water to maintain 100% relative humidity. A 70 ml, wide-based vessel containing 4 ml of 5M or 1M NaOH was placed immediately above the amended soil to absorb CO_2. Preliminary experiments had shown that efficient absorption of CO_2 was achieved by using 5M NaOH during the first 3 days, and a 1M concentration from day 3
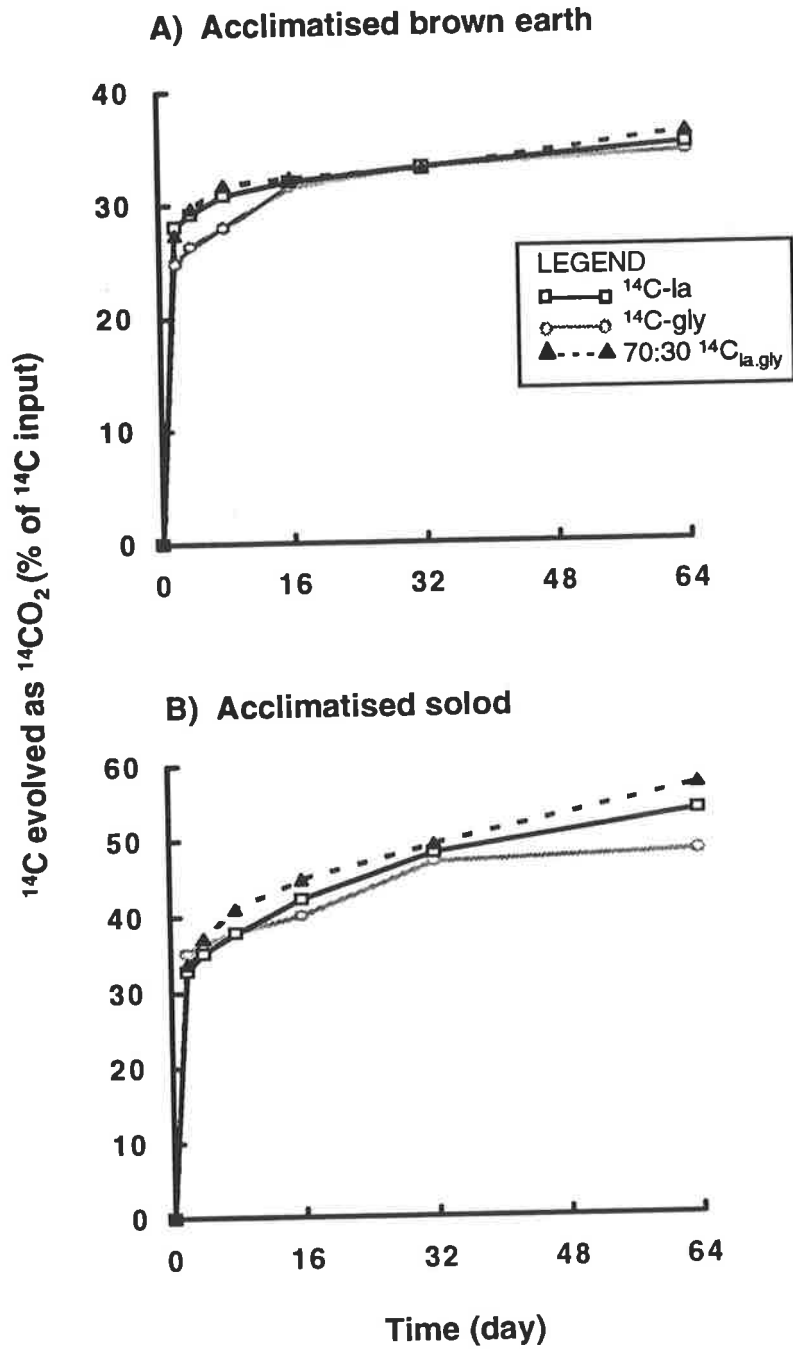
Figure 4.2: Evolution of $^{14}CO_2$ from an acclimatised brown earth and solod amended with L-[U-^{14}C]-lactic acid (la), [U-^{14}C]-glycerol (gly), and a 70:30 (W:W) mixture of la:gly (4 kBq). Error bars were <3%, and hence could not be illustrated.

onwards. Four replicates of the soils were incubated at 25°C for 7 days, which was considered the maximum time allowed between successive irrigations of the field sites with wastewater. Rapid evolution of ^{14}C as $^{14}CO_2$ occurred during the first 2 days after application of the wastewater, with much slower rates of $^{14}CO_2$ evolution occurring thereafter (Figure 4.2).

During the incubation the CO_2-absorbing solutions were removed on days 0.5, 1, 2, 3, and 7, and renewed as appropriate. Soils amended with non ^{14}C-containing wastewater were also incubated to obtain background readings. The frequent changing of the CO_2-absorbing solutions was designed to prevent the development of anaerobic conditions between samplings by replenishment of oxygen from the atmosphere.

The radioactivity of absorbed CO_2 was determined on a 0.13 ml aliquot of the 5M NaOH trapping solution, and on a 0.4 ml aliquot of the 1M NaOH solution. The aliquots were diluted to a total volume of 1 ml, and mixed with 10 ml of PCS^{II} liquid scintillation cocktail. The radioactivity of the ^{14}C-labelled wastewater was determined from a 0.1 ml aliquot mixed with 0.4 ml of 1M NaOH and 0.5 ml of RO water. The mixtures consistently gave a counting efficiency of 91% over 10 min. Cumulative evolution of ^{14}C as $^{14}CO_2$ was expressed as a percentage of added ^{14}C.

4.1.4 Incubation: removal of soluble-^{14}C from soil extracts

Samples equivalent to 10g oven-dried basis, of the soils stored at $0.25W_{-10}$ were placed into 50 ml, wide-neck centrifuge bottles, equilibrated to $0.35W_{-10}$ (24h), and then amended with the ^{14}C-containing wastewater as described in Section 4.1.2, to a final soil moisture content of $0.95W_{-10}$. Four replicates of soil were incubated at 25°C for 0, 0.25, 0.5, 1, 2, or 3 days (by which time <5% of the added ^{14}C could be extracted from the soils). Bottles used for the longer incubations were periodically opened to renew oxygen levels to prevent anaerobic conditions forming.

The remaining soluble-^{14}C was extracted by adding 20 ml of 2M KCl, mixing the soil suspensions for 1h on a rotating shaker, and then centrifuging at 4000 rev/min for 10 min in a 'Heraeus CHRIST Labofuge A' centrifuge. The radioactivity of the supernatant solution was determined from a 1 ml aliquot mixed with 10 ml of PC[II] liquid scintillation cocktail. Recoveries were expressed as a percentage of ^{14}C inputs.

The radioactivity of the ^{14}C-labelled wastewater was determined from a 0.1 ml aliquot mixed with 0.9 ml of the solutions used for the background readings for each type of soil. Background readings were obtained from soil amended with wastewater only. The mixtures consistently gave a counting efficiency of 91% over 10 min.

4.2 Results

About 27% of the added ^{14}C was retained by the brown earth, and 19% of the added ^{14}C was retained by the solod following extraction immediately after amendment with the ^{14}C-labelled wastewater (shown in Figure 4.3 by a drop from 100% to 73% or 81% at t=0 day). This initial reduction in soluble-^{14}C was attributed to adsorption of the substrate ^{14}C-compounds (ie., ^{14}C-labelled lactic acid and glycerol) to clay and/or organic colloids. The initial adsorption of the ^{14}C-compounds was followed by a lag period of variable length where the solution concentration of ^{14}C decreased by <5% of added ^{14}C, followed by a second period of rapid decline in solution-^{14}C due to microbial metabolism, to values reaching an asymptote (<5% of ^{14}C input) as the substrate ^{14}C-compounds added by the wastewater were exhausted.

The decline in solution-^{14}C during the phase of rapid microbial metabolism of ^{14}C-compounds corresponded with the period of maximum mineralisation to $^{14}CO_2$. Evolution of $^{14}CO_2$ continued after the solution concentration of ^{14}C had been reduced to <5% of ^{14}C inputs.
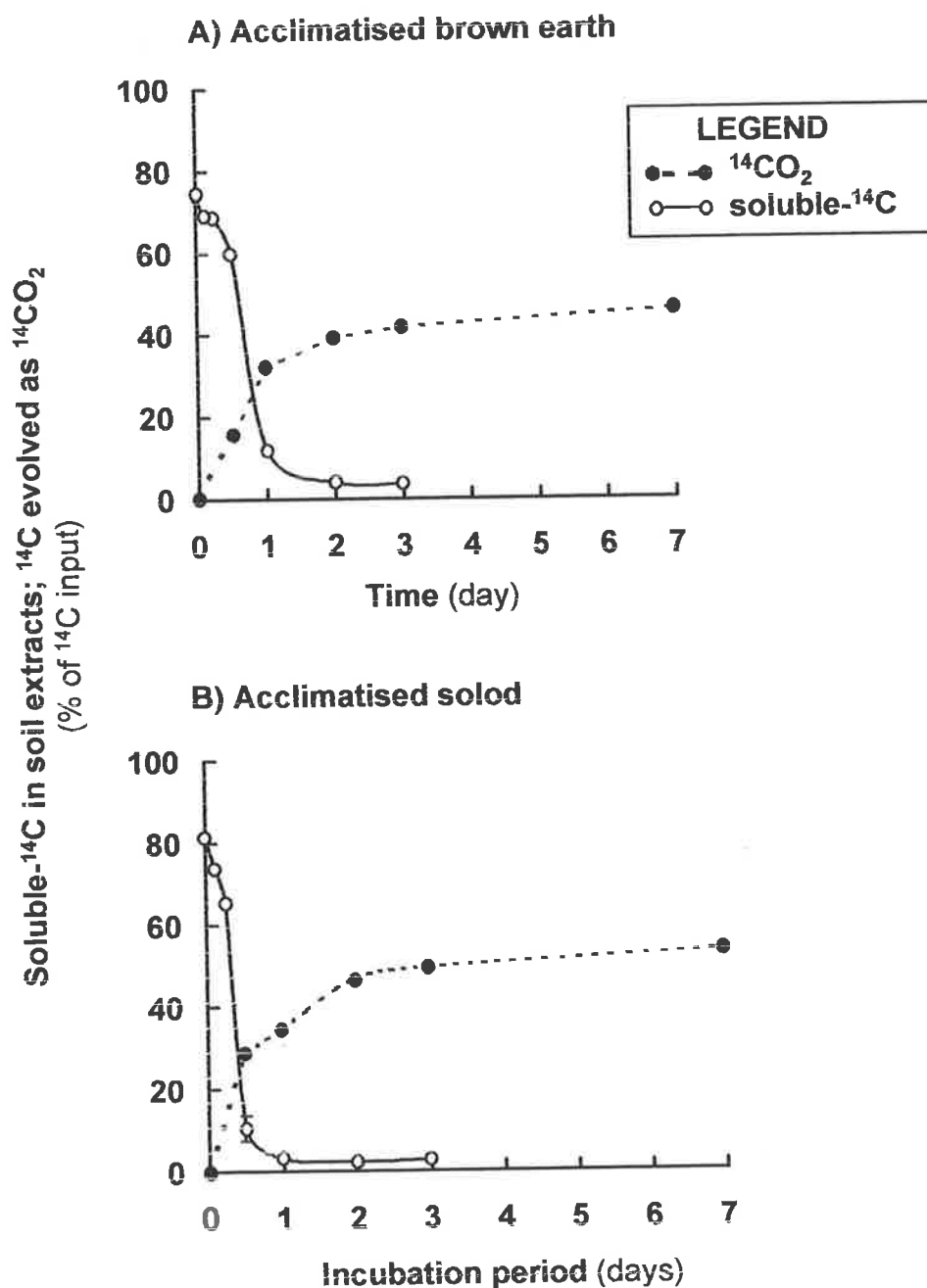
Figure 4.3: Removal of soluble-^{14}C in soil extracts, and the proportion of added ^{14}C evolved as ^{14}CO$_2$ from an acclimatised brown earth and solod incubated with synthetic winery wastewater. Error bars were <2.5%, and hence could not be illustrated.

4.2.1 Model

Both the evolution of ^{14}C as $^{14}CO_2$, and the decline in soluble-^{14}C of soil extracts with time were best described (R^2 >95%) by a logistic model of the form:

$$y = a + \frac{c}{(1 + \exp(-b(t-m)))}$$

where:

y = ^{14}C evolved as $^{14}CO_2$, or the soluble-^{14}C of soil extracts (% of ^{14}C input);

t = time from application (day);

m = the time at which the rate of evolution of $^{14}CO_2$ reached a maximum, or the time at which rate of decline of soluble-^{14}C reached a maximum, during the phase of rapid microbial metabolism of ^{14}C-compounds (% of ^{14}C input/day);

b = the average rate of evolution of $^{14}CO_2$, or the average rate of reduction of soluble-^{14}C, during the phase of rapid microbial metabolism of ^{14}C-compounds (% of ^{14}C input /day).

The curve has two asymptotes, a+c, and a; a and c are both constants (% of ^{14}C input). The asymptote, a, represents proportion of added ^{14}C evolved as $^{14}CO_2$, or the ^{14}C remaining in solution after the substrate ^{14}C-compounds added by the wastewater have become exhausted. The asymptote, a+c, has no biological meaning.

The model was fitted separately to each replicate as recommended by Mead and Currow (1983). An example of the GENSTAT-generated model, and estimated parameters is given in Appendix I.

4.2.2 Definition of parameters

Figure 4.4 shows the parameters estimated from the fitted curves. Added ^{14}C was partitioned into: (I) ^{14}C initially adsorbed by the soil, (II) ^{14}C evolved as $^{14}CO_2$ (not illustrated), (III) ^{14}C microbially metabolised and, (IV) ^{14}C remaining in solution after the substrate ^{14}C-compounds added by the wastewater have become exhausted. The amount
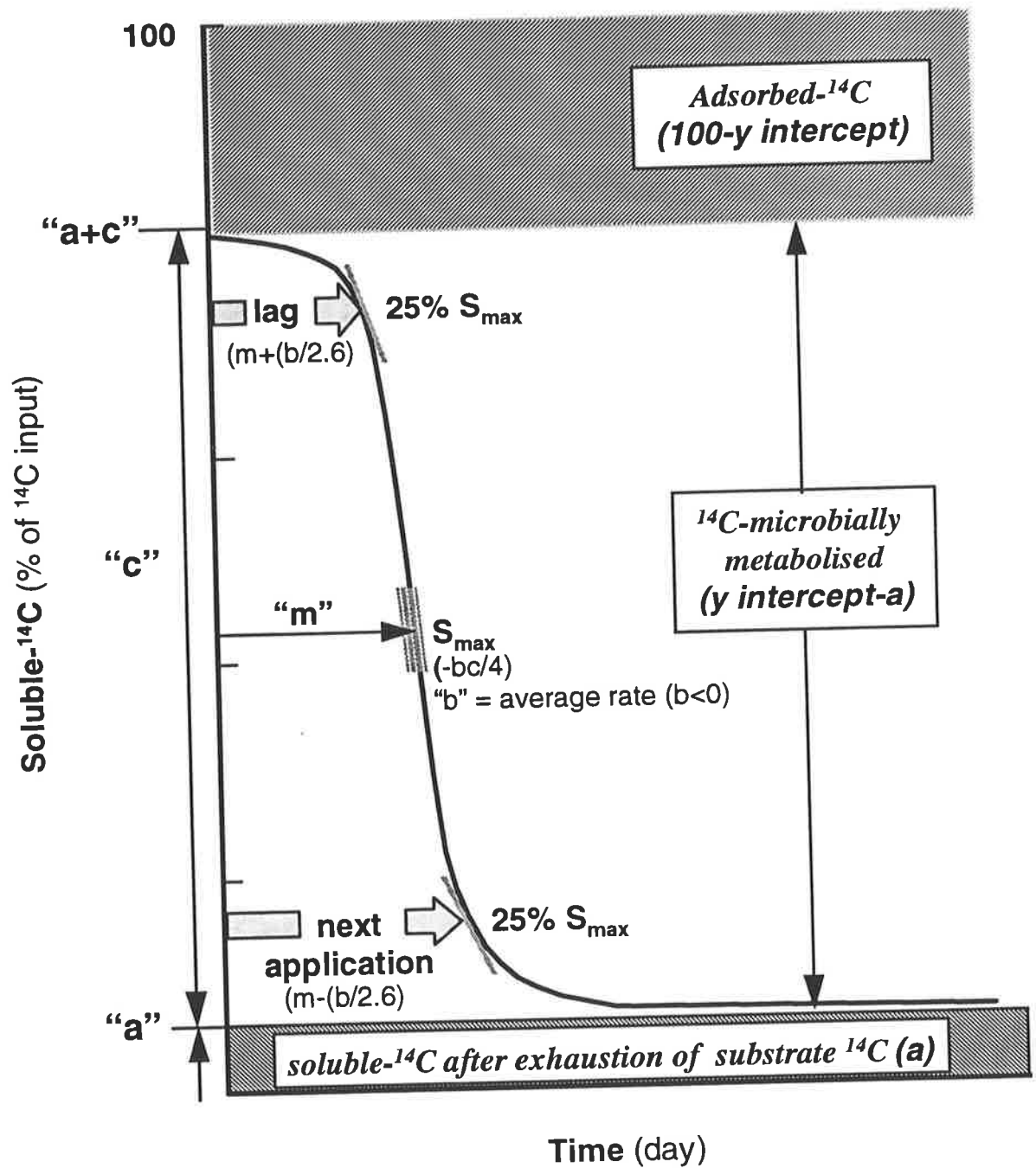
Figure 4.4: Parameters estimated from the model curve of the removal of soluble-^{14}C in soil extracts with time.

Table 4.3: Removal of soluble-^{14}C by an acclimatised brown earth and solod incubated with synthetic winery wastewater.

	Brown earth	Solod	significance at 95% probability
Adsorbed-^{14}C (% of ^{14}C input)			
	28.5	20.0	significant
^{14}C microbially metabolised (% of ^{14}C input)			
	67.5	77.6	significant
^{14}C evolved as $^{14}CO_2$ (% of ^{14}C input)			
	43.2	53.0	significant
Solution-^{14}C after metabolism of the substrate ^{14}C-compounds (% of ^{14}C input)			
	3.9	2.4	not significant

of ^{14}C initially adsorbed by the soils was given by difference between the amount added and the y intercept. The amount of added ^{14}C microbially metabolised was given by difference between the y intercept and "a". The proportions of added ^{14}C evolved as $^{14}CO_2$, or remaining in solution after the substrate ^{14}C-compounds added by the wastewater have become exhausted were defined in Section 4.2.1. The partitioning as designated does not exclude the possibilities that (I) part of the substrate ^{14}C-compounds initially adsorbed by the soils was subsequently metabolised, and (II) part of the ^{14}C extracted at any stage is of microbial origin. The estimates are given in Table 4.3.

As mentioned in Section 2.4, sufficient time must be allowed between successive irrigations with wastewater to allow the soil microorganisms to metabolise the soluble organic carbon. Thus, the minimum time between irrigations was determined by the length of the lag period, and the duration of the period of rapid microbial metabolism of the solution organic carbon (Figure 4.4). Rapid microbial metabolism of ^{14}C-compounds was taken arbitrarily to begin when the rate of removal of soluble-^{14}C in soil extracts had reached 25% of the maximum, and to end when the rate declined to 25% of the maximum. The definition ensured that (I) the concentration of ^{14}C in solution after initial adsorption of ^{14}C-compounds varied by <5% of added ^{14}C during the lag period, and (II) <5% of added ^{14}C could be extracted from the soils following rapid microbial metabolism and exhaustion of substrate ^{14}C-compounds added by the wastewater. Average rates of microbial metabolism were consistently found to be about 70% of the maximum.

Thus, the length of the lag period was defined as the time elapsed from application of the wastewater to the start of rapid microbial metabolism of ^{14}C-compounds. The time elapsed from application of the wastewater to the end of rapid microbial metabolism of ^{14}C-compounds was used to estimate the minimum rest period between successive irrigations with wastewater (Table 4.4). Estimates of mineralisation of added ^{14}C were also obtained (Table 4.4).

Table 4.4: Microbial metabolism of ^{14}C-compounds by an acclimatised brown earth and solod incubated with synthetic winery wastewater.

	Brown earth	Solod	significance at 95% probability
Estimates based on the removal of soluble-^{14}C in soil extracts			
Duration of the lag period (day)			
	0.34	0.33	not significant
Maximum rate of microbial metabolism of ^{14}C-compounds† (% of ^{14}C input/day)			
	118	240	significant
Time to next application (day)			
	1.09	0.57	significant
Estimates based on ^{14}C evolved as ^{14}CO$_2$			
Duration of the lag period (day)			
	0.00	0.17	significant
Maximum rate of microbial metabolism of ^{14}C-compounds† (% of ^{14}C input/day)			
	40	58	significant
Time to next application (day)			
	1.42	1.24	significant

† Average rates of microbial metabolism of ^{14}C-compounds were consistently found to be 70% of the maximum, and hence were not shown separately.

4.2.3 Removal of soluble organic carbon

Although a greater amount of ^{14}C-compounds remained in solution in the brown earth than in the solod following initial adsorption, the solod was able to metabolise the soluble ^{14}C-compounds more rapidly than the brown earth. As a result of the faster rate of metabolism, the solod required less time between irrigations to remove the substrate ^{14}C-compounds added by the wastewater than the brown earth. Once the substrate ^{14}C-compounds were exhausted, <5% of added ^{14}C could be extracted from both soils (Tables 4.3–4.4).

Effects of soil texture on mineralisation of ^{14}C-compounds followed a similar pattern to the removal of soluble-^{14}C from soil extracts, with an exception that evolution of $^{14}CO_2$ began without a lag period in the brown earth. The maximum rate of evolution of $^{14}CO_2$ from both soils was lower than the corresponding rate of removal of soluble-^{14}C from soil extracts.

The lower maximal rate of evolution of $^{14}CO_2$ may be partly responsible for the absence of a lag period in the brown earth, and a shorter lag period in the solod compared with estimates based on the removal of soluble-^{14}C from soil extracts. The time to cessation of rapid evolution of $^{14}CO_2$ was 7.9h greater than the corresponding time taken for the removal of soluble-^{14}C from soil extracts to <5% of ^{14}C inputs for the brown earth, and 16.0h for the solod ("time to next application", Table 4.4).

4.3 Discussion

Both soils had the capacity to rapidly reduce the solution concentration of organic carbon added in synthetic winery wastewater to <5% of input levels. The time for removal of the soluble organic carbon was determined by the length of the lag period before rapid microbial metabolism, and the rate of oxidation obtained.

Greater initial adsorption of ^{14}C-compounds by the brown earth, of higher clay and organic carbon content than the solod, is consistent with the effects of clay and organic carbon contents of soils on adsorption of organic pesticides (Calvert 1989; Weber et al. 1991).

A greater rate of metabolism of ^{14}C-compounds in the solod than brown earth was also in agreement with the effect of clay content on mineralisation of ^{14}C-compounds in soils receiving similar rates of carbon loading observed by Kunc and Stotzky (1974), Van Veen et al. (1985), and Ladd et al. (1992). In contrast, Gregorich et al. (1991) found that during the first day of incubation, decomposition of substrate C was higher in soils with more clay. In their study, Gregorich et al. (1995) emphasised the importance of the active phase of the soil organic matter in providing the main energy source for the biomass. Mineralisation of the higher amounts of organic carbon present in the soils with more clay may partly explain their results.

The role of mineralisation of soil organic carbon during the decomposition of added substrate C requires further clarification in relation to the effect of clay content of soils on the rate of removal of solution organic carbon added by wastewaters, as the former is likely to involve a different group of micro-organisms. Competition for oxygen, and available nutrients, particularly nitrogen, may also influence the ability of soils of different textural groups to rapidly remove the solution organic C added by wastewaters.

Lower maximal rates of evolution of ^{14}CO$_2$ than the corresponding rates of removal of soluble-^{14}C from soil extracts were expected, since metabolism of the ^{14}C-compounds would result in the formation of biomass-^{14}C and other ^{14}C-containing products, as well as ^{14}CO$_2$. Continued turnover of biomass-^{14}C and other ^{14}C-containing products may have contributed to the continued rapid evolution of ^{14}CO$_2$ several hours after the substrate ^{14}C-compounds added by the wastewater had disappeared from solution.

4.4 Conclusions

The removal of soluble organic carbon in winery wastewater by the processes of adsorption and microbial metabolism in soils was quantified at field moisture levels. Between 20-30% of added ^{14}C-compounds was rapidly removed by adsorption. Microbial metabolism was essential for the reduction of the ^{14}C-compounds remaining in solution to <5% of ^{14}C inputs. The duration of the lag period before the phase of rapid microbial metabolism of the organic substrates, and the maximum rate of mineralisation or removal of solution-^{14}C attained by the soils, were both influenced by texture. Thus, the minimum time required between successive irrigations with winery wastewater differed between the two soils.

Based on the time taken for the solution-^{14}C in soil extracts to be reduced to <5% of ^{14}C inputs, at least 1.09 day would be required between irrigations of wastewater to the brown earth, and 0.59 day for the solod. It was concluded that estimates of times required between successive irrigations with wastewater based on the evolution of $^{14}CO_2$ were less reliable, due to the continued turnover of ^{14}C incorporated into microbial biomass and products after the substrate ^{14}C-compounds added by the wastewater had disappeared.

Effects of carbon loading, acclimatisation of microbial populations, and the relative moisture content of the soils at incubation on adsorption and microbial metabolism of soluble organic carbon added in winery and distillery wastewaters by irrigation, will be described in the next Chapter.

CHAPTER 5

REMOVAL OF SOLUBLE ORGANIC CARBON FROM WINERY AND DISTILLERY WASTEWATERS BY IRRIGATION OF SOILS

5.0 Introduction

It was found in Chapter 4 that soluble organic carbon applied in winery wastewater to the topsoils of a solod and brown earth could be rapidly removed to <5% of input values by adsorption, and metabolism to microbial biomass, carbon dioxide and other products. Decreases in the solution concentration of ^{14}C-compounds during the first hour after application of wastewater, containing ^{14}C-labelled lactic acid and glycerol, were ascribed to adsorption. Microbial metabolism was responsible for the removal of the remaining soluble ^{14}C-compounds, with removal times dependent on the duration of a lag period (when present), and the rate of metabolism.

Adsorption and microbial metabolism of the ^{14}C-compounds were influenced by soil texture. The brown earth, of higher clay content than the solod, adsorbed a higher proportion of added ^{14}C-compounds, but metabolised the ^{14}C-compounds remaining in solution more slowly, and hence took more time to remove the soluble-^{14}C to <5% of ^{14}C input than the solod.

It was hypothesised in Chapter 2 that adsorption and/or microbial metabolism of the soluble organic carbon could be influenced by the carbon loading, adaptation of microbial populations to the organic substrates (acclimatisation), and the relative moisture content at which the soils were incubated, as outlined in Section 2.4.3. Experiments designed to test the hypothesis are described below. Composite samples of disturbed soils were used here to allow the greatest combination of the above factors to be tested within a short period of time.

5.1 Materials and methods

5.1.1 Soils

Locations and characteristics of the acclimatised brown earth and solod have been described in Section 4.1.1. Non-acclimatised soils were sampled from pastures which had not been previously irrigated with wastewater, located next to the sites from which the acclimatised soils were sampled. Characteristics of the non-acclimatised brown earth and solod are given in Table 5.1; those of the acclimatised soils in Table 4.1.

Composite samples of the non-acclimatised soils were prepared as described for the acclimatised soils in Section 4.1.1.

5.1.2 Wastewaters

Synthetic winery and distillery wastewaters were prepared based on the dominant organic and inorganic components after separation of solids, as described in Section 4.1.2. The composition of the synthetic distillery wastewater is given in Table 5.2, and of the synthetic winery wastewater in Table 4.2.

Four levels of carbon loading (Table 5.3) were achieved by the application of 2 volumes of synthetic winery or distillery wastewater, each wastewater differing in its carbon content. The volumes of application were equivalent to either 30% or 60% of the gravimetric moisture content of the soils at -10 kPa (W_{-10}).

5.1.3 Incubation: evolution of $^{14}CO_2$

The soils were amended with the wastewaters as described in Section 4.1.3, and if necessary with reverse osmosis water to obtain a relative moisture content for incubation of 0.65-, 0.80-, or 0.95-W_{-10}, for wastewater applied to the soils at $0.3W_{-10}$, and 0.80- or 0.95-W_{-10} for wastewater applied to the soils at $0.6W_{-10}$. The moisture contents were

Table 5.1: Characteristics of the non-acclimatised brown earth and solod.

	Brown earth	Solod
% sand, silt, clay	62/33/5	84/16/0
%W at -10 kPa	35.9	13.9
$pH_{1:5:\ water}$	5.8	6.1
$EC_{1:5:\ water}$ (dS/m)	0.89	0.74
Total Organic Carbon (%)	4.9	1.7
Total Kjeldahl N (mg/g)	55.8	9.0
Bicarbonate P (µg/g)	190	11
Exchangeable Cations (mg/100g)		
Sodium	13	4
Potassium	46	13
Calcium	239	100
Magnesium	17	13
$SAR^{\dagger}$	0.22	0.10

† Sodium Adsorption Ratio $SAR = [Na^+] / (([Ca^{2+}] + [Mg^{2+}])/2)^{1/2}$; concentration of exchangeable cations in millimoles.

Table 5.2: Composition of the synthetic distillery wastewater.

Organic component (g/l)	
Tartaric acid	10.0
Lactic acid	8.0
Acetic acid	4.8
Ethanol	3.1
Glycerol	4.0
Inorganic component (mg/l)	
Sodium	430
Potassium	1400
Calcium	100
Magnesium	80
SAR[†]	7.7
pH	3.5
EC (dS/m)	8.9

[†]   Sodium Adsorption Ratio $SAR = [Na^+] / (([Ca^{2+}] + [Mg^{2+}])/2)^{\frac{1}{2}}$;
concentration of exchangeable cations in millimoles.

Table 5.3: Amounts of organic carbon added to the brown earth and solod by the winery and distillery wastewaters (mg/kg). The volumes of wastewater were applied were equivalent to 30% (0.3) and 60% (0.6)of the gravimetric moisture content at -10 kPa (W_{-10}).

	Winery		Distillery	
	$0.3W_{-10}^{\ddagger}$	$0.6\ W_{-10}$	$0.3W_{-10}$	$0.6W_{-10}$
Brown earth	317	634	1214	2428
Solod	122	245	469	938

chosen to represent normal variations encountered at the field sites. To accommodate the application of wastewater at $0.6W_{-10}$ to obtain a final moisture content of $0.8W_{-10}$, the soils were pre-equilibrated at $0.2W_{-10}$; for all other treatments the soils were pre-equilibrated at $0.35W_{-10}$.

Four replicates of the soils were incubated for 7 days, and amounts of ^{14}C evolved as $^{14}CO_2$ determined, as described in Section 4.1.3. The evolution of $^{14}CO_2$ with time was described by the logistic model outlined in Section 4.3.1. Estimates of the duration of the lag phase, maximum rate of evolution of $^{14}CO_2$, and the time elapsed to the end of rapid evolution of $^{14}CO_2$ were determined from curves fitted separately to each replicate, and subjected to analysis of variance.

After incubation for 7 days, the total residual ^{14}C in the soils and the portion of ^{14}C remaining in microbial biomass were determined. Total ^{14}C was measured after wet combustion of a finely-ground, oven-dried (80°C) subsample (Amato 1983). The proportion of added ^{14}C remaining in the biomass was assayed by the fumigation-incubation method of Jenkinson and Powlson (1976), and its amount was calculated from the difference in radioactivity of $^{14}CO_2$ released from fumigated-incubated and non fumigated-incubated subsamples, divided by 0.45. Non-biomass-^{14}C was then determined by difference between the total residual ^{14}C and the ^{14}C remaining in the biomass. Both biomass- and non-biomass-^{14}C were expressed as percentages of added ^{14}C.

The partitioning of added ^{14}C after incubation for 7 days into ^{14}C evolved as $^{14}CO_2$, ^{14}C remaining in the microbial biomass, and residual non-biomass ^{14}C was subjected to analysis of variance.

5.1.4 Incubation: removal of soluble-^{14}C from soil extracts

The acclimatised and non-acclimatised solod and brown earth were amended with the ^{14}C-labelled wastewaters as described in Section 5.1.2. Four replicate soils were incubated with

the ^{14}C-labelled wastewaters as described in Section 4.1.4, with the exception that soils amended with the synthetic distillery wastewater were incubated for 0, 0.25, 0.5, 1, 2, 3, 5, and 7 days. The remaining soluble-^{14}C was extracted from the soils, and the radioactivity measured as described in Section 4.1.4.

The reduction in solution-^{14}C with time was described by the logistic model outlined in section 4.3.1. Estimates of the partitioning of added ^{14}C into adsorbed-^{14}C, ^{14}C removed during the phase of rapid microbial metabolism, and ^{14}C remaining in solution after the substrate ^{14}C-compounds added by the wastewater had been metabolised were obtained from curves fitted separately to each replicate, and subjected to analysis of variance. The duration of the lag phase, maximum rate of removal of soluble-^{14}C during the phase of rapid microbial metabolism, and the minimum time required between successive irrigations with wastewater were also estimated, as defined in section 4.3.2, and the estimates subjected to analysis of variance.

5.2 Results

5.2.1 Evolution of ^{14}CO$_2$

Evolution of ^{14}CO$_2$ from the soils treated with the synthetic winery wastewater occurred without a lag period, with few exceptions. The main exception was the non-acclimatised solod which always had prolonged lag periods (Table 5.4). Soils treated with the synthetic distillery wastewater had lag periods ranging from a few minutes to a few days. Lag times were greater for soils newly-exposed to the wastewaters (non-acclimatised), than for the acclimatised soils containing microbial populations adapted to the substrates. Increasing the volume of application of the synthetic distillery wastewater increased lag times for all soils. Lag times for all soils and wastewater treatments decreased as the relative moisture content at which the soils were incubated was increased, with few exceptions (Table 5.4).

Table 5.4: Effect of carbon loading and the relative moisture content of incubation of a brown earth and a solod on the duration of the lag phase for acclimatised and non-acclimatised soils.

	Winery		Distillery	
	$0.3W_{-10}$[‡]	$0.6W_{-10}$	$0.3W_{-10}$	$0.6W_{-10}$
Brown earth -acclimatised		lag (day)		
$0.65W_{-10}$	0.00	not determined (ND)	0.56	ND
$0.8W_{-10}$	0.00	0.05	0.06	1.75
$0.95W_{-10}$	<0.05	0.00	<0.05	0.91
-non acclimatised				
$0.65W_{-10}$	0.00	ND	0.73	ND
$0.8W_{-10}$	0.00	<0.05	0.48	0.45
$0.95W_{-10}$	0.00	0.00	0.40	1.69
Solod -acclimatised				
$0.65W_{-10}$	0.00	ND	0.24	ND
$0.8W_{-10}$	0.00	0.33	0.17	1.36
$0.95W_{-10}$	0.08	0.00	0.13	0.85
-non acclimatised				
$0.65W_{-10}$	0.27	ND	1.25	ND
$0.8W_{-10}$	0.09	1.85	1.10	2.39
$0.95W_{-10}$	0.17	1.72	0.38	2.38
				LSD[†]: 1.98

[†] To account for effects of carbon loading on lag times of the different soils the LSD has been calculated from log-transformed data, and hence becomes a ratio between treatment a : treatment b, eg., 0.56/0.6=9.3; significant at 5%.

[‡] W_{-10} = the gravimetric moisture content at -10 kPa.

Average rates of evolution of $^{14}CO_2$ during the phase of rapid mineralisation of the ^{14}C-compounds were consistently 70% of the maximum. The maximum rate of evolution of $^{14}CO_2$ was higher in the solod than in the brown earth, and for acclimatised soils than non-acclimatised soils. A major exception was the application of winery wastewater to the brown earth for which higher rates of evolution of $^{14}CO_2$ were achieved by the non-acclimatised soils (Table 5.5). Both carbon loading and the relative moisture content at which the soils were incubated had a varied effect on the maximum rate of evolution of $^{14}CO_2$. The brown earth pre-equilibrated to $0.2W_{-10}$ and incubated at $0.8W_{-10}$ usually evolved $^{14}CO_2$ more slowly than when pre-equilibrated to $0.35W_{-10}$, and incubated at $0.95W_{-10}$ for the same wastewater treatments ($0.6W_{-10}$).

Times elapsed from the addition of the ^{14}C-labelled wastewaters to the cessation of rapid evolution of $^{14}CO_2$ were greater for the brown earth than solod, and for non-acclimatised soils than acclimatised soils, with some exceptions for the brown earth (Table 5.6). Increasing the carbon loading tended to increase the time elapsed from the addition of the ^{14}C-labelled wastewaters to the cessation of rapid evolution of $^{14}CO_2$ for all soils. Elapsed times were remarkably similar for a given wastewater treatment of the non-acclimatised soils incubated at the different relative moisture contents. The relative moisture content of the acclimatised soils had a variable effect on the time to cessation of rapid evolution of $^{14}CO_2$ for a given wastewater treatment.

5.2.1.1 Partitioning of added ^{14}C into $^{14}CO_2$, biomass-^{14}C, and non-biomass-^{14}C

The partitioning of added ^{14}C after incubation for 7 days into ^{14}C evolved as $^{14}CO_2$, ^{14}C remaining in the microbial biomass, and residual non-biomass-^{14}C, showed no definite trend in response to the relative moisture content of the soils during incubation. Thus, Table 5.7 shows the partitioning of added ^{14}C for soils incubated at $0.95W_{-10}$.

The solod evolved higher amounts of added ^{14}C as $^{14}CO_2$ but retained lower amounts in the microbial biomass than the brown earth. Similar trends were shown by the non-

Table 5.5: Effect of carbon loading and the relative moisture content of incubation of a brown earth and a solod on the maximum rate of evolution of $^{14}CO_2$ for acclimatised and non-acclimatised soils[†].

	Winery		Distillery	
	$0.3W_{-10}$[‡]	$0.6W_{-10}$	$0.3W_{-10}$	$0.6W_{-10}$
maximum rate of evolution of $^{14}CO_2$ (% ^{14}C input/day)				
Brown earth -acclimatised				
$0.65W_{-10}$	75.4	not determined (ND)	44.6	ND
$0.8W_{-10}$	75.4	28.9	41.9	12.3
$0.95W_{-10}$	25.9	39.7	35.0	44.0
-non acclimatised				
$0.65W_{-10}$	89.9	ND	33.9	ND
$0.8W_{-10}$	89.3	34.4	35.1	10.9
$0.95W_{-10}$	74.1	34.8	31.0	24.0
Solod -acclimatised				
$0.65W_{-10}$	79.5	ND	55.0	ND
$0.8W_{-10}$	78.7	68.6	74.2	65.9
$0.95W_{-10}$	31.1	58.0	71.0	44.0
-non acclimatised				
$0.65W_{-10}$	31.1	ND	30.1	ND
$0.8W_{-10}$	34.4	64.8	27.7	43.6
$0.95W_{-10}$	36.0	31.8	23.0	45.0

LSD: 7.4% ^{14}C input/day

† The average rate of evolution of $^{14}CO_2$ during the phase of rapid mineralisation of ^{14}C-compounds was consistently 70% of the maximum, and hence was not given separately.

‡ W_{-10} = the gravimetric moisture content at -10 kPa.

Table 5.6: Effect of carbon loading and the relative moisture content of incubation of a brown earth and a solod on the time elapsed from the addition of wastewater to the end of rapid evolution of $^{14}CO_2$ for acclimatised and non-acclimatised soils.

	Winery		Distillery	
	$0.3W_{-10}$[‡]	$0.6W_{-10}$	$0.3W_{-10}$	$0.6W_{-10}$
	time to cessation of rapid evolution of $^{14}CO_2$ (day)			
Brown earth -acclimatised				
$0.65W_{-10}$	0.91	not determined (ND)	2.26	ND
$0.8W_{-10}$	0.88	2.36	2.09	>7 days
$0.95W_{-10}$	2.41	1.42	2.18	2.69
-non acclimatised				
$0.65W_{-10}$	0.74	ND	2.57	ND
$0.8W_{-10}$	0.69	1.98	2.42	>7 days
$0.95W_{-10}$	0.80	1.97	2.40	4.39
Solod -acclimatised				
$0.65W_{-10}$	0.89	ND	1.80	ND
$0.8W_{-10}$	0.89	1.39	1.25	3.30
$0.95W_{-10}$	2.39	1.24	1.32	2.82
-non acclimatised				
$0.65W_{-10}$	2.82	ND	3.66	ND
$0.8W_{-10}$	2.51	4.34	3.76	4.07
$0.95W_{-10}$	2.47	4.26	3.52	4.10
				LSD[†]: **1.67**

† To account for effects of carbon loading on times to cessation of rapid evolution of $^{14}CO_2$ of the different soils the LSD has been calculated from log-transformed data, and hence becomes a ratio between treatment a : treatment b, eg., 0.91/0.88=1.03; not significant at 5%.

‡ W_{-10} = the gravimetric moisture content at -10 kPa.

Table 5.7: Effect of carbon loading and acclimatisation of soil on the partitioning of added ^{14}C into ^{14}C evolved as ^{14}CO$_2$, ^{14}C remaining in the microbial biomass, and residual non-biomass-^{14}C after incubation of a solod and brown earth at $0.95W_{-10}$[†] for 7 days.

		Winery		Distillery	
		$0.3W_{-10}$[‡]	$0.6W_{-10}$	$0.3W_{-10}$	$0.6W_{-10}$
^{14}C evolved as ^{14}CO$_2$ (% of ^{14}C input)					
Brown earth	-acclimatised	55.5	42.9	55.9	59.3
	-non acclimatised	44.5	55.5	46.8	48.6
Solod	-acclimatised	58.1	53.1	60.3	63.5
	-non acclimatised	57.4	61.2	51.1	59.4
					LSD: 3.9 %
^{14}C remaining in the microbial biomass (% of ^{14}C input)					
Brown earth	-acclimatised	16.1	16.4	11.8	15.2
	-non acclimatised	14.8	15.9	15.4	12.5
Solod	-acclimatised	11.5	12.7	14.3	6.3
	-non acclimatised	10.7	5.7	5.9	
					LSD: 3.8%
Residual non-biomass-^{14}C (% of ^{14}C input)					
Brown earth	-acclimatised	17.0	30.4	21.2	18.9
	-non acclimatised	30.9	22.4	31.8	36.9
Solod	-acclimatised	24.5	27.3	19.1	19.7
	-non acclimatised	22.9	25.7	35.8	30.5
					LSD: 5.0%

† W_{-10} = the gravimetric moisture content at -10 kPa.

acclimatised soils compared with the acclimatised soils (with some exceptions in the brown earth). Carbon loading did not strongly influence the proportion of added ^{14}C evolved as $^{14}CO_2$ or remaining in the microbial biomass of the soils. Total recoveries of ^{14}C exceeded 88% of added ^{14}C.

5.2.2 Removal of soluble-^{14}C from soil extracts

About 20% of the applied ^{14}C was retained by the solod after extraction immediately following amendment with the ^{14}C-labelled wastewaters (Table 5.8). The proportion of added-^{14}C retained by the solod differed little between acclimatised and non-acclimatised soils, and for each level of carbon loading, with two exceptions which showed no definite trend. The brown earth retained 18–49% of applied ^{14}C after extraction immediately following addition of the wastewaters. Higher amounts of ^{14}C were retained by the acclimatised brown earth than the non-acclimatised brown earth, and by both soils at the lower volume of application of the synthetic winery and distillery wastewaters (Table 5.8).

The initial removal of ^{14}C-compounds was attributed to adsorption and microbial uptake (in the absence of a lag period). The amount of ^{14}C remaining in solution after adsorption and microbial uptake of ^{14}C-compounds was often lower than the sum of the amount of ^{14}C evolved as $^{14}CO_2$ and that retained in microbial biomass after incubation of the soils for 7 days (Table 5.7).

The duration of the lag phase ranged from no lag for winery wastewater applied to the soils at $0.3W_{-10}$, to 0.4–2.0 days for distillery wastewater applied to the soils at $0.6W_{-10}$ (Table 5.8). Shorter lag phases were exhibited by the soils at the lower volume of application of the synthetic winery or distillery wastewater, and by the acclimatised soils than non-acclimatised soils, with few exceptions. The duration of the lag phase increased in the general order of acclimatised solod < acclimatised brown earth < non-acclimatised brown earth < non-acclimatised solod.

Table 5.8: Effect of carbon loading on the proportion of added ^{14}C initially removed by adsorption, and remaining in solution after cessation of rapid microbial metabolism of ^{14}C-compounds by an acclimatised and non-acclimatised solod and brown earth incubated at $0.95W_{-10}$[†].

		Winery		Distillery	
		$0.3W_{-10}$[‡]	$0.6W_{-10}$	$0.3W_{-10}$	$0.6W_{-10}$
^{14}C initially removed by adsorption and microbial uptake (% of ^{14}C input)					
Brown earth	-acclimatised	48.5	28.4	40.9	37.7
	-non acclimatised	47.9	18.2	36.8	32.0
Solod	-acclimatised	22.1	20.0	27.9	16.2
	-non acclimatised	21.3	21.6	19.2	20.3
		LSD$_{win}$ 2.0 %		LSD$_{dist}$ 1.7 %	
Solution-^{14}C after cessation of rapid microbial metabolism of ^{14}C-compounds (% of ^{14}C input)					
Brown earth	-acclimatised	<0.1	3.9	1.8	<0.1
	-non acclimatised	<0.1	<0.1	0.9	<0.1
Solod	-acclimatised	2.4	2.4	2.8	1.7
	-non acclimatised	1.6	0.0	2.8	6.8
		LSD$_{win}$ 4.2 %		LSD$_{dist}$ 1.5 %	

† W_{-10} = the gravimetric moisture content at -10 kPa.

Effects of carbon loading and acclimatisation of microbial populations on maximum rates of removal of solution-^{14}C during the phase of rapid microbial metabolism of ^{14}C-compounds were similar to those found for the evolution of $^{14}CO_2$, except that the differences were more pronounced (Table 5.9). For example, the maximum rate of removal of solution-^{14}C increased on average by 158% in acclimatised soils compared with the respective non-acclimatised soils, whereas the average increase in the maximum rate of mineralisation of the ^{14}C-compounds was 25%. Average rates of removal of solution-^{14}C during the phase of rapid microbial metabolism of ^{14}C-compounds were consistently 70% of the maximum.

All but one of the soils were able to reduce the concentration of ^{14}C-compounds in solution to <5% of added ^{14}C during the 7-day incubation (Table 5.8). The exception was the non-acclimatised solod to which distillery wastewater was added at $0.6W_{-10}$, which had removed 93% of the added ^{14}C (the phase of rapid microbial metabolism had not yet finished). Times for removal of soluble-^{14}C to <5% of added ^{14}C for the four soils followed the same order as given for the lag phase, with removal times for all soils increasing with carbon loading (Table 5.9). Times elapsed from the addition of wastewater to the brown earth to the end of rapid metabolism of ^{14}C-compounds in solution were on average 0.52 day shorter than the times to cessation of rapid evolution of $^{14}CO_2$, and 1.7 days shorter for the solod (cf., Tables 5.6 and 5.9).

5.3 Discussion

5.3.1 Factors affecting removal times of soluble-^{14}C

The removal of soluble-^{14}C added in synthetic winery and distillery wastewaters, containing ^{14}C-labelled lactic acid and glycerol, to <5% of ^{14}C inputs was dependent on the capacity of microbial populations in soils to metabolise the organic substrates. Microbial activity, as measured by the duration of the lag phase and the maximum rate of metabolism

Table 5.9: Effect of carbon loading on microbial metabolism of ^{14}C-compounds by an acclimatised and non-acclimatised solod and brown earth incubated at 0.95W.

₁₀.

		Winery		Distillery	
		$0.3W_{-10}$[‡]	$0.6W_{-10}$	$0.3W_{-10}$	$0.6W_{-10}$
		Duration of the lag period[‡] (day)			
Brown earth	-acclimatised	0.00	0.34	0.02	1.05
	-non acclimatised	0.00	0.00	0.35	1.43
Solod	-acclimatised	0.00	0.33	0.21	0.46
	-non acclimatised	0.00	0.07	0.96	2.03
		LSD_{win}[†] 0.86		LSD_{win}[†] 1.48	
		Maximum rate of removal of solution-^{14}C ($\%$ ^{14}C input/day)			
Brown earth	-acclimatised	92	118	95	53
	-non acclimatised	59	91	58	29
Solod	-acclimatised	427	240	115	115
	-non acclimatised	146	57	103	38
		LSD_{win} 19%/day		LSD_{win} 12%/day	
		Time to end of rapid metabolism of ^{14}C-compounds in solution[‡] (day)			
Brown earth	-acclimatised	0.68	1.09	0.80	2.59
	-non acclimatised	1.08	1.55	1.82	4.51
Solod	-acclimatised	0.25	0.57	1.03	1.41
	-non acclimatised	0.65	1.98	1.95	>7
		LSD_{win}[†] 1.12		LSD_{dist}[†] 1.59	

† To account for effects of carbon loading on the duration of the lag period and time to cessation of rapid metabolism of ^{14}C-compounds in solution of the different soils, the LSD has been calculated from log-transformed data, and hence becomes a ratio between treatment a : treatment b, eg., 0.34/0.02=17; significant at 5%.

‡ W_{-10} = the gravimetric moisture content at -10 kPa.

of ^{14}C-compounds, differed between the two types of soil. The concentration of organic compounds remaining in the soil solution after initial adsorption, and previous exposure of the soils to the wastewaters (acclimatisation) strongly influenced microbial activity, and hence removal times of the ^{14}C-compounds.

The amount of ^{14}C remaining in the soil solution after initial adsorption of the ^{14}C-compounds was strongly influenced by soil texture, as outlined in Section 4.3. Greater adsorption of the ^{14}C-compounds by the acclimatised than non-acclimatised brown earth may have been the result of the gradual buildup of organic carbon in the acclimatised soil providing additional surfaces for adsorption. Increasing the carbon loading invariably increased the concentration of organic compounds remaining in solution, although the relative proportion of added ^{14}C adsorbed by the soils was generally not affected.

According to Oades (1989) more immediate and rapid mineralisation of ^{14}C-compounds occur in lighter-textured soils than in heavier-textured soils at similar rates of carbon loading, due to better access of micro-organisms to the substrates in the former soils. The lighter-textured solod used here, however, exhibited rates of mineralisation of ^{14}C-compounds and removal of soluble-^{14}C superior to the brown earth at similar carbon loadings (cf., distillery wastewater added to the solod to winery wastewater added to the brown earth) only when the soils had been previously exposed to the wastewaters. Thus, acclimatisation of microbial populations may be an important determinant in the relative ranking of soils of different textures with respect to metabolism of organic substrates, and hence their effectiveness in the treatment of organic-rich wastewaters used for irrigation. Low levels of total nitrogen and phosphorus in the non-acclimatised solod relative to the other soils may have also limited its capacity to rapidly mineralise the added ^{14}C-compounds.

Removal times of the added ^{14}C-compounds from the soil solution were found to be 2–3 times faster in soils with substrate-adapted micro-organisms than without substrate-adapted micro-organisms. According to Scow (1982) the length of the lag phase and the subsequent

rate of microbial metabolism of organic compounds are governed by the growth and enzymatic adaptation of microbial populations. A regular supply of the organic substrates by irrigating with wastewaters would quickly encourage selection of adapted species and maintain high populations of active cells in soils, resulting in an early start to and higher rates of metabolism of the organic substrates, as found in this study.

Higher solution concentrations of ^{14}C-compounds after initial adsorption and often prolonged lag phases at higher carbon loadings, were not offset by increases in the rates of rapid microbial metabolism, resulting in the soils taking longer to remove the added ^{14}C-compounds from the soil solution. Prolonged lag phases at higher solution concentration may have also been due to nutrient limitations, particularly nitrogen.

5.3.2 Partitioning of added ^{14}C-compounds

At similar rates of carbon loading the solod evolved greater amounts of added ^{14}C as $^{14}CO_2$ and retained less ^{14}C in the microbial biomass than did the brown earth. Marshall (1975) suggested that heavy-textured soils provided better protection of the newly formed biomass from predators than light-textured soils. Sorensen (1981) found that metabolites formed during periods of intense biological activity combined with clay particles which provided partial protection against further decomposition. Thus turnover of carbon through the biomass would be slower in soils with more clay, as found by Kunc and Stotzky (1974), Van Veen et al. (1985), Gregorich et al. (1991) and, Ladd et al. (1992). In this study, however, the amount of ^{14}C remaining in the biomass was relatively low (about 15%) and that in the non-biomass relatively high (about 25%). These differences in partitioning of ^{14}C within the biomass and non-biomass may have been partly due to the addition of much larger amounts of decomposable substrates compared with the abovementioned studies.

Lower rates of evolution of $^{14}CO_2$ than removal of solution-^{14}C during the phase of rapid metabolism of the ^{14}C-compounds were expected given that the latter measurement would also include ^{14}C-compounds incorporated into microbial biomass and other products, as well as ^{14}C evolved as $^{14}CO_2$. The greater sum of added ^{14}C evolved as $^{14}CO_2$ and metabolised

to biomass-^{14}C than concentrations of ^{14}C remaining in solution after initial adsorption, indicated that part of the metabolised ^{14}C-compounds originated from the pool of substrates initially removed by adsorption. Turnover of ^{14}C remaining in the biomass was evident from the continued rapid evolution of $^{14}CO_2$ for at least 10 hours after the solution concentration of ^{14}C-compounds added by the wastewaters had been reduced to <5% of ^{14}C inputs.

5.3.3 Implications for the management of irrigated sites

According to Tcholbanoglous and Burton (1991) the basis of successful management of facilities used for secondary treatment of wastewaters is the maintenance of large populations of active microbial cells. Maintenance of a high population of adapted micro-organisms through regular application of wastewater to the soils would promote immediate uptake of the organic substrates, thereby eliminating lag periods, and promote faster rates of metabolism than shown for this one-off application of wastewater.

A new treatment facility usually has a reduced microbial capacity to metabolise the organic substrates, and hence requires a different management approach during the acclimatisation phase (Tcholbanoglous and Burton 1991). Knowledge of the length of the acclimatisation period as a function of soil depth would allow the development of appropriate strategies of irrigation for new sites.

The effect of repeated applications of wastewater to the solod and brown earth on removal times of the added organic carbon by acclimatised and non-[J1]--- acclimatised soils will be examined in the next Chapter.

In this study, the time elapsed from application of wastewater to the end of rapid mineralisation of ^{14}C-compounds was mostly unaffected by the relative moisture content at which the soils were incubated (Table 5.5). In situations where lag times have been eliminated, slower rates of metabolism at higher relative moisture contents of soils (Table 5.4) may increase removal times of the added organic substrates. Providing the right balance between optimal moisture levels of soils for rapid microbial metabolism of the

organic substrates, and the need to remove the large volumes of wastewater produced by wineries through irrigation will be further discussed in Chapter 8.

CHAPTER 6

EFFECT OF REPEATED IRRIGATIONS WITH WINERY WASTEWATER ON THE REMOVAL OF SOLUBLE ORGANIC CARBON BY SOIL.

6.0 Introduction

The model for management of irrigation systems used for the disposal of organic-rich wastewaters, introduced in Section 2.4, is based on establishing a nominal depth in which an arbitrarily defined percentage of the soluble organic carbon added by the wastewater can be removed within a given period of time. It was found in Chapters 4 and 5 that the solution concentration of organic carbon in winery wastewater could be reduced by >95% of carbon inputs within 1–2 days of application to the topsoils of a brown earth and a solod. Soils newly-exposed to the wastewater (non-acclimatised) were found to have a reduced microbial capacity to metabolise the organic substrates, and hence took longer to remove the soluble organic carbon than soils from existing disposal sites (acclimatised).

Further information is required on the depth to which acclimatised and non-acclimatised soils can achieve 95% removal of the soluble organic carbon in winery wastewater within 1–2 days of application. It is hypothesised that the depth to which the nominal removal of soluble organic carbon is achieved would be greater in acclimatised soils than in non-acclimatised soils upon initial irrigation with the wastewater, but that differences would become less as micro-organisms in the non-acclimatised soils became adapted to the organic substrates upon repeated irrigations with the wastewater. An experiment designed to test the hypothesis is described below.

6.1 Materials and Methods

6.1.1 Soil

A brown earth and a solod from disposal sites, and from adjacent areas not previously exposed to the wastewater, were sampled to a depth of 60 cm and divided into 6 sections. Locations and descriptions of the soils have been given in Section 4.1.1. The upper three sections represented the zone of maximum biological activity. This was estimated to occur within the upper 30 cm of the brown earth and the upper 15 cm of the solod. The remaining soil was divided by depth into three equal sections. The soils were dried at room temperature to 30–35% of their gravimetric moisture contents at -10 kPa as determined on separate samples, before being gently crushed and passed through either a 2 mm sieve (solod) or a 4.75 mm sieve (brown earth). The depths and characteristics of the sections for each type of soil are given in Tables 6.1–6.2.

6.1.2 Incubation

Synthetic winery wastewater (Table 4.2) was applied to the soils at a volume equivalent to 60% of the gravimetric moisture content at -10 kPa (W_{-10}) of the non-acclimatised soil, for each depth of sampling. Wastewater solutions applied to the soils were topped-up with reverse osmosis water to give a final moisture content of $0.95W_{-10}$. Amounts of organic carbon added to the soils during each irrigation are given in Table 6.3.

Following application of the wastewaters, the amended soils were incubated at 25°C for 2 days, then partially dried at room temperature for 2 days to a moisture content of $0.3W_{-10}$. The soil crumbs were gently mixed and turned-over to ensure uniform drying. The cycle of irrigation, incubation, and drying was repeated 16 times.

For the first irrigation, subsamples of the soils were amended with wastewater containing a 70:30 (W:W) mixture of 99.9% pure L-[U-^{14}C]-lactic acid : [U-^{14}C]-glycerol (4 kBq) for

Table 6.1: Characteristics of the brown earth.

Depth (cm)	0–10	10–20	20–30	30–40	40–50	50–60
Acclimatised						
% sand, silt, clay	52/38/10	58/35/7	54/29/17	50/20/30	43/14/43	42/9/49
$W_{-10}^{\dagger}$ (%)	28.1	26.6	29.1	38.9	56.0	59.9
$pH_{1.5, water}$	6.3	6.5	6.6	6.7	76.7	7.0
$EC_{1.5, water}$ (dS/m)	0.95	0.81	0.81	0.80	0.76	0.85
Organic carbon (%)	4.7	2.8	3.1	3.1	2.5	1.5
Total Kjeldahl N (mg/g)	24.7	21.2	19.8	20.1	15.8	10.8
Bicarbonate P (µg/g)	213	128	98	81	73	43
Exchangeable Cations (mg/100g soil)						
Sodium	26	14	5	46	57	103
Potassium	120	88	177	213	204	141
Calcium	276	238	372	393	477	97
Magnesium	35	25	47	57	37	100
SAR[‡]	0.48	0.10	0.06	0.57	0.67	1.06
Non-acclimatised						
% sand, silt, clay	51/42/7	52/39/9	53/37/10	38/22/40	35/17/48	42/18/40
$W_{-10}^{\dagger}$ (%)	27.3	20.8	21.0	19.3	49.3	53.2
$pH_{1.5, water}$	6.0	5.9	6.2	6.1	6.3	6.3
$EC_{1.5, water}$ (dS/m)	0.71	0.71	0.70	0.72	0.72	0.74
Organic carbon (%)	3.7	1.6	1.5	0.9	0.7	0.4
Total Kjeldahl N (mg/g)	20.9	10.4	7.4	5.4	5.9	5.4
Bicarbonate P (µg/g)	175	23	23	8	1	1
Exchangeable Cations (mg/100g soil)						
Sodium	6	9	7	26	40	51
Potassium	57	33	36	54	62	55
Calcium	219	134	133	337	427	454
Magnesium	19	17	13	115	177	206
SAR[‡]	0.10	0.19	0.17	0.31	0.41	0.49

† W_{-10}: the gravimetric moisture content at -10 kPa.

‡ SAR: Sodium Adsorption Ratio

Table 6.2: Characteristics of the solod.

Depth (cm)	0–5	5–10	10–15	15–30	30–45	45–60
Acclimatised						
% sand, silt, clay	93/7/0	95/5/0	96/4/0	98/2/0	99/1/0	99/1/0
W_{-10}[†] (%)	9.1	8.3	6.5	5.5	5.0	2.6
$pH_{1.5, water}$	5.8	5.9	6.1	6.2	6.0	6.3
$EC_{1.5, water}$ (dS/m)	0.75	0.73	0.71	0.75	0.75	0.74
Organic carbon (%)	2.1	1.7	1.5	0.4	0.4	0.2
Total Kjeldahl N (mg/g)	22.3	15.9	13.5	4.5	4.5	3.1
Bicarbonate P (µg/g)	270	61	62	71	65	67
Exchangeable Cations (mg/100g soil)						
Sodium	5	10	4	4	4	5
Potassium	30	17	20	16	15	17
Calcium	124	74	74	29	27	21
Magnesium	23	10	15	3	3	3
SAR[‡]	0.48	0.10	0.06	0.57	0.67	1.06
Non-acclimatised						
% sand, silt, clay	94/6/0	95/5/0	98/2/0	98/2/0	99/1/0	99/1/0
W_{-10}[†] (%)	8.4	7.8	5.6	6.1	3.7	2.5
$pH_{1.5, water}$	6.3	5.8	5.7	5.7	5.7	5.7
$EC_{1.5, water}$ (dS/m)	0.70	0.70	0.70	0.69	0.69	0.69
Organic carbon (%)	1.6	1.1	0.8	0.3	0.1	0.1
Total Kjeldahl N (mg/g)	17.1	9.5	8.1	4.3	2.2	1.8
Bicarbonate P (µg/g)	15	11	4	4	5	1
Exchangeable Cations (mg/100g soil)						
Sodium	6	6	3	33	3	4
Potassium	26	14	11	9	13	7
Calcium	117	66	35	19	11	7
Magnesium	14	10	6	3	3	3
SAR[‡]	0.10	0.19	0.16	0.30	0.41	0.49

† W_{-10}: the gravimetric moisture content at -10 kPa.

‡ SAR: Sodium Adsorption Ratio

Table 6.3: Amounts of organic carbon added to the solod and brown earth during each irrigation with winery wastewater (mg/kg). The volume of wastewater applied was equivalent to 60% of the gravimetric moisture content at -10 kPa.

Depth No.[†]	Brown earth	Solod
1	478	147
2	364	138
3	367	98
4	337	90
5	863	65
6	933	44

† Depths 1–6 for the brown earth and solod as given in Tables 6.1 and 6.2, respectively.

assay of the removal of soluble-^{14}C from soil extracts. The radioactivity of the extracts was determined from three replicate samples for each soil after 0, 0.125, 0.25, 0.5, 0.75, 1, and 2 days incubation at 25°C, as described in Section 4.1.4. Activities of extracts from soils wetted with wastewater containing no ^{14}C were determined as controls. Further assays were conducted following the 2nd, 4th, 8th, and 16th cycle of irrigation, incubation, and drying.

The removal of soluble-^{14}C in soil extracts was described ($R^2 > 0.93$) by a logistic curve, as outlined in Section 4.2.1. Estimates of the parameters described in Section 4.2.2 were obtained from curves fitted separately to each replicate, and subjected to analysis of variance.

6.2 Results

6.2.1 Adsorption of ^{14}C-compounds

Between 10–50% of the applied ^{14}C was retained by the soils after extraction immediately following amendment with the ^{14}C-labelled wastewater (Table 6.4). The proportion of added ^{14}C retained by the soils significantly increased with increased clay and/or organic carbon contents. For the brown earth, greater adsorption of ^{14}C-compounds occurred in the soils sampled from the clay B horizon (30–60 cm). The solod, which has no clay, exhibited greater adsorption of ^{14}C-compounds within the topsoils, which had the higher organic carbon contents.

Adsorption of ^{14}C-compounds by the soils also varied in response to repeated irrigations with winery wastewater. For the non-acclimatised brown earth sampled within the 0–10 cm layer, and the acclimatised brown earth sampled from the 0–30 cm layer, adsorption of ^{14}C-compounds decreased by up to 12% of ^{14}C input during the first 4 irrigations then increased by approximately 10% during the remaining 12 irrigations (Figure 6.1).

Table 6.4: ^{14}C removed by adsorption or microbial uptake by a brown earth and a solod within the first hour after application of winery wastewater to acclimatised and non-acclimatised soils. Means, averaged over 16 cycles of irrigation, and least significant difference at 5% (LSD) are given.

	Brown earth		Solod	
	Non-acclimatised	Acclimatised	Non-acclimatised	Acclimatised
Depth No.[†]	^{14}C removed by adsorption or microbial uptake (% ^{14}C input)			
1	36.4	46.5	24.5	25.6
2	31.9	32.0	24.5	24.9
3	35.6	38.5	24.0	23.8
4	54.9	45.2	22.2	16.7
5	48.3	53.4	9.1	16.2
6	43.9	49.3	8.6	17.7
				LSD 3.1%

† Depths 1–6 as given for the brown earth and the solod in Tables 6.1 and 6.2, respectively.
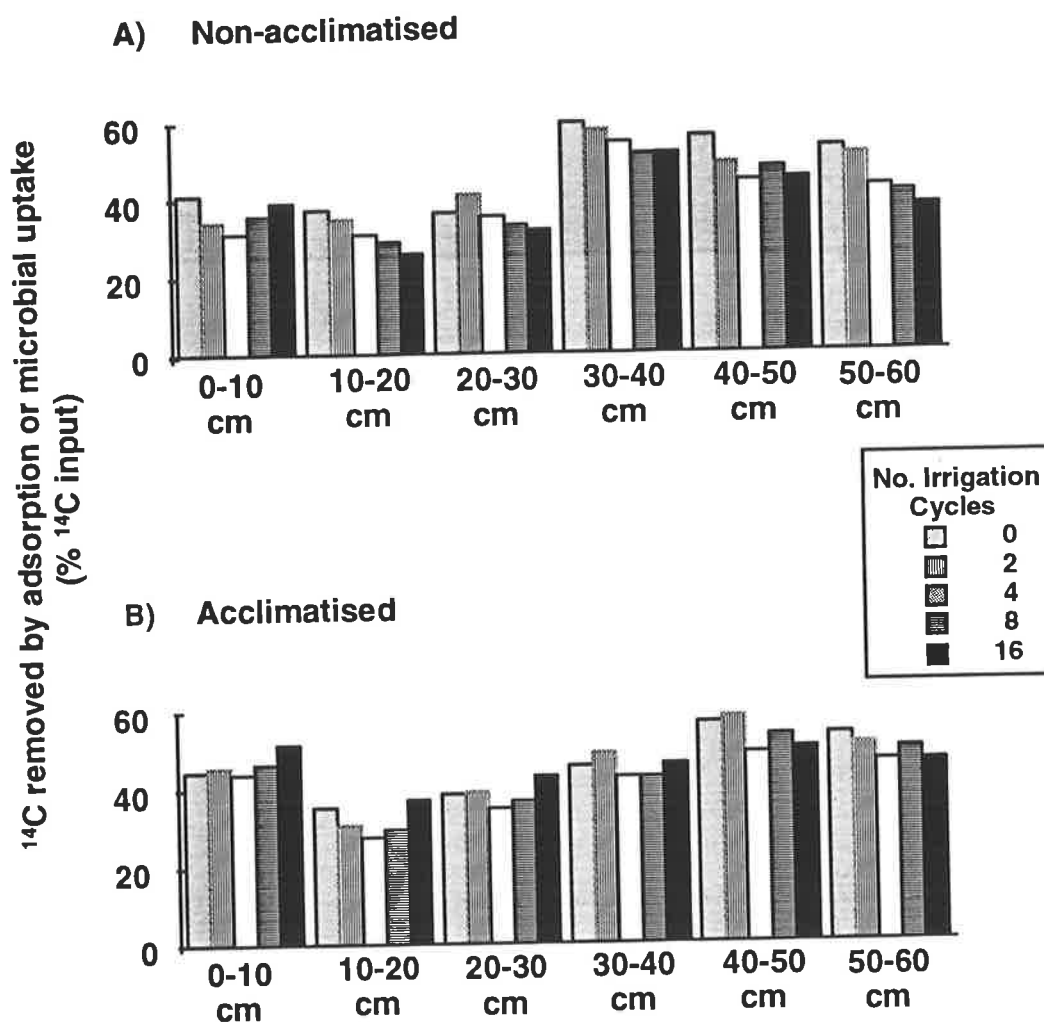
Figure 6.1: Effect of repeated irrigations with winery wastewater on the ^{14}C removed by adsorption or microbial uptake by a non-acclimatised and acclimatised brown earth. The least significant difference of means at 5% was 3.0%.

Adsorption of ^{14}C-compounds by the acclimatised and non-acclimatised brown earth sampled from the lower depths showed either no change, or decreased by up to 15% of ^{14}C input by the end of the 16th cycle of irrigation with wastewater. For the solod, the extent of adsorption of ^{14}C-compounds by a given depth of the acclimatised and non-acclimatised soils varied by less than 5% of ^{14}C input with repeated irrigations with wastewater.

Changes in adsorption of ^{14}C-compounds by the soils in response to repeated irrigations with winery wastewater, however, were poorly correlated with changes in the times taken for the soluble-^{14}C to be reduced by >95% of ^{14}C input.

6.2.2 Removal of ^{14}C-compounds from the soil solution

Figure 6.2 shows the effect of repeated applications of winery wastewater to a brown earth and solod on the times for removal of ^{14}C-compounds from solution to <5% of ^{14}C input by acclimatised and non-acclimatised soils. Lighter textured soils, whether the A horizon of the brown earth (loam) compared with the B horizon (clay), or the solod (sand) compared with the brown earth, took less time to reduce the solution concentration of ^{14}C-compounds to <5% of ^{14}C input following the initial irrigation with wastewater (Figure 6.2, dashed lines). Acclimatised soils, without exception, removed the ^{14}C-compounds more quickly than the non-acclimatised soils sampled at the same depth.

Repeated applications of wastewater led to marked reductions in times for removal of ^{14}C-compounds from the soil solution. Although <10 days elapsed between field sampling and commencement of the assay following the first irrigation with wastewater, the acclimatised soils took longer to remove the ^{14}C-compounds from solution (usually >1 day) than during the equivalent periods of assay after subsequent irrigations with wastewater (≤1 day, Figure 6.2A). After 2 irrigations with wastewater, the removal of ^{14}C-compounds from solution by the acclimatised soils occurred within a 3h range within the 0–10 cm layer, and a 6–10h range for the remaining sampling depths.

A) Acclimatised soils

Brown earth
Time to next application (days)

Solod
Time to next application (days)

No. irrigations	
■··■	0
△—△	2
□—□	4
○···○	8
◇···◇	16

B) Non-acclimatised soils

Brown earth
Time to next application (days)

Solod
Time to next application (days)
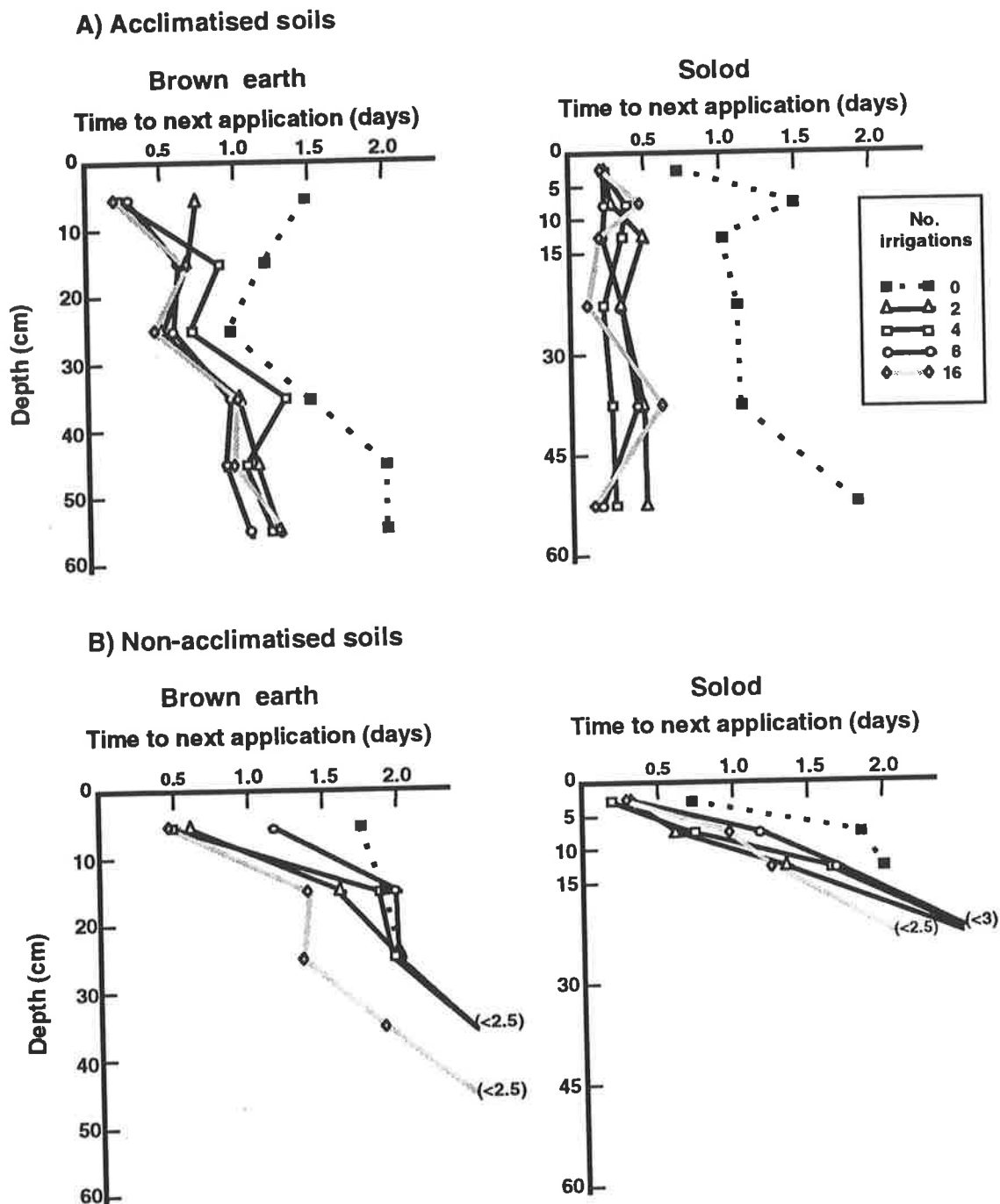
Figure 6.2: Effect of repeated applications of winery wastewater to a brown earth and solod on the times for removal of ^{14}C-compounds from solution to <5% of ^{14}C input by acclimatised and non-acclimatised soils. The least significant difference of means at 5% was 0.09 day.

Adaptation of the non-acclimatised soils was demonstrated by the reduced times for removal of ^{14}C-compounds in solution to <5% of ^{14}C input with increasing numbers of irrigations with wastewater (Figure 6.2B). Adaptation was fastest within the zone of maximum biological activity, viz., the 0–15 cm layer of the non-acclimatised solod, and the 0–30 cm layer of the non-acclimatised brown earth. By the end of the 16th cycle of irrigation with wastewater these upper layers of soil were able to reduce the solution concentration of ^{14}C-compounds to <5% of ^{14}C input within 1.5 days after application. By contrast to the upper layers, non-acclimatised soils sampled beyond the zone of maximum biological activity were very slow to adapt. Only the soil sampled from the 30–40 cm layer of the non-acclimatised brown earth was able to remove the ^{14}C-compounds from solution within the 2-day assay conducted after the 16th irrigation with winery wastewater (Figure 6.2B, grey lines).

Neither the depth of sampling nor the number of irrigations with wastewater affected the general order of times for removal of ^{14}C-compounds in solution by the acclimatised and non-acclimatised soils, which increased in the order: acclimatised solod < acclimatised brown earth < non-acclimatised brown earth < non-acclimatised solod. An exception was the uppermost layer sampled in which the order of removal of soluble-^{14}C was: acclimatised solod < non-acclimatised solod < acclimatised brown earth < non-acclimatised brown earth. Following cessation of rapid microbial metabolism of ^{14}C-compounds in solution <5% of the added ^{14}C could be extracted from the acclimatised soils, or from the non-acclimatised soils sampled from the upper 3 depths, with one exception for the non-acclimatised solod (Table 6.5).

6.2.3 Microbial metabolism of ^{14}C-compounds

Decreased times for the removal of soluble-^{14}C with increased numbers of irrigations with wastewater were due to reductions in lag times and/or increased rates of microbial metabolism of the ^{14}C-compounds in solution. The soluble ^{14}C-compounds may include

Table 6.5: ^{14}C extracted from a brown earth and a solod after cessation of rapid microbial metabolism of ^{14}C-compounds in solution. Means, averaged over 16 cycles of irrigation, and least significant difference at 5% (LSD) are given.

	Brown Earth		Solod	
	non-acclimatised	acclimatised	non-acclimatised	acclimatised
Depth No.[†]	^{14}C extracted after rapid metabolism of ^{14}C-compounds (% ^{14}C input)			
1	0.4 ± 0.5	0.2 ± 0.2	1.5 ± 1.0	2.2 ± 1.1
2	0.4 ± 0.8	0.9 ± 0.8	0.7 ± 0.7	2.0 ± 1.0
3	0.2 ± 0.4	0.9 ± 0.3	6.3 ± 5.4	1.2 ± 1.1
4	$34.8 - 1.3$[§]	0.7 ± 0.5	$57.9 - 7.2$[§]	2.1 ± 1.0
5	$47.8 - 6.9$[§]	2.5 ± 2.4	$88.8 - 59.7$[§]	3.8 ± 0.6
6	$56.2 - 27.3$[§]	0.4 ± 0.6	$88.1 - 72.7$[§]	4.9 ± 5.6
				LSD 4.1%[§]

† Depths 1–6 as given in Tables 6.1 and 6.2, respectively, for the brown earth and the solod.

§ Only partial microbial metabolism of ^{14}C-compounds occurred within the lower three depths of the non-acclimatised soils during the 2-day assay period, as indicated by the range. The soils were not included in the calculation of the LSD.

products formed from metabolism of the ^{14}C-labelled lactic acid and glycerol (cf., Section 4.0).

Lags periods were largely absent in the acclimatised brown earth (Table 6.6). Hence reductions in the times for removal of soluble-^{14}C in this soil resulted from increased rates of microbial metabolism of ^{14}C-compounds only (Table 6.7). After only 2 irrigations with winery wastewater the rates of removal of soluble-^{14}C/day by soils within the A horizon of the acclimatised brown earth had increased by 100–200%, with more modest gains of 50–100% exhibited by soils sampled from the B horizon.

For the acclimatised solod, rates of removal of soluble-^{14}C/day showed even higher relative increases between the initial and subsequent irrigations with wastewater (100–1000%, Table 6.7). As a result of the extremely high rates of metabolism of ^{14}C-compounds exhibited by this soil, the duration of the lag period contributed up to 60% of the total time required for removal of soluble-^{14}C to <5% of ^{14}C input (Table 6.6). By the end of the 16th irrigation with wastewater metabolism of ^{14}C-compounds occurred without lag.

Repeated irrigations with winery wastewater progressively reduced the duration of the lag period of the non-acclimatised soils within each successive depth sampled (Table 6.8). By the end of the 16th irrigation with wastewater metabolism of ^{14}C-compounds occurred without lag in soils sampled from the 0–20 cm layer of the non-acclimatised brown earth and the 0–5 cm layer of the non-acclimatised solod, and had began within the 2-day assay period for all remaining non-acclimatised soils (Table 6.8).

Microbial metabolism of ^{14}C-compounds was faster in the non-acclimatised solod than non-acclimatised brown earth, without exception. After two irrigations with winery wastewater the rate of removal of soluble-^{14}C/day had increased by 300–400% within the 0–20 cm layer of the non-acclimatised brown earth and the 0–5 cm layer of the non-acclimatised solod (Table 6.9). These soils, however, never achieved the same rates of

Table 6.6: Effect of numbers of irrigations with winery wastewater of an acclimatised brown earth and solod on the duration of the lag phase. Means, averaged over 16 cycles of irrigation, and least significant difference at 5% (LSD) are given.

Brown earth

Depth (cm)	0–10	10–20	20–30	30–40	40–50	50–60
No. Irrigations			lag (day)			
0	4.68	0.00	0.00	0.03	1.98	2.65
2	2.41	0.12	0.80	0.03	1.91	0.02
4	0.02	0.00	0.00	0.04	0.05	0.03
8	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00

Solod

Depth (cm)	0–5	5–10	10–15	15–30	30–45	45–60
No. Irrigations						
0	11.03	8.15	0.04	12.61	11.42	0.10
2	2.11	3.13	0.02	1.80	3.29	2.87
4	1.32	3.69	0.12	1.03	1.33	1.39
8	1.80	1.64	1.86	2.45	4.45	0.00
16	0.00	0.05	0.00	0.00	4.76	0.00

LSD[†] 1.84

† To account for effects of carbon loading on lag times of the different soils the LSD has been calculated from log-transformed data, and hence becomes a ratio between treatment a : treatment b, eg., 4.68/2.41=1.94; significant at 5%.

Table 6.7: Effect of numbers of irrigations with winery wastewater of an acclimatised brown earth and solod on the maximum rate of removal of soluble-^{14}C (maxrate) during the phase of rapid microbial metabolism of ^{14}C-compounds in solution[†]. Means, averaged over 16 cycles of irrigation, and least significant difference at 5% (LSD) are given.

Brown earth

Depth (cm)	0–10	10–20	20–30	30–40	40–50	50–60
No. Irrigations		maxrate (% ^{14}C input/day)				
0	57.9	67.8	81.8	45.7	28.1	27.9
2	125.2	147.5	199.9	65.9	50.1	54.8
4	324.9	100.7	117.2	52.2	61.4	54.2
8	235.9	144.2	131.4	74.1	63.6	64.2
16	300.0	133.8	162.8	74.5	67.9	56.9

Solod

Depth (cm)	0–5	5–10	10–15	15–30	30–45	45–60
No. Irrigations						
0	342.4	81.6	98.5	187.7	164.2	36.8
2	620.1	640.9	202.3	417.1	300.4	300.5
4	277.0	530.4	323.9	565.4	510.7	481.2
8	660.2	628.3	672.1	386.9	411.8	529.1
16	444.1	216.8	520.4	901.5	277.5	710.1

LSD 107% ^{14}C input/day

[†] Average rates of removal of soluble-^{14}C/day during the phase of rapid microbial metabolism of ^{14}C-compounds in solution were consistently 70% of the maximum.

Table 6.8: Effect of numbers of irrigations with winery wastewater of a non-acclimatised brown earth and solod on the duration of the lag phase. Means, averaged over 16 cycles of irrigation, and least significant difference at 5% (LSD) are given.

Brown earth

Depth (cm)	0–10	10–20	20–30	30–40	40–50	50–60
No. Irrigations			lag (day)			
0	0.80	19.55	16.24	NR[†]	NR	NR
2	4.02	5.16	15.16	<48	NR	NR
4	2.38	17.37	14.74	<48	NR	NR
8	0.04	0.05	0.07	<24	<48	NR
16	0.00	0.05	2.20	13.61	8.95	<48

Solod

Depth (cm)	0–5	5–10	10–15	15–30	30–45	45–60
No. Irrigations						
0	0.02	0.08	19.11	<48	NR	NR
2	3.74	4.83	11.73	<48	NR	NR
4	0.02	8.58	6.63	<48	NR	NR
8	0.01	0.95	10.53	<24	<48	<48
16	0.01	0.97	2.35	0.25	<12	<24

LSD[‡] 1.84

† NR: not reached by the end of the 2-day assay period; lag values with a < sign were based on extrapolation of the data; the lower 3 depths were not included in the analysis of variance.

‡ To account for effects of carbon loading on lag times of the different soils the LSD has been calculated from log-transformed data (upper 3 depths only), and hence becomes a ratio between treatment a : treatment b, eg., 0.80/4.02=.20; not significant at 5%;

Table 6.9: Effect of numbers of irrigations with winery wastewater of a non-acclimatised brown earth and solod on the maximum rate of removal of soluble-^{14}C (maxrate) during the phase of rapid microbial metabolism of ^{14}C-compounds in solution[†]. Means, averaged over 16 cycles of irrigation, and least significant difference at 5% (LSD) are given.

Brown earth

Depth (cm)	0–10	10–20	20–30	30–40	40–50	50–60
No. Irrigations			maxrate (%^{14}C input/day)			
0	42.8	75.2	58.2	NR[‡]	NR	NR
2	192.5	63.4	41.8	NR	NR	NR
4	223.9	78.8	63.8	NR	NR	NR
8	68.1	43.8	42.2	NR	NR	NR
16	180.3	67.2	73.3	113.5	48.9	NR

Solod

Depth (cm)	0–5	5–10	10–15	15–30	30–45	45–60
No. Irrigations						
0	118.5	56.5	60.4	NR	NR	NR
2	446.6	210.1	202.3	NR	NR	NR
4	296.5	256.8	65.0	NR	NR	NR
8	263.6	93.0	82.8	NR	NR	NR
16	342.0	107.8	83.6	NR	NR	NR

LSD[§] 107% ^{14}C input/day

[†] Average rates of removal of soluble-^{14}C/day during the phase of rapid microbial metabolism of ^{14}C-compounds in solution were consistently 70% of the maximum.

[‡] NR not reached by the end of the 2-day assay period.

[§] The LSD was calculated for data obtained from the upper 3 depths of each type of soil only.

removal of soluble-^{14}C/day as the acclimatised soils sampled at the same depths. The lower depths of both non-acclimatised soils showed no gains or only small gains (<30%) in the rate of removal of soluble-^{14}C/day after repeated irrigations with winery wastewater.

6.3 Discussion

Microbial metabolism of ^{14}C-compounds in solution was essential for the reduction of soluble-^{14}C to <5% of ^{14}C input following repeated irrigation of soils with winery wastewater. Removal times were determined by: (I) the concentration of organic compounds remaining in solution after adsorption and microbial uptake (in the absence of a lag period) during the first hour following application of the wastewater, (II) the duration of the lag period before microbial metabolism, and (III) the rate of metabolism of ^{14}C-compounds in solution.

6.3.1 Adsorption of ^{14}C-compounds

Greater adsorption of ^{14}C-compounds, primarily the ^{14}C-labelled lactic acid and glycerol, with higher clay and/or organic carbon content of the brown earth and solod is consistent with the effects of clay and organic carbon contents of soils on adsorption of organic pesticides (Calvert 1989; Weber et al. 1991). Correlation analysis suggested that organic carbon was more effective than clay content in promoting adsorption (Figure 6.3). The effect may be due to the organic colloids coating the particles of clay thereby occupying surfaces which may otherwise be available for adsorption of solutes, rather than due to differences in the sorptive capacities of the organic colloids and clay minerals. To test this would require observation of thin sections of the soils. In the absence of lag periods, microbial uptake would also be partly responsible for the removal of ^{14}C-compounds during the first hour after application of the wastewater. The proportion of solution ^{14}C-compounds removed by microbial uptake could be estimated by comparing fumigated and non-fumigated soils, although the destruction of microbial cells and subsequent leaking of
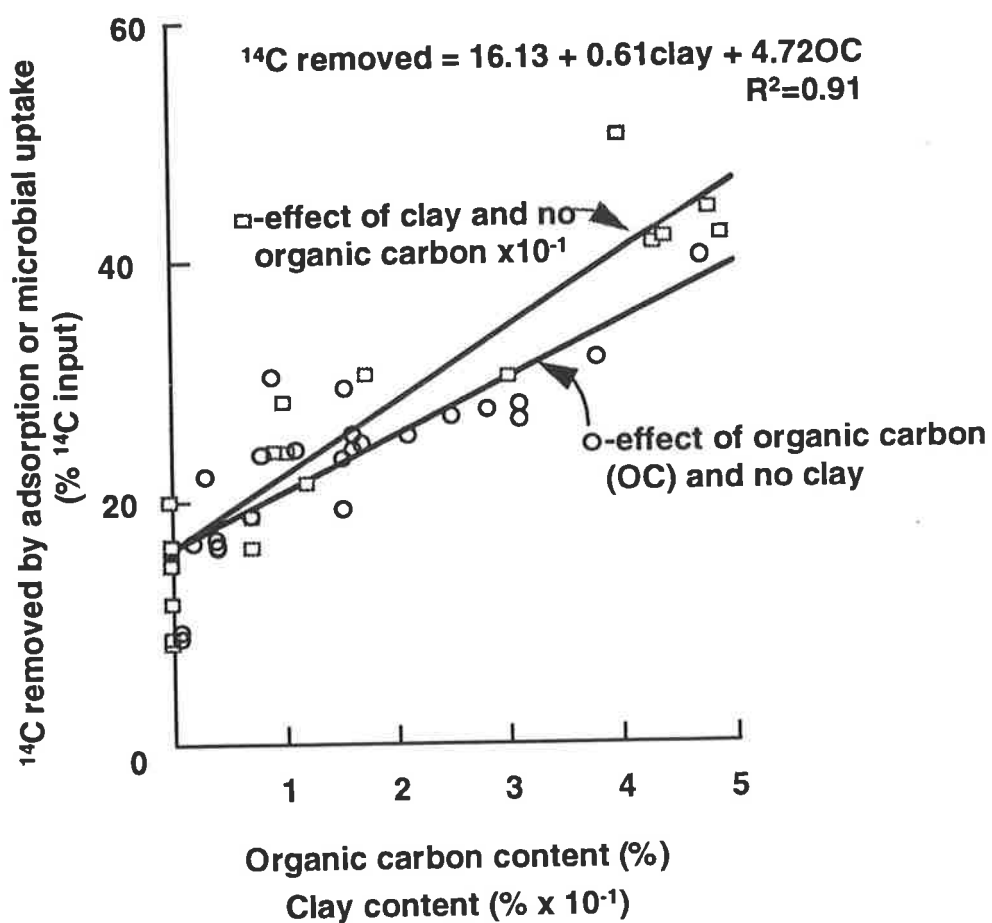
Figure 6.3: Correlation analysis showing the effects of organic carbon and clay contents of soil on adsorption of ^{14}C-compounds in winery wastewater.

their contents during the fumigation process is likely to affect subsequent adsorption of solution ^{14}C-compounds.

Reductions in the solution concentrations of ^{14}C-compounds by adsorption and microbial uptake led to the times taken for removal of soluble-^{14}C to $<5\%$ of ^{14}C input being lower than would be predicted from the rates of microbial metabolism, in the absence of lag periods. The greatest "savings" in removal times of the soluble-^{14}C occurred in soils sampled from the clay B horizon of the acclimatised brown earth, for which adsorption of ^{14}C-compounds was high (40–50% of ^{14}C input), and rates of microbial metabolism were low (removal of soluble-^{14}C/day <50–75% of added ^{14}C). For these subsoils, the soluble-^{14}C was removed approximately 1–2 days sooner than would be predicted from average rates of metabolism of ^{14}C-compounds from solution, which were about 70% of the maximum rates. For the lighter textured soils, initial reductions in the solution concentrations of ^{14}C-compounds were low compared with the rates of microbial metabolism, and hence the former achieved only small savings in the times taken for removal of soluble-^{14}C to $<5\%$ of ^{14}C input.

Continued availability of sites for adsorption may be dependent on the removal of the organic substrates in solution by microbial metabolism promoting desorption of initially adsorbed compounds. It was found in Chapter 5 that the sum of added ^{14}C evolved as $^{14}CO_2$ and metabolised to biomass-^{14}C often exceeded the concentration of ^{14}C remaining in solution after initial adsorption of the ^{14}C-compounds (cf., Tables 5.6 and 5.7), which suggested that part of the ^{14}C-compounds metabolised originated from the pool of substrates initially adsorbed by the soils.

Microbial metabolism of the organic substrates added by the wastewaters may also lead to the formation of new materials which can act as adsorbents, and the gradual buildup of microbial populations in response to higher energy and nutrition levels. Greater initial adsorption of ^{14}C-compounds by the acclimatised soils than by the non-acclimatised soils,

and observed increases in the amounts of ^{14}C-compounds adsorbed by the topsoils of the brown earth between the 4th and 16th irrigations with wastewater may have been due to these reasons.

6.3.2 Microbial metabolism of ^{14}C-compounds

Acclimatised soils could consistently reduce the solution concentration of ^{14}C-compounds, including metabolites of the ^{14}C-labelled lactic acid and glycerol (cf., Section 4.0), by 95% of ^{14}C input within 1 day after application of winery wastewater. This nominal level of treatment was achieved when lag periods were less than 2h in duration, and when microbial metabolism resulted in a loss of solution-^{14}C/day exceeding the ^{14}C input (ie., a maximum rate of removal of solution-^{14}C/day exceeding 140% of the ^{14}C input; Tables 6.7, 6.9). The results of the experiments suggested that acclimatisation of the soils existed at 2 levels.

Repeated application of winery wastewater to newly-exposed soils (non-acclimatised) led to a concurrent decrease in the length of the lag period and an increase in the rate of microbial metabolism, and hence reduced times for removal of ^{14}C-compounds in solution to <5% of ^{14}C input. Measurement of specific activities of isolated micro-organisms would be required to establish whether the changes in microbial activity on repeated irrigations with wastewater, involved both an increase in the numbers of active cells, and an enzymic adaptation of existing microbial cells.

The second level of acclimatisation appeared to involve the maintenance of sufficient numbers of active microbial cells within the groups responsible for immediate and rapid metabolism of the organic substrates, through regular application of wastewater. Successions of microbial populations within the acclimatised soils during the 10 day period between sampling and the first irrigation with the winery wastewater resulted in significantly longer times for removal of ^{14}C-compounds in solution to <5% of ^{14}C input, compared with times shown by the soils after successive applications of wastewater. Thus, weekly or twice-weekly applications of wastewater appeared to be necessary to ensure that sufficiently

high populations of active microbial cells were present in the acclimatised soils to keep removal times of the soluble organic carbon to <1 day.

Microbial metabolism of ^{14}C-compounds in solution by the acclimatised soils fell into 3 distinct groups according to the dominant textural type, viz., the solod (sand), the A horizon of the brown earth (loam), and B horizon of the brown earth (clay). Only the solod and soils sampled from the A horizon of the brown earth could reduce the solution concentration of ^{14}C-compounds by 95% of ^{14}C input within 1 day after application of the winery wastewater. The daily rate of microbial metabolism of ^{14}C-compounds in solution in soils sampled from the B horizon of the brown earth never exceeded the ^{14}C input, even after 16 irrigations with wastewater. Thus the potential for rapid (<1 day) removal of soluble organic carbon in winery wastewater may be partly determined by soil texture. Decreases in the rates of microbial metabolism of ^{14}C-compounds in solution with increasing clay content were consistent with earlier results (Tables 5.4 and 5.8), and effects of soil texture on the rate of evolution of ^{14}CO$_2$ observed by others (Kunc and Stotzky 1974; Marshall 1975; Van Veen et al. 1985; Ladd et al. 1992).

Non-acclimatised soils sampled from the 0–10 cm layer, which were able to reduce the solution concentration of ^{14}C-compounds to <5% of ^{14}C input within 1 day after the 16th irrigation with wastewater, could not achieve the same rates of microbial metabolism as did the acclimatised soils sampled from the same depth. This may have been due to differences between the natural environment of the acclimatised soils and the experimental environment of the non-acclimatised soils, in which acclimatisation of the micro-organisms took place. The organic matter content and contents of the major nutrients, which were considerably higher in the acclimatised than non-acclimatised soils (Tables 6.1–6.2), may be important determinants of the potential microbial activity of soils. Longer removal times of the soluble-^{14}C, and more gradual changes in the duration of the lag periods and rates of metabolism of ^{14}C-compounds in solution for non-acclimatised soils sampled at depth relative to the topsoils, may also be a reflection of lower initial populations of micro-

organisms due to the general lack of energy sources and nutrition compared with the topsoils.

6.3.3 Implications for management of irrigation

Assuming that the rate of disappearance of ^{14}C-compounds in solution to <5% of ^{14}C input is similar to that of the total water-soluble carbon in winery wastewater, removal of the soluble organic carbon can be achieved within 1 day after application when microbial metabolism commences within 2h, and when the daily rate of removal of organic compounds from solution exceeds the input of organic carbon.

For the acclimatised soils, weekly or twice-weekly irrigations with wastewater would ensure that sufficiently high populations of active microbial cells are maintained to keep removal times of the added organic substrates to <1 day. Soils containing micro-organisms not adapted to the substrates would potentially require at least 16 irrigations with wastewater to allow increases in microbial activity to levels shown by the acclimatised soils. During the acclimatisation period penetration of the new chemical front should be restricted to the upper 15 cm, with sites left for a minimum of 2 days.

Soils with a high clay content should not be irrigated, as rates of microbial metabolism are insufficient to allow the nominal level of 95% removal of organic carbon from solution to be achieved within 1 day after application of the winery wastewater.

The above information is based on measurements utilising disturbed soil samples, and thus requires confirmation by measurements utilising undisturbed soils where there will be differences in the distribution of the organic substrates within the upper soil layers during irrigation, and in the supply of oxygen to those layers from the soil surface.

CHAPTER 7

IRRIGATION STUDIES

7.0 Introduction

Studies using disturbed soils have shown that acclimatised soils could consistently reduce the solution concentration of ^{14}C-compounds in winery wastewater by 95% of ^{14}C input within 1 day after application. This nominal level of treatment was achieved when lag periods were <2h in duration, and when microbial metabolism of ^{14}C-compounds in solution resulted in a rate of loss of soluble-^{14}C/day exceeding the ^{14}C input. When oxygen was not limiting, only the lighter textured soils of the acclimatised solod and the A horizon of the acclimatised brown earth were able to metabolise the ^{14}C-compounds quickly enough to remove the soluble-^{14}C within 1 day of application of the wastewater. Regular application of wastewater was also important to maintain sufficient populations of active microbial cells.

The above results require confirmation using undisturbed soils which allow for the effects of: (I) the movement of water during irrigation on the distribution of the organic substrates within the soil matrix, and (II) the ability of the soils wetted to field capacity to transport oxygen to the sites where it is required for microbial metabolism of the organic substrates.

7.1 Materials and Methods

7.1.1 Soils

A solod and a brown earth irrigated with winery wastewater for a minimum of two years were used. Locations and site histories have been described in Section 4.1.1. Properties of the acclimatised solod are listed in Table 7.1, and for the acclimatised brown earth in Table 6.1. Bulk densities were also measured for the 0–15 cm layer on five replicates. The

average bulk density was then used to determine the total pore space (assuming a particle density $P_p = 2.65$ Mg/m^3) for estimation of the maximum oxygen content of the air-filled pore space within a m^2 of the 0–15 cm layer of the solod and brown earth at field capacity. Results are given in Table 7.2.

7.1.2 Irrigation: industrial practices

The industrial practices at the chosen sites have been to remove particulate material from the wastewater by settling for a minimum of 1 day, before passing the effluent through a coarse filter as it enters the irrigation system. The composition of winery wastewater sampled after settling has been described in Chapter 3.

During irrigation, a volume of wastewater equivalent to approximately 10% of the gravimetric moisture content at -10 kPa ($0.1W_{-10}$) of the 0–15 cm layer of each soil type was applied by a sprinkler system and/or by a dripper system at a rate of 5.3 mm/h. Thus the length of irrigation was approximately 45 minutes for the solod and 60 minutes for the brown earth. The time between irrigations varied between 0.5 to 1 day during vintage, and 3 to >7 days during non-vintage. The more frequent applications of wastewater during vintage were a direct result of the larger volumes produced during this period (Figure 3.1).

7.1.3 Sampling and preparation of the soils

Twelve intact columns (15 cm diameter x 100 cm length) were taken at random, using a drilling rig, within an area considered representative of the solod and brown earth. Sheets of rock were often encountered in the brown earth, which led to a high rate of discard of the columns at sampling. The cutting device was designed to provide a tight fit between the outer circumference of the soil column and the smooth inner surface of the supporting tube to minimise the likelihood of edge-flow effects. Access tubes consisting of perforated polycarbonate electrical conduit (0.15 cm diameter) were inserted into the columns at depths of 15, 30 and 60 cm, and at their base. The surface boundary between

Table 7.1: Characteristics of the solod.

Depth (cm)	0–5	5–10	10–15	15–30	30–45	45–60
% sand, silt, clay	73/26/1	83/17/0	78/12/10	52/18/30	56/11/33	50/13/37
W_{-10}[†] (%)	19.9	20.0	17.1	30.4	42.6	42.1
$pH_{1.5, water}$	6.7	6.8	6.9	7.0	7.0	7.0
$EC_{1.5, water}$ (dS/m)	0.81	0.74	0.76	0.82	0.77	0.83
Organic carbon (%)	2.1	1.5	1.1	0.7	0.5	0.5
Total Kjeldahl N (mg/g)	14.0	11.5	9.9	6.8	5.4	3.1
Bicarbonate P (µg/g)	270	200	97	6	10	1
Exchangeable Cations (mg/100g soil)						
Sodium	4	4	6	70	21	80
Potassium	49	49	66	232	45	100
Calcium	181	134	109	100	334	178
Magnesium	27	24	25	82	141	10
SAR[‡]	0.08	0.08	0.13	1.25	0.67	1.17

† W_{-10}: the gravimetric moisture content at -10 kPa.

‡ Sodium Adsorption Ratio

Table 7.2: Estimation of the maximum oxygen content of the air-filled pore space within 1 m^2 of the 0–0.15 m layer of the solod and brown earth at -10 kPa.

Calculation	Solod	Brown earth
Bulk density (Mg/m^3)	1.563 ± 0.461	1.426 ± 0.887
Total porosity[†] (%)	41.02	46.19
$W_{-10 \, (0-0.15 \, m)}$[§] (%)	19.01	27.58
Air-filled porosity$_{(0-0.15 \, m)}$ at -10 kPa (%)	11.32	6.839
Volume of air-filled pores$_{(0-0.15 \, m)}$ (m^3)	1.698×10^{-2}	1.025×10^{-2}
Volume of O_2[‡] (m^3)	3.396×10^{-3}	2.052×10^{-3}
Moles of O_2 at STP* (mol)	1.516×10^{-1}	9.159×10^{-1}
Mass of O_2[ƒ] (g)	4.851	2.931

† Total porosity (%) = 100 - (bulk density (P_b)/particle density (P_p) *100).

§ W_{-10}: the gravimetric moisture content at -10 kPa.

‡ Assume a constant concentration of O_2 of 20% by volume.

* gas constant = 22.4 l/mol at standard pressure and temperature (STP).

ƒ molar mass of O_2=32g.

the soil column and the supporting tube and all other joins were sealed to prevent edge-flow effects and other leakages.

To re-establish the activity of microbial populations within the acclimatised soils *Phalaris* was sown and the soils allowed to stand at 20–25°C for 4 months, during which time winery wastewater was added at twice-weekly intervals. Leachate was collected from the access tube inserted at 30 cm depth, to prevent waterlogging within the A horizon as the result of extremely low rates of drainage through the clay subsoils.

Figure 7.1 shows the design of the column for experimentation. Wastewater was applied through a hole in the cover, and allowed to drip onto a second plastic lid (33 mm diameter) included to absorb the impact energy of the drops. A No. 42 Whatman filter paper was inserted to promote even horizontal distribution of the water without restricting the overall rate of infiltration. The plastic lid and filter paper were added 30 minutes before each irrigation, and removed 30 minutes after.

A 10 mm layer of coarse sand (1–2 mm diameter) covered the surface of the soils to which the *Phalaris* was cut immediately before, and every 3 days during each incubation. The *Phalaris* was allowed to grow between successive incubations. A 15 cm extension tube was joined to the top of the columns to provide a head-space of air for exchange with the soil atmosphere, for the replenishment of oxygen during incubation.

7.1.4 Removal of soluble-^{14}C in leachate

The patterns of flow within the 0–15 cm layer of the columns during irrigation were first determined. For each type of soil, columns with similar flow were then selected for the incubation experiments. It was necessary to reuse the columns for successive experiments, which necessitated re-priming of the soils, as outlined below.
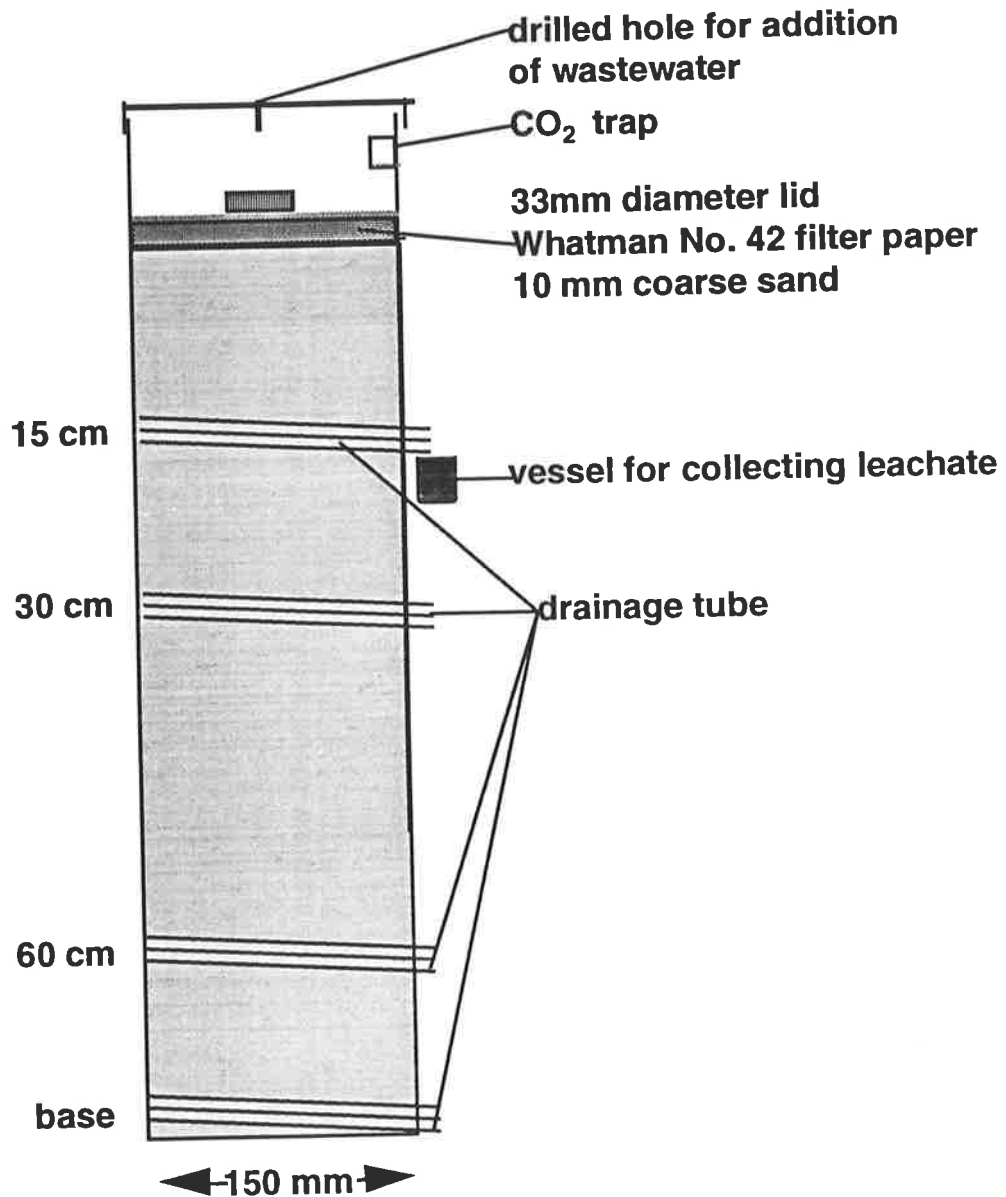
Figure 7.1: Core design for experimentation.

7.1.4.1 Patterns of flow

The soils were primed by daily applications of synthetic winery wastewater equivalent to $0.1W_{-10}$ (0–15 cm), at a rate of 1.68 ml/min. (5.7 mm/h). Thus, the solod was irrigated for 47 minutes, and the brown earth for 62 minutes. Both soils were also irrigated for an extra 2 minutes to account for the volume of wastewater retained by the layer of coarse sand and the filter paper. The columns were sealed between irrigations to minimise evaporation.

The irrigation of an additional volume of wastewater would result in the soil solution being displaced downwards. Leachate was collected from the drainage tubes at both the 15 cm and 30 cm depths. After the 15th daily application of wastewater, flow rates during irrigation were determined by measuring the volumes of leachate collected at 4 minute intervals for 120 minutes from the start of the irrigation. Thirty minutes before the next irrigation, the drains were re-opened to allow measurement of the leachate collected overnight. Measurements were repeated on a daily cycle a further 9 times. The entire procedure was repeated for wastewater applied to 30% of the gravimetric moisture content of the 0–15 cm layer at -10 kPa ($0.3W_{-10}$).

For each type of soil, 6 columns with similar patterns of flow, and for which >85% of the total volume of wastewater added had been collected from the 15 cm access drain were selected for the incubation studies. The typical patterns of flow of the soils from the drain at 15 cm depth are shown in Figure 7.2. The soil solution did not leach from the drain at 30 cm depth until several hours after application of the wastewater.

7.1.4.2 Incubation

The soils were primed over a 2 week period, as described in the previous section. Immediately before irrigation with ^{14}C-amended wastewater, a 70 ml beaker containing 5 ml of 5M NaOH was attached to the side of the extension tube to trap $^{14}CO_2$ evolved from the soils. Synthetic winery wastewater containing a 70:30 (W:W) mixture of L-[U-^{14}C]-lactic acid : [U-^{14}C]-glycerol (40 kBq) was applied to duplicated soil columns. The first
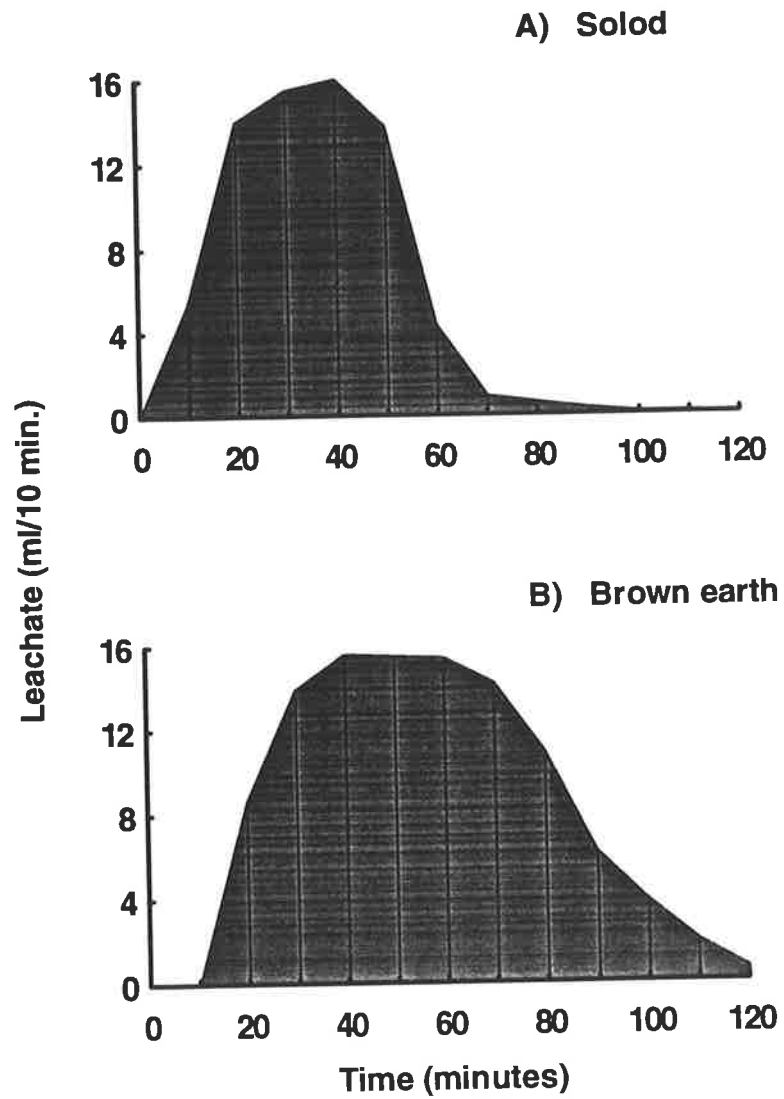
Figure. 7.2: Leachate collected at 15 cm depth from the solod and brown earth during and immediately after irrigation with wastewater at a rate of 5.7 mm/h. The volume of wastewater applied was equivalent to 10% of the gravimetric moisture content of the 0–15 cm layer at -10 kPa.

duplicate set of columns received a volume of wastewater equivalent to $0.1W_{-10}$ (0–15 cm). The second duplicate set received a volume of wastewater equivalent to $0.3W_{-10}$ (0–15 cm). Wastewater containing no ^{14}C was added to the third duplicate set of columns; one column for each volume of application of wastewater. The soils were sealed immediately after irrigation to prevent loss of $^{14}CO_2$. Characteristics of the synthetic winery wastewater are given in Table 4.3; the calculated amounts of organic carbon and the corresponding oxygen demands for biological degradation that would be added to a m^2 of the 0–15 cm layer of the soils during each irrigation are summarised in Table 7.3.

Leachate collected from the drain at 15 cm depth during irrigation, and for 60 minutes after was kept separate. Additional wastewater was then applied to the columns at a rate of 15 ml/min. to leach the soil solution (higher rates of application caused surface ponding). Successive volumes of leachate, each equivalent to $0.5W_{-10}$ of the 0–15 cm layer, were collected every 30–35 minutes for the solod, and 35–42 minutes for the brown earth. Leaching was stopped after a total volume equivalent to $5W_{-10}$ had been collected. The $^{14}CO_2$ traps were also removed at this time.

The ^{14}C contents of the leachates and of the NaOH solution used to trap $^{14}CO_2$ were measured using duplicated aliquots for each column. Background levels were obtained from the soils irrigated with the wastewater containing no ^{14}C. Results were adjusted for the proportion of the total volume of applied wastewater collected by the drain at 15 cm depth and expressed as a percentage of ^{14}C input.

The soils were left for 4 days before being primed for a further 7–10 days, as described in Section 7.1.4.1. The wastewater was re-applied to the columns, and leachate collected from the drain at 15 cm depth during irrigation and for 60 minutes after. The soils were then allowed to incubate at 25°C for 12h from the cessation of irrigation before the soil solutions were leached and their ^{14}C contents measured. The cycle of priming, irrigation, incubation, and leaching was continued until the soils had been incubated for 0.75, 1, 2,

Table 7.3: Amounts of organic carbon added to 1 m² of the 0–15 cm layer of the solod and brown earth during each irrigation with wastewater, and the estimated requirement of oxygen for biological decomposition of the organic carbon (BOD). Volumes of wastewater applied were equivalent to 10% of the gravimetric moisture content at -10 kPa $(0.1W_{-10})$ and $0.3W_{-10}$. The composition of the synthetic wastewater is given in Table 7.2, and of the industry wastewaters in Table 7.4. *(p134)*   *4·2? (p60)*

Wastewater	Solod		Brown earth	
	$0.1W_{-10}$	$0.3W_{-10}$	$0.1W_{-10}$	$0.3W_{-10}$
	amount organic carbon added to the 0–15 cm layer (g)			
Synthetic winery	13.0	39.0	17.2	51.6
Industry winery	12.7	–	16.7	–
Industry distillery	71.6	–	94.9	–
	BOD (g O_2)			
Synthetic winery	22.0	66.1	29.1	87.4
Industry winery	5.9	–	7.0	–
Industry distillery	36.9	–	48.9	–

and 3 days following irrigation. The reservoirs of air in the column head-spaces were periodically renewed during the longer incubations to prevent anoxic conditions from forming; the CO_2 traps were renewed at the same times.

Results from the soils leached for 1h after irrigation indicated that a volume of wastewater equivalent to $2W_{-10}$ contained >90% of the total ^{14}C leached in a volume equivalent to $5W_{-10}$ (Figure 7.3). Thus, for the subsequent incubations, the soils were leached, as described above, until a volume equivalent to $2W_{-10}$ had been collected; a precautionary measure to avoid excessive leaching times. Results were appropriately adjusted for the total amounts of ^{14}C that would be leached in volumes equivalent to $5W_{-10}$.

Background levels of soluble-^{14}C were measured from leachate collected from the previously amended columns during the 24h immediately before each new incubation. The leachate contained <0.5% of ^{14}C input which was considered to be sufficiently low to not affect measurements obtained from subsequent incubations.

Background levels of $^{14}CO_2$ were also calculated from amounts evolved from the previously amended soils during the 24h immediately prior to each new incubation, and used to estimate the amounts of $^{14}CO_2$ expected to be evolved as the result of continued turnover of ^{14}C existing within the biomass from previous incubations. The estimates were subtracted from the total amounts of $^{14}CO_2$ evolved during the subsequent incubation and leaching. Measurements of $^{14}CO_2$ evolved from the cores irrigated with wastewater containing no ^{14}C were used as controls.

The removal of soluble-^{14}C in leachates, and the amount of added ^{14}C evolved as $^{14}CO_2$ were described ($R^2 > 0.93$) by a logistic curve, as outlined in Section 4.2.1. Estimates of the parameters described in Section 4.2.2 were obtained from curves fitted separately to each replicate, and subjected to analysis of variance.

A) 0.1W$_{-10}$ application

B) 0.3W$_{-10}$ application
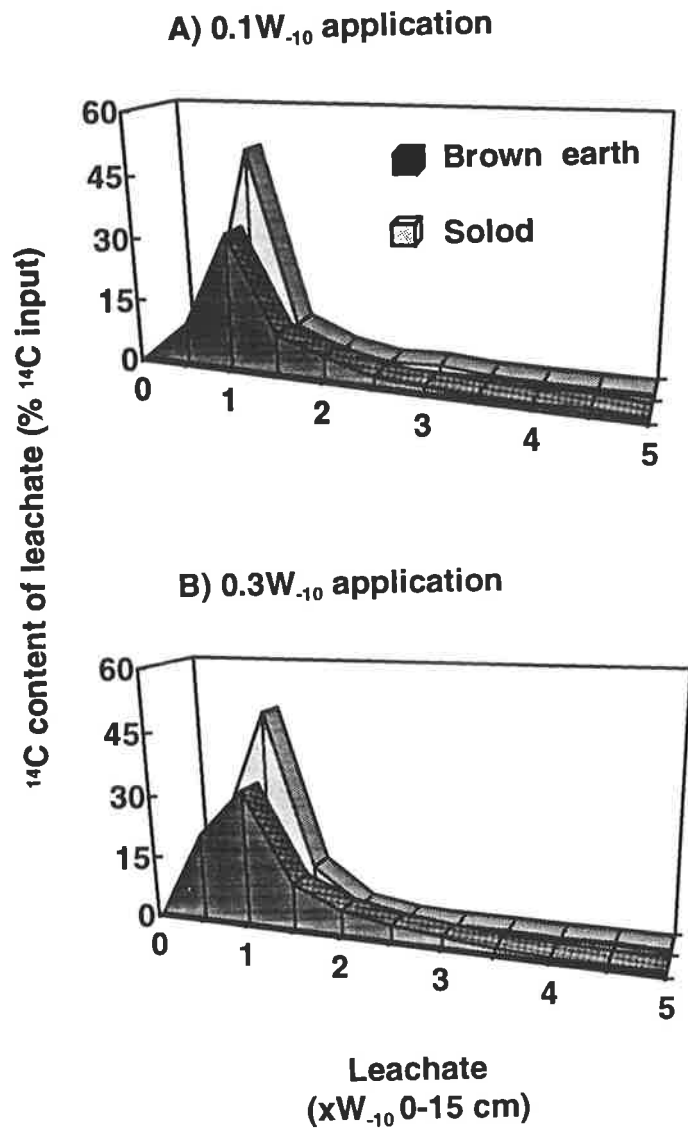
Figure. 7.3: ^{14}C content of incremental volumes of leachate collected at 15 cm following leaching of the solod and brown earth immediately after irrigation with synthetic winery wastewater, containing ^{14}C-labelled lactic acid and glycerol. The volumes of wastewater applied were equivalent to 10% of the gravimetric moisture content at -10 kPa (0.1W$_{-10}$) and 0.3W$_{-10}$ (0–15 cm).

7.1.5 Removal of organic carbon from wastewaters produced by the wine industry

7.1.5.1 Wastewaters

Frozen composite samples of winery and distillery wastewater, retained from analysis of the chemical properties described in Chapter 3, were used. The winery wastewater contained a similar amount of total organic carbon (TOC; 2.86 g/l) to the synthetic winery wastewater (2.92 g/l). Dissolved organic carbon (<0.45 µm) accounted for >92% of the TOC of the winery wastewater. The distillery wastewater had a TOC of 16.1 g/l, of which 48% consisted of dissolved organic materials. Characteristics of the industry wastewaters are given in Table 7.4.

7.1.5.2 Leaching experiment

Following the completion of the incubation-leaching experiment the columns were left for 7 days, and then divided randomly into 2 groups of 3 columns for each soil type. Each group of 3 columns were primed with either winery or distillery wastewater over a 21 day period, as described in Section 7.1.4.1. During the next 8 day period, a 70:30 (W:W) mixture of L-[U-^{14}C]-lactic acid : [U-^{14}C]-glycerol (40 kBq) was added to wastewaters immediately before each irrigation. The amended wastewater was applied to 2 replicate columns of each soil type; the remaining column was irrigated with reverse osmosis water (RO water) as a control. Leachate was collected from the drain at 15 cm depth during irrigation and for 60 minutes after, and from the drains at both 15 cm and 30 cm depths during a 30 minute period prior to the next irrigation. A 5 ml solution of 5M NaOH was used to trap ^{14}CO$_2$ evolved during the 24h period from the beginning of each irrigation. The reservoirs of air in the column head-spaces were periodically renewed to prevent anoxic conditions forming; the CO$_2$ traps were renewed at the same time.

The ^{14}C contents of the leachate and of the NaOH solution used to trap ^{14}CO$_2$ were measured using duplicate aliquots for each column. The total organic carbon content

7.4

Table 6.4: Characteristics of the industry wastewaters.

	Winery	Distillery	
	(vintage)	Dissolved	Total
Organic Component (g/l)			
Tartaric Acid	0.83	1.65	8.80
Lactic Acid	0.27	1.64	2.52
Acetic Acid	0.12	1.25	1.16
Glycerol	0.11	3.25	4.24
Ethanol	1.96	0.00	0.00
Glucose	0.56	0.12	3.08
Fructose	0.72	0.20	2.24
Total Organic carbon	2.86	7.80	16.1
Biological Oxygen demand	1.12	not determined (ND)	8.29
Inorganic Component (mg/l)			
Sodium	286	ND	695
Potassium	52	ND	1583
Calcium	18	ND	139
Magnesium	41	ND	101
pH	4.2	ND	4.5
EC (dS/m)	2.9	ND	6.4

(TOC) of the leachate was measured by potassium dichromate consumption (Nelson and Sommers 1982). Background values were determined from the cores leached with RO water only. Results were expressed as percentages of ^{14}C input or of TOC input for each type of wastewater, and subjected to analysis of variance.

7.2 Results

7.2.1 Patterns of flow

The typical pattern of flow of leachate from the drain at 15 cm depth during irrigation of the solod and brown earth to $0.1W_{-10}$ (0–15 cm) is shown in Figure 7.2. Leachate was collected from the solod within 4 minutes from the commencement of irrigation. Rates of leaching from the solod increased quickly to a maximum, before returning equally quickly to low flows (<0.3 ml/10 min.), 15 minutes after irrigation ceased (Figure 7.2A). In contrast, leaching from the brown earth at the 15 cm depth occurred 10 minutes after irrigation commenced, showed more gradual increases to maximum rates, and more gradual declines to low flows (Figure 7.2B). The maximum rates of flow of leachate from the soils were governed by the rate of application of wastewater. Increasing the volume of wastewater applied to the soils to $0.3W_{-10}$ (0–15 cm) resulted in the period of maximum flow becoming more protracted (>2h). Inspection of the structural arrangement of each soil type following experimentation indicated that the 0–30 cm layer of the solod was a structureless uniform fine sand throughout, whereas that of the brown earth was strongly aggregated and interspersed with larger cracks and biopores.

Leachate began to reach the drains at 30 cm depth 3–10h after commencement of irrigation (based on periodic opening of the drains). The total volume of leachate collected from the drains at the 15 cm and 30 cm depths over a 24h period was >98% of the volume of wastewater applied. This was expected since the columns were sealed to negate water loss by evaporation, and the very slow drainage of water through the clay subsoils would have

promoted lateral flow of water through the 30 cm drain. The proportion of leachate collected from the drain at 15 cm depth ranged from 70–95% of the total volume of wastewater added.

7.2.2 Removal of soluble-^{14}C in leachates during incubation with winery wastewater

Up to 5% of the added ^{14}C passed directly through the 0–15 cm layer of the soils during and immediately after irrigation with the wastewater labelled with ^{14}C-lactic acid and glycerol. Greater amounts of ^{14}C were leached from the brown earth than from the solod, and after application of wastewater to $0.3W_{-10}$ than after application to $0.1W_{-10}$ (Table 7.5).

The ^{14}C content of leachate collected after the soils had been allowed to incubate for 0 to 3 days after irrigation, and the reduction in ^{14}C due to evolution of $^{14}CO_2$ are shown in Figure 7.4. Between 13–33% of the added ^{14}C was retained by the 0–15 cm layer when leaching began 1h after irrigation. Greater amounts of ^{14}C were retained by the soils when the ^{14}C-labelled compounds were added in a volume of wastewater equivalent to $0.1W_{-10}$ of the 0–15 cm layer than when added in wastewater to $0.3W_{-10}$ (Table 7.6). Less than 3% of added ^{14}C was evolved as $^{14}CO_2$ during the combined irrigation and leaching period (7–9 hours from the commencement of irrigation, Figure 7.4).

Microbial metabolism of ^{14}C-compounds in solution began without lag in both soils (Figure 7.4). The maximum rate of removal of soluble-^{14}C/day was higher in the solod than the brown earth, and at the lower level of carbon loading ($0.1W_{-10}$) than at the higher level of carbon loading ($0.3W_{-10}$, Table 7.6). By the end of the phase of rapid microbial metabolism of ^{14}C-compounds in solution 82–90% of the added ^{14}C had been removed from the soil solution. The time elapsed to the end of the phase of rapid microbial metabolism of ^{14}C-compounds increased in the order: solod $0.1W_{-10}$ (0.8 day) < brown earth $0.1W_{-10}$ = solod $0.3W_{-10}$ (1.0 day) < brown earth $0.3W_{-10}$ (1.6 days). By the end of the 3-day incubation, <6% of the added ^{14}C could be leached from the

Table 7.5: ^{14}C content of the soil solution leached at 15 cm during and 1h after irrigation with the synthetic winery wastewater. Volumes of wastewater applied were equivalent to 10% of the gravimetric moisture content at -10 kPa ($0.1W_{-10}$) and $0.3W_{-10}$ (0–15 cm).

Treatment No.[†]		1	2	3	4	5	6
		^{14}C content of leachate (% of ^{14}C input)					
Solod	: $0.1W_{-10}$	0.3	0.2	0.1	0.2	0.3	0.4
	: $0.3W_{-10}$	0.1	1.9	2.9	0.9	0.9	1.7
Brown earth	: $0.1W_{-10}$	0.7	1.0	0.8	1.3	1.5	1.0
	: $0.3W_{-10}$	4.9	4.6	2.5	3.7	3.3	4.2

† Treatment No. 1 corresponds to the t=0 day incubation; No. 2 to t=0.5 day incubation ... No. 6 to t=3 days incubation.
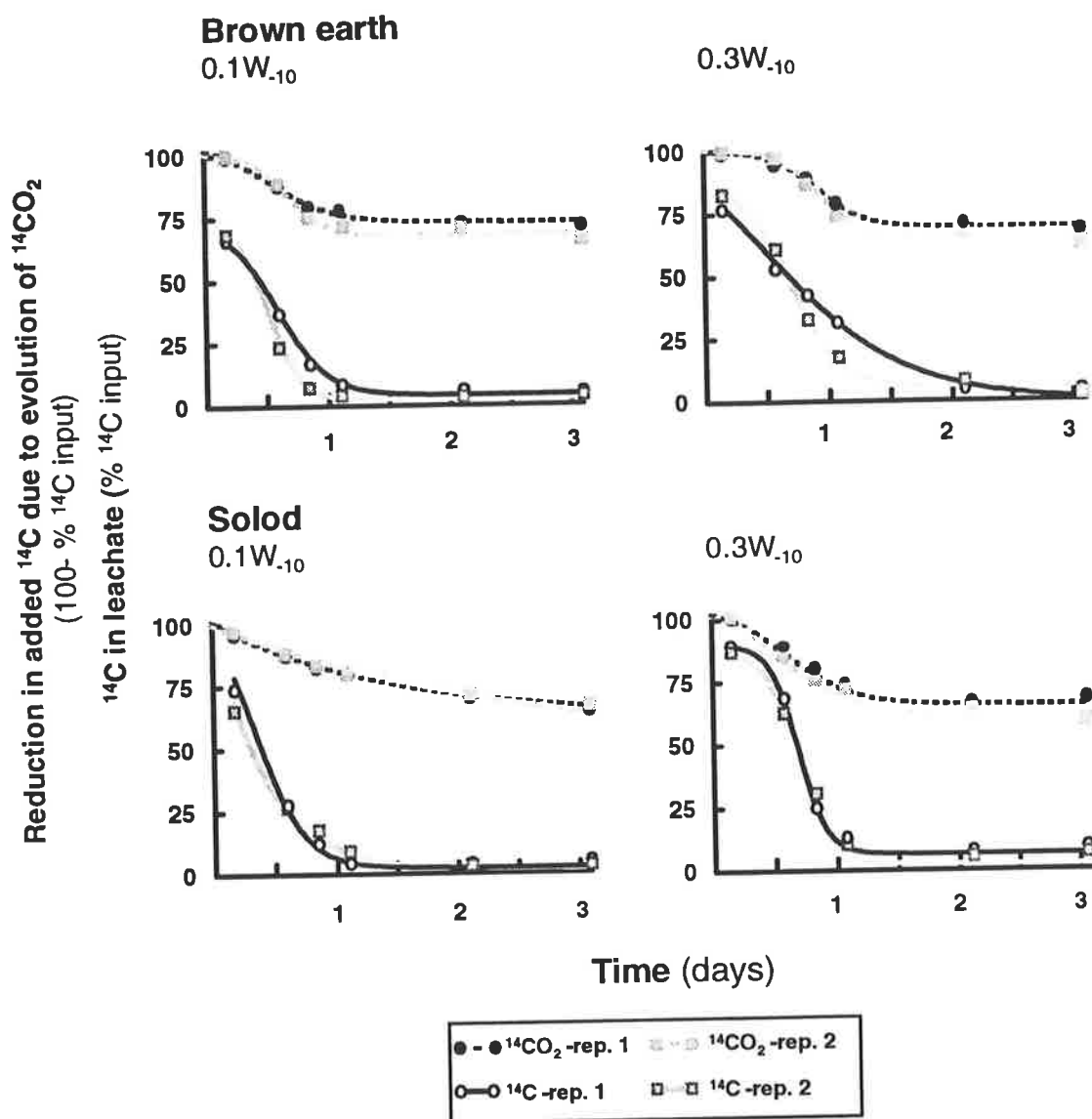
Figure 7.4: ^{14}C content of leachate collected at 15 cm depth, and the reduction in soluble-^{14}C due to evolution of $^{14}CO_2$ after incubation of an acclimatised solod and brown earth with synthetic winery wastewater. Volumes of wastewater applied were equivalent to 10% of the gravimetric moisture content at -10 kPa $(0.1W_{-10})$ and $0.3W_{-10}$ (0–15 cm).

Table 7.6: Leaching behaviour of soil columns. Means and least significant difference between means at the 95% probability level (LSD), are given.

	Solod	Brown earth	LSD
^{14}C retained by the soils after irrigation and leaching (% of ^{14}C input)			
$0.1W_{-10}$[‡]	26.3	33.2	
$0.3W_{-10}$	12.7	19.4	10.6 %
maximum rate of removal of soluble-^{14}C (% of ^{14}C input/day)			
$0.1W_{-10}$	186	113	
$0.3W_{-10}$	170	79	82 %/day
soluble-^{14}C after rapid microbial metabolism of ^{14}C-compounds (% of ^{14}C input)			
$0.1W_{-10}$	17.6	8.1	
$0.3W_{-10}$	10.0	9.9	12.4 %
time elapsed to the end of rapid microbial metabolism of ^{14}C-compounds (day)			
$0.1W_{-10}$	0.76	1.01	
$0.3W_{-10}$	1.06	1.62	1.39[†]
soluble-^{14}C after incubation for 3 days (% of ^{14}C input)			
$0.1W_{-10}$	2.1	3.5	
$0.3W_{-10}$	4.4	2.8	6.02 %
^{14}C evolved as ^{14}CO$_2$ (% of ^{14}C input)			
$0.1W_{-10}$	44.2	29.7	
$0.3W_{-10}$	37.8	33.0	4.6 %

† To account for effects of carbon loading on times elapsed to the end of rapid microbial metabolism of ^{14}C-compounds the LSD has been calculated from log-transformed data, and hence becomes a ratio between treatment a : treatment b, eg., 1.01/1.06=1.0; not significant at 5%.

‡ W_{-10} = the gravimetric moisture content at -10 kPa.

soils. Total amounts of $^{14}CO_2$ evolved from the soils during the 3-day incubation varied between 30–44% of ^{14}C input (Table 7.6).

7.2.3 Removal of organic carbon from wastewaters produced by the wine industry

Less than 0.3% of the ^{14}C added to the solod through daily irrigation with either winery or distillery wastewater to 0.1W-10 was leached from the drain at 15 cm depth. By contrast, leachates sampled from the brown earth varied considerably in their contents of ^{14}C-organic carbon, which ranged from 1–30% of ^{14}C input (Figure 7.5).

The rapid average increase of leachate-^{14}C after the 5th irrigation of the brown earth with winery wastewater was attributed to earthworm activity within one of the replicate columns, which was evident by the appearance of casts at the soil surface. Preferential flow of wastewater through the biopores would have led to a much higher proportion of the ^{14}C-compounds passing through the 0–15 cm layer during irrigation. Variation in the ^{14}C content of leachate collected from the brown earth irrigated with distillery wastewater may had also been partly due to differences in the amount of ^{14}C-compounds passing through the 0–15 cm layer during irrigation.

The total organic carbon (TOC) of leachates from both soils followed similar patterns of variation to those of the ^{14}C-organic carbon with successive irrigations of wastewater (Figure 7.5).

Leachates collected from the solod irrigated with winery wastewater and the brown earth irrigated with distillery wastewater differed significantly in their TOC and ^{14}C-organic carbon contents. For the solod irrigated with winery wastewater the TOC of the leachate was on average 4.8% higher than the ^{14}C-organic carbon content, whereas the ^{14}C-organic carbon content of leachate from the brown earth was on average 7.8% higher than the TOC. The TOC of leachates collected from soils irrigated with reverse osmosis
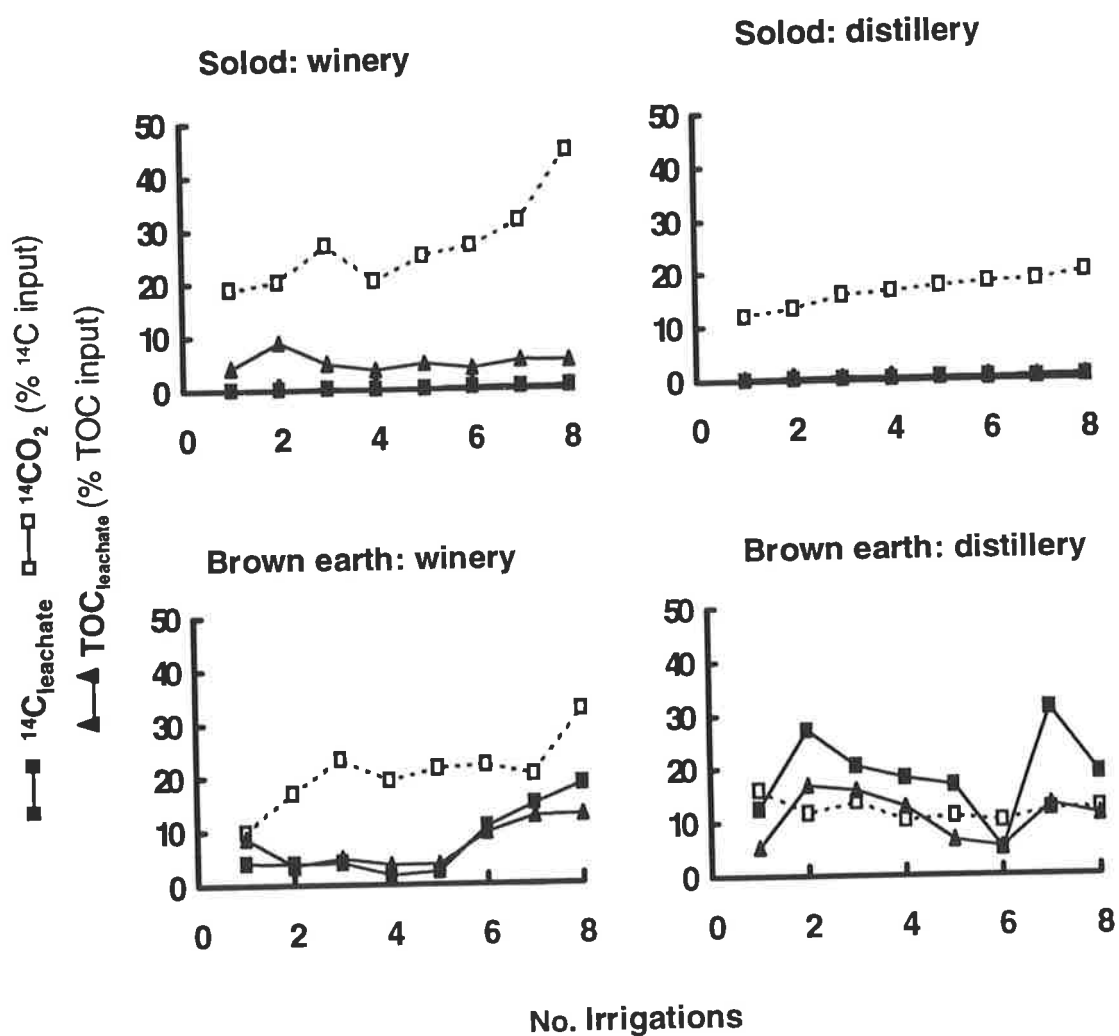
Figure 7.5: Removal of soluble organic carbon (0–15 cm), and the proportion of added ^{14}C evolved as $^{14}CO_2$ following daily application of an acclimatised solod and brown earth with winery and distillery wastewaters containing ^{14}C-labelled lactic acid and glycerol. The volume of wastewater applied was equivalent to 10% of the gravimetric moisture content of the 0–15 cm layer at -10 kPa.

water were equivalent to 3% of the TOC of the winery wastewater, and <1% of the TOC of distillery wastewater.

Evolution of $^{14}CO_2$ from the soils increased rapidly with successive irrigations with winery wastewater to about 30% of ^{14}C input for the brown earth and 45% of ^{14}C input for the solod. By contrast, relatively low amounts of $^{14}CO_2$ were evolved from both soils during irrigation with distillery wastewater (<20% of ^{14}C input; Figure 7.5).

7.3 Discussion

The removal of soluble organic carbon from wastewaters produced by the wine industry by irrigation of soils involved complex relationships between transportation processes and treatment processes. Transportation of the wastewater during and immediately after irrigation determined (I) the distribution of the organic substrates within the 0–15 cm layer of the soils, and (II) the proportion of added organic carbon transported directly beyond 15 cm. Adsorption and microbial uptake during initial transportation and leaching reduced the solution concentration of ^{14}C-compounds by 13–33% of ^{14}C input.

Following irrigation with wastewater the soil solution remained stationary within the 0 to 15 cm layer. Metabolism of ^{14}C-compounds in solution to microbial biomass and products, and mineralisation to carbon dioxide reduced the soluble-^{14}C to <5% of ^{14}C input.

Subsequent irrigation with wastewater would result in a portion of the "treated" soil solution being displaced beyond 15 cm, together with a small portion of the newly-added wastewater.

7.3.1 Transportation of ^{14}C-compounds during and immediately after irrigation

Clothier and Scotter (1985) and Smettem (1986) described a simple mathematical model to explain the role of adsorption in retarding the downward movement of solutes during infiltration. The model was based on displacement of the soil solution ahead of the invading solute front in a "piston" flow, with the downward movement of the solute front being further retarded due to adsorption, as outlined in Section 2.2.1. Examination of a core following cessation of the experimentation indicated that the brown earth was able to retain larger amounts of ^{14}C-compounds within the 15 cm layer than the solod during irrigation with wastewater and immediate leaching of the soil solution. This could be attributed to the higher clay and organic carbon contents of the brown earth, which are associated with larger microbial biomass, and hence solute uptake, and with higher sorptive capacity (Kunc and Stotzky 1974; Calvert 1989; Weber et al. 1991).

Up to 5% of the ^{14}C-compounds, however, passed directly through the 0–15 cm layer of the soils during and immediately after irrigation, which suggested that non-piston flow of the solute front occurred. Preferential flow of wastewater through larger structural pores may have led to the greater leaching of ^{14}C-compounds beyond 15 cm in the brown earth than in the solod during irrigation. Youngs and Leeds-Harrison (1990) found that in saturated conditions, the inclusion of larger structural pores in the infiltration process resulted in greater downward movement of solutes in aggregated soils than in non-structured soils. Increased leaching of ^{14}C-compounds beyond the 15 cm layer of soil as the volume of wastewater applied was increased from $0.1W_{-10}$ to $0.3W_{-10}$ is in agreement with the effects of increased amounts of percolation water on pesticide and nitrate movement in soils observed by Biggar and Nielsen (1978) and Troiano et al. (1993).

Thus the extent by which the ^{14}C-compounds were leached beyond the 0–15 cm layer during application of the wastewater appeared to be dependent on both the management of the irrigation system and transportation processes within the soils. The extent by which the ^{14}C-compounds were retained on the surfaces of clay and organic colloids may also depend

on managing the irrigation system to promote unsaturated flow (Smetten 1986, Youngs and Leeds-Harrison 1990). Although the brown earth had a greater capacity than the solod to adsorb the ^{14}C-compounds from the percolating solution, transportation of the wastewater through preferred pathways within the brown earth would have reduced the opportunity for the solutes to come into contact with the available surfaces.

7.3.2 Microbial metabolism of ^{14}C-compounds in solution

Faster maximum rates of metabolism of ^{14}C-compounds in solution by the solod than by the brown earth concurs with earlier results obtained from measurement using disturbed soils (cf., Sections 5.2.2, 6.2.3). When the different methods of measurement were compared at the same carbon loading, viz. $0.3W_{-10}$, the rates of removal of soluble-^{14}C/day were lower in the intact soil columns (Table 7.4) than in soil extracts sampled from similar depths, and following a single exposure to the wastewater (Table 5.8). More uneven distribution within the soil matrix, and movement of the organic substrates to the lower depths of the intact soils may have resulted in the lower rates of microbial metabolism of ^{14}C-compounds in solution compared with the disturbed soils.

Limitations of oxygen supplies may have also contributed to the lower rates of removal of soluble-^{14}C/day and amounts of added-^{14}C evolved as $^{14}CO_2$ in the intact soils than in the disturbed soils. Rates of evolution of $^{14}CO_2$ were, however, higher for the intact soils than the disturbed soils. According to Oades (1984) and Tisdall and Adem (1985) only pores with diameters >30 μm are expected to remain air-filled following irrigation. Assuming that the levels of oxygen within the 0–15 cm layer of the soils were similar to those determined in Table 7.2, the solod would require at least 7 renewals of the atmosphere within the pores remaining air-filled at -10 kPa to meet the biological oxygen requirement to mineralise 50% of the organic carbon added in the synthetic winery wastewater at $0.3 W_{-10}$ (Table 7.3), while the brown earth would require about 15 renewals (Table 7.7). Competition for the oxygen by microbial populations located closer to the soil surface for metabolism of the

added substrate carbon and mineralisation of soil organic carbon, and uptake by the *Phalaris* roots would further limit its supply to microbial populations at depth.

Thus, the "flush" of $^{14}CO_2$ evolved from the intact soils shortly after application of the wastewater may have been due to mineralisation of ^{14}C-compounds utilising the oxygen existing within the pore spaces, and oxygen transported to the upper layer of soil. With time transportation of oxygen, particularly to microbial populations at depth, may have limited the overall rate of removal of the soluble-^{14}C.

The lower rates of microbial metabolism of ^{14}C-compounds in solution, and hence the longer times taken by the brown earth than solod to remove the soluble-^{14}C to <5% of ^{14}C input could be due to: (I) the higher volume of wastewater, and hence carbon loading applied to the brown earth than solod, (II) deeper and more uneven distribution of the organic substrates within the brown earth as the result of transportation of the wastewater through preferential pathways during irrigation, and (III) the lower air-filled porosity of the brown earth at -10 kPa compared with the solod (Table 7.2). Further experimentation would be needed to establish the relative importance of the above factors.

7.3.3 Removal of organic carbon from wastewaters produced by the wine industry

The small changes in soluble-^{14}C and total organic carbon (TOC) leached from the soils after the first and subsequent irrigations with winery wastewater suggested that both the solod and the brown earth were able to remove the soluble organic carbon retained in the 0–15 cm layer before the solution was displaced beyond 15 cm by successive applications. Increased daily amounts of $^{14}CO_2$ evolved from the soils suggested that mineralisation was a major pathway of removal of the organic carbon (Figure 7.5).

The effect of earthworm activity in one of the replicate cores of the brown earth in allowing preferential flow of wastewater through the newly-formed biopores, and hence a higher proportion of the ^{14}C-compounds and TOC to be transported directly beyond 15 cm

Table 7.7: Number of renewals of the atmosphere within the pore space of the brown earth and solod remaining air-filled at -10 kPa needed to supply oxygen for mineralisation of 50% of the organic carbon added in the synthetic winery wastewater.

	Solod	Brown earth
$0.1W_{-10}$	2.3	5.0
$0.3W_{-10}$	6.8	14.9

during irrigation, however, highlighted the dynamic nature of soils and the potentially important role of soil macrobiota in the leaching process. A low volume sprinkler system, as used under field conditions, would have excluded the biopores from the infiltration process, and hence avoided the problem of preferential flow moving the organic substrates beyond the upper aerated zone of the soil.

By contrast with winery wastewater, a different balance of mechanisms seemed to be involved in the removal of the soluble and the suspended organic materials added to soils by irrigation with distillery wastewater. Suspended solids appeared to be removed by physical filtration. The solod, a fine silty-sand, was able to completely remove the suspended organic solids, as shown by the low TOC content (<1 of TOC input). The more structured brown earth was less efficient at filtering out solids. The reliance on the soil to filter suspended material raises the question of sustainability of sites irrigated with distillery wastewaters when accumulation of organic carbon within the upper soil layer may result in clogging of pores. Cook et al. (1994) concluded that clogging of pores caused a decrease in the infiltration capacity of a volcanic ash used to dispose of wastewater produced by the biological treatment of municipal sewerage.

The low amount of $^{14}CO_2$ evolved from the soils irrigated with distillery wastewater may have been partly due to a reduced capacity to renew the supply of oxygen within the air-filled pores as the result of the clogging of pores at the surface by filtered solids. The high proportion of suspended materials within the distillery wastewater may have also reduced the overall degradability of the organic carbon.

7.3.4 Conclusions

Intact soils containing substrate-adapted micro-organisms were able to reduce the solution concentration of organic carbon in winery wastewater rapidly and extensively by adsorption and microbial oxidation. The irrigation system should be managed to allow rapid microbial oxidation of the organic substrates within the 0–15 cm layer. The present experiments suggested that a volume of wastewater equivalent to 10% of the water holding capacity of

the soil ($0.1W_{-10}$), and no more than $0.3W_{-10}$, can be applied at a given irrigation, due to movement of the organic substrates beyond 15 cm. Saturated flow should also be avoided to minimise problems of preferential movement of water through large structural pores moving the organic substrates beyond 15 cm during irrigation. Under the above conditions, the solod and brown earth were able to reduce the soluble-^{14}C to <5% of ^{14}C input within 1–2 days after application of winery wastewater to $0.1W_{-10}$, and within 2–3 days after application to $0.3W_{-10}$.

Distillery wastewater containing suspended material should not be used for irrigation, as treatment of the organic carbon by microbial oxidation may not be effective, and clogging of pores by filtered solids may limit the sustainability of the irrigated soils.

Judiciously-timed cultivation of the irrigated sites may also be used to (I) remove biopores to minimise preferential flow of the wastewater during irrigation, (II) increase aeration, and (III) expose new organic substrates to microbial population, which, in turn, would lead to more rapid metabolism of the soluble organic carbon added by the wastewater. More rapid removal of soluble-^{14}C/day by the disturbed soils than by the intact soil columns at the same level of carbon loading may have been partly due to the above reasons. Greater evaporative loss of water from the bare surface would also minimise the duration of saturation of the soil following irrigation, hence promote the recovery of aerated conditions.

CHAPTER 8

DEVELOPMENT OF A MODEL FOR THE TREATMENT OF ORGANIC CARBON IN WASTEWATER BY IRRIGATION OF SOIL

8.0 Introduction

The Australian wine industry has sought information to develop protocol for the management of its wastewater as a supplementary source of water to existing natural water resources for irrigation of vineyards. It was envisaged that the wastewater would require minimal pretreatment, i.e., removal of solids. Thus the soil system would be required to remove the organic materials and nutrients present primarily in solution, to prevent contamination of groundwaters. The capacity of soil to remove organic carbon added by the wastewater was evaluted in this thesis.

The purpose of this Chapter is to integrate the analytical and experimental information presented in Chapters 3–7 into a general discussion. A generic model for the treatment of organic carbon in wastewaters produced by the wine industry by irrigation of soil will be developed in accordance with the above aims. Implications of the model and the experimental information on the management of water and organic carbon will be discussed, and conclusions and suggestions for further scientific inquiry made.

It is recognised that the management of wastewaters produced by the wine industry must also consider the environmental impact of other major components especially salts, acidity and sodicity, which has been outlined in Appendix II. The impact of nutrient loading will be briefly outlined in the conclusions.

8.1 General discussion

Incubation studies using disturbed soils demonstrated that the solution concentration of ^{14}C added in winery and distillery wastewaters, containing ^{14}C-labelled lactic acid and glycerol, could be rapidly reduced to <5% of ^{14}C input by the processes of adsorption and microbial metabolism. The patterns of removal of soluble-^{14}C in soil extracts, and mineralisation of ^{14}C-compounds to $^{14}CO_2$, illustrated in Figure 4.3, were both indicative of readily biodegradable substances described by Scow (1982). The results supported the studies of Santos Oliveira et al. (1975) and Mauganet (1978) who found that the organic components of winery and distillery wastewaters, mainly carboxylic acids, sugars, and alcohols, have BOD:COD ratios >0.5 (Table 3.6), and were readily biodegradable.

Between 10–50% of added ^{14}C was adsorbed by the soils during the first hour after application of the wastewaters (Table 6.4). The extent of adsorption of organic substrates added by the wastewaters raised an important question about the possible effect on microbial metabolism of the organic carbon remaining in solution.

It was found in Chapter 6 that reductions in the solution concentrations of the organic substrates by adsorption led to the times taken for removal of soluble-^{14}C to <5% of ^{14}C input being lower than would be predicted from the rates of microbial metabolism of the ^{14}C-compounds, in the absence of lag periods (cf., Figure 6.3 and Tables 6.7, 6.9). Soils sampled from the clay B-horizon of the brown earth, for which adsorption of ^{14}C-compounds was the greatest (40–50% of ^{14}C input), however, exhibited the lowest rates of microbial metabolism; the daily loss of ^{14}C from the soil solution never exceeded the ^{14}C added. Oades (1989) suggested that lower rates of mineralisation of ^{14}C-compounds from heavier textured soils compared with lighter-textured soils was probably due to reduced availability of the substrates to micro-organisms, which may, in-part, be due to the micro-organisms themselves becoming entrapped within the matrix of the clays, as first suggested by Marshall (1975).

Section 6.3.1 raised the question of sustained availability of sites for adsorption possibly being dependent on the removal of the organic substrates in solution by microbial metabolism promoting desorption of initially adsorbed compounds. The sum of added ^{14}C evolved as $^{14}CO_2$ and metabolised to biomass-^{14}C was often found to exceed the concentration of ^{14}C remaining in solution after initial adsorption of the ^{14}C-compounds (cf., Tables 5.6 and 5.7), which suggested that part of the ^{14}C-compounds metabolised originated from the pool of substrates initially adsorbed by the soils.

Microbial metabolism was essential for the removal of ^{14}C-compounds remaining in solution to <5% of ^{14}C input, with removal times dependent on the duration of a lag period (when present), and the rate of metabolism. As discussed in Section 5.3.2, the relative ranking of soils of different textures in the times taken for removal of ^{14}C-compounds in solution to <5% of ^{14}C input was dependent on whether or not the soils contained microbial populations previously exposed to the organic substrates added by the wastewaters. The results of the experiments suggested that "acclimatisation" of the soils existed at 2 levels.

Repeated application of winery wastewater to newly-exposed soils (non-acclimatised) led to a concurrent decrease in the length of the lag period and an increase in the rate of microbial metabolism, and hence reduced times for removal of ^{14}C-compounds in solution to <5% of ^{14}C input (cf., Tables 6.8–6.9, and Figure 6.3B). According to Scow (1982) acclimatisation may involve both the growth and an enzymic adaptation of microbial populations. To prove this would require measurement of specific activities of micro-organisms isolated from the soils.

Continued turnover of organic carbon within the microbial biomass was likely to involve different populations of micro-organisms to the groups responsible for the initial metabolism of the substrate organic carbon added by the wastewater[1]. Thus, the second level of

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[1] J.M. Oades, pers. comm.

acclimatisation appeared to involve the maintenance of sufficient numbers of active microbial cells within the groups responsible for immediate and rapid metabolism of the organic substrates, through regular application of wastewater. As shown in Figure 6.3A, successions of microbial populations within the acclimatised soils during the 10 day period between sampling and the first irrigation with the winery wastewater resulted in significantly longer times for removal of ^{14}C-compounds in solution to <5% of ^{14}C input, compared with times shown by the soils after successive applications of wastewater.

The maintenance of sufficient numbers of active microbial cells within the soils became more critical as the total amount of organic carbon added by the wastewaters was increased. It was found in Chapter 5 that higher organic carbon loadings resulted in higher solution concentrations of ^{14}C-compounds after initial adsorption, and often prolonged lag phases (Tables 5.7–5.8). Repeated application of wastewater to the 0–10 cm layer of the soils quickly eliminated the lag period (Table 6.6).

By contrast to the lag period, which could be eliminated through maintaining high numbers of active microbial cells, the maximum rate of metabolism of the ^{14}C-compounds for a given type of soil and level of acclimatisation of microbial populations appeared to be partly determined by carbon loading and/or the availability of nutrients. For example, it can be seen from Table 6.7 that when the "second level of acclimatisation" had been reached following 2 applications of wastewater, the maximum rate of removal of soluble-^{14}C fell within a 3h range for each soil. It may be possible that for these soils that growth of the microbial populations could no longer be sustained. This is partly supported by the observation that for soils within a given textural group, viz., sand (solod), sandy loam (0–30 cm layer of the brown earth), and clay (30–60 cm layer of the brown earth), the maximum rate of removal of solution-^{14}C increased with the organic carbon content of the soil (cf., Tables 6.7 and 6.1–6.2). For newly-exposed soils, the most rapid decreases in the duration of the lag period, and increases in the rate of microbial metabolism of the ^{14}C-compounds

were exhibited by the topsoils (Tables 6.8–6.9), in which organic carbon and nutrient levels were at their highest (Tables 6.1–6.2).

It was concluded from the incubation studies that acclimatised soils could consistently reduce the solution concentration of ^{14}C-compounds in winery and distillery wastewaters to <5% of ^{14}C input within 1 day after application. This nominal level of treatment could only be achieved when lag periods were <2 hours in duration, and when microbial metabolism resulted in a rate of loss of soluble-^{14}C/day exceeding the ^{14}C input.

Irrigation studies using undisturbed soils demonstrated the possible effects on removal times of the ^{14}C-compounds from the soil solution of: (I) the distribution of the organic substrates within the soil matrix during irrigation, and (II) the ability of the soils wetted to field capacity to transport oxygen to the sites where it is required for microbial metabolism of the organic substrates.

Clothier and Scotter (1985), and Smettem (1986) described a simple mathematical model to explain the role of adsorption in retarding the downward movement of solutes during infiltration. The model was based on displacement of the soil solution ahead of the invading solute front in a "piston" flow, with the downward movement of the solute front being further retarded due to adsorption, as outlined in Section 2.2.1. The acclimatised soils retained 13–33% of the ^{14}C added to the 0–15 cm layer following application of a volume of winery wastewater equivalent to 10% or 30% of the gravimetric moisture content at -10 kPa (W_{-10}), and immediate leaching of the ^{14}C remaining in the soil solution (Table 7.6).

Up to 5% of the ^{14}C-compounds, however, passed directly through the 0–15 cm layer of the soils during and immediately after irrigation (Table 7.5), which suggested that non-piston flow of the solute front occurred. Transportation of the wastewater through preferred pathways during irrigation would have resulted in deeper and more uneven distribution of the organic solutes within the soil matrix. Uneven distribution of the ^{14}C-compounds in

solution within the soil matrix, would, in turn, result in the organic substrates coming in contact with only part of the total population of active microbial cells. Lower rates of microbial metabolism of the ^{14}C-compounds in solution within the intact soils compared with the undisturbed soils at the same carbon loading, viz., winery wastewater applied at $0.3W_{-10}$ (cf., Tables 7.6 and 5.8), may have been partly due to the above reasons.

It was stated in Section 7.3.2 that an oxygen deficit was likely to occur soon after application of the wastewater to the intact soil columns, requiring diffusion processes within the air-filled pores to renew the atmosphere a number of times before aerated conditions were re-established within the 0–15 cm layer. Rates of mineralisation of ^{14}C-compounds in solution were, however, higher for the intact soils than the disturbed soils, following the application of winery wastewater to $0.3W_{-10}$, whereas an extra 20% of added ^{14}C was evolved as [14]CO_2 from the disturbed soils compared with the intact soils. Increasing the carbon loading through daily application of distillery wastewater, resulted in the supply of oxygen within the soil columns becoming rate limiting, evident from the low rates of mineralisation (<20% of ^{14}C input, Figure 7.5).

Quantitative measurement of the distribution of ^{14}C-compounds within the soil matrix following irrigation of the wastewater, and the effect of organic loading within a given depth of soil on the level of diffused oxygen and time taken for the recovery of "aerated" conditions, would provide valuable information about effects on the time required to reduce the concentration of ^{14}C-compounds in solution to <5% of ^{14}C input.

8.2 A model for the treatment of organic carbon in wastewater by irrigation of soil.

A model system for irrigation with wastewaters produced by the wine industry is proposed which will allow acceptable rates of application of the wastewater, and acceptable levels of removal of the soluble organic carbon for improved water quality. The soluble organic carbon in the wastewater is further utilised to improve the fertility and structural stability of soils, through its conversion to humus. The model, illustrated in Figure 8.1, is based on the design of systems used to treat wastewaters by continuous flow through a stationary medium (Tcholbanolglous and Burton 1991), and comprises:

(I) an upper "treatment zone" in which the major reduction in the biological oxygen demand of the wastewater is achieved by adsorption and metabolism by aerobic populations of micro-organisms, resulting in a major loss of carbon as carbon dioxide. The experimental information has indicated that the 0–15 cm layer of soil can achieve a nominal reduction in the solution concentration of organic compounds of >95% of carbon input within 1–3 days of application of the wastewater.

(II) a buffer region >75 cm thick underlying the treatment zone in which a targeted level of organic carbon in solution of 20–50 mg/l is achieved by adsorption and metabolism by anaerobic populations of micro-organisms.

Implications of the model and the experimental information for the management of sites irrigated with wastewaters produced by the wine industry will be discussed in Section 8.3.2.

WINERY WASTEWATER

TOC_{INPUT}

DEPTH (m)   EVAPORATION

0

TREATMENT ZONE
95% reduction of TOC_{INPUT} in 1-2 days

ADSORPTION;
OXIDATIVE DECOMPOSITION

0.15

ZONE OF
PLANT
UPTAKE
OF WATER

TOC_{BUFFER}

Depth of Rootzone
(variable)

BUFFER ZONE

ADSORPTION;
ANAEROBIC
DECOMPOSITION

>1

TARGETED TOC_{OUTPUT} =50 mg/L
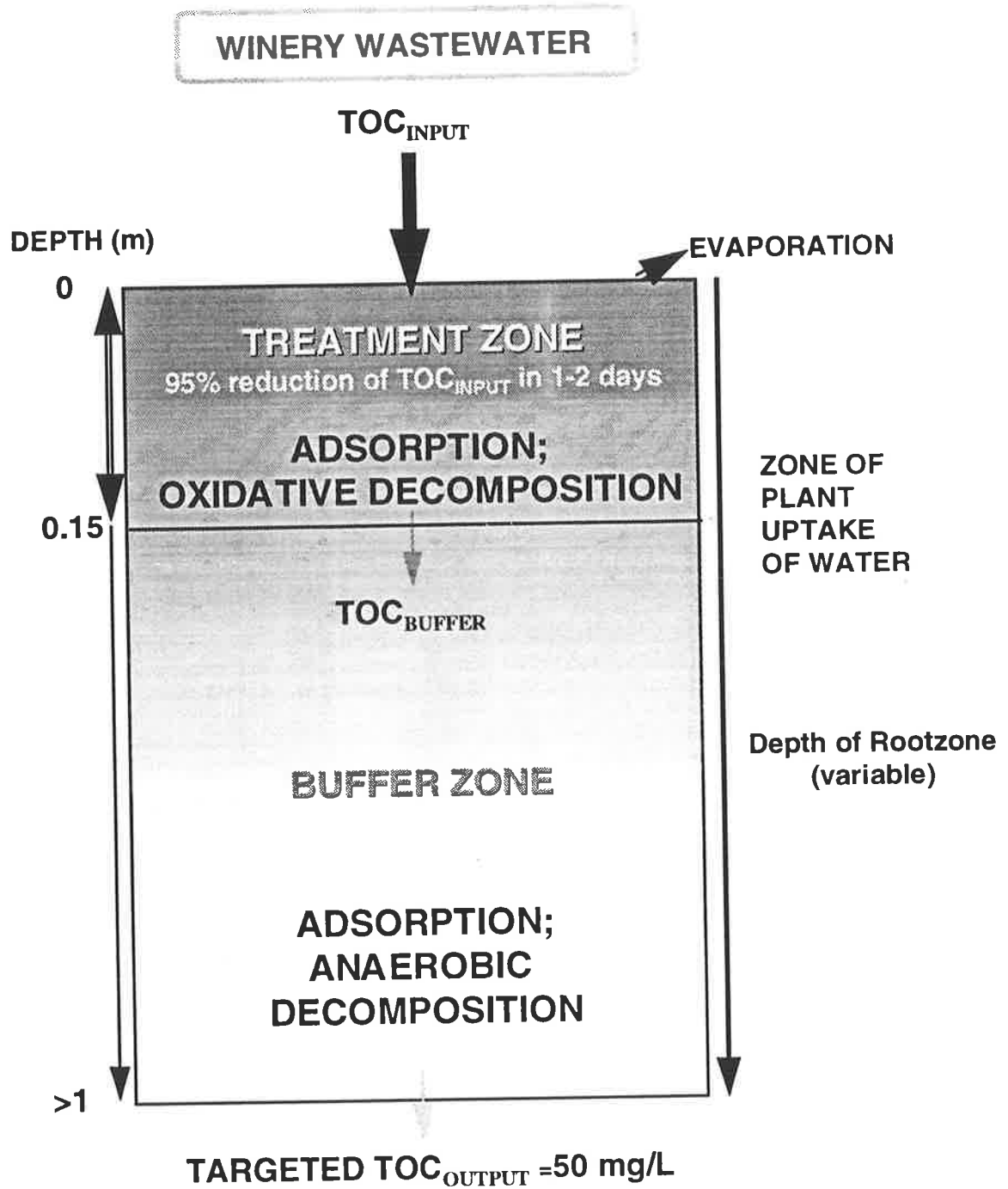
Figure 8.1: A generic model for the treatment of soluble organic carbon in wastewaters produced by the wine industry by irrigation of soils. TOC: total organic carbon in solution.

8.3 Management of wastewaters produced by the wine industry

8.3.1 Principles of management

As mentioned in Section 2.4.1, the basis of successful management of wastewaters used for irrigation is to provide a balance between environmental, social and economic issues. National guidelines for the management of effluents produced by wineries and distilleries list the following principles of management:

- waste minimisation;

- effective and feasible recycling and reuse;

- disposal in a manner that will not cause short- or long-term adverse environmental and/or social impacts.

Adverse environmental impacts include:

- reduced environmental values of surface or groundwaters;

- disruption of ecosystems;

- degradation of soils;

Effluent management can become a public nuisance as the result of:

- the creation of malodours,

- the loss of aesthetic appeal due to unsightly structures.

(NWQMS 1995)

There are four levels of effluent management: (I) production, (II) storage, (III) pretreatment, and (IV) disposal or reuse. Different combinations of the above economic, environmental, and social issues will apply at each level of management, and for different geographical locations and scales of operation of wineries.

8.3.1.1 Principles of management of water

Water is the most important single component of wastewater produced by the wine industry which impacts on the environment, both in terms of quantity, and in its role as a medium for the transportation of the organic and inorganic solutes contained in the wastewater. The management of all organic and inorganic components of the wastewater must be

incorporated into the overall management of water, which is based on obtaining the right balance between quality and quantity considerations as given below:

- minimising the volume of wastewater generated whilst maximising the quality;
- providing sufficient capacity to store the wastewater during times when it is not required for irrigation;
- adopting standards of pretreatment to minimise the effect of the wastewater on the environment;
- managing the irrigation system to use the water as determined by the needs of the crop.

(NWQMS 1995)

8.3.1.2 Principles of management of organic carbon

The impact on the environment of the organic constituents of wastewaters produced by wineries is only second to that of water, and has a major influence on the management of water at all four levels from production to disposal. The high biological oxygen demand of the wastewaters (Table 3.6) quickly results in the generation of malodours during storage. Hence the current approach by the industry to the management of the organic constituents of their wastewaters is almost solely directed at minimising the social impact of malodours, as outlined below:

- minimising the organic carbon content of the wastewater through recovery of useful by-products, eg., potassium-hydrogen bitartrate; lees and residues of wine for distillation;
- minimising the impact of malodours produced during storage on communities through the use of masking agents, covers, and/or siting of lagoons away from communities;
- reducing the biological oxygen demand of the wastewater through the use of biological treatment systems and/or land application after separation of the suspended solids.

(NWQMS 1995)

8.3.2 Implications of the model for management of the irrigation system

Management of sites irrigated with wastewater produced by the wine industry in accordance with the model described in Section 8.2 ensures that malodours will not be produced as the

result of the metabolism of the organic compounds within the soil solution. It is important, however, that managers are aware of the basic assumptions of the model, and that sites are managed accordingly. The model assumes:

(I)   that the soluble organic carbon added in the wastewater during a given irrigation is distributed within the treatment zone;

(II)   a minimum time between successive irrigations with wastewater is allowed to enable the solution concentration of organic compounds within the treatment zone to be reduced by 95% of carbon input by the processes of adsorption and microbial metabolism;

(III)   that the drainage capacity of the irrigated site is not exceeded.

The experimental information suggests that the treatment zone should lie within the upper aerated layer of soil, ideally the 0–15 cm layer, to promote rapid mineralisation of the soluble organic carbon to carbon dioxide. To ensure that the soluble organic carbon is retained within the 0–15 cm layer of soil following irrigation with the wastewater, it is recommended:

- that the volume of wastewater applied to the surface of the soil at a given irrigation should not exceed 30% of the water holding capacity of the 0–15 cm layer. Application of higher volumes of wastewater will result in an unacceptable proportion of the soluble organic carbon moving beyond the 0–15 cm layer of soil.

- Surface ponding should be avoided to eliminate rapid saturated flow through large cracks or biopores, and hence the transport of the soluble organic carbon in the wastewaters beyond the 0–15 cm layer.

Based on the times required by different soils to reduce the solution concentration of organic compounds to <5% of carbon input by adsorption and microbial metabolism, the following minimum rest periods between successive irrigations with wastewater are recommended:

- 1–2 days for soils of light texture (i.e., sands) containing acclimatised populations of micro-organisms;

- 2–3 days for soils of medium texture (i.e., loams) containing acclimatised populations of micro-organisms;

- a minimum of 7 days for soils newly-exposed to the wastewater during the first four months of operation, to allow the acclimatisation of microbial populations;

- a minimum of 3 days for two cycles of irrigation when soils containing acclimatised populations of micro-organisms have not been irrigated with wastewater for 10 days, to allow the populations of active microbial cells to rebuild.

Soils of heavy texture (i.e., clays) should not be irrigated as the rates of microbial metabolism were found to be too slow to allow rapid removal of the soluble organic carbon.

The nominal level of removal of soluble organic carbon could only be achieved within the above times for wastewater produced by a non-distilling winery which had a biological oxygen demand (BOD) <5 g/l, and a total organic carbon content of 2.9 g/l. Thus distillery wastewater will need to be pretreated by an appropriate facility to reduce the BOD to acceptable levels.

Times for removal of the soluble organic carbon can vary under field conditions, particularly by low temperatures during winter which would reduce the rate of microbial metabolic activity. Sites should only be wetted to field capacity, ie., not irrigated during rainfall, as oversaturation of the soil will reduce the effectiveness of treatment of the organic carbon and encourage leaching of contaminants beyond the treatment zone.

The irrigated sites may be cultivated at judicious times to:

- remove biopores and large cracks to minimise preferential flow of the wastewater during irrigation;

- remove compacted layers, especially under tracks near the base of the grape vines, and to increase aeration within the treatment zone;

- reduce the recovery time of aerated conditions via evaporative loss of water;

- encourage breakdown of by-products associated with the accumulation of microbial biomass.

8.3.3 Impact on the environment

The question of whether the model will meet the environmental objectives of management will be crucial to its successful adoption by the Australian wine industry. Regular monitoring of sites irrigated with the wastewaters and of adjacent groundwaters and/or surface waters will therefore be required.

8.3.3.1 Storage

It is generally not possible to irrigate throughout the year, (e.g., grape vines are dormant during winter) requiring the wastewater to be stored for prolonged periods. The volume of wastewater produced during manufacture has a major impact on the required size of storage systems. A small decrease in the volume of wastewater generated by a winery can result in a significant saving in the required capacity of the storage system (Figure 8.2A), and hence costs associated with construction and control of malodours. By contrast, increasing the area of land used for irrigation by a similar percentage only results in a small saving in the required capacity of the storage system (Figure 8.2B).

8.3.3.2 Impact on soil

The large volumes of wastewater generated by wineries, particularly during vintage, and problems associated with malodours produced during storage have resulted in managers opting to use irrigation systems to maximise the disposal of water. Not only is a valuable resource wasted, given that restrictions in the use of groundwater for irrigation affect many wine districts within Australia, I have encountered many sites which have become
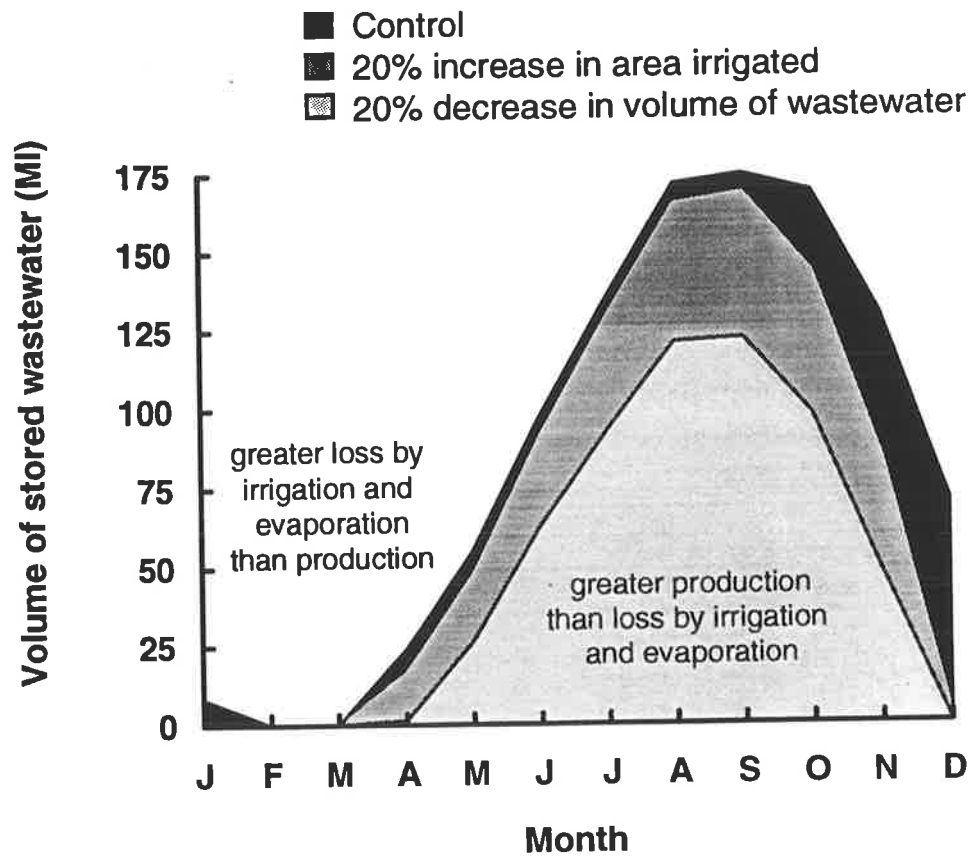
Figure 8.2: Influence of: A) reducing the volume of wastewater generated, and B) increasing the area of land used for irrigation on the required capacity of the storage lagoon of a large-scale distillery. Calculations were based on an average monthly production of wastewater of 27.3 Ml (range 9.9 to 44.6 Ml), and 40 ha of land sown to kikuyu for the control, adjusted for monthly variations in volume of wastewater produced, rainfall, pan evaporation, and evapotranspiration.

Source: the source of the information is acknowledged (to remain anonymous)

waterlogged as a result of over-irrigation. Thus, reducing the volume of wastewater generated by a winery will also reduce the pressure to over-irrigate.

As discussed in Chapter 6, the continued supply of organic substrates through the use of winery wastewater for irrigation will lead to a rise in populations of micro-organisms, and hence biomass. Siegrist and Boyle (1987) found that suspended solids contained in wastewaters, and the by-products of microbes associated with an increase in biomass in soils were responsible for pores becoming partially blocked. Cook et al. (1994) found that pore clogging resulted in a significant decrease in the ponded infiltration and drainage capacity of a volcanic ash soil use for disposal of treated domestic sewerage by rapid percolation. Metcalf and Eddy (1980) recommended that sites used for the disposal of distillery wastewater by flood irrigation (in California) should be cultivated on a regular basis to avoid a "crust" forming at the surface from the settling-out of the suspended solids. This is not a major issue in Australia as most wineries remove the suspended solids before using the wastewater for irrigation, which has since become a recommendation of the NWQMS (1995).

Repeated application of winery wastewater over 17 years has resulted in a 30–75% increase in the total organic carbon content within the 0–15 cm layer of the acclimatised brown earth compared to the non-acclimatised brown earth (Table 5.1). By contrast, the total organic carbon content within the 0–15 cm layer of the acclimatised solod has risen by 30–100% compared to the non-acclimatised solod after only 2 years of irrigation with wastewater from a winery-distillery. Continual irrigation with wastewater has also led to substantial increases in the contents of the inorganic nutrients within the acclimatised soils compared to the non-acclimatised soils.

The improved fertility of the acclimatised soils could be utilised by incorporating a cropping rotation into the management of the irrigated site, when not already planted with grape vines or trees.

8.3.3.3 Impact on water

Based on the experimental model and maximal levels of organic carbon in winery wastewater produced during vintage (Table 3.4), the soil solution will contain about 100–150 mg/l of organic carbon as it drains from the treatment zone to the buffer region. Thus a further 3 to 8-fold reduction in the solution concentration of organic carbon would be necessary within the buffer region to reach a targeted value of 20–50 mg/l. For wastewater produced during non-vintage, which accounts for about 55% of the annual volume of wastewater (Figure 3.1), the soil solution will contain about 60–90 mg/l of organic carbon as it drains from the treatment zone to the buffer region. Substantial reductions in both the total organic carbon content and biological oxygen demand of the wastewaters can also occur during short-term storage (Table 3.4).

8.4 Future lines of scientific inquiry

Increased reliance on alternative sources of water for irrigation will require management approaches based on quantification of (I) the potential resource value of untreated and treated wastewaters for crop production, and (II) the effects of the wastewaters on the soil and water environments. The experimental model could be further improved by quantification of:

- the relationship between the size and activity of the microbial biomass at the time of application, on the time for removal of the soluble organic carbon added by the wastewater within the 0–15 cm layer of soil. A minimum population size of active microbial cells may be required to eliminate lag periods and to ensure that a 95% reduction in the solution concentration of organic carbon is achieved within 1–2 days.

- the relationship between the extent and length of the oxygen deficit within intact soil systems and the time for removal of the soluble organic carbon added by the wastewater.

The rate of diffusion of oxygen as controlled by soil structure rather than the size of the microbial population may limit the rate of metabolism of the organic substrates, and hence the time for removal of the soluble organic carbon. This could have a major influence on the maximum permitted levels of organic carbon and/or biological oxygen demand of winery wastewaters used for irrigation.

- the influence of temperature on the above relationships. Winery wastewater is produced year-round, and hence will be reused or disposed during a wide range of temperature conditions, which may require different approaches to site management.

- the influence of soil organic carbon content and concentrations of major nutrients, particularly nitrogen, on the above relationships. The dynamics of available nitrogen is known to be a critical factor controlling decomposition of soil organic carbon (Griegorich et al. 1991), as well as determine the potential for nitrate contamination of groundwaters.

- the extent and rate of removal of the soluble organic carbon leached to the buffer region where anaerobic conditions predominate. The optimal residency time of the soluble organic carbon within the buffer region needs to be determined.

Extrapolation of information derived from laboratory experiments to field conditions is difficult, and hence the experimental model will require confirmation in the field. For example, the extent to which tillage operations within the vineyard might be used to reduce preferential flow, increase aeration, and encourage breakdown of microbial biomass and by-products could be assessed. The capacity of soils for receiving wastewater, and even the maximum clay content of soils suitable for application of wastewater, may be increased by judiciously-timed cultivation.

The loss of quality of existing natural water resources has resulted in some managers of vineyards substituting winery wastewater for poorer quality bore-water. Furthermore, reductions in yields during seasons of below-average rainfall could also be partially offset by supplementing the rainfall with wastewater to maintain sufficient reserves of sub-soil moisture. Quantification of the possible effects of winery wastewaters on the yield and quality of grapes, and the quality of wine will be required before its use for irrigation of vineyards becomes widely accepted. Site sustainability, particularly the impact of continual additions of organic carbon, nitrogen, and phosphorus on the environment, would also need to be assessed. It can be seen from Tables 6.1, 6.2, and 7.1 that considerable increases in the total levels of organic carbon, nitrogen, and particularly phosphorus have occurred at depth. Thus, site management may require balancing nutrient inputs from the wastewaters with outputs in the harvested grapes.

8.5 Conclusions

On the basis of experiments performed in this thesis, the following major conclusions can be drawn.

- Simple carboxylic acids, sugars and alcohols dominate the organic composition of winery and distillery wastewaters. These compounds are extensively and rapidly decomposed in soils.

- Adsorption and microbial uptake (in the absence of lag periods) are responsible for the decrease in the solution concentration of organic carbon within the first hour after application of the wastewater. Microbial metabolism of organic compounds is responsible for the reduction of the solution concentration of organic carbon to <5% of carbon input. The combined length of the lag period (when present) and the rate of metabolism determine the removal time.

- Soils can achieve a 95% reduction in the solution concentration of organic carbon within 1 day after application of winery wastewater when lag periods are <2 hours in duration, and when the daily rate of removal of organic compounds from solution exceeds the carbon input. Weekly or twice weekly applications of winery wastewater are needed to maintain sufficient populations of active microbial cells in acclimatised soils to remove 95% of the input of organic carbon within 1 day.

- A minimum number of 16 irrigations with wastewater are needed to acclimatise newly-exposed soils. Acclimatisation involves the concurrent reduction in the lag period and an increase in the rate of oxidative decomposition, which are most rapid within the topsoil.

- Increases in organic loading are accompanied by:

  –higher solution concentrations of organic carbon after initial removal by adsorption and microbial uptake;

  –longer lag periods;

  –longer times for the removal of 95% of input concentrations of organic carbon.

- Based on a 95% reduction of input concentrations, removal times of the soluble organic carbon added by winery and distillery wastewaters increase in the general order of: acclimatised solod < acclimatised brown earth < non-acclimatised brown earth < non acclimatised solod; the relative ranking of the soils of different texture is dependent on the extent of acclimatisation of the microbial populations.

- Intact soils containing substrate-adapted populations of micro-organisms are able to achieve >95% reduction in the solution concentration of organic carbon added in winery wastewater to the 0–15 cm layer within 1–3 days.

- The application of large volumes of wastewater, and/or the inclusion of macropores and biopores in the infiltration process through surface ponding can lead to >15% of input concentrations of organic carbon moving beyond the 0–15 cm layer of soil during irrigation, which should be avoided.

- Distillery wastewater will need to be pretreated by an appropriate facility to reduce the total organic carbon content (TOC) and biological oxygen demand (BOD) to levels similar to those of wastewater produced by a non-distilling winery, viz., TOC <3g/l; BOD <7 g/l.

The reuse of the wastewater for irrigation will reduce the reliance of the Australian wine industry on using evaporation lagoons for disposal. Wineries will still need to make provisions to store the wastewater during times when it cannot be disposed of or reused for irrigation. Reducing the volume of wastewater generated, particularly during vintage, will significantly reduce the required capacity of storage systems, as well as reduce the pressure to over-irrigate. Maximising the recovery of organic by-products will reduce the need to pretreat the wastewater for odour control.

APPENDIX I

AN EXAMPLE OF THE GENSTAT-GENERATED MODEL AND ESTIMATED PARAMETERS[1]

```
unit[32]

"Declare factors to describe treatments"
fact[l=4]rep
vari day;!(0,0.125,0.25,0.5,1,2)
fact[la=!t(No,yes)]acclimatize
fact[la=!t(loam,sand)]soil
fact[l=!(30,60)]loading
generate soil,acclimatize,loading,rep

"Declare variates for each replicate of each treatment"
vari[6] left[1...32]

"Read in the amount of ¹⁴C remaining in solution"
open 'winery.dat';2
read[serial=y;end=*;ch=2]left[]
close 2

"Establish the variate parameter into which the estimates of the logistic curve are placed"
pointer param;!p(b,m,c,a)
vari param[]

"Fit the logistic curve to each replicate in turn"
for i=1...32
model left[i]
fitcurv[cu=logistic] day
rkeep left[i];e=est
   if (est$[4]<0)
   fitcurv[cu=logistic;constant=omit] day
   rkeep left[i];e=est3
   vari est;!(#est3,0)
   calc est$[4]=0
   endif

"Store the parameters for future analysis"
for j=1...4
"Note j indexes b,m,c and then a"
calc param[j]$[i]=est$[j]
```

---

[1] Source: Dr. R. Correll pers. comm.

endfor
endfor

Example of the fitted model:
***** Nonlinear regression analysis *****

Response variate: left[1]
Explanatory: day
Fitted Curve: A + C/(1 + EXP(-B*(X - M)))

*** Summary of analysis ***[DH1]

	d.f.	s.s.	m.s.	v.r.
Regression	3	2235.34	745.11	44.87
Residual	2	33.21	16.61	
Total	5	2268.55	453.71	

Percentage variance accounted for 96.3
Standard error of observations is estimated to be 4.07

*** Estimates of parameters ***

	estimate	s.e.
B	-3.55	1.79
M	0.373	0.215
C	64.8	24.1
A	-0.51	4.31

"Print the parameters of the logistic curves fitted to each replicate"
print param[]

"Associate units with the newly-derived parameters"
rest a,b,c,m
calc nunit=nval(a)
unit[nunit]

"Derive other parameters of interest"

"Calculate left0: the amount of soluble-^{14}C extactable from the soils at t=0 day"
calc[s] left0= a + c/(1 + exp(-b*(0 - m)))

" Calculate best x: the time at which the rate of removal was greatest, used to derive other parameters"
calc[s] bestx=(m>0) * m

"Calculate maxrate: the maximum observed rate of removal of C"

```
calc[s] E=exp(-b*(bestx - m) )
calc[s] maxrate=b*c*E/(1+E)/(1+E)
```

"Calculate the duration of the lag period by iteration as the time elapsed from application of the wastewater until the rate of removal of soluble-^{14}C reached 25% of the maximum"

```
calc[s] lag=bestx/2
for iteration = 2...8
calc E=exp(-b*(lag - m) )
calc lagrate=b*c*E/(1+E)/(1+E)
calc sign=1 - 2 * (lagrate <(0.25 * maxrate) )
calc lag = lag + sign *  bestx/2**iteration
endfor
```

"Calculate the amount of soluble-^{14}C extractable from the soils at t=lag"

```
calc[s] lag%= a + c/(1 + exp(-b*(lag - m)))
```

"Calculate the minimum time between irrigations by iteration as the time elapsed from application of the wastewater until the rate of removal of soluble-^{14}C had declined to 25% of the maximum"

```
for iteration = 2...10
calc[s] E=exp(-b*(nextapp-m)
calc fagrate=b*c*E/(1+E)/(1+E)
calc sign=1-(2*(fagrate<(0.25*maxrate)))
calc nextapp=nextapp-sign*(max(day)-bestX)/2**iteration
endfor
```

"Calculate the amount of soluble-^{14}C extractable from the soils at t=nextapp"

```
calc[s] nextapp%= a + c/(1 + exp(-b*(nextapp - m)))
```

"Identify the parameters for analysis of variance within the variate p"

```
pointer derived;!p(left0,lag,lag%,maxrate,fag,fag%,a)
```

"Attach additional label"

```
vari[m=y]derived[];extra=' Winery data on % basis'
```

"Perform analyses of variance"

```
treatment loading*soil*acclimatize
anov derived[]
stop
```

"Example of the analysis of variance"

***** Analysis of variance *****

Variate: left0 Winery data on % basis

Source of variation	d.f.	s.s.	m.s.	v.r.
loading	1	1339.662	1339.662	652.48
soil	1	1745.484	1745.484	850.14

acclimat	1	50.052	50.052	24.38
loading.soil	1	1171.331	1171.331	570.50
loading.acclimat	1	29.016	29.016	14.13
soil.acclimat	1	61.569	61.569	29.99
loading.soil.acclimat	1	76.354	76.354	37.19
Residual	24	49.276	2.053	
Total	31	4522.744		

* MESSAGE: the following units have large residuals.

units 27   3.19   s.e. 1.24
units 32   3.71   s.e. 1.24

***** Tables of means *****

Variate: left0 Winery data on % basis

Grand mean 71.41

loading	30.00	60.00
	64.94	77.88

soil	loam	sand
	64.03	78.80

acclimat	no	yes
	72.66	70.16

loading	soil	loam	sand
30.00		51.51	78.38
60.00		76.55	79.22

loading	acclimat	no	yes
30.00		65.24	64.64
60.00		80.09	75.68

soil	acclimat	no	yes
loam		66.67	61.39
sand		78.66	78.93

soil		loam		sand	
loading	acclimat	no	yes	no	yes
30.00		51.65	51.37	78.83	77.92
60.00		81.68	71.41	78.49	79.95

*** Standard errors of differences of means ***

Table	loading	soil	acclimat soil	loading
rep.	16	16	16	8
s.e.d.	0.507	0.507	0.507	0.716

Table	loading acclimat	soil acclimat	loading soil acclimat
rep.	8	8	4
s.e.d.	0.716	0.716	1.013

Impact of winery effluent on soil

J.A. Chapman

Introduction

Soil is a dynamic system exposed to daily inputs and outputs of materials and energy which maintains a quasi–equilibrium. Continual exposure of the soil system to winery effluent will shift the equilibrium to a new quasi–state.

An outline of environmental, economic and social management principles for the reuse of winery effluent for irrigation has been given. An understanding of the likely interactions between effluent and soil system has been used to develop general management plans for water, organic and inorganic components.

Principles of effluent management

The basis of effluent management is to keep environmental exposures as low as reasonably achievable, given economic and social factors. Hence the effluent management plan must provide a balance between environmental and economic–social issues.

Environmental principles for managing effluent are essentially:

- the prevention of loss of quality to the site to which the waste is applied and of adjacent environments; and

- the avoidance of concentration of contaminants within the food chain.

The principles of economic management of waste include:

- the recovery of all useable components of the waste, for example, bitartrate, water for irrigation and converting organic substances added in irrigation water to soil organic carbon; and

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- the avoidance of unnecessary installation and annual operating costs.

Effluent management can become a public nuisance as the result of:

- the production of malodours during storage or by drift from irrigated sites; and

- loss of aesthetic appeal due to the location of woodlots and other structures associated with effluent management (especially views from residential properties).

The creation of public nuisance conditions should be avoided, as they can sometimes dominate the more important environmental and economic considerations. This involves the participation of the community in developing effluent management plans.

Different combinations of the above environmental, economic and social issues will apply at each stage of effluent management and for each location. Changes in either economic and/or environmental circumstances must be quickly responded to. Hence effluent management plans must be dynamic and continually reviewed.

Management of winery effluent

There are four major components of managing winery effluent—production, storage, reuse, and treatment during storage and/or reuse of the effluent (Figure 1). Production and the preferred option of reuse will have a major impact on the requirement to store and treat the effluent. Production and characteristics of wine industry effluent have been discussed elsewhere in the proceedings. The reuse of the effluent for land application will be considered here.

Water

Water is the most important single component of winery and distillery effluents which impacts on land systems, both in terms of quantity and in its role as transport medium for the organic and inorganic components of the effluent. Hence the principles of water management (Figure 2) are essentially a balance between quantity and quality considerations as given below:

- minimize the volume generated in the winery or distillery whilst maintaining sufficient water quality for irrigation;

- pretreatment of the effluent via an engineering structure is an optional, but necessary, consideration;

- maximize the use of water by the land system without causing degradation of the site;

- contain all surface runoff water from the irrigated site for reuse if deemed of suitable quality for irrigation, or disposal via evaporation when unsuitable for irrigation; and
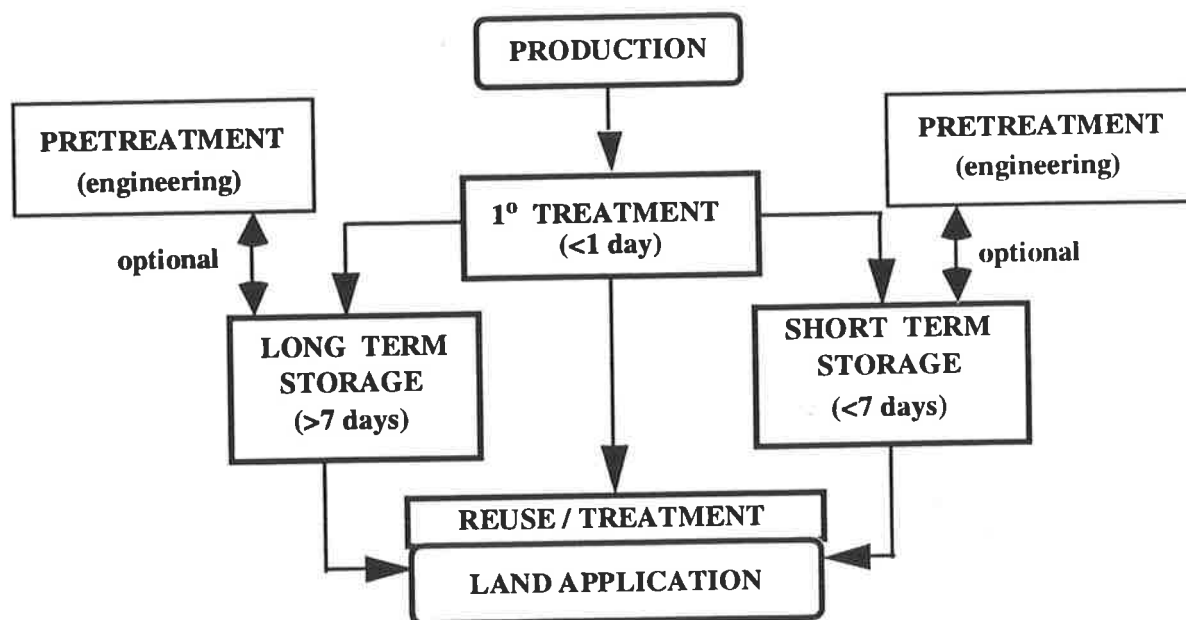
Figure 1. Management of wine industry wastewater.

- contain subsurface drainage water from the irrigated site for reuse if deemed suitable for irrigation, or disposal via evaporation when unsuitable for irrigation.

Determining quality limits is one of the most difficult aspects of effluent management as they are based on three factors:

- quality limits of the irrigated crop—pasture species (for example, kikuyu) and tree lot species have higher tolerance levels to salts and other contaminants than grape vines, however, irrigation of vineyards may be the only option available in many areas due to the lack of available land;

- quality limits of the land system—the quality of the irrigation water may directly affect the sustainability of the land system itself (for example sodicity); and

- quality limits of adjacent environments which receive excess water from the irrigated sites.

Management of organic and inorganic constituents

The underlying principle of management is minimization of unnecessary production of waters high in total salts and/or organic loading within the winery or distillery, as this will ultimately affect the flexibility to manage water quantity in relation to quality, as well as the impact of the contaminants on the land system. Management of organic and inorganic
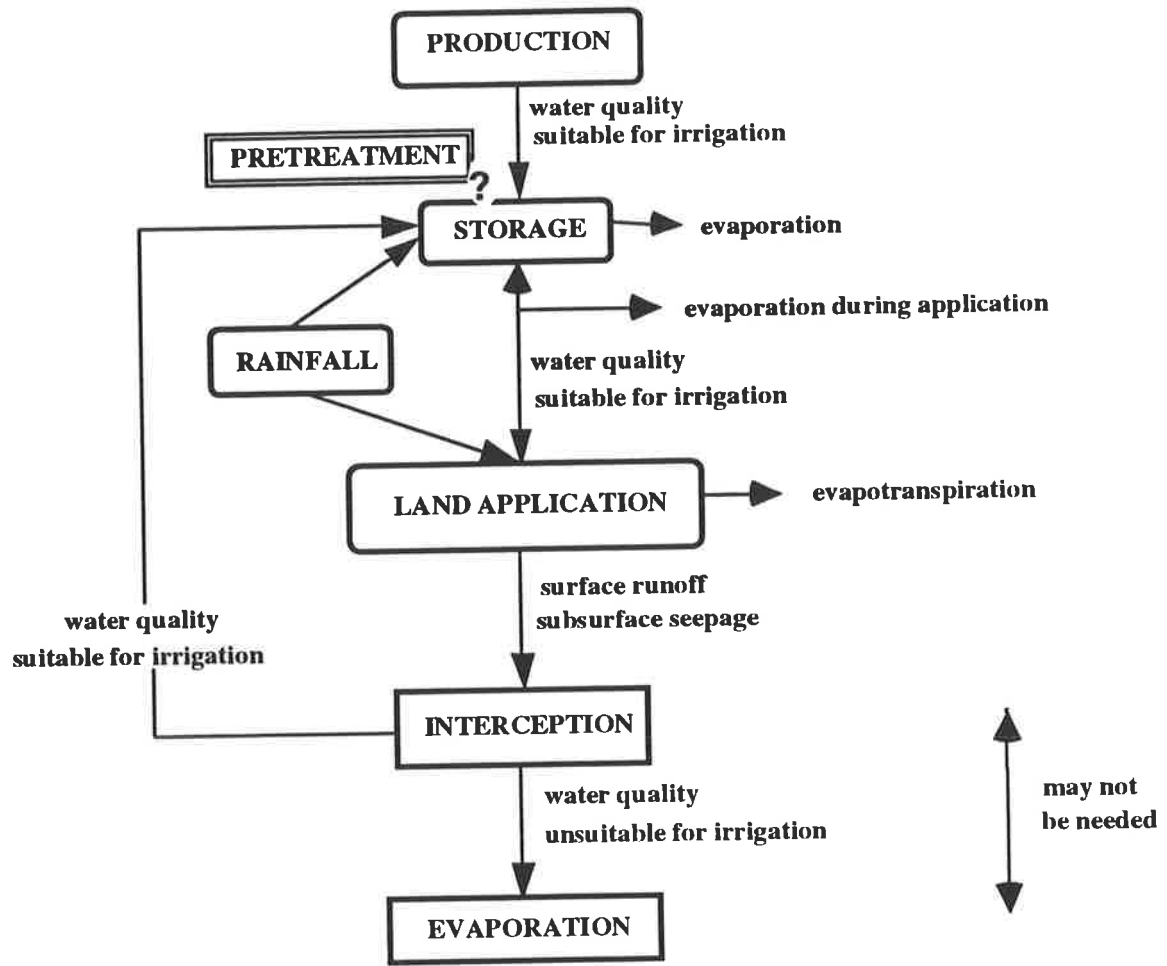
Figure 2. Management of water.

contaminants require separate approaches, as their cycling within and removal from land systems involves different processes.

Inorganics

Generally inorganic elements and their salts are added far in excess than that required by the agrisystem. Hence the principles of management (Figure 3) is one of maintaining tolerable levels within the land system and interception of all surface runoff water and subsurface seepage water, where loss of quality of adjacent environments is likely to be a major problem.

Salt

Each wine producing region has differing degrees of salt-related problems of both normal supplies of irrigation water and of effluent water. Hence for many wineries salt management is not a new issue.

Salt inherited from the winery or distillery effluent may be further concentrated as the result of evaporation during irrigation, or by 'pick-up' of salts as the leachate water seeps through

the soil system. Loss of environmental quality of the irrigation site due to salt can be minimized by:

- ensuring that the salt content in the irrigation water are kept below a critical maximum (which also accounts for possible concentration of salts during storage and\or irrigation);

- by the use of a 'leaching factor' during application of the effluent; and

- by ensuring that soils have sufficient drainage capacity to remove the excess leachate water, and are deep enough to avoid salts from seepage water being dragged towards the surface by evapotranspiration.

Drainage water from the irrigated sites should not be allowed to freely move to other environments, especially rivers and creeks, where salts transported in that water may cause problems. For similar reasons irrigation sites should not be located above important groundwater systems. Where off–site contamination is a problem drainage water should be intercepted by the installation of drains. All surface runoff water from the irrigated site must normally be contained. The intercepted surface and drainage water may be returned to the storage dam for irrigation if the salt content is below the critical maximum (see Figure 3), otherwise disposed via evaporation.

Other inorganic components

The above approach for salt management also applies for the management of sodicity. Sodicity is essentially caused by an excess of sodium in relation to that of calcium plus magnesium. Sodicity causes clays in soil to swell leading to loss of drainage capacity, and in extreme cases subsurface erosion of the clay itself. Again managing sodicity is not a new issue, as about one third of our soils are affected by sodium, a legacy of large areas of land being ancient sea beds.

Sodicity results from several complex interactions making it difficult to predict. Thus management of sodicity is preventative, based on correcting the imbalance of sodium to calcium by adding calcium to the system (normally bought as gypsum). There are many factors which influence the magnitude of the effect of sodicity, particularly the type of clay in the soil, and electrical conductivity of the soil water. Hence it is advisable to seek the assistance of an expert in determining the calcium requirement for the management of sodium.

pH

The optimal pH for most crops and pastures is 6.5, however crops can be successfully grown between pH 5.5 and pH 8.5. The pH of winery and distillery effluents is normally below 5.5, hence their application to land will tend to increase acidity depending on the buffering capacity of the soil. Many wineries choose to adjust the pH of the effluent as the point at which the effluent leaves the storage dam for irrigation. The use of a calcium hydroxide slurry in preference to other hydroxides will also help correct the imbalance of sodium.
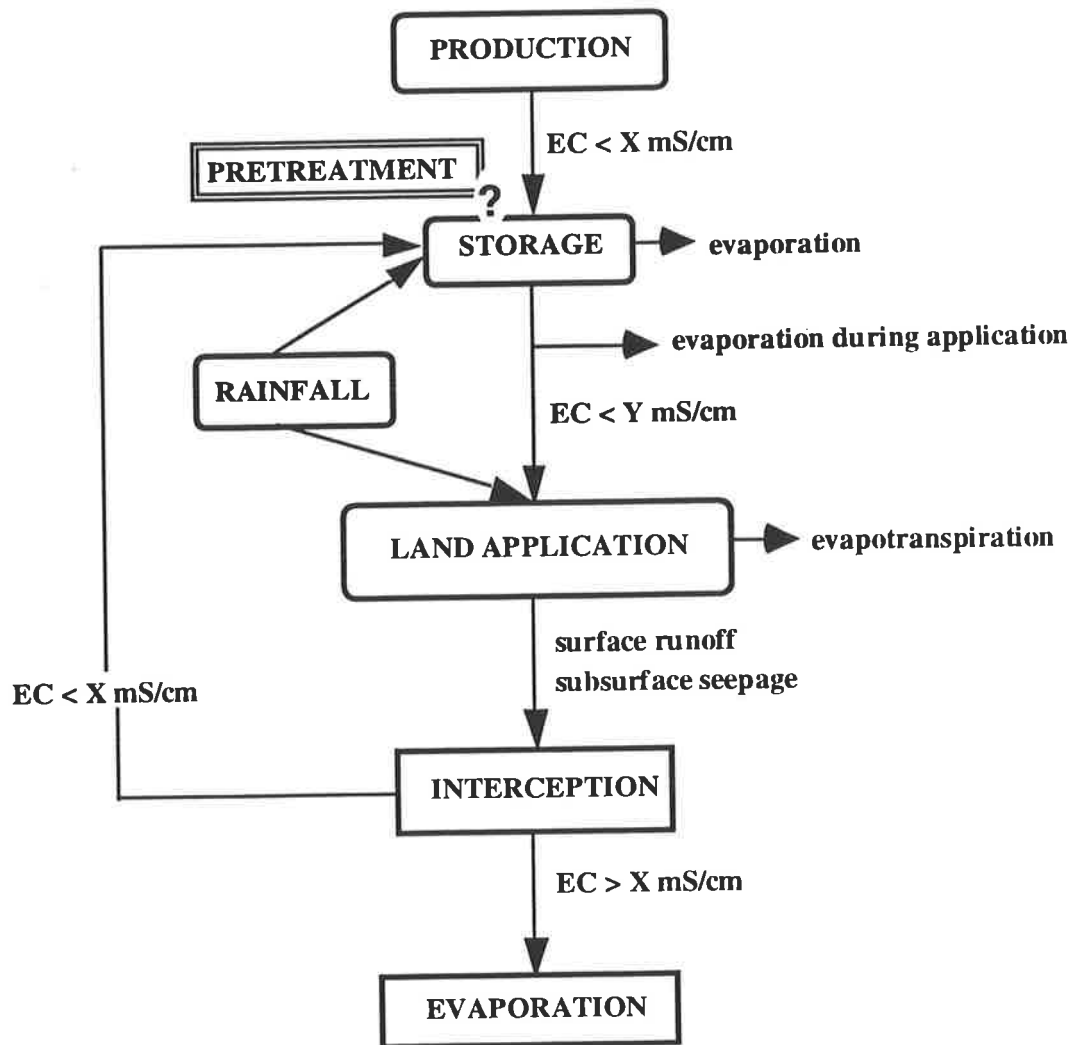
Figure 3. Management of salt (X and Y are quality limits for storage and irrigation, respectively.

Thus both sodicity and pH can be effectively managed via the same system which would consist of an extra 'monitoring' step between effluent storage and land application (Figure 4, option B). Acid conditions also favour the production of hydrogen sulfide (rotten egg gas) which is the dominant malodorous gas. Raising the pH of stored water can greatly reduce the production of hydrogen sulfide, hence lessen the impact of malodours on neighbouring properties (Figure 4, option A).

Organic carbon

The principle of managing organic carbon is to promote its biological degradation to odourless carbon dioxide. But the oxygen required for the degradation must be supplied by the external environment. Diffusion of oxygen in water is about 10 000 times slower than air. Hence the key to low cost treatment of the organic carbon is to apply it to a system which is able to supply the oxygen via diffusion through air.
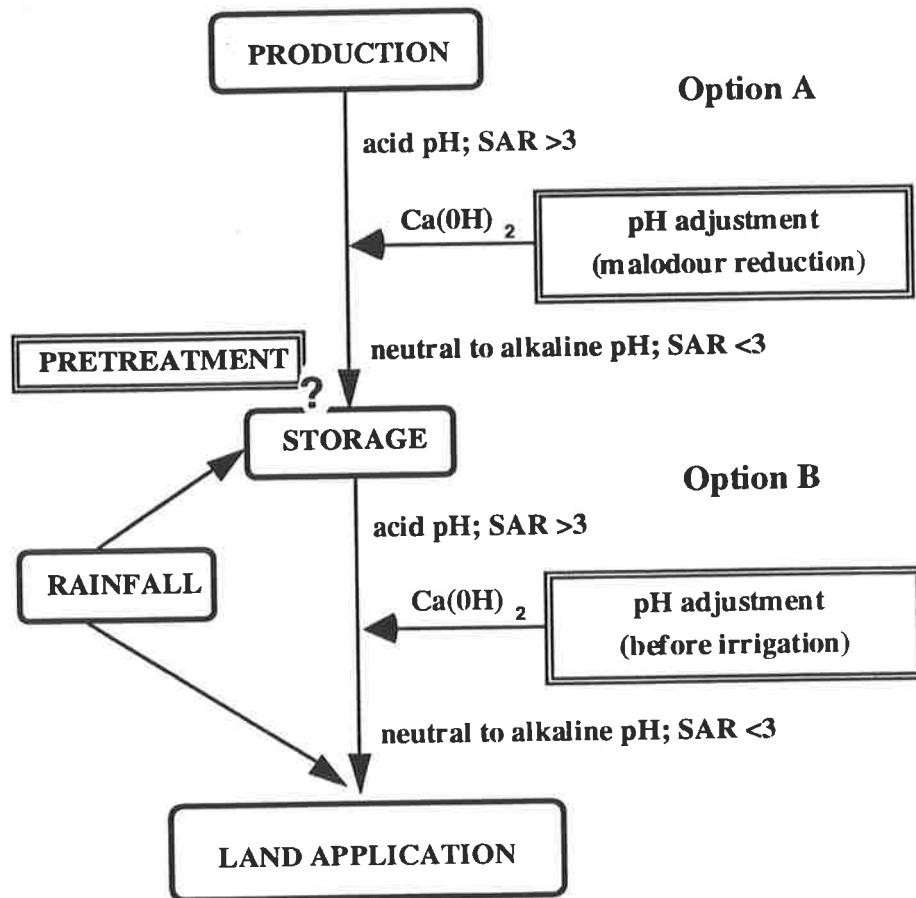
Figure 4. Management of pH and sodicity for (A) control of malodours, and (B) maintenance of water quality for irrigation.

Storage

The rate of diffusion of oxygen in water is too slow to meet the oxygen demand for biological decay of the organic contaminants. Hence the oxygen must be externally supplied to a dam via aerators, a costly exercise, both in installation and annual running costs. Thus it may be more cost effective to operate storage dams anaerobically. Aerobic and anaerobic pretreatment processes and associated costs have been discussed elsewhere in these proceedings.

Land application

Land systems are ideally suited for the treatment of organic carbon contained in winery and distillery effluents. The favoured land system is woodlots, with the main management objective being the removal of water. Sites are often irrigated daily.

Treatment of organic contaminants added in wastewaters by soil is most analogous to batched–flow, fixed–film secondary treatment systems (Figure 5). Wastewater is added to the soil surface via the irrigation system. The movement of water within the soil system transports the organic contaminants to the microbial populations which are supported on a
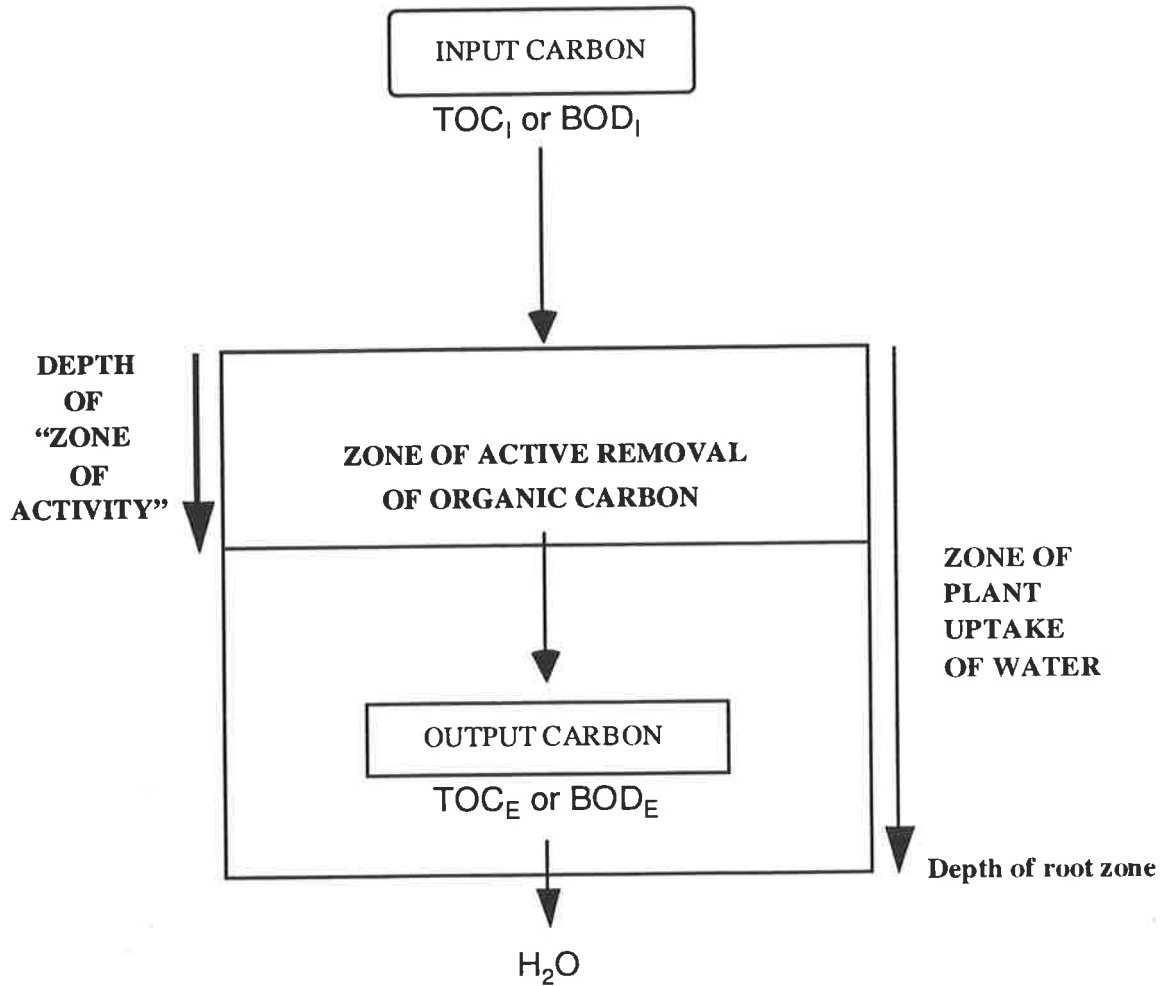
Figure 5. A model for the treatment of organic-rich wastewater by irrigation of soil.

stationary medium. Sufficient contact time is allowed for microbial treatment and removal of the organic contaminants from the soil solution before it is displaced by the next application of wastewater.

The nominal depth of the 'zone of activity' can be defined as the depth which allows the removal of soluble organic carbon from irrigated winery wastewater within 24 h. Once defined the irrigation system can then be managed to keep the newly added organic carbon within this zone. The specific irrigation parameters need to be developed for each location as they are dependent on the soil characteristics of the site and on the organic composition of the effluent.

The increase in microbial biomass and other forms of soil organic carbon is highly beneficial for soil fertility, hence the agrisystem. In ideal conditions about two thirds of the input organic carbon will be converted to carbon dioxide and one third to microbial biomass and other forms of soil organic carbon.

Conclusion

Land application will become an essential component of the management of winery effluent in most locations. The Australian wine industry has moved a long way towards self–regulation of effluent management thereby maintaining control of its own destiny.

Experiences of past 'hit or miss' approaches towards management of irrigated sites have been learnt from. The current approach is informed decision making using a nationally agreed set of management principles as a guideline, and the information derived from targeted research, as that outlined above. In this context the Australian wine industry has 'come of age' in its recognition of the resource value of effluent.

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