IMPACT OF PRE-OZONATION ON DISTILLERY EFFLUENT DEGRADATION IN A CONSTRUCTED WETLAND SYSTEM

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

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ABSTRACT

Distilleries are an example of an agricultural industry that generates large volumes of wastewater. These wastewaters are heavily polluted, and due to the seasonal nature of the product, the amount and composition of the wastewater may exhibit major daily and seasonal variations. Wine-distillery wastewaters (WDWWs) typically are acidic (pH 3.5 - 5.0) and have a high organic content (sugars, alcohol, proteins, carbohydrates and lipids), a COD range of 10 000 – 60 000 $mg.L^{-1}$, have a high suspended solids content as well as containing various inorganic compounds. Additionally refractory compounds present in these wastewaters, such as polyphenols, can be toxic for biological processes, making the selection of a suitable treatment process problematic. Wetlands have been shown to be a feasible treatment for effluent originating from wine, however, they are normally used as a secondary treatment method and not well suited for high volume, high COD (> 5 000 $mg.L^{-1}$) wastewaters. Ozone has been successfully used as a pre-treatment for WDWW due to its oxidising capabilities to partially biodegrade organics and non-biodegradable organics, and reduce polyphenols, which results in an increase in biodegradability. Currently a wetland system is being used on its own at a distillery to treat wastewater from a series of stabilisation dams, but the legal requirement for discharge into a natural resource (COD < 75 $mg.L^{-1}$) is not being met. Additional treatments suited for WDWW are therefore being considered.

Wine-distillery wastewater was characterised and found to show a large variation over time (COD ranging from 12 609 - 21 150 $mg.L^{-1}$). Ozonation of WDWWs was found to be effective in decreasing COD over a wide range of organic loads. For pre-wetland wastewater from the distillery, an average COD reduction of 271 mg $COD.g\ O_3^{-1}$ was found, and for post-wetland effluent, an average of 103 mg $COD.g\ O_3^{-1}$. The effect of ozone on the biodegradability of the wastewater was monitored by activity tests, and a low ozone dose (200 - 400 mg $O_3.L^{-1}$) was found to increase activity in terms of biogas, methane and cumulative gas volumes. By showing an increase in the biodegradability of WDWW, it was concluded that ozone has potential as a pre-treatment step to increase the effectiveness of a biological wetland system.

Lab-scale wetlands were used in trials to determine the effect of pre- and post-ozonation on WDWW. It was found that the efficiency of the wetland receiving the pre-ozonated “off-season” WDWW (2 200 mg $COD.L^{-1}$) had a higher COD reduction (73%) than the wetland fed with untreated (62% COD reduction) WDWW, and the total polyphenol content was reduced by 40 and 31%, respectively. Treatment efficiency in

terms of the reduction of colour, total solids, suspended solids and phosphates were also greatly improved for the pre-ozonated WDWW. Similar results were found when treating high COD “peak season” (7 000 mg $COD.L^{-1}$) WDWW, with higher reduction rates for the wetland treating pre-ozonated WDWW (84% COD reduction) than for the wetland fed with untreated WDWW (74% COD reduction), and the total polyphenol content was reduced by 76 and 72%, respectively. Post-ozonation was also shown to be beneficial in that it improved the final effluent quality leaving the wetland system. Increasing the hydraulic retention time (HRT) of the wetlands from 9 days to 12 days resulted in similar COD reductions for the control and experimental wetland, highlighting the benefits that pre-ozonation has on reducing the acclimatisation period. Therefore using ozone as a pre-treatment could help in reducing the wetland size, HRT and allow increased volumes of wastewater to be treated.

In this study ozone was successfully utilised to reduce COD levels in wine-distillery wastewater, and increase the biodegradability of the wastewater. This study also showed that ozone, used as a pre-treatment to a wetland system, can contribute to improving the performance of a wetland system in terms of higher removal efficiencies. Wetlands are, however, unsuited for treating high strength COD wastewater, and the final effluent was still well above the South African legal limit for direct discharge into a natural resource. The results obtained during this study contributed to developing a method to achieve a more efficient treatment system utilising wetlands for the distillery industry, and can be of value in facilitating efficient environmental management.

UITTREKSEL

Stookerye is ‘n voorbeeld van ‘n landboubedryf wat groot volumes afvoerwater genereer. Hierdie afvoerwater is hoogs besoedel, en uit die aard van die produk is daar groot daaglikse en seisoenale variasie in terme van volumes en inhoud. Wyn-stokery afvoerwater is tipies suur (pH 3.5 - 5.0), het ‘n hoë organiese inhoud (suiker, alkohol, proteine, koolhidrate en lipiedes), ‘n Chemiese Suurstof Behoefte (CSB) variasie van 10 000 - 60 000 mg.L^{-1}, ‘n hoë gesuspendeerde vastestof inhoud, sowel as verskeie anorganiese stowwe. Die opsies vir die selektering van ‘n geskikte behandeling vir die betrokke afvoerwater word ook verder beperk deur die teenwoordigheid van additionele refraktoriese verbindings, soos polifenole, wat toksies kan wees vir biologiese prosesse. Alhoewel daar reeds bewyse is dat vleilande geskik is vir die behandeling van afvoerwater afkomstig van die wyndbedryf, word dit gewoonlik slegs gebruik as ‘n sekondêre behandelingsmetode omdat dit nie geskik is vir hoë volume, hoë CSB (> 5 000 mg.L^{-1}) afvoerwater nie. Osoon (O_3) is al met sukses gebruik as ‘n voorbehandeling vir wyn-stokery afvoerwater omdat dit oor oksiderende vermoëns beskik. Eerstens kan dit organiese stowwe sowel as nie-afbreekbare organiese stowwe gedeeltelik afbreek, en tweedens beskik dit oor die vermoë om polifenole te verminder. Hierdie kan lei tot ‘n toename in bio-afbreekbaarheid in die afvoerwater. Huidiglik word ‘n vleiland sisteem gebruik om afvoerwater, wat van ‘n stokery afkomstig is, te behandel nadat dit eers gestabiliseer is in opgaardamme. Die behandelde water voldoen egter nie aan die wetlike vereiste om water in ‘n natuurlike hulpbron te stort word nie (CSB < 75 mg.L^{-1}) en daarom word addisionele behandeling oorweeg wat geskik sal wees vir stokery afvoerwater.

Wyn-stokery afvoerwater is gekarakteriseer en daar is gevind dat daar ‘n groot variasie oor tyd was (CSB van 12 609 - 21 150 mg.L^{-1}). Daar is gevind dat osonering van wyn-stokery afvoerwater doeltreffend gebruik kan word in die verlaging van van CSB oor ‘n wye reeks van organiese ladings. Vir voor-vleiland afvoerwater van die stokery is ‘n gemiddelde CSB afname van 271 mg CSB.g O_3^{-1} gevind, en vir na-vleiland afvoerwater is ‘n gemiddelde CSB afname van 103 mg CSB.g O_3^{-1} gevind. Die effek van osoon op die bio-afbreekbaarheid van afvoerwater is deur aktiwiteitstoetse gemonitor, en daar is gevind dat ‘n lae osoondosis (200 - 400 mg O_3.L^{-1}) ‘n toename in aktiwiteit veroorsaak het in terme van biogas, metaan en kumulatiewe gas volumes. Deur ‘n toename in die bio-afbreekbaarheid van wyn-stokery afvoerwater te bewys, is daar tot die gevolgtrekking gekom dat osoon potensieel as ‘n voorbehandeling gebruik kan word om die doeltreffendheid van ‘n biologiese vleiland sisteem te bevorder.

Vleilande is op laboratorium skaal in toetse gebruik om die effek van voor- en na-osonering op wyn-stokery afvoerwater te bepaal. Daar is gevind dat die vleilande wat buite seisoen wyn-stokery afwalwater (2 200 mg CSB.L^{-1}) wat vooraf ge-osoneer is ontvang het, 'n groter afname in CSB (73%) toon as die vleiland wat onbehandelde (62% CSB afname) wyn-stokery afvoerwater ontvang het. Die totale polifenole inhoud het ook met 40% en 31% onderskeidelik afgeneem, en die effektiwiteit van die behandeling met betrekking tot die afname in kleur, total vastestowwe, gesuspendeerde vastestowwe en fosfate het ook verbeter vir die vooraf ge-osonerde wyn-stokery afvoerwater. Soortgelyke resultate is ook verkry met die hoë CSB (7 000 mg CSB.L^{-1}) seisoen wyn-stokery afvoerwater. Die vleiland wat vooraf ge-osoniseerde wyn-stokery afvoerwater ontvang het, het 'n groter afname (84% CSB vermindering) getoon as die vleiland wat onbehandelde wyn-stokery afvoerwater ontvang het (74% CSB vermindering). Die totale polifenole inhoud het ook verlaag met 76 en 72%, onderskeidelik. Daar is ook getoon dat na-osonering voordelig is deurdat dit die kwaliteit van die finale afvoerwater wat die vleilande verlaat, verbeter het. Deur die hidroliese retensie tyd (HRT) van 9 dae na 12 dae te verhoog, is soortgelyke CSB velagings vir die kontrole en eksperimentele vleiland waargeneem, wat die voordele van vooraf-osonering toon om die akklimatiseeringstydperk te verminder. Dus kan osoon as 'n voorbehandeling gebruik word om die grote van die vleiland te verminder, HRT te verlaag, en om groter volumes afvoerwater te laat behandel.

In hierdie studie is osoon suksesvol gebruik om die CSB vlakke in wyn-stokery afvoerwater te verminder, sowel as om die bio-afbreekbaarheid van die afvoerwater te verhoog. Verder is daar ook getoon dat indien osoon as 'n voorbehandeling vir 'n vleiland sisteem gebruik word, kan dit bydra tot 'n verbetering in die werkverrigting van 'n vleilandsisteem deurdat dit die verwyderingstempo verhoog. Vleilande is steeds nie geskik om hoë sterkte CSB afvoerwater te behandel nie, en die finale afvoerwater is steeds nie geskik, volgens die Suid-Afrikaanse Wet, om in natuurlike hulpbronne gestort te word nie. Die resultate wat in hierdie studie verkry is, het bygedra tot die ontwikkeling van 'n meer effektiewe behandelingsmetode, deur gebruik te maak van vleilande, vir die stokery bedryf. Hierdie resultate kan van nut wees in die fasiliteering van meer doeltreffende omgewingsbestuur.

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CONTENTS

The language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a number of chapters written as separate investigations, and therefore some repetition is unavoidable.

CHAPTER 1

INTRODUCTION

South Africa is a water-scarce country, with an average annual rainfall of 450 mm per year (DWAF, 2004), well below the world average of 860 mm per year. Water is becoming an increasingly scarce resource. Due to the highly diversified nature of the food industry, various food processing, handling and packaging operations create wastes of different quality and quantities, which if not treated could lead to increasing disposal and pollution problems (Sigge, 2000). With the current emphasis on environmental health and water pollution issues, there is an increasing awareness of the need to dispose of these wastewaters safely and beneficially (Pescod, 1992; Sigge & Britz, 2007). The food and beverage industry can no longer ignore growing environmental pressures from government, consumers, society at large, and most noticeably, their cost (Neall, 2000).

Wine production in South Africa has increased over the past decade (SAWIS, 2007), and this growth places further pressure on our natural resources such as water, soil and vegetation. National legislation and international markets require the responsible management of potential environmental impacts through effective systems, meaning that companies are responsible for waste generated and treatment thereof (Van Schoor, 2000; Sigge & Britz, 2007). Distilleries are an example of an agricultural industry that generates large volumes of wastewater. In spirit distilleries, specific water intake values ranged from 1.8 to 6.2 litres per litre of alcohol produced, with most of the water being used for steam raising, cooling and floor and equipment wash down (Water Research Commission, 1993).

Due to the seasonal nature of the product, the amount and composition of the wastewater may exhibit major daily and seasonal variations (Bezuidenhout *et al.*, 2002). These wastewaters are heavily polluted, and this presents disposal problems. Wine-distillery wastewaters typically have a high suspended solids content and contain residual organic acids, soluble proteins, carbohydrates, as well as various inorganic compounds (Water Research Commission, 1993; Van Schoor, 2000). The wastewater has an acidic pH (3.5 – 5.0) and is characterised by a high organic content (sugars, alcohol, phenols, polyphenols and lipids) with a COD range of 10 000 – 60 000 mg.L^{-1} (Benitez *et al.*, 1999; Van Schoor, 2000; Martín *et al.*, 2002). These wastewaters generally have a brownish colour that is attributed to polymeric pigments, collectively known as melanoidins (Alfafara *et al*., 2000).

Current wastewater treatments for wine distilleries vary in detail, but can broadly be divided into three groups, namely: physical (particle removal, sedimentation, filtering); biological (lagoons, wetlands, UASB reactors, activated sludge) and chemical (ozone, UV, hydrogen peroxide). Biological treatments are one of the most effective ways to treat organic waste (Gilson, 2000) and can be used for the removal of both organic and inorganic contaminants (FAO, 2004). Distillery wastewaters are, however, chemically complex and contain a host of phenolic compounds, some of which resist biodegradation (Martín *et al.*, 2002). Therefore, wine-distillery wastewater can be problematic to treat and may constitute an environmental problem when discharged to surface wastewaters (Beltrán *et al.*, 2001). Investigation into suitable treatment methods for these types of wastewaters is essential.

Wetlands offer utilisation of natural processes, simple construction, simple operation, low maintenance, process stability, little excess sludge production and cost effectiveness (Haberl, 1999) and this is especially suitable for developing countries (Ayaz & Saygin, 1996). Wetlands reduce the chemical oxygen demand (COD) (Shephard, 1998; Mulidzi, 2005), remove chemical nutrients and reduce solids content (Meuleman & Verhoeven, 1999; Schutes, 2001). Wineries utilising wetlands to treat cellar effluent have reported better results with the inclusion of a pre-treatment system; therefore wetlands should be seen as a secondary treatment system (Mulidzi, 2005).

Complete cleansing of wastewater pollutants will not be feasible with the adoption of a single treatment process. Combinations of chemical and biological treatments are often the way to optimise the overall process. The first treatment, if properly chosen, will facilitate the second one, thus leading to a more effective treatment of the waste (Andreozzi *et al*., 1998).

Ozone offers many benefits as a pre-treatment due to its excellent oxidising capabilities. Ozone has many of the oxidising characteristics desired for wastewater treatments: it degrades organic compounds by oxidation; it is readily available; is soluble in water; and leaves no by-products that need to be removed (Acero *et al*., 1999). Ozone has been shown to decrease the COD content (Beltrán *et al*., 2001), decrease colour (Alfafara *et al.*, 2000), and decrease toxic polyphenols (Alvarez *et al*., 2001).

Ozone may therefore facilitate the use of a secondary biological treatment step, resulting in a quicker treatment time and achieving desired final effluent quality. Ozone has been shown to have a pronounced effect downstream in treatment of wastewaters by improving biodegradation (Gottschalk *et al.*, 2000). The efficiency of biological treatment processes can therefore be improved by combining it with a pre-ozonation treatment to reduce the toxicity of polyphenols in the wastewaters.

The objective of this study will be to investigate the impact of ozonation on wetland systems used for wine-distillery wastewater degradation. This will be done, firstly by investigating the effect of ozone on the composition of wine-distillery wastewater, and secondly, by monitoring the efficiency of the wetland process being used to treat wine-distillery wastewater when a pre- and/or post-ozonation treatment is included.

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CHAPTER 2

LITERATURE REVIEW

A. BACKGROUND TO WATER SHORTAGE IN SOUTH AFRICA

South Africa is a water-scarce country with an average annual rainfall of 450 mm per year (DWAF, 2004), which is well below the world average of 860 mm per year. Most of the country can be classified as semi-arid (Pescod, 1992). Few perennial rivers traverse the country and surface water sources are often polluted. Water shortages are, thus, a common occurrence in large parts of South Africa (Olivier & de Rautenbach, 2002). South Africa's water resources are therefore limited and it is essential that they be used as efficiently as possible.

Water use in South Africa is dominated by irrigation, which accounts for around 62% of all water used. Domestic and urban use accounts for about 27%, while mining, large industries and power generation account for some 8% (DWAF, 2004). Wastewater from food processing and agricultural industries generate large volumes of non-desirable waste with a negative environmental impact (Beltrán *et al*., 2000). These industries can no longer ignore growing environmental pressures from government, consumers, society at large, and most noticeably, their growing financial cost (Neall, 2000; Sigge & Britz, 2007).

Distilleries are an example of an agricultural industry that generates large volumes of wastewater. These wastewaters are heavily polluted, and this presents disposal problems. With the current emphasis in the media on water pollution issues, there is an increasing awareness of the need to dispose of wastewaters safely and beneficially (Pescod, 1992; Sigge & Britz, 2007).

B. BACKGROUND ON DISTILLING INDUSTRY IN SOUTH AFRICA

The wine industry in South Africa compromises a group of industrial operations involved mainly in the processing of grapes to a variety of alcoholic and non-alcoholic products. Around 1.3 million tons of grapes per annum were harvested in 2006, and the bulk of this was fermented to produce approximately 1 000 million litres of wine. Of this 15% will be distilled to spirit products (SAWIS, 2007). In 2005, producers income was a gross value of R2.6 billion and wine-industry related firms earned a further R23.7 billion (SAWIS, 2007).

Wine production in South Africa has increased over the past decade, and this growth places further pressure on our natural resources such as water, soil and vegetation. National legislation and international markets require the responsible management of potential environmental impact through effective systems (Van Schoor, 2000). Legislation that is consistent with the National Water Act (Anon, 2004), which emphasizes effective management of our water resources, is affecting wine farms as well as distilleries (Gilson, 2000).

Origins of distillery wastewater

Distillation is the process of converting a liquor to a vapour, condensing the vapour and collecting liquid or distillate. In the wine industry, distillation is used to separate mixtures of different liquids with different boiling points which become either neutral wine alcohol or brandy (Water Research Commission, 1993). The wine making process can roughly be divided into two phases namely, harvest season and off-season. The harvest season can vary from 6 - 20 weeks and during this time the grapes are harvested, pressed and the resulting juice fermented to wine. During the off-season other cellar activities take place such as stabilising, filtering, aging, blending and bottling of the wine (Van Schoor, 2000).

The distillery industry uses water extensively in their processing operations. Large volumes of water are required, mainly for cleaning and cooling purposes. This results in the generation of heavily polluted distillery wastewater (Martín *et al.*, 2002). In spirit distilleries, specific water intake values range from 1.8 to 6.2 litres per litre of alcohol produced, with most of the water being used for steam raising, cooling and floor and equipment wash down. Solid wastes generated by the industry arise largely from the skins and pips of the grape berries. Typically, one ton of harvested grapes generates 0.11 tons of solid waste (Water Research Commission, 1993). Due to the seasonal nature of the distilling industry, the amount and composition of the wastewater may exhibit major daily and seasonal variations (Bezuidenhout *et al.*, 2002).

Characteristics of distillery wastewater

Production of ethanol from wines by distillation processes often releases high strength acidic wastewater that presents significant disposal and treatment problems (Beltrán *et al*., 2000). The average characteristics of wine-distillery wastewater (WDWW) are summarised in Table 2-1. Wine-distillery wastewaters typically have a high suspended solids content and contain residual organic acids, soluble proteins, carbohydrates, as well as various inorganic compounds (Water Research Commission, 1993; Van Schoor, 2000). Vinasse is the name given to wastewater resulting from the production of ethyl alcohol (Benitez *et al.,* 1999). It has an acidic pH (3.5 - 5.0) and is characterised by a high organic content (sugars, alcohol, phenols, polyphenols and lipids) with a COD range of 10 000 - 60 000 $mg.L^{-1}$ (Benitez *et al.*, 1999; Martín *et al.*, 2002). These wastewaters generally have a brownish colour that is attributed to polymeric pigments, collectively known as melanoidins (Alfafara *et al*., 2000).

Solid wastes are produced which cause bad smells and can contaminate soil and water resources, which can lead to further negative impacts on plant growth and crop potential. Additionally, these solid wastes can lead to high mineral content in soil (Van Schoor, 2005).

Wastewaters need to be treated before they can be discharged from the factory, either as re-use for irrigation, or into rivers and sewage drains. The inclusion of a waste treatment system therefore has potential benefits and cost savings over the long run (Gilson, 2000). Cost savings could result from the re-use of water for irrigation, resulting in decreases in penalties from municipalities if wastewater meets required standards. Further potential benefits are a reduction in environmental impact and the creation of a good public image.

Wastewaters from the alcohol industry are particularly high in COD, because for each volume (%) of ethanol remaining in the wastewater, the COD is increased by 20 000 $mg.L^{-1}$ (Wilkie *et al*., 2000). Distillery wastewaters are chemically complex and contain a host of phenolic compounds, some of which resist biodegradation (Martín *et al.*, 2002). Therefore, wine-distillery wastewater can be problematic to treat and may constitute an environmental problem when discharged to surface wastewaters (Beltrán *et al.*, 2001b). Investigation into suitable treatment methods for theses types of wastewaters is essential.

Table 2-1 Characteristics of wine-distillery wastewater (Beltrán *et al*., 1993; Shepherd, 1998; Alfafara *et al*., 2000; Bezuidenhout *et al*., 2002; Martín *et al*., 2002; Dupla *et al*., 2004)

Parameter	Value
COD ($mg.L^{-1}$)	20 000 - 90 000
pH	3.5 - 5.6
Conductivity ($mS.m^{-1}$)	230
Total Polyphenols ($mg.L^{-1}$)	400
Total Suspended Solids ($mg.L^{-1}$)	18 800
Total Dissolved Solids ($mg.L^{-1}$)	11 400
Volatile Suspended Solids ($mg.L^{-1}$)	1 950
Volatile Fatty Acids ($mg.L^{-1}$)	5 500
Total Kjeldahl Nitrogen ($mg.L^{-1}$)	0.63 - 485

C. TREATMENT OPTIONS FOR DISTILLERY WASTEWATERS

Due to the highly diversified nature of the food industry, various food processing, handling and packaging operations create wastes of different quality and quantity, which if not treated, could lead to increasing disposal and pollution problems (Sigge, 2000). The principal objective of wastewater treatment is generally to allow human and industrial effluents to be disposed of without danger to human health or unacceptable damage to the natural environment (Pescod, 1992). The most appropriate wastewater treatment to be applied is that which will produce an effluent meeting the recommended microbiological and chemical quality guidelines, at a low cost and with minimal operational and maintenance requirements (Arar, 1988). Adopting as low a level of technological treatment as possible is especially desirable in developing countries, not only from the point of view of cost but also in acknowledgement of the difficulty of operating complex systems reliably (Pescod, 1992).

The design of wastewater treatment plants is usually based on the need to reduce organic and suspended solids loads to limit pollution of the environment (Hillman, 1988). The first step in the selection of any treatment process for improving water quality is to thoroughly define the problem and to determine what the treatment process is to achieve.

In most cases, either regulatory requirements or the desire to re-use the water will be the driving force in defining the treatment issues to be selected. A thorough knowledge and understanding of these water quality criteria is required prior to selecting any particular treatment process (FAO, 2004).

Wastewater treatments for wine distilleries vary in detail, but can broadly be divided into three groups, namely physical, biological and chemical.

Physical treatments

Certain physical processes are geared to remove suspended particulate matter. These processes might be used in an overall treatment process for the removal of particulates formed in other stages of the treatment, such as removal of bacteria from a biological system or removal of precipitates formed in a chemical treatment process. Particle removal processes may form part of a preliminary or primary step. These steps can consist of the removal of coarse solids by centrifuging, screening, sand filters or sedimentation (Pescod, 1992).

Beltrán *et al.* (2001a) reported a reduction in the COD of wine-distillery wastewater by using sedimentation tanks. Removal of settleable organic and inorganic solids by sedimentation has been shown to remove 25 to 50% of the incoming biochemical oxygen demand (BOD_5), 50 to 70% of the total suspended solids (TSS) and 65% of the oil and grease (Pescod, 1992). Zinkus *et al*. (1998) found that a sedimentation tank could remove TSS by 10 - 50%. Filtration further includes granular media beds, vacuum filters, belt and filter presses (FAO, 2004). For the wine industry, a sand filter has successfully been used (Shepherd *et al*., 2001a) as a pre-treatment to remove solids and reduce the COD of the wastewater.

Biological treatments

Biological treatments are one of the most effective ways to treat organic waste (Gilson, 2000) and can be used for the removal of both organic and inorganic contaminants (FAO, 2004). Biological treatment usually refers to the use of bacteria in engineered reactor systems for affecting the removal or change of certain constituents, such as organic compounds, trace elements and nutrients (Zinkus *et al*., 1998; FAO, 2004). Algae have also been used and natural wetlands systems can be used in some cases to replace conventional reactors. The bacterial reactions involved can be divided into two major categories according to the use of oxygen (O_2) by the bacteria. In aerobic systems, O_2 is provided and used by the bacteria to biochemically oxidise organic compounds to carbon dioxide and water, and possibly to oxidise reduced compounds before their release to the environment. In an aerobic system, oxygen is the electron acceptor and organic carbon sources are usually the electron donors in the biochemical reactions that take place. In an anaerobic system, oxygen is excluded and the bacteria utilise compounds other than molecular oxygen for the completion of metabolic processes (FAO, 2004).

Advantages of biological treatment are that it requires a much smaller land area than is normally used for physical treatment processes, and it may also minimise unpleasant odours. The disadvantages of biological treatment are that it is generally more costly and requires an operator with some technical training. Ponds may also become anaerobic if they are organically overloaded, and pH correction with lime may be required (Water Research Commission, 1993).

Aerobic treatments - Aerobic biological treatment is performed in the presence of oxygen by aerobic microorganisms (principally bacteria) that metabolise the organic matter in the wastewater, thereby producing more microorganisms and inorganic end-products (principally CO_2, NH_3, and H_2O). Several aerobic biological processes are used for secondary treatment, differing primarily in the manner in which oxygen is supplied to the microorganisms and in the rate at which organisms metabolise the organic matter (Pescod, 1992). Beltrán *et al*. (2000) reported that laboratory studies had shown that aerobic biological oxidation will not lead to a complete purification of wine-distillery wastewater. The wastewaters contain some recalcitrant compounds that are not easily biodegraded by microorganisms. Aerobic processes also generate significant quantities of bio-solids that require removal (Zinkus *et al*., 1998).

Activated sludge - Activated sludge is an example of an aerobic treatment process that constitutes the most common approach to achieve a successful treatment for agro-industrial wastewaters (Beltrán *et al*., 2000). The system relies on contact between the organic matter in the wastewater and high concentrations of microorganisms in the presence of dissolved oxygen (Nazaroff & Alvarez-Cohen, 2001). Benitez *et al*. (2003) reported a COD reduction of 85% for wine-distillery wastewater treated in an aerobic activated sludge system.

Irrigation - Irrigation is the preferred method for cellar wastewater disposal (Bezuidenhout *et al*., 2002). Irrigation of wastewaters is attractive for wineries and distilleries with available land and has the additional advantage that grazing can be developed. Kikuyu grass is reported to be suitable for irrigation by winery wastewaters (Water Research Commission, 1993). The purpose of wastewater irrigation should not merely be for disposal, but rather for the beneficial use of water to irrigate crops (Van

Schoor, 2005). Food industry wastewaters typically contain nutrients such as nitrogen and phosphorus (Pescod, 1992). An advantage of using these types of wastewater is that they could reduce or eliminate the requirements for commercial fertilizers and could therefore be utilised to grow crops. Problems may occur if wastewaters accumulate on irrigated land and unpleasant odours may result. Pre-treatment methods are usually necessary (Van Schoor, 2001), such as pH correction prior to irrigation and removal of solids (Van Schoor, 2005; Water Research Commission, 1993) in order to meet the required standards (Table 2-2).

Anaerobic digestion - Anaerobic digestion is used to degrade organic material in wastewater to methane (CH_4) and carbon dioxide (CO_2) (Nazaroff & Alvarez-Cohen, 2001). Four major groups of bacteria function in a synergistic relationship: hydrolytic, fermentative acidogenic, acetogenic and methanogenic (Bitton, 1999). Anaerobic digestion has successfully been used as a treatment process for wine-distillery wastewaters due to sufficient levels of nitrogen, phosphorous and trace elements that are necessary for metabolism being present naturally in the wastewaters (Water Research Commission, 1993; Benitez *et al*., 1999).

For incoming COD levels are in the order of 35 000 $mg.L^{-1}$, COD removals in excess of 90% have been reported for the upflow anaerobic sludge blanket (UASB) technology (Wolmarans & de Villiers, 2002). COD reductions of 93% at an organic loading rate (OLR) of 11.05 kg $COD.m^{-3}.d^{-1}$ and a hydraulic retention time of 14 h were achieved treating a winery wastewater (Ronquest & Britz, 1999). Driessen *et al*. (1994) reported COD reductions of more than 90% at an OLR of 15 kg $COD.m^{-3}.d^{-1}$. McLachlan (2004) treated winery effluent in an UASB reactor, and reported a reduction of 84% at an OLR of 9.75 kg $COD.m^{-3}.d^{-1}$.

In many instances the COD level of the final effluent was still well above the South African legal limit permitted for wastewaters (75 $mg.L^{-1}$) to be directly discharged into a water system (Table 2-2) and a further post-treatment is thus necessary. Anaerobic processes also generate gas such as CH_4, CO_2 and hydrogen sulphide, and these also need to be removed. The CH_4 can, however, be used as an energy source to produce steam and thus reduce the costs of wastewater treatment (Zinkus *et al*., 1998).

Table 2-2 Legislative requirements of wastewater characteristics when utilised for discharge in irrigation or river systems (Bezuidenhout *et al*., 2002; Anon, 2004; Van Schoor, 2005)

Parameter	2000 $m^3.d^{-1}$	500 $m^3.d^{-1}$	50 $m^3.d^{-1}$
COD ($mg.L^{-1}$)	< 75	< 400	< 5000
Electro-conductivity ($mS.m^{-1}$)	< 150	< 200	< 200
pH	5.5 – 9.5	6.0 – 9.0	6.0 – 9.0
Suspended solids ($mg.L^{-1}$)	< 25		
Faecal coliforms (100 mL)	1 000	100 000	100 000
Sodium adsorption ratio	< 5	< 5	< 5

Constructed Wetlands

Wastewaters from intensive agricultural activities typically have significantly higher concentrations of organic matter and nutrients than that treated as municipal effluent. The high pollution loads which are generated pose particular problems and challenges for the industry, if high concentrations of nutrients are allowed to discharge directly to receiving waters. Agricultural wastes must be treated prior to disposal and constructed wetlands (CWs) have been suggested as a potential treatment option prior to land application (Geary & Moore, 1999). Constructed wetlands are natural wastewater treatment systems that combine biological, chemical and physical treatment processes (Crites, 1994). They were initially developed about 40 years ago in Europe and North America to exploit and improve the biodegradation ability of plants (Schutes, 2001). Constructed wetlands are normally used for polishing semi-treated effluents, but the main purpose of CWs is to artificially recreate the filtering capacity of natural wetlands.

Advantages of constructed wetlands - Wetland systems have the following advantages compared with conventional wastewater treatment options: utilisation of natural processes; simple construction; simple operation - they can be established and operated by untrained personnel; lower operating costs; low maintenance requirements; they are robust and stable - being able to withstand a wide range of operating conditions; little excess sludge production; they are environmentally acceptable; and offer considerable potential for conservation of wildlife (Water Research Commission, 1993; Haberl, 1999).

Wetlands are suitable for both small communities and as a final stage treatment in large municipal systems or for industrial and agricultural effluents (Akça & Ayaz, 2000). They can effectively be used as a sewerage system for single houses or small communities, lowering the initial costs by using cheap materials and allowing self-construction, developing a pathogenically safe, as well as aesthetic treatment unit that combines water treatment with hobby garden activities and reuse possibilities, such as for toilet flushing (Ayaz & Akça, 2000 and 2001; Schutes, 2001).

Disadvantages of constructed wetlands - Disadvantages of wetland systems are mainly: their relatively slow rate of operation in comparison to conventional wastewater treatment technology, and the fact that such systems require a large land area. Area requirements will depend on different configurations and different treatment purposes (BOD removal, nitrification, etc.) needed (Ayaz & Akça, 2000). Overloading, surface flooding and media clogging of the subsurface systems are common occurrences in CWs, and this can result in stagnant water and therefore a reduced efficiency (De Gueldre *et al*., 2000; Schutes, 2001). Another weak aspect of wetlands is the periodic washout of suspended solids (Genenens & Thoeye, 2000). Solids washout can increase the COD of the final effluent and falsely indicate poor COD removal rates (Wolmarans & de Villiers, 2002).

Design of a constructed wetland - Typically, a constructed wetland consists of a shallow, lined excavation containing a bed of porous soil, gravel or ash, in which emergent aquatic vegetation is planted. The depth of the bed is generally 0.6 m and is constructed with a peripheral embankment, at least 0.5 m higher than the bed surface, to contain the build-up of decaying vegetation and influent solids (Water Research Commission, 1993).

Wetland designs include a horizontal surface in which the wastewater flows horizontally over the wetland sediment, subsurface flow, vertical flow or infiltration wetlands, in which the wastewater flows vertically through a highly permeable sediment and is collected in drains, and floating raft systems (Meuleman & Verhoeven, 1999). Surface flow wetlands are similar to natural marshes as they tend to occupy shallow channels and basins through which water flows at low velocities above and within the gravel substrate. The basins normally contain a combination of gravel, clay or peat-based soils and crushed rock, planted with macrophytes. In subsurface flow wetlands, wastewater flows horizontally or vertically through the substrate, which is composed of soil, sand, rock or artificial media (Meuleman & Verhoeven, 1999; Schutes, 2001). A subsurface flow wetland consists of channels or basins that contain gravel or sand media which will support the growth of emergent vegetation. The bed of impermeable material is typically sloped between 0 and 2 %. Wastewater flows horizontally through the root zone of the wetland plants and the treated effluent is collected in an outlet channel or pipe (Crites, 1994).

Well sorted gravel is desirable to minimise clogging due to settling of fine material in the pore matrix (Sheperd *et al*., 2001a). For wastewaters with a high COD (eg. cellar wastewaters), coarse gravel must be used, since turbulence may increase the oxygen content of the wastewater. With a lower pH (< 5), limestone or dolomitic lime may be used for pH correction (Van Schoor, 2002).

Plants are chosen for their root length and pH tolerance (Sheperd *et al*., 2001a). Due to the seasonal variation in wine-distillery wastewater, a wide pH tolerance is necessary. In practice mainly cattails (*Scirpus*) and bullrushes (*Typha*), with a plant density of approximately 4 plants.m^{-2}, are used (Van Schoor, 2002). Meuleman & Verhoeven (1999) also recommend helophytes such as *Phragmites* and *Scirpus* spp. as suitable for wetland growth.

Basic function of constructed wetlands - The wastewater fed to a wetland has usually received only a primary filtration through coarse material, but there are also a number of cases in which wetlands are used for final polishing of the effluent from a conventional purification plant. Wetland ecosystems have special characteristics which make them particularly suitable for wastewater purification. They are semi-aquatic systems which normally contain large quantities of water. The flooding caused by wastewater addition is a normal feature of the system; organic matter breakdown takes place through special pathways involving electron acceptors other than oxygen, e.g. nitrate, sulphate and iron. They support highly productive, tall emergent vegetation capable of taking up large amounts of nutrients and responding to enrichment with nutrients with enhanced growth. The helophytes also aerate the soil rhizosphere through aerenchyma in the roots (Meuleman & Verhoeven, 1999; Guimarães *et al.*, 2001; Maestri *et al.*, 2003).

The wastewaters are often mixed with surface water or purified effluent and generally flow through the system with a minimum residence time of a few days. The purification processes include: settlement of suspended solids, diffusion of dissolved nutrients into the sediment, mineralisation of organic material, nutrient uptake by microorganisms and vegetation, microbial transformations into gaseous components, physicochemical adsorption and precipitation in the sediment (Meuleman & Verhoeven, 1999).

The ability of large aquatic plants (macrophytes) in treatment wetlands to assist the breakdown of human and animal derived wastewater, remove disease-causing microorganisms and pollutants has been well-documented by several researchers (Armstrong *et al*., 1990; Burka & Lawrence, 1990; Wood, 1995; Kadlec & Knight, 1996; Brix, 1997). The plants in wetlands are adapted for growing in water-saturated soils. The aesthetic value of the macrophytes can also play a role in acceptability of wetland systems. It is possible to select pleasant looking wetland plants like the Yellow Flag (*Pseudacorus*) or Canna-lilies, and in this way makes CW treatment systems aesthetically pleasing (Brix, 1994). Constructed wetlands can be designed to form an aesthetically pleasing and functional landscape which can be incorporated into residential developments. In addition, they provide a valuable ecological habitat for wildlife (Schutes, 2001).

The wetland plants have many functions related to the treatment of wastewater in CWs (Brix, 1994). These numerous functions include: utilisation of the nutrients, oxygen transfer to the solid medium and support medium for the biofilm on the roots and rhizomes (Guimarães *et al.*, 2001). The purification process occurs during contact with the surface of the media and plant rhizospheres (Schutes, 2001).

The sub-surface plant tissues grow horizontally and vertically and create an extensive matrix which binds the soil particles and creates a large surface area for the uptake of nutrients and ions (Schutes, 2001). Hollow vessels in the plant tissue enable air to move from the leaves to the roots and to the surrounding soil. Aerobic microorganisms flourish in a thin zone (rhizosphere) around the roots and anaerobic microorganisms are present in the underlying soil (Schutes, 2001). Plant root systems are essential as substrates for the development of associations with microorganisms involved in depuration processes (Tanner *et al*., 1995a; Tanner *et al*., 1995b). In this regard, a particular relevance can be attributed to mycorrhizae, mutualistic associations between soil fungi and plant roots, which can be seen in several terrestrial and aquatic plant species (Maestri *et al.*, 2003).

During a study by Guimarães *et al*. (2001) it was found that plants themselves did not contribute significantly to COD reduction when compared to a control wetland without plants (83% vs 79%). Although the plants are the most obvious components of the wetland ecosystem, wastewater treatment is accomplished through an integrated combination of biological, physical, and chemical interactions among the plants, the substrate, and the inherent microbial community (Kadlec & Knight, 1996). In CWs the removal of contaminants and nutrients by the plants is small when compared with those removed by biochemical and photochemical processes in the gravel substrate (Maestri *et al.*, 2003). Maestri *et al.* (2003) reported that absorption by plants was not the main route through which the contaminants were removed or transformed, but that the presence of plants was fundamental for establishing a heterogeneous environment in which chemical or photochemical processes could proceed. Natural filtration in the substrate also assists removal of many pollutants and pathogenic microorganisms (Schutes, 2001).

Factors influencing efficiency - The lifespan of constructed wetlands has been demonstrated as being approximately 20 years for organic waste treatment (Schutes, 2001). The performance of these systems is influenced by their area, length to width ratio, water depth, rate of wastewater loading and the hydraulic retention time (HRT) (Schutes, 2001). Subsurface flow systems are more effective than surface flow systems at removing pollutants at high application rates. However, overloading, surface flooding and media clogging of the subsurface systems can result in a reduced efficiency (Schutes, 2001). Masi *et al.* (2002) noticed that performance increased with a higher retention time. However, an increased HRT may also result in anaerobic conditions and this could be detrimental to plant growth.

Applications of constructed wetlands to food wastes - Maestri *et al.* (2003) investigated the use of CWs to treat rural domestic and dairy parlour effluent. Although the organic load and nutrient contents of influent wastewaters were higher than those of typical domestic wastewater, the high removal efficiency of the wetlands allowed the discharge of effluents into surface waters. The results for removal efficiency obtained for main parameters (COD 91%, nitrogen 50%, phosphorus 60%, total suspended solids 90%, fecal coliforms 99%) were consistent with other experiments reported in the literature (Hunt & Poach, 2001; Olivie-Lauquet *et al*., 2001).

COD reduction - Wetlands generally perform well for reductions in COD, BOD and bacterial pollution, but show limited capacity for nutrient removal. The high removal rates for COD and BOD are caused by sedimentation of suspended solids and by rapid decomposition processes in the water and upper soil layers. Significant reductions in BOD are primarily due to the additional detention provided by further storage and the presence of plants assisting with sedimentation and filtration (Geary & Moore, 1999). As nutrient removal is often also an important objective, knowledge of the various nutrient removal processes and the conditions in which they operate optimally is a prerequisite for enhancement of the nutrient removal function (Geary & Moore, 1999; Meuleman & Verhoeven, 1999).

Research which examined the performance of constructed wetland systems for the treatment of dairy wastewaters showed that significant improvements in effluent quality may be achieved due to the physical, chemical and biological processes which occur in wetland systems (Geary & Moore, 1999). Geary & Moore (1999) reported findings for a wetland constructed at the Tocal Agricultural College, UK, to manage their dairy waste management systems more effectively. The effluent quality was typical for a dairy, with high concentrations of organic matter and nutrients. Reductions in BOD were observed at 61%, which compared favourably to the 68% reported by Kadlec & Knight (1996) for dairy wetlands.

A study of small wastewater treatment plants in Belgium showed that the removal efficiency of organic pollutants was high (COD removal of 89%) in vertical reed beds (De Gueldre *et al*., 2000). At high loading rates wetlands appear to act more as a sink for the pollutants which are removed from the wastewater, initially showing high removal rates, and then as the wetlands became saturated, the nutrients are leached out. Crites (1994) concluded that a treatment wetland would not be suitable as a long term option for dairy waste due to the wetland eventually becoming saturated and no longer showing high removal rates for nutrients.

Mashauri *et al*. (2003) found that wetland systems reduced BOD_5 and total nitrogen (TN) loads by 4.039 $g.m^{-2}.d^{-1}$, which was 82.2% of the influent load, and 0.823 g $N.m^{-2}.d^{-1}$ (56.2% of influent load), respectively. According to the USEPA (1993), CWs can effectively remove 60 - 90% of organic carbon. Kemp & George (1997) reported 73% mass removal of influent BOD_5 (0.442 $g.m^{-2}$ $.d^{-1}$) in their study while using a subsurface flow CW to treat municipal wastewater.

Guimarães *et al*. (2001) found that the applied hydraulic load had very little influence on the COD removal efficiency of a CW. De Gueldre *et al*. (2000) however, found that when the influent flow rate was increased, a stagnant water layer containing sludge formed on top of the gravel bed and caused a drop in removal efficiencies. The high BOD loading of the wastewater can quickly consume available oxygen and can create an anaerobic environment which has negative effects on the wetland (Geary & Moore, 1999).

Nutrient removal - Wetlands generally perform well for COD, BOD and bacterial pollution, but show limited capacity for nutrient removal (Meuleman & Verhoeven, 1999). Nutrient removal efficiency for a CW is normally below 60% (Schutes, 2001). Newman *et al*. (2000) concluded that a CW would not be able to meet operating standards over the long term. The vegetation itself functions as a temporary storage for nutrients (Meuleman & Verhoeven, 1999). At the start of the growing season, large quantities of nutrients are taken up by the root system. If the vegetation is not harvested, most of these nutrients end up in the dead plant matter (Brix, 1994). Therefore storage in accumulating organic matter is another sustainable mechanism of removing nutrients from the wastewater (Meuleman & Verhoeven, 1999).

In autumn and winter, a large part of the nutrients will be gradually released again through leaching and organic matter mineralization. Only a small part of the nutrients taken up stays in the vegetation as additional long-term storage in woody stems or rhizome material (Brix, 1994). If the vegetation is harvested, the amounts of nutrients released in autumn and winter is substantially lower. Harvesting the vegetation in late summer, before retranslocation of nutrients to the root system occurs, can substantially contribute to the nutrient removal capacity of a wetland (Brix, 1994; Kadlec & Knight, 1996).

The microbes decaying the plant matter may also take up large amounts of nutrients from the aqueous environment (immobilisation), which will be released several months, or even years later. In most wetlands, part of the organic matter is broken down at such a slow rate that it accumulates as organic matter in soil. The accumulation of nutrients in organic matter forms a significant removal process in many wastewater wetlands (Verhoeven & Van der Toorn, 1990).

The processes leading to nitrogen (N) removal are mostly through bacterial transformations. Nitrification is the oxidation of ammonium to nitrate by nitrifying bacteria. This process is only operational under aerobic conditions. Denitrification is an anaerobic decomposition process in which organic matter is broken down by bacteria using nitrate instead of oxygen as an electron acceptor (Meuleman & Verhoeven, 1999). The process occurs in two steps: first nitrate is reduced to nitrous oxide, which is subsequently further reduced to atmospheric N. Both end-products are gases which are emitted into the atmosphere. Nitrous oxide is a greenhouse gas and excessive emissions may contribute to the global warming problem. At low pH, the second step of denitrification is inhibited, so that all N is released in the form of nitrous oxide. From an environmental quality perspective, the pH of wastewater wetland soils should therefore remain above 6.0, so that a large percentage of the N denitrified will leave the wetland as atmospheric N (Meuleman

& Verhoeven, 1999). In contrast, Guimarães *et al.* (2001) found no significant pH increases in their use of a CW to treat sewage.

As the N in wastewater is mostly in a reduced state, for a removal into gaseous compounds, nitrification as well as denitrification has to occur (Meuleman & Verhoeven, 1999). In many wetlands, nitrification rates are much slower than denitrification rates, so that the first process determines the actual rates of the second proces. This means that aerobic as well as anaerobic conditions are needed for optimisation of the denitrification process. This can be achieved by using large emergent plants which aerate the soil through leakage of oxygen from their root aerynchyma, such as *Phragmites australis* (Reddy *et al*., 1989; Brix, 1994). Another possibility is to install a water regime of alternating flooded and dry conditions, e.g. a cycle of 2 - 3 days of flooding followed by 4 - 6 days of dry conditions (Meuleman & Verhoeven, 1999).

The major pathway of organic nitrogen removal in a subsurface CW is by sedimentation due to settling or filtration (Newman *et al*., 2000; Mashauri *et al*., 2003). An increase of NH_3-N sometimes occurs due to mineralisation of algae in the subsurface flow CW. Wetlands effectively remove algae in wastewater and subsequently decomposition of those algae produces additional ammonia. This ammonia is not easily nitrified due to insufficient dissolved oxygen occurring in wetlands (Mashauri *et al*., 2003).

Nitrogen removal in a trial wetland with macrophytes showed a total nitrogen removal efficiency of between 59 and 87% (Guimarães *et al.*, 2001). A total nitrogen removal efficiency of 30 - 98% has also been reported (Bastian & Hammer, 1993). This can be attributed to assimilation by microorganisms and macrophytes present in the system and nitrification due to transport of oxygen by the plants (Guimarães *et al.*, 2001). However, it was demonstrated that the amount of oxygen being released by the plants to the immediate environment around the roots is limited (Armstrong *et al*., 1990; Brix, 1994). The limited aeration around the roots ensures that anaerobic conditions will predominate, unless the organic load to the wetland is low and that the wetland is shallow (Ayaz & Akça, 2001).

Phosphorus (P) removal in CWs was found to be high and then to decrease over time (De Gueldre *et al*., 2000). The question of phosphorus removal in a constructed wetland system is therefore problematic. Once the capacity of the soil with respect to adsorption is reached, the system commences to leach phosphorus (De Gueldre *et al*., 2000). The wetland plants only absorb phosphorus needed for growth from the effluent (Geary & Moore, 1999). Guimarães *et al*. (2001) also found phosphorus removal in a CW to be 100% initially and then to decline after 7 months, possibly due to saturation of the sand medium. Adsorption of phosphates to soil particles is an important removal process. The adsorption capacity is dependent on the presence of iron, aluminium or calcium bound to the soil organic matter. Under aerobic, neutral to acidic circumstances, Fe(III) binds phosphates in stable complexes. If the soil turns anaerobic as a result of flooding, Fe(III) will be reduced to Fe(II), which leads to weaker adsorption and therefore release of phosphates (Faulkner & Richardson, 1989). Adsorption of phosphates to calcium only occurs under basic to neutral conditions and therefore this adsorption is reversible by changing pH conditions.

Adsorption is also subject to saturation. Each soil type has only a certain adsorption capacity and as soon as all adsorption sites are occupied, no further adsorption can occur (Kadlec, 1995). Apart from these fast adsorption–desorption processes, phosphates can also be precipitated with iron, aluminium and soil compounds (Nichols, 1983). These processes, which include fixation of phosphate in the matrix of clay minerals and complexation of phosphates with metals, have a much slower rate but are not so easily subject to saturation. If previously adsorbed P is precipitated, the adsorption sites become available again for adsorption of new P (Meuleman & Verhoeven, 1999).

Alkalinity - Alkalinity increases in wetlands over time, possibly due to the elimination (oxidation) of part of the volatile fatty acids and ammonification of organic nitrogen (Guimarães *et al.*, 2001). Therefore the base gravel used in the wetland must be chosen so that carbonates will not leach and contribute to increased alkalinity levels.

Solids removal - High removal of total suspended solids (TSS) has been reported for constructed wetlands. For the removal of organic matter and suspended solids, an efficiency above 80% may be expected (Schutes, 2001). Wetlands have also been reported to be ineffective in the removal of total dissolved solids (TDS) (Sheperd *et al*., 2001a; Mulidzi, 2005). Mean TSS concentrations in dairy wastewaters were reduced by 90% in a study conducted by Newman *et al.* (2000). Masi *et al*. (2000) reported an average TSS removal of 89.1 and 74.7% from two wetlands (a single stage horizontal subsurface flow wetland and vertical flow system followed by a surface flow wetland) monitored treating winery wastewater. Mashauri *et al*. (2003) found that using a wetland system after a primary facultative pond reduced the TSS by 92.5% of influent TSS (9.73 $g.m^{-2}.d^{-1}$), and a further wetland system after a maturation pond reduced the TSS by 89.3% of influent TSS (9.65 $g.m^{-2}.d^{-1}$). In their experiment using a subsurface flow CW of 36 m^2, Kemp & George (1997) reported 84% removal of influent TSS (0.41 $m^{-2}.d^{-1}$).

A good correlation between turbidity and TSS was also found by Mashauri *et al*. (2003). The turbidity decreased by 82.4% after the primary pond and 74.6% after the maturation pond. It has also been seen that an aeration pre-treatment reduces TSS, resulting in greater wetland efficiency and a better quality effluent (Mulidzi, 2005). The decrease in TSS in wetlands can be attributed to the settling of particles due to low water velocities and due to vegetation trapping particles (Kadlec & Knight, 1996). Sheperd *et al*. (2001a and 2001b) reported a direct relationship between TSS reduction and the depth and distance of the CW from the inlet pipe. It seemed that most of the reduction occurred in the first half of the wetland, and that the TSS reduction decreased as the depth of the wetland decreased. Sheperd *et al*. (2001b) used the data to create prediction models for COD removal based on the design parameters of the CW itself, allowing for more suitable wetlands to be built.

Microorganisms - Wetland systems have been shown to significantly remove faecal coliforms (FC). For the removal of disease-causing microorganisms, efficiency above 90% is normally achieved (Schutes, 2001). Mashauri *et al*. (2003) found a reduction of 99.96%, reducing FC from 1.7×10^7 to 7.35×10^3 cfu.100 mL^{-1}, and in another system a reduction of 1.2×10^3 to 131 cfu.100 ml^{-1}, amounting to 89.45% reduction. Newman *et al.* (2000) found similar FC reductions (98%) for wetlands treating dairy milkhouse wastewater. Although these systems gave a high reduction of FC, the effluent still did not meet WHO/FAO standards, which require effluents of < 1000 cfu.100 mL^{-1} (Mashauri *et al*., 2003). The reduction of FC in wetland systems has been attributed to a combination of physical entrapment, filtration, sedimentation and exposure to UV radiation (USEPA, 1993).

Polyphenol reductions - CWs have a potential for removing toxic substances such as phenols from wastewater (Kadlec & Knight, 1996). Newman *et al*. (2000) reported a total polyphenol reduction of 45% for wetland treatment of dairy wastewater. The low removal rate was attributed to the lack of a pre-treatment process. Cooper *et al*. (2005) found a higher removal rate of 77% when treating a paper mill effluent. The reduction was attributed to biodegradation, adsorbtion, plant uptake and volatilisation. However, plant uptake proved to play only a small part when compared to a control wetland without plants. A difference of 4% was found in reduction rates.

Treatment of winery wastewater using constructed wetlands - There are few studies available in the literature on the treatment of this type of wastewater using CWs, probably due to the fact that winery wastewater may contain up to 500 times the organic load when compared to municipal wastewater (Marais, 2001). Shepherd (1998) found that CWs lowered COD, TSS and neutralised pH for winery wastewater. Shepherd (1998) reported a COD inlet of approximately 5 000 $mg.L^{-1}$, which resulted in a COD reduction of 90% when combined with a coarse sand filtering step. Masi *et al*. (2000) monitored three wetlands treating winery wastewaters at three different wineries and found COD reductions of over 90% for all three. The three wetlands also showed high reduction for TSS, total nitrogen and phosphorus. Shepherd *et al*. (2001a) demonstrated the effectiveness of a wetland for treating winery wastewater by using a sand filter as a pre-treatment. This resulted in a COD reduction of inlet COD of 4 850 $mg.L^{-1}$ by 98%. The pH remained between 5 to 7, and TSS was reduced by more than 90%. The studies showed that wetlands were tolerant to variable COD concentrations and produced a consistent effluent quality. The low TN present in the winery wastewater (65 $mg.L^{-1}$) meant that the wetland plants absorbed almost all of it. The final conclusion was that wetlands could handle a higher loading rate than Shepherd had first hypothesised, and that CWs were capable of handling fluctuating water quality without sacrificing good treatment.

In California, USA, a number of wetlands were successfully used to treat winery wastewater (Mulidzi, 2005). Aerators played an important role in pre-treatment of the effluent. The COD of the effluent treated by the aerators was 4 433 $mg.L^{-1}$, and then reduced to 631 $mg.L^{-1}$ before treatment by the wetlands. The final COD from the wetlands was 106 $mg.L^{-1}$.

Chemical treatments

Physico-chemical wastewater treatment systems are often considered as an appropriate alternative or can be applied as an additional treatment to a biological treatment system (Kayser, 1996). When the presence of strong organic compounds in the wastewater will disturb the biological treatment process, such as anaerobic digestion or wetland systems, a physico-chemical step has to be applied as a pre-treatment process (Wang *et al*., 1989). Chemical treatments can also form part of a tertiary treatment step when individual treatment processes are necessary to remove specific wastewater constituents such as nitrogen, phosphorus, additional suspended solids, refractory organics, heavy metals and dissolved solids (Pescod, 1992).

Advanced oxidation processes (AOPs) have been defined as processes which involve the generation of hydroxyl radicals in sufficient quantity to effect water purification by breaking down organic compounds (Gottschalk *et al*., 2000). The hydroxyl radical has a high oxidation potential (2.8 eV) and attacks organic molecules by either abstracting a hydrogen atom or by attacking double bonds. Organic molecules are thus mineralised to non-toxic forms such as carbon dioxide or water (Gulyas *et al*., 1995).

Advanced oxidation processes are commonly based on either the use of hydrogen peroxide (H_2O_2) or ozone in combination with ultraviolet (UV) light to cause radical formation (FAO, 2004). Another type of AOP uses photoactive metal catalysts and UV light to generate the radicals (Suri *et al.,* 1993). Their high cost is the primary disadvantage of most AOPs.

The use of AOPs in the treatment of wastewaters is becoming more commonplace. Wastewaters can be treated chemically to improve biodegradability or simply to reduce their organic and inorganic content, such as for example with UV light, ozone and hydrogen peroxide (Beltrán *et al*., 1997a; Martín *et al*., 2002.). Chemical oxidation may break molecules into small, more biodegradable fragments (Martín *et al*., 2002.). Some organic compounds react rapidly with ozone – leading to destruction, while others are only partially oxidised (Gulyas *et al*., 1995), leading to increased biodegradability. This multiple effect of ozone makes it a highly desirable and readily available form of treatment for wastewaters.

Ozone

Ozone (O_3) is the highly unstable triatomic oxygen molecule that is formed by the addition of an oxygen atom to molecular diatomic oxygen (O_2). Ozone is a strong oxidant that undergoes self-decomposition in water and releases hydroxyl free radicals that have a stronger oxidising capability than ozone (Sotelo *et al*., 1987). Its effectiveness is based upon the multiple effects produced by the oxidative and disinfective activity of ozone and ozone-derived oxidative species (Gottschalk *et al*., 2000). Two of the strongest chemical oxidants are ozone (2.07 eV) and hydroxyl radicals (2.80 eV). Ozone can react directly with a compound or it can produce hydroxyl radicals which then react with a compound (Gottschalk *et al*., 2000).

The potential of ozone for water and wastewater treatment has received increasing attention in recent years and its applications has increased enormously in diversity since the first scale application of ozone for the disinfection of drinking water in Nice in 1906 (Gottschalk *et al*., 2000). The United States Food and Drug Administration (USFDA) granted “Generally Recognised As Safe” (GRAS) status to ozone for use in bottled water in 1982 (Guzel-Seydim *et al*., 2003). After reviewing the worldwide database on ozone, an expert panel from the USFDA in 1997 decreed that ozone was a GRAS substance for use as a disinfectant or sanitiser in foods when used in accordance with good manufacturing practices (Guzel-Seydim *et al*., 2003; USDA, 1997).

Frequently identified by-products from the ozonation of complex organic substances contained in drinking or wastewater are: aldehydes, carboxylic acids and other aliphatic, aromatic or mixed oxidised forms. Such substances are often easily biodegradable and show no significant side effects (Gottschalk *et al*., 2000).

Ozonation is used for the treatment and purification of drinking waters, as well as domestic and industrial wastewaters but it also has a high potential as a pre-treatment method (Gottschalk *et al*., 2000). A characteristic of O_3 is that it is rather selective towards double bonds (Andreozzi *et al*., 1998). Theoretically, it should leave intact the proteins and sugars, which are biodegradable, and attack selectively the double bonds of unsaturated fatty acids and phenols, which resist biodegradation. In this way the total COD may not be significantly changed, because the toxic compounds are usually present in minor concentrations, and biomass potential for biological treatments would not be lost (Andreozzi *et al*., 1998).

Ozone’s multifunctionality thus makes it a promising application for wastewater treatment (Guzel-Seydim *et al*., 2003). However, ozone treatments may increase treatment costs (Baig & Liechti, 2001), and a thorough analysis is needed to determine if ozone is suitable for a specific treatment method and goal. It has been successfully integrated into production processes that utilise its oxidising potential (Gottschalk *et al*., 2000).

Ozone generation - Ozone is an unstable gas which has to be generated at the point of application. In order to generate ozone, a diatomic oxygen molecule must first be split (Rice *et al*., 1981). Ozone is generated commercially by passing oxygen molecules (O_2) through an electrical charge (Bever *et al*., 2004). This process is known as the corona discharge method (Rice *et al*., 1997). Thus, molecular oxygen is split into two atoms of oxygen which are highly reactive free radical moieties. When a free oxygen atom (O^-) encounters molecular oxygen (O_2), it combines to form the highly unstable ozone molecule (O_3). Because ozone is unstable, it rapidly degrades back to molecular oxygen (O_2) with the released free oxygen atom (O^-) combining with another free oxygen atom (O^-) to form molecular oxygen (O_2) or combining with other chemical moieties to cause oxidation. Upon release of the third oxygen atom, ozone acts as a strong oxidizing agent (Bever *et al*., 2004). If air is passed through the generator as a feed gas, 1 - 4% ozone can be produced; however, using pure oxygen allow yields to reach up to 12% ozone (Rice *et al*., 1997).

Ozone advantages and disadvantages - Ozone has many potential advantages for use in wastewater treatment systems. It can allow re-use of wastewater by lowering BOD and COD concentrations of food wastes (Guzel-Seydim *et al*., 2003). Ozone has a number of advantages over conventional technologies, including potential for mineralisation of wastewater constituents, rapid reaction rates, and application too intermittent flows (Duff *et al*., 2002).

Ozone has many of the oxidising characteristics desired for wastewater treatments - it degrades organic compounds by oxidation, it is readily available, is soluble in water and leaves no by-products that need to be removed (Acero *et al*., 1999). In many cases, much of the ozone fed is depleted in the following reaction which shows the high efficiencies of these advanced oxidation processes: $COD + O_3 \rightarrow CO_2 + H_2O$ (Acero *et al*., 1999; Beltrán *et al*., 1997b)

However, ozone application to wastewater has been limited due to excessive ozone consumption for the degradation of compounds of less concern, for example high molecular weight COD (Duff *et al*., 2002). This leads to elevated requirements for oxidants or reductions in the removal of trace organics during adsorption and oxidation. A high ozone utilisation rate is therefore crucial for practical applications.

One way to achieve a high ozone utilisation rate is to apply ozone to the wastewater in as efficiently a manner as possible (Boncz *et al*., 2003). Another way to ensure high ozone utilisation rate is to control the extent of the pre-ozonation contacting process. Efficiency can be monitored by following the degradation of phenolic compounds and the ozone gas outlet concentration. Therefore, excessive ozonation can be avoided, resulting in lower operation cost (Chen *et al*., 2004).

Use of ozone in wastewater treatment and controlling factors - Wine-distillery wastewater is characterised by a high organic content (sugars, alcohols, phenols and polyphenols, lipids) with a high COD value. Frequently these effluents are disposed of through public sewers or evaporation ponds, which cause bad smells and the possibility of pollution of surface waters and underground aquifers (Acero *et al*., 1999). Wastewaters may contain natural organic matter, but concentrations can vary greatly. Organic matter is a direct quality problem due to its colour and odour, and also causes indirect problems, such as bacterial growth and hindering particle separation (Gottschalk *et al*., 2000).

Generally the main areas where ozone is used for treatment of wastewaters are disinfection, oxidation of inorganic compounds, and oxidation of organic compounds, including taste, odour, colour removal and particle removal (Gottschalk *et al*., 2000). The objectives of ozonating organic matter in wastewaters are: removal of colour and thereby decreasing UV-absorbance, increasing the biodegradability of the wastewater, reduction of potential by-product formation - including tri-halomethanes, and direct reduction of organic carbon levels by mineralisation (Gottschalk *et al*., 2000). Ozone is also a powerful antimicrobial substance due to its high potential oxidising capacity (Crawford & Cline, 1990; Guzel-Seydim *et al*., 2004). It has been shown to have a pronounced effect downstream in a treatment sequence, and improving the biodegradation of dissolved organic and inorganic substances (Gottschalk *et al*., 2000).

COD - The COD contents of several food processing wastewaters have been successfully degraded using ozone, or ozone in combination with other factors (Beltran-Heredia *et a*l., 2001; Duff *et al*., 2002; Chen *et al*., 2004). Sigge *et al*. (2002) reported that increasing the ozonation time led to an increase in COD removal from 30% after 5 min, to 55% after 30 min. However, the increase in COD reduction over time was not linear, this being ascribed to the fact that some organic compounds are more susceptible to oxidation than others. This indicated that effluents contain a considerable amount of compounds which are difficult to oxidise. Pratt *et al*. (1990) and Hostachy *et al*. (1997) both found that a higher ozone dose resulted in a higher COD reduction.

Colour - The removal of colour and reduction of UV-absorbance is one of the easier tasks due to quick reactions and low ozone consumptions. Colour can be reduced by 90% or more, while UV-absorbance at 254 nm is commonly reduced to 20 - 50% of the initial value (Gottschalk *et al*., 2000). The reaction mechanism here is primarily the direct ozone attack on C-double bonds in aromatic and chromophoric molecules leading to the formation of ‘bleached’ products, like aliphatic acids, ketones and aldehydes (Gottschalk *et al*., 2000). Alfafara *et al.* (2000) measured colour absorbance at 475 nm and found a reduction of 80% for distillery wastewater which was pre-ozonated by bubbling ozone in a reactor vessel at a rate of 50 mg $O_3.h^{-1}$.

Polyphenol toxicity - Biological oxidation is usually the process through which most of the organic load is removed is wastewater. However, wastewater subjected to biological oxidation should be free of substances resistant to biodegradation or toxic to the bio-culture, which could result in the biological treatment being unable to produce an effluent complying with the environmental regulations (Alvarez *et al*., 2001).

Biological oxidation of wine-distillery wastewater presents some problems due to refractory compounds, such as polyphenols, that are also toxic for microorganisms if present in high concentrations (Alvarez *et al*., 2001). Phenolic compounds are usually toxic and xenobiotic, and therefore the residual amount of phenolic compounds is one of the major criteria on the biodegradability of a solution (Chen *et al.*, 2004). Polyphenols can be precursors to organohalogen compounds (Beltrán *et al*., 1993).

Polyphenols are aromatic compounds that are prone to attack of electrophilic agents like ozone (Alvarez *et al*., 2001). These harmful pollutants need to be degraded before treatment (Benitez *et al*., 1999). A possible way to overcome these problems can be a physico-chemical pre-oxidation step to produce biogenic intermediates (Scott & Ollis, 1995; Boncz *et al*., 2003).

Research has shown that low doses of ozone may ensure sufficient chemical changes of biorefractory compounds to enhance overall wastewater biodegradability (Medley & Stover, 1983). In recent years, the combinations of pre-ozonation with the biological processes for phenolic wastewater treatment have drawn great attention (Chen *et al*., 2004). The ozonation of aromatic compounds usually increases the biodegradability of the wastewater. Depending on the type of wastewater and treatment method, reaction intermediates of greater toxicity than the initial substances may also be produced (Martín *et al.*, 2002). The biodegradability of humic compounds can be improved after ozonation (Bablon, 1991). Ozone has been shown to be capable of destroying several phenolic compounds effectively (Gurol & Nekoulnalnl, 1984). Boncz *et al*. (2003) as well as Singer & Gurol (1983) reported that the reaction of ozone with phenol is a fast reaction, due to phenols being oxidised directly. The decompositions of phenolic compounds may significantly improve the biodegradability of a phenolic solution.

The efficiency of biotreatment process used could therefore be improved by combining it with a pre-ozonation procedure, which would reduce the toxicity of phenolic solutions. Microorganisms could then better utilise and directly degrade the pre-ozonated phenolic solutions. Duff *et al*. (2002) found that tannin levels were reduced much more quickly by ozonation than by biological treatment. A pre-ozonation process can, therefore, reduce the total treatment time of the wastewater (Chen *et al.*, 2004) and provide a considerable cost advantage (Baig & Liechti, 2001).

Wastewater pH - Oxidation rate constants suggest that distillery wastewaters are extremely reactive towards molecular ozone (Beltrán *et al.*, 1997b). The order of reactivity is similar to that of phenols or unsaturated compounds at an acid or neutral pH. At a basic pH, however, the reactivity of these single compounds, especially phenols, with ozone is much higher (Beltrán *et al.*, 1997b).

Ozone efficiency is strongly dependant on the pH of the wastewater. This is due to the fact that the pH affects the double action of ozone on organic matter that may be a direct or indirect (free radical) pathway (Hoigné, 1998). At low pH, ozone exclusively reacts with compounds with specific functional groups through selective reactions such as electrophilic, nucleophilic or dipolar addiction reactions (direct pathway). At basic pH values, ozone decomposes yielding hydroxyl radicals, a highly oxidising species which reacts non-selectively with a wide range of organic and inorganic compounds in the water (indirect pathway).

Oxidation through both reaction mechanisms are limited by the presence of molecular ozone-resistant compounds or hydroxyl radical scavengers (Alvarez *et al.*, 2001). Carboxylic acids and aldehydes, on the one hand, and carbonates, on the other hand, are good examples of these types of compounds. Carboxylic acids and aldehydes are usually final products of direct ozonation reactions, while carbonates accumulate in the water as a result of mineralization of organic compounds at basic pH.

During ozonation the pH of the water decreases due to formation of the carboxylic acids, with the final result in that ozonation becomes inefficient (Alvarez *et al.*, 2001). Alfafara *et al*. (2000) speculated that the pH drop was due to acidic by-products formed from the natural melanoidins present which contain alcohol (-OH) and aldehyde (-CHO) groups.

The COD removal increases with increasing pH due to the fact that ozone undergoes self-decomposition to generate hydroxyl free radicals, which can oxidise the organic compounds more efficiently (Chen *et al.*, 2004). In the absence of carbonates, the ozonation of wastewater at high pH results in an efficient process leading to a significant removal of COD, TOC and TKN (Beltrán *et al.*, 2001b). Although the COD removal rate increases with increasing pH, it is desirable to carry out the pre-ozonation at a pH of 7 in a practical process, as the subsequent biological treatment is generally performed at a pH of 7 and thus further pH adjustment would not be necessary after pre-ozonation (Chen *et al.*, 2004). Boncz *et al.* (1997) indicated that pH value is the most important factor in the ozonation of chlorophenols, because pH determines the degree of dissociation of the phenolic compounds.

Ozone combined use - Ozone has been shown to be a powerful oxidant and disinfectant in water treatment (Bablon, 1991). Previous studies have recommended ozone as a technology to improve the efficiency of wastewater treatment units (Beltrán *et al*., 1997a). Thus, ozone can improve particle flocculation or wastewater biodegradability by removing refractory compounds toxic to microorganisms (Alvarez *et al*., 2001).

Ozone application to wastewater has been limited due to excessive ozone consumption for the degradation of compounds of less concern, for example high molecular weight COD. For this reason Duff *et al*. (2002) rather examined the effectiveness of ozone as a polishing treatment on biologically treated effluent. After 30 min of ozone treatment, COD was reduced by 22%. Biochemical oxygen demand concentration increased by 38% after 30 min of ozonation. This increase in BOD was due to the conversion of a portion of the high molecular weight COD to lower molecular weight compounds capable of exerting a BOD effect (Duff *et al*., 2002).

Doğruel *et al*. (2002) investigated pre- and post-ozonation for a biological treatment. It was found that pre-ozonation may ease biological treatment by converting the more slowly biodegradable COD into simpler compounds or by reducing the amount of inert organic matter. Post-ozonation may have a polishing effect on the effluent quality. It is therefore important to set the basis for the selection of the appropriate location of ozone application.

Pre-ozonation of textile mill effluent by Doğruel *et al*. (2002) resulted in only a limited COD removal (14%) indicating the existence of a selective reaction. The oxidation of readily biodegradable COD was the major mechanism. Soluble inert COD concentration increased at low ozone doses, due to generation of less biodegradable organic byproducts. Post-ozonation provided a higher removal of soluble inert COD. It was concluded that the selective preference of ozone for simpler organic compounds could be avoided by post-ozonation (Doğruel *et al*., 2002).

Kamenev *et al*. (2003) carried out post-ozonation of biologically treated phenolic wastewater in a semi-batch ozonation column. The efficiency of the process was relatively low - the ozone dose consumed during COD reduction was 3 to 8 mg O_3 per mg COD removed. Biological oxygen demand did not change during ozonation, which made the biodegradability of the wastewater, expressed as the BOD/COD ratio, slightly better by ozonation. The improved biodegradability of wastewater made subsequent aerobic bio-oxidation with ozonation in a recycling mode more effective (Kamenev *et al*., 2003).

The main conclusion of Kamenev *et al*. (2003) was that injection of ozone at moderate doses (up to 30 mg.L^{-1}) improved the rate of pollutant removal by 50 - 60%. The improvement in the biological oxidation of phenols by ozone could mean that a combined treatment method could also be used for other effluents containing phenolic compounds. The impact of ozone on combination with aerobic bio-oxidation not only the increased biodegradability of the effluent, but the residual ozone also positively influenced the properties of the activated sludge (Kamenev *et al*., 2003).

Benitez *et al*. (1999) investigated the use of ozone as a pre-treatment to anaerobic digestion. It was found that winery wastewater COD could be reduced up to 20% with ozonation, and coupled with the anaerobic process, achieved higher reductions. In a study by Alfafara *et al.* (2000), it was found that treating wine-distillery wastewater by anaerobic digestion still resulted in a final high COD (mainly organic matter resistant to biodegradation) and a dark brown colour. The residual colour and recalcitrant organic matter in the effluent indicated that a more powerful oxidative process may be needed. Ozone use resulted in a COD decrease of 16%, a biodegradability increase of 40% (BOD:COD ratio) and a colour reduction of 80%. Therefore it was recommended that ozone should be considered for a pre-treatment step due to decolourisation and improvement of biodegradability.

D. GENERAL DISCUSSION

Wine-distillery wastewaters are characterised by the high content of organic material and nutrients, high acidity and large variation in seasonal flow production. Consequently their treatment requires careful consideration of available options. Complete cleansing of wastewater pollutants will not be feasible with the application of a single treatment process and combinations of chemical and biological treatments are often the way to optimise the overall process, leading to a more effective treatment of the waste. It would therefore be desirable to have a pre-treatment method that is capable of converting the inhibitory and refractory compounds by oxidation into simpler molecules that can readily be used as substrates for secondary biological treatment.

Ozonation has been recommended as a pre-treatment to distillery wastewater to increase biodegradability and enhance a further biological treatment process. Ozone offers many benefits as a pre- and post-treatment due to its excellent oxidising capabilities.

The fact that it decomposes quickly back into O_2, and forms CO_2 and H_2O as by-products during reactions, makes it safe to use. Ozone can partially biodegrade non-biodegradable organics, and reduce polyphenols, which results in an increase in biodegradability. This may therefore result in a quicker treatment time and assist in achieving desired effluent quality.

Constructed wetlands is a technology that may offer a low cost and maintenance solution to the wine and distillery industry as a wastewater treatment. Constructed wetlands are suitable for treating effluent quality with low pollution concentrations (COD, BOD, TSS etc.), allowing these wastewaters to be treated to a suitable discharge quality. Wetlands should be an option for distilleries that have sufficient land area for construction of a wetland, and are looking for a treatment process with the above advantages

It is recommended that wetlands, however, should not be fed winery wastewater with a COD higher than 5 000 mg.L^{-1}, since this will result in rapid death of plants at the wetland inlet. For more concentrated wastewaters, such as from distilleries, the long term sustainability and removal efficiencies of CWs is problematic and additional / alternate treatment methods may need to be considered. Therefore a wetland treatment system on its own will not be able to treat WDWW. Studies recommend that wetlands should be seen as a secondary treatment system, and that a pre-treatment system will be needed to achieve desired efficiencies.

It can be concluded that a combination of a chemical and a biological process may lead to greater destruction of organic contaminants in wastewater. Thus, ozonation can be recommended as a pre-treatment for WDWW to increase biodegradability and enhance a further biological treatment process, namely a constructed wetland system. By combining pre- and post-ozonation with a wetland system, and if the ozonation application can be sufficiently optimised, it may reduce the total treatment time of the wastewater, increase the effectiveness of the biological treatment, and may lead to effluent of a quality which may be discharged or reused by complying to legal water standards.

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CHAPTER 3

Investigating the use of ozone as a suitable treatment method for wine- distillery wastewaters

Summary

The effect of ozonation on wine-distillery wastewater was investigated firstly by monitoring the effect of ozonation on the composition of the wastewater, and secondly, by investigating its effect on the biodegradability of the wastewater. For pre-wetland wastewater from a distillery, an average COD reduction of 271 mg COD.g O_3^{-1} was found, and for post-wetland effluent, an average of 103 mg COD.g O_3^{-1}. The activity of stable microbial populations found in upflow anaerobic sludge blanket (UASB) granules can be measured to determine the biodegradability of a substrate. Inhibitory or more biodegradable compounds in wastewaters can affect UASB activity, giving an indication of the effect the wastewater will have on a biological treatment method. Granule activity was measured in terms of biogas and methane production rates, as well as cumulative biogas volume. Low ozone doses (200 - 400 mg $O_3.L^{-1}$) increased granule activity in terms of biogas, methane production, and cumulative gas volumes, thus indicating an increase in biodegradability. Distillery wastewater reduced the activity of granules, most likely due to the presence of polyphenols and other recalcitrant compounds in distillery wastewater. By showing an increase in the biodegradability of WDWW, ozone has potential as a pre-treatment step to increase the effectiveness of a biological wetland system.

Introduction

Production of ethanol from wines by distillation processes results in a high strength acidic wastewater (Beltrán *et al*., 2000). Wine-distillery-wastewaters (WDWW) typically have high suspended solids content and contain residual organic acids, soluble proteins, carbohydrates, as well as various inorganic compounds (Water Research Commission, 1993; Van Schoor, 2000). Wine-distillery wastewater has an acidic pH (3.5 - 5.0) and is characterised by a high organic content (sugars, alcohol, phenols and polyphenols, lipids) with a chemical oxygen demand (COD) range of 10 000 - 60 000 mg.L^{-1} (Benitez *et al.*, 1999; Martín *et al.,* 2002). Ideally the easiest way to dispose of these wastewaters would be by irrigation. In terms of section 39 of the National Water Act of 1998, wastewater must either be treated prior to discharge into a water resource, or disposed of by some alternative method. If left untreated, this wastewater could potentially contaminate natural water resources, contaminate soil and hinder plant growth and create odour problems. For irrigation to take place, the quality of the effluent must comply with stipulated requirements (DWAF, 2004).

Current treatment options include: aerobic systems, activated sludge, aeration of dam wastewaters, anaerobic bacteria, artificial wetlands, physico-chemical or combinations of the above treatments (Van Schoor, 2005). Wastewaters should be free of substances resistant to biodegradation or toxic to microbial populations but WDWW are chemically complex and contain a host of phenolic compounds, some of which resist biodegradation (Martín *et al.,* 2002). Additionally refractory compounds present in these wastewaters, such as polyphenols, can be toxic for microorganisms (Alvarez *et al*., 2001). Therefore, an investigation is needed to establish which treatment methods are best suited to reach specific effluent quality goals.

A physico-chemical step can be applied as a pre-treatment process when there is a presence of strong organic or toxic compounds (Wang *et al*., 1989). Some organic compounds react rapidly with ozone (Gulyas *et al*., 1995; Beltrán-Heredia *et al.*, 2001) and polyphenols are aromatic compounds that are prone to attack by electrophilic agents like ozone (Alvarez *et al*., 2001). The ozonation of aromatic compounds usually increases the biodegradability of the wastewater (Martín *et al*., 2002). Generally the main areas where ozone (O_3) is used for treatment of wastewaters are disinfection, oxidation of organic and inorganic compounds, including taste, odour, colour removal and particle removal (Gottschalk *et al*., 2000).

Duff *et al*. (2002) found that an ozone treatment reduced COD by 22% for treatment of log water run-off, but increased the overall biological oxygen demand (BOD). This was attributed to the conversion of the high molecular weight COD to lower molecular weight compounds capable of exerting a BOD influence. Doğruel *et al*. (2002) also found that pre-ozonation resulted in a limited COD removal (14%) indicating the existence of a selective reaction. The oxidation of readily biodegradable COD was the major mechanism. McLachlan (2004) reported a 20% reduction in ozonation of cellar effluent at a concentration of 73 mg $O_3.L^{-1}$.

Complete cleansing of wastewater pollutants will not be feasible with the adoption of a single treatment process. Combinations of chemical and biological treatments are often the only way to optimise the overall process (Andreozzi *et al*., 1998). Therefore, the aim of this study was to establish whether ozone could be used to optimise degradation of WDWW in constructed wetlands. The effect of ozonation on WDWW was investigated firstly by monitoring the effect of ozonation on the composition of the wastewater, and secondly, by investigating the effect of ozonation on the biodegradability of the wastewater.

Materials and Methods

Characterisation of wastewater streams from distillery

A distillery in the Boland Region of South Africa has an existing treatment system consisting of a primary settling dam, followed by three further holding dams (Fig. 3-1). The first dam receives wastewater directly from the distillery and therefore assists in settling the solids contained in the wastewater. In the second dam, aerobic aeration takes place to lower the organic load of the effluent, while the third and fourth dams allow further settling of suspended solids. In a study by the ARC Infruitec-Nietvoorbij (Mulidzi, 2006), an additional treatment method, namely a pilot, constructed wetland system was being investigated as the final treatment to “polish” the final effluent.

As a start to this investigation, a preliminary study was done to determine the composition of the WDWW, at various points in the treatment system, to determine the efficiency of the current system. Three sampling points were identified, namely: at the distillery outlet, after the three aerobic dams (pre-wetland), and after the pilot wetland (post-wetland).

For the remainder of this investigation, WDWW was obtained from the distillery during the “peak” and “off-seasons” of 2004 and 2005. The sampling was done at the outlet of the 3rd dam but before entering into the wetland (Fig. 3-1). The wastewaters (unozonated and ozonated) were stored in 25 L drums at -18 °C. During the investigation, drums were thawed as required and kept at 4 °C. Tap water was used to dilute the wastewater to the desired COD levels used in the trials.

Ozonation of wine-distillery wastewater

Ozonation was applied by using an O_3 generator (Parc Scientific, Ifafi) that produced O_3 at a concentration of 4.82 g.h^{-1} at a flow rate of 4 L.min^{-1} as determined by the Iodometric Method (APHA, 1998). Diluted and undiluted wine-distillery wastewater was pre-ozonated at room temperature in a glass bubble column. The glass column had a height of 104 cm and a diameter of 10 cm and a volume of 2 L. The glass column contained two sintered discs at the top and bottom of the column. The function of the bottom cinter was to allow better gas bubble distribution, and the function of the top cinter was to reduce the loss of

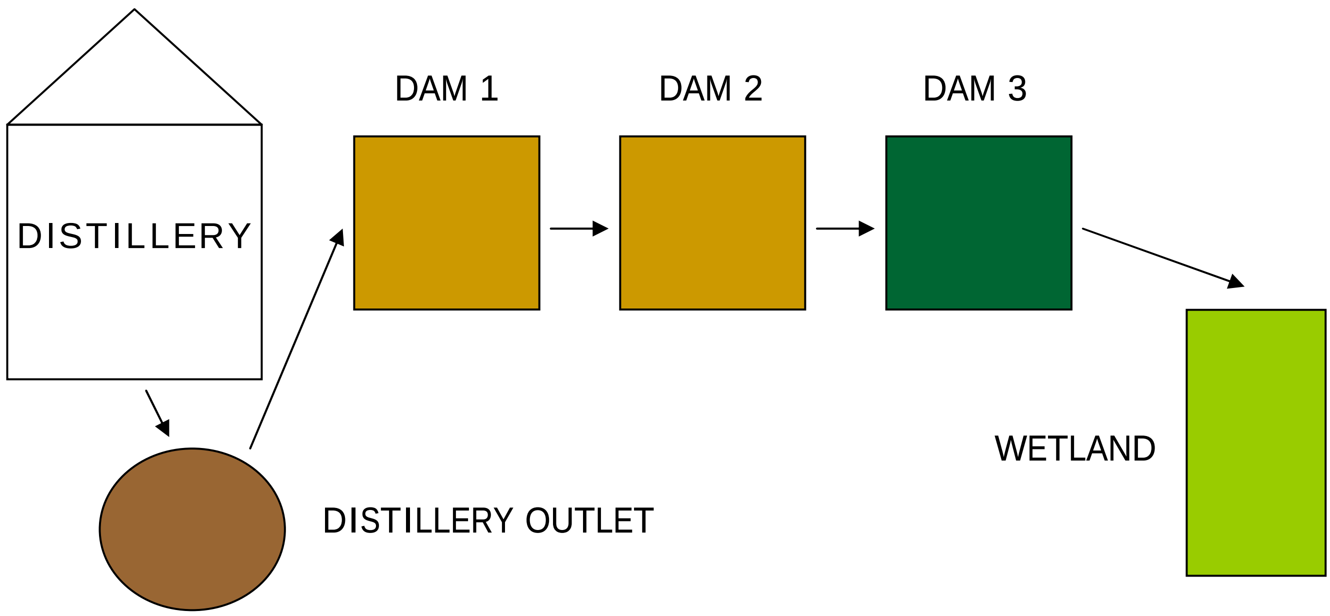

Figure 3-1 Layout of the distillery wastewater treatment system and sampling points for characterisation of the system efficiency.

wastewater through foaming. The sample was poured into the bubble column and was ozonated for the pre-determined time/ozone dose combination.

In order to investigate the efficiency of ozonation on WDWW with different COD loads, a dilution series of pre- and post-wetland WDWW was prepared. Each WDWW dilution for pre-wetland (250, 500, 1000, 1500, 2000, 3500 mg.COD.L^{-1}) and post-wetland (100, 150, 300, 600 mg.COD.L^{-1}) was ozonated with a 400 mg O_3.L^{-1} dose. The COD reduction in each dilution was determined to allow calculation of the ozone efficiency on terms of COD (mg) removed per mg O_3 over a range of COD loads.

Analytical methods

The following wastewater parameters were monitored according to Standard Methods (APHA, 1998): pH, alkalinity, total solids (TS), total suspended solids (TSS), total volatile solids (TVS) and total volatile suspended solids (TVSS). Conductivity was determined using a Hanna Instruments (HI8733) conductivity meter. The COD and orthophosphate phosphorous (PO_4^{3-}) were determined colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (APHA, 1998). Total polyphenol content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965). All analyses were done in triplicate.

The biogas composition was determined using a gas chromatograph (Varian 3300). A 0.2 mL sample of biogas was injected into the gas chromatograph, using helium (He) as a carrier gas at a flow rate of 30 $mL.min^{-1}$ and the oven temperature set at 55 °C. The gas chromatograph was equipped with a thermal conductivity detector and a 2.0 m x 3.0 mm i.d. column packed with Hayesep Q (Supelco, Bellefonte, PA) and a 80/100 mesh.

Granule activity

Granule selection - The presence of certain compounds in complex wastewaters are toxic to sensitive bacteria such as methanogens (Benitez *et al.*, 1999). Therefore, a stable microbial population, such as those found in UASB granules, could be used to determine the toxic effect of a wastewater on the microbial activity.

Activity tests were performed on UASB granules according to a method described by O'Kennedy (2000) and Sigge (2005). Granule activity was measured by the rate of biogas (S_b) and methane (CH_4) production (S_m). Granules which had been obtained from various UASB reactors and stored at 4°C were used. The granules originated from UASB reactors at two different distilleries, a beer waste treatment plant, a fruit canning effluent plant, a winery and a mixture of these granules. Activity of the granules from the various UASB reactors was compared so as to select the most active granule set. The granules with the highest activity would be used in the trial to determine the effect of ozonation on the biodegradability of the WDWW, thereby facilitating more meaningful differences brought about by exposure to possible inhibitory substances in the WDWW.

To activate the granules for the activity tests, 500 g of granules were incubated at 35°C for 48 h in activation media (O'Kennedy, 2000) in a volume ratio of 1:2, which was replaced with fresh activation media after 24 h. This was done to activate the metabolic activity of the various bacterial groups in the granules, which had been kept dormant at 4°C during storage. Once the 48 hour activation period was completed, the granules were drained, and were then prepared for the trials to determine the effect of ozonating WDWW on biodegradability.

Effect of ozonation on biodegradability of WDWW – The drained granules were divided into five 60 g sample portions and placed in five separate 500 mL Schott bottles. Each granule sample (60 g) was exposed to a differently ozonated (in terms of ozone dose) wastewater to determine the effect on activity. The different ozonation doses used to prepare the WDWWs for the activity test trial are summarised in Table 3-1.

Table 3-1 Ozonation treatments of WDWW to determine effect on granule activity

Treatment description	
1	Untreated wastewater
2	Ozone dose = 200 mg $O_3.L^{-1}$
3	Ozone dose = 400 mg $O_3.L^{-1}$
4	Ozone dose = 800 mg $O_3.L^{-1}$
5	Ozone dose = 1 200 mg $O_3.L^{-1}$

After 24 h of exposure to the five different WDWWs, the effluents were decanted off and freshly prepared wastewaters were added. This cycle was repeated four times, thus over a period of 96 h. The initial activity of the UASB granules was determined directly after the activation step and served as a control. The effect of the five different pre-treatments on granule activity was determined by measuring the activity directly after 96 h of exposure to the WDWWs.

Granules from each of the five exposure trials (Table 3-1) were subjected to an activity test. Thus, duplicate granule samples (3 g) for three activity test mediums for each of the five exposure trials were placed into 20 mL glass vials (five treatments in duplicate for three activity mediums = 30 vials). Each vial received 13 mL of the specific activity test medium. The three media used were the basic test media, glucose test media and acetic acid test media and was prepared according to O'Kennedy (2000). Basic test medium (BTM) is not specific for any microbial group and was used to determine the starting granule activity. The glucose test medium (GTM) and acetic test medium (ATM) were used to measure the activity of the acidogens and acetoclastic methanogens, respectively (Sigge, 2005). The vials were sealed with butyl septa and capped with aluminium caps, before being incubated at 35°C. After 5, 10, 25 and 40 h incubation, biogas was sampled by inserting a free moving 10 mL syringe, with a 12 gauge needle through the septa. This allowed the biogas volume to be determined. The biogas composition was determined gas chromatographically.

Results and discussion

Characterisation of distillery wastewater streams

The composition of WDWW has been shown to vary considerably from day-to-day (Bezuidenhout *et al.*, 2002). This is mainly due to the seasonal nature of the product, steam production, cooling and floor and equipment wash down (Water Research Commission, 1993). The composition of the wastewater collected for this study during 2004 at various points of the waste treatment system showed a wide daily variation as well as the impact of other environmental factors. These variations and efficiencies of the treatment system at the distillery are summarised in Table 3-2.

Table 3-2 Composition of three effluent streams from selected points in the overall treatment system

Parameter	Distillery outlet	Pre-Wetland	Post-Wetland
COD ($mg.L^{-1}$)	12 609 - 22 150	3 843 - 7 614	423 - 1 680
pH	4.52 - 4.68	6.75 - 8.51	7.89 - 8.99
Alkalinity (as $mg.L^{-1}$ $CaCO_3$)	325 - 913	1 200 - 2 850	1 338 - 3 650
Phosphates ($mg.L^{-1}$)	254	209 - 245	107 - 180
Polyphenols ($mg.L^{-1}$ GAE)	5.5 - 13.9	3.0 - 4.7	1.3 - 2.3
Total Solids ($g.L^{-1}$)	11.68	7.46	6.59
Total Volatile Solids ($g.L^{-1}$)	8.33	3.62	1.8
Total Suspended Solids ($g.L^{-1}$)	1.43	0.57	0.44
Total Volatile Suspended Solids ($g.L^{-1}$)	1.30	0.44	0.30

Ozonation of WDWW

Due to the daily variation in the composition of the WDWWs at various points in the treatment system as shown in Table 3-2, an investigation was conducted to determine the efficiency of ozonation on WDWW using a COD range (Fig. 3-2 and 3-3). It can be seen from the data in Fig. 3-2 and 3-3 that there is a decrease in the COD reduction efficiency of ozone as the wastewater COD increases. In Fig. 3-2, the COD reduction of the pre-wetland wastewater decreased as the COD content increased. A 38% reduction was obtained with the 250 mg COD.L^{-1} dilution, but decreased to 3% for the 3 500 mg COD.L^{-1} dilution. This would suggest that, as expected, the ozone is rapidly depleted in the oxidation process and no further hydroxyl radical formation occurs to increase the oxidation potential. In Fig. 3-3, the COD reduction of post-wetland wastewater also decreased as the COD content increased. A 28% reduction was achieved for the 100 mg COD.L^{-1} dilution. The COD reduction decreased to 9% for the 600 mg COD.L^{-1} dilution.

The COD reductions achieved in the pre-wetland wastewater equate to an average COD reduction of 271 mg COD.g O_3^{-1}. The COD reductions achieved in the post-wetland wastewater equate to an average of 103 mg COD.g O_3^{-1}. Thus, it can be seen that ozonation had a greater reduction effect on COD content in the pre-wetland wastewaters, than in the post-wetland wastewaters. Therefore it can be calculated that to break down 1 g of COD in pre-wetland wastewater, 3.69 g of ozone will be needed, and to break down 1 g of COD in post-wetland wastewater, 9.68 g of ozone will be needed.

The fact that pre-wetland reduction rates were higher than those for post-wetland ozonated wastewater may be attributed to the efficiency of the wetland treatment system itself. Wetland systems have been shown to reduce COD (De Gueldre *et. al*., 2000; Shepherd, 1998) by the macrophytes utilising nutrients in the wastewater. Therefore, the post-wetland effluent will contain mainly compounds of a more recalcitrant/less biodegradable nature. This would also explain the much lower COD reduction per gram of ozone when ozonating post-wetland wastewater.

Composition of wastewater

The composition of the pre-wetland wastewater batches obtained during 2004 and 2005 are shown in Table 3-3. The variation between "off" and "peak-season" can clearly be seen from the COD's, which range from *ca*. 2 000 mg.L^{-1} in the "off-season" (October 2004 and March 2005), to *ca*. 7 000 mg.L^{-1} in "peak-season" (July 2005). This variation is due to the seasonal nature of production. There is not only "peak" and "off-season" variation, but daily effluent loading also varies (Bezuidenhout *et al.*, 2002). For example solid wastes in the water originate mainly form the skins and pips of the grapes. Other contributing factors are cooling waters, and floor and equipment washing (Water Research Commission, 1993).

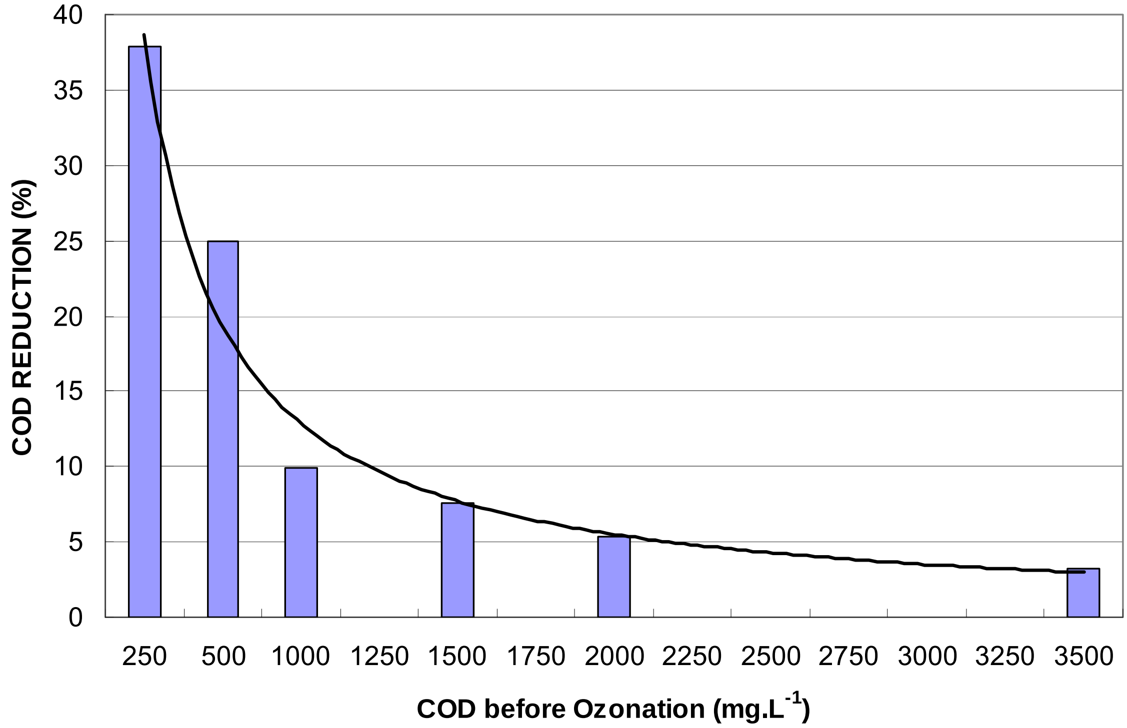

Figure 3-2 COD reduction (%) of dilutions of pre-wetland wastewater after a 400 mg $O_3.L^{-1}$ dose.

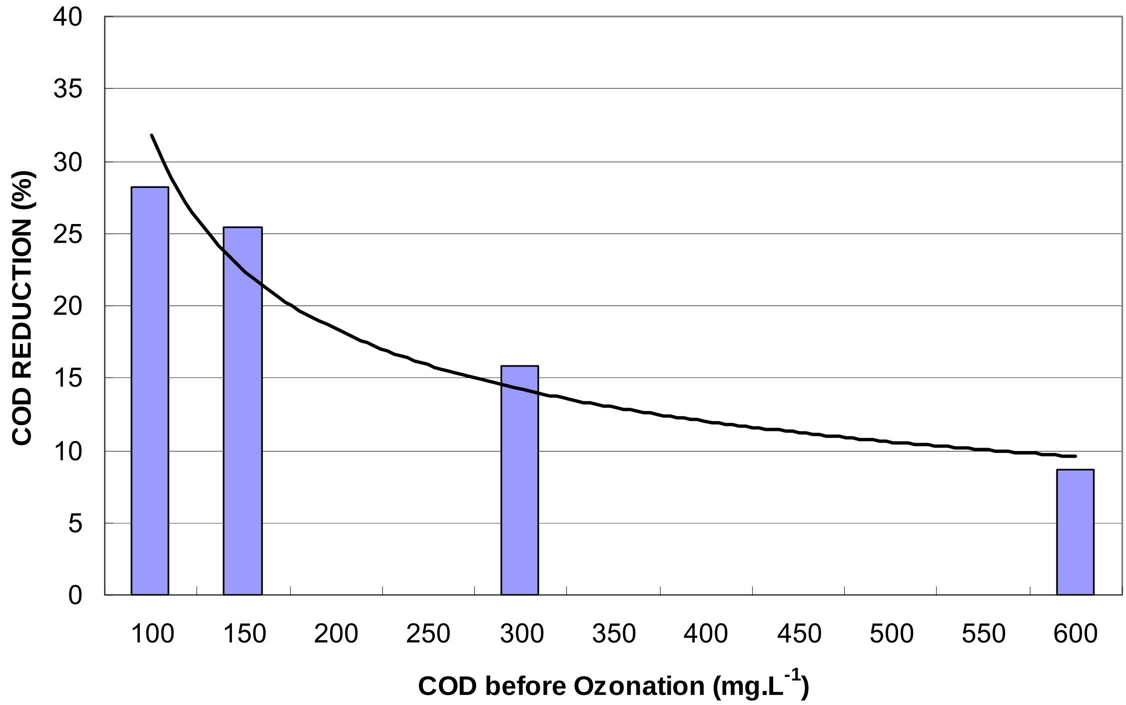

Figure 3-3 COD reduction (%) of dilutions of post-wetland effluent after a 400 mg $O_3.L^{-1}$ dose.

Table 3-3 Composition of wastewater batches taken pre-wetland

	October 2004	March 2005	July 2005
COD ($mg.L^{-1}$)	2 000	2 203	7 000
pH	8.21	9.1	7.51
Alkalinity (mg $CaCO_3.L^{-1}$)	1 150	1 750	2 400
Phosphates (mg PO_4^{3-} $.L^{-1}$)	92.4	378	333
Conductivity ($mS.m^{-1}$)	248	349	533
Polyphenols ($mg.L^{-1}$)	1.64	2.43	5.87
Total Solids ($g.L^{-1}$)	2.70	4.56	7.63
Total Vol. Solids ($g.L^{-1}$)	0.65	1.92	4.23
Total Susp Solids ($g.L^{-1}$)	0.11	0.53	1.07
Total Vol Susp Solids ($g.L^{-1}$)	0.06	0.42	0.81
Colour (254 nm)	2.11	3.82	4.0
Colour (475 nm)	-	1.99	1.90

Activity Tests

Selection of granules - To determine which type of granule would be the most suitable to study the effect of ozonation on the biodegradability of WDWW, a preliminary selection study was done. Granule activity was measured in terms of biogas and methane production rates, as well as cumulative biogas volume. The biogas and methane production rates (S_b and S_m) over the 40 h incubation period in the BTM, GTM and ATM are shown in Fig. 3-4 to 3-9. Cumulative biogas and methane volumes were measured and are shown in Tables 3-4 and 3-5, respectively.

From the data in Fig. 3-4, 3-5 and 3-6 it can be seen that the granules from two different distilleries and the set from a winery were the most active in terms of biogas production rates (S_b) for all three test evaluation media at 5, 10 and 40 h. The granules obtained from the mixture, fruit effluent and beer treatment plants gave lower biogas production rates for all three time periods (5, 10 and 40 h). These differences were further highlighted by the higher cumulative biogas volumes produced by the two distillery and the winery granules in all three test media (Table 3-4).

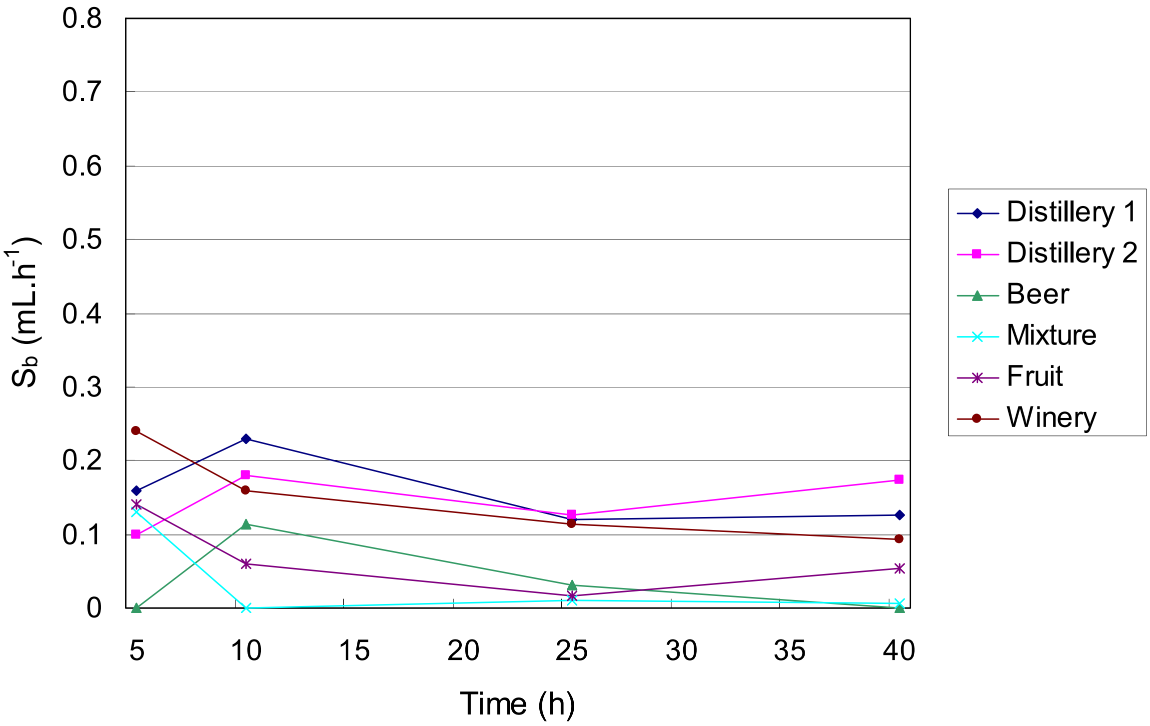

Figure 3-4 Rate of biogas formation (S_b) in the BTM for activated granules from various UASB reactor sources.

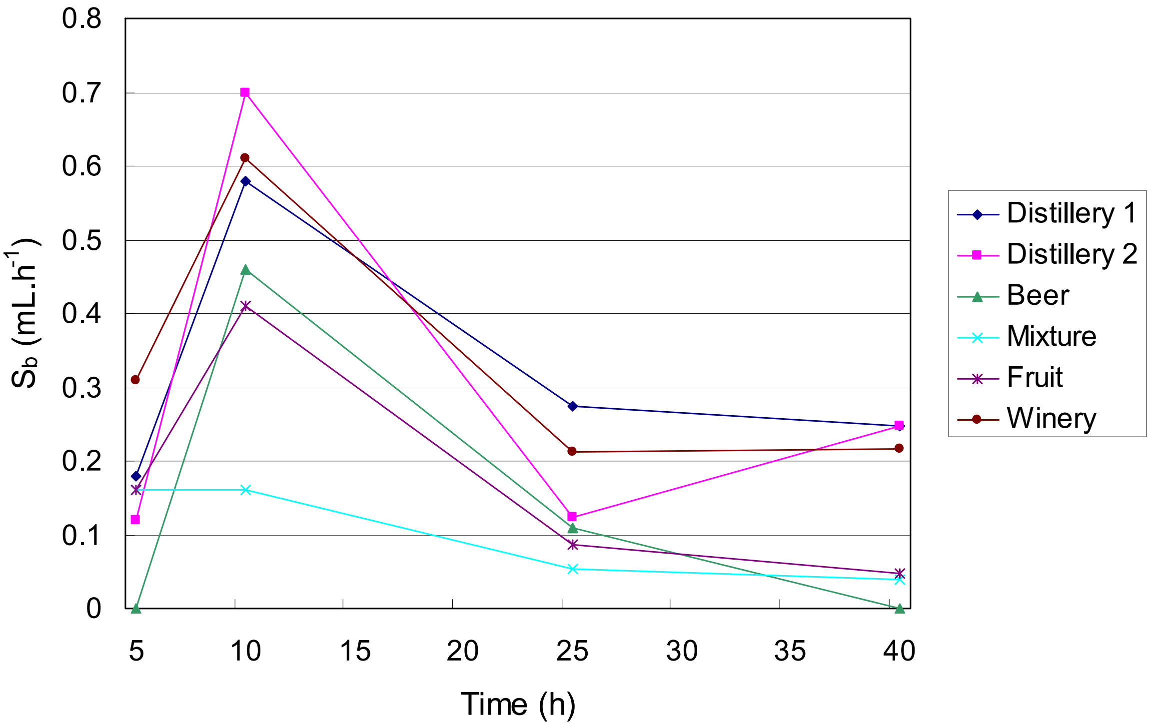

Figure 3-5 Rate of biogas formation (S_b) in the GTM for activated granules from various UASB reactor sources.

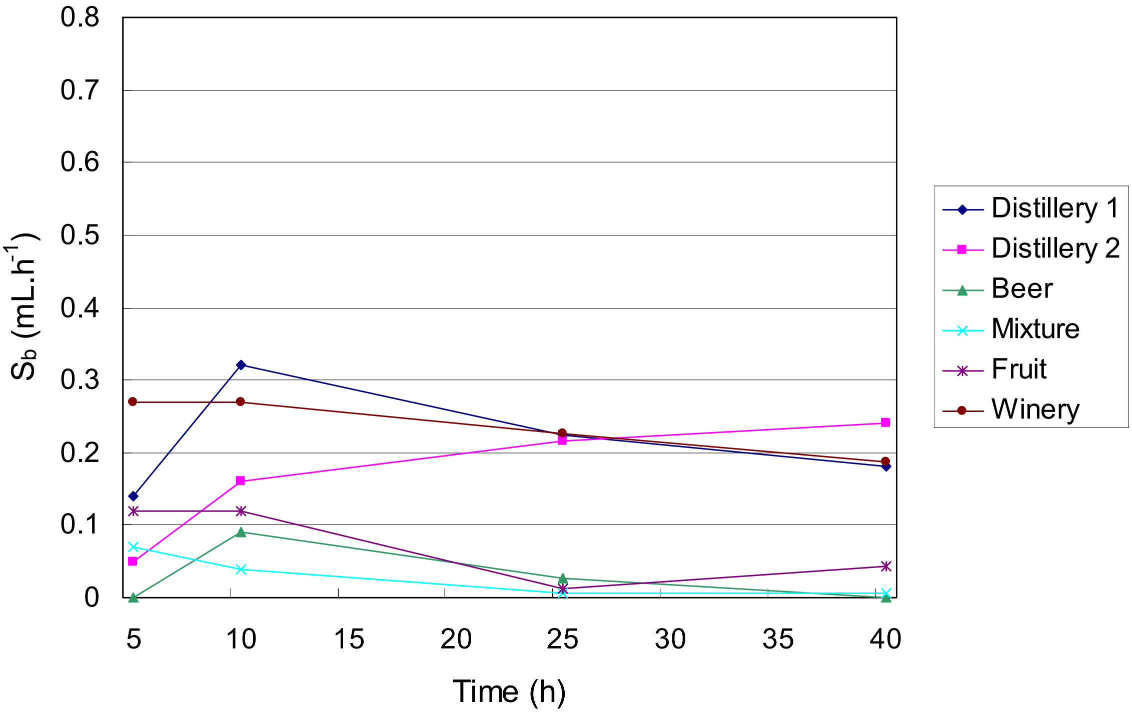

Figure 3-6 Rate of biogas formation (S_b) in the ATM for activated granules from various UASB reactor sources.

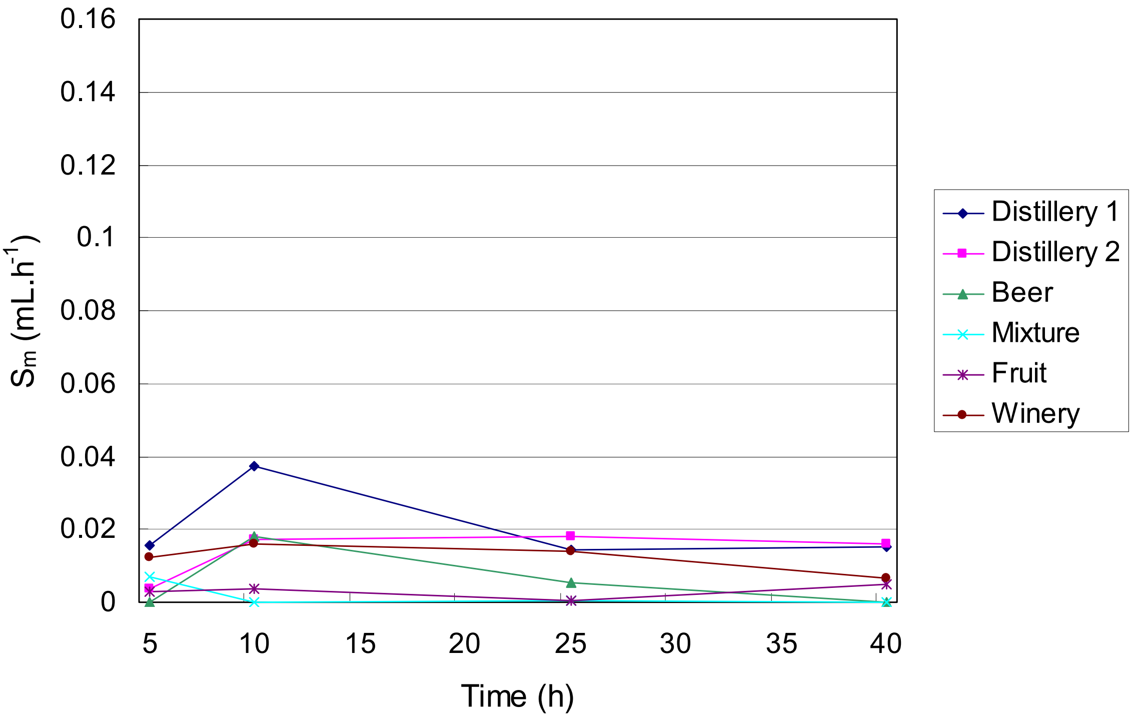

Figure 3-7 Rate of methane production (S_m) in the BTM for activated granules from various UASB reactor sources.

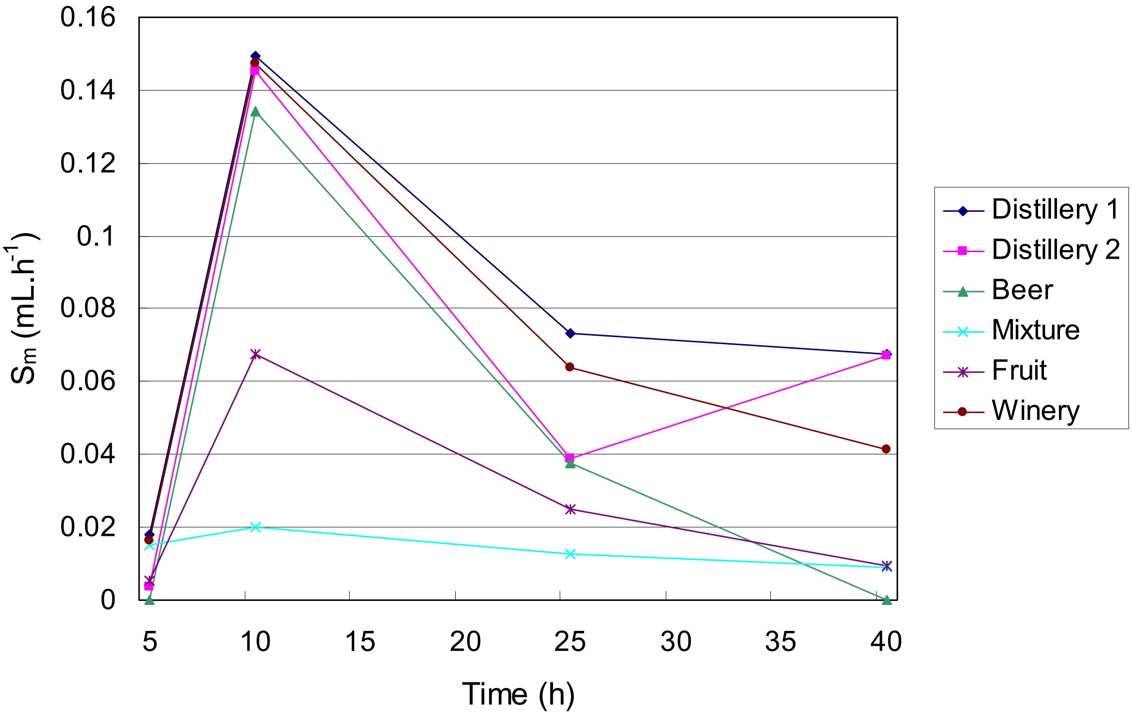

Figure 3-8 Rate of methane production (S_m) in the GTM for activated granules from various UASB reactor sources.

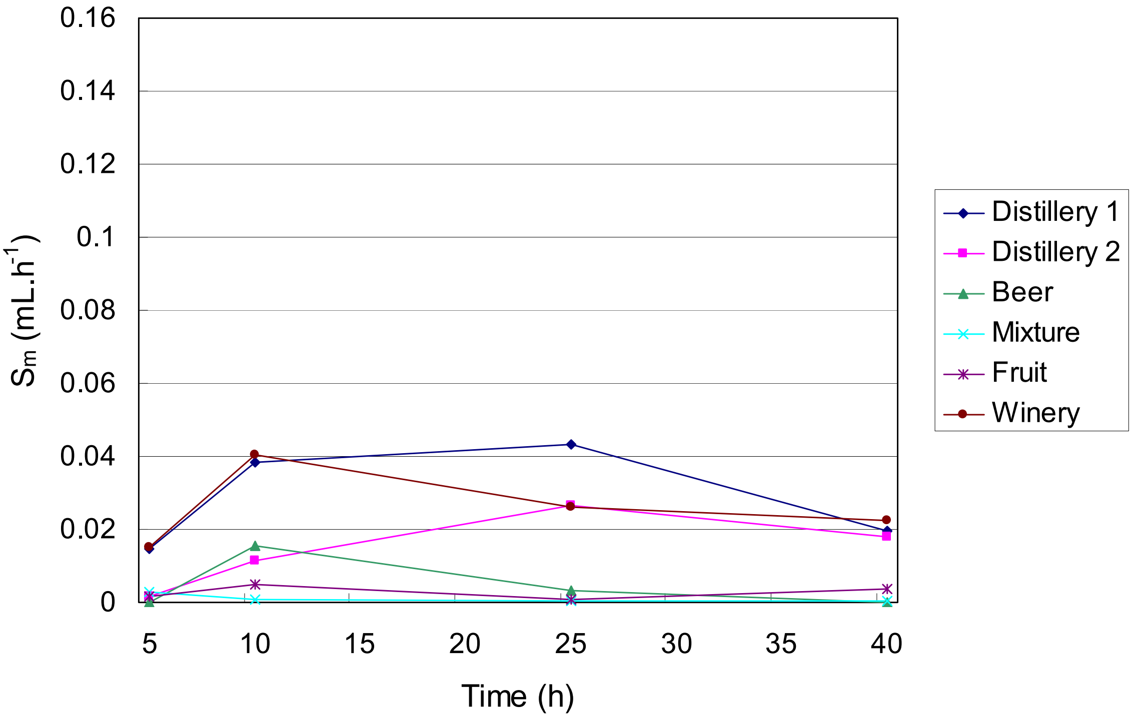

Figure 3-9 Rate of methane production (S_m) in the ATM for activated granules from various UASB reactor sources.

Table 3-4 Cumulative biogas volumes (mL) produced over the 40 h incubation period by granules from various UASB reactor sources. Volumes are reported for the BTM, GTM and ATM activity test media.

Cumulative Biogas Volume					
Granule Source	**Test Medium**	**Incubation time (h)**			
		5	10	25	40
Distillery 1	BTM	0.80	1.95	3.75	5.65
	GTM	0.90	3.80	7.90	11.60
	ATM	0.70	2.30	5.65	8.35
Distillery 2	BTM	0.50	1.40	3.30	5.90
	GTM	0.60	4.10	5.95	9.65
	ATM	0.25	1.05	4.30	7.90
Beer	BTM	0	0.57	1.02	1.02
	GTM	0	2.30	3.95	3.95
	ATM	0	0.45	0.85	0.85
Mixture	BTM	0.65	0.65	0.80	0.90
	GTM	0.80	1.60	2.40	3.00
	ATM	0.35	0.55	0.65	0.75
Fruit	BTM	0.70	1.0	1.25	2.05
	GTM	0.80	2.85	4.15	4.85
	ATM	0.60	1.20	1.40	2.05
Winery	BTM	1.20	2.00	3.70	5.10
	GTM	1.55	4.60	7.80	11.05
	ATM	1.35	2.70	6.10	8.90

Table 3-5 Cumulative methane volumes (mL) produced over the 40 h incubation period by granules various UASB reactor sources. Volumes are reported for the BTM, GTM and ATM activity media.

Cumulative Methane Volume					
Granule Source	**Test Medium**	**Incubation time (h)**			
		5	10	25	40
Distillery 1	BTM	0.078	0.265	0.478	0.706
	GTM	0.090	0.836	1.932	2.947
	ATM	0.073	0.266	0.918	1.210
Distillery 2	BTM	0.019	0.105	0.373	0.614
	GTM	0.019	0.744	1.327	2.332
	ATM	0.007	0.064	0.460	0.731
Beer	BTM	0.000	0.090	0.172	0.172
	GTM	0.000	0.671	1.238	1.238
	ATM	0.000	0.077	0.129	0.129
Mixture	BTM	0.035	0.035	0.043	0.043
	GTM	0.075	0.175	0.366	0.504
	ATM	0.015	0.020	0.024	0.031
Fruit	BTM	0.015	0.033	0.039	0.113
	GTM	0.028	0.365	0.740	0.881
	ATM	0.009	0.033	0.047	0.101
Winery	BTM	0.061	0.142	0.354	0.454
	GTM	0.082	0.819	1.778	2.398
	ATM	0.076	0.277	0.672	1.008

In terms of methane production rates (S_m), the two distillery and the winery granules showed the highest activity at 5, 10 and 40 h for all three test media (Fig. 3-7, 3-8 and 3-9). The differences between these three granule types, in terms of methane production rates and the other granules was less pronounced. The differences in activity are, however, more evident when the cumulative methane volumes are compared (Table 3-5). The two distillery and the winery granules were again the most active.

From the data presented in Tables 3-4 and 3-5, it is evident that the highest cumulative biogas production occurred in the GTM, followed by the ATM. It is expected that the GTM, which is specific for acidogens, should result in the highest gas production. It is interesting to note that for the two distillery and the winery granules, the ATM resulted in a higher biogas and methane production than found for the BTM. As the ATM is specific for the acetoclastic methanogens, these results suggest that this methanogenic population is well developed and represented in these granules. Thus the acidogens and the acetoclastic methanogens are active in these granules, making them suitable to be used in further activity tests to determine the effect of ozonation on biodegradability of WDWW.

Although the granules from Distillery 1, Distillery 2 and the winery UASB reactors were similar in biogas and methane production rates and cumulative gas production, it was decided to use the granules from Distillery 1 UASB reactor for further biodegradability studies.

<u>Effect of ozonation on biodegradability of WDWW</u> - The effect of ozonated wastewater on the activity of anaerobic granules is shown using the biogas (S_b) and methane (S_m) production rates of the granules in BTM, GTM and ATM (Fig. 3-10 to 3-15).

In Fig. 3-10, biogas production rates (S_b) were found to be very similar over the 40 h incubation period, with the exception of the 10 h incubation period. After 10 h incubation, the granules exposed to WDWW (0 mg $O_3.L^{-1}$) and WDWW ozonated at 200 and 400 mg $O_3.L^{-1}$ had higher biogas production rates than the control granules (which had only been exposed to activation medium before activity measurement). The activity, measured as biogas production rate, of the control granules was higher after 5 h incubation, compared to the very low activity of granules exposed to the WDWWs (Fig. 3-10, 3-11 and 3-12). This difference in activity over the first five hours of incubation can be ascribed to the fact that the granules exposed to the WDWWs needed to acclimatise to the specific wastewater.

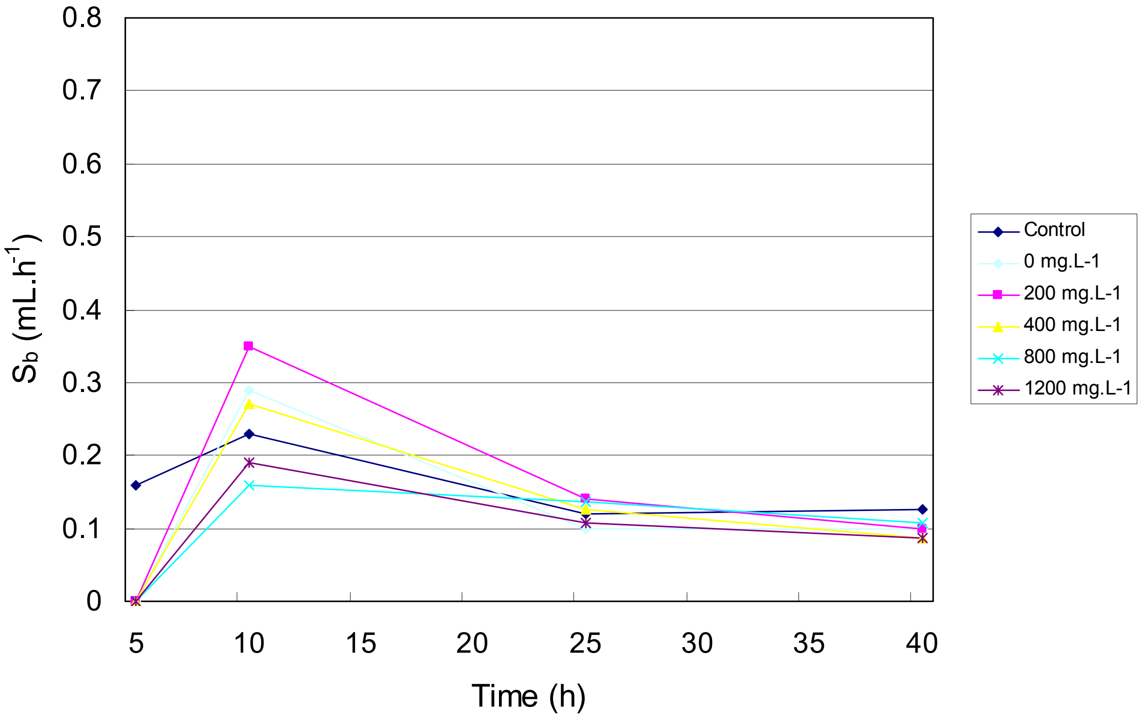

Figure 3-10 Rate of biogas formation (S_b) in BTM for granules exposed to treated wastewater.

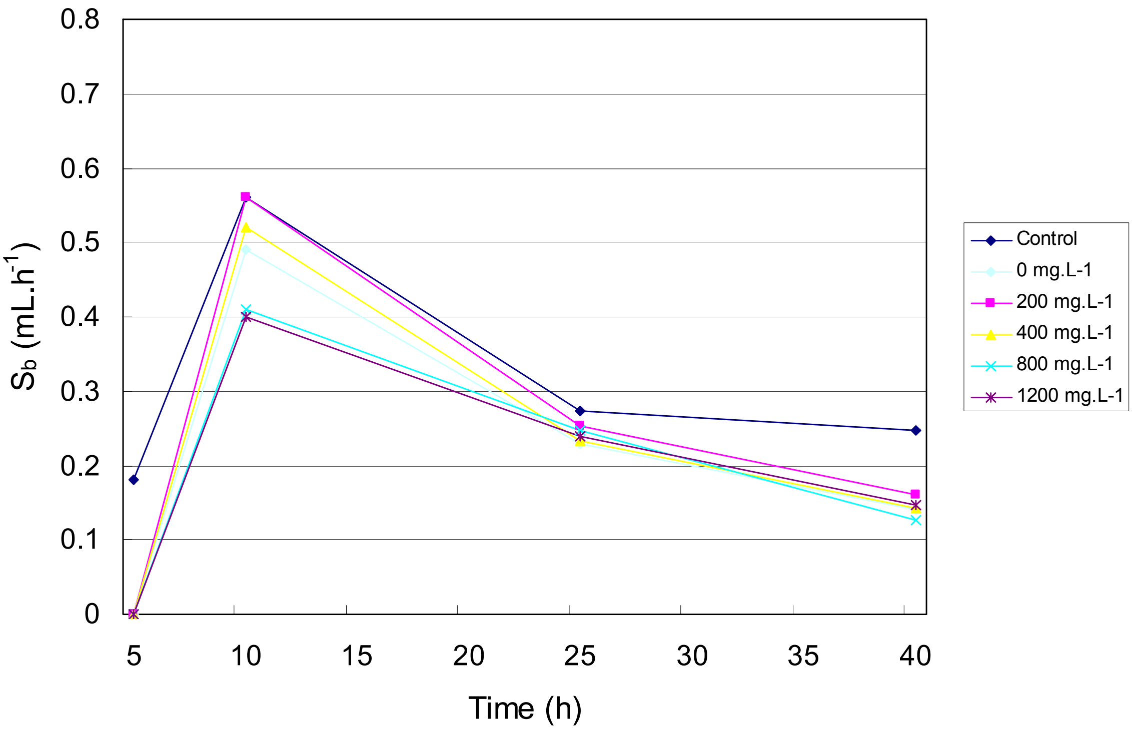

Figure 3-11 Rate of biogas formation (S_b) in GTM for granules exposed to treated wastewater.

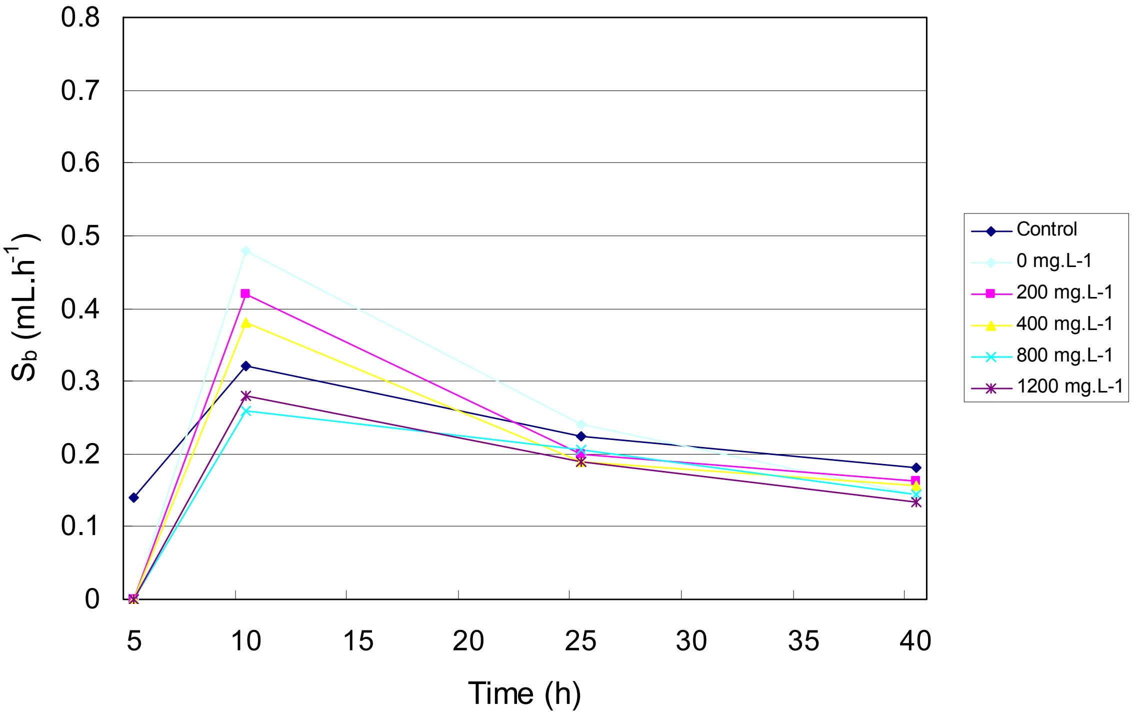

Figure 3-12 Rate of biogas formation (S_b) in ATM for granules exposed to treated wastewater.

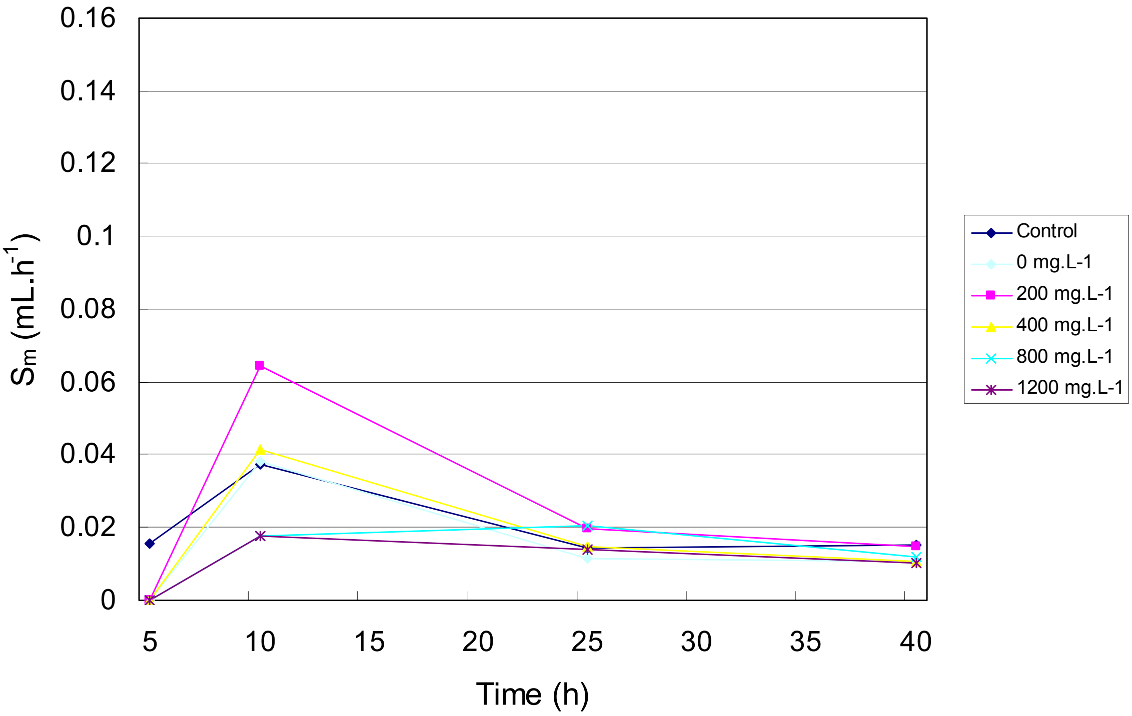

Figure 3-13 Rate of methane production (S_m) in BTM for granules exposed to treated wastewater.

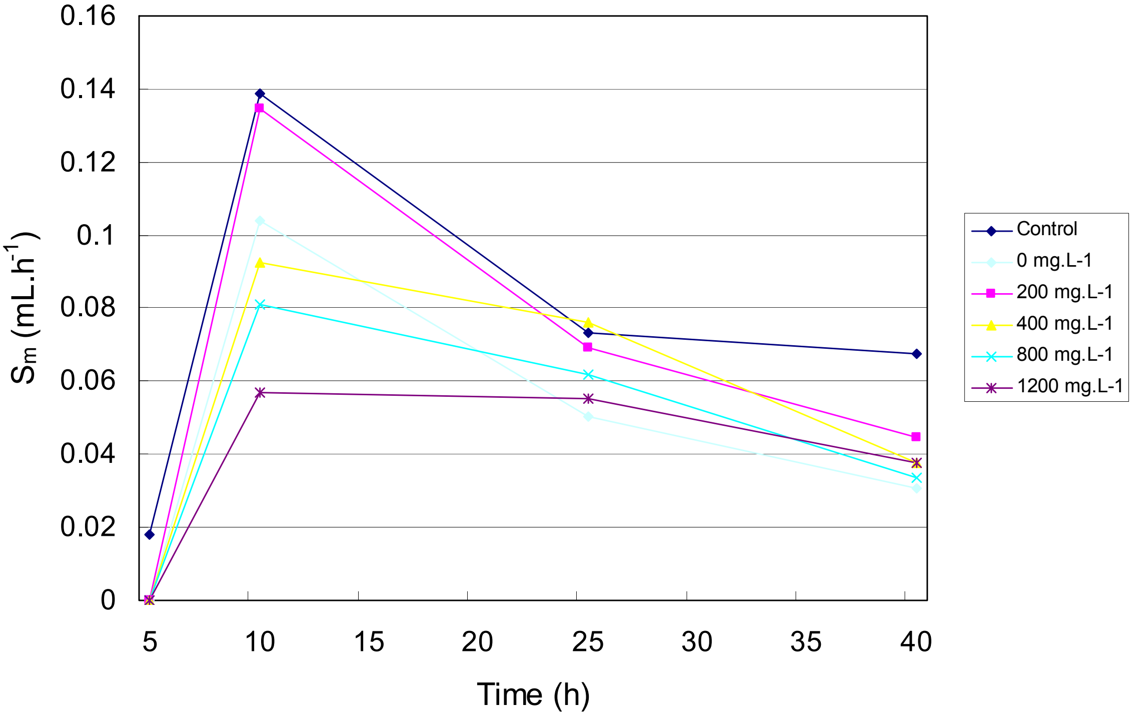

Figure 3-14 Rate of methane production (S_m) in GTM for granules exposed to treated wastewater.

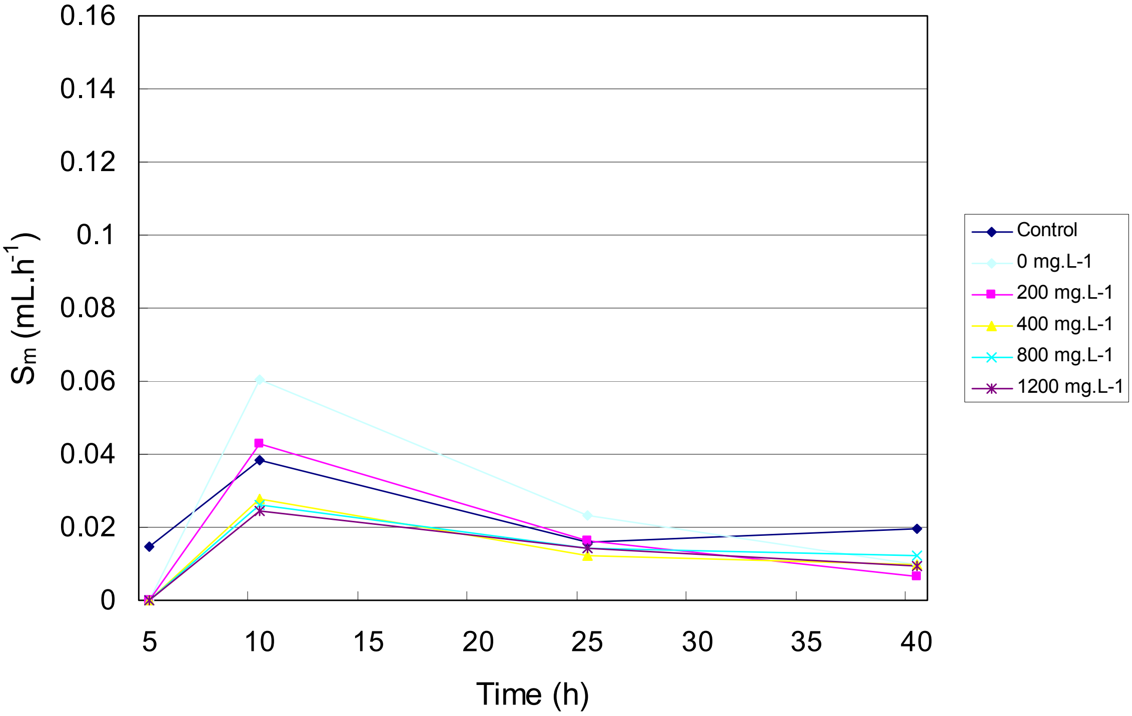

Figure 3-15 Rate of methane production (S_m) in ATM for granules exposed to treated wastewater.

Table 3-6 Cumulative biogas volumes (mL) produced over the 40 h incubation period by granules exposed to treated wastewater in the different activity media

Cumulative Biogas Volume					
Granule Treatment	**Test Medium**	**Incubation time (h)**			
		5	10	25	40
Control*	BTM	0.80	1.95	3.75	5.65
	GTM	0.90	3.70	7.80	11.50
	ATM	0.70	2.30	5.65	8.35
0 mg.L^{-1}	BTM	0.00	1.45	2.95	4.45
	GTM	0.00	2.45	5.90	8.00
	ATM	0.00	2.40	6.00	8.15
200 mg.L^{-1}	BTM	0.00	1.75	3.85	5.35
	GTM	0.00	2.80	6.60	9.00
	ATM	0.00	2.10	5.10	7.55
400 mg.L^{-1}	BTM	0.00	1.35	3.25	4.55
	GTM	0.00	2.60	6.10	8.25
	ATM	0.00	1.90	4.75	7.10
800 mg.L^{-1}	BTM	0.00	0.80	2.85	4.45
	GTM	0.00	2.05	5.75	7.65
	ATM	0.00	1.30	4.40	6.55
1 200 mg.L^{-1}	BTM	0.00	0.95	2.55	3.85
	GTM	0.00	2.00	5.60	7.80
	ATM	0.00	1.40	4.25	6.25

* Control granules activity measured directly after activation step, thus no exposure to WDWW

Table 3-7 Cumulative biogas volumes (mL) produced over the 40 h incubation period by granules exposed to treated wastewater in the different activity media

Cumulative Methane Volume					
Granule Treatment	**Test Medium**	**Incubation time (h)**			
		5	10	25	40
Control*	BTM	0.078	0.265	0.478	0.706
	GTM	0.090	0.783	1.879	2.894
	ATM	0.073	0.266	0.503	0.795
0 mg.L^{-1}	BTM	0.000	0.190	0.364	0.525
	GTM	0.000	0.520	1.272	1.735
	ATM	0.000	0.302	0.648	0.793
200 mg.L^{-1}	BTM	0.000	0.323	0.616	0.835
	GTM	0.000	0.673	1.711	2.378
	ATM	0.000	0.214	0.459	0.557
400 mg.L^{-1}	BTM	0.000	0.207	0.429	0.587
	GTM	0.000	0.462	1.604	2.168
	ATM	0.000	0.139	0.323	0.473
800 mg.L^{-1}	BTM	0.000	0.088	0.399	0.580
	GTM	0.000	0.406	1.336	1.841
	ATM	0.000	0.130	0.342	0.528
1 200 mg.L^{-1}	BTM	0.000	0.088	0.296	0.450
	GTM	0.000	0.285	1.112	1.674
	ATM	0.000	0.121	0.337	0.477

* Control granules activity measured directly after activation step, thus no exposure to WDWW

The biogas production rates (S_b) in GTM (Fig. 3-11) were also similar over the 40 h incubation period. After 10 h incubation the control granules and granules exposed to treated WDWW (0, 200 and 400 mg $O_3.L^{-1}$) had slightly higher gas production rates than the granules exposed to WDWW ozonated at 800 and 1 200 mg $O_3.L^{-1}$. The activity of the control granules was again higher after 5 h and also had a higher production rate after 40 h than found for the other granules. The difference in activity after 5 h can again be ascribed to these granules not needing to acclimatise to the WDWW. The higher rate after 40 h is most likely due to the fact that the GTM has a higher concentration of easily degradable carbon and that the control granules did not have to acclimatise to the wastewater.

The granules exposed to treated WDWW (0, 200 and 400 mg $O_3.L^{-1}$) had slightly higher biogas production rates (S_b) after 10 h incubation in ATM (Fig. 3-12) than the control granules and granules exposed to higher dose ozonated WDWWs (800 and 1 200 mg $O_3.L^{-1}$). Similar to the production rates in the BTM and GTM, the rate of biogas production of the control granules was higher after 5 h incubation.

The effect of WDWW (ozonated and unozonated) can also be seen when examining the cumulative biogas volume data given in Table 3-6. The overall activity, in terms of biogas production in BTM, GTM and ATM, of the control granules was higher than that of the granules exposed to the WDWWs over the 40 h incubation period. This is mainly due to the higher gas production rates achieved after 5 h incubation (Fig. 3-12 to 3-14). Cumulative biogas volumes in BTM, GTM and ATM for granules exposed to ozonated WDWW (200 and 400 mg $O_3.L^{-1}$) were slightly higher than granules exposed to unozonated WDWW (0 mg $O_3.L^{-1}$). This would suggest that lower ozonation doses are beneficial to increasing biodegradability of WDWW.

Although similar trends to the biogas production rates (S_b) were observed for the methane production rates (S_m), the rates were much lower and thus differences are more difficult to differentiate. In all three activity media (BTM, GTM and ATM) the control granules exhibit higher methane production rates (S_m) after 5 h incubation. This can be ascribed to the fact that the granules did not need to first acclimatise to the WDWW.

As was the case with the cumulative biogas volumes, the effect of WDWW (ozonated and unozonated) can be seen from the cumulative methane volumes listed in Table 3-7. The overall activity, in terms of methane production in BTM, GTM and ATM, of the control granules was higher than that of granules exposed to WDWW over the 40 h period. It can also be seen from Table 3-7 that the overall activity, in terms of methane production in BTM, GTM and ATM, of the granules exposed to ozonated WDWW (200 and

400 mg $O_3.L^{-1}$) was slightly higher than the granules exposed to unozonated WDWW (0 mg $O_3.L^{-1}$) and higher dosages of ozonation (800 and 1 200 mg $O_3.L^{-1}$). This could again indicate a beneficial application in terms of biodegradability when applying low ozone doses to WDWW.

Wine-distillery wastewater is known to be toxic to microbial populations as a result of the often high polyphenol concentrations (Alvarez *et al*., 2001; Martín *et al.*, 2002). The WDWW used in this study contained polyphenols (Table 3-2) and therefore it is reasonable to assume that the control granules should have exhibited the highest activity due to not being pre-exposed to polyphenol containing WDWW. As expected, the cumulative biogas and methane volumes showed that the control granules produce more biogas over the 40 h period for BTM, GTM and ATM (Tables 3-6 and 3-7). However, exposure of granules to WDWW ozonated at 200 mg $O_3.L^{-1}$ resulted in a higher cumulative biogas and methane volume than the 0 mg $O_3.L^{-1}$ dose.

From the data for biogas and methane production rates (S_b and S_m) (Fig. 3-10 to 3-15) and cumulative gas volumes (Tables 3-6 and 3-7), it would appear that lower ozone doses (i.e. 200 and 400 mg $O_3.L^{-1}$) were beneficial in increasing the biodegradability of WDWW. It can also be deduced that WDWW itself reduces the activity of granules, most likely due to the presence of polyphenols and other recalcitrant compounds in WDWW. This is evident from the lower activity of the unozonated WDWW compared to the control granules.

Conclusions

The WDWW used in this study was characterised and found to show a large variation over a time. These variations were mainly ascribed to the impact of the production cycle of the distillery.

In this study ozone was successfully utilised to firstly, reduce COD levels in wine-distillery wastewater, and secondly to increase the biodegradability of the wastewater. Ozonation of WDWWs was found to be effective in decreasing COD over a wide range of organic loads. For pre-wetland wastewater from the distillery, an average COD reduction of 271 mg COD.g O_3^{-1} was found, and for post-wetland effluent, an average of 103 mg COD.g O_3^{-1}. The difference in efficiencies was attributed to the biodegradation ability of the wetlands. Therefore based on the data from the study it was concluded that ozone has the highest efficiency in terms of COD oxidation in pre-wetland wastewater.

Granule activity was measured to determine the effect of ozone on biodegradability of WDWW and a low ozone dose was found to increase biodegradability of WDWW. A suitable granule source was selected based on activity in three activity media and then further activity trials carried out by exposing the granules to WDWW which had received different ozone doses. It was shown that a low ozone dose (200 - 400 mg $O_3.L^{-1}$) increased granule activity in terms of biogas, methane production, and cumulative gas volumes. It is known that WDWW is toxic to microbial populations due to high COD and polyphenols concentrations (Alvarez *et al*., 2001; Martín *et al.* 2002), and this would have an effect on a biological treatment method. Thus if an increase in biodegradability can be shown, it can be argued that ozone would have potential as a pre-treatment to a biological wetland system.

In this study it was shown that the use of ozone has potential as a pre-treatment of WDWW but the effect of ozone still needs to be demonstrated on a more practical scale than just the activity tests. Therefore, the next step would be to investigate the use of ozone as a pre- and/or post-treatment in combination with a biological treatment, namely constructed wetlands.

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CHAPTER 4

Use of wetlands combined with ozonation as a suitable option for the treatment of wine-distillery wastewaters

Summary

The effect of an ozonation treatment on the efficiency of a lab-scale wetland system treating wine-distillery wastewater (WDWW) being treated in a lab-scale wetland system was evaluated. Four trials were done to compare and quantify the differences between ozonated and unozonated wastewater. In the first trial two different ozonation modes were evaluated to determine whether either had an impact on the wetland efficiencies. The results showed that there were no major differences in terms of wetland efficiency between a continuous mode and a batch mode ozonation system. The second trial evaluated the effect of pre-ozonation on wetland efficiency treating a low COD wastewater (2 200 mg COD.L^{-1}). It was found that pre-ozonation resulted in an increase in the treatment efficiency from 62% to 73% COD reduction. Treatment efficiency in terms of the reduction of polyphenols, colour, total solids, suspended solids and phosphates were also greatly improved. Similar results were found when treating high COD wastewater obtained during the "peak season" (7 000 mg COD.L^{-1}), with pre-ozonation resulting in an increase in the treatment efficiency from 78% to 84% COD reduction. These results clearly showed that the use of pre-ozonated WDWW as feed for wetland systems is beneficial to the wetland efficiency. COD reduction increased towards the end of the 81 day trial period, possibly indicating that the wetlands required a longer acclimatisation period for higher COD wastewaters. Thus, the hydraulic retention time (HRT) was increased to 12 days in the fourth trial, investigating the efficiencies of the wetland treating high COD (7 000 mg.L^{-1}) WDWW. Increasing the HRT resulted in smaller differences in treatment efficiencies between the ozonated and un-ozonated wastewaters and this is probably due to the lengthened acclimatisation period. This, however, highlights the benefits of pre-ozonation which allows shorter HRT's to be used. The inclusion of ozone treatments can thus eliminate the need to construct a larger wetland, while maintaining a constant wastewater volume or by increasing the efficiency of a current wetland system and thus allowing the wastewater volume being fed, to be increased.

Introduction

Agricultural industries generate wastewaters that pose particular problems and challenges for the industry. These wastewaters contain high concentrations of nutrients and contribute to waste management problems when discharged directly to receiving waters. Wastewaters originating from distilleries are typically acidic (pH 3.5 - 5.0) and are characterised by a high organic content (sugars, alcohol, phenols and polyphenols, lipids) with a chemical oxygen demand (COD) range of 10 000 - 60 000 mg.L^{-1} (Benitez *et al.*, 1999; Martín *et al.,* 2002). Agricultural wastes must be treated prior to disposal and constructed wetlands (CWs) have been suggested as a potential treatment option prior to land application (Geary & Moore, 1999). The purpose of constructed wetlands is to artificially create natural wetlands, which are specifically chosen to be suitable for treating the desired effluent.

Constructed wetlands are natural wastewater treatment systems that combine biological, chemical and physical treatment processes (Crites, 1994). Wastewater treatment occurs through a combination of complex interactions between plant (bioflora) uptake, the filtering capacity of the substrate, and the activity of the inherent microbial community (Kadlec & Knight, 1996). Thus, the biodegradation ability of plants and microorganisms is utilised to break down organic components in the wastewater. The purification process in a wetland typically includes: settlement of suspended solids, uptake of dissolved nutrients into the sediment, plants and microorganisms, and mineralization of organic material (Meuleman & Verhoeven, 1999).

An artificial wetland consists of a lined excavation containing a bed of porous gravel or ash, in which aquatic vegetation is planted. The gravel must be chosen to minimize clogging, and plants are chosen for their root length and pH tolerance (Sheperd *et al*., 2001). Due to the seasonal variation in wine-distillery wastewater (WDWW), a wide pH tolerance is necessary. A sub-surface flow is typically utilised, where the wastewater will enter on one side of the wetland and flow through the gravel and root zone until exiting and being collected on the outflow side.

Constructed wetlands are desirable due to the utilisation of natural processes, simple construction, operation and maintenance, process stability, little excess sludge production and cost effectiveness (Haberl, 1999). Wastewater treatment performance is influenced by the volume of the wetland, rate of wastewater loading and the hydraulic retention time (HRT) (Schutes, 2001). Disadvantages of wetland systems are their slow rate of operation in comparison to conventional wastewater treatment technology, and the large amount of land required (Ayaz & Akça, 2000). Another disadvantage is that overloading can result in a reduced efficiency and thus the necessary loading rates need to be calculated for each application when constructing a wetland for a specific treatment. It is a well known fact that wetlands struggle to maintain efficiency under high load influent, such as when treating wastewater from a distillery.

Ozonation has been shown to have a high treatment potential when used in combination with a biological system as a pre-treatment option to improve biodegradation ability. The ozonation of wastewaters usually increases biodegradability (Martín *et al.*, 2002) by means of toxic polyphenol reduction (Alvarez *et al*., 2001). Polyphenol reduction is a major criterion for increasing biodegradability and these harmful pollutants need to be degraded before most biological treatments are applied (Benitez *et al*., 1999). Polyphenols are aromatic compounds that are prone to attack of electrophilic agents like ozone (Alvarez *et al*., 2001). Therefore an ozonation process can improve efficiency and reduce the total treatment time of a wastewater treatment process (Chen *et al.*, 2004).

The aim of this study was to determine whether pre-ozonation could improve the biodegradability of wine-distillery wastewater (WDWW) to be treated by a wetland system, and thereby improve the organic component removal efficiency. This will be done by monitoring the effect on components present in the wastewaters before and after ozonation, as well as after pre-ozonated wastewater has been treated in a wetland.

Materials and Methods

Wastewater and substrate

Wine-distillery wastewater was obtained from a distillery in the Boland region of South Africa during the peak and off-seasons of 2004 and 2005. The wastewaters (unozonated and ozonated) were stored in 25 L drums at -18°C. During the investigation wastewater drums were defrosted as required and kept at 4°C. Tap water was used to dilute the wastewater to the desired COD levels used in the trials.

Ozonation of wastewater

Ozonation was applied by using an ozone generator (Parc Scientific, Ifafi) that produced O_3 at a concentration of 4.82 $g.h^{-1}$ at a flow rate of 4 $L.min^{-1}$ as determined by the Iodometric Method (APHA, 1998). Diluted and undiluted wine-distillery wastewater was ozonated in a continuous mode through a venturi system on a 50 L recirculating contacting system for pre-determined time/ozone dose combinations. For smaller amounts of wine-distillery wastewater, ozonation was applied in a glass bubble column, with a volume of 2

L. The chemical oxygen demand (COD) removal was determined before and at various time intervals during ozonation.

Analytical methods

The following wastewater parameters were monitored according to Standard Methods (APHA, 1998): pH, alkalinity (expressed as mg $CaCO_3.L^{-1}$), total solids (TS), total suspended solids (TSS), total volatile solids (VS) and total volatile suspended solids (VSS). Conductivity was determined using a Hanna Instruments (HI8733) conductivity meter. Chemical oxygen demand and orthophosphate phosphorous (PO_4^{3-}) were determined colorimetrically using a DR 2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (APHA, 1998). Total polyphenol content was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965). All analyses were done in triplicate.

Pilot scale wetland trials

Two pilot scale wetlands were constructed by ARC Infruitec-Nietvoorbij to simulate the effect of large scale wetlands. The wetlands were built from Perspex with a total volume of 30 L (0.5 m × 0.3 m × 0.4 m). Washed gravel stones were used as a substrate for nine plants in each wetland. The hydraulic retention time (HRT) was set to 9 days. Wastewater with a standardized COD was used for each trial and was prepared by dilution and kept frozen until required. Therefore during each trial, <u>untreated wastewater</u> refers to the standardised diluted wastewater, and <u>treated wastewater</u> refers to pre-ozonated standardised diluted wastewater. Pre-ozonated wastewater was prepared by ozonating raw wastewater in a continuous mode through a venturi system on the 50 L recirculating contacting system using an ozone generator producing 4.82 $g.h^{-1}$ O_3 at a flow rate of 4 $L.min^{-1}$.

The wastewater used in these trials was not wastewater directly from the distillery (COD = 10 000 - 30 000 $mg.L^{-1}$), but wastewater obtained from the 3rd settlement dam of the treatment works (Fig. 3-1). By the time the wastewater reached this dam, the COD content had been reduced by anaerobic degradation, aerobic degradation and settling of solids. Thus, the wastewater being used in this study was representative of the typical wastewater that was being treated in a full-scale wetland system on the site. For WDWW, COD, polyphenols, pH, conductivity, colour and solids content are of great significance, and thus the study will focus on these components. The end goal of the trials was to be able to compare and quantify the differences in using ozonated and unozonated wastewater.

Trial 1 - The first trial was designed to determine whether the mode of ozone application affected the efficiency of the wetland treatment. Two ozonation modes were compared, namely a continuous mode (simulating an in-line ozonation), and a batch mode. Continuous mode (CM) ozonated wastewater was defrosted and ozonated in the bubble column, for small quantities, directly prior to being fed to the wetland. Batch mode (BM) ozonated wastewater was ozonated in the continuous recirculating system, for large quantities, and aliquots were then frozen until required.

The first wetland received wastewater treated by the CM, and the second wetland received wastewater that had been ozonated in the BM process. During Trial 1, both wetlands were first fed untreated wastewater (unozonated) for a period of 27 days so that the biological part of the wetland setup could acclimatise to the WDWW. Hereafter, treated wastewater from the continuous (CM) or the batch modes (BM), was fed to the wetlands. Parameters, including COD, pH, alkalinity, phosphates, conductivity, polyphenols, solids and colour in the wetland effluents were monitored over a total 63 day period at regular 9 day intervals.

Trial 2 – In this trial the effect of pre-ozonation on wetland efficiency was investigated. A COD concentration of 2 200 $mg.L^{-1}$, representative of the “off-season” period of the distillery, was used as feed. During Trial 2 both wetlands were again operated for a period of 27 days on untreated wastewater, where after one wetland was designated as the <u>Control Wetland</u> and the feed continued on <u>untreated wastewater</u> while the second wetland, the <u>Experimental Wetland</u>, was fed <u>treated wastewater</u>. The same parameters as in Trial 1 were monitored over a total 72 day period at 9 day intervals.

Trials 3 and 4 – In these trials the effect of pre-ozonation on wetland performance at a higher COD concentration (7 100 $mg.L^{-1}$) similar to the concentration typically obtained during the “peak-season”, were investigated. A HRT of 9 days was used in Trial 3, and a HRT of 12 days during Trial 4. During Trials 3 and 4 both wetlands were first fed (1st retention time) on untreated wastewater at a COD of 3 750 $mg.L^{-1}$, then (2nd retention time) at a COD of 5 500 $mg.L^{-1}$, and then (3rd retention time) at a COD of 7 100 $mg.L^{-1}$. Thereafter, the Control Wetland was fed with only untreated wastewater while the other wetland was fed treated wastewater (both at *ca*. 7100 $mg.L^{-1}$) for the rest of the trial period. The same wastewater efficiency parameters as in Trial 1 were monitored over the total trial period at each HRT.

RESULTS AND DISCUSSION

TRIAL 1- Mode of ozone application

The data in Table 4-1 summarises the average effect of the direct ozonation on the untreated wastewater. The ozonation resulted in a COD reduction of 37%, reducing the COD from 2 000 to 1 260 mg.L^{-1}. It is known that ozonation tends to increase alkalinity due to precipitation of carbonates as a result of mineralization of organic compounds (Alvarez *et al*., 2001), and this could be the reason for the increase in alkalinity from 1 150 to 1 737 mg.L^{-1}. The precipitation of carbonates may also contribute to the increase in solids content, which increased from 2.70 to 3.55 g.L^{-1}. Polyphenol reduction was only 1%, and this is due to the fact that the initial levels were very low in the untreated wastewater. Colour decreased from an UV 254 nm absorbance level of 2.11 to 1.80, and this can probably be attributed to ozone's ability to directly attack the C-double bonds in aromatic and chromophoric molecules leading to the formation of 'bleached' products, like aliphatic acids, ketones and aldehydes (Gottschalk *et al*., 2000). Based on the data obtained in this first section of Trial 1 it was decided to determine if the ozonation delivery mode has an impact on the reduction efficiencies.

The results of Trial 1, done over a period of 63 days, on the mode of ozonation are summarised in Table 4-2 and Fig. 4-1. The effluent of day 63 was chosen to be representative of the stabilisation of the wetlands and these are the results given in Table 4-2. The data showed that the batch mode (BM) resulted in a higher (30%) COD reduction (232 mg.L^{-1}) than the continuous mode (CM) (302 mg.L^{-1}). This can primarily be attributed to the BM using the more efficient recirculating ozonation method, and the CM using the bubble column, in applying ozone. During this study the alkalinity also increased to 2 400 mg.L^{-1}. This large increase in alkalinity was attributed to carbonate washout from the dolomitic gravel that was used in the wetland.

The pH is high (pH 8 to 9), which is atypical for a wetland system (Tables 4-1 and 4-2), and this may be the main cause of the dying of the plants, which occurred towards the end of the trial in both wetlands. In the study it was found that the conductivity increased, and the levels were still well above the legal requirements of below 150 mS.m^{-1} (Van Schoor, 2005). Conductivity increases during ozonation because salts in the WDWW are oxidised which in turn liberates ions (Gottschalk *et al*., 2000). Polyphenol reduction was found to increase with pre-ozonation, but these values were low. Phosphates increased, and this may be attributed to ozone degrading complex compounds to free phosphate ions.

Table 4-1 The effect of ozonation (106 mg.O_3.L^{-1}) on untreated wastewater (Trial 1)

Parameters	Untreated Wastewater	Treated Wastewater	Change (%)
COD (mg.L^{-1})	2000	1260	- 37
pH	8.21	8.40	na
Alkalinity (as mg $CaCO_3$.L^{-1})	1150	1737	51
Phosphates (mg PO_4^{3-} .L^{-1})	92	122	33
Conductivity (mS.m^{-1})	248	309	25
Polyphenols (mg.L^{-1})	1.64	1.62	- 1.0
Total Solids (g.L^{-1})	2.70	3.55	31
Total Volatile Solids (g.L^{-1})	0.65	1.15	77
Total Susp. Solids (g.L^{-1})	0.1	0.2	100
Total Volatile Susp. Solids (g.L^{-1})	0.06	0.20	333
Colour 254 nm	2.11	1.80	na

na = not applicable

The total, volatile and suspended solids generally showed little change, but these results can be influenced by the wetlands themselves. It is possible that due to the constant algal growth and plant matter shedding that took place in the wetland system, the filtering capacity of the wetlands may quickly have become saturated, leading to an increase in solids content.

The data in Fig. 4-1 illustrates the effect of the two pre-ozonation modes (BM and CM) on the wetland effluent. After the 27 days acclimatisation period for both wetlands on untreated wastewater (Table 4-1), the wetlands had an effluent with an average COD of 890 mg.L^{-1}, which gives a COD reduction of about 56%. In contrast, pre-ozonation of the raw effluent (Table 4-1) and then using this as the influent (day 27) resulted in wetland effluents with a CODs as low as 232 mg.L^{-1}, giving a COD reduction of 82% by day 63 (Table 4-2). Both treatment modes resulted in similar COD reduction trends (Fig. 4-1), therefore the treatment modes did not appear to strongly impact wetland efficiency. The difference in final COD can be attributed to the difference in the method that ozone was applied for the two treatments.

Table 4-2 The influence pre-ozonation using the batch and continuous modes on the composition of effluent from both wetlands (Trial 1 on day 63)

Parameters	Treated Wastewater Influent (Day 27)	Batch Ozonated Mode (Day 63)	Continuous Ozonated Mode (Day 63)	Difference between Modes (%)
COD ($mg.L^{-1}$)	1260	232	302	30
pH	8.40	8.99	9.04	na
Alkalinity (as mg $CaCO_3.L^{-1}$)	1737	2400	2375	na
Phosphates (mg PO_4^{3-} $.L^{-1}$)	122	122	123	0
Conductivity ($mS.m^{-1}$)	309	425	427	0
Polyphenols ($mg.L^{-1}$)	1.62	1.16	1.28	1
Total Solids ($g.L^{-1}$)	3.55	4.25	4.18	- 2
Total Volatile Solids ($g.L^{-1}$)	1.15	0.79	0.86	9
Total Susp. Solids ($g.L^{-1}$)	0.2	0.045	0.055	22
Total Volatile Susp. Solids ($g.L^{-1}$)	0.20	0.035	0.045	29
Colour 254 nm	1.80	2.27	2.56	na

na = not applicable

However, the use of treated wastewater (pre-ozonated) did lead to a much lower COD in the wetland effluents, as can be seen in the difference between the day 27 effluent and the day 63 effluents (Fig. 4-1). A post-ozonation investigation was also done on the wetland effluents that had received the BM pre-ozonated influent. This was done in order to determine whether post-ozonation would further increase efficiency. Post-ozonation (Table 4-3) gave only a 10% further COD decrease. The polyphenols, total solids and colour also showed further reductions. This can be attributed to the wetlands probably utilising more available biodegradable components present in the wastewater. It was also possible that the post-wetland effluents would probably consist of mainly compounds that are more recalcitrant and less biodegradable.

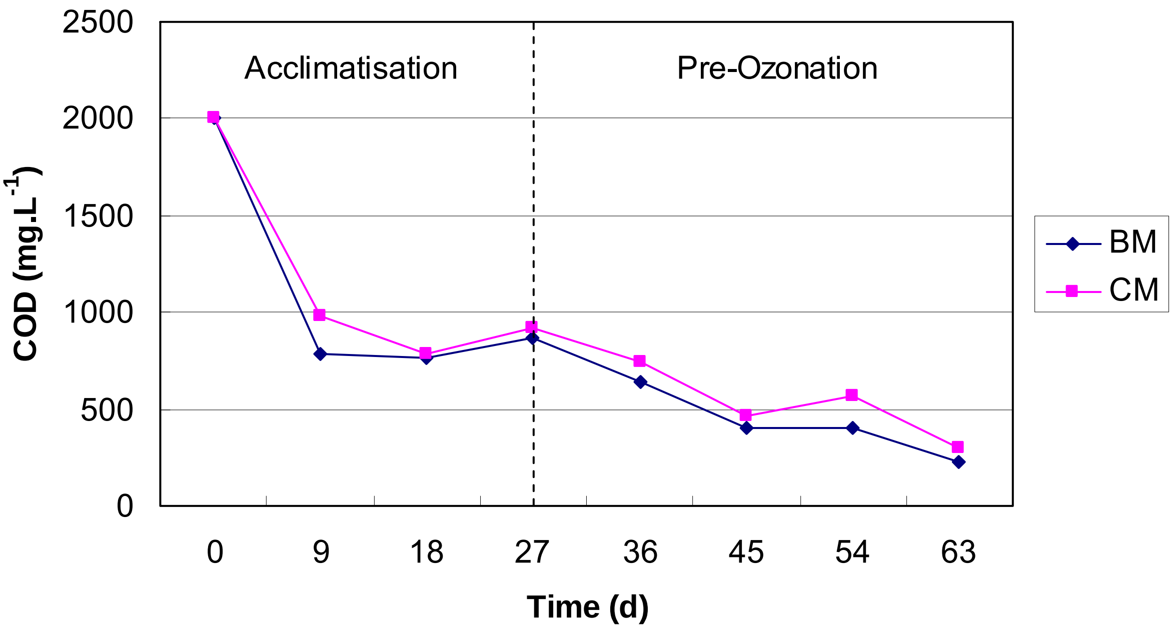

Figure 4-1 The influence of ozonation of the influent using either a batch mode (BM) or a continuous mode (CM) on the COD of the wetlands effluent over the 63 day period of Trial 1. (Acclimatisation phase – both wetlands fed untreated wastewater. Pre-ozonation phase – the two wetlands were fed batch and continuous mode ozonated wastewater. The values plotted are the average of duplicate samples with a variation was less than 5%).

The Trial 1 study was of value in allowing the parameters for the further trials to be established in terms of wastewater storage method, mode of ozonation application, characterisation of components, setting of a suitable hydraulic retention time, acclimatising the wetlands to WDWW and general indication of how wetlands perform when fed with untreated and treated influent as well as the impact of post-ozonation on the composition of final wetland effluent.

Table 4-3 The effect of post-ozonation (400 mg.O_3.L^{-1}) in Trial 1 on effluent from the wetland receiving batch ozonated (BM) influent wastewater after the 63 day trial period

Parameters	Batch Ozonated Effluent	Post-Ozonated Effluent	Change (%)
COD (mg.L^{-1})	232	209	- 10
pH	8.99	8.76	na
Alkalinity (as mg $CaCO_3$.L^{-1})	2400	2031	- 15
Phosphates (mg PO_4^{3-} .L^{-1})	122	105	- 14
Conductivity (mS.m^{-1})	425	371	- 13
Polyphenols (mg.L^{-1})	1.16	0.90	- 24
Total Solids (g.L^{-1})	4.25	3.41	- 20
Total Volatile Solids (g.L^{-1})	0.79	0.81	3
Total Susp Solids (g.L^{-1})	0.045	0.05	11
Total Volatile Susp Solids (g.L^{-1})	0.035	0.025	- 40
Colour 254 nm	2.27	0.79	na

na = not applicable

TRIAL 2 – The effect of pre-ozonation of WDWW on wetland efficiency

The aim of Trial 2 was investigate the effect of pre-ozonation when compared to a Control Wetland. Due to the COD levels still showing a decreasing trend (Fig. 4-1) in Trial 1, it was decided to extend the hydraulic retention time in Trial 2 to 72 days, giving the wetlands more time to reach maximum removal efficiency. Additionally, as the aim of this study was to monitor the wetlands over time, and it should firstly be possible to establish if pre-ozonation contributes positively to the degradation of the wastewater, and secondly, if the ozonation has any undesirable effect on the treatment efficiency.

The results of the investigation into the effect of pre-ozonation on the parameter efficiency of WDWW (COD, pH, alkalinity, phosphates, conductivity, polyphenols, solids and colour), are given in Tables 4-4 to 4-8. After the initial 27 day acclimatisation period (Fig. 4-3 to 4-7), the Control Wetland was only fed with untreated wastewater (Table 4-4) and the Experimental Wetland with treated wastewater (Table 4-5) for the remainder of the 72 day trial.

The data (Table 4-5) showed that pre-ozonation resulted in a higher total COD reduction (73%) for the Experimental Wetland, while the Control Wetland fed with the unozonated wastewater showed only a 62% reduction (Table 4-4 and Fig. 4-3). From this improved COD reduction it was concluded that the application of ozone led to an increase in the Experimental Wetlands COD removal efficiency.

The high pH levels (8.8 – 9.1) (Tables 4-4 and 4-5) were similar to those obtained in Trial 1, but in this case did not appear to have a negative effect on the wetland plant life. Alkalinity was also found to increase by 24% in the Control Wetland effluent to 2 175 $mg.L^{-1}$ (Table 4-4), while in the Experimental Wetland increased by only 6% to 2 517 $mg.L^{-1}$. As was found in Trial 1, this was attributed to the possibility of carbonates leaching out from the dolomitic gravel used in the wetland construction. Phosphate reduction was found to be higher for the Experimental Wetland with a 62% reduction when compared to the 38% reduction for the Control Wetland (Fig. 4-4). Conductivity was also found to increase for both wetlands, which was similar to the data for Trial 1 and may be attributed to leaching of salts from the gravel, but the values still remained well above the legal requirement.

Polyphenol reduction also showed larger variations, with 40% reduction being achieved in the Experimental Wetland, compared to the 31% reduction for the Control Wetland. It is known that polyphenols are highly toxic for most biological systems (Alvarez *et al*., 2001), and good polyphenol reductions may contribute to the long term efficiency of a wetland system.

Table 4-4 Compositional changes in the effluent of the Control Wetland fed with untreated wastewater for 72 days (Trial 2)

Parameters	Wetland Influent	Wetland Effluent	Change (%)
COD ($mg.L^{-1}$)	2 204	839	- 62
pH	9.1	8.9	na
Alkalinity (as mg $CaCO_3.L^{-1}$)	1 750	2 175	24
Phosphates (mg $PO_4^{3-}.L^{-1}$)	378	236	- 38
Conductivity ($mS.m^{-1}$)	350	440	27
Polyphenols ($mg.L^{-1}$)	2.4	1.7	- 31
Total Solids ($mg.L^{-1}$)	4 560	4 620	1
Total Vol. Solids ($mg.L^{-1}$)	1 920	1 230	- 36
Total Susp. Solids ($g.L^{-1}$)	530	420	- 21
Total Vol. Susp. Solids ($mg.L^{-1}$)	420	370	- 12
Colour (254 nm)	3.8	3.8	na
Colour (475 nm)	2.0	1.1	na

na = not applicable

Table 4-5 Compositional changes in the effluent of the Experimental Wetland fed with treated (120 mg.O_3.L^{-1}) wastewater for 72 days (Trial 2)

Parameters	Wetland Influent	Wetland Effluent	Change (%)
COD (mg.L^{-1})	2 327	628	- 73
pH	8.9	8.8	na
Alkalinity (as mg $CaCO_3$.L^{-1})	2 375	2 517	6
Phosphates (mg PO_4^{3-} .L^{-1})	415	157	- 62
Conductivity (mS.m^{-1})	460	530	14
Polyphenols (mg.L^{-1})	2.2	1.3	- 40
Total Solids (mg.L^{-1})	5 870	5 290	- 10
Total Vol. Solids (mg.L^{-1})	2 110	1 270	- 40
Total Susp. Solids (mg.L^{-1})	480	210	- 56
Total Vol. Susp. Solids (mg.L^{-1})	430	150	- 65
Colour (254 nm)	4.0	3.2	na
Colour (475 nm)	1.6	0.5	na

na = not applicable

The Experimental Wetland had a higher total solids content and lower suspended solids content (Table 4-5) than the Control Wetland system (Table 4-4). It was also found that the Control Wetland effluent had a higher suspended solids content (420 mg .L^{-1}) when compared to 210 mg .L^{-1} for the Experimental Wetland. This was also evident from the appearance of the two effluents colour (Fig. 4-2). In this study colour absorbance was also measured at UV 254 and 475 nm. It is known (Gottschalk *et al*., 2000) that at these wavelengths humic substances and dopachrome are measured, which are precursors to more toxic substances and give an indication of organic pollution. In other studies it has been shown that ozone effectively breaks down these substances (Gottschalk *et al*., 2000). This is confirmed by the data obtained in this study as shown in Fig. 4-6 and 4-7. Phosphate reduction can be attributed to uptake by the plants in the wetlands for growth.

Figure 4-2 Colour of the effluents from the Control Wetland (left) and the Experimental Wetlands (right) during Trial 2.

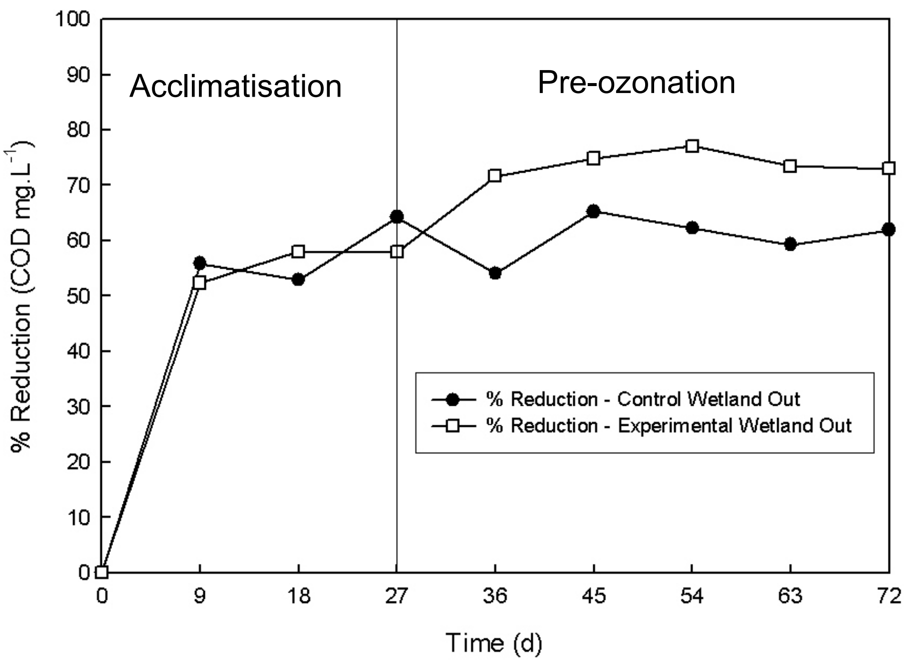

Figure 4-3 Reduction of COD (%) over time for Trial 2. (During the acclimatisation phase, untreated wastewater was fed to both wetlands, and for the trial phase, treated wastewater was fed to the Experimental Wetland).

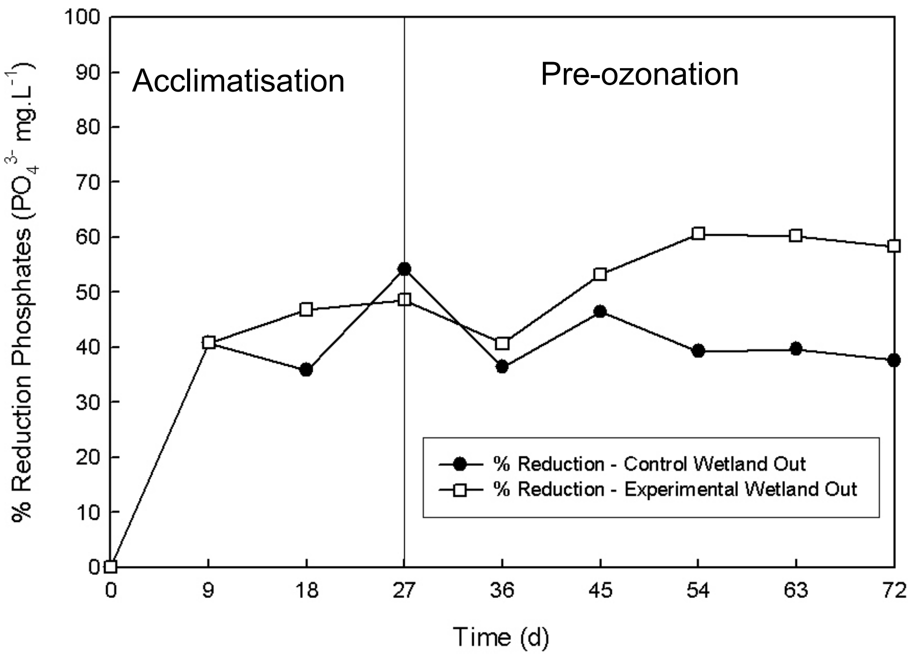

Figure 4-4 Reduction of phosphate (%) over time for Trial 2. (During the acclimatisation phase, untreated wastewater was fed to both wetlands, and for the trial phase, treated wastewater was fed to the Experimental Wetland).

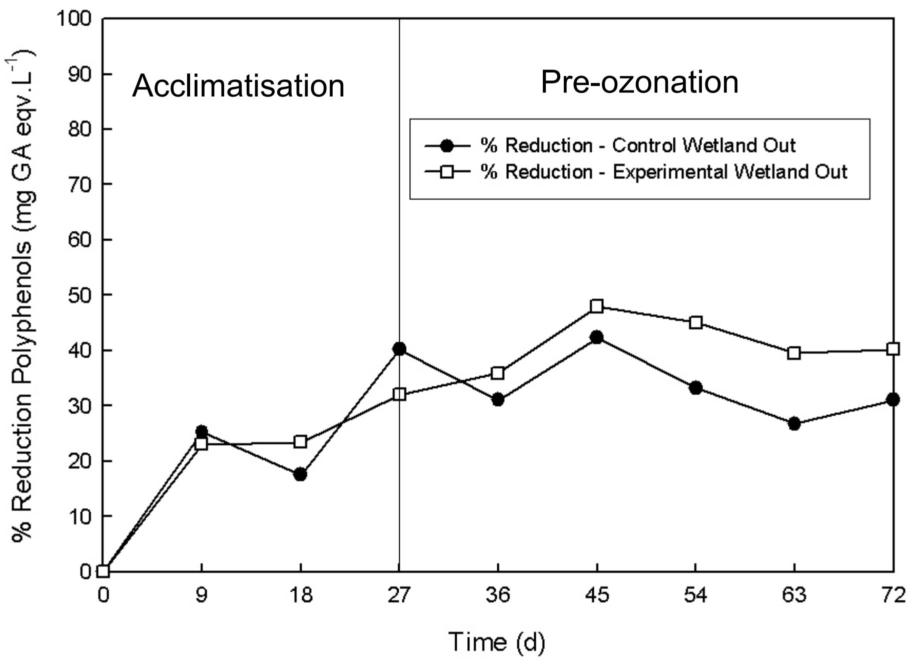

Figure 4-5 Reduction of polyphenols (%) over time for Trial 2. (During the acclimatisation phase, untreated wastewater was fed to both wetlands, and for the trial phase, treated wastewater was fed to the Experimental Wetland).

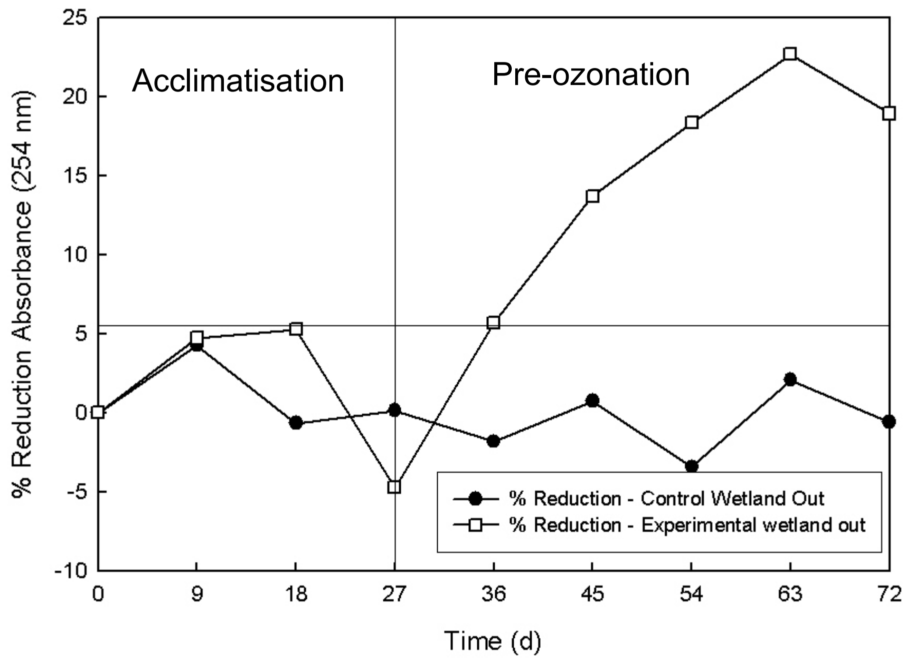

Figure 4-6 Change of absorbance at UV 254 nm (%) over time for Trial 2. (During the acclimatisation phase, untreated wastewater was fed to both wetlands, and for the trial phase, treated wastewater was fed to the Experimental Wetland).

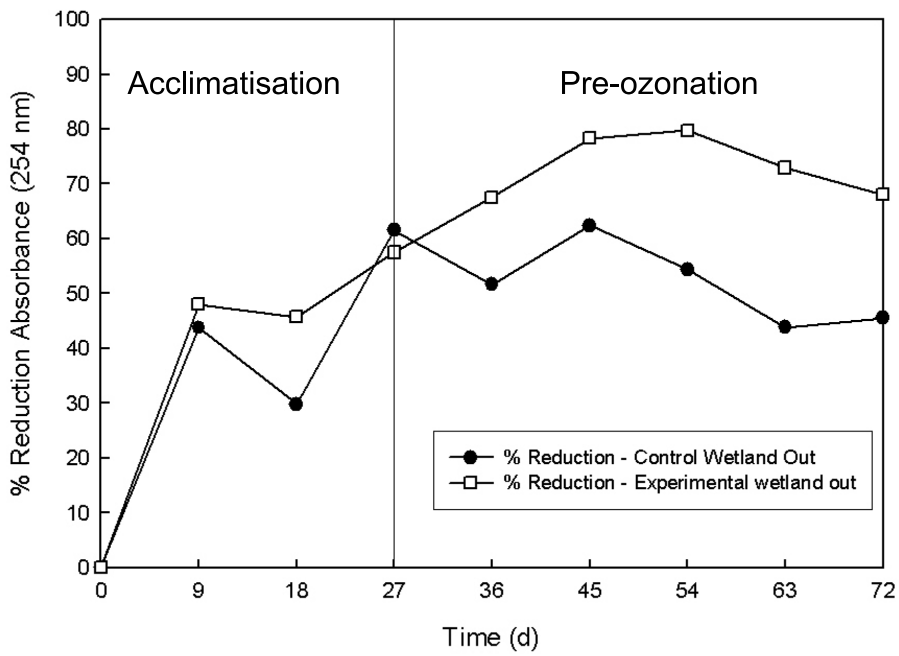

Figure 4-7 Change of absorbance at UV 475 nm (%) over time for Trial 2. (During the acclimatisation phase, untreated wastewater was fed to both wetlands, and for the trial phase, treated wastewater was fed to the Experimental Wetland).

In Fig. 4-3 to 4-7, a discernable effect can be observed from day 27 onwards when the feed of treated wastewater was initiated to the Experimental Wetland. By day 72 of the trial period, the wetlands appeared to have stabilised (more consistent results). It was also clear that the Experimental Wetland gave higher removal rates (higher COD, phosphate, polyphenol and colour reductions) than the Control Wetland. It was thus concluded that the higher reductions obtained showed that pre-ozonation did lead to an increase in the efficiency of the Experimental Wetland. The data showed that polyphenol reduction (Fig. 4-5) was higher for the Experimental Wetland and may have resulted in a lower toxicity level of the WDWW feed. Colour reduction was also found to be higher for the Experimental Wetland (Fig. 4-6 and 4-7). This was expected since ozone has is know to be a bleaching agent (Alfafara *et al.*, 2000; Gottschalk *et al*., 2000). Based on especially the increased colour reduction, the excellent efficiency of the Experimental Wetland can probably be attributed to an increase in biodegradability as a result of the ozone breaking down the more complex organic components in the wastewater (Kamenev *et al*., 2003).

The results of the post-ozonation of the Control and Experimental Wetland effluents are summarised in Tables 4-6 and 4-7. In this section of the study the Control Wetland effluent showed higher reductions, especially in terms of COD (32% vs 11%), polyphenols (43% vs 35%) and phosphates (45% vs 22%) respectively. The higher reductions of the Control Wetland effluent after post-ozonation was attributed to the fact that the Control Wetland still contained biodegradable components, which had already been removed in the Experimental Wetland during the pre-ozonation step. However, the final concentration of components in the effluents was very similar. The Control Wetland effluent, after post-ozonation, reached a COD value of 569 $mg.L^{-1}$, while the effluent from the Experimental Wetland receiving the pre-treated wastewater reached 559 $mg.L^{-1}$. Polyphenol reduction values were also similar, with the Control Wetland effluent reaching a value of 0.95 $mg.L^{-1}$ and effluent from the Experimental Wetland reaching 0.87 $mg.L^{-1}$ respectively. Phosphate reduction may be attributed to ozone oxidising the remaining phosphate ions which have already been degraded by the wetlands.

Table 4-6 Compositional changes in the effluent of the Control Wetland effluent when post-ozonated (400 mg.L^{-1}) after day 72 (Trial 2)

Parameter	Effluent on day 72	Post-ozonated effluent from day 72	Change (%)
COD (mg.L^{-1})	839	569	- 32
pH	8.96	8.54	Na
Alkalinity (as mg $CaCO_3$.L^{-1})	2175	1933	- 11
Phosphates (mg PO_4^{3-} .L^{-1})	236	131	- 45
Conductivity (mS.m^{-1})	443	412	- 7
Polyphenols (mg.L^{-1})	1.68	0.95	- 43
Total Solids (g.L^{-1})	4.62	4.19	- 9
Total Vol Solids (g.L^{-1})	1.23	1.16	- 6
Total Susp Solids (g.L^{-1})	0.42	0.097	- 77
Total Vol Susp Solids (g.L^{-1})	0.37	0.085	- 77
Colour (254 nm)	3.84	1.63	na
Colour (475 nm)	1.081	0.15	na

na = not applicable

The overall wetland efficiency including the post-ozonation is summarised in Table 4-8. The very similar overall removal efficiencies obtained for the Control and Experimental Wetlands (Table 4-8) may be an indication that there is a minimum level to which ozonation is effective, and thus further ozonation is unnecessary or may even be ineffective.

Table 4-7 Compositional changes in the effluent of the Experimental Wetland effluent when post-ozonated (400 mg.L^{-1}) after day 72 (Trial 2)

Parameter	Effluent on day 72	Post-ozonated effluent from day 72	Change (%)
COD (mg.L^{-1})	628	559	- 11
pH	8.78	8.31	Na
Alkalinity (as mg $CaCO_3$.L^{-1})	2517	2304	- 8
Phosphates (mg PO_4^{3-} .L^{-1})	157	122	- 22
Conductivity (mS.m^{-1})	531	483	- 9
Polyphenols (mg.L^{-1})	1.33	0.87	- 35
Total Solids (g.L^{-1})	5.29	4.72	- 11
Total Vol Solids (g.L^{-1})	1.27	1.13	- 11
Total Susp Solids (g.L^{-1})	0.21	0.067	- 68
Total Vol Susp Solids (g.L^{-1})	0.15	0.043	- 71
Colour (254 nm)	3.24	1.41	na
Colour (475 nm)	0.5	0.076	na

na = not applicable

Table 4-8 Comparison between the overall removal efficiencies of the Control and Experimental Wetlands of Trial 2 in combination with a final post-ozonation treatment step after day 72

Parameter	Control Wetland	Experimental Wetland
COD ($mg.L^{-1}$)	- 74	- 75
Alkalinity (as mg $CaCO_3.L^{-1}$)	10	32
Phosphates (mg $PO_4^{3-}.L^{-1}$)	- 65	- 68
Conductivity ($mS.m^{-1}$)	18	38
Polyphenols ($mg.L^{-1}$)	- 61	- 64
Total Solids ($g.L^{-1}$)	- 8	4
Total Vol. Solids ($g.L^{-1}$)	- 40	- 41
Total Susp. Solids ($g.L^{-1}$)	- 82	- 87
Total Vol. Susp. Solids ($g.L^{-1}$)	- 80	- 90

TRIAL 3 – Higher organic loading rate

The aim of Trial 3 was to establish if pre-ozonation would affect the reduction rates of components in wastewater during the high load "peak-season" experienced by the wine distillery industry. By monitoring the trial over time, it should be possible to establish if pre-ozonation could contribute positively to the degradation of wastewater with higher COD concentrations. The characteristics of the wastewater used during Trial 3 are summarised in Table 4-9. The reason for adopting a "step-wise" increasing of the influent COD was so that the plants and microbial communities would have time to adapt to higher organic loads. Once both wetlands had acclimatised (day 36), the Experimental Wetland was fed treated wastewater for the remainder of the trial.

The data in Tables 4-10 and 4-11 show the differences between the Control and Experimental Wetlands after being fed with full load influent from day 36 till day 81. The COD was reduced in the Control Wetland by 78% and by 84% in the Experimental Wetland. As was found in Trial 2, the increased COD reduction efficiency can be attributed to an increase in the biodegradability of the wastewater as it is known that the application of ozone improves biological treatment methods (Beltrán *et al.*, 1997). Once again, as found in Trials 1 and 2, the alkalinity in both effluents remained high in the region of 2 600 $mg.L^{-1}$ (Tables 4-10 and 4-11). Phosphate reduction was higher for the Experimental Wetland (62%) than for the Control Wetland (53%). Conductivity remained fairly stable and reached similar levels for both wetlands.

The polyphenol content in the effluent of the Experimental Wetland was reduced by 76%, while for the Control Wetland the effluent showed a reduction of 72% (Tables 4-10 and 4-11). The residual phenolic compound concentration has been reported to be one of the major criteria determining the biodegradability of a solution (Chen *et al.*, 2004). Therefore it was concluded that the lower polyphenols content of the Experimental Wetland may also have contributed to increased biodegradability of the wastewater.

Both wetlands had similar solids and suspended solids concentrations (Tables 4-10 and 4-11). However, the Control Wetland appeared to have a finer type in contrast to the solids from the Experimental Wetland which consisted of large pieces (Fig. 4-8). Colour (at 275 and 475 nm), showed greater reductions in the Experimental Wetland than in the Control Wetland (Tables 4-10 and 4-11). These higher colour reductions for the Experimental Wetland is in agreement with the observations made in Trial 2.

One aspect of a wetland that was not characterised in this study, but would be extremely important in terms of treatment efficiency, is the health of the plant material present in the wetland. Plant material could be harvested and measured to quantify differences between plant growth In this study it was without a doubt found that better plant growth took place in the Experimental Wetland which had been fed with treated WDWW. It can be seen in Fig. 4-9 how the wetlands looked, and the better plant growth (difference in heights) in the Experimental Wetland is clearly visible.

Table 4-9 Composition of feed used to acclimatise wetlands to higher COD loads. Both wetlands were fed untreated wastewater for the first three retention times (up to day 36). After day 36 the Control Wetland was fed with full strength untreated wastewater and the Experimental Wetland with treated wastewater

Parameter	Both Wetlands	Both Wetlands	Control Wetland	Experimental Wetland
	½ Strength influent (Day 0 – 8) (1st retention)	**¾ Strength influent (Day 9 – 18) (2nd retention)**	**Full strength influent (Day 19 – 81) (3rd retention)**	**Treated influent (Day 36 – 81) (3rd retention)**
COD ($mg.L^{-1}$)	3762	5447	7000	7150
pH	7.46	7.47	7.51	7.65
Alkalinity (as mg $CaCO_3.L^{-1}$)	1317	1958	2400	2700
PO_4^{3-} (mg PO_4^{3-} $.L^{-1}$)	208	301	333	358
Conductivity ($mS.m^{-1}$)	290	408	533	566
Polyphenols ($mg.L^{-1}$)	3.43	4.42	5.87	3.85
Total Solids ($g.L^{-1}$)	8.84	5.85	7.63	4.62
Total Vol. Solids ($g.L^{-1}$)	4.88	3.23	4.23	2.69
Total Susp. Solids ($g.L^{-1}$)	0.55	0.83	1.07	1.28
Total Vol. Susp. Solids ($g.L^{-1}$)	0.42	0.68	0.81	0.83
Colour 475 nm	1.22	1.58	1.90	1.88

Table 4-10 Composition of the influent (day 36) and effluent (day 81) of the Control Wetland

Parameter	Influent from day 36	Effluent on day 81	Change (%)
COD ($mg.L^{-1}$)	7000	1528	- 78
pH	7.51	7.74	na
Alkalinity (as mg $CaCO_3.L^{-1}$)	2400	2567	7
Phosphates (mg PO_4^{3-} $.L^{-1}$)	333	155	- 53
Conductivity ($mS.m^{-1}$)	533	535	0
Polyphenols ($mg.L^{-1}$)	5.87	1.64	- 72
Total Solids ($g.L^{-1}$)	7.63	5.55	- 27
Total Susp Solids ($g.L^{-1}$)	1.07	0.23	- 78
Colour 254 nm	4.00	3.68	na
Colour 475 nm	1.90	0.62	na

na = not applicable

Table 4-11: Composition of the influent (day 36) and effluent (day 81) of the Experimental Wetland

Parameter	Influent from day 36	Effluent on day 81	Change (%)
COD ($mg.L^{-1}$)	7150	1152	- 84
pH	7.65	7.91	na
Alkalinity (as mg $CaCO_3.L^{-1}$)	2700	2633	- 3
Phosphates (mg PO_4^{3-} $.L^{-1}$)	358	136	- 62
Conductivity ($mS.m^{-1}$)	566	531	- 6
Polyphenols ($mg.L^{-1}$)	3.85	0.91	- 76
Total Solids ($g.L^{-1}$)	4.62	5.50	19
Total Susp Solids ($g.L^{-1}$)	1.28	0.11	- 91
Colour 254 nm	4.00	3.18	na
Colour 475 nm	1.88	0.37	na

na = not applicable

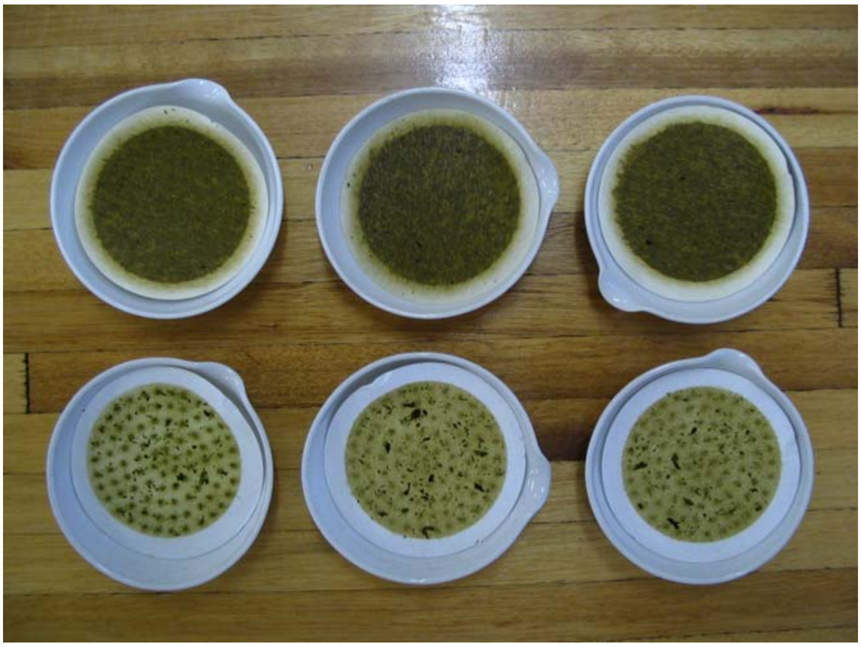

Figure 4-8 Difference in suspended solids composition for Control Wetland effluent (top) and Experimental Wetland effluent (bottom) from Trial 3.

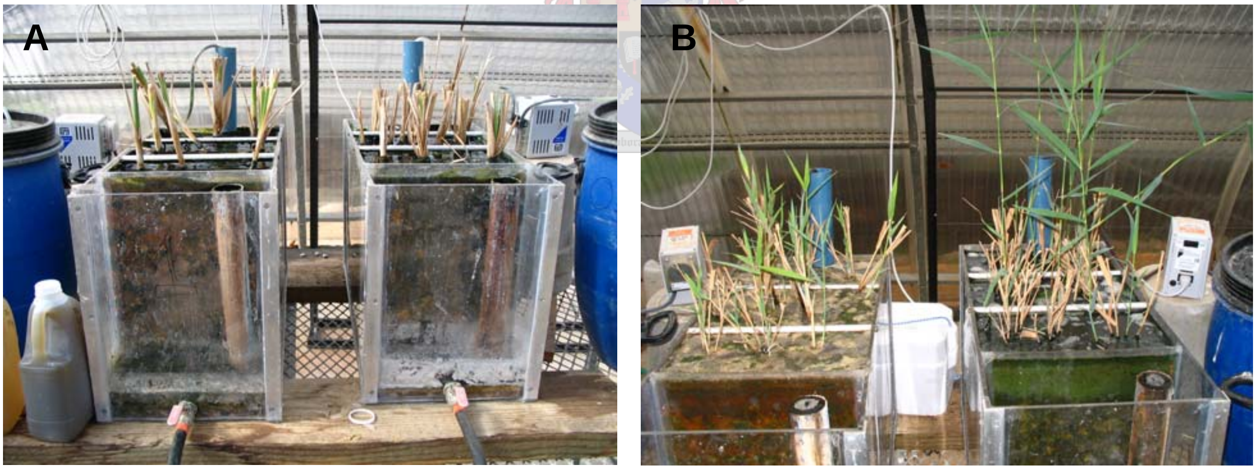

Figure 4-9 The Control and Experimental Wetlands at the start (A), and at the end of Trial 3 (B). Untreated substrate was fed to the Control Wetland (left unit in both A and B), and treated substrate to the Experimental Wetland (right unit of both A and B).

The impact of post-ozonation of the final effluents (Table 4-12) from both wetlands units showed further reductions in components for both the Control and Experimental Wetland effluents. The higher residual COD in Trial 3 than Trial 2 showed that the COD reduction (29%) was higher in the Experimental Wetland (Table 4-13), while the Control Wetland effluent was reduced by 24%. This also indicates that the organic components in the Experimental Wetland were probably more biodegradable and this may also be attributed to the COD content consisting more of plant matter. In Fig. 4-8 it can clearly be seen that the Experimental Wetland effluent consists of plant matter and this could be primarilyl due to the better plant growth in the wetland (Fig. 4-9). The final polyphenol content was lower in the post-ozonated Experimental Wetland effluent, reaching 0.32 $mg.L^{-1}$ compared to 0.52 $mg.L^{-1}$ for the Control Wetland. However, the Control Wetland achieved a better post-ozonation reduction (68% vs 65%, Table 4-13). This was ascribed to the fact that the initial ozone pre-treatment on the Experimental Wetland effluent (Table 4-9) had probably already led to the degradation of most complex compounds. When seen as a whole, the Experimental Wetland resulted in higher removal efficiencies for COD, phosphates, polyphenols and colour (Table 4-13).

An interesting fact that was noted during this Trial was that the effluent composition showed increasing reduction rates towards the end of the 81 day trial period. The fact that both the wetlands were showing increasing efficiency, may mean that the wetlands took much longer to acclimatise to the higher influent load than was originally thought. Thus, for the next trial (Trial 4) it was decided to extend the retention time to 12 days. In practise, the large-scale wetland at the distillery was also using a 12 day retention time, so the data from the small-scale wetlands may therefore be more representative when applying a 12 day hydraulic retention time.

Table 4-12 Composition of the post-ozonated effluent from the Control and Experimental Wetlands after 81 days (Trial 3)

Parameter	Control Wetland effluent after post-ozonation	Experimental Wetland effluent after post-ozonation
COD ($mg.L^{-1}$)	1163	821
pH	8.3	8.4
Alkalinity (as mg $CaCO_3.L^{-1}$)	2600	2575
Phosphates (mg PO_4^{3-} $.L^{-1}$)	119	97
Conductivity ($mS.m^{-1}$)	522	522
Polyphenols ($mg.L^{-1}$)	0.52	0.32
Total Solids ($g.L^{-1}$)	5.5	5.7
Total Vol. Solids ($g.L^{-1}$)	1.60	1.71
Total Susp. Solids ($g.L^{-1}$)	0.21	0.09
Total Vol. Susp. Solids ($g.L^{-1}$)	0.18	0.07
Colour (254 nm)	3.33	1.51
Colour (475 nm)	0.253	0.09

Table 4-13 Summary of the reductions (%) after the post-ozonation step and the overall efficiencies for the Control and Experimental Wetlands (Trial 3)

Parameter	Post-O_3 reduction (%)		Overall Reduction (%)	
	Control	Experimental	Control	Experimental
COD	- 24	- 29	- 83	- 89
Alkalinity	1	- 2	8.5	- 5
Phosphates	- 23	- 29	- 64	- 73
Conductivity	- 2	- 2	- 2	- 8
Polyphenols	- 68	- 65	- 91	- 92
Total Solids	- 1	4	- 28	25
Total Vol. Solids	- 10	4	- 62	- 36
Total Susp. Solids	- 9	- 19	- 81	- 93
Total Vol. Susp Solids	- 10	- 18	- 78	- 91

TRIAL 4 – Influence of *higher organic loading rates and an extended HRT*

The purpose of Trial 4 was to supplement the results from Trial 3 and to establish if pre-ozonation would affect the reduction rates of components of the WDWW during a higher loading which was considered representative of the "peak-season". The HRT was also extended from 9 to 12 days so as to determine whether with a longer HRT, pre-ozonation would have a greater effect on biodegradability and effluent quality.

The composition of the WDWW used during Trial 4 is summarised in Table 4-14. The same effluent was used as in Trial 3, and the wetlands were once again acclimatised to the higher influent load by stepwise increasing the influent load (Table 4-14) so that the plants and the microbial community would have time to adapt to the environmental changes. Once both wetlands were acclimatised, the Experimental Wetland was fed treated wastewater for the remainder of the trial (from day 36 to 72).

The data in Tables 4-15 and 4-16 show the results of Trial 4. The results confirm the hypothesis that the wetlands required more time to acclimatise as shown by the higher COD removals, even though the influent composition was similar to that used during Trial 3. In this study the COD removal increased, with the effluent having a final COD content of 568 $mg.L^{-1}$ for the Control Wetland, and 539 $mg.L^{-1}$ for the Experimental Wetland. Alkalinity also increased dramatically up to 3 600 $mg.L^{-1}$ for both wetlands, and the leaching of carbonates from the gravel was again attributed as the cause. Phosphate reduction was very similar (51% – 58%) to Trial 3 (53% – 62%), and therefore showed good consistency over the trials. Polyphenol content for the Control Wetland was 1.96 $mg.L^{-1}$ with a 67% removal and 1.61 $mg.L^{-1}$ with a 58% removal for the Experimental Wetland. The polyphenols content in the wetland effluent was was higher than in Trial 3 and was attributed to the wetlands reaching a maximum holding capacity and no longer being able to filter these components out. De Gueldre *et al*. (2000) also reached the conclusion that wetlands act as a sink for nutrients, and will eventually start to leach components out after having reached their saturation levels. The total solids of both wetlands (6.63 and 7.26 $g.L^{-1}$) measured in the effluent increased in Trial 4 (Tables 4-15. 4-16, 4-17), when compared to the residual value in Trial 3. This supports the idea that the wetlands have a maximum holding capacity (or saturation point) and when exceeded, washout occurs. Colour showed poor reductions at 254 nm, but high reductions at 475 nm.

Table 4-14 Composition of WDWW feed used to acclimatise the wetlands in Trial 4 to higher COD loads. Both wetlands were fed untreated wastewater for the first three retention times (up to day 36), and then the Control Wetland continued being fed with full strength untreated wastewater for the remainder of the 72 days. The Experimental Wetland was fed treated wastewater from day 36 onwards

Parameter	Both Wetlands	Both Wetlands	Control Wetland	Experimental Wetland
	½ Strength influent (Day 0 – 11) (1st retention)	¾ Strength influent (Day 12 – 36) (2nd retention)	Full strength influent (Day 36 – 72) (3rd retention)	Treated influent (Day 36 – 72) (3rd retention)
COD (mg.L^{-1})	3762	5447	7000	7150
pH	7.46	7.47	7.51	7.65
Alkalinity (as mg $CaCO_3$.L^{-1})	1317	1958	2400	2700
PO_4^{3-} (mg PO_4^{3-} .L^{-1})	208	301	333	358
Conductivity (mS.m^{-1})	290	408	533	566
Polyphenols (mg.L^{-1})	3.43	4.42	5.87	3.85
Total Solids (g.L^{-1})	8.84	5.85	7.63	4.62
Total Vol. Solids (g.L^{-1})	4.88	3.23	4.23	2.69
Total Susp. Solids (g.L^{-1})	0.55	0.83	1.07	1.28
Total Vol. Susp. Solids (g.L^{-1})	0.42	0.68	0.81	0.83
Colour 475 nm	1.22	1.58	1.90	1.88

Table 4-15 Composition of the influent (day 36) and effluent (day 72) of the Control Wetland

Parameter	Influent on day 36	Effluent on day 72	Change (%)
COD ($mg.L^{-1}$)	7000	568	- 92
pH	7.51	8.20	na
Alkalinity (as mg $CaCO_3.L^{-1}$)	2400	3583	49
Phosphates (mg PO_4^{3-} $.L^{-1}$)	333	165	- 51
Conductivity ($mS.m^{-1}$)	533	683	28
Polyphenols ($mg.L^{-1}$)	5.87	1.96	- 67
Total Solids ($g.L^{-1}$)	7.63	6.63	- 13
Total Susp. Solids ($g.L^{-1}$)	1.07	0.14	- 87
Colour 254 nm	4.00	3.64	na
Colour 475 nm	1.90	0.42	na

na = not applicable

Table 4-16 Composition of the influent (day 36) and effluent (day 72) of the Experimental Wetland

Parameter	Influent on day 36	Effluent on day 72	Change (%)
COD ($mg.L^{-1}$)	7150	539	- 93
pH	7.65	8.33	na
Alkalinity (as mg $CaCO_3.L^{-1}$)	2700	3608	34
Phosphates (mg PO_4^{3-} $.L^{-1}$)	358	149	- 58
Conductivity ($mS.m^{-1}$)	566	753	33
Polyphenols ($mg.L^{-1}$)	3.85	1.61	- 58
Total Solids ($g.L^{-1}$)	4.62	7.26	57
Total Susp. Solids ($g.L^{-1}$)	1.28	0.092	- 93
Colour 254 nm	4.00	3.61	na
Colour 475 nm	1.88	0.29	na

na = not applicable

Table 4-17 Summary of the reductions (%) after the post-ozonation step and the overall efficiencies for the Control and Experimental Wetlands (Trial 4)

	Post-O_3 reduction		**Overall Reduction**	
	Control Wetland	**Experimental Wetland**	**Control Wetland**	**Experimental Wetland**
COD	- 10	- 19	- 93	- 94
Alkalinity	- 2	- 8	68	60
Phosphates	- 22	- 18	- 64	- 65
Conductivity	- 1	10	45	51
Polyphenols	- 36	- 35	- 79	- 68
Total Solids	13	18	17	107
Total Vol. Solids	- 77	93	- 29	14
Total Susp. Solids	- 54	- 44	- 85	- 94
Total Vol. Susp. Solids	1	- 64	- 89	- 95

Figure 4-10 Pre-ozonated WDWW (left), Experimental Wetland effluent (middle), post-ozonated effluent (right) for Trial 4.

The impact of post-ozonation of the final effluents from both the Control and Experimental Wetland units, as summarised in Table 4-17 and shown in Fig. 4-10, showed further reductions in most components. COD reduction was found to be higher (94%) in the Experimental Wetland that had been fed with treated wastewater (Table 4-12), while the Control Wetland effluent was reduced by 93%. This suggests that post-ozonation of the Control Wetland effluent was just as effective as the pre- and post-ozonation of the Experimental Wetland wastewater. Alkalinity, phosphate, polyphenol and conductivity reduction were also similar for the Control and Experimental Wetlands. The solids and colour reductions were also similar for both wetlands.

A further difference in the wetlands was the health of the plants (macrophytes). During Trial 3 the plants in the Experimental Wetland treating pre-ozonated wastewater showed a much greater height and healthy appearance than the plants in the Control Wetland being fed the untreated wastewater. This is clearly visible in Fig. 4-9 for Trial 3. A similar result, as shown in Fig 4-11, was obtained in Trial 4 where final plant heights of 95 and 118 cm were measured in the Control Wetland and the Experimental Wetland, respectively. These differences between the Control and Experimental Wetlands in Fig. 4-9 and 4-11 can only be attributed to the characteristics of the wastewaters fed, since all other parameters (temperature, light, flow rate) were the same. Therefore it was concluded that the plants in the wetlands grew better when fed with pre-ozonated wastewater. The increase in total solids (Table 4-17) of 107% for the Experimental Wetland may be attributed to the increased plant matter.

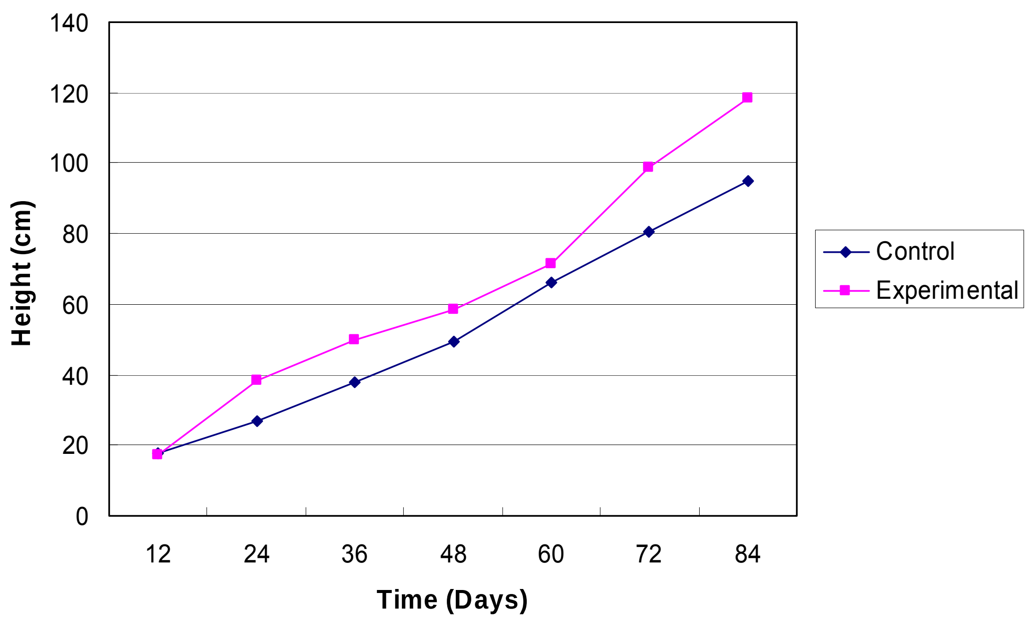

Figure 4-11 Plant heights of the Control and Experimental wetlands used in Trial 4.

CONCLUSIONS

Ozonation as a pre-treatment of WDWW before being fed to a wetland was shown to increase the efficiency of the wetland system especially in terms of improving the biodegradability of the WDWW. Improved biodegradability allowed greater utilisation of the components in the wastewater by the wetland system. Post-ozonation was also shown to be feasible in increasing the final effluent quality emanating from the wetland system.

In this study two different ozonation modes were evaluated to determine whether either had an impact on the wetlands treating ozonated WDWW. The results showed that there were no major differences in terms of wetland efficiency between the two modes.

In Trial 2 where the efficiency of wetlands fed with either unozonated, but low COD load ("off-season") WDWW or ozonated ("off-season") WDWW, was evaluated, it was found that the efficiency of the wetland receiving the treated effluent was much better (73% COD reduction) than the wetland fed with untreated (62% COD reduction) WDWW. Treatment efficiency in terms of the reduction of polyphenols, colour, total solids, suspended solids and phosphates were also greatly improved. In Trial 3 similar results were found when treating high COD load "peak season" (7 000 mg COD.L^{-1}) WDWW, with pre-ozonation resulting in an increase in the treatment efficiency from 78% to 84% COD reduction. These results clearly show that the use of pre-ozonation WDWW as feed for wetland systems is beneficial to the wetland efficiency.

From a practical side the data from the studies showed that the COD content of the final wetland effluent was still above the legal requirements (Anon, 2004) and thus Trial 4 was initiated to determine whether the wetland efficiency could be further increased by extending the hydraulic retention time 9 to 12 days. The results clearly showed that with the extended HRT it was possible to produce a final wetland effluent with lower COD levels. It was concluded that the increase in HRT allows the biological component of a wetland system to better acclimatise to the WDWW. At the extended HRTs the system thus has time to metabolise even the less biodegradable WDWW components. Although this would seem to negate the inclusion of a pre-ozonation treatment, it would in effect necessitate either larger wetlands to be constructed or smaller volumes of wastewater to be fed to the wetland to achieve the longer HRT. The inclusion of ozone treatments can thus be of use in such scenarios, by either eliminating the need to construct a larger wetland, while maintaining a constant wastewater volume or by increasing the efficiency of a current wetland system and thus allowing the wastewater volume being fed, to be increased.

Throughout the trials done in this study it was found that ozonation did not negatively impact the biological part of the wetland systems. What was extremely positive about the ozonation treatments was the visual and physical measurable increases in the plant growth. This was attributed to the wastewaters being less toxic and containing a more biodegradable carbon source. Wetlands rely on plant growth, and improving the health and growth potential of the plants will lead to greater removal efficiencies in the long term. The high pH for the WDWW in Trials 1 and 2 may also need to be adjusted to facilitate plant growth.

Another practical aspect that was observed during the studies was that acclimatisation of the wetlands to WDWW was of importance in maintaining wetland efficiency. Sheperd *et al*. (2001) recommended that wetlands should not be fed winery wastewater with a COD higher than 5 000 $mg.L^{-1}$, since this would result in rapid death of plants at the wetland inlet. Wineries with a pre-treatment system perform better than those without such a system; therefore wetlands should be seen as a secondary treatment (Mulidzi, 2005).

The cost of ozonation is an important factor in evaluating the feasibility of a combined treatment system. Sigge *et al*. (2006) investigated the difference between lab-scale and industrial scenarios. For a lab-scale circulating contactor, as used in these trials, the cost per m^3 of wastewater to oxidise 1 500 mg $COD.L^{-1}$ was found to be about R 0.21. In contrast for an industrial scenario to ozonate 5 000 L of wastewater per day to achieve 15% reduction on a COD of 10 000 $mg.L^{-1}$ using a 10 g $O_3.h^{-1}$ generator, it would cost R1.03 per m^3 of wastewater.

It is important to note that a high ozone utilisation rate is crucial for practical applications. The efficiency of the ozone contacting processes used in this investigation, namely a 2 L bubble column and a 50 L continuous ozonation system, can be substantially improved. A higher ozone efficiency would be possible by making use of static mixer technology. Static mixers are efficient due to the finer ozone bubble dispersion, allowing better mixing and larger surface to volume ratios for improved contact. Static mixers during ozone generation may be up to 95% more efficient (Chen *et al*., 2004). By avoiding excessive ozonation and increasing the contacting process, lower operating costs can be achieved.

The positive results obtained during this study with the use of ozone to improve the treatment efficiency of wetlands can be of value in facilitating efficient environmental management in the wine distillery industry. It is also recommended that further studies be done to investigate the feasibility of ozone treatment on a larger scale and making use of improved ozone generation and static mixing technology.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Background to wine-distillery wastewater problems in South Africa

The various food and agricultural industries create non-desirable wastewaters of different qualities and quantities, which lead to increasing disposal and pollution problems for companies and the environment. South Africa has limited water resources; therefore there is an increasing awareness of the need to decrease wastewater, limit pollution, dispose and reuse these wastewaters efficiently and safely. Distilleries are an example of an agricultural industry that generates large volumes of wastewater. These wine-distillery wastewaters (WDWWs) are heavily polluted and due to the seasonal nature of the product, the amount and composition of the wastewater may exhibit major daily and seasonal variations. Additionally, refractory compounds present in these wastewaters, such as polyphenols, can be toxic to microorganisms (Alvarez *et al*., 2001). This makes the selection of a suitable treatment process problematic. National legislation of South Africa and potential international markets require the responsible management of wastewaters through effective systems to minimise the environmental impact.

A number of treatment options are available which may be suitable for WDWWs, but simple, low tech systems are also effective to help with disposal problems (lagoons, wetlands and irrigation). However, it has be shown that combinations of chemical and biological treatments are often the way to optimise the overall process. Ozone has been used to treat wastewaters by increasing biodegradability. Wetlands have successfully been used to treat wine cellar effluent, but are normally regarded as a secondary treatment system. Therefore wetland use for treating WDWWs has not been recommended due to the high COD levels encountered. Mulidzi (2006) also found high COD wastewater to be unsuitable for wetlands.

The use of ozone as a suitable treatment method for WDWW

In this study ozone was successfully utilised to firstly, reduce COD levels in wine-distillery wastewater, and secondly to increase the biodegradability of the wastewater. Characterisation of the wastewater showed that there is a high seasonal variation in terms of composition, and this can be attributed to the production cycle. Ozonation of the wastewater destined for the wetland resulted in an average COD reduction of 271 mg COD.g O_3^{-1}, while for post-wetland effluent, an average COD reduction of 103 mg

COD.g O_3^{-1} was achieved. A maximum effectiveness to ozone use was observed, and and it was found that to increase the ozone dose was ineffective to increase COD reductions. This was attributed to wastewater containing recalitrant compounds resistant to oxidation.

Granule activity was used in this study to measure and determine the effect of ozone on the biodegradability of WDWW. It was shown that at low ozone doses (200 - 400 mg $O_3.L^{-1}$) granule activity increased in terms of biogas, methane and cumulative gas volumes, thus indicating an increase in biodegradability of the WDWW. By showing an increase in the biodegradability of WDWW, it was concluded that ozone has potential as a pre-treatment step to increase the effectiveness of a biological wetland system.

Combinations of wetlands and ozonation as a suitable treatment for WDWW

Ozone was utilised as a pre-treatment to improve biodegradability of WDWW, so as to allow greater utilisation of the wastewater components by a constructed wetland system. It was found that the efficiency of the wetlands receiving low (2 200 mg $COD.L^{-1}$) and high COD (7 000 mg $COD.L^{-1}$) WDWW with a 9 day retention time improved when the wastewater was pre-ozonated. A COD reduction of 73% was achieved by the wetland treating the low COD pre-ozonated wastewater, while only a 62% reduction was achieved with the control wetland. A COD reduction of 84% was achieved by the wetland treating the high COD pre-ozonated wastewater, while only a 74% reduction was achieved with the control wetland. Wetland treatment efficiency in terms of reduction of polyphenols, colour, total solids, suspended solids and phosphates also increased with the pre-ozonation steps.

Post-ozonation of the effluent from the wetland treating the low COD load resulted in a further 11% COD reduction, while only a further 10% reduction was achieved in the wetland treating the high COD load. From the data obtained it was concluded that the use of pre-ozonated WDWW as feed for wetland systems is beneficial to the wetland efficiency. Post-ozonation was also shown to be beneficial in that it improved the final effluent quality leaving the wetland system.

In the studies it was found that the COD content of the final wetland effluent was still above the legal requirements and thus a further trial was initiated to determine whether the wetland efficiency could be further increased by extending the wetland hydraulic retention time (HRT) from 9 to 12 days. The results showed that with the extended HRT it was possible to produce a final wetland effluent with a lower COD level, with 92 and 93% COD reduction for the wetland treating the un-ozonated and pre-ozonated wastewater, respectively. Post-ozonation showed little effect on the effluents from both wetlands in terms of COD reduction. The similar and much higher COD reductions may be attributed to the increase in HRT allowing more time for the biological component of the wetland system to better acclimatise to the WDWW. With the extended HRTs, the system thus has time to metabolise even the “less biodegradable” WDWW components, leaving little biodegradable components for post-ozonation. Although this would seem to negate the inclusion of an ozonation treatment, it would in effect necessitate either larger wetlands to be constructed, or smaller volumes of wastewater to be fed to the wetland to achieve the longer HRT.

Ozone can thus be of use in such WDWW treatment scenarios, by either eliminating the need to construct a larger wetland, while maintaining a constant wastewater volume or by increasing the efficiency of a current wetland system and thus allowing the wastewater volume being fed, to be increased. In this study ozone was also observed to benefit plant and algal growth in the wetlands based on visual observation as well as total solids increase for the wetland receiving pre-ozonated WDWW, and it was concluded that by improving the health and growth potential of the biological part of a wetland, it will directly lead to greater removal efficiencies over the long term. The study thus clearly proved that better acclimatisation is therefore of importance in maintaining wetland efficiency.

From the results in this study it can be concluded that ozone, used as a pre-treatment, improves wetland performance by increasing efficiency. However, the final effluent was still well above the South African legal limit the standard of 75 $mg.L^{-1}$ permitted for wastewaters to be directly discharged into a water system, necessitating further investigations in order to increase removal efficiencies for the total system.

Concluding remarks

The positive results obtained during this study can be of value in facilitating efficient environmental management in the wine distillery industry. The use of a biological wetland system as a primary treatment method is a feasible possibility, if a pre- and post-treatment system is used. A number of parameters must be taken into account for each site to choose the correct combination of suitable treatment methods: the available area, COD, volumes, treatment time, inhibitory substances, land zoning, production seasons etc. The results from this study can be used in designing a full treatment system in order to reach the desired effluent quality.

It is recommended that making use of improved ozone generation and static mixing technology will allow much greater ozone usage efficiencies, resulting in higher COD reductions, improved biodegradability and cost reduction. The WDWW composition could also be evaluated to determine which components resist degradation by ozone and the wetlands in order to evaluate other possible treatment methods, as well as determining which components are contributing to the final COD. Wetlands shed plant and algal organic matter, and if this is contributing to the COD in the final effluent, a filtering step may be an easy option to greatly reduce COD. The effect of the gravel on conductivity and alkalinity of the wastewater also needs to be investigated. The final investigation would be to undertake a study to investigate the feasibility of ozone treatment on an industrial scale with a full size ozone generator and constructed wetland system at a distillery.

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