# Winery Project Team Draft Literature Review Report
# (August 15, 2007)

## Executive Summary

(forthcoming in final)

## Introduction

### Study Purpose

In August, 2004, the Wine Institute (www.wineinstitute.org) issued a report by Kennedy/Jenks Consultants (www.kennedyjenks.com) (hereafter WI/KJ), *Land Application of Winery Stillage and Non-Stillage Process Water: Study Results and Proposed Guidelines* (Wine Institute, Kennedy/Jenks Consultants, 2004) (hereafter WI/KJ Study and Guidelines). The Study consisted of a two-year, two-site field trial for developing new guidelines for the use of spreading basins for the treatment and discharge of winery process water from both non-stillage (wine) and stillage (wine distillation) production. The scope of the Study did not include issues related to crop irrigation with wastewater and other process water pre-application treatments. The objective of the field study was to provide basic data for use in (1) revising existing stillage wastewater land application guidelines, and (2) developing new non-stillage wastewater application guidelines. The objective of the WI/KJ Study and Guidelines report was to summarize the field study results, analyze the dataset, and develop draft revised guidelines for management of winery wastewater land application (Wine Institute, Kennedy/Jenks Consultants, 2004, p.1-5).

After completion of the WI Study and Guidelines, a controversy developed between the staff of the Central Valley Regional Water Board (CVRWQCB) and WI/KJ regarding the validity and applicability of the study results, and the efficacy of the proposed guidelines for the protection of groundwater quality. In an attempt to resolve the controversy, the State Water Resources Control Board (SWRCB) requested a panel of scientists and engineers convened by the University of California Davis (UCD) to conduct a technical and scientific peer review of the WI/KJ Study and Guidelines. The panel completed the peer review (PR) in 2005. The review was critical of the WI Study and Guidelines in a number of areas. WI/KJ prepared a detailed, point-by-point response to the peer review (hereafter WI/KJ Response) in December, 2005, presenting justifications and alternative interpretations to many of the criticisms of the PR panel.

Following the WI/KJ Response, discussion centered on additional research necessary to address the remaining unanswered questions and unresolved issues. An in-depth, worldwide literature review was proposed, to determine what additional light could be shed on the remaining questions and issues by other research that may have been conducted on the land application of winery waste, with the intent of avoiding the large cost and time required for additional research that may have already been conducted by others. In addition, the objective of the scientific literature review (hereafter LR) was to lay a foundation for revising existing guidelines for the land application of winery waste to spreading basins and croplands, while using best existing scientific information to judge a resolution to the present questions and issues. The revised guidelines could then be used by the wine industry for assessing the suitability of new and existing land application sites and by the Regional Water Quality Control Boards as a reference in issuing Waste Discharge Requirements.

The following were all considered possible results of the LR, in whole or in part:

(1) information gathered substantiates the conclusions and recommendations of the *Land Application Study and Proposed Guidelines*;
(2) information gathered suggests modifications of the conclusions and recommendations of the *Land Application Study and Proposed Guidelines*;
(3) information gathered contradicts the conclusions and recommendations of the *Land Application Study and Proposed Guidelines* and suggests alternative conclusions and recommendations;
(4) information gathered suggests and recommends alternatives to land application of winery waste to spreading basins and croplands;
(5) information gathered is considered insufficient to address/resolve the present questions and issues regarding the land application of winery waste to spreading basins and croplands with additional studies suggested or recommended.

**Project Team**

The LR was organized and directed by a Project Team consisting of Wine Institute representatives and Sate and Regional Board executives and staff. The Project Team members were:

Karl Longley, Central Valley Regional Water Quality Control Board member
Mike Falasco, Wine Institute
Robert Chrobak, Kennedy/Jenks Consultants
Robert Calvin, Constellation Wines U.S.
Sue Giampietro, Wine Group
Kathy Meechan, Foster's Wine Estates Americas
Chris Savage, Gallo Winery
Wendy Wyels, Central Valley Regional Water Control Board Board staff
Gordon Innes, State Water Resources Control Board staff
Wayne Verrill, State Water Resources Control Board staff

**State Water Board Staffing**

The LR was conducted by SWRCB staff, Wayne Verrill, Environmental Scientist, and Khalida Fazel, UC Davis engineering student and SWRCB Engineering Student Assistant.

**Literature Review Process**

Step 1: The Project Team identified significant unanswered questions and unresolved issues with respect to the regulation of land application of winery waste to spreading basins and croplands. The questions and issues were refined, prioritized, and sequentially ordered to facilitate analysis.

Step 2: For each issue topic, State Water Board staff researched, collected, and distributed to all members selected database search terms, complete results of the database literature searches, and selected articles that were most relevant to resolving the topic questions or issues. Emphasis was placed on scientific research published in peer-reviewed journals. The UC on-line bibliographic database of the UC Library system was accessed and utilized in the documentation search by a UC Student Assistant.

Step 3: A summary of the most pertinent information and conclusions derived from each selected research article was presented to Project Team members in a series of periodic meetings, followed by a collective discussion. SWRCB staff conducted an analysis of the data and information contained in the research articles, and prepared a draft report containing conclusions and recommendations pertaining to the unanswered questions, unresolved issues, and WI/KJ Study proposed guidelines. The draft report was submitted to the Project Team for review and approval.

**Definition of Primary Unresolved Issues**

The following issues were identified as the major questions that would be addressed in the LR:

(1) Are the draft guidelines for land application of winery waste equally applicable to loams and silt loams (<20% clay) as well as loamy sands and sandy loams that characterized the study sites in the *Land Application Study and Proposed Guidelines*?
(2) Is the method of determining waste nitrogen removal by denitrification through measuring soil solubility of iron and manganese verifiable and reproducible? Are the procedures, data, and explanations of waste nitrogen transformations within the soil sufficient to assure protection of groundwater quality under the proposed land application guidelines?
(3) Is the recommended upper limit of BOD loading and the recommended BOD/TN ratio appropriate to assure sufficient decomposition, absorption, volatilization, and uptake of potential contaminants for the protection of groundwater quality?
(4) Is there sufficient basis to assume or verify that potential groundwater contaminants infiltrating below the five-foot depth of soil will be transformed in vadose zone processes to assure the protection of groundwater quality?
(5) What monitoring programs should be established for land application sites to ensure that the systems are functioning as intended? Will vadose zone monitoring by lysimeters produce representative samples? What type of lysimeter design is appropriate?
(6) Are the resolution of issues and application of guidelines for land application of winery waste equally applicable to the reuse of winery wastewater for crop irrigation with resultant agronomic uptake of dissolved solids and decomposition products as crop nutrients?
(7) To protect underlying groundwater quality, should waste exceeding specified limits of contaminant concentrations be pre-treated before land application? If so, what should the concentration limits and treatment methods be?
(8) Does the data collected to date show that the volatile component of TDS is fully removed within the top five feet of soil? If not, how should the land application unit be managed to provide full removal?
(9) What precise definition of a level of confidence is satisfactory to the Water Boards to ensure protection of groundwater quality under land application sites?
(10) What finding can be made from existing groundwater quality monitoring data from land application sites regarding the nature and extent of groundwater quality impacts?

Although the accumulation of salt in the soil and leaching to groundwater as a result of the land application of winery waste and other organic wastes is a significant issue, it was not within the scope of the WI/KJ Study and therefore was not addressed in the PR and not included in the list of issues to be addressed in this LR.

**Information Collection/Scientific Literature Review**

The primary resource for conducting the scientific literature review was the on-line bibliographic and electronic journal access available through the UC Davis Library system. The use of this resource was intended to provide the largest possible source of relevant references and published articles, including the most recent of international publications. Additional resources included conventional library resources and hard copy books and journals. A description of the bibliographic databases used in the literature search follows:

**AGRICOLA (Agricultural Online Access)**

The AGRICOLA database contains bibliographic records of materials acquired by the National Agricultural Library (NAL) and cooperating institutions in the agricultural and related sciences. Ninety percent of the records describe journal articles and book chapters, while the remaining ten percent describe monographs, series, microforms, audiovisuals, maps, and other types of materials. Together they provide worldwide coverage of the agricultural literature. The database describes publications and resources encompassing all aspects of agriculture and allied disciplines including: animal science; veterinary science; entomology; plant science; forestry; aquaculture and fisheries; farming, farming systems and crops; agricultural economics; extension and education; food and human nutrition; and earth sciences and environmental sciences. The Bibliography of Agriculture is the print index to agricultural literature going back to 1942.

The National Agricultural Library is one of four national libraries of the United States, with locations in Beltsville, Maryland and Washington, D.C. It houses one of the world's largest and most accessible agricultural information collections and serves as the nexus for a national network of state land-grant and U.S. Department of Agriculture field libraries.

**CAB Abstracts**

CAB Abstracts is the largest and most comprehensive agricultural literature database. Produced by CABI Publishing, the database contains citations and abstracts to the international agricultural literature, including veterinary medicine, human and animal nutrition, rural development, as well as other related topics such as tourism and human ecology. The database covers over 11,000 journals and conference proceedings and selected books in agriculture, from 1910 to the present.

**FSTA (Food Science and Technology Abstracts)**

FSTA is produced by the International Food Information Service (IFIS) and contains over 500,000 references; annual updates add approximately 18,000 references per year. The database covers all areas of food science, food technology, and human nutrition, including basic food science, biotechnology, toxicology, packaging, and engineering. Some 1800 publications in over 40 languages are scanned regularly, including journals, reviews, standards, legislation, patents, books, theses, and conference proceedings. Abstracts with complete bibliographic details are produced from these original sources. Sources of all the materials abstracted are listed together with instructions for full text document delivery.

**WRA (Water Resources Abstracts)**

WRA includes abstracts of current and past journal articles, monographs, reports, and other publication formats covering the development, management and research of water resources, from 1967 to the present.

**Relationship to Other Ongoing and Proposed Studies**

A number of other studies are completed or ongoing which are closely related to the LR Study, the results and conclusions of which may have relevance in comparison to the results and conclusions of the LR. Other studies include:

(1) Wine Institute funded BPTC study by Kennedy/Jenks Consultants, to develop specific guidelines for source control winery waste reduction as a continuation to the ***Land Application Study and Proposed Guidelines***; report completed but not yet released;
(2) Hilmar Cheese Company sponsored Supplemental Environmental Project, a study entitled *Management of Salinity in Wastewater in the California Food Processing Industry*; http://www.hilmarsep.com/index.html, projected completion date November, 2007;
(3) Final *Manual of Good Practice for Land Application of Food Process/Rinse Water*, California League of Food Processors, March, 2007, prepared by Brown and Caldwell, and Kennedy/Jenks Consultants; http://www.clfp.com/Gmanual.html, see especially Chapter 7: Loading Rates and System Design Approach;
(4) Literature review prepared by a Research Team for the Stanislaus County Department of Environmental Resources to address unresolved issues regarding the renewal of a waiver for the discharge of food processing waste within the County, unreleased draft completed December 29, 2006;
(5) Continuing program on Central Valley Salinity Alternatives for Long-Term Sustainability, Central Valley regional Water Quality Control Board, http://www.waterboards.ca.gov/centralvalley/cv-salts/index.html

**Results and Format of LR Study**

A comprehensive review article on the treatment and land application of worldwide fermentation industry waste was written by Mark Grismer and colleagues from UC Davis (Grismer 1999). Increased wastewater generation from rapidly expanding wineries in California was specifically cited as a growing concern (805). However, most research on wastewater treatment comes from Europe, Australia, and South Africa (805). Specifically, published research on winery wastewater treatment continues to receive minimal attention in the United States as opposed to Europe and other producing countries where disposal of wastewater poses particular challenges (807). One of the reasons for the greater emphasis on wastewater treatment in Europe is the limited land availability for direct application of wastewater (Shephard 2001, 394). This necessitates discharge to municipal wastewater systems, or even directly to surface water bodies, which in turn requires a higher level of pre-treatment before discharge.

In line with the perspective above, our literature review found almost no research on winery wastewater done in the U.S., except for that by Grismer and Shephard. Extensive research of winery wastewater land application and related subjects of food processing wastewater land application, and the process and fate of organic matter decomposition in soil, were found originating from Spain, France, Italy, Greece, Israel, South Africa, Australia, New Zealand, and other countries.

Recognizing the extent of published literature on relevant topics and recognizing the importance of emphasis on the most recent research, our scientific literature database searches were mostly limited to publications since the year 2000. Even when our search terms were narrowed to more specific terminology, we sometimes received on the order of hundreds to thousands of results. On the basis of abstracts, we selected the most promising papers and articles for electronic or hardcopy retrieval. The selected papers were then reviewed in detail.

Each of ten issues above is specifically addressed in this report, under one or more of four headings, with all four headings for most issues. Under the first heading, Issue Analysis, the substance of the controversy is presented with reference to the WI/KJ Study and Guidelines, the Peer Review, and the WI/KJ Response. The Analysis is intended to define the nature of the controversy and consider some possible solutions. The next heading, Literature Review Results, presents in detail that most relevant and pertinent results from the review of recent scientific research literature. The next heading, Literature Review Conclusions, identifies the conclusions that can be drawn with respect to the Issue Analysis. The final heading, WI/KJ Study and Guideline Conclusions, specifies point-by-point recommendations as to substantiation or modification of the recommendations of the WI/KJ Study and Guidelines report, including unresolved issues and areas for further study, where appropriate.

## Literature Review Issue Analysis, Results, Conclusions, and Recommendations

### Issue 1. Are the draft guidelines for land application of winery waste equally applicable to loams and silt loams (<20% clay) as well as loamy sands and sandy loams that characterized the study sites in the *Land Application Study and Proposed Guidelines*?

#### Issue Analysis

The WI/KJ Study and Guidelines study site soils were Atwater sandy loam (Fresno County stillage site), Dinuba sandy loam, Hanford sandy loam, and Tujunga sandy loam (Stanislaus County non-stillage site), with an available water-holding capacity of 10 inches per 5 feet for all soils (2-1). The Atwater series soil is sandy loam throughout with no restrictive layers. The Dinuba series soil has a subsoil layer of stratified silt and very fine sand that is weakly cemented with lime. The Hanford series soil is sandy loam throughout with no restrictive layers. The Tujunga series soil may range to loamy sand and coarse sand throughout the soil depth, with no restrictive layers.

With respect to water flow into and within the soil profile, three terms and conditions need to be distinguished. Infiltration refers to the capacity (in volume and time) of the soil surface to allow the influx of water applied onto the surface to enter the soil volume or pedon. Percolation refers to the porosity and permeability of the soil that allows the downward flow of water through the soil under saturated or unsaturated soil conditions. Saturated hydraulic conductivity refers to the capacity as depth per unit time of the surface and subsoil layers or horizons to allow the continuous percolation of water under near saturated conditions. Saturated hydraulic conditions occur when the soil water pressure is positive, which is when the soil matric potential is (theoretically) zero. In more practical terms, this condition occurs when about 95% of the total soil pore space is filled with water, with the remaining 5% filled with trapped air (Soil Survey Manual 1993, 102-103). Under saturated hydraulic flow, applied wastewater may be expected to

flow through the soil depth to the vadose zone and groundwater table in an amount of the applied wastewater that exceeds the water-holding capacity of soil capillary attraction against the downward pull of gravity. Given that the intent of applying winery wastewater in the context of the WI/KJ Study and Guidelines is to apply an amount in a single application that at least equals the soil water-holding capacity, the assumption may be made that the wetting front of the applied wastewater will continue percolation through the soil profile under saturated hydraulic conditions.

All soil water flow functions may be either impeded or enhanced by soil conditions and wastewater application management. Natural soil conditions, such as dense clay layers or hardpans, may set limits on these functions. Issues pertaining to management practices intended to enhance or restore soil water flow capacity, specifically deep ripping of subsoil layers and rototilling of the soil surface, were raised by the PR.

The PR argued that the deep ripping of the non-stillage test site between the two study years "indicates that there was already an infiltration problem at this site." Even though the PR refers to an infiltration "problem," it is argued that correction of the "problem" will itself be a problem as "deep ripping will cause short circuiting and improper treatment of the process water." Since both restricted percolation and its alleviation were cited as problems, the PR concluded that "site selection may have been inappropriate." In their response, WI/KJ observed that "ripping is a common but not annual occurrence at spreading basin sites." WI/KJ point out that the test results indicated a reduction in denitrification for the non-stillage site following deep ripping (WI/KJ Response, 11). This leads to the following prescription under annual basin maintenance in the WI/KJ Study and Guidelines: "deep ripping can be prescribed but must be conducted so that excessive drainage does not result. In fine-textured soils or soils with hard pans, deep ripping may be useful. In coarse-textured soils commonly used for spreading basins, deep ripping may create drainage conditions that prevent the temporary anaerobic conditions essential to nitrogen removal by denitrification" (5-7 to 5-8). Likewise in initial site selection, the WI/KJ Study and Guidelines indicate that a balance of permeable and impermeable conditions is necessary (5-4).

As to whether impeded infiltration and percolation was induced by wastewater application or was of natural origin, of the three soils present on the non-stillage test site, the Dinuba soil does have a moderately restrictive natural subsoil layer, deep ripping of which could result in improved or excessive percolation independent of soil changes resulting from use.

The PR contended that the necessity for frequent rototilling of the land application site was possibly indicative of excessive "parameter" (constituent?) loading and "inappropriate cycling." WI/KJ responded that rototilling is a "standard procedure for maintaining spreading basins … to eliminate surface crusting." In addition to the organic matter in the applied wastewater, diatomaceous earth (composed of silica), an additive used in wine filtration, was also identified as a factor in the formation of soil crust (WI/KJ Response, 13).

The PR commented that the generally sandy loam texture of the test site soils means "the results would still be site specific and it would be extremely difficult to extrapolate them." Further, "it is likely the results produced on coarse-textured soils will overestimate wastewater application on fine-textured soils". In response, WI/KJ states that areas with fine-textured soil "are not likely to be the best choice" for spreading basins because "low percolation rates and long resting/drying times will limit the use … because … the acreage requirement would be as large or larger than other discharge alternatives." The respondents stated further that "the loading rates and application cycles used for coarse-textured soils would not be appropriate for fine-textured soils. The authors anticipated this in the guidelines by providing limits based on soil and site-specific information for application amount and timing" (WI/KJ Response, 3, Major Comment 2).

In the WI/KJ Study and Guidelines Flowchart, three soil criteria are established for land application suitability. The first is that the soil clay content should be less than 20% (5-4 and Figure 13). In soil texture classification, sandy loams do have a maximum clay content of 20%. Additional soil texture classifications can also have clay content less than 20%: loams with a clay content ranging from 7-27%, silt loams with a clay content ranging from 0-27%, and silts with a clay content ranging from 0-12% clay (Soil Survey Manual 1993, Figure 3-16). Silts are of very rare occurrence in California, but loams and silt loams are common in some regions. Loams and silt loams have a higher percentage of silt relative to sand, and therefore have overall finer-textures and lower percolation rates than sandy loams.

**FIGURE 3-16**

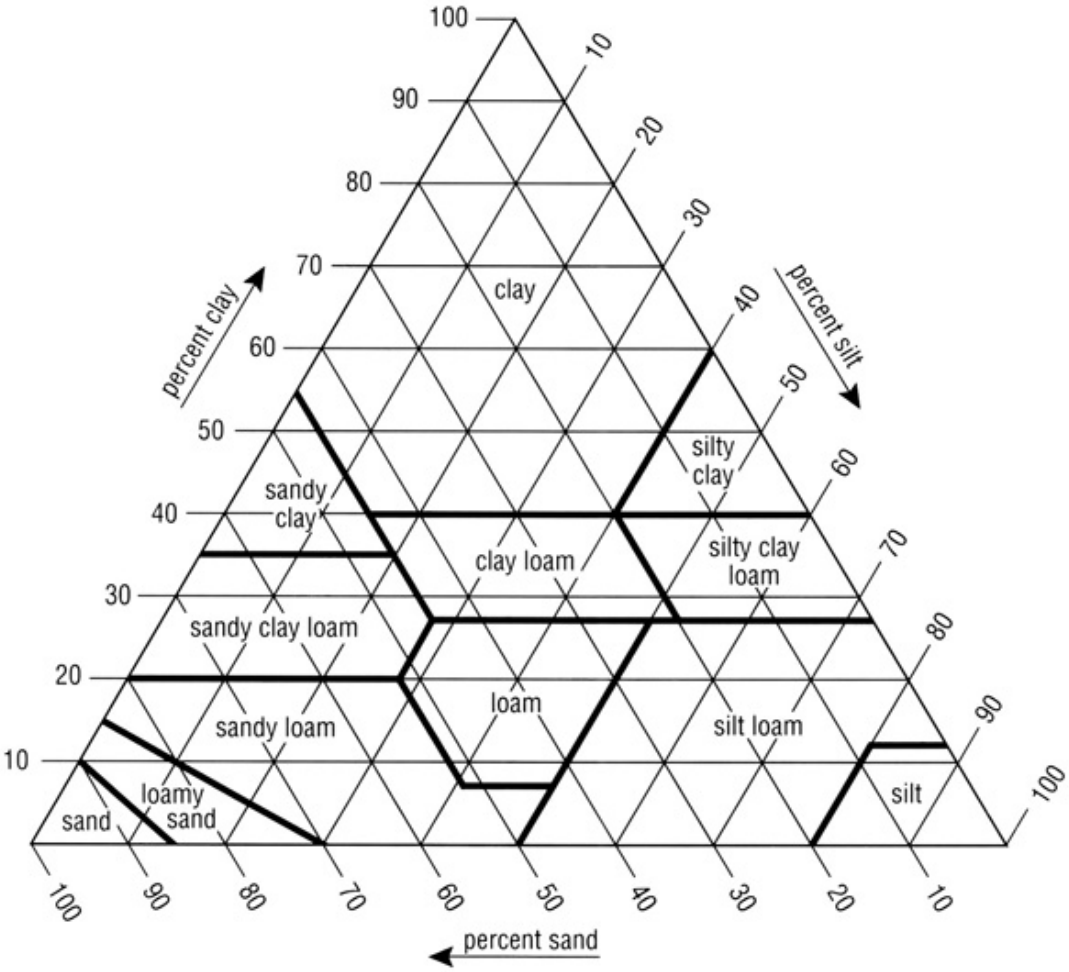



Chart showing the percentages of clay, silt, and sand in the basic textural classes.

The WI/KJ Study and Guidelines can therefore be considered both site specific and generally applicable as stated, with the exception of the question as to whether loams and silt loams with less than 20% clay are also suitable soils for land application. Further questions not raised by the

PR include (1) should there be a minimum clay content as well as a maximum, and (2) is the 20% clay limitation more conservative than necessary?

The WI/KJ Study and Guidelines second soil criteria is a recommended soil site selection based on an infiltration rate >0.6 inches/hour (5-4 and Figure 13). Guidelines are not proposed for an upper limit infiltration rate. However, the report states that "a site with uniform coarse textured soils such as loamy sands and sands may not be suitable for complete process water treatment …" (5-4). The process water treatment referred to is denitrification under anaerobic conditions (5-4). The WI/KJ Study and Guidelines state that "the primary measurement-based limitation on site selection is measured infiltration rate." The infiltration rate >0.6 inches/hour is proposed as the "lower limit for automatic site suitability," even though this limit is "not a direct outcome of the 2002 study" (5-4). The WI/KJ Response states that "we provided a permeability value (>0.6 inches/hour) that represents soil textures not commonly used for spreading basin application. The intent was to suggest a permeability value that would be found for a soil that may have limitations related to slow water flow (i.e., < 0.6 inches/hour) … if a soil has lower permeability, it is not eliminated from consideration (as a suitable site), it simply requires further analysis. The authors would welcome the suggestion of an alternative value – either for a minimum or a maximum value" (Response, 22). In the Literature Review Conclusions below, alternatives for both minimum and maximum values will be proposed.

The third soil criterion is that the single-event hydraulic loading rate should not exceed the soil water-holding capacity between the surface and five-foot depth. "This volume establishes the amount of water that can be applied without immediate percolation of the applied water. Applications to a spreading basin should not exceed this value or there will not be sufficient residence time in the soil to accomplish process water treatment for nitrogen" (5-5). It is this criterion that actually determines the minimum acreage requirement, except for exceptionally high BOD wastewater.

**Literature Review Results**

Substantial information on the soil treatment benefits of increased silt and clay content in land application site soils is presented under Issue 3 below.

For a field study in Italy, vinasse (stillage) was applied to a 15-year-old vineyard, with a clay loam soil, that was poor in organic matter, only about 1%. The described soil characteristics have a general similarity to CA Central Valley soils (Tano 2005, 200). Good results were obtained in the grape harvest from the vinasse-applied vineyard (see question 6). A field study of land application of winery waste mulches was conducted on established vineyards in New Zealand (Agnew 2005), with sandy clay loam, silty clay loam, and silty clay soils, with generally good results in terms of soil suitability (see question 6).

Soil zones with high levels of silt, clay, and consequently moisture, had denitrification rates one to two orders of magnitude higher than sand zones (Artiola 1997, 808). Potential denitrification in poorly drained clay soils is seven times higher than in well-drained sandy soils (Delgado 2002).

Organic carbon sequestration of the heavy-fraction of applied and decomposed organic matter as humus is stabilized in silt and clay-sized organo-mineral complexes, where the highest concentrations of soil organic carbon are found (Post 2000:318, see Issue 3). Studies suggest that three different classes of organic matter: persistent, transient, and temporary, are associated with

three physical soil fractions: macro-aggregates, micro-aggregates, and silt and clay (Kong 2005, 1078).

Vadose zone preferential flow paths are particularly dominant in sandy unsaturated sediments (Harter 2005, 129).

**Literature Review Conclusions**

None of the land application research studies found in the literature search sought to specifically define upper and lower limits of soil characteristics for wastewater land application suitability. The WI/KJ Study was also not designed to specifically define upper and lower limits of soil characteristics. Several research studies did however indicate an expanded extent of soil characteristics which would support or enhance percolation, denitrification, decomposition, and humus formation in organic waste application.

Sandy soils with clay contents as low as 5% were found to be suitable mediums for soil treatment of winery wastewater. A minimal amount of clay content is necessary for the minimal formation of soil structure, porosity, and micro-aggregates for the enhancement of denitrification and humus formation. Therefore, 5% clay content may be proposed as a minimum soil criteria guideline.

Good results in research studies were obtained with finer-textured soils such as sandy clay loam, clay loam, and silty clay loam. Reference to the standard soil texture classification triangle (Soil Survey Manual, 1993,138, and 8 above) shows that clay content suitability may be elevated at least to a maximum of 30%. Soils with a clay content of 30% may likely be even better mediums for the treatment of winery wastewater than coarser-textured soils.

Texture alone does not determine the soil infiltration and percolation rates. Other factors that are important for determining the rates are soil structure, porosity, and micro-aggregate formation, all of which strongly influence and can be measured collectively as the soil bulk density. In general, the lower the soil bulk density, the better the soil as a medium for wastewater treatment. Lower bulk densities are indicative of higher porosity, more developed structure, higher organic matter contents (in the form of humus), and higher water-holding capacities, all of which also indicate a more conducive environment for soil decomposing micro-organisms.

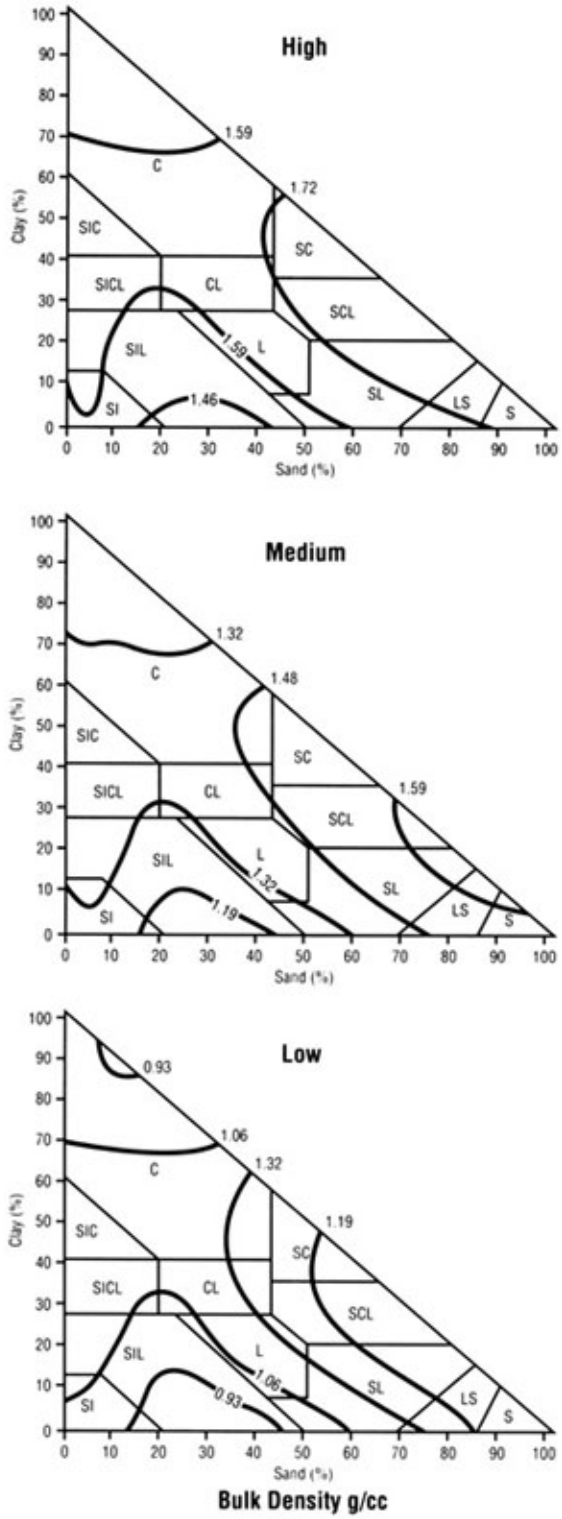
The WI/KJ Study and Guidelines recommended lower limit infiltration rate of 0.6 inches/hour is about the mid-point value for the USDA moderately high saturated hydraulic conductivity class (Soil Survey Manual, 1993, 106-107). Assuming this conductivity rate remains constant throughout the full five-foot depth of a soil profile, wastewater applied to the soil could pass through the soil into the vadose zone in 100 hours or about 4 days. For the upper limit of the moderately high saturated hydraulic conductivity, 1.4 inches/hour, the time required to pass through a uniform five-foot soil profile would be just under 2 days. For the lower limit of the moderately high saturated hydraulic conductivity, 0.14 inches/hour, the time required to pass through a uniform five-foot soil profile would be just under about 17 days, or about 3 and one-half days to pass through the first foot of soil.

In a very detailed winery wastewater study below (Chapman 1995, see Issue 3), the minimum time required to achieve the less than 5% level of soluble organic carbon in the applied wastewater remaining in soil solution ranged from 0.3 to 2.6 days for acclimatized soils and 0.7 to 4.8 days for non-acclimatized soils. The highest micro-organism concentration is in the approximately one-foot depth of the soil profile. In order for decomposition, absorption, and

humus formation, sufficient to reduce soluble organic carbon to 5% or less, to occur after wastewater application, sufficient transit time through the soil depth must be assured. The percolation limits for the moderately high saturated hydraulic conductivity classification provide this assurance and meet the approximate upper limit of time required for reduction of soluble organic carbon.

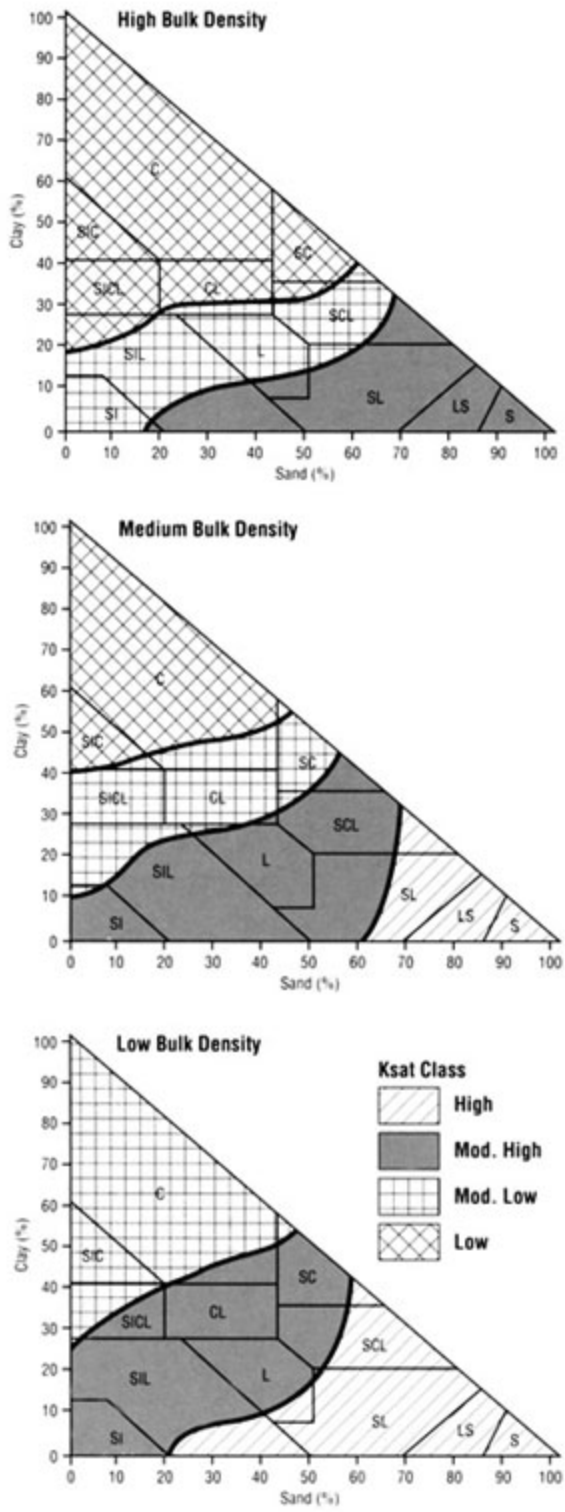
The range of soil textures and bulk densities that meet the moderately high saturated hydraulic conductivity range have been determined (Soil Survey Manual, 1993, 107-110). Figures 3-11 and 3-12 show that the bulk density ranges and saturated hydraulic conductivity ranges do not correspond exactly with the soil texture classifications and clay contents, but approximations may be made as follows.

**FIGURE 3-11**




Saturated hydraulic conductivity classes (Rawls and Brakensiek, 1983). A clay loam with a bulk density of 1.40 g/cc and 35 percent both sand and clay falls in the medium bulk density class.

## FIGURE 3-12




**Saturated Hydraulic Conductivity Classes**

Bulk density and texture relationships.

For medium bulk densities, 1.30 g/cc to 1.55 g/cc, soils with sand content approximately > 60% would be excluded from suitability, or soils with clay content approximately > 30% would be excluded from suitability.

For low bulk density soils, < 1.30 g/cc, soils with approximately >30% sand or >20% clay would be excluded from suitability.

For high bulk density soils, >1.55 g/cc, soils with approximately <20% sand or >15% clay would be excluded from suitability.

For sands and sandy loams, a bulk density >1.65g/cc is required in order to have moderately high saturated hydraulic conductivity.

The above approximations may be determined more precisely by reference to Soil Survey Manual, 1993,109-110, Figures 3-11 and 3-12. Soil layers that have a bulk density that exceeds the low, middle, and high values for its texture type, must be considered to have an unsuitable density or compaction as a result of soil formation processes that places them outside the moderately high saturated hydraulic conductivity range. Soil layers that slightly exceeed the established range of bulk densities as a result of adverse management practices, could be brought into range as a result of deep ripping, as explained below. A bulk density >1.80 g/cc would have to be considered unsuitable for all textures.

Both textures and bulk densities are variable between the different pedogenically (by soil-weathering and formation processes) or depositionally formed soil layers or horizons within the five-foot soil profile. If textures and bulk densities are highly variable, then saturated hydraulic conductivities will also be highly variable. Such high variability with depth could cause undesirable consequences for the land application of wastewater. For example, if a dense clay layer or hardpan layer occurs in the subsoil, beneath a surface soil layer with suitable saturated hydraulic conductivity, then the downward flow of wastewater may be impeded, thereby causing saturated and anaerobic conditions for an excessive time period in the soil layer above the impeding layer. If an excessively sandy layer occurs in the subsoil, beneath a surface soil layer with suitable saturated hydraulic conductivity, then the flow of infiltrating wastewater may be accelerated, resulting in excessively rapid drainage and possibly allowing pollutants to pass into the vadose zone and groundwater before complete soil treatment. By this line of reasoning, it may be concluded that in the determination of site soil suitability, all distinct soil layers should fall within the proposed suitability guidelines.

The primary effect of deep soil ripping is to create substantial zones of preferential flow (see issue 4). Such zones are likely to allow for the rapid flow of untreated wastewater into the vadose zone. However, there are different reasons for the occurrence of dense subsoil layers that would be candidates for deep ripping. If the dense subsoil layer exists under natural soil forming conditions, then that layer would likely preclude the site under the initial site suitability investigation. Ripping such a layer would not be acceptable as an alteration of the natural condition to allow an otherwise unsuitable soil to be classed as suitable. However, deep ripping of a soil subject to excess compaction as a result of land use (such as use of heavy equipment when the soil is wet), for a soil that otherwise does meet the suitability guidelines under natural (uncompacted) conditions, would act to restore the suitability of the soil as a land application site. In this case, a reduced application of wastewater below the calculated soil water-holding capacity would be advisable at least for one-year, to allow for a re-establishment of more uniform subsoil conditions.

The rototilling of the soil surface to break up a surface crust, whether mineral or organic in nature is a beneficial restorative practice that would not have any adverse effect on the surface infiltration rate nor the soil saturated hydraulic conductivity within the soil profile. Bare California soils with low organic matter do have a strong tendency for crusts to form sealing the surface after wetting, even with low or no BOD application water.

The third soil criterion that the single-event hydraulic loading rate should not exceed the soil water-holding capacity between the surface and five-foot depth is important in allowing for complete soil treatment of wastewater to occur. Sandy soils have the lowest water-holding capacity and the highest saturated hydraulic conductivities. Soils with a significant mineral-fraction proportion of silt and clay typically have more pronounced soil structure and a higher water-holding capacity, as well as lower saturated hydraulic conductivities. However, the limitation of single-event wastewater application to soil water-holding capacity was not fully observed in the WI/KJ Study as antecedent soil moisture conditions were not taken into account, and in some trial applications the soil water-holding capacity was deliberately exceeded in order to test and measure the result. Strictly limiting the single-event hydraulic loading rate for soils with higher saturated soil hydraulic conductivities is especially important to prevent rapid infiltration of wastewater through the five-foot soil depth before adequate soil treatment has occurred. Conservative assumptions of allowing for a minimum of 3 days flow-through for acclimatized soils, and 5 days flow-through for non-acclimatized soils, allow for calculation of the minimum saturated hydraulic conductivities for strict adherence to the antecedent water-holding capacity application limitation. The minimum values are 0.83 inches/hour for acclimatized soils and 0.50 inches/hour for non-acclimatized soils. For soils with saturated hydraulic conductivities below these values, the depth or volume of wastewater application would be limited by the occurrence of ponded conditions for excessive periods of time (and total BOD application), not by the antecedent soil water-holding capacity.

**WI/KJ Study and Guideline Conclusions**

On the basis of the literature review, the following recommendations are made:

1-1. WI/KJ Study and Guidelines recommends that for a site selection soil criterion of >20% clay, a site specific evaluation needs to be conducted to determine if proposed management practices can be successful (Figure 13). The recommendation is made to conduct the site specific evaluation by determination of site soil texture and bulk density, thereby determining soil saturated hydraulic conductivity in accord with Soil Survey Manual, 1993, p.109-110, Figures 3-11 and 3-12.

1-2. WI/KJ Study and Guidelines recommend that for a site selection soil criterion infiltration rate of <0.6 inches per hour a site specific evaluation needs to be conducted to determine if proposed management practices can be successful (Figure 13). The recommendation is made to limit site suitability by the selection criterion of having a moderately high saturated hydraulic conductivity throughout the five-foot soil depth, within the range 1.4 inches per hour to 0.14 inches per hour.

1-3. The recommendation is made to use Figures 3-11 and 3-12 in the Soil Survey Manual to determine soil saturated hydraulic conductivity on the basis of texture and bulk density. The following approximates the limitations thereby established:

For medium bulk densities, 1.30 g/cc to 1.55 g/cc, soils with sand content approximately > 60% would be excluded from suitability, or soils with clay content approximately > 30% would be excluded from suitability.

For low bulk density soils, < 1.30 g/cc, soils with approximately >30% sand or >20% clay would be excluded from suitability.

For high bulk density soils, >1.55 g/cc, soils with approximately <20% sand or >15% clay would be excluded from suitability.

For sands and sandy loams, a bulk density >1.65g/cc is required in order to have moderately high saturated hydraulic conductivity.

A bulk density >1.80 g/cc would be unsuitable for all textures.

1-4. The WI/KJ Study and Guidelines recommendation that the single-event hydraulic loading rate should not exceed the soil water-holding capacity between the surface and five-foot depth should be expanded to include the calculation for antecedent soil moisture conditions. For soils with saturated hydraulic conductivities below 0.83 inches/hour for acclimatized soils, and 0.50 inches/hour for non-acclimatized soils, strict adherence to antecedent soil moisture conditions is not required. The depth or volume of single-event applications for such soils is then limited by excessive surface ponding (or BOD loading).

1-5. Regardless of other considerations above, a minimum clay content of 5% should be required for soil suitability in order to have at least minimal conditions necessary for the formation of soil structure, porosity, and micro-aggregates for the enhancement of denitrification and humus formation.

**Issue 2. Is the method of determining waste nitrogen removal by denitrification through measuring soil solubility of iron and manganese verifiable and reproducible? Are the procedures, data, and explanations of waste nitrogen transformations within the soil sufficient to assure protection of groundwater quality under the proposed land application guidelines?**

**Issue Analysis**

One of the assumed most important safeguards in the land application of winery wastewater, and a major objective of the WI/KJ Study, was to demonstrate the reduction in nitrate through denitrification during soil treatment and thereby prevention of the leaching of nitrate into the groundwater. Total wastewater nitrogen (TN) loading rates were highly variable, more due to the variable TN concentration of wastewater than the variable hydraulic loading (WI/KJ Study and Guidelines, Table 3). The maximum TN loading rate was 411 lb/acre on the stillage test site in 2003 and the minimum TN loading rate was 5.1 lb/acre, also on the stillage test site, in 2002. The TN daily loading rates were equally variable, from a high of 45.7 lb/acre/day on the stillage test site in 2003, to 0.3 lb/acre/day on the same test site in 2002.

Over two seasons at each of the two test sites, TN in soil water sampled by lysimeter was substantially reduced at the 5-foot depth relative to the applied concentration in all except the 2003 season at the non-stillage test site (Table 6). Reduction in TN could be due to denitrification or incorporation into micro-organisms and humus, or both. Monitoring methods for determining anaerobic conditions for denitrification included direct measurement of oxygen status, measurement of soil water content to determine whether saturated conditions occurred at depth, and measurement of soluble iron and manganese (p. 5-10). Low TN levels in lysimeter

samples were achieved only when iron plus manganese concentrations were temporarily greater than approximately 10 mg/l (3-12, Figure 10). No monitoring measurements were made to determine the portion of nitrates that was incorporated into micro-organisms and humus. The WI/KJ Study cited earlier studies that demonstrated a significant fraction of the N present (up to 80 to 95%) can ultimately be transformed to gas and lost to the atmosphere (1-4).

For the three seasons that a reduction in TN occurred, other indicators (Fe, Mg, SO4, alkalinity) showed that a reducing environment was achieved. For the 2003 non-stillage test site, the indicators do not show that a reducing environment was achieved. Ironically, the highest hydraulic loadings, and the only ones to exceed the site soil water holding capacity (not accounting for antecedent soil water status), was the 2003 non-stillage season (Table 3).

Two reasons were given to possibly explain the increased concentrations of nitrate at the 5-foot depth in 2003, in contrast to 2002, at the non-stillage test site. The site was deep-ripped between the 2002 and 2003 seasons, and corn was planted and removed in 2003 before land application of wastewater, which may have resulted in preferential flow and re-aeration through existing root channels (3-9 and 3-10).

In both seasons at the non-stillage test site, total nitrate in the leachate spiked during the first and second wastewater applications. As a result, the WI/KJ Study made the recommendation that removal of nitrogen stored in the soils prior to crush season application (i.e., by cropping) would greatly reduce N mobilization beyond the five-foot depth (3-10).

The WI/KJ Study and Guidelines proposed an average process water BOD:TN >20 in order to achieve sufficient oxygen demand to accomplish near complete denitrification. The WI/KJ Study and Guidelines also recommended that if BOD:TN <20, the process water stream should either be pre-treated to remove some nitrogen or less cycles applied to the soil may be required to achieve adequate treatment during cropping prior to additional loading (5-5 and Figure 13). Elsewhere, the WI/KJ Study and Guidelines observed that BOD:TN in winery wastewater is higher than customarily required to support denitrification, and therefore not limiting (3-3 and 3-4).

The PR questioned whether increases in soluble iron and manganese were reliable indicators of denitrification, calling this a “little-accepted notion” because of the “low correlation,” and suggested that putting forth the idea was itself an indicator of a “poor understanding of the soils scientific literature.” In response, WI/KJ cites a number of peer-reviewed research articles supporting the correlation (Response, 7).

The PR expressed concern that the maximum study site TN loading rate of 343 lb/acre was “very high” and “may work using high N demanding crops.” The actual maximum TN loading rate was 411 lb/acre. WI/KJ replied that since most of the applied N was removed in denitrification, a cropping program may be more easily matched to the loading rate (Response, 7).

The PR expressed concern that soil conditions conducive to optimal denitrification would result in “unacceptable levels” of iron and manganese. The PR further questioned the WI/KJ Study and Guidelines assumption that re-oxidation and immobilization of iron and manganese will occur in the vadose zone sufficient to achieve an acceptable level of iron and manganese in the groundwater, stating that “no studies were conducted to determine how much vadose zone is required … nothing in the report evaluates whether the reactions hoped for in this vadose zone actually take place. In this sense the studies are incomplete” (Response, 9).

There are two questions involved in this analysis by the PR and WI/KJ. First is what constitutes an "unacceptable" concentration of iron and manganese in the vadose zone percolate and in the groundwater. In response, WI/KJ point out that the PR took the drinking water Secondary Maximum Contaminant Level (SMCL) which is protective of drinking water odor, taste, and appearance as the standard for determining "unacceptable" concentrations (Response, 9 and 20). WI/KJ questioned the appropriateness of this standard as a comparison, noting that "the soils of the Central Valley contain vast reservoirs of iron. This supply is an important factor in maintaining the reduction-oxidation status of the soils" (Response, 20).

The second question concerns the occurrence of oxidizing conditions in the vadose zone and subsoil sufficient for iron and manganese immobilization below the "unacceptable" level. WI/KJ explained that the study was "explicitly defined … to be a characterization of process water treatment in the surface 5 feet" so that groundwater or deep vadose zone measurements were not made, but "such studies should be conducted" (Response, 9). Further, in making the Guideline recommendations, the WI/KJ Sudy makes a trade off between high nitrate levels and high iron and manganese levels in the vadose zone, as "the authors believe that nitrogen treatment in the upper 5 feet is more important that iron and manganese mobilization at this depth. Iron and manganese will be swiftly returned to their oxidized immobile state when oxygen is encountered below 5 feet" and "denitrification requires anaerobic conditions that cannot be assured at greater depths and, although denitrification has been observed at greater depths, this location should not be depended upon for efficient denitrification" (Response, 23). BOD and hydraulic loading levels also must be factored in (see below).

Other major questions not debated by the PR and WI/KJ include the determination of lower limit conditions for the occurrence of sufficient denitrification (short of the reduction of iron and manganese), the magnitude of the contribution of microbial uptake and humus formation to N removal from the soil solution, and the identification of a specific optimal BOD:TN or C:N range for denitrification.

**Literature Review Results**

Scientists have identified at least six different soil processes that reduce nitrate. The most prevalent soil process is termed dissimilatory respiratory denitrification (Tiedje 1994, Chapter 14: Denitrifiers).

No standard or absolute method for measuring denitrification exists; there is no consensus as to a best method for field measurement (Mosier 1994, Chapter 44: Measuring Denitrification in the Field, 1048). To measure denitrification in the field, large temporal and spatial variation must be considered (1047). Factors controlling the process include: (1) soil air oxygen concentration, which is dependent on the O diffusion rate and consumption rate, and in turn dependent on soil structure and presence of easily degradable organic C; (2) soil water content, with an O diffusion rate 10,000 times slower in water than air (1047). For the determination of a quantitative measure of denitrification, two biological methods are most commonly used: the acetylene inhibition method and nitrogen-15 method. Both of these methods are used in conjunction with soil cores (1 m in length and 3-8 cm in diameter) or closed chambers (10-60 cm in length and 15-30 cm in diameter) (Mosier 1994 and Hauck 1994, Chapter 40: Practical Considerations in the Use of Nitrogen Tracers).

Most reduction reactions of N species are not reversible, are biologically mediated, and therefore are not well defined by thermodynamic pe-pH relationships, with a wide range in pe for reduction of each of the intermediates (James 2000, B-182). The overall reduction of nitrate is almost

identical to that for O; the pe overlap for O and nitrate reduction intermediates indicates that denitrification and aerobic respiration may occur simultaneously under certain conditions when organic C is used as the electron donor (B-183).

The most common method for quantifying electron activity of soils and natural waters is to measure the potential difference between electrodes. Generally this potentiometric measurement is unreliable for accurate assessments of redox status, especially of aerobic soils. Other methods that employ analysis of soil solution analytes indicative of redox status may prove more reliable for calculating pe ranges for aerobic and anaerobic soil systems (James 2000, B-186). Given the uncertainty associated with measured electrode potentials in soils to quantify Eh or pe, actual measurements of reductant and oxidant activities, along with a reliable pH measurement, may be a better approach. Since Mn and Fe are relatively easy to measure accurately by atomic absorption or colorimetric methods, assessing pe for Mn and Fe oxide-dominated systems could be reliable. Detailed research is needed to prove this hypothesis. In contrast, dissolved gases are harder to measure accurately, and qualitative estimates may be sufficient to obtain accurate evaluations of pe (James 2000, B-190). Data from 760 private domestic wells in El Dorado, Yuba, and Tehama Counties show a very strong negative correlation between concentrations of nitrate and manganese and iron, indicative of denitrification (presentation on SWRCB Groundwater Ambient Monitoring and Assessment Program (GAMA) at the 2006 "Nitrate in California's Groundwater" Symposium).

Denitrification had long been considered as a strictly anaerobic process, but it is now well established that it can also occur in apparently aerobic environments because many soil denitrifying micro-organisms are able to produce $N_2O$ over a wide range of oxygen pressures (Khalil 2004, 688). Water filled pore space (WFPS) > 60% is required for denitrification, with a substantial increase > 80% WFPS, above which $N_2$ emission dominates over nitrous oxide (Mosier 2002). Denitrification takes place with oxygen concentration < 0.2 mg/l (Hissock 1991) and pressure < 0.35 kPa (Khalil 2004). Anoxic conditions occur in soil if the saturation aggregate radius exceeds a critical value, 1.0 mm at 0.35 kPa to 3.5 mm at 20.4 kPa of $O_2$ (Khalil 2004).

Potential denitrification in poorly drained clay soils is seven times higher than in well-drained sandy soils (Delgado 2002). Denitrification is strongly variable, both spatially and temporally, because of the heterogeneity of soil conditions (Delgado 2002, Hofstra 2005). Heterogeneous anaerobic microsites explain the large variation in measured N gas emission rates (Mosier 2002).

Denitrification activity is concentrated in active soil sites within soil aggregates or on decomposing organic material (Mosier 1994, Chapter 44: Measuring Denitrification in the Field, 1048). Nitrate accumulated during fallowing is more susceptible to loss by denitrification than that which is produced when plants are present (Mosier 2002, 510).

Denitrification in a semi-arid Arizona soil was strongly enhanced with increasing C:N ratios and warm temperatures with percent soil moisture a less important factor. Denitrification following waste applications was higher in soils with low infiltration rates and with high moisture and organic matter contents; most field studies involving either sludge or wastewater applications indicate that the process of denitrification is greatly enhanced in the zone of incorporation or the top 50 cm (20 in) of soil (Artiola 1997, 799).

Denitrification of 80-90% was observed with C:N 1 (Hissock 1991). A significant rate of denitrification, > 0.89 lb/acre/day, was observed with an N concentration > 2ppm in the top 10 cm of soil (Mosier 2002). Addition of ethanol at the rate of about 575 lb/acre increased denitrification ten-fold (Mosier 2002).

Denitrification potential increased 100-fold with the addition of 200 ppm each of N and C (Paramasivam 1999, 307). Nitrate concentrations in the soils likely limit denitrifier activity only in situations where denitrification rates exceed the rate of nitrate resupply; addition of nitrate resulted in a 1- to 40-fold increase in denitrification activity in soil samples (Paramasivam 1999, 314-315). Dissolved organic C content varied by one order of magnitude in different monitoring well locations and varying depths in the soil profile; dissolved organic C contents were highly correlated to denitrification capacity; denitrification activity increased by 1- to 4-fold with the addition of readily available C to soil samples and 2- to 38-fold with addition to groundwater samples (Paramasivam 1999, 315). However, the incorporation of residues with high C:N into soils immobilizes mineral N, thereby making N available for later mineralization (Mosier 2002, 511).

Composting is most successful when the mixture of component organic materials has C:N 25-40. This ratio best meets the needs of the decomposing micro-organisms for high rates of decomposition while minimizing the loss and maximizing the availability of other nutrients. If N needs to be added to adjust the ratio for high C:N compost materials, N in organic form is recommended to reduce losses due to leaching or volatilization (denitrification) (Power 2000, 320). The converse would be true, added mineralized N would lead to greater denitrification, but also potential leaching. Finished composts with relatively high N content, C:N <15, can accelerate mineralization of other soil organic C pools and in low doses can actually reduce soil organic C levels. Composts with relatively high C content, C:N >30, will increase soil organic matter levels but can also induce N immobilization, which in turn can result in an increase in undecomposed organic material (or as in the case of wastewater, continued high levels of soluble C) (Power 2000, 158).

In a study on composting of winery waste, a decline in an initial C:N 35-40 to a finished C:N 18-20 was found to be indicative of an advanced degree of compost stabilization and useful as a compost maturity index (Inbar 1992, 38-39).

The typical mineralization rate from composted organic material and relatively stable soil humus is 8%-20%/year (Power 2000, 158).

The optimum pH range for denitrification has variously been shown to range between 7.0-8.0 (Hissock 1991), and 6.6-8.3 (Simek 2002). Neutral to alkaline pH was first demonstrated to be optimal for denitrification over fifty years ago (Khalil 2004). More recently, acidic soils have been shown to have a high potential for denitrification, with an optimum pH at or near the prevailing (or unmodified) soil pH (Simek 2002). Above pH 7, $N_2$ is the primary denitrification product (Simek 2002), thereby lessening the emission of gases ($N_2O$) that contribute to global warming.

The optimal pH for composting is 6.7 – 9 (Power 2000, 321).

A doubling of the denitrification rate occurs with a 10 degree C rise in temperature (Hissock 1991). There is an optimum temperature, above and below which denitrification rates decrease (Hofstra 2005).

**Literature Review Conclusions**

Soil solution analysis for the presence of soluble, reduced Fe and Mn is a relatively easy and accurate method for determining the soil redox status. Data indicate a strong negative correlation

between nitrate in soil solution, and Fe and Mn in soil solution. Therefore, a concentration of Fe and Mn in solution is strongly indicative that a high degree of denitrification has occurred. However, this method does not measure partial degrees of denitrification, which must be determined by other methods. Yet no standard or absolute method exists for measuring the degree of denitrification; there is no consensus as to a best method for field measurement. Nevertheless, two biological methods are presently most commonly used by scientific researchers: the acetylene inhibition method and the nitrogen-15 method. The measurement of increased Fe and Mn concentration in the soil solution in the WI/KJ Study is indicative of a lowered redox potential sufficient to maximize denitrification under existing soil conditions. However, increased Fe and Mn concentration is not indicative of the percent of nitrate in solution that was denitrified and volatilized with respect to the percent of nitrate in solution that was incorporated into the exponentionally increasing soil microbial biomass in response to the addition of readily decomposable organic compounds in wastewater solution which was subsequently incorporated into long-term stable humus.

Denitrification and aerobic respiration may occur at the same time under certain conditions when organic C is used as the electron donor. Denitrification can also occur in aerobic environments because many soil denitrifying micro-organisms are able to produce $N_2O$ over a wide range of oxygen pressures. Denitrification may occur in the range of 60-100+% water-filled pore space (WFPS); soil with 80% WFPS may provide the simultaneous optimization of the processes of decomposition and humification of organic matter, and denitrification. Above 80% WFPS, the emission of $N_2$ over $N_2O$ is optimized, thereby also reducing the emission of gases associated with global warming. Soil saturation occurs effectively at 95% WFPS. Therefore, a soil water content of 15% WFPS less than saturation may be considered optimal for simultaneous denitrification, decomposition and humification. A volume of wastewater application intended to achieve substantially above 80% WFPS may be unnecessary to remove nitrate from the soil solution and may have the unnecessary negative consequence of increased Fe and Mn in solution, with the potential for leaching into the vadose zone and groundwater. Maintaining soil water status within the range of about 60% - 85% WFPS may be optimal for achieving all of the objectives of environmentally sustainable winery wastewater land application.

The seemingly contradictory WI/KJ Study result of the highest hydraulic loading rate resulting in the lowest denitrification rate may be a result of application of a loading rate in excess of the soil water-holding capacity for a soil with a high saturated hydraulic conductivity resulting in insufficient time for denitrification to occur. Assuming that the WI/KJ Study site soil had a saturated hydraulic conductivity at the upper end of the optimal range, 1.4 inches/hour, the resulting 1.75 days time of passage, through five feet of soil, of the wastewater in excess of soil water-holding capacity may be insufficient for denitrification even in an acclimatized soil.

Denitrification activity is concentrated in active soil sites within soil aggregates. Soils with higher clay content relative to sand content have higher denitrification rates by several fold. Soils with higher clay content also can form more highly developed and stable soil aggregates with greater porosity and more active denitrification sites. Further, denitrification is enhanced through increased retention time of organic C and N and nitrates within the upper soil profile region of greatest microbiological activity. Soils with higher clay content will have reduced infiltration rates and saturated hydraulic conductivities, again with associated enhancement of denitrification.

The assumption may be made that finer-textured soils, and soils with higher clay content within the lower part of the moderately high saturated hydraulic conductivity range, as defined under Issue 1 above, may be expected to have substantially higher denitrification rates. The converse would also be true, coarser-textured soils with higher sand content within the upper part of the

moderately high saturated hydraulic conductivity range may be expected to have substantially lower denitrification rates. In that case, greater emphasis needs to be placed on the decomposition and humification of soluble carbon in applied wastewater. This enhancement may be largely accomplished by the upward adjustment of the C:N ratio (see below).

Denitrification activity is also concentrated in active soil sites on decomposing organic material. Denitrification is enhanced through the increased application of fresh organic material with dissolved organic C and N and nitrates in wastewater application. The application of organic matter can enhance the formation of soil micro-aggregates, thereby enhancing denitrification through the expansion of sites for anaerobic microbial processes. In simpler terms, the more winery wastewater with soluble organic C and N and the more solid organic waste, especially in the form of finished compost, that is added to a soil, the more efficient that soil will be in accomplishing both denitrification and humification, a condition otherwise referred to as acclimatization.

Conditions optimal for the protection of groundwater are directly linked to C:N in applied wastewater. If applied C:N is to low, denitrification may be inhibited by the reduction of oxygen depletion from decomposition. In addition, reduced decomposition may limit the microbial uptake of available nitrate, which serves as a counterpart to denitrification in the removal of nitrate from the soil solution. If applied C:N is too high, microbial uptake of solution nitrate may preclude denitrification even if reduced dissolved oxygen is otherwise conducive, and more significantly may leave undecomposed and unincorporated dissolved carbon in the soil solution that may be subject to leaching. The optimal C:N range for applied wastewater is 25-40; the optimal C:N range for finished soil organic matter and soil solution is 15-30. The finished C:N range will also be optimal for maintaining the humus content against further decomposition, and preventing excessive N mineralization and availability during the wastewater non-application season, which is generally co-incident with the growing season.

In the WI/KJ Study and Guidelines, the specification was given as BOD:TN, rather than C:N. BOD:C is a medium specific empirical relation, that may be determined for each type of wastewater. In the case of winery wastewater, research results indicate a C:BOD ratio of 1.25. Given this ratio, the WI/KJ Guideline of BOD:TN > 20 would be equivalent to C:N >25, the lower end of the proposed range for applied wastewater established through the LR.

Finally, the denitrification rate is optimized in the pH range of about 6.5-8.0, the typical range for Central Valley soils. Maintaining the soil pH within this natural range will help ensure sufficient denitrification. The emission of nitrogen gas over nitrous oxide is optimized in pH 7-8 range within the optimized denitrification range, thereby gaining further benefits by reducing the emission of gases associated with global warming.

**WI/KJ Study and Guideline Conclusions**

On the basis of the literature review, the following recommendations are made:

2.1 WI/KJ Study and Guidelines should specifically include growing a perennial cover crop on wastewater application sites. Continuous cover cropping will result in nitrate uptake (especially during growing season mineralization) and help reduce the nitrate leaching flush in the first yearly wastewater application, as well as establish and maintain soil characteristics that enhance denitrification, such as soil aggregation, porosity, infiltration capacity (through reduced surface crusting), and microbiological activity.

2.2 WI/KJ Study and Guidelines should include an optimal specified range C:N 25-40 for applied wastewater and a post soil treatment range C:N 15-30, prior to subsequent wastewater application.

2.3 WI/KJ Study and Guidelines should include a single application wastewater hydraulic loading rate of 100% soil-water holding capacity and maintenance of a soil water-filled pore space within the range of approximately 60% - 85% for simultaneous optimization of denitrification, decomposition, and humification. Antecedent soil moisture status prior to application should be taken into account for soils with a saturated hydraulic conductivity approximately exceeding 0.83 inches/hour for acclimatized soils and 0.50 inches/hour for non-acclimatized soils.

2.4 WI/KJ Study and Guidelines should include maintenance of soil pH within 6.5-8.0, following soil treatment after wastewater application, and pH 7.0-8.0 if the soil is naturally slightly alkaline.

**Issue 3. Is the recommended upper limit of BOD loading and the recommended BOD:TN ratio appropriate to assure sufficient decomposition, absorption, volatilization, and uptake of potential contaminants for the protection of groundwater quality?**

**Issue Analysis**

In the WI/KJ Study, the BOD loading rate was highly variable over both seasons at both sites, more due to the variable BOD concentration of wastewater than the variable hydraulic loading rate. The highest BOD loading rate, 10,280 lb/acre, was on the non-stillage site in 2003. The lowest BOD loading rate, 54 lb/acre, was on the stillage test site in 2003. Loading rates per day were also highly variable, ranging from a high of 885 lb/acre/day on the stillage test site in 2002 to a low of 2.7 lb/acre/day on the same site in the same year (Table 3).

The WI/KJ Study and Guidelines proposed that an application rate of 7,000 lb/acre be used as a temporary upper BOD loading limit, at least "pending additional experimental verification." This rate is "per cycle" or per individual process water application event and is extrapolated from Figures 15 and 16, where for applications below this rate "iron and manganese concentrations do not reach excessive values," but "correlates with a high total nitrogen removal" through denitrification (WI/KJ Study and Guidelines, 5-6). "Field study results showed that neither BOD oxidation nor odor generation limited BOD loading rate" (5-4).

Therefore, in order to not exceed the recommended upper limit BOD loading rate, the hydraulic loading rate for an individual process water application event must be calculated as the lower of the maximum soil water storage or holding capacity from ground surface to the five-foot depth, or the maximum wastewater that can be applied before the 7,000 lb/acre BOD limit is reached (5-6).

In conjunction with the upper BOD application limit, the WI/KJ Study and Guidelines recommended an average process water BOD:TN >20 in order to achieve "sufficient oxygen demand to accomplish near complete denitrification" (5-5 and Figure 13). The Manual recommended that if the BOD:TN <20, "the process water stream should either be pre-treated to remove some N or less cycles applied to the soil may be required to achieve adequate treatment during cropping prior to additional loading."

The WI/KJ Study and Guidelines suggested that "a reasonable criterion for resting cycle duration, in order to allow for complete soluble organic carbon decomposition, would be sufficient time to create unsaturated soil conditions to at least 1-foot depth. Monitoring at two feet would allow an indirect determination of aeration conditions at 1 foot as well as direct measurement of conditions at 2 feet" (p. 5-10).

In addition to these recommended Guidelines, additional management practices are indicated, including growth of a cover crop on the application site, and tillage of the surface soil of the site with annual cover crops. In the WI/KJ Study, a field corn crop was grown and harvested on the non-stillage site in the summer of 2003 and a winter grain crop was grown and harvested on the stillage site in 2003, prior to land application of wastewater in the fall. Planting an annual summer crop in spreading basins was stated to be a standard practice (2-2). Test basins were rototilled or disked at the end of each cycle just prior to the next application. In later cycles, percolation rates were observed to be much slower, with standing water remaining on the test basins for several days (3-2).

The PR was concerned about the suitability of the BOD application rate in the proposed Guidelines, where it was stated that "no data was provided on the removal of BOD in the near-surface soil layer where it is likely that most of the BOD removal occurred. It is likely that near-surface BOD removal was responsible for soil plugging that required tilling and deep ripping of the soils" (Response, 6). The WI/KJ response was that the data from a single one-foot depth lysimeter shows the majority of BOD removal in the first foot. This was consistent for both the non-stillage and stillage sites for 2002 and the data also showed that BOD removal was significant in the one- to five-foot depth of soil, except for the inexplicable increase in BOD concentration in the one- to five-foot depth during two application cycles (1 and 2) at the stillage site (Response, Tables C-1 and C-2). Further, WI/KJ replied that soil plugging constituted the formation of surface "leathers," and that these were alleviated by shallow tillage. It is unclear why the PR stated that BOD removal was responsible for near-surface soil plugging rather than BOD accumulation, or how this could be alleviated by deep ripping.

The PR disputed the WI/KJ Study and Guidelines proposed temporary upper BOD loading limit of 7,000 lb/acre, proposing a limit of 3,000 lb/acre instead. This difference is based on the interpretation of Figures 15 and 16. The PR stated that the priority was to maintain Fe and Mn concentrations below 20 mg/L (Response, 7, 23, and 24). WI/KJ argued that the priority was to enhance denitrification within the upper five feet of the soil, as both re-oxidation of Fe and Mn and reduction through decomposition of BOD are more likely to occur than nitrate reduction below a depth of five feet, stating that "denitrification requires anaerobic conditions that cannot be assured at greater depths and, although denitrification has been observed at greater depths, this location should not be depended upon for efficient denitrification" (Response, 23).

**Literature Review Results**

There are three soil processes that can account for the reduction in BOD from applied wastewater to the percolate at the five-foot depth: decomposition, aggregation, and humification. Four general soil processes can lead to soil organic carbon (SOC) sequestration: (1) humification – conversion of biomass into humic substances; (2) aggregation – formation of stable organo-mineral complexes within soil micro-aggregates; (3) leaching of carbonates into groundwater; (4) translocation of dissolved C and humus into the subsoil (Lal 2003:831). Of these four processes, three (1, 2, and 4) are simultaneously protective of water, soil, and air quality, assuming subsequent uptake and humification of dissolved organic C reaching the vadose zone. Decomposition into carbon dioxide and water, with the diffusion of $CO_2$ back into the

atmosphere, may not be protective of groundwater quality if there is leaching of mineralized decomposition products in the soil solution into the vadose zone and groundwater table. Decomposition alone does not result in carbon sequestration. Carbon sequestration is a major process by which excess carbon can be removed from the atmosphere, thereby reducing the rise in greenhouse gases contributing to climate warming. The optimal objective of land application of winery wastewater could be established as the simultaneous optimization of water quality, soil quality, and air quality through humification of applied OM, and formation of soil micro-aggregates, resulting in carbon sequestration. Before the WI/KJ Study and LR results can be evaluated for indicated carbon sequestration benefits, the nature of soil organic matter, its characterization and component fractionation, must be understood.

Physical fractionation of SOC pools into light and heavy OM fractions emphasizes the role of soil minerals and soil structure in SOC turnover and relates more directly to SOC dynamics in soil than in chemical fractionation (Post 2000:318, Figure 1).

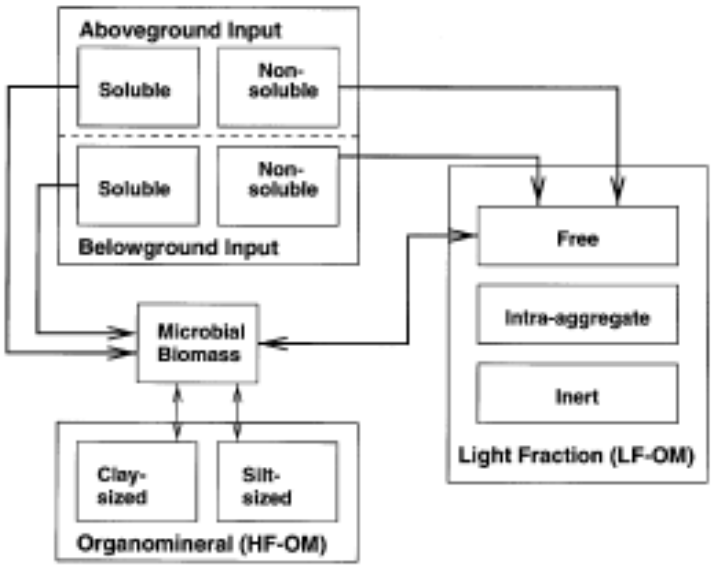



Fig. 1 Mineralization and transfer of organic matter in soil (Christensen 1996).

The light fraction (LF) is particulate plant residues (such as winery and food-processing waste) undergoing decomposition (by micro-organisms). Part of the LF can be physically stabilized in macro-aggregates as intra-aggregate particulates. This fraction is highly decomposable and can show seasonal fluctuations and spatial variation. The dynamic LF OM turnover time (months to a few years) is linked to macro-aggregate formation and is greatly impacted by cropping (enhanced by perennial grass cover) and tillage (enhanced by reduced tillage) (Post 2000:318).

Soil microbial biomass mediates the OM transformation producing the heavy fraction (HF) OM from the LF (Figure 1). HF OM is stabilized in silt and clay-sized organo-mineral complexes, where the highest concentrations of SOC are found. Following the addition of simple substrates, new SOC is associated with a range of mineral particle sizes, but clay-sized organo-mineral complexes often show greater accumulations and subsequently more rapid loss rates that silt-

sized complexes, which have higher stability. HF turnover time is on the order of decades (Post 2000:318).

Tillage breaks up aggregates and exposes organo-mineral surfaces otherwise inaccessible to decomposers, reducing the intra-aggregate LF and some HF. The refilling of depleted fast-turnover LF pools and the active portions of organo-mineral pools may result in much higher rates of SOC storage (Post 2000, 319).

Turnover time of C in particulate OM is 3-4 years, while carbon in mineral associated OM is ten-fold greater, with a literature review median value of 56 years (Table 8). A significantly greater stock and fraction of C in mineral associated OM indicates a greater stability of surface soil C under pastures (Garten and Ashwood 2002, 10). The time period for SOC sink saturation/new SOC equilibrium to be reached after a land-use change is about 100 years in a temperate climate (Smith 2004, 215). An equilibrium would be formed when the annual rate of mineralization of the stored HF OM equals the rate of humification of applied LF OM.

LF OM is associated with (temporary) N immobilization (in the form of microbial biomass and OM such as lignins that are resistant to decomposition); HF OM is the primary contributor to potentially mineralizable soil N. These two components correlate with particulate and mineral-associated OM (Garten and Ashwood 2002:11). (As stated above, mineral-associated OM has a ten-fold greater turnover time.)

<u>UC Davis Carbon Sequestration Study</u>

In a long-term study at UC Davis, increases in SOC levels have been found to be directly linked to the return of fresh organic material to soil and soil aggregate dynamics. Studies suggest that three different classes of OM: persistent, transient, and temporary, are associated with three physical soil fractions: macro-aggregates, micro-aggregates, and silt and clay (Kong 2005, 1078).

A strong linear relationship has been found between sequestered SOC and cumulative C input over a 10 year period, which suggests that site soils are not C saturated. SOC increased over all input rates, indicating a large SOC deficit and therefore great potential for C sequestration (1082-1083). Comparison with other long-term agricultural experiment sites suggests that California cropping systems have a lower efficiency in converting residue-C to SOC, a 7.6% average rate (compared with up to 21% rate). The UCD study site must receive C inputs in excess of 3.1 Mg C/ha/yr (1.4 tons C/acre/yr, 3.5 tons OM/acre/yr) to maintain existing SOC levels, 11 times greater that that found in Montana, for example. Hot Mediterranean climate summers combined with irrigation likely accounts for the differences (Kong 2005, 1083).

Results suggest that residue quality may be directly linked to the amount of SOC sequestered; some systems disproportionately accumulated SOC relative to their C input. The organic system with crop residues, winter legume cover crop, and composted manure received 1.7 times more C input than the conventional system, but resulted in 14 times greater SOC sequestration. This and other results indicate that the growth of winter legume cover crops enhanced the rate of C sequestration, irrespective of irrigated (as opposed to rainfed) systems. Study data implies that C quality, as governed by legume and compost addition, is as influential on SOC sequestration as the total quantity of added C (Kong 2005, 1083-1084).

The majority of the increase in sequestered SOC occurs preferentially in micro-aggregates formed within macro-aggregates. The micro-aggregate fraction is an ideal diagnostic indicator of long-term SOC sequestration (Kong 2005, 1084).

Methods for Enhancing Soil Organic Carbon Sequestration and Storage

There are four important factors for increasing SOC storage (Post 2000, 325):
(1) increasing OM input rate;
(2) increasing decomposable LF input;
(3) placing organic matter deeper in the soil profile either directly (by infiltration of soluble OM) or indirectly by enhancing mixing by soil organisms (specifically earthworms);
(4) enhancing physical protection through intra-aggregate and organo-mineral complex formation (coupled with reduced tillage).
Conditions favoring these processes occur generally when soils are converted from cultivated use to permanent perennial vegetation (Post 2000, 322).

In a more recent article (Post 2004), the list of factors for increasing SOC storage are expanded. In general, SOC sequestration can be enhanced by (1) increasing rates of OM input, (2) partitioning C to longer-lived pools, and (3) increasing the longevity of C pools (896). Variations occur in the length of time the enhancement method is effective (time to saturation), in the average SOC residence time, and in SOC susceptibility to disturbance leading to decomposition and atmospheric release (897). The expanded list of methods for enhanced SOC sequestration is as follows:

(1) Increased crop residue input resulting from cropping intensification engenders a limited increase in long-term soil carbon because the residue is readily decomposed and the soil carbon reaches saturation in a few decades (or longer depending on local conditions). Much greater increases in SOC are frequently obtained if manures (as biologically altered inputs) are applied, because these inputs appear to contain a larger fraction of organic materials that are more resistant to decomposition than unaltered plant material (897).

(2) Soil structure controls microbial access to substrates, microbial turnover processes, and decomposer food web interactions. Relatively labile material may become physically protected from decomposition if it is incorporated into soil aggregates or deposited in micro-pores that are inaccessible even to bacteria. Macro-aggregates > 0.25 mm in diameter are sensitive to soil disturbance such as tillage, but micro-aggregates < 0.25 mm are generally more stable and resistant to disturbance and appear to turn over more slowly. Recent research has shown that micro-aggregates facilitate the creation of chemically resistant organo-mineral associations with relatively long residence times. Reduction in tillage allows aggregation processes to re-establish through an increase in fungal-dominated OM recycling and an increase in mycorrhizal biomass (897-898).

(3) Perennial grassland establishment on previously tilled land results in substantial increases in SOC, through increased micro-aggregate formation, increased fungal decomposition, and greater OM inputs especially in the root zone. Perennial grassland may also be harvested as a biomass crop, thereby obtaining additional carbon sequestration credit through savings on fossil fuel emissions (898-899).

(4) Transformations involving the formation of melanin-like humic compounds increase SOC resistance to decomposition and are promoted by phenoloxidase enzymes and abiotic oxidants. Stability and activity of the latter can be significantly enhanced by maintaining soil pH >7 (899).

(5) The formation of humic compounds is maximized under partly oxidizing conditions; higher soil oxygen levels result in full mineralization, while lower soil oxygen levels inhibit oxidative polymerization. Frequent wet-dry cycles avoid the stagnation that occurs under either oxidizing or reducing conditions and promote the oxidative polymerization reaction that stabilizes SOC (899).

(6) Optimizing the amount of minerals containing iron and manganese oxides have the potential to stimulate formation of humic materials. A decrease in the rate of mineralization can also be promoted by the development of chemical and physicochemical associations between decomposable compounds and soil mineral components, such as the sorption of organics polymers to the surfaces of soil clay minerals facilitated by polyvalent cations including calcium, magnesium, and iron, thereby protecting the polymers from further microbial and chemical decomposition. Therefore, the addition of divalent liming agents (calcium and magnesium) and iron and manganese fertilizers, coupled with the management of drainage conditions, can do much to enhance the net rate of SOC sequestration and storage (899).

(7) Soil anions such as sulfate and especially phosphate can effectively compete for dissolved organic carbon (DOC) sorption sites, releasing DOC into soil pore water. In soils with deep profiles and limited lateral flow (as for many Central Valley soils), this process could actually serve to enhance SOC sequestration, since the DOC would be reabsorbed onto mineral particles deeper in the soil (or in the vadose zone). Because subsurface mineral-stabilized carbon pools are significantly less dynamic than SOC in upper soil horizons, manipulating the geochemical environment to move carbon from upper to lower soil layers, through desorption and re-absorption of DOC, is a potential means of enhancing carbon sequestration in the subsurface (vadose zone) (899).

(8) Future management of specific microbial populations and processes involved in carbon sequestration, through inoculation or use of biocides, or through manipulation of vegetation, pH, or added amendments, etc., has the potential to enhance the production of biologically resistant compounds (900).

SOC sequestration is greater under pasture than under forest because of a greater allocation of soil C to mineral associated OM, which is a long-term storage pool (Garten and Ashhwood 2002, 1,13). Long-term pastures and maintained grasses are an ideal land cover for rhizosphere development, soil aggregate formation, and aggregate stabilization, all of which promote soil carbon storage. Litter (or residue) with low C:N will produce greater amounts of stabilized OM, with decomposition resulting in a higher fraction of recalcitrant organic componds (such as humics and lignin) that can be stabilized through associations with soil silt and clay (Garten and Ashwood 2002, 10).

C:N is the most common index for the effect of residue composition on decomposition, with N being the most limiting nutrient for decomposition. C:N 20-30 is the threshold above which decomposition is suppressed with N immobilization exceeding mineralization, or in other words, N demand exceeds supply. The threshold ratio may be decreased with increased moisture, higher rates of residue application, and higher proportions of readily available C. C:N rather than BOD:TN is the preferred indicator. Decomposition also depends on lignin, polyphenol, and carbohydrate content (Sumner 2000: C-110).

The optimum temperature range for nitrification is 25-35 degrees C (Videla 2005, 4). The warmer the soil the faster the decomposition by about a factor of 2 for a 10 degree C rise in temperature. The optimal pH for decomposition is 6 – 7.5 (Sumner 2000: C-111).

Winery Wastewater Land Application Studies

In Australia, winery wastewater is usually ponded to allow solids to settle before land application. After ponding, winery wastewater typically contains 500-2000 mg/l C and distillery wastewater typically contains 3000-15,000 mg/l C. The remaining soluble organic C in the applied wastewater can be converted into soil organic C (humus) for improved soil quality and fertility, while removal of the C (through settling and humus conversion) will prevent anoxic conditions in underlying groundwater (Chapman 1995, 1).

The most detailed study of the land application of winery wastewater was conducted in Australia (Chapman 1995A). Samples from the upper soil layer of two sites that had been receiving winery wastewater for 17 years and 2 years respectively, were collected for laboratory trials. Samples were also collected from adjacent sites with the same soil types that had not received winery wastewater. The samples together constituted soils acclimatized with adapted micro-organisms and non-acclimatized to winery wastewater. The soil textures were sandy loam and loamy sand respectively for the 17 and 2-year wastewater application sites, with corresponding 5% and 0% clay content, although both site soils had a dense clay subsoil with poor permeability as shallow as 0.2 – 0.3 m depth. The 2-year acclimatized site was growing trees, the rest of the sites were in pasture (1-2). For the purpose of the experiment, synthetic winery and distillery wastewaters were made from the "natural" wastewater, for the purpose of having wastewater of "reproducible composition" (2, Table 2). Wastewater was applied to the two soil samples at rates of 30% and 60% of the water-holding capacities (WHC). Given that the sandy loam soil had about 20% less sand and double the silt content of the loamy sand, the sandy loam had over twice the WHC of the loamy sand (2, Table1), resulting in about 2.5 times more organic C added to the sandy loam than the loamy sand (3, Table 3).

As a result of the experiments, a graph of change in soil solution soluble organic C with time after wastewater application was developed (4, Figure 1 shown below). Three distinct phases were identified: (1) a rapid initial decrease in soluble C (as a percent of added C) during the first hour, ascribed to microbial uptake and adsorption; (2) a period of variable length with a decrease in soil solution soluble C less than 5% of added C; (3) a second period of rapid decline in C, approaching a low constant value, as the added C is metabolized by oxidative microbial decay, coinciding with rapid evolution of $CO_2$.

In this experiment, both types of soil had the capacity to rapidly reduce high concentrations of soluble organic C to less than 5% of that added in applied wastewater (7). The minimum times required to achieve the less than 5% level ranged from 0.3 to 2.6 days for acclimatized soils and 0.7 to 4.8 days for non-acclimatized soils. Measurements of soluble C were found to be a more reliable indicator of the length of the required rest period between successive irrigations with wastewater than was the evolution of carbon dioxide (8).

Lighter-textured soils (more sand) have a more immediate and rapid microbial decay of soluble organic C than do heavier-textured soils (more silt and clay). However in this experiment, the lighter-textured soil exhibited superior rates of decay only when acclimatized (7). A regular supply of organic C would quickly encourage selection of adapted species and maintain high populations of active cells resulting in an early start of rapid applied substrate decay, by a factor of 2-3 times (8). Lighter-textured soils retain less substrate C in the microbial biomass than

heavier-textured soils. A higher proportion of substrate C was assimilated into the microbial biomass in acclimatized soils (8).

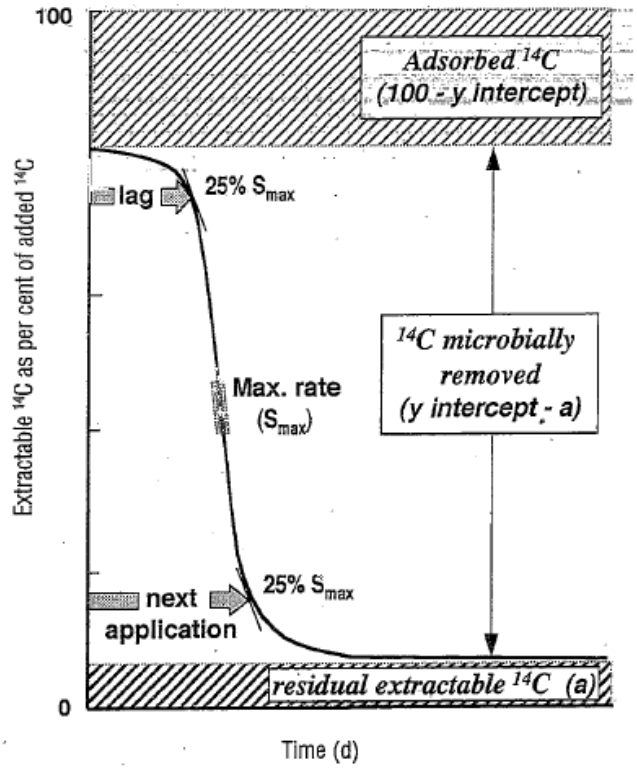



**Figure 1. Model curve of the disappearance from soil of extractable $^{14}C$ contained in applied wine industry wastewaters.**

Increases in organic C loading resulted in higher concentrations of soluble C remaining after the initial rapid removal phase and lag phase. This higher residual could not be fully offset by the concurrent increase in the rate of rapid microbial decay, resulting in longer soluble C removal times overall. The rapid microbial uptake or adsorption of soluble C in applied wastewater may play an important role in helping to keep the substrate close to the soil surface where microbial populations and oxygen levels are relatively high. Another study referred to found that rapid immobilization of solutes slowed the rate of downward movement of the chemical front, relative to the water front (8).

The lighter-textured soil was found to retain less substrate C in the microbial biomass than the heavier-textured soil, and to release greater amounts of $CO_2$, a result that confirms the result of a number of earlier studies. Acclimatized soils also retain more substrate C in the microbial biomass. Long-term increases in TOC in acclimatized soils provides more available energy and nutrients to sustain larger microbial populations, in a self-sustaining and augmenting cycle. Regular applications of wastewater will maintain a high population base of adapted micro-organisms, promote more rapid decomposition, and more rapid uptake of substrate C. In contrast, a new land application site would require different management of wastewater application (8).

In a continuation of the same study (Chapman 1995B), soil samples from each of the soil horizons within the five-foot depth, including the dense claypan of the acclimatized sandy loam.

These soil samples were then subjected to 17 wet-dry cycles, ranging from 95% to 35% of water-holding capacity. The same pattern of soluble C removal over time was observed (Figure 1 above) with the initial rapid decrease of 10% – 50% in the first hour attributed to soluble C absorption by soil particles, increasing in percentage with increasing clay and/or organic C contents, even for clay contents approaching 50% (79). Repeated wastewater applications reduced the percent absorption for some soil layers, but increased absorption in others. Repeated wastewater applications decreased or eliminated the lag period before the onset of rapid microbial metabolism and increased the rate of metabolism, with significant reductions in the time for removal of soluble C from the wastewater applied to the soil (76).

Acclimatization of new wastewater application soils was most rapid in the surface and upper soil layers. For acclimatized soils, added soluble C could be reduced to below 5% within 1 day, when the lag time was less than 2 hours, to the extent that daily removal of soluble C could even exceed that of the wastewater input (76), with regular applications of wastewater on the order of 1-2 times a week required to maintain rapid removal (84). High rates of microbial metabolism also desorbed and consumed C previously absorbed by soil particles, thereby making absorption sites available for the next wastewater application (83).

For unacclimatized soils, the recommendation is made to restrict the depth of percolation of applied wastewater to 15 cm, with applications spaced at least 2 days apart (84). The time required for soluble C removal to below 5% was observed to increase with increasing clay content, leading to the recommendation that soils with an unspecified high clay content would not be suitable soils for wastewater application (or would just require more careful management).

A second lab study of the application of winery waste treatment sludge to soil was conducted in Italy (Saviozzi 1994). As of 1994, direct land application of winery waste without pretreatment was prohibited in Italy, but digested treatment sludge could be land applied if meeting criteria established in 1992 (173). Sludge (Table 1) was added to lab soil at rates of 0%, 0.5%, and 2.5% dry weight (173). The lab soil was “representative of an Italian wine-producing region”, but was not identified as acclimatized to winery waste, and otherwise was not characterized (173). High initial levels of carbon dioxide generation indicated rapid decomposition of easily decomposed components (177), as water-soluble sugars rapidly decomposed in all soils (175-176). Elevated phenolic compounds with sludge addition dissipated as quickly as simple sugars; a separate germination test with the sludge showed no effect (176). Subsequent reduced carbon dioxide generation indicated the decomposition resistance of (other) recalcitrant compounds (177). A carbon decomposition rate curve (Figure 1) and equation was developed. The lack of an initial lag in decomposition confirms the lack of a toxic effect (177). The initial substantial soil increase in COD upon application disappeared after 5-19 days (175-176). Soil water-holding capacity was maintained at 50% throughout experiment (174).

Two measures of soil microbial biomass were tested. The activity of dehydrogenase, the enzyme responsible for bio-oxidation of all organic compounds, increased with highest sludge addition, rapidly decreased for five days, then slowly decreased to near equal the control after 6 months. Levels of fluorescein diacetate (FDA), a measure of total microbial activity, also increased with the highest sludge addition and remained high (176). Total soil biomass carbon increased substantially for the largest sludge addition; biomass carbon continued to increase substantially through 3 months, but stabilized with the control after 6 months (176-177).

Organic carbon increased substantially with sludge addition, and in all three soils declined over time, but after 6 months, 85% of the sludge added C remained, suggesting that winery sludge can raise OM levels in soils (175-176). The total loss of carbon in the form of carbon dioxide over

the six month period was only 3% of the added sludge carbon. Winery sludge seems to be a promising material for improving the organic matter level of soil and maintaining soil biologic activity (177).

Soil nitrate concentrations increased steadily in both the control and treated soils. The low initial C:N may have been a contributing factor. The constant 50% soil water-holding capacity would have prohibited denitrification (174-175).

Addition of sludge resulted in P increase, which then declined over time due to soil adsorption, and resulted in a slight increase in K which remained stable over time. Addition of sludge increased sulfate in all three soils relative to the control, and sulfate increased over time, as did nitrate (175).

Although the sludge was highly alkaline (pH 8.9), the soil pH never increased, and slightly decreased within 5-19 days. EC significantly increased for both sludge addition soils over control, but the control soil increased in EC also (174-175). The highest sludge incorporation rate (2.5%) increased soil salinity to a possibly hazardous level for salt-sensitive crops and the environment (173, 177).

Effect of Soil Water Content and Wet-Dry Cycles on Decomposition

In general, the optimal soil water potential for decomposition is -0.01 to -0.05 MPa. With decreasing water potential the soil becomes too dry, although fungi can survive at -4 to -10 MPa. With increasing water potential above -0.01 MPa, decomposition is limited by low oxygen diffusion. Aerobic decomposition declines when O concentration falls below 10%, but is also dependent on the rate of O replenishment/diffusion (Sumner 2000, C-111).

A number of studies investigated soil biochemical changes in frequent wet-dry cycling. A flush of C and N into soil solution occurs on rewetting of dry soil and is the result of microbial death (Mikha 2005, 339) releasing readily degradable cell solutes that can be utilized by surviving microorganisms, contributing to the pulse of soil respiration after rewetting (340). Repeated wet-dry (WD) cycles significantly reduced cumulative C and N mineralization. The flush of mineralized C significantly decreased with repeated WD cycles; reduction in cumulative mineralized C increased with additional WD cycles (339). Repeated WD cycles did not significantly reduce soil microbial biomass (SMB). Therefore the size of the microbial biomass was not the limiting factor for C and N mineralization, more likely a change in microbial species composition was indicated (345). Reduction in available inorganic N with wetting indicates a surge in microbial growth and activity (345). Overall, there was a significant reduction in available N in WD cycles, as compared to soil with constant water content (345), along with a significant increase in SMB-N in WD cycles (346).

Divergent results with other studies showing an increase in mineralization in WD cycles may be due to soil physical disruption through aggregate destruction and consequent release of protected soil organic matter, whereas in the present study, techniques allowed soil structure to remain intact (hence importance of no-tillage soil management). Lack of soil aggregate destruction with rewetting, as observed in other studies, may be due to higher clay and soil organic matter content; lack of soil aggregate destruction may also be due to the formation of a soil crust and a residue cover (346). These results confirm the same results in an earlier study (Magid 1999).

Another study establishes the existence of limiting ranges for N mineralization, with reduced N mineralization at lower and upper limiting soil water contents, along with a least limiting water

range with N mineralization largely independent of water content (Drury 2003, 1388). This study apparently does confirm that 80% water-filled pore space is optimum for simultaneous mineralization and denitrification (1403). The study also proposed methods for determining mineralization and denitrification capabilities of specified soils (1404).

Another study on the effect of soil water content on N mineralization, nitrification, and immobilization found that at 100% field capacity (FC), N mineralization was only 34% of that at 85% FC, with 75% FC the optimum water content for mineralization. For nitrification, at 85% FC, the rate is 88% less than at 100%FC (Videla 2005. 4). At 100% FC, there is significant immobilization of N, and also likely denitrification, along with an increase in SMB-N (5).

**Literature Review Conclusions**

The proper management of the application of organic waste to soil for optimization of soil organic carbon sequestration and humification will result in simultaneous enhancement and protection of soil, water, and air quality, with substantial benefits to be realized. Optimization of the humification of organic waste by soil micro-organisms has numerous soil quality benefits: increased biomass activity, increased water-holding capacity, lowered bulk density, increased cation exchange capacity, increased buffering capacity, and increased fertility, to name some of the most important. Water quality is enhanced by the incorporation of nutrients, as potential groundwater quality contaminants, into the growing microbial biomass and newly formed humus, instead of remaining available in soil solution for eventual leaching from the soil into the vadose zone and groundwater. Air quality is enhanced by the sequestration of carbon into relatively stable, long-lasting soil humus, rather than decomposition and emission of $CO_2$ into the atmosphere, where it only adds to the overall increase in global warming gases. Management of denitrification to result in the emission of nitrogen gas rather than nitrous oxides is a further enhancement to global warming gas air quality. These triple beneficial results of the land application of organic waste could apply to not just winery waste, but also to the land application of food processing waste, biosolids, manure, and green waste. Management practices leading to the restoration of soil depleted of organic matter from natural levels offers a potential for substantial reduction of atmospheric carbon dioxide by soil sequestration of carbon.

In a long-term study at UC Davis, increases in soil organic carbon levels have been found to be directly linked to the return of fresh organic material to soil and soil aggregate dynamics. Central Valley soils are typically low in organic matter as a result of conventional agriculture, and have a large soil organic matter deficit and great potential for soil carbon sequestration with an unknown upper-limit capacity for the accumulation of stable soil organic matter.

Optimization of environmental quality through the land application of organic matter waste is further enhanced through methods and management for the physical stabilization of the light-fraction of organic matter in soil macro-aggregates, and the stabilization of the heavy-fraction of organic matter in silt and clay-sized organo-mineral complexes and micro-aggregates.

BOD is a measure of organic matter potentially subject to decomposition, a measure most applicable to the design of engineered wastewater treatment facilities. Measurement of wastewater C:N is the most effective wastewater application management tool. Measurement of soil microbial biomass, through multiple possible methods that need to be evaluated for best applicability to California soils, is most important to the evaluation of the humification and stabilization of soil applied organic matter waste, the measurement of the beneficial end result.

Although no study has yet confirmed by direct observation, research indicates the likelihood that given optimal wastewater C:N, most or all available nitrate in solution could be taken up by soil microbial biomass shortly after land application, leaving no nitrate available for denitrifying organisms.

Research results show that after initial acclimatization, a self-perpetuating cycle is established where the application of ever increasing amounts of organic waste to the soil results in ever increasing soil microbial biomass, increasing humification and carbon sequestration, increasing soil structure and porosity, increasing organic carbon absorption sites, increasing soil water-holding capacity, and other factors all of which are conducive to the application of even larger amounts of organic waste.

A certain percentage of soil organic matter stored in the form of humus will be decomposed by soil micro-organisms on an annual basis, primarily during the warm summer months of the growing season, prior to the season of winery wastewater production and land application. The growth of an annual crop or perennial cover crop on the land application site will enhance the uptake of the gradually released nutrients in soil solution. Maintaining summer fallow on the land application site with no application of irrigation water will retard the decomposition of soil humus. Pre-irrigation of land application sites after summer fallow will serve to "recharge" soil micro-organisms in preparation for the first application of winery wastewater in the fall. At some point, likely 100 years or more for typical California soils, a state of equilibrium will be reached where the annual decomposition rate of soil humus will be equal to or exceed the annual humification rate. At that point the soil has reached a maximum limit of organic matter storage as a percent of total soil mass. However, the soil would still remain an excellent site for the continued application of winery wastewater.

Additional conclusions are given in the next section.

**WI/KJ Study and Guideline Conclusions**

On the basis of the literature review, the following recommendations are made:

3.1. The application of winery wastewater to land may be managed to optimize the humification of wastewater organic matter into long-term stable soil organic matter and microbial biomass for the purpose of carbon sequestration. This process will simultaneously result in benefits to water quality, soil quality, and air quality.

3.2. Land applying winery wastewater after achievement of C:N 25-40 will likely result in rapid uptake of nitrate in solution by soil micro-organisms, so that establishment of conditions conducive to denitrification may be unnecessary. In any case, limiting wastewater application to no more than 100% soil water-holding capacity after accounting for antecedent soil water status, for soils above the limits of saturated hydraulic conductivity established above, will optimize the potential for both humification and denitrification, while being protective of groundwater quality by assuring sufficient time for complete soil treatment.

3.3. A large variety of methods are available to enhance and optimize the humification and sequestration of organic matter waste applied to soil directly as a benefit to water, soil, and air quality. At present, ten methods have been identified, including (a) increasing the application of decomposition resistant organic matter; (b) reducing tillage and other soil disturbance to promote the formation and maintain the micro-aggregate soil structure, where alleviation of surface crusting is not required; (c) establishing perennial grassland on land application sites; (d) maintaining soil pH between 7-8; (e) applying wastewater in an amount

3.4. There is no theoretical basis, nor research literature review basis, for limiting the BOD mass loading of winery waste per unit of time, nor resolving the alternative recommendations for limiting BOD mass loading to 7000 lb/acre or 3000 lb/acre. The issue of the degree of reduction of Fe and Mn and the leaching of soluble Fe and Mn into the vadose zone and groundwater could be rendered moot by reducing the wastewater loading rate to no more than 100% of soil water-holding capacity including antecedent conditions, given the ability of the soil to maintain air exchange with the atmosphere and sufficient oxygen in the soil air. The most important factor in the BOD mass loading is not the establishment of an upper limit, but the maintenance of C:N 25-40 in applied wastewater. The BOD mass loading limit would then best be established empirically on a site-specific basis by factors including observed infiltration time and surface ponding.

3.5. A soil not previously used for application of winery wastewater must be gradually acclimatized to accepting winery waste over a period of at least one-year (or application season).

3.6. For soils that have been acclimatized to winery wastewater, the acclimatization must be maintained by frequent applications of winery wastewater.

**Issue 4. Is there sufficient basis to assume or verify that potential groundwater contaminants infiltrating below the five-foot depth of soil will be transformed in vadose zone processes to assure the protection of groundwater quality?**

**Issue Analysis**

As the WI/KJ Study did not make direct observations of the vadose zone and underlying groundwater, the question of the treatment potential of the vadose zone was covered in a literature review (Section 4). In the literature review, a comparison was made between studies on the land application of secondary and tertiary treated municipal wastewater for the purpose of groundwater recharge, where high hydraulic applications and unimpeded flow to the water table is preferred, and the land application of winery wastewater for the purpose of disposal, where sufficient soil residence time for near complete treatment in the upper soil profile is required. Secondary and tertiary treated municipal wastewaters typically are low in BOD and BOD:TN relative to winery wastewater.

The WI/KJ literature review concentrated on three recent treated municipal wastewater groundwater recharge studies. These studies measured wastewater carbon content as dissolved organic carbon (DOC) or biodegradable dissolved organic carbon (BDOC). In one study, the largest decrease in DOC (approximately 60-80%) was observed within the upper part of the soil profile. Nearly continuous lower rates of removal were observed in the vadose zone over 120 feet of depth, and over six years of wastewater application, until the DOC concentration in the ambient groundwater was reached (Figure 11). In the second study, DOC concentrations were

reduced 50% in the soil profile, and about 70-80% in the vadose zone (4-5). In the third study, consistent conditions and data could not be obtained (4-6).

Study results indicated that the reduced oxygen and anaerobic conditions generally occurring in the vadose zone, combined with at least minimal DOC in solution, result in continued denitrification (4-7). Another process, anaerobic ammonia oxidation, has been hypothesized to result in nitrate reduction when conditions are not conducive for primary denitrification.

At two of the study sites, a phenomenon called "nitrate spiking" was observed in the lower part of the soil profile and upper part of the vadose zone. In one study, nitrate levels increased over ten-fold from the surface to the five-foot depth, but then decreased over ten-fold between 17-40 feet below ground surface. In another study, "nitrate spiking" was observed to occur 5-10 feet below ground surface, but was substantially decreased by 20 feet below surface, where dissolved manganese was detected (4-7 to 4-8).

In the PR analysis, the WI/KJ literature review was dismissed as being a "recitation of examples." Citing the substantial difference between secondary and tertiary treated municipal wastewater and winery wastewater, the PR concluded that "this chapter has little, if any, relevance to the treatment of winery wastewater." The PR then cited examples of two sources for information deemed to be more relevant. In the WI/KJ response, both sources were said to have been examined in preparing the literature review, and that the (biogeochemical) mechanisms presented in the review are relevant (Response, 5).

The WI/KJ Study and Guidelines recommended that the hydraulic loading rate for an individual process water application event be calculated as the lower of the maximum soil water storage capacity from ground surface to the five-foot depth, or the maximum wastewater that can be applied before the 7,000 lb/acre BOD limit is reached, for a single loading event. The WI/KJ Study concluded "this hydraulic limitation ensures that, on average, applied water will be retained in the soil profile for treatment instead of immediately percolating into the subsurface. There is almost certainly additional treatment capacity below the surface five-feet of soil, especially for sites with at least 15 feet of unsaturated zone above the water table. This, however, is treated as additional capacity for the purpose of these guidelines. The basic treatment capacity is assumed to be in the surface 5 feet" (5-6). The WI/KJ Study and Guidelines recommended depth to groundwater >15 feet as a site selection criterion (Figure 13).

The issue of the extent of vadose zone denitrification and treatment of potential groundwater contaminants (specifically BOD and N) percolating or leaching beyond the five-foot soil depth presupposes that percolating wastewater passes below that depth prior to soil treatment. The WI/KJ Study and Guidelines propose that by limiting hydraulic loading for each single application to no more than the soil water-holding capacity for the five-foot depth, that sufficient soil treatment will be assured. The PR contended that the failure to take account of soil water storage status antecedent to each application event lead to the "possibility of significant leaching beyond 5 feet" (Response, 8).

The PR referred to Figures 4 and 5 in stating that "no effort was made to determine percolation beyond 5 feet." WI/KJ responded that percolation was determined in Figures 4 and 5 (Response, 8). The Figures do confirm the determination of percolate for each wastewater application and for yearly cumulative totals: 18 inches in 2002 and 28 inches in 2003 for the non-stillage site, and 12 inches in 2002 and 11 inches in 2003 for the stillage site. The WI/KJ Study even calculated the mass per acre and percent of application mass for each percolate constituent in Table 6.

Whereas the PR regarded the percolate as polluting of groundwater, the WI/KJ Study reasoned that "the applied water displaces any water already in the soil profile and the displaced water percolates. The water in the soil profile undergoes treatment while it is held in the soil. The rest period between applications is intended to allow time for BOD removal and nitrogen transformations. It is not part of spreading basin management to adjust loading rates based on soil moisture. Basins are managed to allow applied water to percolate after a residence time in the soil" (Response, 8).

**Literature Review Results**

Vadose zone preferential flow paths are heterogeneous and occupy only a relatively small area of the vadose zone; soil moisture is not a sensitive indicator for location of preferential flow paths; location of preferential flow paths does not change over time (Harter 2005, 128). Preferential flow paths are responsible for most of the water and solute transport from the root zone to the water table and the quick flushing of nitrate with little or no denitrification; lower nitrate would occur in stagnant water zones outside preferential flow paths where subject to a small amount of denitrification, (however in this study) soluble carbon was found to be very low, not favoring high rates of microbial degradation of nitrogen (by denitrification) (Harter 2005, 130). Low average nitrate concentration in vadose zone pore water should not be interpreted as an indicator for high denitrification and low nitrate impact on groundwater, rather a result of swift, unattenuated nitrate transport to the water table (Harter 2005, 131).

In alluvial sediments, the often used assumption of uniform flow in the deep vadose zone is inadequate to predict nitrate levels in the deep vadose zone below the root zone. Actual nitrate levels are potentially much lower due to rapid N transport in preferential flow paths of limited spatial extent. Measured nitrate levels below the root zone (typically through use of a lysimeter) should not be used to validate a long-term potentially leachable N analysis or to close the mass balance, as doing so would lead to significant underestimation of nitrate leaching rates to groundwater. Denitrification may locally occur throughout the deep vadose zone, but is not likely to be a major process (Onsoy 2005, 52).

The Harter/Onsoy study cited above did not include the addition of organic matter to the study site soil. Others studies conducted with the addition of organic matter have observed an increase in denitrification in the vadose zone.

In general, denitrification activity within and below the root zone is limited by C availability (Artiola 1997, 804). Laboratory measured denitrification rates were one to two orders of magnitude higher (from 2-14 m depth) in a sludge-amended plot than in a fertilizer-amended plot (805). The type and amount of organic C supplied by sludge may significantly impact nitrate losses within the root and vadose zone due to the leaching of more soluble and readily available forms of organic C. Field and laboratory data indicate that nitrate below the root zone is significantly impacted by the source of soluble C available as well as the amount of C that is leached below the root zone; a sludge-amended soil showed significantly higher denitrification rates below the root zone, correlated to the actual amounts of soluble organic C from 8-14m depth. Another referenced study showed that the denitrifying activity (and reducing power) of various naturally occurring C substrates, such as sucrose, citrate, and malate, vary by a factor of three (on a mass basis): one gram of citrate would generate three times more nitrous oxide gas than the same amount of glucose (810).

In one study, data showed a poor linear correlation between nitrate concentration in surficial groundwater (top of the groundwater table), and depth of the vadose zone and depth of

percolation; instead differences in conditions conducive for denitrification in the soil profile and the soil/groundwater interface appears to be the major factor for variation in surficial groundwater nitrate (Paramasivan 1999, 312-313, 314).

The deeper the vadose zone, the lower the concentration of DOC in groundwater near the water table, indicating considerable attenuation of surface-derived DOC occurred in the vadose zone. DOC concentrations also decreased exponentially with increasing depth below the water table, most notably in the upper two meters, implying continued attenuation in the upper layer of the saturated zone. Nitrate was depleted where DOC was high providing evidence of denitrification. DOC concentrations in shallow groundwater showed considerable spatial variability, but stability over temporal variability, therefore the largest source of variation in DOC concentration in groundwater was spatial rather than temporal, suggesting that local heterogeneities play an important role. The results highlight the importance of shallow vadose areas and the need to distinguish where samples are collected in relation to flow paths. Substantial loses of DOC in the vadose zone and in shallow depths of the aquifer suggest active biogeochemical processes in these boundary environments (Pabich 2001, 247).

Two factors can explain the limited decrease of nitrate concentrations in the field (50 times slower relative to laboratory results, where field tests are done in decomposed granitic soil):

1. The variability of bacterial activity was found to be correlated with water content and clay content, the higher for both the higher the bacterial activity. In illuviation processes, zones of transport of clay particles are also zones of transport of water, dissolved carbon and nitrates. Sites of illuviation are therefore sites for increased denitrification. However, dissolved oxygen could also be preferentially transported to the same sites or fingering zones, thereby contributing to aerobic nitrification processes and offsetting denitrification. (Legout 2005, 346).

2. Since denitrification must be heterogeneous in the vadose zone and must occur at the aggregate or mineral scale, only small microsites with ideal redoxic conditions must be biogeochemically active. Denitrification could be limited by the bioavailability of N and C substrates, which are controlled by the water content that allows diffusion processes. However, when the soil is saturated, the convective movements of solutes may be too rapid for denitrification to be completed (347-348).

A high degree of measurement variability was found in a site with undeveloped sandy soil with minimal soil structure and uniform fertilizer and irrigation management practice. The denitrifier population varied by three orders of magnitude for different monitor well locations, but was greater in the stagnant water samples than in the purged water samples, and was lower in the stagnant water samples than in the soil matrix in the capillary fringe region. This is the reason for greater denitrification activity in the aquifer porous matrix/capillary fringe/soil-groundwater interface compared to the groundwater matrix (Paramasivam 1999, 314).

Dissolved organic C content varied by one order of magnitude in different monitoring well locations and varying depths in the soil profile and was highly correlated to denitrification capacity. Denitrification activity increased 1- to 4-fold with the addition of readily available C to soil samples and 2- to 38-fold with addition to groundwater samples (315).

The composition of groundwater should attain some equilibrium over a long period of time unless localized soil conditions at the soil/groundwater interface can induce some reactions which can alter nitrate concentrations (308). Denitrifier populations were greater in stagnant well water compared to five well-volume purged well water, with corresponding much lower nitrate

concentration. This study demonstrated that denitrification occurs in well drained sandy soils particularly at the soil/groundwater interface (317).

**Literature Review Conclusions**

Denitrification activity within and below the root zone is determined by type, availability, and solubility of the carbon substrate. Denitrification activity is greatly enhanced by the addition of a soluble carbon source to the soil. Where soluble carbon is very low in percolating soil solution, the vadose zone denitrification rate is also very low, potentially resulting in a swift, unattenuated nitrate transport to the water table. In the absence of any soluble carbon substrate, vadose zone denitrification will likely be non-existent. In this context, the PR was largely correct that the treated municipal wastewater studies were "irrelevant", because of the relatively lower levels of DOC. They were not correct in the implied assumption that denitrification would not be as effective in the vadose zone given the substantially greater concentration of both C and N in applied winery wastewater. In fact, vadose zone denitrification may be expected to be up to several orders of magnitude higher on sites of winery wastewater application in comparison to treated municipal wastewater application.

The largest source of variation in dissolved organic C concentration in groundwater is spatial rather than temporal. One consequence of this principal is that substantial loses of DOC in the vadose zone and in shallow depths of the aquifer suggest active biogeochemical processes in these boundary environments. Greater denitrification activity occurs in the aquifer porous matrix/capillary fringe/soil-groundwater interface in comparison to the groundwater matrix. Therefore, the vadose zone denitrification capacity may not be dependent on the depth to groundwater, and hence the length of time necessary for percolate to pass through the vadose zone. This circumstance also indicates that the traditional practice of purging groundwater monitoring wells before collecting water samples for regulatory compliance purposes may be inadvertently short-circuiting denitrification in the groundwater interface, thereby resulting in a false measurement of elevated nitrate in the groundwater.

Given the above circumstance, the need for a > 15 foot depth to groundwater may be obviated, as a degree of vadose zone flow through time may have no particular benefit. Some research even suggests that denitrification rates may be higher in shallower vadose zones. Possibly a better rule would be that a site that has sufficient depth to groundwater to grow grapes may also be considered suitable for application of winery wastewater.

All soil types (including and perhaps especially sandy soils) can exhibit preferential flow paths in the vadose zone that may be responsible for most of the water and solute transport from the root zone to the water table. This condition may explain the observations of "nitrate spiking". The recognition of this phenomenon emphasizes the importance of maximum soil treatment within the generalized five-foot depth below ground surface. Optimization of soil treatment can only be assured if sufficient wastewater residence time is assured. For soils with saturated hydraulic conductivity > 0.83 inches/hour for acclimatized soils, and > 0.50 inches/hour for non-acclimatized soils, strict adherence to wastewater hydraulic loading applications not to exceed soil water-holding capacity, including accounting for antecedent soil water status, would be essential to limit the possible impact of preferential flow.

There is no evidence presented, nor found in the LR, to support the WI/KJ contention that "applied water displaces any water already in the soil profile and the displaced (and therefore treated) water percolates." This contention was made in the WI/KJ Response to the PR analysis, so the peer reviewers did not have an opportunity to comment on it. Other comments by the peer

reviewers do make clear their concern that wastewater application rates should not exceed the soil water-holding capacity including antecedent soil water status. Therefore, this adjustment in the WI/KJ Guidelines needs to be made, along the criteria proposed in the preceding paragraph. The water in the soil profile undergoes treatment while it is held in the soil. Only by maintaining this requirement can application sites be managed "to allow applied water to percolate after (sufficient) residence time in the soil," and not by failure or neglect to adjust loading rates based on soil (antecedent) moisture.

Given that denitrification is heterogeneous in the vadose zone and must occur at the aggregate or mineral scale, the importance of the water-holding capacity limit management measure is further indicated by the observation that only preferential flow small microsites with ideal redoxic conditions are biogeochemically active. Further, given that denitrification is limited by the bioavailability of N and C substrates, that availability is controlled by the water content and infiltration rate. Therefore, when the soil is saturated, such as under application of wastewater that exceeds the soil water-holding capacity, the downward movements of solutes may be too rapid for complete denitrification.

**WI/KJ Study and Guidelines Conclusions**

On the basis of the literature review, the following recommendations are made:

4-1. The WI/KJ Study and Guidelines could be established on the basis of validated vadose zone and groundwater capillary fringe zone denitrification, assuming the sufficient presence of dissolved organic carbon and nitrate. However due to the expected presence of preferential flow paths, particularly in sandy soils, every effort must be made to retain applied winery wastewater within the upper soil zone for sufficient time for treatment by soil micro-organisms.

4-2. Sufficient soil treatment time may be assured by wastewater application rates that do not exceed the soil water-holding capacity, with allowance for antecedent soil water status. For soils with saturated hydraulic conductivity values approximately > 0.83 inches/hour for acclimatized soils, and > 0.50 inches/hour for non-acclimatized soils, strict adherence to wastewater hydraulic loading applications not to exceed soil water-holding capacity, including accounting for antecedent soil water status, would be essential to limit the possible impact of preferential flow.

4-3. Depth to existing groundwater as a site selection criterion for application of winey wastewater may be determined by sufficient depth to groundwater to grow grapes.

4-4. Site soils with higher clay contents and more well-developed soil structure, within the moderately high saturated soil conductivity classification, may be expected to have substantially higher denitrification rates, all other factors being equal.

4-5. In order to correctly measure denitrification at the groundwater surface, groundwater monitoring samples should be collected from unpurged wells.

**Issue 5. What monitoring programs should be established for land application sites to ensure that the systems are functioning as intended? Will vadose zone monitoring by lysimeters produce representative samples? What type of lysimeter design is appropriate?**

**Issue Analysis**

A specific monitoring protocol was not developed and presented in the WI/KJ Study and Guidelines. Rather the presumption seemed to be that the measurements used in the WI/KJ Study, and the measurements needed for the optimal management of winery wastewater application, would best serve as the monitoring protocol. The PR did not specifically question or amplify further on this presumption, other than the WI/KJ protocol itself. Likewise, most LR research was not specific to development of a monitoring protocol. Again, a monitoring protocol was suggested or implied based on the research measurement parameters.

Two research papers did specifically address a comparison study of microbial biomass activity measures, and these papers are detailed in the next section. Results of a few other studies lend insight into what may be the limitations of some possible monitoring protocols, and will also be presented below.

The overall results and conclusions of the LR do however suggest a monitoring protocol that has the advantages of relative simplicity, quantitative over qualitative measures, upper and lower limits to demonstrate optimization of groundwater quality protection, monitoring of changes in soil parameters over time that may impact wastewater application suitability, and measurements that are necessary for proper wastewater application management, regardless of regulatory requirements. This proposed monitoring protocol will be presented in the sections on Literature Review Conclusions and monitoring Guidelines Conclusions below.

**Literature Review Results**

Two comprehensive studies have recently been conducted that evaluate a large number of biological soil quality parameters for evaluating water quality and environmental impacts. In one, 22 methods of soil microbial biomass quantification under five categories were evaluated for suitability as indicators for the protection of groundwater quality from organic carbon inputs (Rauch and Drewes 2005, 910-911). In the other, over 20 soil biological parameters where evaluated by five groups of scientists (in five countries) on 49 soil sites varying with four types of soil use, multiple soil types, and varying degrees of pollution or its absence, for suitability and sensitivity as indicators for measuring the degree of environmental degradation and ability of the soils to function as a pollution remediator (Filip 2002, 170-171). BOD was not one of the parameters evaluated in either study.

In the first comprehensive study (Rauch and Drewes 2005) on methods of measuring soil microbial biomass, five categories of soil biomass quantification methods were evaluated: (1) direct total counts by microscopy, (2) extraction of biomass cell constituents, (3) molecular biological methods, (4) biomass enzyme activity, and (5) microorganism culture methods. Twenty-two specific methods within the five general categories were evaluated and the best were selected based on the following criteria: (1) recovery and reproducibility, (2) limit of detection, (3) specificity, (4) applicability to different soil textures (including surface and vadose zone soil), (5) simplicity and cost, and (6) information value with respect to microbial metabolic activity (Rauch and Drewes 2005, 910-911, Table 1). Based on the study paper literature review, three methods, one from one category, and two from another category, were selected as best meeting the six criteria. From the second category, extraction of biomass cell constituents, the method of phospholipid extraction was selected. From the fourth category, biomass enzyme activity, the methods of dehydrogenase activity and substrate induced respiration were selected (910).

Phospholipids are components of cell membranes and their extraction estimates the amount of viable microbial biomass in the soil regardless of its physiological activity, such as decomposition, nitrification, or denitrification. Substrate induced respiration is a simple

measurement for the presence of metabolically active biomass that is involved in the decay of easily degradable carbon sources under aerobic conditions. Dehydrogenase is a universal enzyme that acts as a catalyst in the decomposition of organic matter (as a food or energy source). Quantification of dehydrogenase activity measures microbial biomass activity independent of prevalent soil redox conditions, and therefore is sensitive to denitrifying bacteria (910).

In the second comprehensive study (Filip 2002), 20 soil microbiological and biochemical parameters grouped under four categories (Figure 1) were evaluated for sensitive indicators of soil quality and environmental changes using 49 soil sites in Eastern Europe (169), representing many different soil types and present uses (170). The basis for the study was the adoption of the Federal Soil Protection Act in Germany in 1999. The implementation of this Act required the development of well-justified standards and reliable monitoring methods. The purpose of the study was to estimate whether some microbiologically-based soil characteristics would be capable of indicating anthropogenically-caused alterations in soil quality (170).

The paper characterizes soil physico-chemical parameters as indicative of end-point values in a soil "retrospective", meaning measures of the after effects of active soil processes already concluded. In contrast, biochemical soil parameters are key indicators of sustainability of natural soil functions under different uses, and measure the actual flux of organic matter and mineral elements, and transfers between soil pools, and other environmental mediums, such as water and air. Biochemical parameters tested were grouped into four catagories: (1) microbial biomass, (2) microflora composition, (3) mineralization processes, and (4) synthesizing processes (171). The relative numbers and functions of soil macro- and micro-organisms were represented graphically in Figure 3 (173).

As the result of the study, five soil biochemical parameters were selected as the most promising sensitive soil quality indicators: (1) N-fixing bacteria, (2) total microbial biomass, (3) soil respiration, (4) dehydrogenase activity, and (5) humification activity (169). The humification process was found to be mainly suppressed in polluted soils where most of the added organic substrate (lucerne/alfalfa meal) was either mineralized or remained non-utilized during the incubation period (172). In addition to these single analytical values, a number of ratios appeared to be useful indicators: (1) microbial biomass to soil organic carbon, (2) the metabolic quotient (the amount of $CO_2$ evolved per weight of microbial biomass), (3) total aerobic bacteria to spore-forming bacteria, (4) total actinomycetes to fungi, and (5) humic to fulvic organic acids (172). The overall conclusion was that further research is needed to identify parameter ranges and limits with respect to soil types, uses, and seasonal oscillations (169).

The extensive San Joaquin Valley study (Harter 2005, Onsoy 2005) of soil/groundwater relationships discussed above (Issue 4), came to a number of conclusions regarding the accuracy and significance of established monitoring methods. The observed predominance of preferential flow conduits included observations that recharge water may constitute only a minor portion of vadose zone water. Therefore, treatment changes in recharge water quality are not effectively represented by composite soil samples, from which composite or average measures of nitrate concentrations are not appropriate for estimating the amount of denitrification (Harter 2005, 131). Measured nitrate values were found to vary by several orders of magnitude over relatively short distances. The variability of nitrate concentrations served to underscore the importance of high spatial sampling frequencies when monitoring field-scale solute leaching with suction lysimeters that measure relatively small volumes of soil water (Onsoy 2005, 52).

In this context, it should be remembered that lysimeter data from the WI/KJ Study showed considerable variation. Specifically, in the 2002 stillage test site lysimeter data, each of three five-foot depth lysimeters behaved differently (3-5).

The questionable nature of lysimeter data was not found to apply to groundwater sampling data. Groundwater quality measurements at the water table were found to be a viable monitoring tool, as travel times through deep vadose zones may be shorter than previously assumed (Onsoy 2005, 52).

**Literature Review Conclusions**

The LR focus on recent research since 2000 showed the nearly universal trend of researchers to place the highest value on soil microbiological and biochemical data as the most accurate measure of changes in soil quality, and by direct impact, groundwater quality, as a result of the application of organic waste and other wastes to the soil. The soil is recognized as the essential natural medium for the recycling of organic waste. The soil's essential natural function of waste recycling may be strained by an excessive unnatural level of organic waste input. In either case, direct measurements and monitoring of soil microbiologic populations and functions represents the most significant and accurate measure of soil environmental changes, changes in sustainability of soil ecologic function, and the overall health and improvement in soil quality, and related water and air quality. Specifically, measurements of soil microbial biomass and functions are the most direct measure of the fate of organic matter in land applied winery wastes, and hence the potential for groundwater quality impacts.

Recent research based on measures of soil microbial mass and functions in response to the application of organic wastes highlights a very important realization. The proper management of the application of organic waste to soil will result in simultaneous improvement of soil, water , and air quality, with substantial benefits to be realized (see Issue 3).

A great variety of possible methods and parameters exists for monitoring of soil microbial biomass and functions as a result of organic waste application. Many of these parameters and indices derived from the parameters have proven to be sensitive measures of environmental quality change in specific circumstances, but no single measure or group of measures has been found to be best applicable to all situations. Attempts to compare the different measures under similar conditions have resulted in varying recommendations for the best methods. Generally recommended and applied measurement methods include total soil microbial biomass, soil microbial biomass carbon and nitrogen, phospholipid extraction, dehydrogenase activity, humification activity, substrate induced respiration, and fumigation incubation and extraction, as well as others. A detailed exposition of the LR results on the pros and cons, applicabilities and limitations of the various methods and their derived indices could be presented, but that would not conclusively resolve the issue of the best method to use for the monitoring of winery waste application. Therefore, this question is the one that can best be recommended, as a result of this LR, as an important and essential topic for further research.

While awaiting a definitive assessment of the best soil microbial monitoring parameters, the resolution and recommendations of outstanding issues regarding winery waste application, as detailed above, provide a relatively simple, inexpensive, practical, and necessary set of monitoring parameters. These parameters are the ones that are necessary to determine if the winery waste application is being conducted according to the best management practices that have been identified. These parameters include:

(1) soil bulk density, measured for the surface soil layer on an annual basis, for the purpose of monitoring changes in soil saturated hydraulic conductivity;
(2) soil water status or content, as a percent of water-holding capacity, prior to each application of wastewater;
(3) BOD concentration and C:N ratio of the wastewater after each mixing in a holding tank or pond and prior to land application;
(4) pH of the surface soil, on an annual basis, at the same time of the year after the conclusion of wastewater application;
(5) total soil organic matter content, measured on an annual basis.

One time determinations of soil texture and bulk density of all identified soil horizons, presence of hardpans, total soil water-holding capacity, existing organic matter content, and depth to groundwater table constitute determination of baseline conditions and site suitability prior to initial wastewater application or resumption of application.

Groundwater quality measurements at the top of the water table are a viable monitoring tool, and since the composition of groundwater may be expected to attain a degree of equilibrium over a period of time, and reflect the result of leaching, denitrification, and other soil biogeochemical processes, groundwater monitoring samples should be collected in stagnant well water rather than purged well water samples.

Because of a very high degree of spatial variability, soil solutions collected by lysimeters are an inaccurate and unreliable means of monitoring land application of winery wastewater for regulatory purposes.

**WI/KJ Study and Guideline Conclusions**

On the basis of the literature review, the following recommendations are made:

5-1. The quantitative monitoring measures that are necessitated by the best management practices identified in the WI/KJ Study and Guidelines and as modified by the LR, also provide the most straightforward quantitative measures for the purpose of regulatory compliance. These monitoring parameters include (1) surface soil bulk density on an annual basis, (2) soil water content as a percentage of water-holding capacity on a single wastewater application basis; (3) wastewater BOD concentration and C:N ratio on a single wastewater application basis; (4) pH of surface soil on an annual basis, and (5) soil total organic matter content on an annual basis.

5-2. Site selection suitability criteria and baseline soil data require the determination of the following parameters prior to initiation or resumption of wastewater application: (1) soil texture of all horizons, (2) bulk density of all horizons, (3) presence of hardpans, (4) total soil water-holding capacity, (5) soil organic matter content, (6) depth to groundwater table, and (7) pH of all soil horizons.

5-3. Groundwater quality monitoring of unpurged groundwater surface samples may be continued as a reliable regulatory monitoring parameter.

5-4. Soil percolate sampled by lysimeter may be used for site management purposes, but lysimeter data is too variable and inaccurate for regulatory compliance monitoring purposes.

5-5. A primary need for future research is the selection of one or more soil microbial biomass and function parameters and indices suitable for measurement of winery wastewater application impacts to California soils. This future monitoring tool would serve as a

direct measure of the end-point of the decomposition of applied organic waste resulting in environmental quality protection.

**Issue 6. Are the resolution of issues and application of guidelines for land application of winery waste equally applicable to the reuse of winery wastewater for crop irrigation with resultant agronomic uptake of dissolved solids and decomposition products as crop nutrients?**

**Issue Analysis**

There are two alternatives for use of winery wastewater for crop irrigation: (1) growth of an annual or perennial crop such as grass on the spreading basin, and (2) irrigation of the vineyard with wastewater. An existing crop use of the spreading basin for growth and harvest of an annual crop during the spring and summer growing season prior to the seasonal application of wastewater is "a standard practice for spreading basin management" (Response, 11). Growth and harvest of both an annual and perennial crop in the spreading basin would have beneficial effects in maintaining soil structure and soil quality, maintaining soil permeability, and uptake of residual and applied nutrients contributed by the wastewater. The growth of a perennial crop in the spreading basin would have additional benefits over an annual crop, but could conflict with the need to rototill the surface after formation of a crust as a result of wastewater application.

On this issue, the WI/KJ Study and Guidelines states that "though crop irrigation was not evaluated directly as part of this study, the same fundamental biochemical reactions (Refer to Appendix E) apply to land application processes regardless of the method of process water application" (6-2).

The central question is whether rates of BOD and wastewater application, and C:N ratios of wastewater applied to vineyards, is beneficial or detrimental to grape growth, or could result in water quality impacts relative to land application to spreading basins of bare soil or with other annual or perennial crop growth. These issues are addressed directly and indirectly in the research studies described below.

**Literature Review Results**

A field study was conducted in Italy (Tano 2005) on the land application of exhausted wine vinasses (after by-product extraction), which was described as an ancient use or practice, along with application of leaves, pruning wood, and other winery by-products (199). The field trials consisted of applying three rates of vinasse, average 2.75 t/ha, 5.55 t/ha, and 8.35 t/ha dry weight (1.2 tons/acre, 2.5 tons/acre, and 3.7 tons/acre dry weight), tilled into the vineyard soil in January over a four year period (200). The vinasse application rates were calculated to apply N at rates of 50, 100, 150 kg/ha (45, 89, and 134 lb/ac). Vinasse N varied from 1.66 % to 1.90%, vinasse C varied from 47% to 51%, and vinasse C/N varied from 26 to 28. Ureic N was added as additional fertilizer (0, 50, 100 kg/ha; 0, 45, 89 lb/ac, respectively) just before bud break in early April (200). The optimal grape yield in this specific trial resulted from the lowest vinasse application rate, 1.2 tons/acre dw, 50 kg/ha N (45 lb/ac N) (as compared to a vinasse production rate of 0.9 to 1.0 tons/acre) (201-202). The grapes benefited from the enhanced summer nitrogen mineralization from the vinasse supplied organic matter, thereby also resulting in a reduction in risk of nitrate leaching to groundwater as a result of irrigation. Addition of N mineral or ureic fertilizer may be necessary in some circumstances, although the lack of an additive effect

indicates that high doses of N should be avoided to limit possible N microbiological immobilization and consequent N competition between soil bacteria and grape vines (203).

In addition to the field study, the paper (Tano 2005) has a good review paragraph of previous studies, almost all of which were published in Italian (199). Past studies recommended the blending of vinasse with manure, or composting with other additives, use as fertilizer in calcareous soils (typical of the Central Valley), and soil application along with other wine grape residue to counteract the annual mineralization of soil organic matter. Polyphenols in the vinasse were found to be more restrictive of crop growth in the laboratory than in the field, possibly depending on the type of crop grown.

A field study of land application of winery waste mulches was conducted on established vineyards in New Zealand (Agnew 2005). Mulch of four variant compositions, including vineyard prunings, marc, green waste, pine bark, manure and mussel shells (Tables 1 and 2) was applied one-time, 15 cm thick (about 6 inches) to four vineyards along with a no mulch control, in six replicates, tested over three seasons (2). The C:N ratio varied from 21-28 for the four mulch compositions (3, Table 2). Benefits of mulching identified in the trials included increased soil moisture retention enabling an irrigation reduction, increased nutrient release enabling a chemical fertilizer reduction, weed suppression enabling an herbicide reduction, buffering of soil temperature changes, increased soil fungi, and diversion of organic wastes from landfills (1). The amount of mulch applied did not adversely effect grapevine growth, but the amount applied was in excess of the amount that could be practically used in a vineyard without excessively high soil nutrient concentrations (8). A moderate to substantial increase in soil phosphorus occurred at all four sites during the second year. Phosphorus is relatively immobile, and was retained within the soil (4). Soil potassium increased up to 400% in the second year, as grape marc is high in potassium; for this reason the researchers recommended against applying raw (uncomposted) grape marc by itself as mulch (4). Soil calcium did not increase significantly; magnesium increased (statistically) significantly at one site, and sodium increased (statistically) significantly at three sites (5). Soil organic matter showed a significant increase only at one site, a result that could not be explained. That site however, had a significant increase in cation exchange capacity and base saturation (along with two other sites) indicating an increased capacity to retain potassium, magnesium, and sodium (5).

In a comprehensive review article on winery wastewater treatment and land application by Mark Grismer and colleagues from UC Davis (Grismer 1999), several studies were cited on the effects of applying wastewater to vineyards and other croplands. A study in France determined that wastewater application in excess of recommended levels resulted in accumulation of potassium, sodium, and copper in the soil with subsequent deleterious effects on crop production. A study in Australia found that wastewater application to a vineyard resulted in a soil total organic carbon increase of less than 1% for one season (which will have a substantial beneficial effect over the course of several seasons of application). Another study found that wastewater application had little nutrient value in adding to soil fertility and resulted in non-detectable accumulations of heavy metals and other toxicants. The primary concern was found with detergent use, with the recommendation to minimize the use of lyes, acids, peroxides, and alcohols for cleaning and washing operations (809). In another review article by UCD researchers, winery wastewater is said to be typically stored and treated in aerated ponds, then disposed via postharvest vineyard irrigation (Shephard 2001, 394).

Another comprehensive review article (Bustamonte 2005, 146, second paragraph) contains a review of studies on the effects of winery wastewater (non-stillage) and vinasse (stillage) on crop growth and water quality. The review notes that in general more studies have been done on the

application of vinasse to crops than on wastewater application. Vinasse has been shown to have a significant fertilizer value, especially in its potassium and calcium content, and also overall organic matter content. However, vinasse is also noted to contain phytotoxic, antibacterial, and recalcitrant compounds (such as phenols and heavy metals), and wide variations in composition. In one long-term comprehensive study, vinasse application to cropland maintained soil fertility over a 15-year period, but resulted in an increase in groundwater nitrate concentration.

In contrast, wastewater may have a high organic load, but more moderate content of plant nutrients, and value as an additional source of irrigation water. However, the review concludes that there is insufficient data on wastewater composition and agricultural use (146).

**Literature Review Conclusions**

Both winery wastewater (non-stillage) and vinasse (stillage) contain valuable contents of organic matter and plant nutrients for improved soil quality and fertilizer for vineyards and other crops. Wastewater is a valuable addition to irrigation water supplies, especially where the latter are short or expensive (an increasing occurrence in California). While concerns have been expressed over possible toxicity, detailed studies have not shown toxic effects from nutrient or other element or compound concentrations. However studies have shown that wastewater, and particularly vinasse application directly to cropland must be done more conservatively than may be necessary for application to spreading basins or to perennial grasses or pasture.

Specifically, application of uncomposted winery waste may result in competition between grapes or other crops and soil micro-organisms for available nutrients during the decomposition and humification process. Wastewater should not be applied in amounts in excess of standard crop water requirements. Wastewater and vinasse should not be directly applied to grapes or other crops in substantial excess of crop nutrient requirements for any given constituent. The most detailed studies indicate that the best production of grapes results from the minimally tested amounts of wastewater and uncomposted organic matter application. These limitations in wastewater application do not apply to finished compost.

Application of wastewater to crops will require annual leaching of accumulated salt in order to maintain soil productivity. This practice must also be performed on any irrigated cropland, whether using primary source irrigation water or wastewater. In some cases, leaching is accomplished naturally through rainfall, where precipitation occurs in sufficient amount. The same essential process occurs in natural ecosystems, and where it is naturally deficient, either in precipitation or drainage, gradually increasing salinity changes vegetation type, and ultimately renders the soil completely infertile. In wastewater application sites growing salt-tolerant grasses or other salt-tolerant plants, the site soils may be managed to retain some salts.

**WI/KJ Study and Guideline Conclusions**

On the basis of the literature review, the following recommendations are made:

6-1. Wastewater should not be applied to grapes or other agricultural crops in excess of standard crop water requirements.
6-2. Wastewater should not be applied to grapes or other agricultural crops in nutrient concentrations in substantial excess of standard crop requirements.
6-3. Applied wastewater should have a C:N range of 15-30 as the optimal range for a nutrient availability balance between crops and soil micro-organisms. Application of high-

strength wastewater or wine production solid organic waste should best be done after composting of the solid or settled waste and attainment of the optimal C:N range.

6-4. Although sufficient research has not been done to establish a specific range or limits, research suggests that wastewater application to vineyards be limited to a single annual application, depending on wastewater strength, in contrast to multiple applications to spreading basins or perennial pasture. This restriction would not restrict the separate application of finished compost made from winery waste alone, or in combination with other organic wastes.

6-5. Grapes and other agricultural crops will require annual leaching of salts as a necessary agricultural practice to maintain soil productivity. In contrast, spreading basins, and especially salt-tolerant pasture may be managed to retain some salts within the soil root-zone and allow uptake, harvest, and removal of some salts.

**Issue 7. To protect underlying groundwater quality, should waste exceeding specified limits of contaminant concentrations be pre-treated before land application? If so, what should the concentration limits and treatment methods be?**

**Issue Analysis**

One important observation about winery wastewater is the very high degree of chemical and constituent variability even over very short periods of time. The wastewater BOD concentration at the WI/KJ Study non-stillage test site varied from 1900 mg/l to 4,500 mg/l over the two seasons of the study. The wastewater BOD concentration at the stillage test site varied from 120 mg/l to 18,000 mg/l over the two seasons of the study (Table 3). Historic data from the study sites indicates that non-stillage BOD concentration could be as low as 52 mg/l, and stillage BOD concentration could be as low as 3 mg/l (Table 1, p.2-1). Data from other California wineries indicates that stillage BOD concentration can be as high as 30,000 mg/l, and wastewater from centrifuge desludging can have a BOD concentration as high as 70,000 mg/l (Region 5 communication 01/12/07).

The wastewater total nitrogen (TN) concentration at the non-stillage test site varied from 14.9 mg/l to 149 mg/l over the two seasons of the study. The wastewater TN concentration at the stillage test site varied from 9 mg/l to 572 mg/l over the two seasons of the study (Table 3). Historic data from the study sites indicates that non-stillage TN concentration could be as low as 2 mg/l, and stillage TN concentration could be as high as 1540 mg/l (Table 1, p.2-1). Data from other California wineries indicates that wastewater from tank washing can have a TN concentration as low as 0.6 mg/l (Region 5 communication 01/12/07).

The wastewater BOD:TN ratio at the non-stillage test site varied from 30 to 127 over the two seasons of the study. The wastewater BOD:TN ratio at the stillage test site varied from 11 to 156 over the two seasons of the study (Table 3). Historic data on BOD:TN ratios from the study sites is not given. Data from other California wineries indicates that BOD:TN ratio can be as high as 609 from centrifuge desludging and 590 from tank washing, and as low as 15 for non-stillage (Region 5 communication 01/12/07).

The WI/KJ Study and Guidelines does not recommend either maximum or minimum concentrations of BOD and TN for direct land application, but does recommend that if the BOD:TN <20, “the process water stream should either be pre-treated to remove some nitrogen or less cycles applied to the soil may be required to achieve adequate treatment during cropping

prior to additional loading." Test site data showed that BOD:TN <20 occurred three times in the stillage wastewater, with a low of 11, and also had occurrences of 20, 21, and 23. The WI/KJ Study and Guidelines does not recommend a maximum BOD:TN ratio.

With respect to the highly variable nature of winery wastewater over short periods of time and from one land application to the next, the WI/KJ Study and Guidelines states that "this is representative of large wineries in California … concentrations vary throughout the day as well as seasonally." As to the value of temporary storage or treatment of wastewater for the purpose of blending or reduction of the wide range in constituent characteristics, the WI/KJ Study and Guidelines reasons that "most process water managers do not take this step because process water equalization occurs during spreading basin application" (Response, 13).

**Literature Review Results**

A comprehensive review article on the treatment and land application of worldwide fermentation industry waste was written by Mark Grismer and colleagues from UC Davis (Grismer 1999). In general comments, fermentation wastewater is stated to be amenable to biodegradation processes, without problems associated with strong redox reactions, but some problems are encountered with treatment of phenolics, or particular (unspecified) inorganic species. A related article states more clearly that little research has been directed toward on-site wastewater treatment and removal of compounds identified as "particular" to winery wastewater: sulfur, phenols, tannins, and lignins (Shephard 2001, 394). Wastewater must often be pretreated before discharge to municipal systems, or eventual local secondary treatment (Grismer 1999, 805). Outside of the U.S., several strategies for managing winery wastewater have been investigated including separation of wastewater components, use of physico-chemical pretreatment systems, use of aerobic-anaerobic treatment systems, and eventual land application of the treatment products (807).

A standard value for the average quantity of wastewater produced in wine production per volume of wine produced has been determined for California: 3 L of wastewater per L of wine, with 5000 mg /L of BOD (or 8330 mg/L of COD). However, in the course of research one investigator found that the 3:1 ratio may be two high, as a ratio of 2.2:1 was found in the study, and ratios as low as 0.44:1 for white wine production, and 0.2:1 for red wine production have been reported elsewhere in the literature for winemaking in France. Wastewater production as a ratio to wine produced varies from winery to winery, and within a given winery depending on the type of wine produced, and whether the grapes are crushed on-site or off-site. Development of water conservation measures may further reduce the ratio (Shephard 2001, 394, 398-399).

In California, there is typically a lull in winery activity during mid-December after the harvest and crush, but bottling in January results in an increased in wastewater COD concentrations to near crush-season levels. Observed peak and average COD concentrations during the research period, 45,500 mg/L and 15,400 mg/L respectively, were substantially higher than the stated average value of 8330 mg/L (Shephard 2001, 398-397).

The variable nature of winery wastewater was identified as a persistent issue in regarding the need for pre-treatment before discharge to municipal sewage systems, to the extent that some studies have suggested individual treatment systems be designed for each winery (808), and that individualized wastewater monitoring was necessary to successfully address winery wastewater management (Shephard 2001, 395). Wineries that separate lees from the wastewater had about a 50% reduction in wastewater COD (Grismer 1999, 807). The development in Europe and elsewhere of additional physical methods of pre-treatment for reducing COD and suspended

solids levels are described, as well as a method of enhanced evaporative concentration prior to land application (808). A variety of aerobic and anaerobic treatment methods have been developed to reduce BOD, COD, total suspended solids, total nitrogen, and total phosphorus, with some storage necessary prior to treatment to counteract the effects of highly variable organic loading to the treatment systems (808-809). The presence of sulfate compounds in winery wastewater was identified as needing appropriate treatment prior to ultimate land disposal (805).

Since almost all research papers on land application of winery waste are from outside the US, it is important to compare the nature of the winery waste from other wine-growing regions to see if it is comparable to that of California. One detailed study of winery wastewater characteristics comes from Greece (Vlyssides 2005). The paper gives average values for non-stillage wastewater, and distinguishes between white wine and red wine production. Average BOD for white wine wastewater is 1740 mg/l, and red whine wastewater is 1970 mg/l (56, Table 2). These values are at the low end or below those for the WI/KJ test site. The paper gives the ranges for BOD wastewater concentration for 20 different stages of wastewater production in the wine-making process, with ranges of 210 mg/l to 19,620 mg/l for white wine wastewater, and 150 mg/l to 21,110 mg/l for red wine wastewater (57, Table 3). These values considerably exceed both ends of the range for the test site, but not for California winery wastewater overall. Average TN for white wine wastewater is 67 mg/l, and 71 mg/l for red wine wastewater, well within the range for the test site. Average BOD:TN in Greece is 26 for white wine, and 28 for red wine (56, Table 2). Both values are slightly below the range for the test site, but not out of the range for other California winery wastewater. The only significant difference between white wine and red wine wastewater is in total phenolic compound concentration, 280 mg/l and 1450 mg/l respectively (56, Table 2).

Average values of wastewater concentrations are also given for vinasses, the waste product from distillation, comparable to the stillage wastewater of the WI/KJ test site. The paper further distinguishes vinasse wastewater from wines and from wine lees, the latter being the solid material, primarily yeast cells, that settles out in the wine-making process and is removed by filtration and clarification. Stillage can be made either from wine or wine lees. Vinasse BOD concentrations average 16,300 mg/l from wine, and 67,500 from wine lees (59, Table 4). The concentration for vinasse from wine is within the stillage test site range, but the concentration for vinasse from wine lees substantially exceeds the stillage test site range, but not the range for all California winery wastewater generating processes. Average TN concentrations are 650 mg/l from wine, and 17,400 mg/l from wine lees (59, Table 4). The concentration for vinasse from wine is slightly above the stillage test site range, but the concentration for vinasse from wine lees exceeds by over a factor of ten the highest historic value for the stillage test site. Average C:N is 32 from wine, and 4.8 from wine lees. Average BOD:TN is 25.1 from wine, and 3.88 for wine lees (59, Table 4). A BOD:TN to C:N conversion factor can only be determined empirically for a given organic waste. This data suggests a winery wastewater conversion factor of about 1.25 to convert BOD:TN to C:N. The average BOD:TN from wine is in the low end of the range from the stillage test site, but the very low BOD:TN from the wine lees is below any reported value for California winery wastewater or stillage.

Another detailed study of winery wastewater characteristics comes from Spain (Bustamonte 2005). This study provides extensive data on winery wastewater and vinasse characteristics, but does not distinguish between vinasse from wine and vinasse from wine lees. Grape marc is identified as an additional source material for distillation. Specifically, winery wastewater is described as including the remains of grape pulp, skin, and seeds and different compounds used in filtration, precipitation and cleaning processes. Vinasse is distillation wastewater that includes exhausted wine and wine lees, water-soluble compounds of grape marc, and hydrochloric or

sulfuric acid (145). From wineries and distilleries throughout Spain, 8 wastewater samples and 13 vinasse samples were collected and then analyzed (146-147, Table 1). Non-stillage wastewater BOD ranged from 125 mg/l to 130,000 mg/l, with a mean value of 22,418 mg/l. The mean value exceeds the maximum WI/KJ non-stillage study site value by a factor of five. The maximum value exceeds by almost a factor of 2 any value reported for California wineries. Stillage wastewater BOD ranged from 4,767 mg/l to 32,500 mg/l with a mean value of 14,542 mg/l. The mean and maximum stillage values are less than the non-stillage values in Spain, but are comparable to the WI/KJ stillage site values.

Non-stillage wastewater TN ranged from 0.0 mg/l to 142.8 mg/l, with a mean value of 35.4 mg/l. Stillage wastewater TN ranged from 21.3 mg/l to 252.5 mg/l with a mean value of 104.9 mg/l. These values are generally comparable to the WI/KJ test sites, although the maximum stillage wastewater value is less than half the maximum stillage test site value.

The mean C:N is 61.0 for non-stillage wastewater, and 41.9 for stillage wastewater. The mean BOD:TN is 633 for non-stillage wastewater, and 138 for stillage wastewater. Since these are calculated from mean values rather than direct measurements, they may not be representative of actual ratio values.

Polyphenol concentrations ranged from 29 mg/l to 474 mg/l, with a mean value of 140 mg/l, for non-stillage wastewater, and ranged from 65 mg/l to 766 mg/l, with a mean value of 318 mg/l, for the stillage wastewater. Even the maximum concentration is only about half of the concentration reported above (Vlyssides 2005) for red wine wastewater. The paper commented that the presence of polyphenols in wastewater and vinasse considerably increased BOD, with detrimental effects on soil micro-organisms and plants, necessitating a conditioning treatment (147-148).

Another paper (Bustamonte 2005) identified the likely source for elevated winery and vinasse wastewater concentrations of various mineral constituents. Higher N in vinasse may be due to the presence of lees, a by-product of fermentation and clarification in wine-production which contains OM high in N from yeast and bacteria, and grape and added proteins. Higher K in vinasse may be due to release of K in tartaric acid production. Higher Mg in vinasse may come from soluble magnesium salts in the by-products used in alcohol production (wine, wine lees, and grape marc). Higher Mn in vinasse is naturally present in the solid part of the grape, especially the seed. Higher Zn in vinasse may be due to Zn precipitation from use of potassium hexacyanoferrate for Fe elimination. Higher Pb in both wastewater and vinasse may be mainly due to acidic pH dissolution of Pb from production equipment (147-148).

The paper reviewed the different methods for pre-treatment of winery (non-stillage) wastewater and vinasse (stillage) wastewater before land application. Non-stillage pre-treatment includes physical and chemical methods based on natural and enhanced evaporation in ponds. Non-stillage pre-treatment biological methods are based on aerobic or anaerobic digestion. In contrast with non-stillage, stillage physical/chemical pre-treatment may include four to six steps of evaporation concentration, and biological pre-treatment may include aerobic fermentation to produce protein, and alkaline treatment to produce ruminant feed (146). The paper observed that the Spanish wine industry generates six times more wastewater than France or Italy, mainly due to the low cost of the disposal fee, and that the drawback to treatment is that the methods are very expensive and there is still the need to dispose of treatment sludge or other by-products (146).

The paper concluded that given the wide range in constituent concentrations, BOD SS, VS, TS, polyphenols, organic C, total N, P, Na, Ca, etc., from one to three orders of magnitude (147), that waste constituent concentrations do not favor direct application to agricultural soils, necessitating

prior conditioning treatment (150). Further, the great variation found in most parameters, and the high degree of randomness, necessitates complete effluent characterization before use and treatment (148, 150). The paper went on to derive equations for estimating constituent parameters (148-150).

Composting of Winery Waste Preceding Land Application

Composting of winery waste prior to land application mimics the same soil processes of decomposition and humification in direct land application, with the benefit of allowing greater control over the processes, and the ability to prevent the leaching of premature components into the vadose zone and groundwater. Studies of winery waste composting also reveal greater insight into what happens in direct application soil processes. Composting of winery waste is a widespread practice in California, but typically the solid waste is sold to composting companies who move the waste off-site of the vineyards and compost the winery waste with other agricultural wastes. At least some of the finished product is then returned to the vineyards as fertilizer (communication from WI). At least one report of on-site composting in California is indicative of the substantial benefits that can be gained from this practice.

In one composting study from Spain, the objective was to develop the simplest and least expensive composting program, using winery sludge and grape stalks, to produce the highest quality compost for vineyards (Bertran 2004, 204). The composition and characteristics data on stalks and sludge is given in Tables 1 and 2 (204).

Observed changes in the waste material as a result of the composting process are given in Table 3: (1) the waste alkaline pH decreased towards neutral; (2) EC increased as a result of decomposition and mineralization; (3) the proportion of VS declined; (4) the percent N decreased, but the majority of organic nitrogen was retained; (5) C/N tended to decrease with decomposition; (6) germination tests in finished compost showed no phytotoxicity (206-207). In composting, low C/N causes the loss of N as ammonia and odor problems, high C/N lengthens the decomposition process (203). Three months was demonstrated as sufficient time to attain an adequate compost for soil application (206).

A better quality compost was obtained from the combination of treatment sludge and stalks, in a ratio of one part to two respectively, over stalks only compost. The 1:2 ratio resulted in higher pile temperatures, VS percentage more readily stabilized with low mineralization, more N retained, and C/N similar to existing soil. Grinding of the stalks before composting was found to be advantageous (206-207).

A comparison of winery waste compost was made with other organic waste composts (Table 4). Calcium was found to be significantly higher in the winery waste compost, possibly due to the nature of the wine making process; other constituent values tended to be average or lower (particularly for EC) compared to other composts (206-207).

Low vineyard soil N extraction, and great sensitivity of young vineyards to excess N, makes compost preferable as fertilizer, with stabilized OM and medium to long-term N release. Compost can be added to vineyards at a rate of 30-50t/ha (about 13-22 t/ac) to improve soil quality, with larger rates applied to soils on slopes with erosion risk, especially as a mulch (204).

In conclusion, there are several benefits of composting as a pre-treatment prior to application to vineyard soil, over direct land application of waste: (1) composting facilitates soil incorporation and increased soil water-holding capacity (the latter identified as an especially important factor

for wine production); (2) composting facilitates gradual release of organic N after application; (3) higher amounts of K is advantageous to wine production (acidity control); (4) two-waste compost matches all requirements of vineyard fertilizer for production of quality wines (206-207).

A study on the composting of grape marc in Israel was intended to develop an alternative to what was described as a "universal practice" of direct incorporation of grape marc into vineyard soils, as "serious problems" were alleged because degradation products inhibit root growth (Inbar 1992, 36, see also Inbar 1991 abstract). This study used chemical fractionation rather than physical fractionation of organic matter, into the two components of humic acid (HA) and fulvic acid (FA). The non-humic fraction (NHF) of grape marc was found to decrease rapidly during the first 70 days of composting and remained unchanged thereafter (38).

One intent of the study was to find a suitable measure or index for evaluating compost maturity. First, C:N can be used as a compost maturity index. A decline in C:N from 35-40 to 18-20 was found to be indicative of an advanced degree of compost stabilization. The grape marc compost C:N decreased from 26 to 20 during the composting process, with a high correlation with composting time, and organic matter concentrations of humic substances and the non-humic fraction, a function independent of composting system and design (38-39). Two indices of the changes in the organic matter fractions were identified as applicable. First is the Humification Index (HI) = NHF/HA+FA. The HI for grape marc compost decreased linearly from 0.33 to 0.08 during the composting process (Figure 1), a higher rate than for some manures and other composts due to the higher simple sugar content and more rapid degradation (39-40); an HI <0.1 may be indicative of NHF decomposition (46). Second is the Hummification Ratio (HR) = HA/FF or = HA/FA. HR increased for grape marc compost, with the biggest change occurring during the last stage of composting (40), after C:N dropped below 21 (46).

The research paper refers to other studies that found that compost made from grape marc and cattle manure could serve as a high-quality peat substitute for container-grown plants (36), thereby avoiding the concern over inhibition of root growth.

Another study of composting winery waste, this time in Italy (Ranalli 2001) was intended to find a use for winery and olive waste, regarded as toxic due to the presence of phenols, polyphenols, long chain fatty acids, and tannins as waste constituents (416). In this study, compost was made from dairy sludge 35%, grape-dregs 30%, grape-stalks 25%, and rice husks 10% (417, for composition of raw materials see Table 2 on 421). Physical, chemical, organic, biochemical, microbial, enzymatic, and phytotoxic tests were performed and compared (417-420, see Table 1, 419), again with the intent of finding a suitable measure or index for compost maturity. For the cured or finished compost (compare Tables 2 and 4), there was a good reduction in C:N, a slight rise in pH, good reduction in lignin and hemicellulose percent, and a good percent of organic matter as humic and fulvic acids.

The paper observed that many parameters have been proposed for monitoring the compost process, but a wide increasing consensus is that the most effective parameter for determining compost stability is microbial activity (416). Two measures of microbial activity were recommended as a result of this study. First, Fourier Transform Infared Spectroscopy (FTIR) monitors changes in humic and fulvic acids (general organic matter components) and molecular composition changes (418). This test is a simple non-destructive and rapid technique useful for the characterization of the main functional groups of a complex matrix. The resulting absorption bands (Figure 5, 424) can be considered molecular markers of the degree of humification in direct relation to the degree of maturity and quality of the compost (423-424). This test has good applicability for the rapid characterization of the structural features of the humic matter and

biotransformations occurring during composting (433). Second, thermal analysis was found to be a meaningful complementary tool to FDIR (433), providing a fingerprint of the compost materials, and indicating the degree of the composting process (424), and the degree of humification (426). Thermal analysis confirms the FDIR results (426). These two techniques offer a good alternative to traditional methods of monitoring compost, requiring a shorter time for analysis, and allowing for optimizing management of the composting system (433).

The overall conclusion was that after 150 days, a mature and stable compost with good hygienic characteristics was produced (433, include last paragraph on 433). The paper included a discussion on the relation between phytotoxic effects of compost materials and volatile organic acid production, immaturity of compost, anoxic conditions, and formation of organic and acetic acids. Studies conducted on the nature of phytotoxic organic compounds show that volatile acids represent only transitory phytotoxic substances (432-433). The only problem was an increase in EC to a fairly high level, with grape stalks and dregs being the largest contributors among the raw materials (430-431).

A non-peer review article about Napa Valley winery compost operation (Peterson 2002) described the operation of the Napa Wine Company/Yount Mill Vineyards, a 600-acre organic vineyard and 6,000 ton/year crush facility. Available materials used for on-site composting included 55% pomace (crush residue), 25% manure, 10% finished compost, 10% clay, 3% straw, 3% soil, 3% trimmings (there is an error somewhere in these numbers as the total equals 109%; stalks are not mentioned and may be included in trimmings). Finished compost application rates are 10t/ac for new vineyards, and 4t/ac for established vineyards. As a result of compost addition, vineyard soil organic matter has risen from 2.5% to 4.5% (number of years of application is not given). The Company reported good results and production of world-class wines.

Wetland Treatment of Winery Waste

A wetland treatment system for winery wastewater has been developed in California by UC Davis working in conjunction with environmental consultants (Shephard 2001, Grismer 2001). While winery wastewater is considered relatively simple in composition compared to municipal wastewater, which has been proven to be amenable to wetland treatment, wetland response to high and widely variable organic loading rates characteristic of winery wastewater was judged uncertain (Shephard 2001, 395).

A pilot-scale wetland tank was constructed in 1995, with subsurface flow and dimensions of 6.10 x 2.44 x 1.20 m. Pea-sized gravel was placed in the tank to a depth of 0.95 m. The tank was then planted with cattails, bulrushes, arrowheads. Winery wastewater representative of the variety of winery processes, including racking, bottling, and tank washing from both white and red wine production and of typically high quality variability, was collected and mixed in a storage tank. The mixed wastewater was adjusted and discharged to the wetland with a specified COD concentration and volume per unit time (396-397).

Four experiments were conducted with introduction of wastewater to the constructed wetland with average COD concentrations 1.5x (993 mg/L), 2.5x (1650 mg/L) , 3.5x (2010 mg/L), and 8x (4720 mg/L) the maximum recommended USEPA organic loading rate to constructed wetlands for wastewater treatment, 110 kg BOD/ha-d or 200kg COD/ha-d (395, 397-398). Large nitrate concentrations in the wastewater result from proteins in the grapes, and yeasts used in winemaking, but the high nitrates correlate with high organic loading rates with resulting high C:N (398). At the highest COD application rate, the plants nearest the discharge point into the

wetland did not grow with as much vigor as plants in the center. After a three-day accidental discharge at rates ranging from 12,800 mg/L COD to 16,800 mg/L COD, the plants nearest the discharge immediately yellowed and died within a short period. As a result, a maximum wetland input COD concentration of approximately 5000 mg/L was determined (399).

After a wetland retention and treatment time of approximately 10 days, average outflow COD concentrations of 21, 32, 57, and 51 mg/L COD were obtained, corresponding to COD removal rates of 99.0%, 98.5%, 97.3%, and 99.0%, respectively (398, Table 5). Reductions in other wastewater constituents were total suspended solids 97%, nitrogen 78.2%, sulfide 98.5%, ortho-phosphate 63.3%, volatile fatty acids 99.9%, phenols 100%, and tannins and lignins 77.9% (394). The phenols were most likely to have been all converted to tannins and lignins. In contrast, TDS concentrations increased considerably during wetland treatment, up to 50% or more as a result of evapotranspiration (400). The wetland treatment system did neutralize the pH of high-strength acidic winery wastewater (394).

In conclusion, the researchers stated that the observed resultant high degree of wastewater treatment was unexpected, and exemplified the degree to which natural treatment systems can adapt. Given that winery wastewater constituents are generally ubiquitous in nature, it is therefore expected that microorganisms capable of decomposing and incorporating wastewater constituents are abundant in the natural environment. The constructed wetland system did not exhibit any negative responses to high organic loading rates, and was capable of withstanding fluctuating water quality without sacrificing good treatment (401).

In a follow-up study (Grismer 2001), mean wastewater detention times for wetland treatment were determined to be 8.6 days for a system that had been in operation for three years, and 11.1 days for a newly constructed system (476, Table 3).

A large, full-scale operating winery wetland treatment system has been tested in South Africa (Mulidzi 2006). The dimensions of the system are 45, 4, and 1.2 m. The system was constructed with an impermeable liner. Wastewater retention time was 14 days (283). The system had an average COD removal rate of 83% in winter, and 80% in summer for 2004/2005 (284), and 88% in winter and 77% in summer for 2005/2006 (288). Results showed a consistently low COD concentration in the outflow, regardless of the range of COD concentration from low to high in the inflow. A reasonable rate of removal of Na, P, K, and Ca was reported, and the outlet wastewater was always in the neutral range, pH 6.5 to 7 (289).

A procedure for harvesting the wetland plants was developed, in order to keep the wetland plants green and growing throughout the year. This improved the winter performance of the wetland. Routine removal of excess plant material and captured sediment assured continuous effective functioning and good performance throughout the year (289). Cabbage crops were successfully grown with only the outflow used as irrigation water, with no added fertilizer (291).

**Literature Review Conclusions**

The extensive literature on management and treatment of winery waste originating from outside the U.S. contains a large percentage of articles on pre-treatment processes prior to disposal. For the purposes of this LR, most of the pre-treatment literature was not reviewed nor included within this report, as much of it was written on the assumption of the need for treatment, rather than determining the need for treatment in the first place. This assumption seems to be largely based on the fact that prior to the widespread advent of pre-treatment, untreated winery waste was not land applied, but rather was disposed of directly to surface flowing water bodies or to municipal

wastewater treatment systems, two circumstances that were assured of causing eventual water quality and other environmental problems.

As the course of this LR has made clear, the land application of winery waste under carefully selected site parameters and carefully managed conditions will result in expected environmental and water quality benefits. The carefully selected and controlled conditions presumes a degree of consistency or uniformity of applied winery waste, not only to stay within the defined parameters, but in recognition of the value of soil and soil micro-organism acclimatization to the characteristics of winery waste for natural decomposition and assimilation. Yet winery waste characteristics typically, if not universally, vary over a very wide range of several orders of magnitude, not just for different wine growing regions, but within each individual winery, and not just over the course of a wine-making season, but even within time periods as short as twenty-four hours. Such wide variation of winery wastewater biochemical characteristics over such short time periods would be expected to cause major disruptions to soil microbial species and populations if applied directly to soil, with a consequent time period of adjustment of unknown length during which the desired soil treatment of applied waste may be hindered.

The solution to this problem would be storage/retention of winery wastewater for a sufficient length of time to accomplish blending or adjustment of biochemical characteristics to provide a certain degree of consistency in the characteristics of the applied wastewater. The most important characteristic for consistency is C:N, to be blended/adjusted to the range of 25-40. Wastewater that is below this range can be blended with high C:N wastewater; wastewater that is above this range can have added N in an organic form, such as from urea. Maintaining C:N within the optimum range for decomposition, humification, and denitrification is essential for assurance of groundwater quality protection, as is maintaining the soil water content within the 60-100% range of the soil water-holding capacity.

The WI/KJ Response to the PR that “most process water managers do not take this step (temporary storage or treatment of wastewater for the purpose of blending or reduction of the wide range in constituent characteristics) because process water equalization occurs during spreading basin application” (Response, p.13) is questionable particularly in regard to C:N. Given that this statement was made in the Response and not in the WI/KJ Study and Guidelines, the PR did not have an opportunity to respond to it. While C:N does adjust toward the middle from the extremes over a period of time following land application, it is the processes (or lack thereof) that can occur in the soil during the adjustment time period that may be contributory to eventual groundwater quality degradation.

Storage /retention of wastewater also provides the opportunity for other beneficial treatment measures in addition to C:N adjustment. One would be the settling/separation of organic solids, which then may be available for composting. Another would be evaporation, allowing for greater ability to land apply wastewater within the limit of the soil water-holding capacity including antecedent conditions.

LR results show that multiple advantages may be gained from the composting of solid winery waste prior to land application, and that the application of finished compost to land application sites will result in improved soil conditions on those sites for the soil treatment of applied wastewater. Therefore, wherever feasible, winery solid waste and recovered solids from settling of wastewater should be composted before land application.

The development originating in California of an on-site wetland treatment system offers a viable potential alternative to the direct land application of wastewater, particularly for small wineries

with limited land, for wineries with soil that is unsuitable for direct land application, and for land application sites that have a history of developing groundwater quality problems.

**WI/KJ Study and Guideline Conclusions**

On the basis of the literature review, the following recommendations are made:

7-1. Given the highly variable nature and composition of winery wastewater, the wastewater should be blended in a storage tank or holding pond until achieving C:N 25-40, either through mixing of different batches of on-site winery wastewater, or wastewater from a separate winery or food-processing facility. Wastewater with excessively high C:N, may have urea or other high N organic waste added to reduce C:N to the desired range.

7-2. Wherever feasible, winery solid waste and recovered solids from settling of wastewater should be composted before land application. This will avoid actual or perceived problems associated with the direct land application of uncomposted solid waste. The application of finished winery waste compost, made alone or in combination with other organic waste products, to land application site soils will result in improved soil quality and soil capacity for direct treatment of winery wastewater. Materials suitable for use as mulch would not be composted before application.

7-3. Where a winery site is unsuitable for the direct land application of wastewater, construction and operation of a wetland treatment system may provide a viable alternative to on-site waste treatment and disposal.

**Issue 8. Does the data collected to date show that the volatile component (VDS) of total dissolved soilids (TDS) is fully removed within the top five feet of soil? If not, how should the land application unit be managed to provide full removal?**

**Issue Analysis**

WI/KJ Study and Guidelines concluded that VDS concentrations in the applied process water are significant while those in the lysimeter samples are markedly lower indicating that VDS is largely removed. The good correlation between VDS and BOD levels for all samples also indicates that BOD and VDS reductions are linked (Response, p. 3-5).

In the wastewater constituent analysis (Table 3), values for IDS are given, but not for TDS or VDS. Again, IDS concentration values are reported for the lysimeter samples (Table 5), and total load values for applied wastewater and percolate (Table 6), but not corresponding values for TDS and VDS. Soil sample data for VDS concentrations are reported along with TDS and IDS concentrations for both the non-stillage and stillage test sites (Appendix C).

The PR did not comment on this issue other than to say that VDS could have been used as a "quick" measure of BOD and likely total organic carbon (TOC) as well. WI/KJ replied that TOC data was not collected (Response, 3-5).

**Literature Review Results**

Typical wastewater concentrations from a Greek Winery are total volatile solids (TVS) in white wine production wastewater, 3400 mg/l, in red wine production wastewater, 3750 mg/l; suspended volatile solids (SVS), 128 and 200 mg/l, for white and red wine respectively. Typical vinasse concentrations are TVS in vinasse from wine, 32,200 mg/l, and in vinasse from wine lees, 152,700 mg/l; SVS in vinasse from wine, 890 mg/l, and in vinasse from wine lees, 140,800 mg/l (Vlyssides 2005, Tables 2 and 4).

TVS in winery wastewater in Spain ranged from 661 mg/l to 54,952 mg/l, with a mean of 12,385 mg/l. The concentration in vinasse ranged from 4,956 mg/l to 75,184 mg/l, with a mean of 24,197 mg/l (Bustamante 2005, Table 1).

**Literature Review Conclusions**

Insufficient data is reported in the WI/KJ Study and Guidelines to evaluate the Issue question and to evaluate the WI/KJ Study conclusion. The LR found VDS concentration data for wastewater and vinasse, but did not find any research studies where the fate of VDS in land application was considered a significant issue. Accordingly, no conclusions or recommendations can be reached for BMPs to assure full VDS removal in land application, nor any data to even indicate that this is an issue needing further investigation. The PR did not regard this question as one deserving of comment.

**Issue 9. What precise definition of a level of confidence is satisfactory to the Water Boards to ensure protection of groundwater quality under land application sites?**

**Issue Analysis**

While a satisfactory answer to this question is pertinent to knowing how to proceed with implementation of the WI/KJ Study and Guidelines report, combined with the recommendations for modified guidelines contained in this LR, the answer is not amenable to a literature review. Rather, this is a policy issue to be resolved by the State and Regional Water Boards collectively and individually. The LR did find reference to the resolution or attempted resolution of this issue in other countries and wine growing regions, but what may be the best resolution of the issue in one region is not necessarily the best resolution in another region.

The most recent specific documentation on the current resolution of this issue may be found in the following:

1. *Peer Review of Wine Institute's Proposed Guidelines for the Land Discharge of Stillage and Non-Stillage Process Water* (Central Valley Regional Water Quality Control Board, no date), page 6, sub-heading *Regulatory Context of Winery wastewater Discharges in the Central Valley Region*.
2. *Regulation of Food Processing (and Winery) Waste Discharges to Land* (Central Valley Regional Water Quality Control Board, January, 2005).
3. *Update Regarding the Regulation of Food Processing (and Winery) Waste Discharges to Land* (Central Valley Regional Water Quality Control Board, February, 2006).
4. *Questions and Answers About Water Quality Regulation and the Food Processing (and Winery) Industry* (State Water Resources Control Board, Office of the Chief Counsel, January, 2006)

**Issue 10. What finding can be made from existing groundwater quality monitoring data from land application sites regarding the nature and extent of groundwater quality impacts?**

**Issue Analysis**

The intent of Issue 10 was to review a comprehensive set of groundwater quality monitoring data from California winery wastewater land application sites under permit, and analyze the data in light of what has been learned from the LR. Up to the present, this data has not yet been made available.

Through the application of the guideline below, determination may be made as to reasons for degradation of groundwater quality at some existing winery wastewater land application sites. For example, winery wastewater land application guidelines established and in use prior to the WI/KJ Study and Guidelines specified a minimum permeability of 2 inches/hour for the first ten feet below ground surface (5-4). In light of the LR results and conclusion that the maximum permeability rate for land application soils should be 1.4 inches/hour for the first five-feet below ground surface, any wastewater application site based on the old guideline would be a high probability site for existing groundwater impacts.

**WI/KJ Study and Guideline Conclusions**

10-1. The WI/KJ Study and Guidelines report, combined with the recommendations for modified guidelines contained in this LR, provide a basis for evaluating the present performance of winery wastewater land application systems. The recommendation is made that groundwater quality monitoring data be analyzed in view of the degree of implementation of BMPs developed in these two documents, for determination of where improvements in BMP implementation can be made where monitoring data show groundwater impacts, and for determination of where appropriate implementation of BMPs has occurred where monitoring data show no groundwater quality impacts. This analysis will help provide guidance to wineries in BMP implementation, and guidance to Regional Water Board regulators in the development of Waste Discharge Requirements protective of groundwater quality.

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