SOIL PROCESSES TO REMEDIATE DAIRY EFFLUENT USING
MSL (MULTI-SOIL-LAYER) SYSTEMS

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DEDICATION

I dedicate this thesis to my parents, Pratap Ranjan Pattnaik and Lily Pattnaik. Without their patience, understanding, encouragement, and most of all love, the completion of this work would not have been possible.
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I would like to express my appreciation to Dr. Russell Yost, my adviser, for the extreme support and guidance he provided me during the completion of this study. Dr. Yost was always there to listen and give advice to me. He taught me how to write academic papers, had confidence in me, encouraged me and brought out the good ideas in me. Without his encouragement and constant guidance, I could not have finished this thesis. I would like to thank my committee members, Dr. Roger Fujioka and Dr. Jonathan Deenik who gave insightful comments and reviewed my work. Thanks to the USDA T-STAR Program of the University of Hawaii at Manoa for providing the funds to support me during my study. I owe special thanks to Mr. Guy Porter for his hard work and assistance with my fieldwork. I would like to extend my special thanks to all my colleagues Aminata, Antonio, Rowena, Hamidou, and Richard for their support and help. I would also like to thank Andrea, Billy, and Joe for their help with my field work. Thanks must also go to my aunties Rili and Tuli, for making the hard times little easier and for helping me when I was lost. Finally, I would like to thank my family members and Lakshman for their patience and encouragement to complete my thesis.
ABSTRACT

The disposal of dairy effluent in Hawaii is a current concern because of possible contamination of surface, subsurface and coastal water as well as increased costs resulting in the closure of many dairies. This study was conducted to assess the removal of inorganic N, phosphate, organic matter (COD) and fecal coliform in dairy effluent and reduce the costs of doing so using MSL (Multi-Soil-Layer) systems. Four MSL systems were constructed with two replications of two treatments. Treatments were Perlite or the Leilehua soil in the aerobic layer, and a mixture of charcoal, sawdust, iron filings and Honouliuli soil in the anaerobic layer. Dairy effluent was applied to each system and the MSL-treated effluent was collected every week and analyzed for inorganic N, phosphate, organic matter (COD) and fecal coliform.

The first phase of the study revealed that the removal of inorganic N was similar for the Leilehua and Perlite MSL system which was 22-93% and 21-96% respectively. The phosphate removal was higher in the Leilehua MSL system (64-99%) compared to the Perlite MSL system (9-97%). The removal of organic matter (COD) in Perlite MSL system (4-37%) was greater than in the Leilehua MSL system (3-30%). The removal of fecal coliform was similar for the Leilehua and Perlite MSL system which was 6-99% and 29-98% respectively. The percentage removal of inorganic N by both the MSL systems and phosphate by the Perlite MSL system decreased over time in the first phase.

Three possible improvements were made in the second phase of the study to increase the removal efficiency of the MSL systems. Additional aeration increased the removal of phosphate by the Leilehua MSL system. Sucrose application with a constant rate of
aeration increased the removal of inorganic N, organic matter (COD), and fecal coliform in the Leilehua and Perlite MSL systems. The removal of phosphate by the Perlite system increased with sucrose additions. The different rates of aeration with constant rate of sucrose enhanced the removal of organic matter (COD) and fecal coliform.

This study demonstrated that MSL systems have the potential to remove inorganic N, phosphate, organic matter and fecal coliform in dairy effluent. Some adaptations, such as application of sucrose with constant aeration increased the removal efficiency of the MSL systems. The systems can be very economical due to the nominal cost of the materials used in it.
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CHAPTER 1

INTRODUCTION

1.1. The Problem and Justification

The dairy industry generates wastewaters characterized by high nutrients, organic contents, and pathogens (USDA-SCS, 1992). Furthermore, the dairy industry is one of the major sources of waste effluents in Hawaii and in the Continental U.S. (USDA-SCS, 1992). Dairy waste effluents are concentrated in nature, and the main contributors of nutrient to these effluents are nitrogen (N), and phosphorus (P). Since dairy effluents contain high concentrations of nutrient, organic matter, and pathogens, they may cause serious environmental problems when discharge into receiving water. Effluents high in N and P concentrations cause eutrophication (explosive growth of algae) in receiving waters, degrading water quality (Smith et al., 1999). Organic matter contamination of water bodies can degrade the aquatic habitat through depletion of dissolved oxygen (Shah et al., 2002). Pathogens in effluents can impact human health, other livestock, and aquatic life when introduced into the environment (USEPA, 1992).

Dairy effluent disposal is a serious problem in Hawaii and many island dairies. The current method used in Hawaii to dispose dairy effluent is large settling lagoons. Dairy productions establish multiple lagoons to accumulate and store effluent. Occasionally, the lagoons overflow leading to the transfer of high nutrients, such as N and P, and other contaminants like organic matter and pathogens in effluent, which can pollute surface, subsurface, and coastal waters. The two nutrients, N and P have a
detrimental environmental effect on natural water sources and together with organic matter and pathogens comprise the majority of the pollutive influences of agriculture and food production in the environment. The Environmental Protection Agency (EPA) and State Department of Health (DOH) have restrictive rules and regulations for the disposal of dairy effluent (Hawaii State Department of Health Wastewater Branch, 1996). Proper management of dairy effluent is currently a serious problem in Hawaii which has increased the operation costs and reduced profitability of many island dairies. The inability of many dairy operators to properly manage the effluent has forced more than 50% of them to close their business during the last 10 year (C. N. Lee, personal communication, 2006).

Producers need solutions to continue the production yet reduce the contamination of associated streams and water bodies. Attempted solutions have included:

1. Multiple lagoons to increase residence time to improve and maximize both retention capacity and improve in situ processing. This, unfortunately, increases the risk factor, should unusually large storms occur causing overflow, which leads to catastrophic contamination of the associated waterbodies.

2. Multiple lagoons to provide maximum surface area to promote evaporative removal of the water. This, unfortunately, concentrates the solutes and dissolved materials in the remaining effluent and thus does not permit reuse of the nutrients and organic materials in the effluent. This may, in fact, complicate the use of effluents with exceedingly high contents of salinity and nutrients.
3. Land application of effluent has been attempted, but effluent application schemes have been designed as disposal facilities rather than to maximize beneficial uses. These options might be a problem if managed improperly.

The result is that producers are challenged to maintain profitable enterprises while dealing with the increasing restrictions from the EPA and society. A growing number of producers are overwhelmed and are phasing out of production leaving the State more dependent on external food supplies. It is becoming imperative that new ways of using lagoon effluents be found. Alternative methods are needed to reduce excessive nutrients, organic matter, and pathogens from dairy effluent and also reduce cost and improve profitability. The Multi-Soil-Layer (MSL) system is a promising alternative, which displays potential for increasing the profitability of dairy industry by eliminating environmental problems associated with dairy effluent disposal.

1.2. Background

The MSL system is a new technology which has been successfully developed in Japan and Thailand to treat domestic and restaurant wastewater as well as polluted river water (Wakatsuki et al., 1993; Luanmanee et al., 2001). The system reduces inorganic contaminants such as nitrate, ammonium, and phosphate, as well as organic contaminants as measured by high COD (chemical oxygen demand) and BOD (biological oxygen demand). This is a biphasic layered system that uses locally available materials such as soil, iron particles, jute or sawdust, charcoal, and zeolite/perlite to clean wastewater (Attanandana et al., 2000; Luanmanee et al., 2001). The two layers of the MSL system are aerobic and anaerobic. Aerobic layers consist of zeolite/perlite alternated with
anaerobic layers of soil mixture blocks. The efficiency of the MSL system in purifying wastewater depends on the relative distribution of aerobic and anaerobic conditions (Wakatsuki et al., 1993; Attanandana et al., 2000). Aerobic conditions occur in the zeolite / perlite inter-layer, which has a high proportion of coarse pore spaces. Anaerobic conditions occur in the soil mixture block, which has a high adsorption capacity and is saturated with wastewater. In the anaerobic layer of the soil mixture block, nitrate is transformed into nitrous oxide and nitrogen gas (denitrified) and ferric iron is reduced to the more mobile ferrous iron, which moves out of the anaerobic layer (Wakatsuki et al., 1993). Aerobic conditions enhance nitrification, oxidation and precipitation of mobile ferrous iron to high surface area ferric oxide, enhancing phosphorus sorption (Wakatsuki et al., 1993). Organic materials are added as food for the microorganisms and as electron donors for denitrification. The maintenance of the MSL system is simple and the durability of the system was estimated to be longer than 10 years (Luanmanee et al., 2001).

1.3. Literature Review

1.3.1. Dairy Wastewater Characteristics

Dairy wastewaters are characterized by high biological oxygen demand (BOD) and chemical oxygen demand (COD) concentrations representing their high organic content (Orhon et al., 1993). The organic and nutrient content of dairy wastewaters depends upon the size, lactation, and diet of the cow. In addition, dairy wastewater composition is significantly influenced by the wastewater management, climate, operating conditions, and types of flushing. Table 1 shows the levels of major nutrients in
dairy wastewater. In industrial dairy wastewaters, nitrogen originates mainly from milk proteins, and is present in various forms; either as organic nitrogen (proteins, urea, nucleic acids), or as ions such as \( \text{NH}_4^+ \), \( \text{NO}_2^- \), and \( \text{NO}_3^- \). Phosphorus is found mainly in inorganic forms, as orthophosphate \( (\text{PO}_4^{3-}) \) and polyphosphate \( (\text{P}_2\text{O}_7^{4-}) \), as well as organic forms (Guillen-Jimenez et al., 2000).

Table 1. Dairy Wastewater Characteristics (Wright, 1996)

<table>
<thead>
<tr>
<th>Potential pollutant source</th>
<th>Biochemical Oxygen Demand ( \text{mg L}^{-1} )</th>
<th>Nitrogen ( \text{mg kg}^{-1} )</th>
<th>Phosphorus ( \text{mg kg}^{-1} )</th>
<th>Volume gallons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milking Center waste</td>
<td>400-10,000</td>
<td>80-900</td>
<td>25-170</td>
<td>73,000</td>
</tr>
<tr>
<td>Silage Leachate</td>
<td>12,000-90,000</td>
<td>4,400</td>
<td>500</td>
<td>105,000</td>
</tr>
<tr>
<td>Barnyard Runoff</td>
<td>1,000-10,000</td>
<td>50-2,500</td>
<td>5-500</td>
<td>80,000</td>
</tr>
<tr>
<td>Dairy Manure</td>
<td>20,000</td>
<td>5,600</td>
<td>900</td>
<td>660,000</td>
</tr>
</tbody>
</table>

1.3.2. \( N \) and \( P \) in Animal Waste and their Impact in the Environment

Nitrogen from animal waste originates mostly from dairies, open feedlots, confined feeding operations, and other facilities for raising and holding animals. There are primarily two forms of nitrogen found in animal waste, organic nitrogen, and inorganic nitrogen (ammonium, nitrate). In fresh manure the organic N is normally 60 to 80 percent of the total N, where as in anaerobic lagoons it is 20 to 30 percent of the total N (USDA-SCS, 1992). Nitrogen in lagoon waste is mostly in the form of ammonium (up to 70%) (Baumgarten et al., 1999). Phosphorus appears in animal waste mainly in its inorganic forms, as orthophosphate \( (\text{PO}_4^{3-}) \), polyphosphate \( (\text{P}_2\text{O}_7^{4-}) \), and organically
bound P. Generally, about 70% of the P ingested is excreted (Church, 1979). Faeces generally contain more phosphate than urine and this can be further categorized into inorganic, acid soluble, lipid and residual P (Barnett et al., 1994).

Wastewater containing excess N and P, when discharged into receiving waters may lead to serious environmental problems. Eutrophication is one such problem caused by excess nutrient discharge, which is a common and growing problem in lakes, rivers, estuaries, and coastal water (Carpenter et al., 1998; Smith et al., 1999). Typically, the eutrophication process leads to a change in the structure of the algal community, including severe algal blooms for extended periods of time (Smith et al., 1999). In turn, the decomposition of these large algal blooms usually leads to a depletion in dissolved oxygen concentrations, which affects the aquatic ecosystem causing fish dieback, and a loss of biodiversity (Carpenter et al., 1998; Smith et al., 1999).

Nitrate (NO₃⁻) is the primary constituent of animal waste that reduces ground water quality as a direct health threat to human and other mammals (Carpenter et al., 1998). Nitrate in water is toxic at high concentrations and has been linked to methemoglobinemia in infants and toxic effects on livestock (USDA-SCS, 1992). The Environmental Protection Agency has set a maximum contaminant level of 10 mg L⁻¹ for nitrate (NO₃⁻-N) and 1 mg L⁻¹ for nitrite (NO₂⁻-N) in public water supplies (USDA-SCS, 1992).
1.3.3. Organic Matter in Wastewater

Agricultural and animal wastewaters often contain high levels of organic matter. The organic matter dissolved in wastewater is an important source of carbon for microorganisms and is considered a water pollutant. Microorganisms in streams and ponds live and grow by consuming dissolved organic matter, utilizing the oxygen that fish and other aquatic organisms need, consequently stressing and even killing fish (Metcalf and Eddy, 1991). Chemical Oxygen Demand (COD) is often measured as a rapid indicator of organic pollutants in water. It is defined as "a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant" (APHA, 1989). The COD method determines the quantity of oxygen required to oxidize the organic matter in a waste sample, under specific conditions of oxidizing agent, temperature and time (Sawyer and McCarty, 1978).

1.3.4. Fecal coliform in Wastewater

Fecal coliform bacteria are a sub-group of total coliform which contains the genera of Escherichia coli, Citrobacter and Klebsiella (Leclerc et al., 2001). Fecal coliforms are facultative anaerobes, capable of aerobic and anaerobic respiration (Leclerc et al., 2001). Fecal coliform bacteria are often used as indicators to test water quality for pathogens. It is difficult to identify all the enteric pathogens present in the water and also it is expensive and hazardous. Researchers use indicator organisms like fecal coliform to assess the possibility of fecal contamination. Being warm-blooded animals, domesticated animals carry fecal coliforms and pathogenic microbes (Thelin and Gifford, 1983). The fecal coliform group grow mainly in the intestines of warm-blooded animals (Thelin and
Gifford, 1983). Fecal coliform bacteria have been cultured from fecal deposits after extended periods of time, even as long as one year if conditions are favorable (Thelin and Gifford, 1983). Kress and Gifford (1984) found that fecal coliform persist in cow feces at least seven weeks under hot, dry summer range conditions. Thus, fecal deposits are capable of providing a long-term source of potential pollution to surface water (Thelin and Gifford, 1983). The fecal deposit appears to act as a protective medium for the bacteria within by forming a crust, thus decreasing interaction of the bacteria with the soil and atmosphere (Stoddard et al., 1998). Thelin and Gifford (1983) have found that a large population of fecal coliform still exists in a fecal deposit long after the deposit has been thoroughly dried. Not only have coliform bacteria been found to survive outside of the intestinal tract, but they also have been found to exhibit initial regrowth in certain conditions. Moist conditions, mild temperatures, and manure crusting are believed to contribute to regrowth (Stoddard et al., 1998). Organic nutrients present within the feces may also provide a favorable environment for growth (Van Donsel et al., 1967). Additionally, fecal bacteria are able to obtain nutrients associated with the sediment particles (Davies et al., 1995).

1.3.5. Different systems for the Treatment of Animal Wastewater

Within the last decade, various technologies have been applied to treat dairy wastewaters. Among these, land treatment and biological treatment can be counted. These treatments can be achieved either alone or in combination. Selection of an appropriate treatment technology basically depends on demand and supply of inhabitants, geographical situation, economic welfare, and the discharge limits of the farm.
1.3.5.1. Land treatment

Many processes of land treatment of dairy wastewater have been developed. Some of the processes which are widely used are surface and subsurface application (Caro-Costas et al., 1972; Valencia-Gica et al., 2004), vegetative filter strips (Ikenberry and Mankin, 2000) and wetlands (Payne et al., 1992).

Surface spreading and subsurface injection are two of the most common land-application methods. Dairy wastewater can be stored in a holding pond and applied to croplands and pastures by portable irrigation equipment. The options for use of animal wastewaters are substantial and particularly good in tropical environments where cropping systems are perennial and plant growth and nutrient absorption is not interrupted in any part of the year (Caro-Costas et al., 1972; Valencia-Gica et al., 2004). The amount of livestock effluent that can be applied to land is limited either by crop irrigation requirements or by the amount of nutrients, pathogens, salts or other components of the effluent solution. Woodard et al. (2002) used waste effluent spray fields for forage systems to prevent loss of N to ground water. By using different loading rates of effluent N, nitrogen removal from soil was accomplished. Valencia-Gica et al. (2004) used subsurface drip irrigation for tropical grasses to assess the effects of effluent irrigation on the retention, accumulation, and movement of P in soil. By using different grasses and applying different irrigation rates, it was found that the extractable soil P and soil solution total P could be maintained at acceptable loads. Frequently, soil P loads increase excessively in effluent irrigated pastures. Although surface and subsurface application have some advantages, for example dairy effluent has value as a soil conditioner and fertilizer, it has also some disadvantages. Surface and subsurface
application have a high capital cost. The applications require accurate design, close monitoring and regular maintenance.

Vegetative filter strips (VFS) are increasingly being viewed as practical, low-cost management options for improving the quality of surface runoff from pollutant sources as well as providing protection. Ikenberry and Mankin (2000) presented a good review of VFS effectiveness at reducing the pollution potential from feedlot runoff. Vegetative filter strip have been shown to substantially reduce nutrient and sediment runoff from cultivated agricultural areas (Ikenberry and Mankin, 2000). They enhance filtration of suspended sediment by vegetation, provide adsorption on soil and plant surfaces, and enhance adsorption of soluble pollutants by plants (Fajardo et al., 2001). The removal of fecal coliform was between 64% and 87% when using small scale simulated runoff events with stockpiled manure (Fajardo et al., 2001). Ikenberry and Mankin (2000) reported that the removal of total N, and P by vegetative filter strips was more than 85% and ranged from 12-97% respectively. Ikenberry and Mankin (2000) also suggest that not enough research exists to support wide spread use of the many proposed Vegetative filter strip methods.

Constructed wetlands have received considerable attention for the last 5 or 6 years as a treatment method for treating animal waste (Payne et al., 1992). They have been designed and constructed to utilize natural processes involving wetland vegetation, soils and the associated microbial groups to assist in treating wastewaters (Knight et al., 2000). They provide fish and wild life habitat, drinking water supply, ground water recharge, flood control, protection from erosion, improvement of water quality, and nutrient recycling (Ancell et al., 1998). They improve water quality through numerous physical
and biological events (Ancell et al., 1998). In a review of wetland literature Ancell et al. (1998) reported removals varied from 60-99% for BOD, 43-97% for N, and 28-99% for P. Bacteriological removals are sometimes reported, and showed that wetlands are very effective at reducing indicator organism counts. Constructed wetlands may result in zero discharge to waterways due to effluent evaporation and can be easily added on as an additional treatment to existing systems. But constructed wetlands have limitations for treating animal waste. Effluent flowing into a constructed wetland system requires pretreatment. The system can take up considerable land area and may have to cut across existing pasture and fence lines. The system will also require much time and expertise to ensure that wetland plants are established successfully.

1.3.5.2. Biological treatment

The basic mechanisms of biological treatment are the same for all treatment processes. Microorganisms, principally bacteria metabolize organic material and inorganic ions present in wastewater during growth, which results in the removal of dissolved organics and inorganic ions (Metcalf and Eddy, 1991). Some of the commonly used biological processes are: 1) aerobic processes, 2) anaerobic processes, and 3) bioremediation.

Most conventional wastewater treatment processes are aerobic, where the bacteria break down the waste products and take in oxygen to perform their function. This results in the high energy requirement (oxygen has to be supplied) and a large volume of waste bacteria (sludge) is produced, which makes the processes complicated to control, and costly (Metcalf and Eddy, 1991). Carta et al. (1999) have investigated a continuous flow
experiment to purify effluents with low COD and a minimum ammonium-nitrogen concentration. Air injection was applied to the system for aeration. The result showed a decreased concentration of COD and ammonium-nitrogen. It also showed a similar decrease in nitrate and nitrite. Cheng and Liu (2001) studied a continuous-flow intermittent aeration for nitrogen removal from anaerobically digested swine wastewater with high ammonium content and found an average of 91% total Kjeldahl nitrogen and 92% NH₄⁺-N removed with an alteration of 1-h aeration and 1-h non aeration.

The bacteria in anaerobic processes do not use oxygen. Excluding oxygen is relatively easy, and the energy requirements and sludge production is much less than with aerobic processes, which makes the anaerobic processes cheaper and simpler (Andrews and Graef, 1970; Parkin and Owen, 1986). Manariotis and Grigoropoulos (2003) studied an upflow anaerobic filter to evaluate the direct treatment of low-strength wastewater over a long period of operation by using four complex synthetic-type wastewaters with a low solid content and found 72-80% removal of COD. The main disadvantages of anaerobic processes are that they are much slower than aerobic processes, and they generally like steady effluents (Metcalf and Eddy, 1991). They are not good with variations in flow of composition.

Bioremediation is a recent technology for treating wastewater. This approach involves bacteria, fungi and yeast that need nutrients (such as carbon, nitrogen, phosphate, and trace metals) to survive. They break down organic (carbon-containing) compounds found in nature to obtain energy for growth (Paul et al., 2005). For example, soil bacteria use petroleum hydrocarbons as a food and energy, changing them into harmless substances of carbon dioxide, water and fatty acids. They usually do not
produce toxic by-products and destroy the target chemicals. One of the primary goals for bioremediation is the removal of organic material from wastewater so that excessive oxygen consumption would not become a problem when the wastewater is released to the environment (Paul et al., 2005). A wheat straw biofilter was studied by Shah et al. (2002) for reducing pollutants in dairy wastewater. This was a 14-day study during which period a biofilter was operated in a sequential aerobic-anaerobic mode while the range of temperatures was 8-14°C. The biofilter was very effective for the removal of total suspended solids and removal of chemical oxygen demand (37% removal). It was ineffective in the removal of nitrate, and removed relatively small amounts of ammonium (20%) and total Kjeldahl nitrogen (15%). It was not effective for the removal of orthophosphate, total P, and fecal coliform. Dubeski et al. (2001) studied lab-scale sequencing batch reactors to treat wastewater that had high chemical oxygen demand (5980-8990 mg L\(^{-1}\)) and found 30-41% reduction of COD with 1 hour settling and 53-62% reduction of COD with 4 hours settling. Within the first 16 hours of aeration most of the oxygen demand reduction occurred. Prochaska and Zouboulis (2003) used a sand filter treatment method to remove some common pollutants from wastewaters. They found that a significant reduction of COD was possible, more than 50% of the COD was removed. Some of the disadvantages of bioremediation are: 1) the range of contaminants on which bioremediation is effective is limited, 2) the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate, and 3) considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result.
1.3.6. Multi-Soil-Layer System

Some of the recent research to restore and clean wastewater has developed using the Multi-Soil-Layer (MSL) system. This is a new technology that uses natural soil in an unit to facilitate wastewater treatment (Wakatsuki et al., 1993). The MSL system is inexpensive to build and needs very little maintenance. The estimated system lifetime is between 10 to 15 years (Attanandana et al., 2000; Luanmanee et al., 2001).

1.3.6.1. Structure and Operations of the MSL System

Wakatsuki et al. (1993) and Luanmanee et al. (2002) described the MSL system structure and its operation in detail. The MSL system is a bi-phasic layered system, consisting of anaerobic layers of soil mixture blocks alternated with aerobic layers of Zeolite / Perlite inter-layer in a brick-like pattern (Figure 1) (Wakatsuki et al., 1993; Luanmanee et al., 2002). The soil mixture blocks consist of soil, iron particles, jute or sawdust, and charcoal (Luanmanee et al., 2002).

Figure 1. Structure and dimensions of the MSL system constructed at Kasetsart University, Thailand (Luanmanee et al., 2002).
The wastewater is initially pre-treated using different settlement tanks before discharging to the MSL system (Luanmanee et al., 2002). Different loading rates of wastewater are applied depending on the surface area of the MSL systems (Luanmanee et al., 2002). Different rates of aeration may or may not be used depending on the systems requirement and operation (Luanmanee et al., 2002). The wastewater passes through the alternative layers of soil mixture blocks and Zeolite/Perlite, and in the process different reactions can take place such as nitrogen removal (nitrification and denitrification), phosphorus removal, organic matter (COD) removal, and many others (Wakatsuki et al., 1993; Luanmanee et al., 2002).

1.3.6.1.1. Aerobic Layer Processes

Nitrification

Nitrification takes place in the aerobic layer with the help of nitrifying bacteria, free oxygen (O₂), and carbon (Wakatsuki et al., 1993; Luanmanee et al., 2002). Nitrification is the biochemical oxidation of ammonium to nitrate. It is a process carried out by a series of bacterial populations that sequentially oxidize ammonium to nitrate with intermediate formation of nitrite. In the first step of nitrification, ammonium-oxidizing bacteria oxidize ammonium to nitrite (eq. 1.1) (Sharma and Ahlert, 1977; Brady and Weil, 1996).

\[ 2\text{NH}_4^+ + 3\text{O}_2 \rightarrow 2\text{NO}_2^- + 2\text{H}_2\text{O} + 4\text{H}^+ + \text{energy} \]  

(1.1)
Nitrosomonas is the most frequently identified genus associated with this step, although other genera, including Nitrosococcus, and Nitrosospira can also oxidize ammonium (Watson et al., 1981). In the second step of nitrification, nitrite-oxidizing bacteria oxidize nitrite to nitrate (eq. 1.2) (Brady and Weil, 1996; Gerardi, 2002).

\[2\text{NO}_2^- + \text{O}_2 \rightarrow 2\text{NO}_3^- + \text{energy}\] (1.2)

Nitrobacter is the most frequently identified genus associated with this second step, although other genera, including Nitrospina, Nitrococcus, and Nitrospira can also oxidize nitrite (Watson et al., 1981). Some of the major factors that influence microbial nitrification are: temperature, moisture content, and pH. The nitrification process is very dependent on temperature and occurs over a range of approximately 4 to 45°C (39 to 113°F) (Gilliam and Gambrell, 1978). Nitrification rates increase with temperature. If the temperature is cold (below 4°C), then there is no growth of Nitrosomonas or Nitrobacter and the denitrification rates decrease with temperature (Gilliam and Gambrell, 1978). Nitrifying bacteria remain active in very dry conditions, but all are inactive in waterlogged soil. Wet soils do not usually contain enough \(\text{O}_2\) to supply nitrifying bacteria (Gerardi, 2002). Nitrification decreases dramatically below pH 6.0. Its optimum range is between pH 6.6 and 8.0 (Gilliam and Gambrell, 1978).

**Oxidation of ferrous to ferric iron and P sorption**

Oxidation of ferrous to ferric iron takes place in aerobic layer adsorbing P and removing it from the wastewater (Wakatsuki et al., 1993; Luanmanee et al., 2002). Adsorption of phosphates to soil particles is an important removal process for P. The adsorption capacity is dependent on the presence of iron (Fe) in clay minerals or bound to
soil organic matter. Under aerobic, neutral to acidic circumstances, Fe (III) binds phosphates in stable compounds. The high concentrations of phosphorus as orthophosphate are adsorbed to the soil iron oxide surfaces through a process of ligand exchange forming a binuclear bridge by a two-step process (Bohn et al., 1985). First a phosphate ion displaces a hydroxide from the soil iron mineral micelle and bonds to the iron molecule, the ion then follows with the displacement of a proton forming a very soluble "binuclear" bridge (Figure 2).

![Figure 2. Representation of $\text{H}_2\text{PO}_4^-$ penetration into an iron oxide surface and subsequent formation of a stable binuclear bridge. (Bohn et al., 1985)](image)

Phosphate can also be precipitated with iron, and soil compounds (Nichols, 1983). These processes, which include the retention of phosphate in the matrix of clay minerals and complexation of phosphate with metals, have a much slower rate but are not so easily subject to saturation. If previously absorbed P is precipitated, the adsorption sites become available again for adsorption of new P (Nichols, 1983).
1.3.6.1.2. Anaerobic Layer Processes

**Denitrification**

Denitrification takes place in the anaerobic layer with the help of denitrifying bacteria, nitrate, carbon, and no free oxygen (Wakatsuki et al., 1993; Luanmance et al., 2002). Denitrification is the biochemical reduction of nitrate to nitrogen gas in the absence of oxygen (eq. 1.3) (Brady and Weil, 1996; Gerardi, 2002).

\[ 2\text{NO}_3^- \rightarrow 2\text{NO}_2^- \rightarrow 2\text{NO}^\uparrow \rightarrow \text{N}_2\text{O}^\uparrow \rightarrow \text{N}_2^\uparrow \] (1.3)

It is the key process concerned in removing nitrogen from wastewater. It occurs when the oxygen concentration in the wastewater becomes low enough that the bacteria begin to utilize nitrate as an electron acceptor (Focht and Chang, 1975; Gerardi, 2002). The nitrate is then reduced by heterotrophic bacteria to the intermediate nitrite and then to nitrogen gas. The nitrogen is then able to leave the wastewater as inert nitrogen gas (Focht and Chang, 1975; Gerardi, 2002). Although there are numerous genera of denitrifying bacteria, the genera *Alcaligenes, Bacillus* and *Pseudomonas* contain the largest number of denitrifying bacteria (Gerardi, 2002). There are a number of factors that influence microbial denitrification. These factors include oxygen, temperature, pH, nutrients, and carbon. Free molecular oxygen inhibits denitrification by competing with nitrate as an electron acceptor in the energy metabolism of cells. It is generally accepted that an anaerobic environment is required for the microbial denitrification (Focht and Chang, 1975; Gerardi, 2002). Temperature is a significant controlling factor on denitrification. Minimum temperature for denitrification is \( \geq 5^\circ \text{C} \) (Gerardi, 2002). Maximum temperature for denitrification appears to be about \( 75^\circ \text{C} \) (Gerardi, 2002).
Denitrification is relatively insensitive to acidity but may be slowed at low pH. The optimal pH range for denitrification is 6.5 to 8.5 (Gerardi, 2002). The availability of nutrients is an important requirement in supporting biological cell growth. According to Payne (1985), the two major nutrients necessary for anaerobic bacteria are nitrogen and phosphorus. Organic carbon availability is one of the most important factors that affect denitrifying bacteria. Organic carbon acts as both a source of cellular material for biological respiration and an electron donor for dissimilatory nitrate reduction (Payne, 1985). While denitrification is enhanced by carbon found in the natural environment (e.g., straw, mulch, sawdust and woodchips) it can also be increased with external additions of carbon (e.g., methanol, ethanol, acetate, and sucrose) (Payne, 1985).

**Reduction of Ferric to Ferrous iron**

Reduction of ferric to ferrous iron takes place in anaerobic layer increasing the mobility of ferrous iron to the aerobic layer (eq. 1.4) (Bohn et al., 1985; Wakatsuki et al., 1993; Luanmanee et al., 2002).

\[
Fe^{3+} + e^- \rightarrow Fe^{2+}
\]  

(1.4)

1.3.6.1.3. Organic Matter Removal

Organic matter (BOD and COD) from the wastewater is first physically and chemically adsorbed at the soil surfaces and subsequently decomposed by microorganisms (Wakatsuki et al., 1993). Organic matter removal depends on the operational conditions of the MSL system and the quality of the wastewater. Aeration enhances the activity of microorganisms which decompose the organic matter in the wastewater (Luanmanee et al., 2002).
1.3.6.2. Advantages and Disadvantages of the MSL System

Masunaga and Wakatsuki (1999) note many of the advantages of using an MSL system. First, it removes the N, P, and organic pollutants (BOD/COD) from wastewater simultaneously. Compared to conventional soil trench systems (10 to 40 L m\(^{-2}\) d\(^{-1}\)), the MSL system has a high loading rate capacity, 1000 to 4000 L m\(^{-2}\) d\(^{-1}\). Another example of the flexibility of MSL system relates to the effect of aeration. Luanmanee et al. (2002) demonstrated how aeration affected the MSL system treating domestic wastewater. They showed the different rates of aeration in the system could play a major role in the efficiency of the treatment. Low aeration decreased the efficiency of the system. By adjusting rate of aeration, the efficiency of the system increased. In addition, since MSL systems use locally available soils and materials, they are inexpensive and easy to maintain. Also the materials used for water treatment can be reused as a soil conditioner for agricultural land (Masunaga and Wakatsuki, 1999). One of the disadvantage of the MSL system is the rate of aeration to the system (Luanmanee et al., 2002). The system needs an appropriate rate and a proper length of time for the aeration. Excess or low aeration affects the efficiency of the system. Another disadvantage is the clogging in the MSL system during operation (Masunaga and Wakatsuki, 1999).
1.3.6.3. Removal of N, P, and COD in MSL System

Various studies have been done to improve the performance of MSL system in wastewater treatment. Wakatsuki et al. (1993) used an on-site domestic wastewater treatment system to remove Biological Oxygen Demand (BOD$_5$), COD, total nitrogen (TN), and total phosphorus (TP) using Multi-Soil-Layering method. During 1 year of operation, the results showed that the removal rates of BOD$_5$, COD, TN, and TP were 84.5, 72.5, 88.9, and 87.5%, respectively. Luanmanee et al. (2001) further investigated the effect of aeration on the efficiency of the same system after continuous use for 10-years. They found that the system was still effective at removing BOD$_5$ and TP. In the tenth year of operation, the removal rates of BOD$_5$ and TP increased up to 95.2 ± 3.8 and 82.9 ± 11.9%, respectively. A continuous aeration of 24 h d$^{-1}$ ($\equiv 2.8 \times 10^4$ L m$^{-3}$ d$^{-1}$) of the MSL system was effective in facilitating the removal of BOD$_5$ and TP (Luanmanee et al., 2001). However, the system was not effective for the removal rate of TN with aeration. The removal rate of TN was improved considerably to 89% after aeration was stopped (Luanmanee et al., 2001).

Attanandana et al. (2000) used a similar system as that used by Wakatsuki et al. (1993) and Luanmanee et al. (2001) for a cafeteria wastewater treatment system. They modified the system using different mixtures in the soil block, such as sawdust plus iron scrape and kenaf plus corncob. The results showed that the percentage removal of BOD, COD, total N, NH$_4^+$-N, total P and dissolved P was 90, 70, 91, 76, 90 and 89 respectively by sawdust with a loading rate of 230 L m$^{-2}$ day$^{-1}$, and was more effective than kenaf plus corncob (Attanandana et al., 2000). The results also showed that the
efficiency of wastewater treatment increased significantly with an aeration of 24 h, at 50 L min\(^{-1}\) aeration for 14 days (Attanandana et al., 2000). Luanmanee et al. (2002) further investigated the same system with different intermittent aeration rates. Two different aeration rates were applied at 4000 and 20,000 L m\(^{-3}\) d\(^{-1}\) and the result showed that the latter rate was more effective in enhancing the BOD\(_5\), COD, TN and SRP removals than the former rate. The removal percentages of BOD\(_5\), COD, TN and SRP were 92.2, 73.3, 15.0 and 74.7, respectively (Luanmanee et al., 2002). They also noticed that the MSL system showed a better performance with the aeration rate of 20,000 L m\(^{-3}\) d\(^{-1}\) alternated with 2 months of non-aeration (Luanmanee et al., 2002).

Sato et al. (2005) investigated the characteristics of the treatment processes inside the MSL system using a laboratory-scale MSL system. They found the treatment processes in the MSL system were different for the COD, P, and N in different layers. The removal rate of COD was 80% in the first layer and was increased with the downward movement of water and reached 90% removal at the last layer. The P concentration was lower under the soil mixture layers and slowly decreased in the lower layers of the system. Nitrification occurred in the upper part of the system and denitrification occurred mainly in the soil mixture layers.
1.4. Information Gaps

1.4.1. Conventional method and limitation

Much research has been carried out to remediate dairy effluent using different treatment methods. Some of the methods such as land application (Caro-Costas et al., 1972; Valencia-Gica et al., 2004), vegetative filter strips (Ikenberry and Mankin, 2000), constructed wetlands (Payne et al., 1992), aerobic and anaerobic processes (Manariotis and Grigoropoulos, 2003), and bioremediation (Carta et al., 1999; Prochaska and Zouboulis, 2003) have performed well, but their widespread use is limited because they are either costly, require regular maintenance, require large areas of land, or the wastewater must be pre-treated.

1.4.2. Possible new methods

Despite a large amount of research to remediate dairy effluent using different treatment methods, a limited amount of research has been undertaken so far where research uses soil as a remediation media for dairy effluent. The MSL system is a new technology that has been developed in Japan and adapted in Thailand to remediate domestic and cafeteria wastewater using soil as a remediation media. To-date, no MSL system has been tested or adapted for the remediation of untreated dairy effluent. There is not much information available on the reliability, consistency, and nutrient removal efficiency of the system. Also, the system has not yet been tested for its ability to remove indicator organisms (fecal coliform). Thus, it is of interest to determine whether the MSL
system can remediate dairy effluent and improve the efficiency in removal of nutrient and indicator organisms.

1.5. Objectives and Hypotheses

1.5.1. Objectives

The following are the primary objectives of this study:

1. Investigate the effectiveness of the MSL systems in remediating dairy effluent.

2. Determine whether the Leilehua or the Perlite MSL system is more effective in removing inorganic N, phosphate, organic matter (COD) and fecal coliform in dairy effluent.

3. Evaluate the effect of aeration and sucrose additions on inorganic N, phosphate, organic matter (COD), and fecal coliform removal efficiency.

1.5.2. Hypotheses

There are three hypotheses involved in this study:

1. The dairy lagoon effluent can be remediated using the MSL systems.

2. The Leilehua MSL system can be more effective than the Perlite MSL system in removing inorganic N, phosphate, organic matter (COD), and fecal coliform in dairy effluent.

3. Optimization of aeration and sucrose applications is a key to improving MSL systems efficiency and consistency in removing inorganic N, phosphate, organic matter (COD), and fecal coliform in dairy effluent.
CHAPTER 2

MATERIALS AND METHODS

2.1. Experimental Site

The experimental site was located in Waianae, latitude 21°27', longitude 158°11' on the west shore of the island of O'ahu, Hawaii. The experiment was conducted using dairy effluent from the third settling lagoon of an effluent waste management system. Average maximum and minimum daily temperatures of the area are 28° C (83° F) and 16° C (61° F) (Hobo Weather Station, 2001-2003). Average annual precipitation is 50-190 cm (20-75 in). The maximum and minimum solar radiation is 13,000 and 8,000 w m$^{-2}$ (Hobo Weather Station, 2001-2003). Average sunshine is 70%. The soil moisture regime is ustic reflecting intense dry periods interspersed with rainfall.

2.2. Experimental Design

The experiment consisted of two replications of two aeration treatments for a total of four MSL systems (Figure 3 and Figure 4). One treatment consisted of aerobic layers of the Leilehua soil and anaerobic layers of soil mixture blocks that included the Honouliuli soil. The other treatment consisted of aerobic layers of Perlite and the similar anaerobic layers of soil mixture blocks that included the Honouliuli soil. The four MSL systems received the same amount of filtered dairy effluent. The units were arranged in a completely randomized design (CRD) (Figure 3).
In the above figure L1 and L2 represent the treatment Leilehua and P1 and P2 represent the treatment Perlite.

Figure 3. Schematic of treatment layout
Figure 4. Four MSL Systems
2.3. MSL Construction

2.3.1. Aerobic Layer

2.3.1.1. Perlite

Natural Perlite was used as an aerobic layer in two of the MSL systems. The Perlite particles that were smaller than 4mm were discarded. This was to assure an adequate pore size to increase aerobic conditions. Perlite is a type of volcanic glass with high water content in its molecular structure. It is a silicate rock, which means that it has a high percentage of silica. Perlite is an important commodity in the horticulture industry where it is mixed with soil to increase the amount of air (i.e., oxygen) held in the soil, as well as the amount of water retained by the soil. The physical properties of the Perlite are given below in Table 2.

Table 2. Physical properties of Perlite

<table>
<thead>
<tr>
<th></th>
<th>White</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>White</td>
</tr>
<tr>
<td>Free Moisture, Maximum</td>
<td>0.50%</td>
</tr>
<tr>
<td>pH (of water slurry)</td>
<td>6.5 - 8.0</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>2.2 - 2.4</td>
</tr>
<tr>
<td>Bulk Density (loose weight)</td>
<td>As desired but usually in the 2-25 lb ft(^{-3}) range (32-400 kg m(^{-3}))</td>
</tr>
<tr>
<td>Mesh Size Available</td>
<td>As desired, 4-8 mesh and finer</td>
</tr>
</tbody>
</table>

Source: The Perlite Institute
2.3.1.2. Aerobic Layer Soil

The type of soil used in the aerobic layer of this study was the Leilehua (Very-fine, mixed, isothermic Humic Rhodic Kandiudox) soil series (Soil Survey Staff, 2006). It was used as an aerobic layer in two of the four MSL systems. The Leilehua soil was screened using a 4mm screen and soil particles less than 4mm were discarded thus assuring an adequate pore size for aerobic microbiological processes. The Leilehua soil series is a silty clay, and fine granulated soil. The Leilehua soil series is well suited to the aerobic layer because it is well-aggregated, well-drained, and contain high amounts of free oxides. These properties make the Leilehua soil series a good candidate for the aerobic layer in the MSL system. The high aggregation and well drained property help to make the system well aerated, enhancing the nitrification, organic matter decomposition, oxidation of ferrous iron to ferric iron, facilitating phosphorus sorption and the removal of COD (Luanmanee et al., 2001). The physical and chemical properties of the Leilehua soil are given in Table 3 and Table 4.

2.3.2. Anaerobic Layer

2.3.2.1. Anaerobic Layer Soil

The type of soil used in the anaerobic layer of this study was the Honouliuli (Fine, kaolinitic, isohyperthermic Typic Chromustert) soil series (Soil Survey Staff, 2006). It was used as an anaerobic layer in all of the four MSL systems. The Honouliuli soil series is a clayey, and medium granulated soil. The Honouliuli soil series is well suited for the anaerobic layer because it is well-drained, has a high water retention capacity, and has high surface area. These properties make the Honouliuli soil series a good candidate to
use in the anaerobic layer of the MSL systems. The water retention capacity of the soil helps the soil system become anaerobic enhancing denitrification (Luanmanee et al., 2001). The physical and chemical properties of the Honouliuli soil are given in Table 3 and Table 4.

Table 3. Selected physical properties of Leilehua and Honouliuli soils

<table>
<thead>
<tr>
<th>Series</th>
<th>Clay</th>
<th>Silt</th>
<th>Sand</th>
<th>Water holding capacity</th>
<th>Bulk density</th>
<th>Particle density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;.002</td>
<td>.002-.05</td>
<td>.05-2</td>
<td>33 kPa</td>
<td>1500 kPa</td>
<td></td>
</tr>
<tr>
<td>Leilehua</td>
<td>58.5</td>
<td>33.6</td>
<td>7.9</td>
<td>n/a</td>
<td>33.2</td>
<td>0.97</td>
</tr>
<tr>
<td>Honouliuli</td>
<td>58.4</td>
<td>34.8</td>
<td>6.8</td>
<td>30.2</td>
<td>22.3</td>
<td>1.31</td>
</tr>
</tbody>
</table>

Source: Soil Survey Staff, 2006

Table 4. Chemical properties of Leilehua and Honouliuli soils

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH ($H_2O$, 1:1)</th>
<th>OC$[^a]$</th>
<th>TN$[^b]$</th>
<th>Dithionite Fe$[^c]$</th>
<th>Oxalate Fe$[^d]$</th>
<th>P sorbed$[^*]$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% of &lt;2mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lilehua</td>
<td>4.8</td>
<td>2.61</td>
<td>0.233</td>
<td>6.4</td>
<td>1.04</td>
<td>1400</td>
</tr>
<tr>
<td>Honouliuli</td>
<td>6.9</td>
<td>0.74</td>
<td>0.11</td>
<td>7.5</td>
<td>n/a</td>
<td>100</td>
</tr>
</tbody>
</table>

Source: Soil Survey Staff, 2006 *Guo and Yost (1998)

2.3.2.2. Soil Mixture Blocks

The anaerobic layer of the systems consisted of seven layers of soil mixture blocks. Each of the soil mixture blocks consisted of Honouliuli soil mixed with finely ground charcoal, fine sawdust, and approximately 1mm diameter iron filings at the ratio of 7:1:1:1 by dry weight. The soil mixture was evenly mixed using an electrical concrete mixer and packed into two sizes of pre-stitched burlap bags to form the blocks of the anaerobic layer. The sizes of the burlap bags were approximately 5 x 10 x 22 cm$^3$ and 5 x 10 x 38 cm$^3$. Charcoal was used as the absorber of various pollutants, sawdust served as a carbon source of microorganisms, and iron filings as the P fixer (Attanandana et al., 2000).

2.3.3. MSL Assembly

Four MSL systems were constructed, each based on an High Definition Poly Ethylene (HDPE) corrugated sewage pipe with 45.72cm interior diameter by 1m height with a cross-sectional area of approximately 0.1648 m$^2$ (Figure 5). The bottom of the HDPE pipe was sealed with plastic bases (2cm thick PVC foam board). At the base of each of the upright HDPE pipes, a 25.4mm PVC discharge pipe was installed for discharging the treated effluent from the system. Also a layer of gravel (≈ 5 cm) was placed at the bottom of the upright pipes to facilitate the system discharge. Above this, the first aerobic layer was introduced, consisting of a 5cm thick soil/perlite layer. The second layer was then installed, consisting of four soil mixture blocks, two 5 x 10 x 22 cm$^3$ blocks and two 5 x 10 x 38 cm$^3$ blocks. Then again the third aerobic layer of 5cm
thick soil/perlite was installed followed by a fourth layer of soil mixture blocks, which consisted of three $5 \times 10 \times 38$ cm$^3$ blocks. In this way, the systems were assembled using 7 alternative layers of soil mixture blocks (anaerobic layers) and 8 layers of screened soil or Perlite (aerobic layers) (Figure 5). An aeration pipe (Figure 6) was installed in between the $9^{th}$ and $10^{th}$ layer of the system ($\approx 50$ cm from the bottom) for the infusion of air whenever it was necessary. An array of emitters (Figure 7) was installed on the top ($\approx 80$ cm from the bottom) of the aerobic and anaerobic layers through which the dairy effluent was discharged into the system.
Figure 5. Cross sections of the MSL systems (Leilehua and Perlite)
2.3.4. Aeration Pipe

The aeration pipe was made from 1.9 cm (3/4 inch) diameter PVC pipe with 0.3 cm (1/8 inch) holes spaced 2.5 cm (1 inch) apart along the pipe (Figure 6). The holes were facing up towards the aerobic layer approximately 70 cm depth from the bottom of the MSL system. An air pump was connected to the aeration pipe to provide the air.

2.3.5. Emitters

In the beginning, an array of four emitters (Figure 7) was placed in each system and later it was changed to accommodate different application rates. The array of emitters was made from 0.6 cm (1/4 inch) diameter drip irrigation tube. Four pieces of drip irrigation tubes, 30 cm each were joined together in a square shape using 90° plastic angles. In the beginning each piece was having an emitter in the middle of the tube and later the number of emitters changed according to the application rates. The arrays were placed on top of each MSL system and connected to 1 inch diameter of PVC pipe through which the untreated but filtered effluent was pumped into the systems. Each emitter emitted 0.98 L (0.26 gal) of effluent per hour.

2.3.6. Flow Meters

A flow meter (Figure 8) was placed for each pair of treatments to measure the actual effluent application rate of the systems. The flow meters were placed at the end of each PVC pipe, which was connected to the array of emitters at one end and the main PVC pipe at the other end through which the untreated effluent pumps to the system.
Figure 6. Aeration Pipe

Figure 7. Array of four drip emitters

Figure 8. Flow meters
2.4. MSL Operating Conditions and Management

The dairy effluent was directly pumped from the lagoon, filtered using a 0.0254m plastic disc filter (140 mesh) to remove the larger particles, and discharged into the MSL system at the specified rates and during the specified time periods.

2.4.1. Application Rates and Residence Times

Three application rates of effluent were applied to the system according to the performance of the system. An initial flow rate of 80 L day$^{-1}$ was applied to each of the system from April 18 to November 3, 2005. This was equal to a hydraulic loading rate of 505 L m$^{-2}$ day$^{-1}$. The loading rate of MSL system is calculated on a surface area of the MSL system. The surface of the MSL is used rather than the volume because MSL systems can be scaled up by increasing surface area. The retention time of the effluent in the MSL system at a loading rate of 505 L m$^{-2}$ day$^{-1}$ was ≈ 48hr. A layer of ponding effluent was observed on the top of the two Leilehua systems. Subsequently, the hydraulic loading rate of the MSL system was reduced to 252 L m$^{-2}$ day$^{-1}$ on November 3, 2005 and continued until April 20, 2006. Correspondingly, the retention time of the effluent in the MSL system increased to 95 hr. The Leilehua systems started ponding again on April 18, 2006. Thus, from April 20 to July 10, 2006 the loading rate of the effluent was decreased to 178 L m$^{-2}$ day$^{-1}$. This corresponded to a retention time of 135hr.
2.4.2. Aeration Rates

Different aeration rates were applied to the systems. Initially, aeration was not applied to the systems. The aeration started during the 10th month of the experiment. February 10 to April 13, 2006, the MSL system was aerated at a rate of 28 L min\(^{-1}\). From April 14 to 27, 2006, the aeration was increased to 31 L min\(^{-1}\). Because of the possibility of disruption of the system due to the large amount of air, the aeration rate was decreased to 17 L min\(^{-1}\) for one week, from April 28 to May 4, 2006 followed by a rate of 11 L min\(^{-1}\) until May 18, 2006. The system did not perform well for the aerobic layer during the periods of low aeration. Therefore, from May 19 until July 10, 2006, the aeration rate was increased to 23 L min\(^{-1}\).

2.4.3. Sucrose Application

An additional source of carbon in the form of a sucrose solution was applied to the MSL system beginning at the end of the 12th month of the study because of the poor performance of the removal of N. Two different application methods were used to apply the sucrose solution to the systems. The initial application of sucrose solution started from April 27, 2006 and continued until May 30, 2006. During this one month period, sucrose solution was applied three times a week with a two-day interval, designed to approximately match the effluent retention time in the MSL system. The concentration of sucrose solution added during this period was 19g (0.055 moles per 500 mL) for the Leilehua system and 22g (0.064 moles 500 mL) for the Perlite system (Table 5). Sucrose additions were based on amounts needed to reduce the expected O\(_2\) content of the MSL system. Later the duration of the application of sucrose solution changed to once in a
week from May 30 until July 10, 2006. Although the amount of sucrose solution increased from May 30 to July 10, 2006, the sucrose concentration and the amount per week was same as the previous application. A detailed description of the calculation of the amount of sucrose is given in Table 5. The percent pore spaces were calculated first from the bulk densities and particle densities of Leilehua and Honouliuli soil, and Perlite. Then the amount of air spaces was calculated from the volume of each system. The amount of oxygen was calculated from the amount of air space and the amount of oxygen in the air. Finally the amount of sucrose was calculated based on the stoichiometric reaction equation (eq. 2), which shows how much sucrose is needed for the microorganisms to consume the specific amount of oxygen.

\[
C_{12}H_{22}O_{11} + 12 \, O_2 \rightarrow 12 \, CO_2 + 11H_2O
\]  

(2)
Table 5. Calculation procedures for estimating MSL system porosity and amount of sucrose

<table>
<thead>
<tr>
<th></th>
<th>Leilehua</th>
<th>Honouliuli</th>
<th>Total (LH)</th>
<th>Honouliuli</th>
<th>Perlite</th>
<th>Total (PH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density (g cm(^{-3}))</td>
<td>0.97</td>
<td>1.31</td>
<td>1.31</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle density (g cm(^{-3}))</td>
<td>2.88</td>
<td>2.93</td>
<td>2.93</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Pore space</td>
<td>66.32</td>
<td>55.29</td>
<td>121.61</td>
<td>55.29</td>
<td>86.96</td>
<td>142.25</td>
</tr>
<tr>
<td>No. of Layers in MSL</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height of each layer (cm)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
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<tr>
<td>Width of each layer (cm)</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume of each layer (cm(^3))</td>
<td>7948</td>
<td>7948</td>
<td>7948</td>
<td>7948</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume of MSL in cm(^3)(No. of layers * Vol. of each layer)</td>
<td>63585</td>
<td>55637</td>
<td>119222</td>
<td>55637</td>
<td>63585</td>
<td>119222</td>
</tr>
<tr>
<td>Air spaces (cm(^3))</td>
<td>42169</td>
<td>30762</td>
<td>72931</td>
<td>30762</td>
<td>55291</td>
<td>86053</td>
</tr>
<tr>
<td>Amount of Oxygen (cm(^3))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15315</td>
<td>18071</td>
</tr>
<tr>
<td>Oxygen (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21.89</td>
<td>25.82</td>
</tr>
<tr>
<td>Oxygen (mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.68</td>
<td>0.81</td>
</tr>
<tr>
<td>Sucrose (mol)**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Amount of Sucrose (g)**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19.51</td>
<td>23.02</td>
</tr>
</tbody>
</table>

\(* 1 \text{ cm}^3 = 1 \text{ mL} = 1/1000 \text{ L}; \text{ Density of Oxygen} = 1.429 \text{ g L}^{-1} \)

\(**C_{12}H_{22}O_{11} \,(\text{mol}) = x \text{ mol O}_2 / 12 \text{ mol O}_2\)

\(***C_{12}H_{22}O_{11} \,(g) = y \text{ mol C}_{12}H_{22}O_{11} \times 342.30 \text{ g mol}^{-1} \,(\text{Mol. Wt. of sucrose} = 342.30 \text{ g mol}^{-1})\)

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2.5. Sample Collection

The dairy effluent input and MSL treated effluent samples were collected using acid washed and autoclaved 250 ml Nalgene bottles, labeled, and immediately placed in a cooler on ice. Upon returning to the lab, they were processed the same day for some of the parameters (COD, fecal coliform, and phosphate) and kept frozen and processed for the rest of the measurements (ammonia nitrogen and nitrate nitrogen) as soon as possible (typically within 1 week).

2.6. Analytical Methods

Samples were taken every week from May to Dec 2005 and Jan to Jul 2006 and analyzed for the following parameters: ammonia nitrogen (NH$_4$\(^+$\)-N), nitrate nitrogen (NO$_3$\(^-\)-N), inorganic phosphate, chemical oxygen demand (COD) and fecal coliform.

Initially for one month (May 2005) the samples were submitted to agricultural diagnostic service center University of Hawaii at Manoa for the analysis of total N, NH$_4$\(^+$\)-N, NO$_3$\(^-\)-N, and total P. Later the samples were analyzed in the lab for NH$_4$-N, NO$_3$\(^-\)-N, and inorganic phosphate. Before processing for these three parameters, each sample was filtered thorough Whatman no. 42 filter paper to reduce the effect of turbidity. Ammonia nitrogen was measured using the salicylate method (Mulvaney, 1996a). Nitrate nitrogen was measured using the cadmium reduction method (Mulvaney, 1996b). Total effluent nitrogen consisted of 98% ammonium and about 1% nitrate. The total inorganic nitrogen (Inorganic N) was approximated as the summation of NH$_4$\(^+$\)-N and NO$_3$\(^-\)-N. The Ascorbic acid method was used to measure inorganic phosphate (Kuo,
A Unico 1100RS Spectrophotometer was used to make all colorimetric determinations.

The COD was determined using the micro-COD test method at a standard range (Bioscience, 2000). The prepackaged COD vials (Accu-TEST® twist-cap Standard Range) and a 15-tube Bioscience® COD reactor were used in this method to digest the sample for 2hr at 150 ± 2° Celsius. The concentration of the sample was measured using a Unico 1100RS Spectrophotometer at 600nm. The COD was used as a measurement of organic matter in the dairy effluent.

The standard membrane filtration method was used to determine Fecal coliform (APHA, 1989). An appropriate volume of sample was filtered through 47 mm membranes of 0.45 μm porosity and the filters were placed on mFC agar with rosolic acid and incubated for 24hr at 44.5 ± 0.2° C. Blue colonies were recorded as fecal coliform.

In addition to weekly measurements of the above parameters, a single time measurement of phosphate sorption (Fox and Kamprath, 1970) was made in Jan 2006 and a two time measurement of total suspended solid (APHA, 1989) were made in May 2005 and July 2006.
2.7. Calculation

Weekly treatment efficiency for the removal of inorganic N, phosphate, COD, and FC were calculated as:

\[
\% \text{ Removal} = \frac{RC - Co}{RC} \times 100
\]

Where:
- \( RC \) = concentration of the parameter in untreated effluent (\( \mu g \) mL\(^{-1} \)), and
- \( Co \) = concentration of the parameter in treated effluent (\( \mu g \) mL\(^{-1} \)).

2.8. Statistical Analysis

The percentage removal of inorganic N, phosphate, COD, and fecal coliform between the Leilehua and Perlite MSL systems were compared using Sigma Plot version 9 (Sigma Plot, 2004). Data were also analyzed using the Statistical Analysis Software, SAS (SAS, 2004). The PROC MIXED procedure was used to analyze percentage removal of inorganic N, phosphate, COD, and fecal coliform using the repeated measures model (autoregressive covariance structure) (Littell et al., 1996; Littell et al., 1998; SAS, 2004). The General Linear Model (GLM) was used to calculate the least significant difference (LSD) between treatments.

For 2005 data, a repeated measures model was tested for overall treatment effects, the effect of time, and the treatment x time interaction (APPENDIX). For 2006 data, a repeated measures model was used to test for overall treatment effects, the effect of aeration, the effect of sucrose, the effect of aeration and sucrose, and lastly the
interactions of treatment x aeration, treatment x sucrose, and treatment x aeration within sucrose (APPENDIX). Least square means (LSMEANS) were used for 2005 and 2006 data to determine estimated effects between treatments and interactions. Least squares means under the PROC MIXED procedure adjust for unbalanced data as well as for both treatment and replication effects. In addition, tests of significance of the least squares mean use the appropriate error term provided by PROC MIXED, and thus are more accurate than the LSMEANS tests under PROC GLM.
CHAPTER 3

RESULTS AND DISCUSSION

The characteristics of effluent used in this experiment were compared with other dairy effluents in Hawaii (Table 6). The percentage removal of inorganic N, phosphate, COD and fecal coliform are given and discussed in two phases. The first phase contains the data from May to Oct 2005 and the second phase contains the data from Jan to July 2006. Although samples were collected in the first phase from Oct to Dec 2005, the data were not included in the analysis because of drastically differing conditions of the aeration. There was a six week pause (Dec 2, 2005 to Jan 14, 2006) between the two phases due to mechanical problems and also due to a suspected build up of biofilms. In the second phase aeration and sucrose additions were compared in an attempt to increase the efficiency of the MSL systems. Three possible improvements were tested in the second phase between Jan 19 to Jul 10, 2006 – 1. Effect of increased aeration, 2. Effect of sucrose addition with a constant rate of aeration, and 3. Effect of different rates of aeration with sucrose.

3.1. Characteristics of Dairy Effluent

The analysis of the effluent was compared with other dairy effluents in Hawaii (Fukumoto et al., 1995; Valencia-Gica et al., 2004) (Table 6). The concentration of total N, NH$_4^+$-N, and NO$_3^-$-N was lower in the effluent use in this experiment than the other dairy effluent. This might be a result of using effluent from the third and last settling of the lagoon system, which was more diluted than that from the first lagoon.
Table 6. Dairy effluent used in this experiment, in comparison with data from other dairy lagoons in Hawaii

<table>
<thead>
<tr>
<th>Source</th>
<th>pH</th>
<th>EC</th>
<th>TSS</th>
<th>TN</th>
<th>NH₄⁺-N</th>
<th>NO₃⁻-N</th>
<th>TP</th>
<th>IP</th>
<th>COD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mS cm⁻¹</td>
<td>mg L⁻¹</td>
<td>µg mL⁻¹</td>
<td></td>
<td></td>
<td>mg L⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy a</td>
<td>8.2</td>
<td>6.4</td>
<td>n/a</td>
<td>395.7</td>
<td>183.8</td>
<td>n/a</td>
<td>14.5</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Dairy b</td>
<td>8.1</td>
<td>3.0</td>
<td>~1000</td>
<td>119</td>
<td>108</td>
<td>5.76</td>
<td>16.8</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>This experiment†</td>
<td>8.4</td>
<td>3.6</td>
<td>400</td>
<td>44.6</td>
<td>43.16</td>
<td>1.30</td>
<td>21.39</td>
<td>13.21</td>
<td>482</td>
</tr>
<tr>
<td>This experiment§</td>
<td>7.77</td>
<td>3.22</td>
<td>n/a</td>
<td>20</td>
<td>17.05</td>
<td>2.86</td>
<td>17.76</td>
<td>5.72</td>
<td>447</td>
</tr>
<tr>
<td>This experiment**</td>
<td>8.90</td>
<td>3.62</td>
<td>320</td>
<td>5.39††</td>
<td>5.26</td>
<td>0.12</td>
<td>NA</td>
<td>6.08</td>
<td>710</td>
</tr>
</tbody>
</table>

EC: electrical conductivity; TSS: total suspended solid; TN: total nitrogen; IP: inorganic phosphate;
COD: chemical oxygen demand.

* Analysis of lagoon effluents from various nutrient streams (Fukumoto, G. K. et al., 1995)
† Valencia-Gica, R. B. et al., 2004
‡ One month before running the experiment, Mar 3, 2005
§ The beginning of the experiment, May 2, 2005
** The end of the experiment, July 10, 2006
†† Total inorganic N (summation of NH₄⁺-N and NO₃⁻-N)
3.2. Removal of Inorganic N

3.2.1. Year 2005

The effectiveness of the MSL systems in removing inorganic N was compared over a six month period (May 05 – Oct 05) (Figure 9). The MSL systems were not significantly different in percentage removal of inorganic N (P > 0.1) (Table 7). However, the percentage removal of inorganic N was significantly different over time for both the MSL systems (P < 0.0001) (Table 7). The non significant interaction indicates that the MSL systems behaved similarly in percentage removal of inorganic N (P > 0.1) (Table 7).

Table 7. A comparison of the effect of time and MSL system on inorganic N removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.8303</td>
</tr>
<tr>
<td>Time</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MSL systems x Time</td>
<td>0.9474</td>
</tr>
</tbody>
</table>

The inorganic N removal by the Leilehua MSL system and the Perlite MSL system ranged from 22-93% (lsmean of 61.94) and 21-96% (lsmean of 63.40) respectively (Figure 9). It appears from the result that the two MSL systems were equally effective in removal of inorganic N. Almost all of the nitrogen in the lagoon effluent comes to the system in the form of ammonium (Table 6). Ammonium is nitrified to nitrate in the aerobic layer of the systems (Wakatsuki et al., 1993; Attanandana et al., 2000). The nitrate is transformed to gaseous nitrogen (presumably N₂) in the anaerobic
layer and in the process inorganic N is removed from the effluent and leaves the MSL system as a gas (Wakatsuki et al., 1993; Attanandana et al., 2000).

The percentage removal of inorganic N by both the MSL systems was initially high (>90%) and then decreased over time (Figure 9). We hypothesized that the decrease might be due to the reduced aeration in the aerobic layer or decreased microorganism-available carbon in the anaerobic layer. We tested the hypothesis by adding supplemental aeration and sucrose (as carbon source) in the second phase of this experiment (2006).

There were some pauses in effluent delivery during this period (May 05 – Oct 05). The sudden decrease in removal of inorganic N that appears in Figure 9 seems to be related to these pauses. There was a two day pause before the sampling on June 7 (see label 2D), a three day pause on June 30 (see 3D), a six day pause on July 26 (see 6D), an eight day pause on Aug 29 (8D), and a 12 day pause on Sep 22, 2005 (12D), respectively (Figure 9). It appears from Figure 9 that the percentage removal of inorganic N decreased sharply after each pause in effluent delivery. This might be because the lack of effluent inside the system during the pause allowed the system to dry and become aerobic. Denitrifying bacteria become inactive in aerobic conditions (Golterman, 1985). So, the lack of effluent was expected to limit the denitrification process and thus reduce the removal of nitrogen. An increase in the concentration of NO$_3^-$-N in MSL treated effluent seems to be associated with the pauses and would be expected if the denitrification process were limited or shut down because of aerobic conditions (Figure 10). The percentage removal of inorganic N increased after the effluent delivery resumed (Figure 9). Furthermore it appears that once there is a pause in effluent delivery, it takes several days for the systems to regain the efficiency in removing inorganic N. Although
the MSL systems received effluent continuously from the Oct 13 - 20, 2005, the
efficiency of the systems in percentage removal of inorganic N remained low. The
decreased percentage of inorganic N removal in this period (Oct 13 – 20, 2005) appears
to be associated with the effect of the long pause of one week before the system started
operating again.
Figure 9. Removal of inorganic N in the Leilehua and Perlite MSL systems as affected by time.

In this figure "2D" indicates a 2 day pause; "3D" — a 3 day pause; "6D" — a 6 day pause; "8D" — a 8 day pause; "12D" a 12 day pause.
Figure 10. Concentrations of NO$_3^-$-N in effluent input and MSL-treated effluent.
Figure 11. Concentrations of $\text{NH}_4^+$-N in effluent input and MSL-treated effluent.
3.2.2. Year 2006

3.2.2.1. The Effect of Aeration

A comparison was made between no aeration and two different rates of aeration (28 L min\(^{-1}\) and 31 L min\(^{-1}\)) in removal of inorganic N during a sampling period of Jan 19 to April 27, 2006 (Figure 12). The percentage removal of inorganic N was not significantly different between the MSL systems (\(P > 0.1\)) (Table 8). There was no significant difference in percentage removal of inorganic N with aeration for both the MSL systems (\(P > 0.1\)) (Table 8). The non significant interaction indicates that the MSL systems behaved similarly in percentage removal of inorganic N (\(P > 0.1\)) (Table 8).

Table 8. A comparison of the effect of aeration and MSL system on inorganic N removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
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<tr>
<td>MSL systems</td>
<td>0.8008</td>
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<tr>
<td>Aeration</td>
<td>0.8214</td>
</tr>
<tr>
<td>MSL systems x Aeration</td>
<td>0.4330</td>
</tr>
</tbody>
</table>

The removal of inorganic N by the Leilehua system and the Perlite system ranged from 8-61\% (lsmean of 29.34) and 10-73\% (lsmean of 33.10) respectively, which indicates that both the MSL systems were similarly effective in percentage removal of inorganic N (Figure 12). It seemed that the MSL systems did not increase efficiency with the increased aeration. However, it appears from Figure 12 that the percentage removal of inorganic N increased initially (Feb 23 to Mar 9, 2006) followed by a rapid decline. This suggests that initially the application of aeration enhanced the percentage removal of
inorganic N by both the MSL systems which might be due to the increased aeration inside the systems. A decrease in concentration of NH₄⁺-N in MSL treated effluent seems to be associated with the increased aeration and would be expected due to the high nitrification process in the aerobic layer (Figure 13). On the other hand the decline in the percentage removal of inorganic N (April 13 – 20, 2006) might be due to the excessive nitrification which was indicated by high concentration of NO₃⁻-N by the MSL treated effluent (Figure 14). The increased concentration of NO₃⁻-N suggests that the systems were nitrifying the effluent N but were not denitrifying it, which decreased the overall inorganic N removal efficiency of the MSL systems.

3.2.2.2. The Effect of Sucrose with Aeration

A comparison was made between the non sucrose and sucrose application with constant aeration in removal of inorganic N from Feb 16 to April 13 and May 25 to July 10, 2006 (Figure 12). The MSL systems were not significantly different in percentage removal of inorganic N (P > 0.1) (Table 9). However, there was a significant increase in percentage removal of inorganic N with sucrose additions for both the MSL systems (P < 0.1) (Table 9). The non significant interaction indicates that the MSL systems behaved similarly in percentage removal of inorganic N (P > 0.1) (Table 9).
Table 9. A comparison of the effect of sucrose and MSL system on inorganic N removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
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</tr>
</thead>
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</tr>
<tr>
<td>Sucrose</td>
<td>0.0539</td>
</tr>
<tr>
<td>MSL systems x Sucrose</td>
<td>0.8913</td>
</tr>
</tbody>
</table>

The inorganic N removal by the Leilehua and the Perlite MSL system ranged from 9-89% (lsmean of 48.77) and 10-92% (lsmean of 53.36) respectively (Figure 12). The MSL systems were similarly effective in percentage removal of inorganic N. The results revealed that application of sucrose with constant aeration increased the efficiency of the MSL systems by increasing the removal of inorganic N. The increased inorganic N removal might be due to enhanced microbial activity and increased denitrification in the systems, suggested by the low concentration of NO₃⁻-N in the MSL treated effluent (Figure 14). Although sawdust was supplied as a carbon source while implementing the system, it seems that eventually it did not provide sufficient carbon to sustain the microbial activity in the denitrification process. The systems appear to need additional carbon to ensure sustained microbial activity. Thus by providing sucrose as a carbon source, the system seemed to enhance the microbial activity and thus enhancing the denitrification process. So the hypothesized decrease in microorganism-available carbon in 2005 over time seems to be supported by the sharp increase in percentage removal of inorganic N by both the MSL systems with sucrose applications.
3.2.2.3. The Effect of Different Rates of Aeration

Three different rates of aeration (11 L min\(^{-1}\), 17 L min\(^{-1}\), and 23 L min\(^{-1}\)) were compared in removal of inorganic N between a sampling period of May 4 to July 10, 2006 when sucrose was added (Figure 12). The MSL systems were not significantly different in the percentage removal of inorganic N (P > 0.1) (Table 10). There was no significant difference in percentage removal of inorganic N with different rates of aeration for both the MSL systems (P > 0.1) (Table 10). The non significant interaction indicates that the MSL systems behaved similarly in percentage removal of inorganic N (P > 0.1) (Table 10).

Table 10. A comparison of the effect of different rates of aeration and MSL system on inorganic N removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.8640</td>
</tr>
<tr>
<td>Aeration</td>
<td>0.3546</td>
</tr>
<tr>
<td>MSL systems x Aeration</td>
<td>0.3249</td>
</tr>
</tbody>
</table>

The removal of inorganic N by the Leilehua and the Perlite system ranged from 31-89% (lsmean of 60.33) and 43-92% (lsmean of 62.24) respectively (Figure 12). It appears that the MSL systems were similarly effective in removing inorganic N. The systems did not increase efficiency in removal of inorganic N with the different rates of aeration. This might be due to a pause in the system from June 13 - 20, 2006 (Figure 12). The pause is expected to interrupt the denitrification process of the system. Also as discussed earlier, the system takes several days to regain the efficiency after the pause.
and that might be another reason for no change in effectiveness of the systems with different rates of aeration.
Figure 12. Removal of inorganic N in the Leilehua and Perlite MSL systems as affected by sucrose addition and different rates of aeration.
Figure 13. Concentrations of NH$_4^+$-N in effluent input and MSL-treated effluent.
Figure 14. Concentrations of NO$_3^-$-N in effluent input and MSL-treated effluent.
3.3. Removal of phosphate

3.3.1. Year 2005

The effectiveness in removal of phosphate by the MSL systems was compared over a six month period (May 05 – Oct 05) (Figure 15). There was a significant difference observed in percentage removal of phosphate between the MSL systems ($P < 0.05$) (Table 11). The Leilehua MSL system was more effective than the Perlite MSL system. There was a significant decrease in percentage removal of phosphate over time ($P < 0.001$) (Table 11). The significant interaction indicates that the percentage removal of phosphate in the Perlite MSL system decreased at a faster rate than the Leilehua MSL system ($P < 0.1$) (Table 11).

Table 11. A comparison of the effect of time and MSL system on phosphate removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.0292</td>
</tr>
<tr>
<td>Time</td>
<td>0.0004</td>
</tr>
<tr>
<td>MSL systems x Time</td>
<td>0.0997</td>
</tr>
</tbody>
</table>

The percentage removal of phosphate by the Leilehua MSL system (64 - 99%) (lsmean of 92.70) was greater than the Perlite MSL system (9 - 97%) (lsmean of 59.41) (Figure 15). This was probably because the high capacity of the Leilehua series soil to adsorb phosphate (1600 µg P g⁻¹ soil) (Figure 16). The Leilehua series soil contains a large amount of iron oxide (Table 4), which is present in the ferric oxidation state with aeration and sorbs phosphate from the effluent (Wakatsuki et al., 1993; Attanandana et
al., 2000). In case of the Perlite MSL system the ferric iron ($Fe^{3+}$) is expected to be reduced to ferrous iron ($Fe^{2+}$) in the anaerobic layer and then transported by the effluent to the aerobic layer where it is then oxidized to ferric iron in the aerobic layer and, consequently, precipitates phosphate from effluent (Wakatsuki et al., 1993; Attanandana et al., 2000). This is the primary mechanism for phosphate removal by the Perlite MSL system.

The Leilehua MSL system consistently removed phosphate with time except Sept 15 and Oct 6, 2005 (Figure 15). This might be because of the long pauses in delivery of effluent before sampling on Sept 15 and Oct 6, 2005. The decrease in removal of phosphate by the Perlite MSL system was more rapid with time (Figure 15). The decrease in phosphate removal by the Perlite MSL system might be a result of decreased microorganism-available carbon in the anaerobic layer. The organic matter provided in the initial phase provides a carbon source for the microorganism-mediated chemical reduction of iron. As indicated in the above section on N removal, it appears that the sawdust was insufficient to maintain microbial activity and thus inadequate to maintain chemical reducing conditions in the soil blocks. Thus a decrease in soluble carbon in the anaerobic layer is thought to decrease ferric iron availability in the aerobic layer. As a result, the phosphate fixation capacity of the aerobic phase of the Perlite MSL system decreased, resulting in less removal of phosphate. In other words, it appears that the Perlite system was much more dependent on obtaining anaerobic conditions in order to effectively remove phosphate than was the Leilehua MSL system.
Figure 15. Removal of phosphate in the Leilehua and Perlite MSL systems as affected by time.

In this figure, "2D" indicates a 2 day pause; "3D" - a 3 day pause; "6D" - a 6 day pause; "8D" - a 8 day pause; "12D" - a 12 day pause.
Figure 16. The relationship between P sorbed and soil solution P in Leilehua and Honouliuli soil.
3.3.2. Year 2006

3.3.2.1. The Effect of Aeration

To assess the effects of aeration on removal of phosphate, a comparison was made between no aeration and two different rates of aeration (28 L min\(^{-1}\) and 31 L min\(^{-1}\)) during a sampling period from Jan 19 to April 27, 2006 (Figure 17). The percentage removal of phosphate was significantly different between the MSL systems (P < 0.01) (Table 12). The Leilehua MSL system was more effective in percentage removal of phosphate than the Perlite MSL system. Changes in aeration resulted in a significant difference in percentage removal of phosphate (P < 0.1) (Table 12). The significant interaction indicates that the MSL systems behaved differently in the removal of phosphate (P < 0.05) (Table 12).

Table 12. A comparison of the effect of aeration and MSL system on phosphate removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.0088</td>
</tr>
<tr>
<td>Aeration</td>
<td>0.0782</td>
</tr>
<tr>
<td>MSL systems x Aeration</td>
<td>0.0141</td>
</tr>
</tbody>
</table>

The removal of phosphate by the Leilehua MSL system ranged from 42-91% (lsmean of 66.64) was greater than the Perlite MSL system (11-41%) (lsmean of 27.22) (Figure 17). Efficient phosphate removal by the Leilehua system would be expected due to the high phosphate sorption capacity of the Leilehua soil.
The application of aeration seemed to enhance the efficiency in removal of phosphate by the Leilehua system. This might be because of the otherwise insufficient aeration in the system to oxidize ferrous iron from the anaerobic zone (Feb 16 to Apr 27, 2006). As discussed earlier, the ferrous iron oxidizes to ferric iron in the aerobic zone, then precipitates and sorbs the effluent P (Wakatsuki et al., 1993; Attanandana et al., 2000). The decreased removal of phosphate by the Perlite system might be the result of too much aeration in the Perlite system. The additional air may have increased aeration in the anaerobic zone, decreasing its effectiveness in chemically reducing the Fe$^{3+}$ to Fe$^{2+}$ and minimizing its movement out of the soil blocks and resulting in less Fe$^{3+}$ deposition and less phosphate removal.

3.3.2.2. The Effect of Sucrose with Aeration

A comparison was made between the non sucrose and sucrose application with a constant rate of aeration on removal of phosphate from Feb 16 to April 13 and May 25 to July 10, 2006 (Figure 17). The MSL systems significantly differed in the percentage removal of phosphate (< 0.05) (Table 13). There was no overall significant difference in percentage removal of phosphate with the sucrose application (P > 0.1) (Table 13). However, the significant interaction indicates that the percentage removal of phosphate increased by the Perlite system (P < 0.1) (Table 13).
Table 13. A comparison of the effect of sucrose and MSL system on phosphate removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.0342</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.1325</td>
</tr>
<tr>
<td>MSL systems x Sucrose</td>
<td>0.0980</td>
</tr>
</tbody>
</table>

The removal of phosphate by the Leilehua MSL system ranged from 59-93% (lsmean of 76.31) was more effective than the Perlite MSL system (11-75%) (lsmean of 46.13) (Figure 17).

The sucrose application did not increase the already high removal rate of phosphate by the Leilehua system with an lsmean of 74.99 compared to non-sucrose lsmean of 77.63 (Figure 17). Application of sucrose, however, did increase removal of phosphate by the Perlite system with an lsmean of 60.68 compared to non-sucrose lsmean of 31.57 (Figure 17). During the period of 30 May - 10 July, 2006 the removal of phosphate by the Perlite system increased to about as high as that of the Leilehua system (Figure 17). This might be because of the addition of sucrose provided a carbon source for the microorganisms increasing their activity resulting in more O₂ consumption and enhanced reducing conditions in the anaerobic zone moving iron into the aerobic zone where it could precipitate the phosphate in effluent. The hypothesized decrease in carbon source for microorganisms in 2005 seems to be supported by the sharp increase in percentage removal of phosphate by the Perlite systems when sucrose was added.
3.3.2.3. The Effect of Different Rates of Aeration

A comparison was made among three different rates of aeration (11 L min\(^{-1}\), 17 L min\(^{-1}\), and 23 L min\(^{-1}\)) on removal of phosphate from May 4 to July 10, 2006 where sucrose was added (Figure 17). There was a significant difference observed in percentage removal of phosphate between the MSL systems (P < 0.1) (Table 14). There was no significant difference in removal of phosphate with different rates of aeration (P > 0.1) (Table 14). The non significant interaction indicates that the MSL systems behaved similarly in removal of phosphate (P > 0.1) (Table 14).

Table 14. A comparison of the effect of different rates of aeration and MSL system on phosphate removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
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<tr>
<td>MSL systems</td>
<td>0.0937</td>
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<tr>
<td>Aeration</td>
<td>0.1373</td>
</tr>
<tr>
<td>MSL systems x Aeration</td>
<td>0.2538</td>
</tr>
</tbody>
</table>

The percentage removal of phosphate by the Leilehua MSL system ranged from 59 – 93% (lsmean of 73.71) was greater than the Perlite MSL system (17 – 75%) (lsmean of 46.77) (Figure 17).

The application of different rates of aeration (May 4 – Jul 10, 2006) did not result increased removal of phosphate by both the MSL systems. The Leilehua system consistently removed phosphate with all levels of aeration (Figure 17). However, the higher application rates of aeration increased the removal of phosphate by the Perlite system with an lsmean of 55.34 (17 L min\(^{-1}\)) and 59.96 (23 L min\(^{-1}\)) compared to lower
application rate of aeration with an lsmean of 25.02 (11 L min⁻¹) (Figure 17). This increased aeration of the Perlite MSL system, may increase oxidation of ferrous iron (Fe²⁺) to ferric iron (Fe³⁺) in the aerobic layer, increasing the precipitation of phosphate in effluent (Wakatsuki et al., 1993; Attanandana et al., 2000).
Figure 17. Removal of phosphate in the Leilehua and Perlite MSL systems as affected by sucrose additions and different rates of aeration.
3.4. Removal of Organic Matter

3.4.1. Year 2005

The efficiency of the MSL systems in removing organic matter in effluent was measured as percentage reduction of COD in MSL-treated effluent. The effluent concentration and the percentage reduction of COD by the MSL systems (Leilehua vs Perlite) were observed over a six month period (May - Oct 2005) (Figure 18). The percentage reduction of COD was significantly different between the MSL systems, with the Perlite MSL system being more effective than the Leilehua MSL system ($P < 0.05$) (Table 15). There was a significant increase in percentage reduction of COD over time for both the MSL systems ($P < 0.0001$) (Table 15). The significant interaction indicates that the increase in reduction of COD by the Perlite MSL system was faster than the Leilehua MSL system ($P < 0.01$) (Table 15).

Table 15. A comparison of the effect of time and MSL system on chemical oxygen demand reduction as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.0306</td>
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<tr>
<td>Time</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MSL systems x Time</td>
<td>0.0075</td>
</tr>
</tbody>
</table>

The reduction of COD by the Perlite MSL system ranged from 4 – 37% (lsmean of 21.82) was greater than the Leilehua MSL system (3 – 30%) (lsmean of 11.17) (Figure 18). This was probably because of the highly porous nature of the Perlite, particularly the large macropores resulting in the Perlite system being more aerobic. Aeration assists
microorganisms to decompose organic matter in the effluent (Luanmanee et al., 2001; Luanmanee et al., 2002).

The reduction of COD by both the MSL systems gradually increased with time (Figure 18). The increase in COD reduction might be due to the high concentration of the organic matter in the dairy effluent (> 600 mg L⁻¹) (Figure 18). Since the organic matter in the dairy effluent is an important source of energy for microorganisms, the increased organic matter may have increased microbial growth and activity decomposing more organic matter.
Figure 18. Concentration of COD in effluent input, and reduction of COD in MSL-treated effluent (Leilehua and Perlite) as affected by time.

In this figure, "2D" indicates a 2 day pause; "3D" - a 3 day pause; "6D" - a 6 day pause; "8D" - a 8 day pause; "12D" - a 12 day pause.
3.4.2. Year 2006

3.4.2.1. The Effect of Aeration

A comparison was made between no aeration and two different rates of aeration (28 L min\(^{-1}\) and 31 L min\(^{-1}\)) on percentage reduction of COD during a sampling period of Jan 19 to April 27, 2006 (Figure 19). The percentage reduction of COD was significantly different between the MSL systems, with increased reduction by the Perlite system (\(P < 0.05\)) (Table 16). However, there was no significant difference in reduction of COD with aeration (\(P > 0.1\)) (Table 16). There also appeared to be no difference between MSL systems in response to aeration (\(P > 0.1\)) (Table 16).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.0170</td>
</tr>
<tr>
<td>Aeration</td>
<td>0.2526</td>
</tr>
<tr>
<td>MSL systems x Aeration</td>
<td>0.1432</td>
</tr>
</tbody>
</table>

The reduction of COD by the Perlite MSL system ranged from 16-36% (lsmean of 29.87) was higher than the Leilehua MSL system (6-32%) (lsmeans of 17.08) (Figure 19). This might be due to the high porosity of the Perlite particles in the Perlite MSL system.

The reduction of COD by both the MSL systems did not change with the application of aeration during the Jan 19 to April 27, 2006 period (Figure 19). As
mentioned earlier there was a six week pause between the sampling period of Dec 2005 and Jan 2006. When the MSL systems started operating again on Jan 14, 2006, it is assumed that there was sufficient air inside the systems even though the systems were not aerated. The aeration inside the system might have helped the microorganisms to decompose organic matter in the effluent (Luanmanee et al., 2001; Luanmanee et al., 2002). Thus, the efficiency of the MSL systems in reducing COD was similar with and without aeration.

3.4.2.2. The Effect of Sucrose with Aeration

The effect of sucrose additions with a constant rate of aeration in reduction of COD is shown in Figure 19 (Feb 16 to April 13 and May 25 to July 10, 2006). The MSL systems were significantly different in percentage reduction of COD. The Perlite MSL system was more effective in reducing COD than the Leilehua MSL system (P < 0.05) (Table 17). There was a significant increase in COD reduction with the application of sucrose for both the MSL systems (P < 0.05) (Table 17). The significant interaction indicates that the increased COD reduction behaved differently by both the MSL systems (P < 0.1) (Table 17).

Table 17. A comparison of the effect of sucrose and MSL system on chemical oxygen demand reduction as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.0256</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.0255</td>
</tr>
<tr>
<td>MSL systems x Sucrose</td>
<td>0.0631</td>
</tr>
</tbody>
</table>
The reduction of COD by the Perlite MSL system ranged from 24-42% (lsmean of 34.13) was greater than the Leilehua MSL system 11-43% (lsmean of 22.84) (Figure 19). As discussed earlier, the increased reduction of COD by the Perlite system might be due to the high porosity of the Perlite particle in the Perlite MSL system.

The efficiency of the MSL systems increased with the application of sucrose. This might be due to the additional carbon, which is expected to increase the microbial activity inside the system and help to decompose the organic matter. Application of sucrose increased the efficiency in reduction of COD by the Leilehua MSL system with an lsmean of 32.09 compared to non-sucrose lsmean of 13.59 (Figure 19). This might be because of the microorganism-available carbon source provided by sucrose additions, which increased the activity of microorganisms in the Leilehua MSL system.

3.4.2.3. The Effect of Different Rates of Aeration

Three different rates of aeration (11 L min⁻¹, 17 L min⁻¹, and 23 L min⁻¹) were compared in reduction of COD between a sampling period of May 4 to July 10, 2006 when sucrose was added (Figure 19). The MSL systems were significantly different in percentage reduction of COD (P < 0.1) (Table 18). There was a significant increase in percentage reduction of COD with aeration (P < 0.05) (Table 18). The non significant interaction indicates the percentage reduction of COD by both the MSL systems behaved similarly (P > 0.1) (Table 18).
Table 18. A comparison of the effect of different rates of aeration and MSL system on chemical oxygen demand reduction as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.0890</td>
</tr>
<tr>
<td>Aeration</td>
<td>0.0072</td>
</tr>
<tr>
<td>MSL systems x Aeration</td>
<td>0.1996</td>
</tr>
</tbody>
</table>

The reduction of COD by the Perlite MSL system ranged from 11-42% (lsmean of 31.66) was more effective than the Leilehua MSL system (2-43%) (lsmean of 20.95) (Figure 19). It seemed that the efficiency in percentage reduction of COD by both the MSL systems was gradually increased with increased aeration rates between a sampling period of May 4 to July 10, 2006 (Figure 19). This might be due to the application of proper amount of aeration and sucrose to the system, which helps to increase microbial activity inside the system decomposing organic matter.
Figure 19. Reduction of COD in the Leilehua and Perlite MSL systems as affected by sucrose additions and different rates of aeration.
3.5. Removal of Fecal coliform

3.5.1. Year 2005

The fecal coliform colonies (cfu/100mL) in effluent input and MSL-treated effluent output are presented in Figure 20. Using the effluent input as the starting point, the percentage removal of fecal coliform was calculated for the Leilehua and the Perlite MSL system. Figure 21 shows a relation between the removal of fecal coliform and the MSL systems over a six month period (May 05 – Oct 05). There was no significant difference observed in percentage removal of fecal coliform between the MSL systems (P > 0.1) (Table 19). However, the percentage removal of fecal coliform was significantly different over time for both the MSL systems (P < 0.01) (Table 19). The non significant interaction indicates that the MSL systems behaved similarly in percentage removal of fecal coliform (P > 0.1) (Table 19).

Table 19. A comparison of the effect of time and MSL system on fecal coliform removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.3766</td>
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<tr>
<td>Time</td>
<td>0.0358</td>
</tr>
<tr>
<td>MSL systems x Time</td>
<td>0.9058</td>
</tr>
</tbody>
</table>

The fecal coliform removal by the Leilehua MSL system and the Perlite MSL system ranged from 6-99% (I(2)mean of 50.53), and 29-98% (I(2)mean of 60.83) respectively (Figure 21). It appears from the results that both the MSL systems were similarly
effective in the removal of fecal coliform. The removal of fecal coliform varied considerably by both the MSL systems over the entire sampling period.

As discussed in removal of inorganic N, there were some pauses in effluent delivery during this period. There was a two day pause before the date of sampling on June 7 (see label 2D, Figure 13), a three day pause on June 30 (see 3D), a six day pause on July 26 (see 6D), an eight day pause on Aug 29 (8D), and a 12 day pause on Sep 22, 2005 (12D) (Figure 21). It appears from Figure 21 that the removal of fecal coliform decreased after each pause in effluent delivery. This might be because the lack of effluent inside the system during the pause allowed the system to dry, which is expected to be deficient in nutrients and probably there was a lack of predator microorganisms. Among others, the two factors, competition for nutrients and predation by other organisms are considered to be controlling the rate of decay in fecal coliform (Enzinger and Cooper, 1976). It also noticed that the removal of fecal coliform increased after the effluent delivery resumed (Figure 21). Furthermore it appears that once there is a pause in effluent delivery, it takes several days for the systems to regain the efficiency of removing fecal coliform. Restoration of removal efficiency appears to take much longer than does its interruption. It observed from Figure 21 that the percentage removal of fecal coliform decreased sharply by the Leilehua MSL system compared to the Perlite MSL system. This might be because of the expected high levels of fecal coliform in the Leilehua soil. Studies in the tropical soil have shown that proliferation of \textit{E. coli} can occur in soil in the absence of fecal contamination (Hardina and Fujioka, 1995).
Figure 20. Fecal coliform counts in effluent input and MSL-treated effluent.

In this figure, "2D" indicates a 2 day pause; "3D" - a 3 day pause; "6D" - a 6day pause; "8D" - a 8 day pause; "12D" - a 12 day pause.
Figure 21. Removal of fecal coliform by the Leilehua and the Perlite MSL systems as affected by time.

In this figure "2D" indicates a 2 day pause; "3D" - a 3 day pause; "6D" - a 6 day pause; "8D" - a 8 day pause; and "12D" - a 12 day pause.
3.5.2. Year 2006

3.5.2.1. The Effect of Aeration

The fecal coliform colonies (cfu/100mL) in effluent input and MSL treated effluent output are presented in Figure 22. A comparison was made between no aeration and the two different rates of aeration (28 L min⁻¹ and 31 L min⁻¹) in removal of fecal coliform during a sampling period of Jan 19 to April 27, 2006 (Figure 23). The percentage removal of fecal coliform was not significantly different between the MSL systems (P > 0.1) (Table 20). There was no significant difference in percentage removal of fecal coliform with aeration by both the MSL systems (P > 0.1) (Table 20). The non significant interaction indicates that the MSL systems did not differ with aeration (P > 0.1) (Table 20).

Table 20. A comparison of the effect of aeration and MSL system on fecal coliform removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.1806</td>
</tr>
<tr>
<td>Aeration</td>
<td>0.1596</td>
</tr>
<tr>
<td>MSL systems x Aeration</td>
<td>0.2716</td>
</tr>
</tbody>
</table>

The removal of fecal coliform by the Leilehua MSL system and the Perlite MSL system ranged from 6-93% (lsmean of 60.36) and 56-100% (lsmean of 79.95) respectively (Figure 23). There was almost no change in removal of fecal coliform with or without aeration by the Perlite MSL system (Figure 23). This might be because of the sufficient air inside the systems even if there was no supplemental air. The sufficient air
inside the systems is expected to be due to the six weeks of pause before applying effluent to the system on Jan 14, 2006.

3.5.2.2. The Effect of Sucrose with Aeration

A comparison was made between the application or not of sucrose with constant aeration in removal of fecal coliform from Feb 16 to April 13 and May 25 to July 10, 2006 (Figure 23). There was no significant difference observed in percentage removal of fecal coliform between the MSL systems ($P > 0.1$) (Table 21). However there was a significant increase in percentage removal of fecal coliform with sucrose addition by both the MSL systems ($P < 0.1$) (Table 21). The non significant interaction indicates that fecal coliform removal by the MSL systems increased about the same with the application of sucrose ($P > 0.1$) (Table 21).

Table 21. A comparison of the effect of sucrose and MSL system on fecal coliform removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.2453</td>
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<tr>
<td>Sucrose</td>
<td>0.0649</td>
</tr>
<tr>
<td>MSL systems x Sucrose</td>
<td>0.2637</td>
</tr>
</tbody>
</table>

The removal of fecal coliform by the Leilehua 13 – 100% (lsmean of 83.00) and the Perlite MSL systems 75 – 100%(lsmean of 90.27) (Figure 23). It appears from Figure 23 that there was an increase in percentage removal of fecal coliform which corresponds to the addition of sucrose with aeration. The sucrose addition is expected to increase the
bacterial population inside the systems. It may be difficult for the fecal coliform to survive with all other bacteria and as a result there was reduction in fecal coliform.

3.5.2.3. The Effect of Different Rates of Aeration

Three different rates of aeration (11 L min⁻¹, 17 L min⁻¹, and 23 L min⁻¹) at a constant application rate of sucrose were compared in removal of fecal coliform between a sampling period of May 4 to July 10, 2006 (Figure 23). There was no significant difference observed in percentage removal of fecal coliform between the MSL systems (P > 0.1) (Table 22). However, there was a significant increase in removal of fecal coliform with the additional aeration by both the MSL systems (P < 0.05) (Table 22). The non significant interaction indicates that the percentage removal of fecal coliform in MSL systems increased about the same with the different rates of aeration (P > 0.1) (Table 22).

Table 22. A comparison of the effect of different levels of aeration and MSL system on fecal coliform removal as analyzed by SAS Proc Mixed repeated measures analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Probability of significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL systems</td>
<td>0.2597</td>
</tr>
<tr>
<td>Aeration</td>
<td>0.0227</td>
</tr>
<tr>
<td>MSL systems x Aeration</td>
<td>0.3675</td>
</tr>
</tbody>
</table>

The removal of fecal coliform by the Leilehua MSL system 22 - 100% (lsmean of 52.25) was similar to that of the Perlite MSL system 51 - 100% (lsmean of 73.46) (Figure 23). There was an increase in removal of fecal coliform with the increased aeration rates (Figure 23). Studies from the literature show that aeration plays a major role in fecal coliform removal (Dewedar and Baghat, 1995). Fecal coliform decay rates in
aerobic environments are higher than in anaerobic environments (Dewedar and Baghat, 1995). Figures 24 and 25 illustrate a significant positive correlation ($r = 0.9258$ and $0.8646^{**}$) between the application of different rates of aeration and percent removal of fecal coliform within sucrose addition. A high rate of aeration tended to give a high percentage removal (Figure 24 and 25). The low rate of aeration in this experiment probably was insufficient for optimal removal of fecal coliform (Figure 24 and 25).
Figure 22. Fecal coliform counts in effluent input and MSL-treated effluent.
Figure 23. Removal of fecal coliform by the Leilehua and the Perlite MSL systems as affected by sucrose additions and different rates of aeration.
Figure 24. The relationship between different rates of aeration within sucrose application and percentage removal of fecal coliform by the Leilehua MSL system.

Figure 25. The relationship between different rates of aeration within sucrose application and percentage removal of fecal coliform by the Perlite MSL system.
3.6. Overall efficiency of the MSL systems (Leilehua and Perlite)

3.6.1. Inorganic N removal

The efficiency of the MSL systems in removing inorganic N from dairy effluent was not significantly different throughout the study (Year 2005 and 2006). Both the MSL systems, Leilehua and Perlite were similarly effective in removing inorganic N. However, the MSL systems were significantly different in removal of inorganic N over time in 2005. The rate of removal in effluent application decreased over time. We hypothesized the decrease might be due to inadequate aeration in the aerobic layer or decreased microorganism-available carbon in the anaerobic layer and tested this by adding supplemental aeration and sucrose (as carbon) in 2006. The systems were not significantly different in removal of inorganic N with supplemental aeration. However, the removal of inorganic N was significantly increased in both the MSL systems with the application of sucrose. The increased removal rate was likely due to the additional carbon provided by sucrose applications, which enhanced microbial activity and thus increased the denitrification in the MSL system. The hypothesized decrease in microorganism-available carbon in 2005 over time seems to be supported by the increase in removal of inorganic N by the MSL systems with sucrose applications. The removal of inorganic N was not significantly different with different rates of aeration in 2006. There were some pauses in the MSL systems in 2005 and sudden drops in removal of inorganic N seems to be due to these pauses.
3.6.2. Phosphate removal

The efficiency of the MSL systems in removing phosphate from dairy effluent was significantly different throughout the study (Year 2005 and 2006). The Leilehua MSL system was more effective in removing phosphate than the Perlite MSL system. This was probably because of the high sorption capacity of the Leilehua soil in the Leilehua MSL system which adsorbs and precipitates phosphate from the effluent. The removal of phosphate was significantly decreased over time by the Perlite MSL system in 2005. We hypothesized that the decrease in removal of phosphate by the Perlite system might be a result of decreased microorganism-available carbon in the anaerobic layer. Supplemental aeration and sucrose (as carbon) were applied in 2006 to increase the efficiency of the MSL systems in removing phosphate. The removal of phosphate was significantly increased with supplemental aeration by the Leilehua MSL system. This might be because sufficient aeration in the Leilehua MSL system oxidized ferrous iron to ferric iron in the aerobic layer, leading to higher adsorption of phosphate by the soil colloids. The sucrose application did not increase the already high removal of phosphate in the Leilehua MSL system. However, the percentage removal of phosphate was significantly increased with the application of sucrose in the Perlite MSL system. This might be because of the additional carbon provided by sucrose application which increased the activity of microorganisms resulting in more oxygen consumption and enhanced reducing conditions in the anaerobic layer moving iron into the aerobic layer where it could precipitate the phosphate in effluent. The hypothesized decrease in microorganism-available carbon in 2005 seems to be supported by the sharp increase in percentage removal of phosphate by the Perlite systems with sucrose applications. The
removal of phosphate was not significantly different with different rates of aeration by the MSL systems in 2006. The systems consistently removed phosphate with different rates of aeration. Thus from the results of supplemental aeration and sucrose applications it appears that the phosphate removal mechanism is likely different between the MSL systems. The removal of phosphate in the Leilehua MSL system was mainly due to sorption by iron in the aerobic layer, whereas in the Perlite MSL system it was due to the iron moving out of the anaerobic layer.

3.6.3. Organic matter removal

The efficiency of the MSL systems in removing organic matter (as measured by COD) from the dairy effluent was significantly different throughout the study (Year 2005 and 2006). The Perlite MSL system was more effective in removing organic matter (COD) than the Leilehua MSL system. This was probably because of the highly porous nature of the Perlite (86.96 %) in the Perlite MSL system compared to the Leilehua system (porosity of 66.32%), which increase O2 in the aerobic layer and assists microorganisms to decompose organic materials in the dairy effluent. The MSL systems significantly increased the removal of organic matter (COD) over time in 2005. The increase in removal rate was attributed to the quality of dairy effluent which contained a high concentration of organic matter during this period. The systems were not significantly different in removing organic matter (COD) with supplemental aeration in 2006. However, the removal of organic matter (COD) was significantly increased by both the MSL systems with the application of sucrose in 2006. The increased removal rate might be due to the addition of a highly available carbon source to the system, which
caused as an increase in microbial activity in the system and help to decompose the organic matter. The removal rate of organic matter (COD) was significantly increased by both the MSL systems in 2006 when different rates of aeration applied with a constant rate of sucrose. The increased organic matter (COD) removal during this period might be due to the need for additional aeration and carbon inside the system, which assists the microorganism to decompose more organic matter.

3.6.4. Fecal coliform removal

The efficiency of the MSL systems in removing fecal coliform from the dairy effluent was not significantly different throughout the study (Year 2005 and 2006). The MSL systems were similarly effective in removal of fecal coliform. However, the systems were significantly different in removal of fecal coliform over time in 2005. There were some pauses in the MSL systems in 2005 and the sudden drops in removal of fecal coliform seems to be due to these pauses. It also appears that the removal of fecal coliform decreased sharply by the Leilehua MSL system compared to the Perlite MSL system in 2005. This might be a result of additional fecal coliform in the Leilehua soil which is a tropical soil and studies show that tropical soil can multiply large numbers of fecal coliform (Hardina and Fujioka, 1995). The systems were not significantly different in removal of fecal coliform with aeration in 2006. However, the removal of fecal coliform was significantly increased in both the MSL systems with the application of sucrose in 2006. The increased removal rate might be due to the sucrose application, which enhanced the growth and activity of microbial population and the increased microbial population limited the survival of fecal coliform inside the system.
removal of fecal coliform was significantly increased with different rates of aeration by both the MSL systems in 2006. This might be because of the additional aeration which makes the system more aerobic and the aerobic condition helps with the decay of fecal coliform (Dewedar and Baghat, 1995).

3.7. Comparison of the MSL Systems

The removal rate of inorganic N, phosphate, and COD in our study for the Leilehua MSL systems was 22 – 93%, 64 – 99%, and 3 – 30% respectively, and that to Perlite MSL system was 21 – 96%, 9 – 97%, and 4 – 37%, respectively, during the first part of operation (May – Oct 2005). The study of total N, total P and COD removal in the MSL system in Matsue City, Japan (Wakatsuki et al., 1993) during the first year of operation were 88.9%, 87.5%, 72.5%, respectively. If we compare the first part of our study with the MSL system in Japan, the percentage removal of inorganic N and phosphate seem to be most likely similar, whereas the percentage removal of COD is higher in the MSL system in Japan. The increased COD removal might be due to the high concentration of organic matter in the wastewater they used for their study. Although the primary mechanism in removing N, P, and COD by the MSL systems in our study and MSL system in Japan was the same, the structure of the system, the materials used in the system and the application rate of wastewater were different. It's not possible to precisely compare the removal rates of N, P, and COD in our study with different MSL systems. In the second part of our study (Jan – July 2006), we applied aeration and sucrose (carbon source) to increase the efficiency of the MSL systems. The removal rate of inorganic N, phosphate, and COD in our study for the Leilehua MSL system was 8 – 61%, 42 – 99%,
and 6 – 32% respectively, and for the Perlite MSL system was 10 – 73%, 11 – 41%, and 16 – 36%, respectively, during the application of aeration. Luanmanee et al. (2001) investigated the different rates of aeration in the MSL systems in Thailand and found a high rate of aeration (20,000 L m\(^{-3}\) d\(^{-1}\)) for 3 days was more effective for enhancing COD, total N and soluble reactive P (SRP) removal at the rate of 73.3%, 15.0% and 74.7%, respectively. It seems the removal rate of N in our study is similar or greater than the study in Thailand. The removal rate of P in our study by the Leilehua MSL system is similar or greater than the study in Thailand, but the removal of COD in Thailand is greater than our study, which might be due to the high concentration of the organic matter in the wastewater they used for their system. The structure, materials, and the application rate of wastewater and aeration were different in our study from the study in Thailand. A precise comparison is not possible in this case. Overall it appears that the efficiency of the MSL systems in removing N and P in our study was similar to the study in Japan, and similar or greater than the study in Thailand. However, the removal of COD was low in our study, which might be due to the low concentration of the organic matter in dairy effluent.

3.8. Use of MSL-treated Effluent

The Hawaii State Department of Health has three different categories of recycled water – R-1, R-2, and R-3 water which are listed in Table 23 with specific criteria (Hawaii State Department of Health, 2002). R-1 is the highest quality recycled water. It has been filtered and disinfected. It can be used in any form of irrigation served by fixed irrigation systems supplied by buried piping for turf and landscape irrigation of golf
courses, parks, elementary schools, roadsides, and residential property where managed by an irrigation supervisor (Hawaii State Department of Health, 2002). R-2 is a slightly lower quality recycled water. It is secondary (biologically) treated wastewater that has also been filtered and disinfected (Hawaii State Department of Health, 2002). Its use requires more caution and restrictive controls than R-1 water. R-3 is the least pure class of recycled water. R-3 quality water is wastewater that has been treated to the secondary level. It can only be used for irrigation at places where people rarely go (Hawaii State Department of Health, 2002).

The average concentration of NO$_3^-$-N and phosphate, and fecal coliform colonies in MSL-treated effluent of our study is given in Table 24. If we compare our study with the recycled water requirements of State Department of Health in Hawaii, the MSL-treated effluent comes under R-3 water. MSL-treated effluent meets the criteria of nitrate and fecal coliform (May – July 2006) of R-2 water and approach the criteria for R-1 water. Improvements in efficiency of the type examined in this study are needed to meet the phosphate criteria. In addition a process, such as chlorination is needed to disinfect the treated effluent.
<table>
<thead>
<tr>
<th>Type of Recycled Water</th>
<th>Treatment</th>
<th>Recycled Water Quality</th>
<th>Recycled Water Monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>Oxidized$^{12}$</td>
<td>≤23 fecal coliform/100mL</td>
<td>Coliform – no more than one sample in any 30-day period</td>
</tr>
<tr>
<td></td>
<td>Filtered$^{88}$</td>
<td>Nitrate ≤10mg L$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disinfected$^{***}$</td>
<td>Total Phosphorus ≤ 1.0 mg L$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>R-2</td>
<td>Oxidized</td>
<td>≤200 fecal coliform/100mL</td>
<td>Coliform – no more than one sample in any 30-day period</td>
</tr>
<tr>
<td></td>
<td>Filtered</td>
<td>Nitrate ≤10mg L$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disinfected</td>
<td>Total Phosphorus ≤ 1.0 mg L$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>R-3</td>
<td>Oxidized</td>
<td>Secondary Undisinfected</td>
<td></td>
</tr>
</tbody>
</table>

$^{12}$ Wastewater in which the organic matter has been stabilized

$^{88}$ The passing of wastewater through natural undisturbed soils or filter media such as sand

$^{***}$ The destruction, inactivation, or removal of pathogenic microorganisms by chemical, physical, or biological means. Disinfection may be accomplished by chlorination, ozonisation, other chemical disinfectants, UV radiation, membrane processes, or other processes.
Table 24. Concentrations of MSL-treated effluent

<table>
<thead>
<tr>
<th></th>
<th>NO₃⁻ - N µg mL⁻¹</th>
<th>Phosphate ± SD</th>
<th>Fecal coliform cfu/100mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>May – Oct 2005†††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leilehua MSL system</td>
<td>2.15 ± 3.25</td>
<td>0.46 ± 0.56</td>
<td>658 ± 1321</td>
</tr>
<tr>
<td>Perlite MSL system</td>
<td>3.81 ± 5.35</td>
<td>2.16 ± 1.72</td>
<td>459 ± 674</td>
</tr>
<tr>
<td>May – Jul 2006†††</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leilehua MSL system</td>
<td>2.48 ± 2.68</td>
<td>2.83 ± 1.38</td>
<td>64 ± 95</td>
</tr>
<tr>
<td>Perlite MSL system</td>
<td>5.04 ± 9.24</td>
<td>5.25 ± 2.74</td>
<td>36 ± 53</td>
</tr>
</tbody>
</table>

††† First phase of data without aeration and sucrose addition (mean ± SD, n = 21)
††† Second phase of data with different rates of aeration and constant rate of sucrose, considered as the optimal management of the system (mean ± SD, n = 9)
CHAPTER 4

CONCLUSIONS

Dairy effluent disposal has been a serious problem in Hawaii and many Island dairies. Accumulating and storing dairy effluent in lagoons has been problematic for both economic and environmental reasons. The dairy effluent from overflowing lagoons can pollute surface, subsurface, and coastal water with high concentrations of N, P, organic matter and pathogens. Alternative methods are needed to decrease the excessive nutrients and pathogens from dairy effluent which would reduce the risk of environmental pollution and can be developed a more sustainable and environment-friendly dairy production system. The present study tested MSL systems as a strategy to manage and remove high concentrations of inorganic N, phosphate, organic matter (COD) and fecal coliform (pathogen indicator organisms) from dairy effluent.

Results of this study indicate that the removal percentages of inorganic N and fecal coliform were high and similar in both the MSL systems. The percentage removal of phosphate was high to very high in the Leilehua MSL system and it removed considerably more phosphate than the Perlite MSL system. Organic matter (COD) removal was reduced by both the MSL systems. The Perlite MSL system, however, removed more organic matter (reduced COD) compared to the Leilehua MSL system. Three possible improvements were tested to increase the efficiency of the MSL systems in the second phase of the study. The results indicated that supplemental aeration did not significantly improve the removal of inorganic N, organic matter (COD), and fecal
coliform. The removal of phosphate, however, increased in the Leilehua MSL system with additional aeration. Application of sucrose with constant aeration was crucial for removing inorganic N, phosphate, organic matter (COD), and fecal coliform. It appears that sucrose additions increased the microbial activity in the MSL systems which helped to increase the removal of inorganic N, phosphate, organic matter (COD), and fecal coliform. The increasing rates of supplemental aeration with constant sucrose addition further enhanced the removal of organic matter (COD), and fecal coliform.

The present research showed that both the MSL systems have the potential to remediate dairy effluent. Some adaptations, such as those proposed in this study would substantially increase removal efficiency and consistency. The installation of MSL systems is simple and basically requires only electricity, freshwater, a constant supply of effluent and a very small amount of land. The materials used in the system are inexpensive and easily obtainable. The MSL-treated effluent approaches R-1 water criteria, with improvements in pathogen and P removal still needed. A number of questions, however, remain unanswered: (i) How can we improve the removal of organic matter (COD)? (ii) How much carbon needs to be added while installing the MSL system? (iii) Does mixing the Leilehua soil and Perlite that used in the aerobic layers increased the efficiency of the MSL system? Further research in the MSL systems can provide the information to answer these important questions.
Recommendations

Several recommendations can be made for further work in development and improvement of MSL systems. A few of them are: (i) the MSL system should operate continuously without any interruption to avoid the pauses in effluent delivery (ii) further investigation is needed to determine optimal quantities of carbon to ensure reducing conditions in the anaerobic layer (iii) further investigation is needed to develop higher removal mechanisms of organic matter (as measured by COD) (iv) further study of different rates of aeration are needed to enhance the effectiveness of the MSL system and (v) since indicator organisms such as Enterococci, Clostridium, and FRNA coliphages are currently preferred for the state of Hawaii (R. Fujioka, personal communication, 2006), a study should be conducted to quantify the effect of MSL on Enterococci or Clostridium or FRNA coliphages.
Repeated measures code for inorganic N, phosphate, COD, and fecal coliform in 2005:

data one; set new.filename;

proc mixed;
   class trt rep time;
   model remd = trt time trt*time;
   repeated time/ type=ar(1) sub=rep(trt);
   estimate 'MSL system Leilehua' intercept 1 trt 1 0;
   estimate 'MSL system Perlite' intercept 1 trt 0 1;
run;

Repeated measures code for inorganic N, phosphate, COD, and fecal coliform in 2006:

Effect of Aeration

data one; set new.filename;
if date='04MAY2006'D then delete;

proc mixed;
   class trt aeration rep time;
   model remd = trt aeration trt*aeration;
   repeated time/ type=ar(1) sub=rep(trt);
   estimate 'MSL system Leilehua' intercept 1 trt 1 0;
   estimate 'MSL system Perlite' intercept 1 trt 0 1;
run;

Effect of Sucrose with Aeration

data one; set new.filename;

proc mixed;
   class trt sucrose rep time;
   model remd = trt sucrose trt*sucrose;
   repeated time/ type=ar(1) sub=rep(trt);
   estimate 'MSL system Leilehua' intercept 1 trt 1 0;
   estimate 'MSL system Perlite' intercept 1 trt 0 1;
run;
Effect of Different Aerations with Sucrose

data one; set new.filename;
if date <= '27APR2006'D then delete;

proc mixed;
  class trt aeration rep time;
  model remd = trt aeration trt*aeration;
  repeated time/ type=ar(1) sub=rep(trt);
  estimate 'MSL system Leilchua' intercept 1 trt 1 0;
  estimate 'MSL system Perlite' intercept 1 trt 0 1;
run;
REFERENCES


