Field Demonstration of the Performance of the L4DB® Microbial Treatment System to Reduce Phosphorus and Other Substances from Dairy Lagoon Effluent

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Texas Water Resources Institute
make every drop count

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EXECUTIVE SUMMARY

Two upper North Bosque River segments were designated as impaired in 1998 due to point source and nonpoint source (NPS) pollution of phosphorus (P) to these segments of the watershed. As a result, two Total Maximum Daily Loads (TMDLs) were applied which called for the reduction of annual loading and annual average soluble reactive P (SRP) concentrations by about 50%. Under Clean Water Act (Section 319(h)), a new technologies demonstration project was funded by the USEPA Region 6 and administered by the Texas State Soil and Water Conservation Board (TSSWCB) for reducing water pollution associated with dairy animal production systems. As part of this demonstration, the efficacy of a prospective new technology (i.e.L4DB® microbial treatment system) was evaluated, which may aid dairy farmers in reducing P from lagoon effluent. In many cases, this effluent is applied to waste application fields (WAFs) as irrigation water; thus reducing P in the effluent can have a direct impact on NPS pollution in the watershed.

Beginning in May 2006 a dairy's anaerobic lagoon was treated with L4DB® microbes at an average application rate of 65 gallons (246 L) of microbial solution/month for a period of 12 months. Lagoon samples were collected monthly or bi-monthly from two different profiles: lagoon supernatant (LS, sampled from top of the liquid level to 2ft (61 cm) depth) and lagoon profile (LP, sampled from the entire depth of the lagoon) using a sludge judge (a sampling tube with a check valve at the bottom to take lagoon sample at different depths). For

each LP and LS, 30 samples (3 samples per location × 10 locations) were collected during each sampling event. A set of 15 LP and 15 LS samples were mixed separately to get two composites of each for nutrients including P, solids, pH, conductivity and metals. In addition, 60 samples of lagoon effluent (hereafter IR) used to irrigate a nearby pasture were collected bi-monthly from a riser located just upstream from the big gun irrigation unit. Fifteen IR sub-samples were grouped together to get four IR composite samples. The IR composite samples were also analyzed for the above mentioned physical and chemical constituents.

L4DB® microbial treatment reduced average sludge depth by 24% as compared to its pre-treatment level (however, this reduction was 16%, when sludge measurement anomaly in August 2006 was excluded). The microbial treatment also reduced averaged total solids (TS) and total suspended solids (TSS) by 43 and 45%, respectively, for the LP, and 60 and 71%, respectively, for LS. Conversely, these values increased by 124% for IR effluent over times.

This microbial treatment system was effective in reducing average total phosphorus (TP) by 27 and 52% for the LP and LS, respectively, but not effective in reducing TP concentration for IR effluent. Overall, no clear soluble reactive phosphorus (SRP) reduction trends were observed for any sampling locations. Similar to the effect on TP, the L4DB® treatment was effective in reducing total Kjeldahl nitrogen (TKN) from the LP (36%) and LS (48%), but not effective in reducing potassium (K) for LP and LS. No clear trend of reducing these nutrients from IR effluent was observed over time.

This microbial treatment system was effective in reducing metals concentration as well. The average concentration reductions of Al, Ca, Cu, Fe, and Mn for LP were over 50%, while the reduction seen in Mg was only 22%. Similarly, the concentration reductions observed in LS samples were over 60% for the same suite of elements while Mg was reduced

by about 42%. No clear metal concentration reduction trends were observed for IR effluent. As a result, it can be inferred that most of these solids, nutrients, and metal reductions were likely due to microbial treatment, dilution of lagoon slurry by excessive rain and runoff as well as settling of dead and degraded bacterial mass accumulated at the bottom of lagoon. Additional measurements of lagoon sludge accumulation rate and constituents are warranted to assess possible increase in nutrients and solids due to accelerated solids settling and increased accumulation of microbial mass at the lagoon bottom.

INTRODUCTION

The bulk of the manure from animal feeding operations (AFOs) in the USA is applied to crop and pastureland. Although manure is an excellent resource for plant nutrients and soil conditioning, excessive land application rates and improper uses of manure can lead to environmental concerns. Manure phosphorus (P) that is not utilized by plants represents one of these concerns and can significantly impact surface water quality. Water quality degradation due to nonpoint source phosphorus (P) contribution from effluent and manure applied to waste application fields (WAFs) is a major concern in the Bosque River watershed. In 1998 two upper North Bosque River segments (Upper North Bosque River – Segment 1255; North Bosque River – Segment 1226) were designated as impaired segments on the Texas Clean Water Act, Section 303(d) list (TNRCC, 2001). This designation was the result of excessive nutrient loading and aquatic plant growth in those segments. The changes in the status of the Bosque River segments prompted the Texas Commission on Environmental Quality (TCEQ) to develop TMDLs that address P loading to the designated segments. In December of 2002, TCEQ approved the implementation plan for these TMDLs; these plans were also approved by the Texas State Soil and Water Conservation Board (TSSWCB) in January 2003. The TMDLs call for a reduction of the annual loading and annual average soluble reactive P (SRP) concentrations by about 50%.

The TCEQ has cited pollution from nonpoint source agricultural operations (by way of runoff) as the main source of contamination to these segments. As a result, reducing P from dairy effluent applied to WAFs is vital step in protecting the quality of these water bodies. Runoff from WAFs is not currently regulated because it is considered a nonpoint source, but it's impact on water bodies can be minimized by using on farm management practices to

reduce potential pollutants in dairy lagoon effluent applied to WAFs. There are currently a number of dairy operations in the watershed using best management practices (BMPs) to remove P and SRP from the wastewater; however, to meet the goals of the established TMDLs, new, more effective and efficient BMPs are needed. One prospective BMP is the use of L4DB® microbial treatment to remove P and other constituents from the effluent being stored and treated in dairy lagoons.

This report outlines the performance of a patented liquid-borne L4DB® microbial treatment (hereafter L4DB®) introduced by *Envirolink® LLC*, Greeley, Kansas. The demonstration evaluated under this project was set-up to treat a single cell anaerobic lagoon at a 300-head lactating cow free-stall dairy in the Bosque River watershed. Free-stall alleys were flushed 4 times per week and scraped in the remaining time. During each flushing, 10,000-12,000 gallons (37,854-45,425 L) of effluent was washed into the lagoon. As needed, this effluent was used to irrigate hay and cropland at the dairy using a big gun irrigation system.

L4DB® treatment system

According to Envirolink®, the patented liquid-borne L4DB® microbial treatment is derived from milk. Some of the physical and chemical properties of the L4DB® are listed in Table 1. Prior to its application to the lagoon, the L4DB® was thoroughly mixed and applied at an average rate of 65 gallons/month (246 L/month), which was predetermined by Envirolink® based on the lagoon size, depth of water and solids in the effluent; monthly L4DB® inputs are listed in Table 2. The L4DB® treatment was applied by spraying along the perimeter of lagoon while continuously agitating the liquid surface using a water sprinkler and lagoon effluent.

Table. 1 Properties of L4DB® used in this study

Product name	L4DB®
Manufacturer	Envirolink® LLC, KS
Active ingredient	Lactobacillus acidolphilus and lactobacillus gasseri
Boiling point	212°F (100°C)
Vapor pressure	Same as water (760 mg Hg at 100°C)
Specific gravity	1 (gravity of H_2O = 1 at 4°C)
Evaporation rate	Same as water
Solubility in water	Total soluble
Appearance and odor	Light tan/ slight odor
Flash point	None
Health hazard	None
Toxicity	None
pH	7.0

Source: MSDS, US Department of labor (provided by Envirolink®) and technology provider

Table 2. Lagoon treatment date and L4DB® treatment application rate

Lagoon treatment date	Application rate, gallons(liters)
5/22/06	100 (378)
06/02/06	50 (189)
07/01/06	50 (189)
08/02/06	50 (189)
09/03/06	50 (189)
10/02/06	50 (189)
11/03/06	50 (189)
12/01/06	75 (284)
01/02/07	100 (378)
02/03/75	75 (284)
03/01/07	75 (284)
04/02/07	50 (189)
05/05/07	75 (284)

As shown in Table 2, the L4DB® application rate was adjusted from time to time based on the ambient temperature and amount of precipitation since the last treatment. In colder months application rate was higher than in a warmer month; this is done to offset lower microbial activity during cooler temperatures. Similarly, when monthly precipitation was greater, application rate was reduced slightly due to increased dissolved oxygen in the lagoon from rain water.

Additionally, two large tanks (volume of liquid in T1 and T2 was 539 gal (2,040 L) and 528 gal (1,998 L), respectively) were filled with untreated flushed manure to assess the L4DB® treatment effect on flushed manure from the free-stall (Fig. 1). Tank T1 was used as the control (no treatment was applied) and T2 was treated with L4DB® at a rate of 1 gal/month (3.78 L/month).
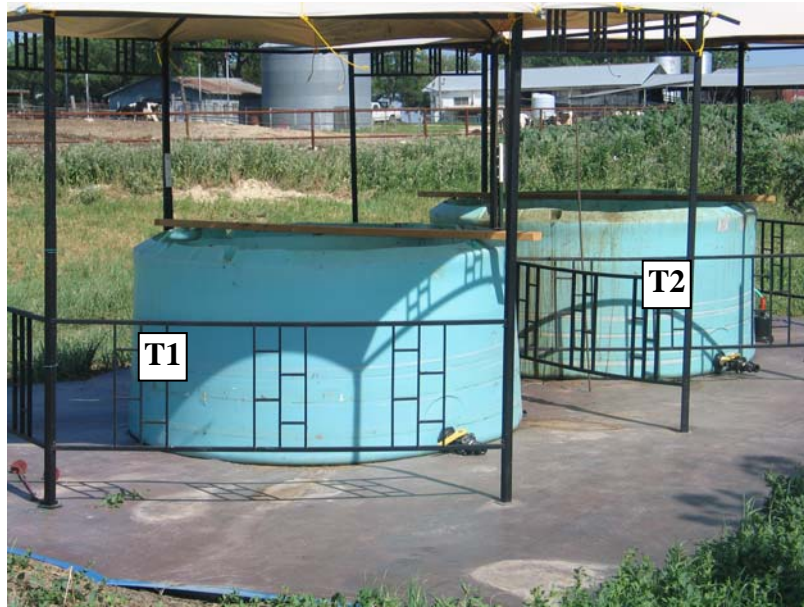
Fig.1. Tanks T1 (control) and T2 (treated) used in this study

METHODS

Layout of sampling scheme

Prior to sampling, the lagoon was divided into three roughly equal sections by transect lines running the width and length of the lagoon (Figs. 2). The location of each transect was marked permanently using a steel post (Fig. 2a) and each intersection was noted as sampling location 1 through 9 (Fig. 2b). In addition, the 10th sampling location was chosen near the irrigation pump (Fig. 2b).

(a)
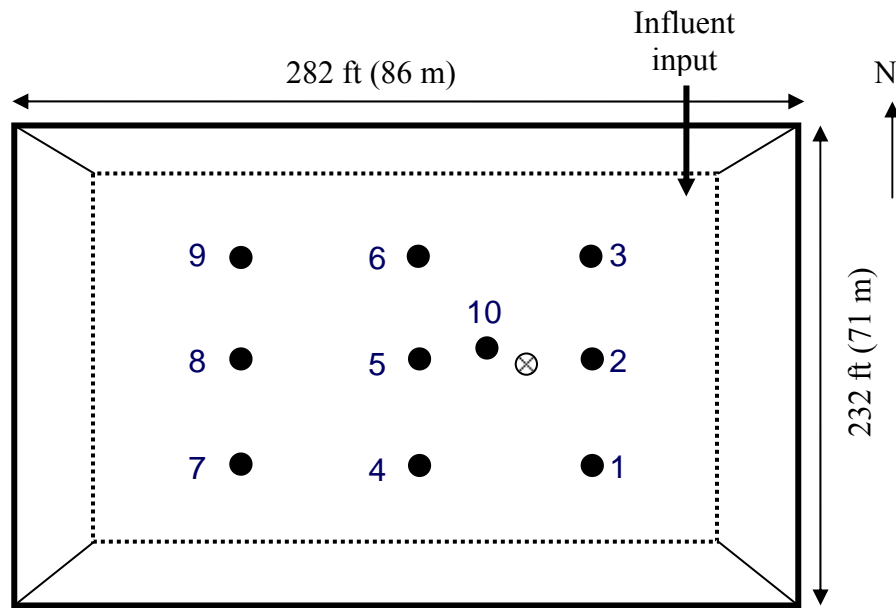
(b)

Fig. 2. a) Transect line running the width and length of the lagoon along with sampling location, and b) schematic of lagoon sampling layout. ● Indicates lagoon sampling locations and sludge depth measurement locations; ⊗ indicates irrigation pump locations (not to scale)

At each location three lagoon supernatant (from top of the liquid level to two ft (61 cm) depth, hereafter, LS) and lagoon profile (from the entire depth of the lagoon, hereafter, LP) samples were taken (Fig. 3) for analysis. Summary of sampling events is listed in Table 3.
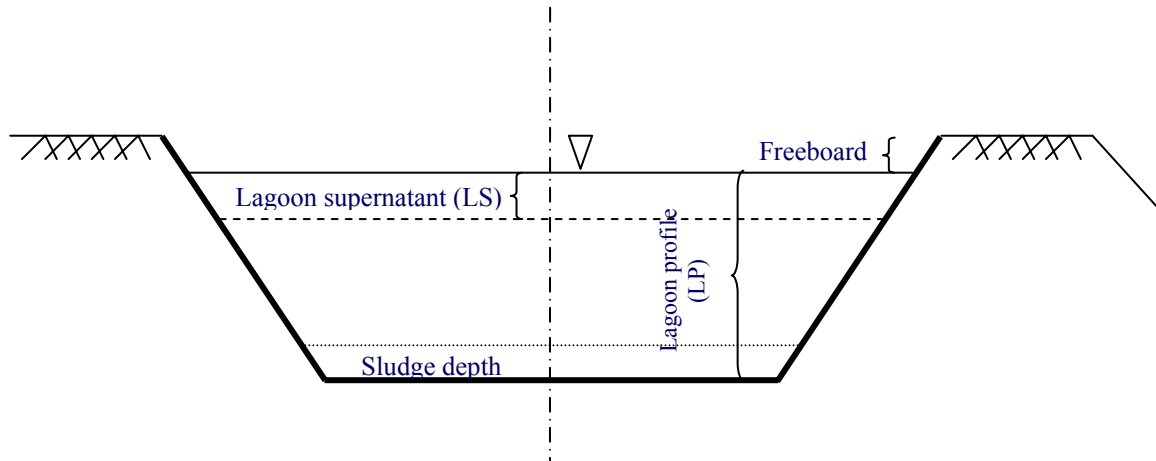

Fig. 3. Schematic of lagoon and sampling profile (not to scale)

Table. 3. Sampling events

Component/Date	May, 06	June, 06	July, 06	Aug, 06	Oct, 06	Dec, 06	Feb, 07	Mar, 07	May, 07	June, 07
Irrigation effluent (IR**)	√		√		√	√	√			√
Lagoon profile (LP)	√	√	√	√	√	√	√			√
Lagoon supernatant (LS)	√	√	√	√	√	√	√			√
Tank supernatant (TS)	√	√	√	√*	√	√		√*	√	
Tank profile (TP)	√	√	√	√	√	√		√	√	

* Tanks were re-filled and pre-treatment samples were collected
** Irrigation effluent was not sampled during every sampling event due to sampling and analysis cost constraints

Two composite samples each for tank supernatant (from top of the liquid level to 1 ft (30 cm) depth, hereafter, TS) and tank profile (from the entire depth of the tank, hereafter, TP)

samples were taken diagonally from 15 locations, respectively, for each tank during each sampling event (Fig. 4). Due to high evaporation losses from the tanks, they were both emptied and refilled twice with flushed manure during the course of this monitoring study. In phase 1 (hereafter P1), the tanks were filled in May 2006 and sampled in May, June and July 2006. During phase 2 (hereafter P2), both tanks were emptied and refilled in August and sampled in August, October and December 2006. No tank samples were taken in February 2007 due to presence of thick crust on the surface of manure in tanks as well as insufficient tank water depth for TS and TP samples. Low tank volumes were replenished in March 2007 (hereafter, P3) and sampled in March and May 2007. Due to intermittent sampling, tank parameters were evaluated and compared within each phase instead of comparing among phases.
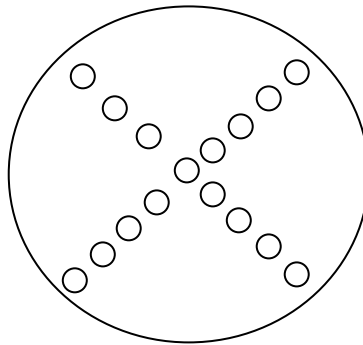
Fig. 4. Approximate tank sampling locations

As listed in Table 2, lagoon effluent (hereafter, IR) irrigated to nearby pasture land (Coastal Bermuda grass) was collected bi-monthly from a riser located just upstream from the big gun irrigation system. The big gun irrigation used a 20 HP centrifugal pump and a 4 inch (10 cm) dia hose for effluent irrigation. Irrigation samples were collected every three minutes for three hours yielding a total of 60 samples. Sample preparation and analysis for IR samples

have been discussed in the sample preparation and analysis section. For December 2006 sampling, the irrigation pump was moved from its original location (Fig. 2b) for repairs and installed close to the shore of the lagoon. Once repaired, the pump was moved back to its original location and stayed there for the remainder of the project. During IR sampling, flow rate was monitored using a Greyline PDFM 4 Doppler flow meter (Massena, NY). Flow rates were recorded on three minute intervals and ranged from 136-185 gpm (515-700 lpm) during sampling events. At these rates, a total of 24,391 gallons (92,330 L) to 35,043 gallons (132,651 L) IR effluent was pumped during that time.

Sludge depth (SD) measurement

Typically, reduction of TSS in lagoon supernatant is accompanied by reduction of P, and a potential change in sludge depth. Therefore, accurate tracking of sludge depth is important to evaluate the performance of L4DB® treatment effectively. During each sampling event, total depth (TD) and the depth above dense sludge (DADS) for the lagoon and tanks were measured using a graduated plastic conduit fitted with an end cap (Fig. 5). All depth measurements in the lagoon were taken at the same location as liquid samples were collected. Sludge depth (SD) of lagoon and tanks was estimated by subtracting the DADS from the TD of the lagoon and tanks, respectively.

Lagoon, tank and irrigation effluent sample collection

In order to ensure consistent sampling and monitoring, lagoon sampling locations and the sampling profile were predetermined (Figs. 2 & 3). At each lagoon sampling location, 3 LS and 3 LP samples were taken in 250 ml bottles. Samples were collected using the "Ultra Sludge Judge" (Nasco, Fort Atkinson, WI), which consisted of three 5 ft (1.52 m) sections of 1.25 inch (3 cm) diameter acrylic tube and a ball check valve at the bottom end (Fig. 6).
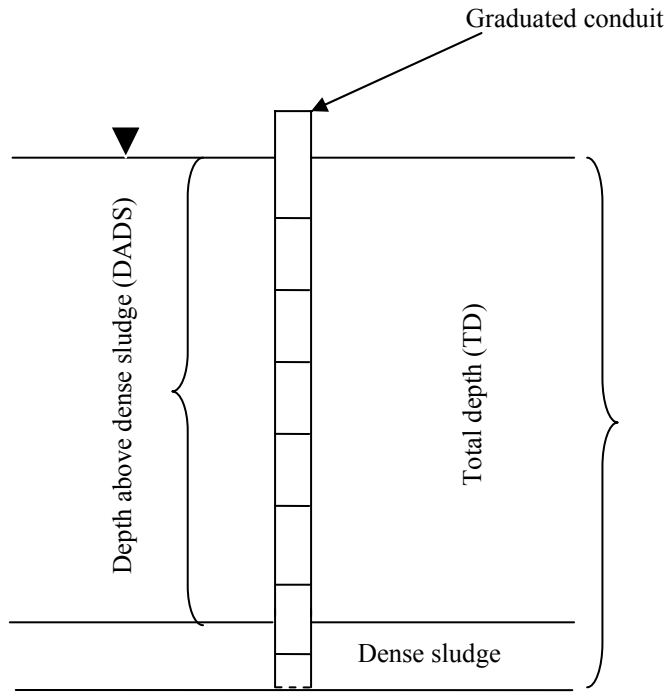
(a)

(b)

Fig. 5. a) Schematic of lagoon depth measurement, b) actual depth measurement

For LS sampling, the sampler was lowered slowly to the desired depth (2 ft, or 61 cm), while for LP sampling, the sampler was lowered slowly until it rested above the dense sludge at the bottom of lagoon. After lowering the tube at desired depth, it was gently pulled out of the lagoon as straight as possible. A total of 30 LS (3 samples per location × 10 locations) and 30 LP (3 samples per location × 10 locations) samples were collected from lagoon during each sampling event. Sample preparation and analysis for LS and LP will be discussed in the following section.

Fig. 6. Lagoon sampling using a sludge judge

Following the same sampling procedures used in the lagoon, 15 TS and 15 TP samples were collected from each tank using a sludge judge (Fig. 7). Thus, total 60 (15 samples per tank × 2 tanks × 2 profiles) samples were collected from two tanks during each sampling event.

Fig. 7. Tank supernatant sampling using a sludge judge

In addition, samples of lagoon effluent (IR) used to irrigate a nearby pasture were collected bi-monthly from a riser located between the irrigation pump and a big gun irrigation system (Fig. 8). Samples were collected every three minutes for 3 hours yielding a total of 60 IR samples were collected during each sampling event.

Fig. 8. Sampling of irrigation effluent (IR) from a riser

Within an hour of sampling, bottles kept on ice were transported to the Texas Institute for Applied Environmental Research (TIAER) laboratory, at Tarleton State University in Stephenville, Texas, for physiochemical parameter (i.e., nutrients, solids, metals, pH and conductivity) analysis.

Sample preparation and analysis

After each sampling event, 15 LS samples were mixed together to obtain one LS composite sample. Similarly, 15 LP samples were mixed together to obtain one LP composite sample. In this way, two LS and two LP composite samples (LS1 & LP1 composited samples from locations 1 through 5 and LS2 & LP2 composited samples from locations 6 through 10) were prepared for analysis. Similarly, each set of 15 TS and 15 TP samples were mixed separately to get two TS (T1S and T2S) and two TP (T1P and T2P) composite samples of each for analysis. Also, 15 IR sub-samples were mixed separately to get one IR composite sample. In this way, four IR (hereafter IR1, IR2, IR3, and IR4) samples were prepared for subsequent analysis from each sampling event

Using EPA laboratory procedures (Budde, 1995) and Standard methods (APHA, 2005) (Table 4) all composited samples were analyzed for: Total Solids (TS), Total Volatile Solids (TVS), Total Fixed Solids (TFS), Total Suspended Solids (TSS), Soluble Reactive Phosphorus (SRP), Total Phosphorus (TP), Nitrate/Nitrite-Nitrogen (NNN), Total Kjeldahl Nitrogen (TKN), Potassium (K), Aluminum (Al), Calcium (Ca), Magnesium (Mg), Sodium (Na), Manganese (Mn), Iron (Fe), and Copper (Cu). Concentrations of Total Dissolved Solids (TDS) were found by subtracting the concentrations of TSS from TS. Also pH and conductivity were measured for each composite sample.

Table 4. Laboratory analytical methods

Parameter	Method	Equipment Used
Nitrite+Nitrate Nitrogen	EPA 353.2 and SSSA 38-1148	Perstorp® or Lachat® QuickChem Autoanalyzer
Total Kjeldahl Nitrogen	EPA 353.2, modified	Perstorp® or Lachat® QuickChem Autoanalyzer
Potassium	EPA 200.7	Spectro ® ICP
Calcium	EPA 200.7	Spectro ® ICP
Magnesium	EPA 200.7	Spectro ® ICP
Sodium	EPA 200.7	Spectro ® ICP
Manganese	EPA 200.7	Spectro ® ICP
Iron	EPA 200.7	Spectro ® ICP
Copper	EPA 200.7	Spectro ® ICP
Orthophosphate Phosphorus	EPA 365.2	Beckman® DU 640 Spectrophotometer
Total Phosphorus	EPA 365.4, modified	Perstorp® or Lachat® QuickChem Autoanalyzer
Total Suspended Solids	EPA 160.2	Sartorius® AC210P or Mettler® AT261 analytical balance, oven
Total Solids	SM 2540C	Sartorius® AC210P or Mettler® AT261 analytical balance, oven
Total Volatile Solids	SM 2450G	Sartorius® AC210P or Mettler® AT261 analytical balance, oven, muffle furnace
Total Volatile Solids	EPA 160.4	Sartorius® AC210P or Mettler® AT261 analytical balance, oven, muffle furnace
Potential Hydrogen	EPA 150.1 and EPA 9045A	Accument® AB15 Plus pH meter
Conductivity	EPA 120.1 and EPA 9050A	YSI® 3200 conductivity meter
Aluminum	EPA 200.7	Spectro ® ICP

  EPA = Methods for Chemical Analysis of Water and Wastes, March 1983 and version 2, June 1999.
  There is no difference between EPA methods 200.7 and 6010B. Method 200.7 is a newer version and will yield the same results.

Statistical analysis

Analysis of variance (ANOVA) was performed to examine the treatment effects on lagoon slurry and irrigated effluent solids, nutrients and metals at different sampling profiles (LP, LS, and IR) using a general linear model in SAS. The differences among mean groups were compared using the Duncan's multiple range tests (Steel & Torrie, 1997) at a significance level *P* of *0.05*.

RESULTS AND DISCUSSION

Average daily ambient temperature and evapotranspiration (ET) data of the Stephenville area (about 12 miles or 20 km from the dairy) was used to assess environmental conditions during the monitoring period. Total monthly precipitation data for the dairy was provided by the producer.

During the monitoring, period tank evaporation losses were not compensated by addition of lagoon slurry; therefore, it was difficult to maintain a consistent TS and TP sampling depth in tanks between scheduled tank effluent sampling events. As a result, both tanks were re-filled twice during the monitoring period (Table 2); pre-treatment and treated tank slurry samples were taken during each sampling event. Refilling the tanks with flushed manure led to substantial variations in tank constituents; therefore, tank effluent physiochemical characteristics were compared for the period between each tank filling sampling event rather than among refilling of tanks. No clear and consistent trends for solids, nutrients and metals were observed in tank effluent samples. Consequently, tank data and physiochemical characteristics were not a true representation of lagoon environmental

conditions and sampling replication due to extreme outdoor environmental conditions were not included in this report.

Environmental conditions

Monthly precipitation and evapotranspiration (ET) are presented in Fig. 9, and daily ambient temperatures are presented in Fig. 10. It is evident from Figs. 9 and 10 that although there was no precipitation recorded in September 2006, June-August of 2006 were the warmest and driest months. During this period, the study area received low amounts of precipitation and had the greatest ET losses. Conversely, in May 2007, the study area had the highest precipitation with only moderate ET losses. Average ambient temperature for July and August 2006 were 83.2 (±3.5 °F) and 85.5°F (± 4.2 °F), respectively, while the lowest average temperature occurred in January 2007 and measured 38.6 °F (± 8.67 °F).
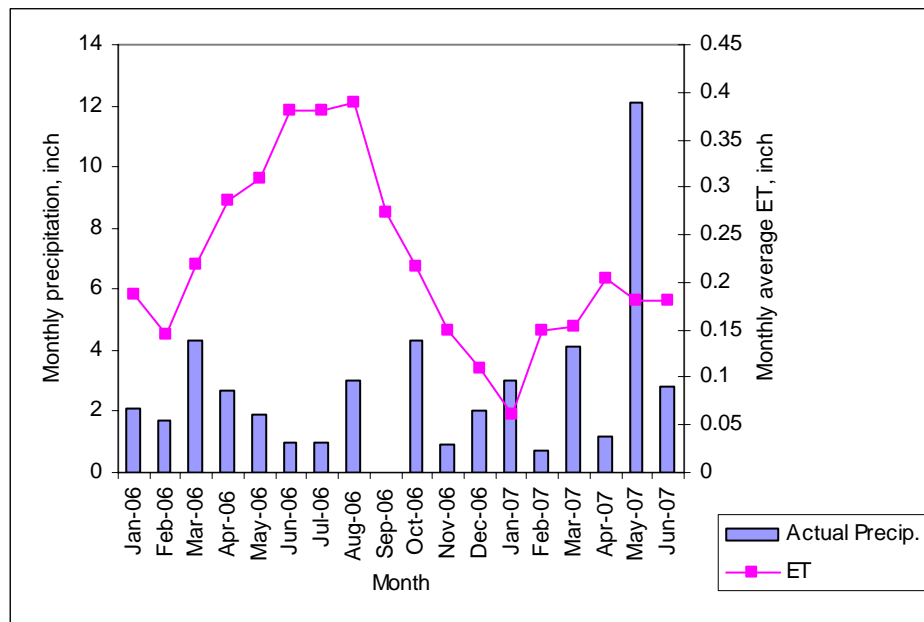

Fig. 9. Precipitation and evapotranspiration (ET) trend in the study area (Note: ET values were taken from the nearest weather station in Stephenville, TX)
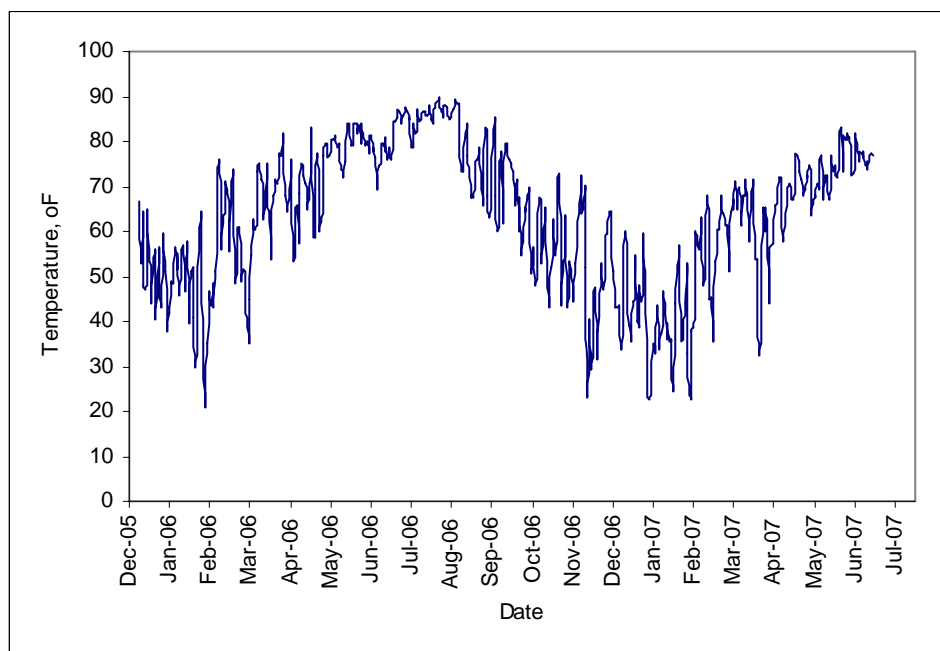
Fig. 10. Daily mean ambient temperature for the study area (Note: ambient temperature was taken from the nearest weather station in Stephenville, TX)

Lagoon Performance

Sludge depth

Average TD and SD in the lagoon during each sampling event are shown in Fig. 11. TD fluctuation was likely due to variations in precipitation, volume of effluent used for irrigation, and ET during monitoring while the variation in DASD was likely due to variation of settling and re-suspension of solids from microbial activities (Fig. 12). Following the first treatment in May 2006, the sludge depth decreased by as much as 21% until July 2006; however, in August 2007, an anomaly was observed (Fig. 11) where the SD decreased sharply by 69%. This drastic decrease in SD was likely due to depth measurement errors. Thereafter, lagoon depths fluctuated at the end of the demonstration, but SD remained lower than the pre-treatment sludge depth (Fig. 11). The likely cause of this reduction is that microbes obtained energy by consuming organic matter, which resulted in reduced solids and eventually reduced

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SD. Since sludge accumulation is composed of TFS and slowly degradable volatile solids (Chastain et al., 2001), variations in SD are likely due to variation in these solids for this lagoon. In addition, high variability in sludge depth was also likely due to re-suspension of sludge from microbial activities as well as by wind-driven turbulence and gas lift (Reed et al., 1995), annual cycle of storage, heating and organic matter accumulation (Hamilton et al., 2006; Westerman et al., 2006). Overall, L4DB® treatment was effective in reducing sludge depth by 24% (however, this reduction was 16%, when measurement anomaly in August 2006 was excluded) to its pre-treatment level. This reduction of SD due to microbial treatment is likely to improve lagoon effluent characteristics, increase lagoon capacity and reduce maintenance cost for this lagoon. Average SD for this lagoon was 34% of the TD. Greater sludge depth means higher loading rate which is associated with higher TSS, TVS, TKN, as well as conductivity of the lagoon (Sukias et al., 2001). Overall TD, DADS, and SD for this lagoon during the monitoring period were 10.75 ft (±1.2), 7.11 ft (±1.06), and 3.64 ft (±0.098), respectively.

Further analysis of sampling locations revealed that in a given sampling event no significant differences in TD were observed among locations except for sites L1 and L4 (Fig. 2). Significant differences in DADS and SD measurement were observed among locations, despite measuring these depths at nearly the same locations during all sampling events (Fig. 12). The overall large variation of SD measurement among locations indicates the difficulties in measuring sludge accumulation in the lagoon.
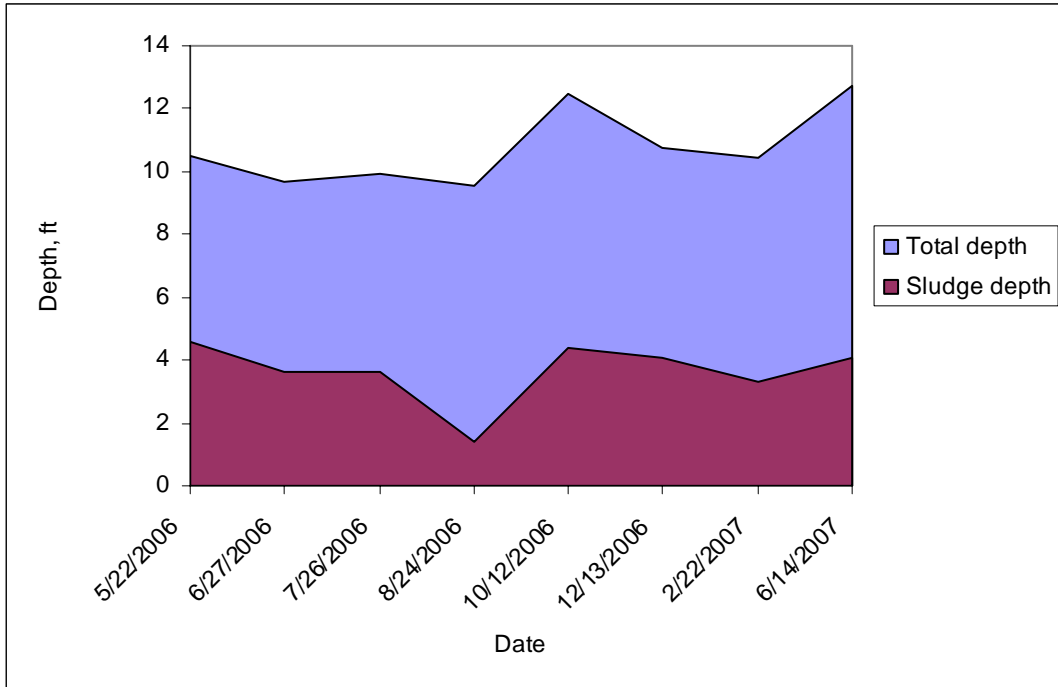
Fig. 11. Total and sludge depths of the lagoon (Note: May 2006 sampling is the pretreatment depth)
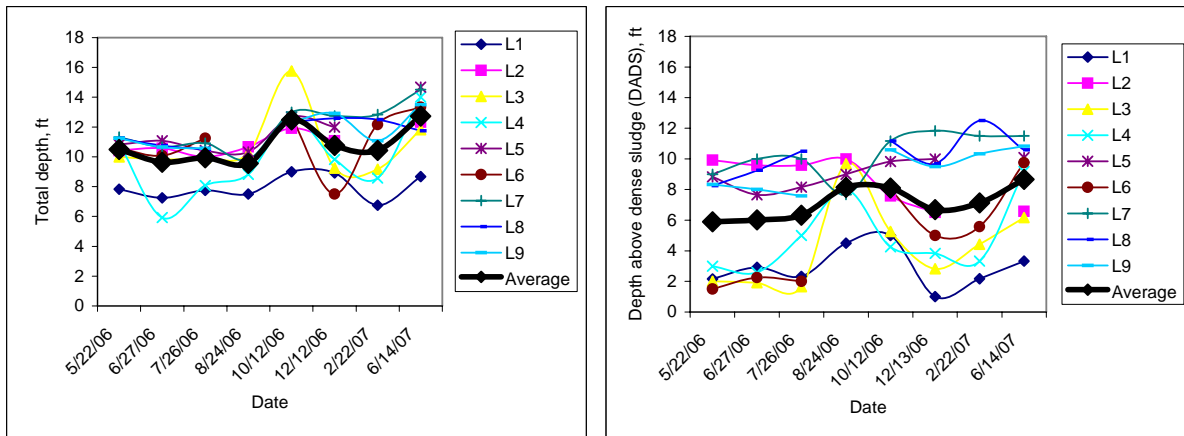
Fig. 12. Total depth (TD) and depth above dense sludge (DADS) at different sampling locations of the lagoon (Note: May 2006 sampling is the pretreatment depth)

Physicochemical characteristics of lagoon

In this section, physicochemical parameters (solids, nutrients and metals) analyzed for LP, LS and IR samples (untreated and treated with bacteria) have been compared among sampling events as well averaged over sampling events. During the monitoring period, lagoon water volume varied considerably (Fig. 13) due to above average natural precipitation, runoff to the lagoon and effluent pumping for irrigation use. To demonstrate the effect of increased lagoon liquid volume due to excessive rains (potentially diluting lagoon slurry), a few results (i.e., TS and TP concentration) are also reported in this section to show treatment and dilution effects.
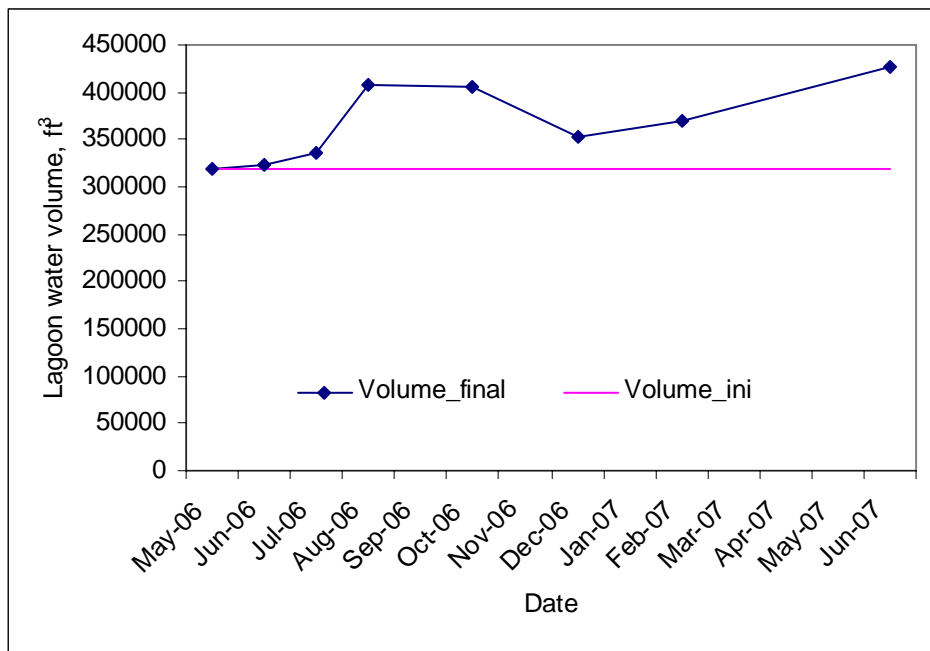
Fig. 13. Lagoon volume changes over time. Volume_ini: Initial volume; Volume_final: Final volume of the lagoon (Note: May 2006 is the initial lagoon depth measurement)

During one of the IR sampling events, four additional irrigation samples (IR_field) were collected using a freezer bag placed inside a coffee can to check whether effluent being

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applied to the land had the same chemical make-up as the effluent sampled from the riser. Coffee cans were placed at four random locations within the irrigated area. The IR_field samples were analyzed individually for TS, TSS, TVS, SRP, TP and TKN and were compared with IR samples collected simultaneously for the same sampling event. Results suggested that, except SRP, IR_field showed higher concentrations than all other measured parameters compared to IR effluent samples (Table 5). These differences between IR effluent and IR_field were likely due to foaming that occurred during IR sampling through the riser. As a result, TS, TSS, TVS, SRP, TP and TKN for IR were reported as corrected values whereas the values of other parameters for IR were not corrected since they were not analyzed for IR_field samples.

Table. 5. Comparison of selected parameters in IR effluent, lagoon grab samples at different depths and IR samples collected from the field (IR_field)

Parameter	IR[1]	IR_field[1]
TP (mg/L)	67.6b±4.7	76.3a±0.6
SRP (mg/L)	14.9a±0.7	7.1b±0.6
TS (%)	0.46b±0.005	0.52a±0.004
TSS (%)	0.06b±0.008	0.10a±0.01
TVS (%)	0.19b±0.006	0.23a±0.002
TDS (%)	0.39b±0.005	0.42a±0.006
TFS (%)	0.27b±0.004	0.29a±0.003
TKN (mg/L)	481.5a±22.0	503.0a±14.1

[*]Averages within a row followed by different letters are significantly different at $P \leq 0.05$ according to Duncan multiple range tests.
[1] Both IR and IR_field samples were collected on the same day simultaneously (6/14/07)

pH

Lagoon profile (LP) samples showed significantly lower pH than the IR, whereas no significant differences in pH were observed between LS and IR and LP and LS. IR had

slightly higher pH as compared to LS and LS had slightly higher pH than the LP. Similarly, significant differences in pH were observed among sampling events and pH trends in LP, LS and IR were presented in Fig. 14. Average pH for LP, LS and IR were 7.46 (±0.14), 7.55 (±0.17), and 7.57 (±0.12), respectively, indicating that this microbial treatment slightly increases pH in the LS and IR in this lagoon.
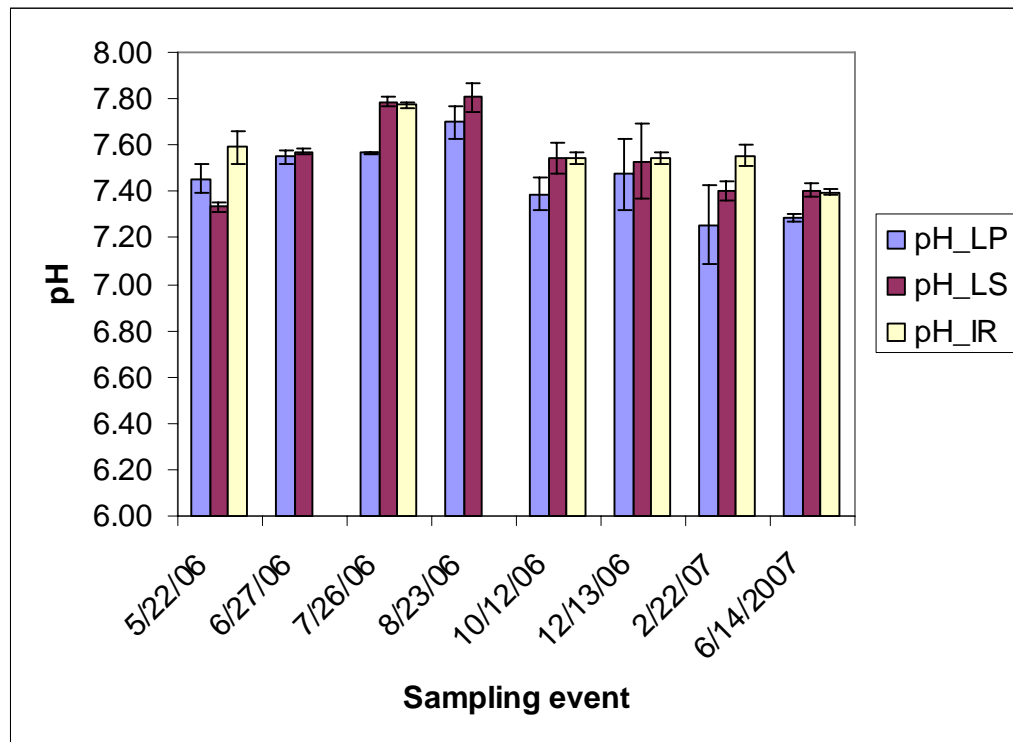
Fig. 14. Average pH trends over time for the L4DB® treatment. LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May 2006 sampling is the pretreatment sampling)

Although pre-treatment pH for the LP was slightly higher than LS, the pH of LS increased slightly following microbial treatment and remained relatively higher until the end of monitoring. Conversely, pH for IR was slightly higher than that of LP and LS and maintained the same trend until the end of the demonstration. Higher pH for the LS and IR

was likely due to lesser amount of organic matter in IR and LS samples as compared to LP. All pH values as received from TIAER are listed in tables I through III in Appendix A.

Solids

Average TS concentrations during each sampling event are shown in Fig. 15a and overall concentration of TS in LP, LS, and IR are listed in Table 6. All solids concentrations as received from TIAER lab are listed in tables IV through VI in Appendix A. TS concentration in LS decreased following first microbial treatment in May 2006 and continued to decrease throughout the monitoring period with a small amount of fluctuation at the end of the demonstration (Fig. 15a). Overall reduction of TS in LS was 60% while the TS concentration for LP did not show significant reduction until August 2006 after the third treatment had been applied; at this point, TS concentration of LP was reduced by 56%. Throughout the course of the demonstration, the overall reduction of TS in the LP was 43%. The higher TS reduction for LP and LS were observed when temperatures were favorable to microbial activities.
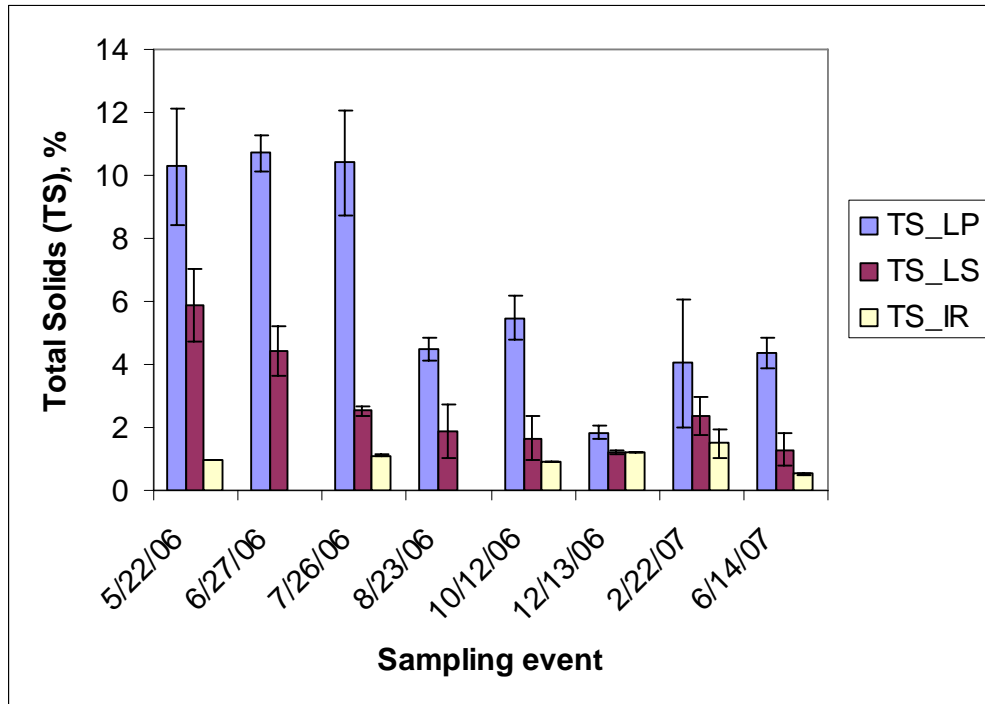
(a)
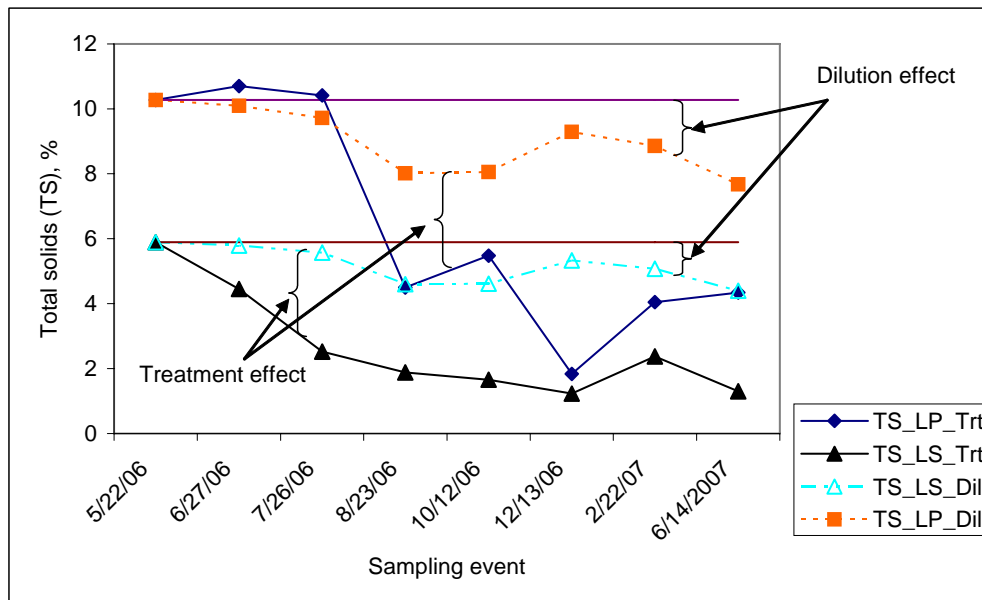
(b)

Fig. 15. L4DB® treatment effects on: a) Total solids (TS) and b) dilution and treatment effect on TS. LP: liquid profile, LS: Liquid supernatant; Trt: Treatment, Dil: Dilution (Note: May 2006 sampling is the pretreatment sampling)

Table 6. Average TS, TSS, TDS, TVS and TFS for lagoon and irrigated effluent samples averaged over sampling events

Parameter[1]	Sampling location		
	LP	LS	IR
Total solids (TS)	6.45a[*] ±3.47	2.66b±1.70	1.04c±0.34
Total suspended solids (TSS)	5.33a±3.64	1.93b±1.77	0.35c±0.42
Total dissolved solids (TDS)	1.30a±1.28	0.85ab±0.38	0.70b±0.17
Total volatile solids (TVS)	3.13a±1.41	1.49b±0.95	0.52c±0.24
Total fixed solids (TFS)	3.32a±2.14	1.16b±0.77	0.49b±0.12

[*] Averages within a row followed by different letters are significantly different at $P \leq 0.05$ according to Duncan multiple range tests.
[1] parameter is in %

The majority of TS concentration reduction in LP and LS samples occurred when temperatures were favorable for microbial activity. As a result, the reduction of TS may be caused by an increase in biological uptake. Conversely, average TS for IR showed a slight increase (21%) as compared to its pre-treatment concentration (Fig. 15a), which could not be explained.

To explore further whether this reduction of solids for LP and LS was likely due to treatment or dilution effect, lagoon water volume changes were taken into account and TS values were adjusted. As seen in Fig. 15b, changes in lagoon water volume can reduce TS concentrations considerably as compared to pretreatment TS concentration due to a dilution effect (TS concentration differences between pretreatment and adjusted for dilution). On the other hand, TS for LP increased slightly following microbial treatment until July 2006 (third treatment) (Fig. 15b). This was likely due to re-suspension of solids resulting from microbial biodegradation of sludge; this phenomenon has also been observed by other researchers (Converse and Karthikeyan, 2004). After July 2006, measured TS levels were always significantly lower than the adjusted TS for dilution. Hence, the differences between the

measured and adjusted for dilution TS concentrations were likely due to microbial digestion of solids, as well as solids settling at the bottom of the lagoon. In addition, volatile losses of solids due to microbial activities might also contribute to reduction of TS from LP as indicated by Zhu et al. (2000).

Overall, average TS for LP, LS and IR (Table 6) were greater than TS concentration observed by Mukhtar et al. (2004), Barker et al. (2001; cited in Mukhtar et al., 2004), and Converse and Karthikeyan (2004). Solids concentration for LS were also higher than the typical 1% found in the supernatant of most anaerobic dairy lagoons suggesting that this lagoon had a much higher solids loading than other lagoons. This could contribute to greater sludge accumulation if this lagoon is not managed properly.

Total suspended solids (TSS) for LP, LS and IR followed a trend similar to TS concentration for these sampling locations. Average TSS concentration for each sampling event and overall concentration averaged for all sampling events are presented in Fig. 16 & Table 6, respectively. The TSS concentration for LP did not show significant reduction following treatment until August 2006 (third treatment) (Fig. 16), when TSS concentration for LP was reduced by 59%. Overall, the reduction of TSS for the LP was 45%. In LS samples, TSS concentration reduced gradually throughout the treatment with the highest reduction occurring in June 2007 (94%); the overall TSS reduction for LS was 71%. TSS concentration for IR increased significantly (123%) as compared to pre-treatment concentration (Fig. 16). For this lagoon, TSS exhibited 83 and 73% of the TS for LP and LS, respectively, while the overall TSS was 63% of TS. Therefore, most TS reductions for LP and LS in this lagoon were apparently reductions of TSS indicating that the treatment system was effective in reducing TSS significantly for LP and LS, but not IR effluent.

As expected, TS and TSS concentrations of LP were significantly greater than those of LS and IR (Table 6). Averaged TSS for the LP was higher than the LS since suspended solids degrade slowly and remain suspended in the entire LP. In addition, accumulated dead and degraded bacterial mass at the bottom of lagoon might also contribute to increased solids content for LP.
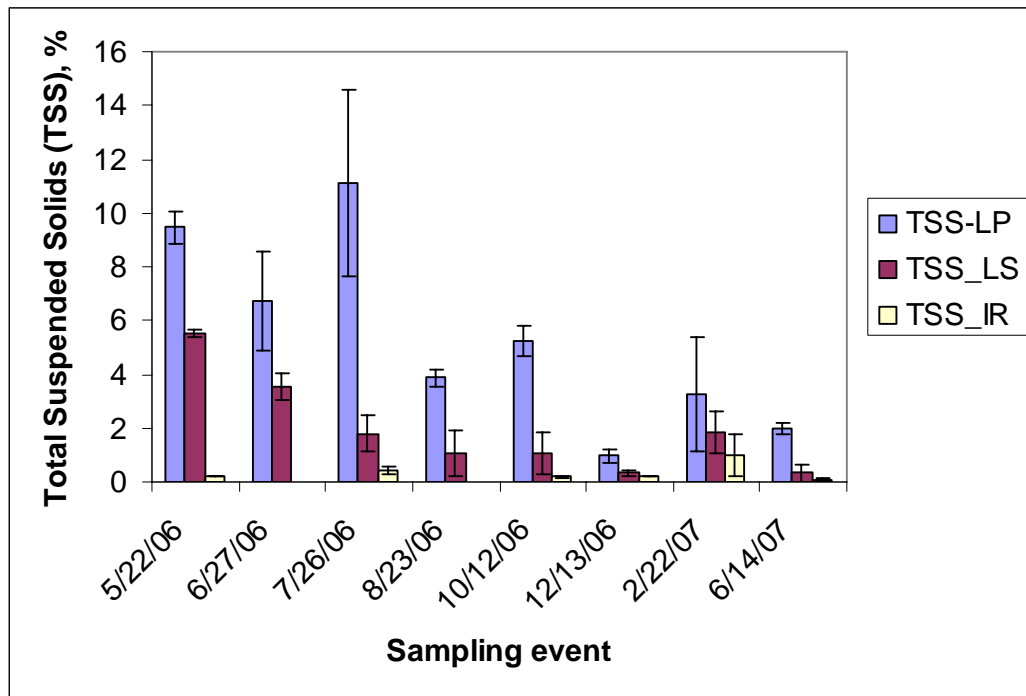
Fig. 16. L4DB® treatment effects on: a) Total suspended solids (TSS). LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May 2006 sampling is the pretreatment sampling)

Total dissolved solids (TDS) are easily degradable organic matter and a measure of total materials that are dissolved in water. Following microbial treatment of the lagoon, TDS concentration for LS decreased slightly until October 2006 and fluctuated slightly near the end of the demonstration (Fig. 17); overall, TDS in LS samples decreased by 44%. Conversely, following the first treatment TDS for LP increased significantly in June 2006 (280%). This

drastic increase in TDS for the LP was likely due to rapid conversion of suspended solids into dissolved solids by the microbes following the first treatment in the lagoon (Zhu et al., 2000). Thereafter significant TDS reductions were observed in LP until October 2006 (75%), but following October sampling, TDS fluctuated and its concentration increased by 125% in June 2007 from its pre-treatment (Fig. 17). Overall, TDS increased by 28% to its pre-treatment level for LP, however excluding June 2006 and 2007 sampling events TDS decreased by 42% from its pre-treatment in LP profile.
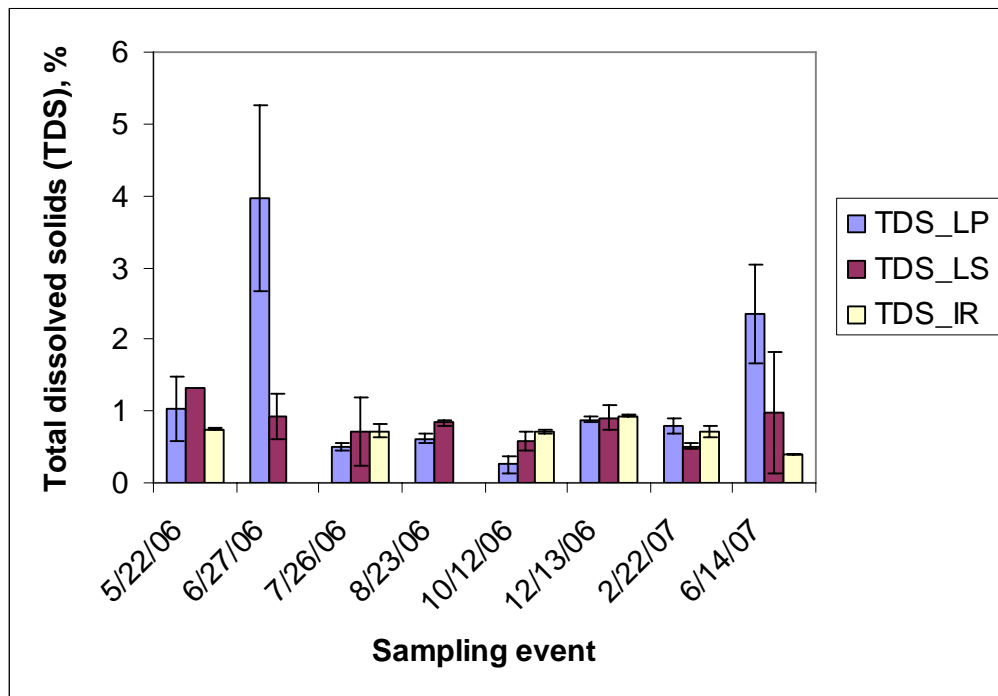
Fig. 17. L4DB® treatment effects on: a) Total dissolved solids (TDS). LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent. (Note: May 2006 sampling is the pretreatment sampling)

Similarly, TDS concentration for IR fluctuated throughout the monitoring period but increased slightly (3%) as compared to the IR pre-treatment concentration. Overall, the ratio of TDS/TS was much higher in IR (0.67) than it was in LS (0.32) and LP (0.20). This implies

that about 67, 32 and 20% of TS in IR, LS and LP were dissolved solids, respectively. Therefore, greater solids for IR are likely due to greater TDS content. This suggests that microbes are more active in the supernatant as compared to the entire profile, where most of the solids reduction was observed.

Total volatile solids (TVS) and TFS are presented in Figs. 18 and 19. Just as TS, TVS did not show significant reduction in LP following treatment until August 2006 (third treatment). After August, TVS concentration in LP decreased by 44% while December 2006 exhibited the highest single TVS reduction (78%). In total, TVS for the LP was 31% and it constituted 48% of the TS. TVS concentration in LS responded similarly and gradually decreased until December 2006; thereafter, values fluctuated slightly. The overall TVS reduction for LS was 58% and TVS represent 56% of TS. IR samples showed no clear TVS trends; overall TVS increased by 37%. This variation in TVS was likely due to variation in the rate and extent of microbial biodegradation of organic compounds and the influence of flushed water added to the lagoon (Wilkie, 2005).

Total fixed solids (TFS) for LP, LS and IR followed a trend similar to TSS (Fig 19). The TFS concentration for LP did not show significant reduction following treatment until August 2006 (third treatment) when TFS concentration for LP decreased by 64%; the overall reduction of TFS in LP was 51%. Total fixed solids (TFS) concentration in LS reduced gradually throughout the monitoring period with the highest reduction occurring in June 2007 (85%) and the overall TFS reduction was 62%. Total fixed solids concentrations for IR fluctuated throughout the monitoring period and showed an overall reduction of 9% (Fig. 19). Typically, TFS is neither chemically reactive nor biologically degradable and theoretically it

should stay unchanged (Zhu et al., 2000). In this case, TFS fluctuated in the lagoon suggesting that variability in sludge depth was partly due to variation in these solids.
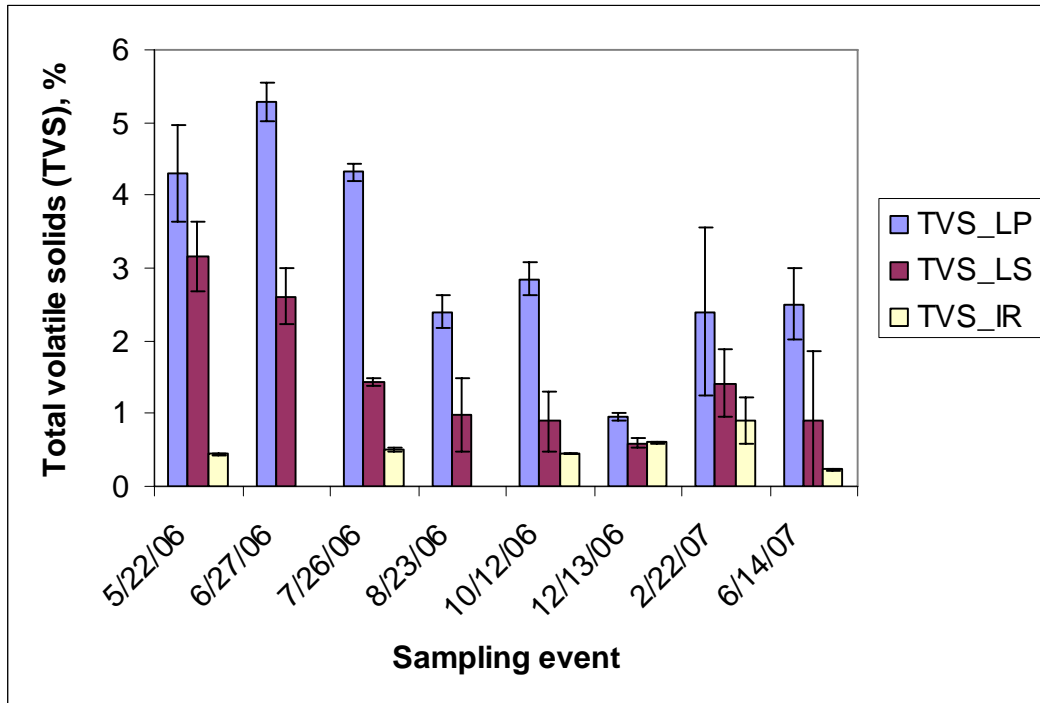
Fig. 18. Total volatile solids (TVS) trend over time for the L4DB® treatment. LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May 2006 sampling is the pretreatment sampling)

Both TDS and TFS for LP were significantly greater than those from LS and IR, while they were statistically similar between LS and IR. Additionally, all other solids for LS were significantly greater than those for IR. The difference in solids concentration between LS and IR was un-expected because the irrigation pump inlet was located at a depth of 15 inches (46 cm), which is within the LS samples collection depth range (0-24 inches).
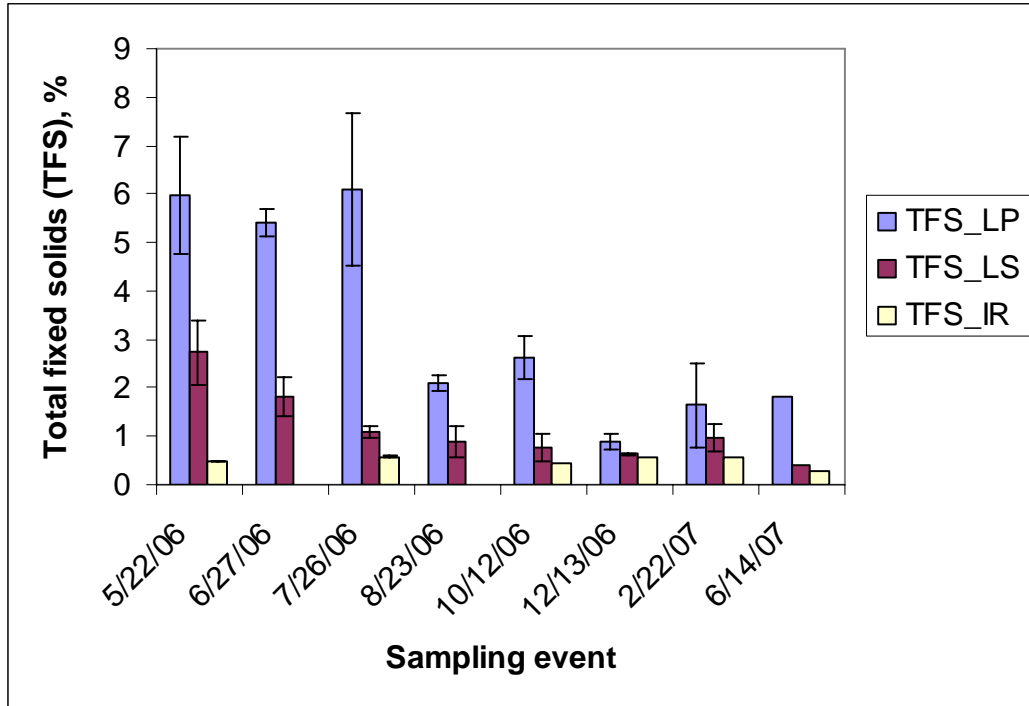
Fig. 19. Total fixed solids (TFS) trend over time for the L4DB® treatment. LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May 2006 sampling is the pretreatment sampling)

Nutrients

Average total P for LP, LS and IR for each sampling event are presented in Fig 20a. Total P concentration in LP increased slightly until July 2006 and was likely due to re-suspension of solids resulting from microbial degradation of sludge (Converse and Karthikeyan, 2004). In December 2006, TP concentrations in the LP were reduced by 72% from its pretreatment concentration; thereafter, TP concentration fluctuated considerably until the end of demonstration (Fig. 20a).
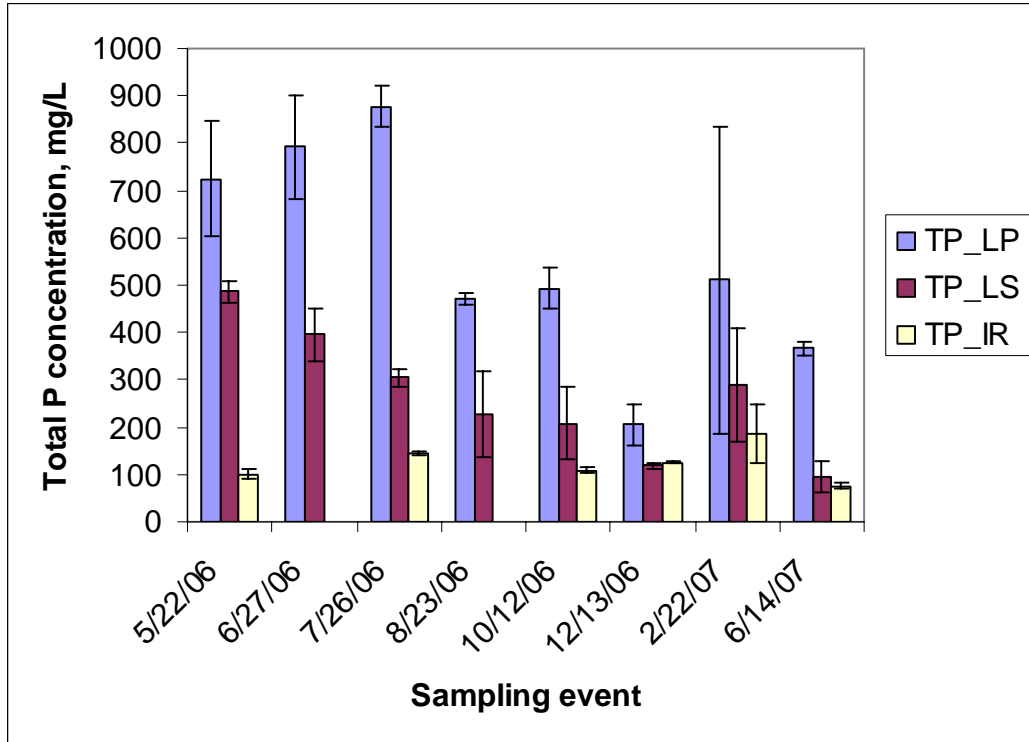
(a)
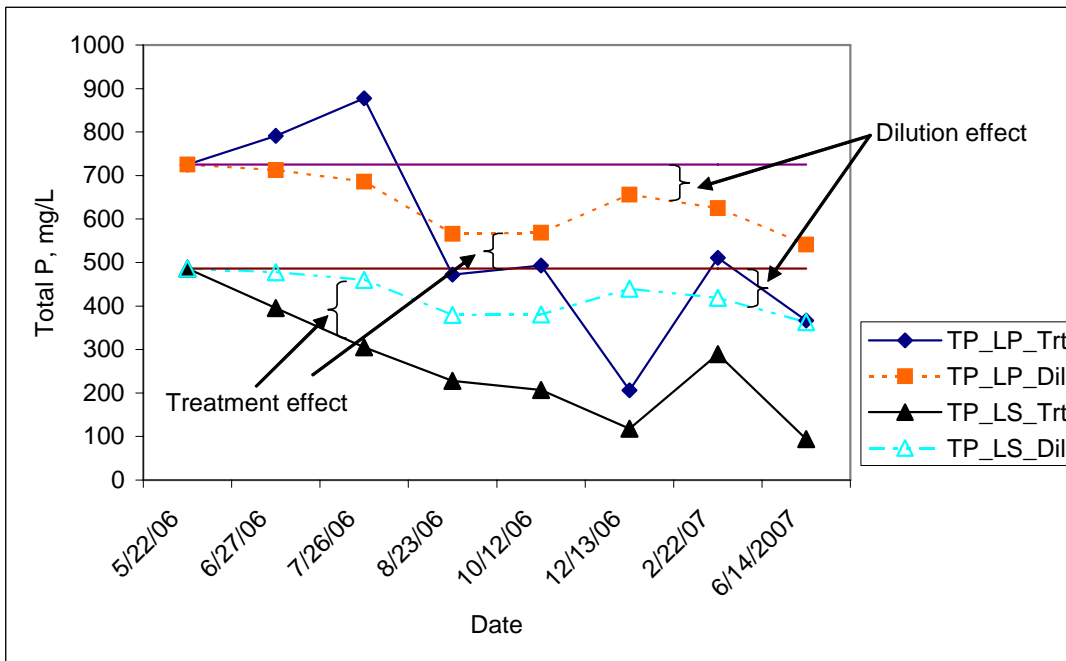
(b)

Fig. 20. a) Total phosphorus (TP) trend over time for the L4DB® treatment and b) treatment and dilution effect on Total P. LP: liquid profile, LS: Liquid supernatant; Trt: Treatment, Dil: Dilution (Note: May 2006 sampling is the pretreatment sampling)

Microbial consumption of suspended solids is the likely reason for TP reductions; sedimentation of particulate P and degraded microbial cells at the bottom of the lagoon could also influence TP levels in the lagoon. Throughout the course of the demonstration, TP was reduced by an average of 27%.

Total P concentration for LS decreased gradually following microbial treatment until August 2006 when it began to fluctuate somewhat for the remainder of the demonstration (Fig. 20a). The highest single reduction in TP was 81% for LS samples and was observed in June 2007 with the average reduction totaling 52% for the entire demonstration. The high TP reduction in June was likely due to combination of increased microbial activities at a favorable environmental condition, dilution resulting from runoff water contribution, as well as the low evapotranspiration rate during that time (Fig. 20a).

In the case of IR effluent, overall TP concentration increased by 28% compared to its pretreatment concentration and could be the cause of greater dissolved solids in the IR effluent. No clear trend in TP levels was observed in IR samples; however, a weak correlation (R^2= 0.20) was observed between TP and TDS for IR effluent.

A dilution effect could have influenced reductions in TP for LP and LS. To evaluate this theory, lagoon water volume changes were taken into account and TP concentration were adjusted accordingly. As shown in Fig. 20b, dilution itself can reduce TP concentration substantially from its pretreatment concentration as indicated by the dilution effect. After adjusting samples for dilution, it was revealed that differences between treatment and dilution adjusted TP concentrations were likely due to L4DB® microbial treatment (Fig. 20b). No significant reduction in TP concentration was observed for the LP until August 2007, when TP measured was significantly lower than TP adjusted for dilution. Although, measured TP

concentrations varied towards the end of monitoring, but these values were much lower than those adjusted for dilution. Dilution analysis shows that the differences between treatment TP and TP adjusted for dilution were likely due to treatment effects. Overall, significant differences in TP concentration were observed among LP, LS and IR effluent (Table 7).

Table 7. Average TP, SRP, TKN, NNN and K concentration (mg/L) for lagoon and irrigated effluent samples averaged over all sampling events

Parameter[1]	Sampling location		
	LP	LS	IR
Total phosphorus (TP)	555a[*]±239	265b±137	124c±43
Soluble reactive phosphorus (SRP)	9.24a±3.79	9.28a±3.89	4.28b±1.83
Total Kjeldahl nitrogen (TKN)	2023a±801	1288b±586	775c±223
Nitrate-Nitrite Nitrogen (NNN)	0.22a±0.10	0.23a±0.10	0.20a±0.06
Potassium (K)	1228a±294	1129ab±289	992b±312

* Averages within a row followed by different letters are significantly different at $P \leq 0.05$ according to Duncan multiple range tests.
[1] parameter is in mg/L

As expected, higher TP concentration for LP was likely due to higher TS and TSS as compared to LS and IR (Table 6). In addition, degraded microbial cells accumulate at the bottom of the lagoon and runoff water added might also contribute to increased TP concentration for LP. In this study no quantitative or qualitative assessment of runoff water added to the lagoon was conducted, therefore we can not quantify the effects of runoff on the lagoon. TP was also strongly tied to TS ($R^2 = 0.91$) and TSS ($R^2 = 0.87$) (Fig. 21). A similar correlation for TP versus TS and TSS was also reported by McFarland et al. (2003). A stronger relationship was observed in LS samples between TSS and TP ($R^2=0.92$) as compared to TS and TP ($R^2= 0.90$). This suggests that most of the TP in LS is adsorbed to

suspended materials (i.e., TSS), while it is adsorbed to larger particulate matter for the LP. Therefore, without measuring the sludge's P content, the reduction of P from the entire profile due to treatment can not be unequivocally determined.
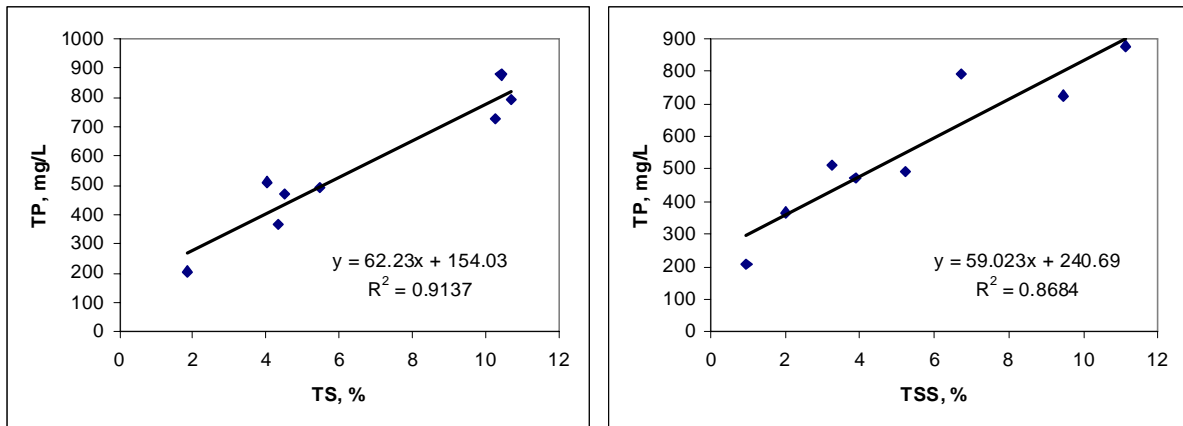
Fig. 21. Relationship between TP vs. TS and TP vs. TSS for LP

Conversely, TP for IR effluent increased by 28% from its pre-treatment concentration, which was likely due to loosening of sludge and dead microbial cells from the bottom of the lagoon to the upper profile as well as mixing of slurry due to impeller action at pumping depth. This loosening of sludge phenomena is also observed by other researchers (Converse and Karthikeyan, 2004), indicating that loosening of the settled solids from the lagoon bottom caused them to rise to the upper profile, carrying the P associated with them.

Average SRP for LP, LS and IR during each sampling event is presented in Fig. 22. Following the first microbial treatment, SRP concentration for these sampling locations reduced gradually until August 2006; thereafter its concentration fluctuated considerably, especially at the end of sampling (June 2007) when SRP concentration increased significantly as compared to pre-treatment concentrations (Fig. 22). This increased SRP concentration was

likely due to excessive runoff water contribution to the lagoon. Average SRP for IR was significantly lower than in LP and LS; however, it was statistically similar to LP and LS (Table 7). Overall, no clear SRP reduction trends were noticed for any of these locations.

Fig. 22. Orthophosphate phosphorus (SRP) concentration trends over time for the L4DB® treatment. LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May 2006 sampling is the pretreatment sampling)

Total Kjeldahl nitrogen (TKN) in LP, LS and IR samples followed a trend similar to TP concentration. No significant changes in TKN concentration were observed in LP until August 2006; thereafter, little variation of TKN was observed (Fig. 23). The highest TKN reduction for LP occurred in December 2006 (67%) and the overall reduction was 36%. The highest TKN reduction in LS samples was observed in June 2007 (74%) and the overall reduction was 48%. Total Kjeldahl nitrogen fluctuated in IR (Fig. 23) and over time, TKN concentration in IR increased slightly (6%). The highest TKN concentration for LP was likely

due to higher TSS in the LP, since TKN is strongly correlated with TSS in LP ($R^2 = 0.78$) and
LS ($R^2 = 0.89$). This is comparable to the findings of McFarland et al. (2003), where they
reported a correlation coefficient of 0.85 between TSS and TKN.

Fig. 23. Total Kjeldahl nitrogen (TKN) concentration trends over time for the L4DB®
treatment. LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May
2006 sampling is the pretreatment sampling)

Significant differences in TKN concentration were observed among LP, LS and IR
(Table 7) as well as among sampling events for LP, LS and IR (Fig. 23). The reduction of
TKN concentration for LP and LS were likely due to a combination of treatment effects,
added flush water and ammonia volatilization. Higgins et al. (2004) reported that reductions in
TKN concentration are also likely due to ammonia volatilization caused by higher lagoon
temperature and wind velocity. Scotford et al. (1998) also suggested that a flushing system
may dilute the slurry and thereby reduce TKN concentrations. These findings fail to explain
the observed increases in TKN concentration in the IR effluent.

Average Nitrate-Nitrite Nitrogen (NNN) concentrations for LP, LS, and IR are presented in Fig. 24. Following the pre-treatment sample in May 2006, NNN concentration fluctuated considerably for both LP and LS, especially towards the end of the treatment where significant reduction of NNN concentration were observed for all sampling locations (Fig. 24). Overall, no clear trends of NNN concentration reduction were observed for LP and LS, although its concentration was reduced by 11% for the IR effluent. Variation in NNN concentrations was likely due to flush water added to the lagoon. Findings from Bicudo et al. (1999) support this; their studies show that 60-70% of the soluble NNN is contained in the effluent. Overall, no significant differences in NNN concentration were observed among LP, LS and IR effluent (Table 7) suggesting that this treatment was not effective in reducing NNN concentrations.
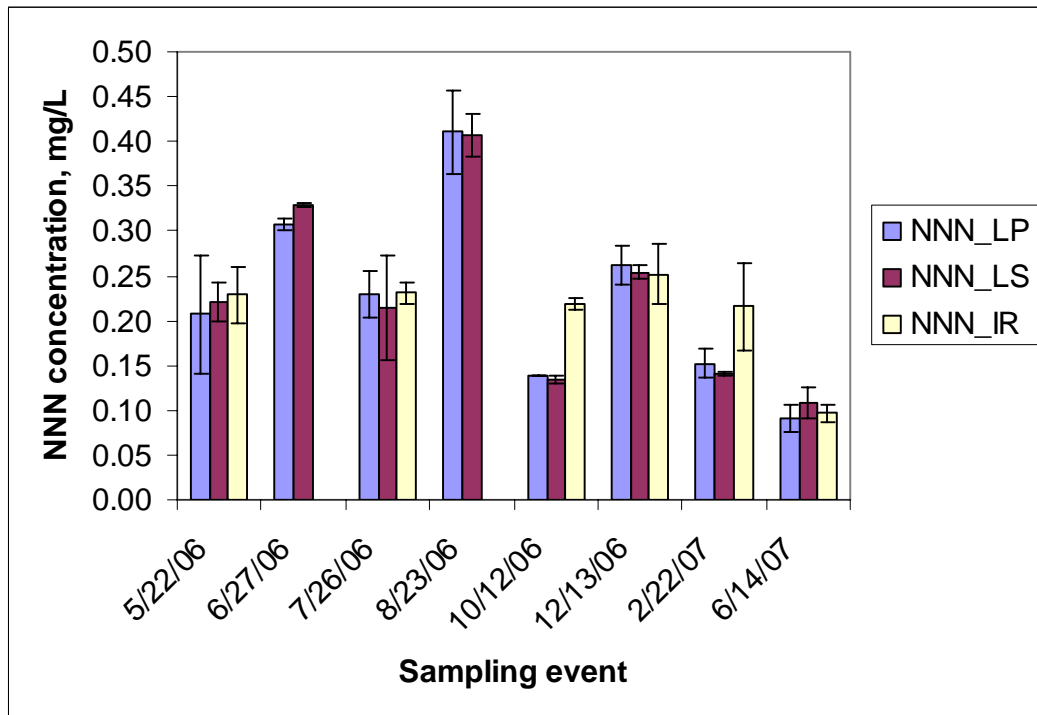
Fig. 24. Nitrite-Nitrate Nitrogen (NNN) concentration trends over time for the L4DB® treatment. LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May 2006 sampling is the pretreatment sampling)

Average K concentration for LP, LS and IR during each sampling event is presented in Fig 25. The highest K concentration was observed in December 2006 and no significant changes in the concentration of K occurred until the end of sampling. This variation in concentration was likely due to runoff water contribution and variation in flush water added to the lagoon and K's high water-solubility (Gustafson et al., 2007). Average K concentrations are listed in Table 7 and show no significant differences concentration in any sample set. It is apparent that this microbial treatment was not effective in reducing the concentration of K.
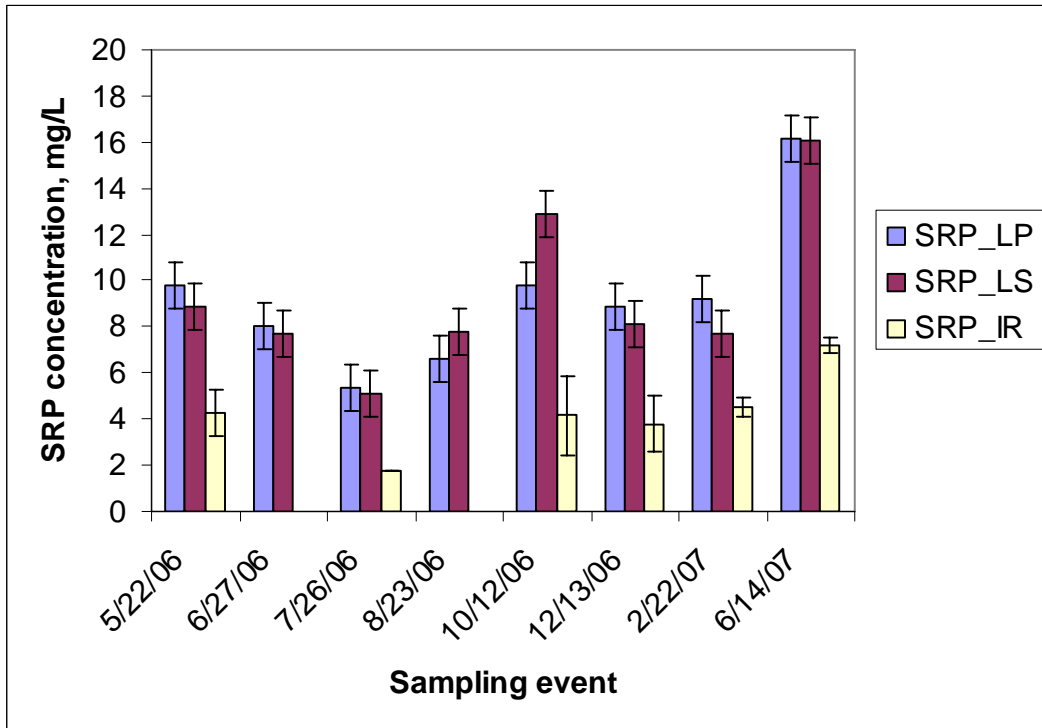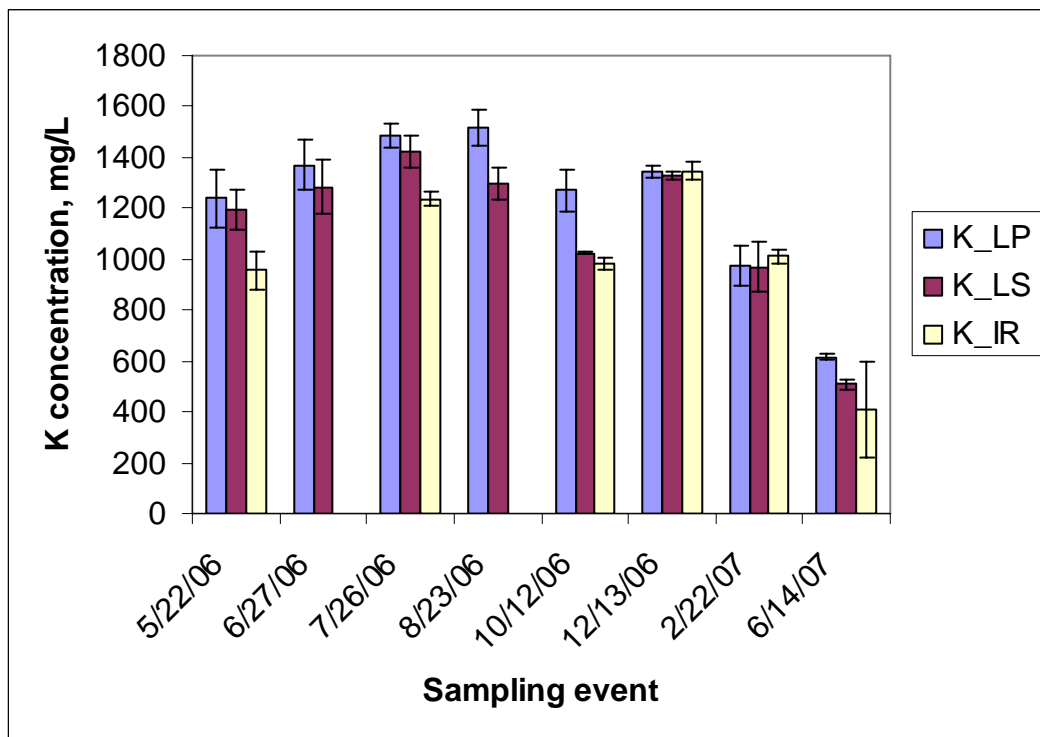

Fig. 25. Potassium (K) concentration trends over time for the L4DB® treatment. LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May 2006 sampling is the pretreatment sampling)

Nutrient data analyses suggest that L4DB® treatment was somewhat effective in reducing TP, TKN, but not SRP, K and NNN concentration in LP, LS and IR. This implies

that this microbial treatment was not highly effective in reducing nutrients that are water soluble. Without accurate measurements of sludge nutrient content, it was difficult to ascertain that the reduction of nutrients from these profiles was likely due to settling of solids including dead and degraded bacterial mass accumulated at the bottom of lagoon. All nutrient concentrations received from TIAER are also listed in tables I through III in Appendix A.

Metals

Metals in animal manure largely reflect the metals concentration in feeds that the animals consumed (Nicholson et al., 1999). Following microbial treatment, aluminum (Al) concentration in LS decreased gradually until December 2006 but then fluctuated toward the end of the demonstration (Fig. 26). The highest Al concentration reduction in LS was observed in December 2006 (96%) and the overall reduction was 82%. Aluminum concentration in LP fluctuated considerably throughout the monitoring period, but remained significantly lower than the pre-treatment concentrations; overall, Al concentrations were reduced by 62% in LP.

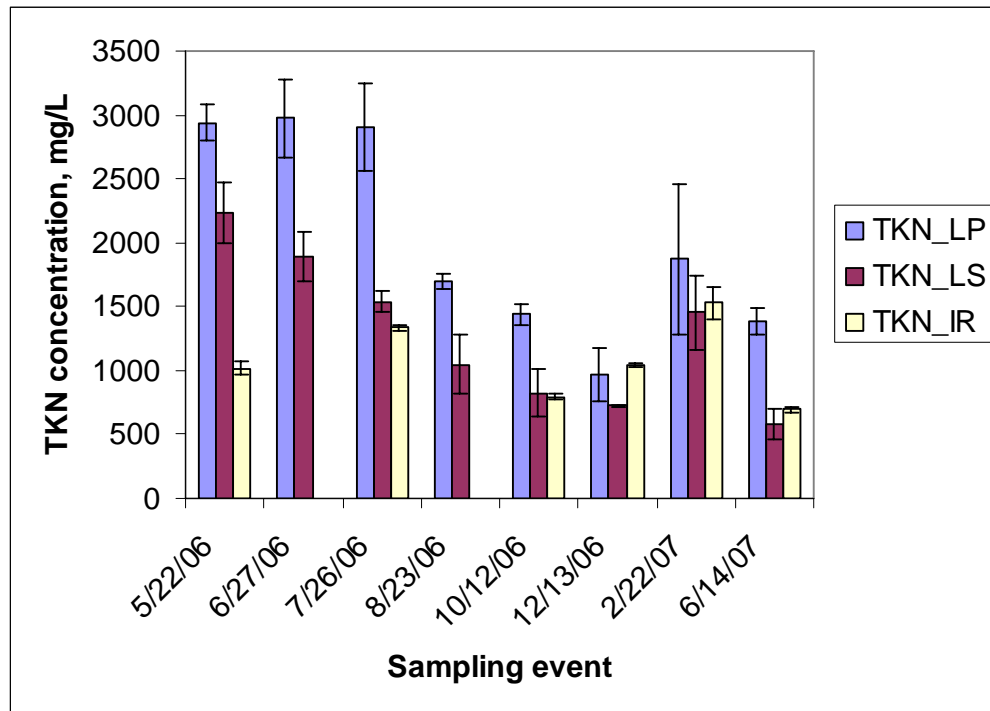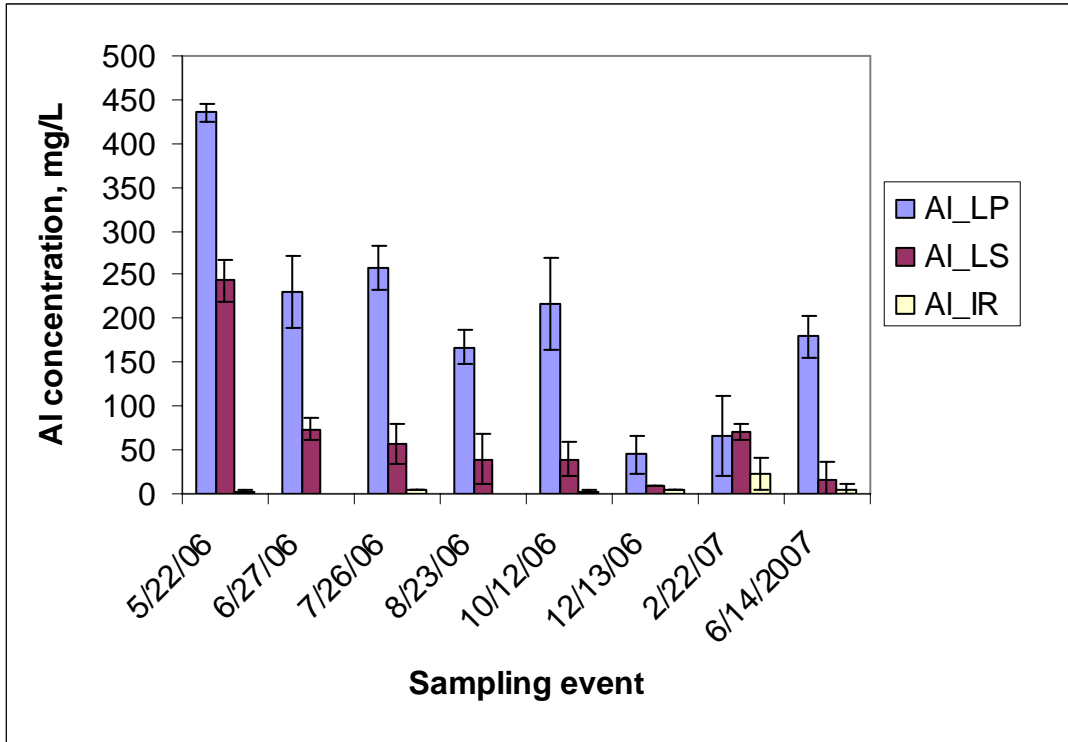Fig. 26. Aluminum (Al) concentration trends over time for the L4DB® treatment. LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May 2006 sampling is the pre-treatment sampling)

Similar or greater reductions than the average Al concentrations in LP, LS and IR samples were observed in all metals except Mg. Average metals concentrations at different sampling events for LP, LS and IR are listed in Tables 8 & 9. Overall Al, Ca, Cu, Fe, Mg, and Mn concentration reduction for the LP were 62, 57, 58, 56, 22 and 55% respectively while these values for LS were 82, 70, 80, 81, 42, and 62% respectively. These concentration reductions are likely due to microbial activities as well as variation in feed composition, which was not explored in this study and was beyond the scope work.

Table. 8. Average concentration of aluminum (Al) Calcium (Ca), Copper (Cu) concentration for LP, LS and IR at different sampling events

Date	Al (mg/L)			Ca (mg/L)			Cu (mg/L)		
	LP	LS	IR	LP	LS	IR	LP	LS	IR
5/22/06	435.50a ±10.60	244.00a ±24.04	3.10b ±0.43	5785a ±120	3140a ±665	325cd ±25	16.80a ±0.98	11.29a ±3.26	1.00b ±0.0
6/27/06	230.00cb ±41.01	73.60b ±12.58	N/A	3575cb ±530	1450b ±339	N/A	12.45b ±0.49	3.26b ±1.04	N/A
7/26/06	258.50b ±24.74	56.85cb ±22.69	4.52b ±0.43	4315b ±233	1320cb ±127	396cb ±11	14.75ba ±1.20	2.68b ±0.65	0.46c ±0.04
8/23/06	167.50c ±19.09	39.95cbd ±29.62	N/A	2200ed ±99	861cbd ±423	N/A	5.95dc ±0.25	2.07b ±1.37	N/A
10/12/06	216.00cb ±52.32	39.65cbd ±19.44	3.26b ±0.26	2810cd ±594	813cbd ±307	304cd ±14	6.71c ±1.45	1.58b ±0.82	1.00b ±0.00
12/13/06	44.55d ±20.85	8.60d ±0.57	4.72b ±0.79	1078f ±313	577cd ±11	505b ±11	1.62e ±0.87	1.00b ±0.00	1.00b ±0.00
2/22/07	65.75d ±45.74	70.60b ±9.47	22.20a ±18.52	1433f ±829	1225cb ±64	657a ±190	3.33de ±2.34	3.28b ±0.47	1.565a ±0.77
6/14/07	179.50cb ±23.33	16.55cd ±19.73	5.38b ±6.14	2099edf ±239	354d ±177	209d ±65	4.30dce ±0.53	2.00b ±0.00	2.00a ±0.00

*Averages within a column followed by different letters are significantly different at P ≤ 0.05 according to Duncan multiple range tests.

Table. 9. Average concentration of iron (Fe), magnesium (Mg), and manganese (Mn) for LP, LS and IR at different sampling events

Date	Fe (mg/L)			Mg (mg/L)			Mn (mg/L)		
	LP	LS	IR	LP	LS	IR	LP	LS	IR
5/22/06	385.50a ±2.12	213a ±31.11	3.08b ±0.26	597ba ±117	524a ±55.15	210b ±15.43	20.05a ±3.89	13.35a ±2.89	1.01c ±0.02
6/27/06	269b ±33.23	73.50b ±21.07	N/A	591ba ±28.28	392b ±52.32	N/A	14.690a ±1.27	6.02b ±1.55	N/A
7/26/06	303b ±31.82	61.65b ±18.03	4.48b ±0.23	694a ±12.02	374b ±26.16	256a ±4.32	16.65a ±1.34	5.50b ±1.06	1.66b ±0.03
8/23/06	140c ±2.82	36.45cb ±31.18	N/A	504bc ±30.40	321cb ±38.18	N/A	8.67b ±0.29	3.97cb ±1.13	N/A
10/12/06	193c ±49.49	36.50cb ±19.09	2.041b ±0.10	502bc ±46.66	261cd ±37.47	195b ±6.73	5.79b ±4.73	11.55a ±1.63	1.08cb ±0.04
12/13/06	39d ±15.90	8.45c ±0.24	4.38bb ±0.57	304d ±16.26	261cd ±0.70	260a ±10	3.26b ±0.60	1.70c ±0.04	1.40cb ±0.03
2/22/07	57d ±38.89	60.25b ±7.28	18.03a ±13.77	334d ±129	298c ±0.70	222b ±27.19	5.41b ±3.15	5.44b ±0.77	2.41a ±0.96
6/14/07	178c ±16.97	10.15c ±11.52	4.95b ±5.90	352dc ±18.38	189d ±13.43	131c ±45.49	7.99b ±0.84	1.021c ±0.30	1.00c ±0.00

[*]Averages within a column followed by different letters are significantly different at P ≤ 0.05 according to Duncan multiple range tests

For LP and LS, metals concentrations were highly correlated with solids (R^2= 0.77 to 0.92 for LP and R^2= 0.63 to 0.93 for LS), but no clear trends for metals were observed in IR. Overall, significant differences in metals concentration (i.e., Al, Ca, Cu, Fe, Mg, Mn) were observed among LP, LS and IR; Na was the only metal to show a decrease (Table 10). It is apparent from the low percentage reduction in Na that this treatment system was not effective in reducing Na and other soluble constituents (i.e., SRP, NNN, TDS etc.) in this lagoon.

Table 10. Average metals concentration (mg/L) for lagoon and irrigated effluent samples averaged over all sampling events

Parameter[1]	Sampling location		
	LP	LS	IR
Aluminum (Al)	19.669a*±120	68.73b±73	7.20c±9.87
Calcium (Ca)	2912a±1556	1218b±868	399c±166
Copper (Cu)	8.24a±5.53	3.39b±3.33	1.18b±0.58
Iron (Fe)	195.47a±117	62.49b±67	6.23c±7.69
Manganese (Mn)	10.46a±6.30	6.09b±4.30	1.43c±0.62
Magnesium (Mg)	485a±146	330b±104	212c±49
Sodium (Na)	470a±140	465a±124	424a±146

* Averages within a row followed by different letters are significantly different at P ≤ 0.05 according to Duncan multiple range tests
[1] parameter is in mg/L

Nicholson et al. (1999) reported that the mean Cu concentration in dairy cattle slurry collected from commercial farms in England and Wales was 4.73 mg/L (62.3 mg/kg dm; dry matter 7.6%). Ullman and Mukhtar (2007) reported Cu concentrations in dairy lagoons in central Texas in the range of 8.1-19.2 mg/L depending on management practices applied at the specific dairy. In this study, average Cu concentration for LP was 8.24 mg/L and was similar to concentrations found in other studies. Cu concentration in manure is related to Cu added as

a supplement to feed (Li et al., 2005). In general, manures will contain higher Cu concentration if feeds contained higher concentrations of Cu (Nicholson et al. 1999). In this study feed composition was not analyzed; however, average concentration of metals (i.e., Ca, Mg, Fe, etc.), except Mn, was much higher than those reported by Ullman and Mukhtar (2007). All metals concentrations as received from TIAER are also listed in tables VI through IX in Appendix A.

Conductivity

The average conductivity for LP, LS and IR are presented in Fig. 27, where L4DB® microbial treatment appeared to cause little or no reduction in EC levels until the end of the demonstration. A sharp increase in EC during December 2006 was observed in LP and LS samples and was likely due to greater amount of nutrients present during that time (due to lower irrigation frequency and additional solids loading) compared to the previous sampling, since dissolved mineral salts (Stevens et al., 1995; Scotford et al., 1998; Yayintas et al., 2007) change conductivity. Typically, when salinity increases, conductivity increases. Conductivity and K, for this lagoon, exhibited good correlation in IR (R^2= 0.57) and LS (R^2= 0.53) samples, but were somewhat correlated in LP (R^2= 0.22). Scotford et al. (1998) also observed strong correlation (R^2 = 0.80) between K and EC. Although conductivity exhibited some variability in this study, no significant differences were observed among LP, LS and IR samples.
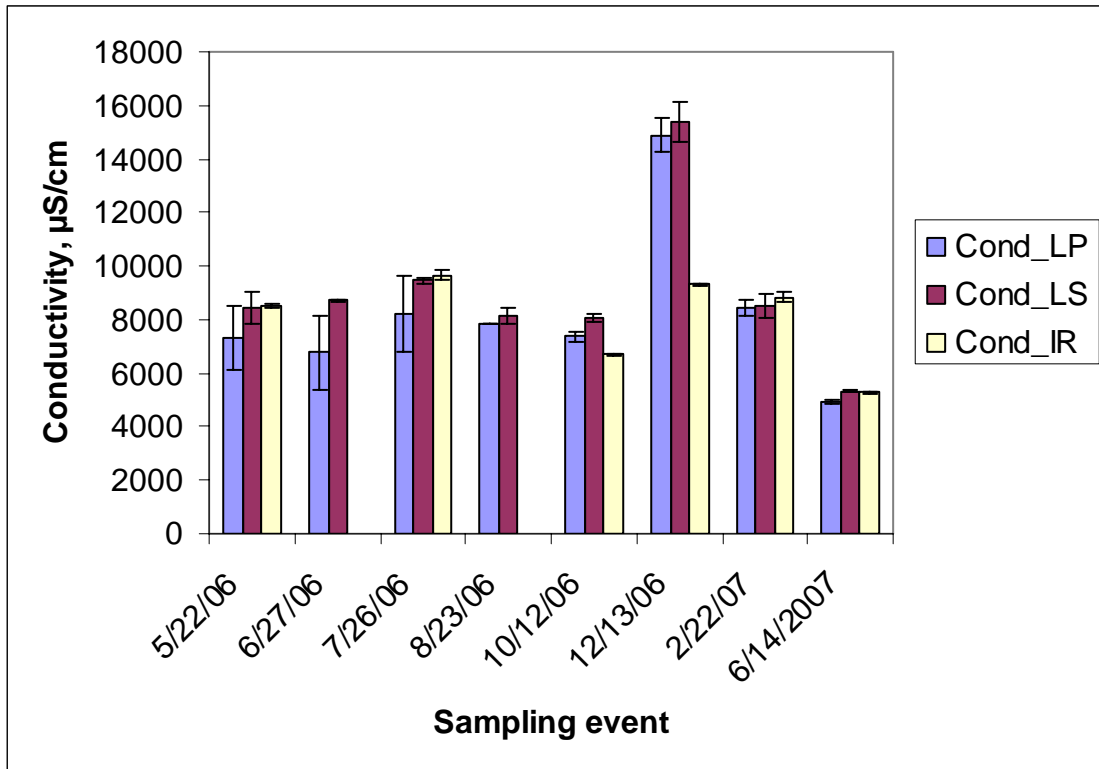
Fig. 27. Conductivity trends over time for the L4DB® treatment. LP: liquid profile, LS: Liquid supernatant; IR: Irrigation effluent (Note: May 2006 sampling is the pretreatment sampling)

While statistically similar, the average conductivity for LS (9,184±2,052 μS/cm), was slightly higher than LP (8,379±2,193 μS/cm) and IR (8,356±1,360 μS/cm). Safley et al. (1993) reported that EC value of 8,000 μS/cm can inhibit bacterial population in livestock treatment lagoon. In this lagoon, EC was higher than this suggested threshold value and might have impacted L4DB® microbial performance in reducing physiochemical parameters of slurry. All conductivity values as received from TIAER are also listed in tables I through III in Appendix A.

TREATMENT COSTS

Costs to implement this lagoon treatment method varied based on the daily amount of manure and wastewater that is added to the lagoon, the existing lagoon capacity and sludge depth, prior wastewater treatment (e.g., pretreatment of flushed manure for solids separation before it flows to the lagoon), lagoon depth, and the number of lagoon cells in the wastewater management system. In addition, the treatment costs will also vary with the type of manure alley cleaning system used, such as flushing or vacuuming. The following cost matrix was also provided by the technology provider:

Table 11. Cost to treat a lagoon with L4DB® microbial treatment

Herd size	Unit cost ($/cow/month)	$/cow/year
1000	1.00	12
1001-7000	0.60 ~ 0.90	7.2 ~ 10.8
>7001	0.30 ~ 0.60	3.6 ~ 7.2

Based upon the information in Table 11, for this 300-head dairy, the total cost to treat the lagoon was estimated at $3900 for a 13 months period or $12/cow/year.

CONCLUSIONS

Effectiveness of L4DB® microbial treatment on an anaerobic lagoon was monitored for one year. It appears that L4DB® microbial treatment was somewhat effective in reducing solids and resulted in reducing sludge depth by 24% (however, this reduction was 16% excluding the measurement anomaly in August 2006). The L4DB® treatment was also highly

effective in reducing TS, TSS, TVS and TFS in the LS, but less effective in reducing these solids from LP and no clear trends were observed for irrigation effluent (IR). Over time, L4DB® treatment reduced TS (43%), TSS (45%), TDS (42%), TVS (31%), and TFS (51%) in LP samples, while they were reduced by 60, 71, 44, 58, and 62% respectively for LS samples. Similarly, reductions of phosphorus were likely due to microbial uptake of P from LS and LP; however, P continues to be mobile until settling occurs (Farve et al., 2004). The trend shown in this report confirms that due to microbial activities P was very mobile in LP profile as compared to LS. Overall, L4DB® treatment was somewhat effective in reducing TP, TKN, but was not effective in reducing SRP, NNN and K concentrations. Average concentrations of TP and TKN in the LP were reduced by 27 and 36%, respectively while these constituents were reduced by 52 and 48% in the LS. Significant metal concentration reductions were observed for the LP (ranged from 22 to 62%) and the LS (ranged from 42 to 82%), while metals concentration increased slightly for IR over time. Although conductivity exhibited considerable variability, no significant differences in conductivity were observed among LP, LS and IR samples.

Variable performance and poor reduction of nutrients in few cases were likely due to over loading of the lagoon as well as varied treatment application rates. The technology provider pre-determined the application rate for this lagoon based on experiences, but not by measuring environmental conditions of the lagoon. It might be useful to conduct a lab-scale study to determine the effective application rate based on varying conditions of temperature, manure nutrient and metals loading and existing sludge level in lagoons to be treated.

Therefore, it could be inferred that most of these solids, nutrients, and metal reduction were likely due to microbial treatment, dilution of lagoon slurry due to excessive rain and

runoff water as well as settling of dead and degraded bacterial mass accumulated at the bottom of lagoon. Additional measurements of lagoon sludge accumulation rate and constituents are warranted to assess possible increase in nutrients and solids due to accelerated solids settling and increased accumulation of microbial mass at the lagoon bottom.

CHALLENGES

Tanks were used to mimic the repeatability of lagoon treatment with microbes and to get additional information on treatment effectiveness. Tank evaporation losses caused significant difficulty in maintaining a consistent TS and TP sampling depth in tanks. As a result, it remains a challenge to obtain replicated data on treatment effectiveness in outdoor environmental conditions under tank environment. It is apparent that microbial treatment was more effective in the lagoon supernatant than the entire profile but, without accurate assessment of pre- and post-treatment sludge characteristics, it is premature to conclude how effective the treatment was in reducing nutrient, metals and solids in the lagoon. The foremost challenge is to collect and monitor the lagoon sludge sample for an extended period of time prior to, during and after treatment to determine solids, nutrients and metal content of the lagoon that will enable a determination to be made regarding the effectiveness of the applied treatment.

ACKNOWLEDGEMENT

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APPENDIX A

Table I. pH, conductance (μS/cm), and nutrients concentration (mg/L) in LP samples at different sampling events

Site ID	Collection Date	pH	Conductance	NNN	ORP	TP	TKN
LP1	5/22/2006	7.5	8140	0.254	7.83	810	3040
LP1	6/27/2006	7.57	5900	0.312	7.04	713	2760
LP1	7/26/2006	7.57	9570	0.21	5.49	847	2660
LP1	8/23/2006	7.64	9510	0.451	8.27	464	1740
LP1	10/12/2006	7.34	7440	0.139	11.1	462	1380
LP1	12/13/2006	7.38	13300	0.249	13	237	1120
LP1	2/22/2007	7.13	8650	0.141	11.4	741	2290
LP1	6/14/2007	7.27	5630	0.078	18.5	356	1307
LP2	5/22/2006	7.41	6470	0.16	11.7	640	2840
LP2	6/27/2006	7.53	7870	0.303	9.09	870	3190
LP2	7/26/2006	7.56	7480	0.248	5.24	908	3140
LP2	8/23/2006	7.76	9540	0.37	5.02	480	1660
LP2	10/12/2006	7.44	7220	0.138	8.52	524	1500
LP2	12/13/2006	7.57	12500	0.275	4.77	175	825
LP2	2/22/2007	7.38	9070	0.164	7.06	281	1460
LP2	6/14/2007	7.3	5780	0.103	13.8	377 [SR]	1455

Table II. pH, conductance (μS/cm), and nutrients concentration (mg/L) in LS samples at different sampling events

Site ID	Collection Date	pH	Conductance	NNN	ORP	TP	TKN
LS1	5/22/2006	7.32	8880	0.206	9.05	470	2060
LS1	6/27/2006	7.58	8910	0.331	7.08	356	1760
LS1	7/26/2006	7.8	9910	0.172	4.88	292	1480
LS1	8/23/2006	7.86	9630	0.387	9.7	293	1210
LS1	10/12/2006	7.5	7920	0.131	14.1	261	957
LS1	12/13/2006	7.43	13800	0.249	12.3	113	719
LS1	2/22/2007	7.37	8620	0.139	9.85	374	1660
LS1	6/14/2007	7.38	6100	0.095	17	69.9 [SR]	499
LS2	5/22/2006	7.35	8020	0.236	8.69	502	2400
LS2	6/27/2006	7.56	8840	0.327	8.28	435	2030
LS2	7/26/2006	7.77	9730	0.257	5.4	318	1600
LS2	8/23/2006	7.75	10100	0.427	5.92	163	889
LS2	10/12/2006	7.59	8150	0.138	11.6	153	693
LS2	12/13/2006	7.63	12900	0.258	3.96	123	730
LS2	2/22/2007	7.43	9250	0.142	5.51	205	1250
LS2	6/14/2007	7.43	6190	0.123	15.2	118 [SR]	666

Table III. pH, conductance (μS/cm), and nutrients concentration (mg/L) in IR samples at different sampling events

Site ID	Collection Date	pH	Conductance	NNN	ORP	TP	TKN
IR1	5/22/2006	7.69	8450	0.209	6.33	82.6	684
IR1	7/26/2006	7.78	10100	0.242	3.69	132	928
IR1	10/12/2006	7.54	8070	0.211	5.26	103	579
IR1	12/13/2006	7.54	8070	0.277	4.77	111	730
IR1	2/22/2007	7.58	9400	0.14	9.92	127	971
IR1	6/14/2007	7.41	6130	0.093	14.6	61.1	455
IR2	5/22/2006	7.53	8610	0.204	10.2	81.4	647
IR2	7/26/2006	7.79	10100	0.231	3.62	132	940
IR2	10/12/2006	7.58	8060	0.219	5.96	100	553
IR2	12/13/2006	7.58	8060	0.275	10.4	111	718
IR2	2/22/2007	7.6	9380	0.236	8.23	132	1020
IR2	6/14/2007	7.4	6040	0.114	14.2	67.2	472
IR3	5/22/2006	7.55	8520	0.229	10.9	99.9	738
IR3	7/26/2006	7.77	10100	0.225	3.75	130	943
IR3	10/12/2006	7.53	8120	0.228	11.3	97.7	548
IR3	12/13/2006	7.53	8120	0.221	9.19	114	731
IR3	2/22/2007	7.5	9000	0.246	10.1	247	1240
IR3	6/14/2007	7.4	6070	0.092	15.1	70.5	497
IR4	5/22/2006	7.6	8480	0.273	8.07	95.3	759
IR4	7/26/2006	7.76	10100	0.226	3.62	125	897
IR4	10/12/2006	7.53	8080	0.216	12.2	89.9	527
IR4	12/13/2006	7.53	8080	0.233	7.36	111	708
IR4	2/22/2007	7.54	9320	0.242	9.32	162	1020
IR4	6/14/2007	7.38	6080	0.088	15.9	71.7	502

Table IV. Concentration of solids (%) in LP samples at different sampling events

Site ID	Collection Date	TS	TSS	TVS	TDS	TFS
LP1	5/22/2006	11.6	9.89	4.78	1.68	6.82
LP1	6/27/2006	10.3	5.43	5.09	4.89	5.21
LP1	7/26/2006	9.23	8.68	4.24	0.548	4.99
LP1	8/23/2006	4.76	4.1	2.56	0.658	2.2
LP1	10/12/2006	4.99	4.82	2.69	0.166	2.3
LP1	12/13/2006	1.99	1.14	0.994	0.85	0.996
LP1	2/22/2007	5.47	4.76	3.22	0.713	2.25
LP1	6/14/2007	4	2.14	2.16	1.86	1.84
LP2	5/22/2006	8.95	9.04	3.83	0.4044	5.12
LP2	6/27/2006	11.1	8.03	5.48	3.05	5.62
LP2	7/26/2006	11.6	13.6	4.4	0.4675	7.2
LP2	8/23/2006	4.23	3.66	2.24	0.567	1.99
LP2	10/12/2006	5.96	5.62	3.01	0.339	2.95
LP2	12/13/2006	1.69	0.78	0.914	0.908	0.776
LP2	2/22/2007	2.62	1.74	1.58	0.875	1.03
LP2	6/14/2007	4.69	1.86	2.86	2.83	1.83

Table V. Concentration of solids (%) in LS samples at different sampling events

Site ID	Collection Date	TS	TSS	TVS	TDS	TFS
LS1	5/22/2006	6.7	5.62	3.49	1.08	3.21
LS1	6/27/2006	3.89	3.19	2.34	0.697	1.55
LS1	7/26/2006	2.41	1.35	1.4	1.06	1.01
LS1	8/23/2006	2.46	1.64	1.35	0.816	1.11
LS1	10/12/2006	2.14	1.64	1.18	0.498	0.96
LS1	12/13/2006	1.19	0.4	0.544	0.788	0.646
LS1	2/22/2007	2.9	2.42	1.75	0.48	1.15
LS1	6/14/2007	0.463	0.084	0.205	0.379	0.258
LS2	5/22/2006	5.08	5.45	2.82	0	2.26
LS2	6/27/2006	5.01	3.87	2.89	1.14	2.12
LS2	7/26/2006	2.64	2.26	1.48	0.382	1.16
LS2	8/23/2006	1.3	0.43	0.627	0.866	0.673
LS2	10/12/2006	1.17	0.5	0.599	0.673	0.571
LS2	12/13/2006	1.27	0.24	0.641	1.03	0.629
LS2	2/22/2007	1.85	1.3	1.09	0.547	0.754
LS2	6/14/2007	2.14	0.56	1.58	1.58	0.56

Table VI. Concentration of solids (%) in IR samples at different sampling events

Site ID	Collection Date	TS	TSS	TVS	TDS	TFS
IR1	5/22/2006	0.843	0.11	0.358	0.733	0.485
IR1	7/26/2006	0.972	0.366	0.401	0.606	0.571
IR1	10/12/2006	0.843	0.152	0.377	0.691	0.466
IR1	12/13/2006	1.07	0.11	0.495	0.958	0.575
IR1	2/22/2007	1.08	0.3	0.593	0.782	0.49
IR1	6/14/2007	0.456	0.054	0.188	0.405	0.268
IR2	5/22/2006	0.878	0.128	0.376	0.75	0.502
IR2	7/26/2006	1	0.324	0.424	0.676	0.576
IR2	10/12/2006	0.826	0.122	0.374	0.704	0.452
IR2	12/13/2006	1.06	0.145	0.492	0.919	0.568
IR2	2/22/2007	1.08	0.33	0.591	0.749	0.487
IR2	6/14/2007	0.459	0.064	0.184	0.395	0.275
IR3	5/22/2006	0.881	0.13	0.382	0.751	0.499
IR3	7/26/2006	0.997	0.195	0.42	0.802	0.577
IR3	10/12/2006	0.82	0.096	0.37	0.724	0.45
IR3	12/13/2006	1.06	0.135	0.498	0.92	0.562
IR3	2/22/2007	1.92	1.32	1.15	0.601	0.767
IR3	6/14/2007	0.457	0.062	0.191	0.395	0.266
IR4	5/22/2006	0.877	0.127	0.377	0.75	0.5
IR4	7/26/2006	1.02	0.2	0.431	0.822	0.589
IR4	10/12/2006	0.826	0.11	0.371	0.736	0.455
IR4	12/13/2006	1.07	0.135	0.508	0.935	0.562
IR4	2/22/2007	1.25	0.56	0.701	0.686	0.545
IR4	6/14/2007	0.469	0.074	0.199	0.395	0.27

Table VII. Metals concentration (mg/L) in LP samples at different sampling events

Site ID	Collection Date	Al	Ca	Cu	Fe	K	Mg	Mn	Na
LP1	5/22/2006	428	5870	16.1	387	1320	680	22.8	499
LP1	6/27/2006	201	3200	12.1	245	1300	571	14	497
LP1	7/26/2006	241	4150	13.9	280	1450	703	16.7	581
LP1	8/23/2006	154	2130	5.77	142	1470	483	8.46	247
LP1	10/12/2006	179	2390	5.69	158	1210	469	2.44	499
LP1	12/13/2006	59.3	1300	2.24	50.4	1360	316	3.69	630
LP1	2/22/2007	98.1	2020	4.99	84.6	1030	425	7.64	377
LP1	6/14/2007	196 SR	E2268 D	4.68 SR	190 D, SR	626	365 D, SR	8.59	255
LP2	5/22/2006	443	5700	17.5	384	1160	515	17.3	411
LP2	6/27/2006	259	3950	12.8	292	1440	611	15.8	550
LP2	7/26/2006	276	4480	15.6	325	1520	686	18.6	587
LP2	8/23/2006	181	2270	6.13	138	1570	526	8.88	654
LP2	10/12/2006	253	3230	7.74	228	1330	535	9.14	538
LP2	12/13/2006	29.8	857	1	27.9	1330	293	2.84	602
LP2	2/22/2007	33.4	847	1.68	29.6	918	243	3.18	349
LP2	6/14/2007	163 SR	1930 D	3.93	166 D, SR	606	339 D	7.39	239

Table VIII. Metals concentration (mg/L) in LS samples at different sampling events

Site ID	Collection Date	Al	Ca	Cu	Fe	K	Mg	Mn	Na
LS1	5/22/2006	261	3610	13.6	235	1250	563	15.4	481
LS1	6/27/2006	64.7	1210	2.52	58.6	1210	355	4.92	449
LS1	7/26/2006	40.8	1230	2.22	48.9	1380	375	4.75	591
LS1	8/23/2006	60.9	1160	3.05	58.5	1250	348	4.78	519
LS1	10/12/2006	53.4	1030	2.16	50	1030	288	10.4	425
LS1	12/13/2006	8.19	585	1	8.62	1320	261	1.73	589
LS1	2/22/2007	77.3	1270	3.62	65.4	898	297	5.99	348
LS1	6/14/2007	2.60 ^{SR}	229 ^{D}	< 2 ^{SR}	< 2 ^{SR}	495	179 $^{D, SR}$	<1	213
LS2	5/22/2006	227	2670	8.98	191	1140	485	11.3	443
LS2	6/27/2006	82.5	1690	4	88.4	1360	429	7.12	502
LS2	7/26/2006	72.9	1410	3.14	74.4	1470	412	6.25	598
LS2	8/23/2006	19	562	1.1	14.4	1340	294	3.17	542
LS2	10/12/2006	25.9	596	1	23	1020	235	12.7	451
LS2	12/13/2006	9.01	570	1	8.28	1340	260	1.67	643
LS2	2/22/2007	63.9	1180	2.95	55.1	1040	298	4.9	421
LS2	6/14/2007	30.5 ^{SR}	480 ^{D}	< 2	18.3 ^{SR}	520	198 ^{D}	1.43	227

Table IX. Metals concentration (mg/L) in IR samples at different sampling events

Site ID	Collection Date	Al	Ca	Cu	Fe	K	Mg	Mn	Na
IR1	5/22/2006	3.57	316	1	3.41	920	204	1	379
IR1	7/26/2006	4.44	388	0.507	4.42	1210	252	1.68	513
IR1	10/12/2006	2.93	318	1	2.27	987	197	1.02	399
IR1	12/13/2006	5.52	521	1	4.94	1400	273	1.43	658
IR1	2/22/2007	9	550	1.25	9.35	1040	211	1.79	417
IR1	6/14/2007	14.6 ^{SR}	307 ^{D}	< 2 ^{SR}	13.8 ^{SR}	130	62.4 ^{SR}	<1	64.9
IR2	5/22/2006	2.57	360	1	2.79	1060	232	1	426
IR2	7/26/2006	4.32	394	0.429	4.2	1250	256	1.62	505
IR2	10/12/2006	3.18	313	1	2.44	1010	203	1.1	454
IR2	12/13/2006	4.94	502	1	4.62	1320	250	1.42	584
IR2	2/22/2007	11	519	1.1	9.4	981	199	1.82	403
IR2	6/14/2007	2.51 ^{SR}	176	< 2 ^{SR}	< 2 ^{SR}	497	151 ^{SR}	<1	211 ^{SR}
IR3	5/22/2006	2.93	322	1	2.99	960	210	1.02	383
IR3	7/26/2006	5.15	391	0.442	4.77	1220	254	1.65	510
IR3	10/12/2006	3.43	289	1	2.45	954	187	1.11	475
IR3	12/13/2006	4.8	500	1	4.35	1340	253	1.4	621
IR3	2/22/2007	49.1	935	2.79	38.3	1030	261	3.83	392
IR3	6/14/2007	2.09 ^{SR}	175	< 2 ^{SR}	< 2 ^{SR}	508	154 ^{SR}	<1	212 ^{SR}
IR4	5/22/2006	3.32	302	1	3.16	888	196	1.05	352
IR4	7/26/2006	4.16	412	0.461	4.56	1270	262	1.71	513
IR4	10/12/2006	3.52	295	1	2.5	989	193	1.09	436
IR4	12/13/2006	3.63	498	1	3.6	1330	263	1.35	663
IR4	2/22/2007	19.7	623	1.46	15.1	1000	215	2.22	407
IR4	6/14/2007	2.34 ^{SR}	180	< 2	< 2 ^{SR}	510	155 ^{SR}	<1	201 ^{SR}