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CHARACTERIZATION OF DAIRY MILK HOUSE WASTE WATER IN KENTUCKY

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ABSTRACT. This study focuses on characterization of milk house waste water from eight different farms in Kentucky. The farms were separated into three groups based on the number of cows: small (20-30), medium (30-60), and large (over 60 cows). Samples were collected once a month from four farms and twice a month from the remainder. Samples were analyzed for chemical, biochemical, and microbiological characteristics. Results indicated a large and significant variation in the chemical and microbiological characteristics between the farms. Farm size had a significant effect on the nutrient content of the waste water. Though samples exhibited seasonal variation, there was no trend. Based on the results we will investigate the use of anaerobic-aerobic-anoxic-aerobic sequencing batch reactor (SBR) to treat milk house waste water on a pilot scale. **Keywords.** Dairy waste water, Nutrients, Bacteria, Bacteriophage.

ilking centers with a milking parlor setup require regular equipment and floor washing which produces a large volume of waste water. Recent estimates of waste water volumes from milking systems and milk houses range from 11 to 32 L/cowday (Christopherson et al., 2003). Generally, as the cow number increases, the amount of waste water produced per cow goes down, but it is safe to assume that at least 1000 L of waste water with milk and wash chemicals are being disposed daily from dairy milk houses with less than 200 cows (Havard et al., 2003). This waste water, which has high organic load and large amounts of cleaning and disinfectant chemicals, is usually discharged down the floor drain and is directed either to a lagoon, or discharged on to the ground depending on the farm situation. Although storing milk house waste water along with livestock slurry has been a common practice, the method needs to be re-evaluated because its large volume unnecessarily dilutes the slurry and therefore increases the cost of manure storage, distribution, and land application. Many dairy farms have installed septic tanks to treat milk house waste water in the past. Over the years, most of these systems have failed because they were not designed to treat the large amount of organic matter contained in these wastes, and milk fats caused leach lines to seal up, forcing waste water to come to the surface. Frequently, the discharge is to a stream, or road ditch leading to a stream, or a field drain tile leading to a stream resulting in water pollution.

Various methods like low rate irrigation of dairy waste water, construction of wetlands, and soil-based filtration have been employed to treat waste water from milking centers. However, these methods were inadequate to treat the waste water effectively. The constructed wetland systems reduced the biochemical oxygen demand (BOD) efficiently, but were less efficient at removing nitrates and phosphates (Tanner et al., 1995a, b). Low rate irrigation of dairy waste water caused problems such as clogging of soil (Williams and Nicholson, 1995). Soil based filtration systems effectively removed molybdate reactive phosphorus through adsorption to exchange sites on soil particles, but resulted in high nitrate concentrations in leachates, especially at high application rates. Due to the fast accumulation of phosphorus in the topsoil, the capacity of ion exchange declined drastically so the topsoil layer has to be replaced frequently. This is the major disadvantage of such a system (Chadwick et al., 2000).

Aerobic treatment can remove not only ammonia nitrogen but also phosphorus (P) by precipitation with or without chemical treatment, depending upon the waste water characteristics (Bicudo et al., 1999; Svoboda et al., 2000; Zhu et al., 2001). Recently, Christopherson et al. (2003) evaluated the suitability of aerobic treatment units commonly used in individual sewage systems for the treatment of milk house waste water. Despite several operational challenges with the treatment units, removals of organic matter and total suspended solids ranged from 30% to 95%. Little phosphorus reduction was observed and no effective nitrification of the effluent was obtained. Variable performance and poor removal of nutrients were probably due to organic over loading since units are designed for lower strength waste water. Because these units are designed to treat low strength waste waters, there is a need to investigate both design and operational parameters of aerobic systems in order to adapt and optimize units to treat milk house waste water and other wastes with similar strength.

Sequencing batch reactors (SBR) have been used to remove nutrients and organic matter from municipal, industrial, and animal waste water (Irvine et al., 1987; Surampalli et al., 1997; Bicudo et al., 1999; Zhang and Zhu, 2005). The system consists of four steps: fill, react, waste, and settle.

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These steps can be adjusted to provide anaerobic, anoxic, and aerobic phases in a certain sequence to achieve desired nutrient and organic matter removal. During the aerobic phase, nitrification is achieved, while the anoxic phase results in denitrification. Phosphorus removal occurs during the anaerobic phase. These phases are used in a cycle to remove nitrogen, phosphorus, and carbon from the waste water. Depending on the characteristics of the waste water, the cycle time and the duration of each phase can be adjusted to treat the waste water. The advantage of this method is that both phosphorus and nitrogen can be removed in the same process. It is easy to operate and the cycle and the phases in the system can be adjusted according to the characteristics of the waste water. Since our aim is to develop a sequencing batch reactor (SBR) technology to treat milk house waste water on a pilot scale by simultaneous removal of both carbonaceous and nitrogenous pollutants, it is very important to characterize the waste water. Based on the waste water characteristics we will design the SBR operation system to reduce nutrient pollution to the environment. Hence, the objective of this study was to characterize milk house waste water.

MATERIALS AND METHODS

FARM SELECTION

A survey was conducted to identify dairy farms in and around Lexington, Kentucky, with varying numbers of cows and milking/washing practices. Eight farms were identified and characterized as small- (20-30), medium- (30-60), and large- (60 and more cows) sized farms based on the number of cows. The cows were milked twice a day on all farms except for Farm 8 where the cows were milked three times a day. Farm characteristics are summarized in table 1.

On most of the farms the sampling location was located outside the milking building. Two farms had two waste outlets, one for washings from the milking parlor which had manure and the other one carried waste water from the milking unit. Waste water from the milking unit consisted of milk, sanitizers, and other cleaning agents. The remaining farms had just one outlet, containing waste from the milking parlor and the milking unit.

WASTE WATER SAMPLES

Samples were collected from November 2004 through June 2005, once a month for half of the farms (3, 4, 5, and 7) and twice a month from the remaining farms (1, 2, 6, and 8).

The amount of water used to clean the milking parlor and the resulting waste composition varied on each farm and from day to day. On the other hand, the amount of water used to clean the milking unit remained constant. Cleaning of the milking parlor was followed by cleaning of the milking unit. It was observed that about two thirds of the water was used to clean the milking parlor and one third for cleaning the milking unit. The composite sample thus collected for each farm was such that it had two thirds of the water from the milking parlor and one third from the milking unit. A representative sample was collected every 2 min, from the time the water started flowing in to the drain until the cleaning of the milking parlor and the milking unit was completed, in a bucket using a dipper. The sample was then mixed thoroughly and collected in four different bottles for analysis. The samples were transported in coolers to the Department of Biosystems and Agricultural Engineering, University of Kentucky. The following parameters were analyzed for each sample using standard methods (APHA, AWWA, WEF, 1999): pH, total solids (TS), chemical oxygen demand (COD), biological oxygen demand (BOD₅), and fat. Total Kjeldahl nitrogen (TKN) and total phosphorus (TP) were determined by adaptations of AOAC 990.3, AOAC 985.01, and EPA 6010a methods (Peters et al., 2003), respectively. Chloride, sulfate, nitrate, nitrite, E. coli, Enterococci, and total coliform were determined according to standard methods (APHA, AWWA, WEF, 1999) in the Environmental and Research Training Laboratory (ERTL), Civil Engineering, University of Kentucky. Occurrence of somatic and F+ specific coliphages were analyzed according to methods recommended by USEPA (2001). Phages that infect the suppressive host Salmonella typhimurium LT2 (RP1) were analyzed using modifications to host type and agar composition as applied to the basic method described by USEPA (2001). The modified top agar contained 15-g tryptic soy broth, and 7-g agar dissolved in 500-mL de-ionized water. The bottom agar contained 18.4-g nutrient agar, and 4-g sodium chloride dissolved in 800-mL de-ionized water.

STATISTICAL ANALYSIS

For statistical analysis, data were grouped together by season, namely Fall (November and December), Winter (January-March), and Spring (April-June) and by farm size (small, medium, and large) and farms. Analysis of variance (ANOVA) was performed with farm size, seasonal variation and farms as main factors. All statistical analysis was conducted using MINITAB® Statistical Software with a level of significance of 5%.

Table 1. Farm characteristics for the eight farms located in Kentucky.						
Farm ID	County ID	No. of Milking Cows	Milking per Day	Milking Unit Cleaning System	Sampling Location	Total No. of Samples
F1	Fayette	100	2	Automatic	Drain located outside the building	15
F2	Garrad	103	2	Automatic	Drain located outside the building	15
F3	Garrad	85-100	2	Manual	Drain located outside the building	8
F4	Anderson	140-180	2	Automatic/ manual	Two separate drains (from milking parlor and from washings of the milking unit) located inside the building	8
F5	Madison	40	2	Automatic	Drain located outside the building	8
F6	Garrad	55	2	Automatic	Drain located outside the building	14
F7	Garrad	24	2	Manual	Drain located outside the building	8
F8	Garrad	28	3	Automatic	Two separate drains (from milking parlor and from washings of the milking unit) located inside the building	15

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Table 2. Characteristics of milk house waste water from eight different farms in Kentucky, [a]

		Large-siz	ze Farms		Mid-size Farms		Small-size Farms	
Parameter	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8
n	15	15	8	8	8	14	8	15
рН	6.5 ± 1.3	7.7 ± 0.9	6.9 ± 2.4	7.7 ± 0.9	6.5 ± 1.2	9.3 ± 1.2	6.3 ± 0.5	8.2 ± 0.9
Total Solids (g/L)	15.8 ± 10.1	7.8 ± 3.9	14.2 ± 5.6	10.3 ± 5.3	10.1 ± 5.5	13.2 ± 8.8	8.2 ± 2.9	6.6 ± 1.7
COD (g/L)	21.1 ± 6.7	7.8 ± 4.4	15.6 ± 4.3	14.2 ± 4.1	13.5 ± 7.4	8.5 ± 3.7	11.6 ± 5.6	6.2 ± 1.7
BOD (g/L)	0.07 ± 0.05	0.02 ± 0.01	0.03 ± 0.04	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.05	0.02 ± 0.01	0.01 ± 0.01
Fat (%)	0.4 ± 0.3	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.01	0.1 ± 0.1
Chloride (mg/L)	128.6 ± 78.8	138.8 ± 93.4	254.1 ± 137.6	163.6 ± 56.7	227.1 ± 137.2	504.5 ± 194.3	250.9 ± 205.4	118.9 ± 38.5
Sulfate (mg/L)	63.7 ± 46.3	75.3 ± 48.1	99.7 ± 51.9	83.1 ± 49.4	68.2 ± 43.1	283.5 ± 143.7	57.4 ± 31.8	54.7 ± 50.9
Nitrate (mg/L)	0.2 ± 0.3	0.8 ± 1.5	0.5 ± 0.7	0.8 ± 1.6	0.5 ± 1.3	1.3 ± 1.9	1.9 ± 2.1	2.6 ± 2.8
TKN (mg/L)	635.1 ± 186.4	596.9 ± 290.0	630.4 ± 319.2	505.1 ± 227.6	556.1 ± 252.5	472.3 ± 511.2	242.7 ± 141.3	191.0 ± 80.8
TP (mg/L)	143.7 ± 51.7	65.4 ± 35.9	175.1 ± 62.6	155.2 ± 55.9	104.7 ± 47.6	117.9 ± 72.4	62.0 ± 24.0	63.9 ± 29.1
E.coli	10 ^{8.9}	108.3	106.3	10 ^{6.3}	106.3	10 ^{2.1}	107.6	10 ^{4.4}
(cfu/100mL)	(52,600,000- 2,779,000,000)	(26,750,000- 6,018,500,000)	(0- 3,448,000,000)	(0- 1,918,000,000)	(0- 3,873,000,000)	(0- 58,000,000)	(4,040,000– 771,000,000)	(0- 410,155,000)
Total Coliform	109.2	10 ^{8.8}	106.6	10 ^{8.8}	106.5	10 ^{2.8}	108.5	10 ^{5.1}
(cfu/100mL)	(3,770,000- 5,909,000,000)	(40,550,000- 8,666,942,000)	(0- 6,131,000,000)	(8,400,000- 3,873,000,000)	(0- 6,131,000,000)	(0- 230,550,000)	(2,723,000- 11,199,000,000)	(0- 1,421,110,000)
Enterococci	107.5	107.3	10 ^{5.8} (0-	10 ^{5.8} (0-	10 ^{5.6} (0-	10 ^{1.7}	107.0	10 ^{3.5}
(cfu/100mL)	(6,250,000- 236,500,000)	(3,150,000- 365,550,000)	285,000,000)	121,100,000)	112,400,000)	(0-855,000)	(2,000,000- 39,300,000)	(0-3,100,000)
Somatic	10 ^{5.1}	104.4	10 ^{2.8}	104.1	10 ^{3.8}	10 ^{1.5}	10 ^{3.1}	10 ^{2.6}
coliphage (pfu/100mL)	(23,000-40,600)	(7,000-96,000)	(0-49,000)	(0-460,000)	(0-290,000)	(0-130,000)	(0-350,000)	(0-12,000)
F+ specific	10 ^{2.1}	$10^{1.8}$	$10^{2.0}$	$10^{1.2}$	$10^{1.6}$	10 ^{1.2}	10 ^{1.7}	10 ^{1.5}
coliphage (pfu/100mL)	(0-6,200)	(0-6,000)	(0-25,000)	(0-6,000)	(0-13,000)	(0-19,000)	(0-180,000)	(0-5,000)
Phage isolated on	10 ^{3.4}	10 ^{3.8}	10 ^{2.9}	10 ^{2.7}	10 ^{2.3}	$10^{1.7}$	10 ^{2.5}	$10^{1.9}$
S. typhimurium LT2 (RP1) (pfu/100mL)	(500-11,000)	(400-97,000)	(0-86,000)	(0-340,000)	(0-4,000)	(0-9,800)	(0-640,000)	(0-9,000)

[a] All chemical values are mean and standard deviation of all samples collected from each farm over a period of eight months from November 2004 to June 2005. Microbial values are reported as the geometric mean with the ranges presented in parenthesis.

RESULTS AND DISCUSSION

The mean data with standard deviations for each parameter for all the farms are given in table 2.

The variations in pH among all farms except Farm 6 are small but statistically significant. Alkalinity of Farm 6 waste water samples is probably due to usage of a very high concentration of chlorine to clean the milking unit which is evident from the chloride ion concentration. There was a significant effect of seasonal variation on the pH of the waste water at 0.05 probability level; however farm size did not have any effect on the pH of the waste water (table 3 and 4).

Total solids concentration showed significant (p < 0.05) variation among samples collected from different farms (table 3) with concentrations ranging from 6.96 to 16.3 g/L. There was a wide variation in the samples collected from the same farm, varying with the total amount of manure produced during milking and the subsequent amount of water used to wash the manure away. Among the large size farms, Farm 2 had very low TS concentration, 7.8 ± 3.9 g/L. This was because this particular farm utilized a large volume of water to clean the milking parlor. However, comparing the means of the samples from different farms showed that the samples collected from the large-sized farms had higher TS concentration followed by mid-sized and small farms though the effect was statistically insignificant (table 4). These values reflect the general expectation that as the number of

Table 3. Significance levels (p-values) of the main effect of different
farms and season on milk house waste water characteristics.

Parameter		Farms ^[a]	Seasonal Variation
pН	< 0.0001	0.025	
Total solids (g	/L)	0.027	0.010
COD		< 0.0001	NS
BOD		0.004	NS
Fat (%)		< 0.0001	< 0.0001
Chloride (mg/l	L)	< 0.0001	NS
Sulfate (mg/L)	1	< 0.0001	NS
Nitrate (mg/L)		0.028	0.001
Total Kjeldahl	nitrogen (mg/L)	0.009	NS
Total phosphor	< 0.0001	NS	
Bacteria	Log E. coli (cfu/100mL)	< 0.0001	NS
	Log total coliform (cfu/100mL)	0.001	NS
	Log enterococci (cfu/100mL)	< 0.0001	NS
Bacterionhage	Log somatic colipahge (pfu/100mL)	< 0.0001	NS
Bacterrophage	Log F+ specific coliphage (pfu/100mL)	NS	NS
	Log phage isolated on <i>S. typhimu- rium</i> LT2 (RP1) (pfu/100mL)	<0.0001	<0.0001

 [a] NS denotes the main effect was not significant at the 0.05 probability level.

Table 4. Significance levels (p-values) of the main effect of
farm size on milk house waste water characteristics.

Parameter		Farm Size ^[a]		
pН	NS			
Total solids (g/	NS			
COD		0.002		
BOD		NS		
Fat (%)		NS		
Chloride (mg/l	L)	< 0.0001		
Sulfate (mg/L)		< 0.0001		
Nitrate (mg/L)	0.005			
Total Kjeldahl	< 0.0001			
Total phosphor	0.001			
Bacteria Log E. coli (cfu/100mL)		0.001		
	Log total coliform (cfu/100mL)	0.009		
	Log wnterococci (cfu/100mL)	0.002		
Bacteri-nhage	Log somatic colipahge (pfu/100mL)	0.034		
Bueten phage	Log F+ specific coliphage (pfu/100mL)	NS		
	Log phage isolated on <i>S. typhimurium</i> LT2 (RP1) (pfu/100mL)	NS		
[a] NS denotes the main effect was not significant at the 0.05 probability level				

cow increases, the amount of waste water produced per cows goes down, thus increasing the TS concentration. Both pH and total solids exhibited a significant effect of season variation at a 5% significance level (table 3).

Both BOD and COD values indicate the level of pollution of the waste water. BOD is the major criteria used in water pollution control and is an important factor in the design of treatment facilities. There were wide variations in both these parameters varying from farm to farm and day to day. COD ranged from 6.6 to 15.8 g/L whereas the range of BOD was very low (0.01 to 0.07 g/L). This huge difference between the COD and BOD values (table 2) is probably due to the presence of biologically resistant organic matter in the waste water. Statistical analysis (ANOVA) of the data showed that statistical analysis (ANOVA) of the data showed that seasonal variation did not affect the BOD and COD of the waste water. However COD values were significantly affected by different farms and farm size.

The amount of fat in the waste water sampled depended on the amount of milk disposed of due to mastitis. Its concentration ranged from 0.1% to 0.4% and was significantly different among different farms. Analysis of variance (ANOVA) indicated a seasonal variation but there was no particular trend. Farm size did not have any effect on the fat concentration in the waste water samples.

Dairy farms utilize large amounts of cleaning and disinfectant chemicals which are a rich source of chloride, sulfate, and phosphate ions. The amount of detergent and chemicals used to clean the milking unit is constant when an automatic system is employed. However, a few of the farms had a manual system whereby the amount of detergents and chemicals used varied considerably, which accounts for wide variation in these ion concentrations in the samples from different farms. Chloride, sulfate, and ortho-phosphate concentration varied significantly with different farms. Farm 6 had the highest concentration of chloride and sulfate ions which is probably due to excess usage of disinfectant chemicals and cleaning agents. Sulfates and chloride concentration were significantly influenced by farm size whereas seasonal variation had a significant effect on nitrate concentration. High concentrations of total phosphorus (TP) in waste water samples are of particular concern since phosphorus is usually the limiting nutrient in fresh water. Significant differences in Total Kjeldahl nitrogen (TKN) and Total phosphorus (TP) were detected at the 5% probability level between samples from different farms (table 3). As expected, large-sized farms had higher concentration of TKN and TP followed by mid- and small-sized farms. Seasonal variation did not have any significant effect on TN and TP concentrations.

Both fecal coliform and bacteriophage levels are an indicator of water quality with respect to its potential to carry and cause disease input by fecal material. Coliforms are naturally present in the environment and in fecal materials and their significance is best understood when their numbers are compared to those of the thermo-tolerant fecal coliforms, such as E. coli which come from human and animal fecal waste. Their presence is not a health threat in itself, but it is used to indicate whether other potentially harmful bacteria may be present as there are defined relationships between the numbers of these indicators and other bacterial pathogens. The milk house waste water had very high concentration of coliforms, close to the levels normally seen in domestic human sewage, indicating that if this water is discharged without treatment, the result would be streams that would not meet the recommended standards for water contact or use as a potable source depending upon the level of dilution (table 2). The majority of Total Coliforms isolated from the waste water were thermotolerant E. coli, a subgroup of coliforms that possess the enzyme to split lactose into glucose and are hence more closely related to fecal materials rather than naturally occurring soil microbes. The relationship between the numbers of E. coli. and Enterococci present was variable with Enterococci concentrations less numerous than the coliforms, but again in levels that would violate recommended surface water standards.

It is well known that the bacterial indicators are not appropriate indicators for other types of pathogens that survive longer in the environment. That is why analysis for different groups of bacteriophage, viruses that infect bacteria, is useful in estimating the fate and transport of pathogenic viruses during treatment processes. While there has been some study on the prevalence of bacteriophages in fecal material directly gathered from animals, their presence in milk house waste is not as well studied. Therefore their use as a treatment evaluation device needed to be substantiated. The concentrations of coliphages were less than for the bacteria, with somatic coliphage present at higher numbers, and isolated with greater frequency than for F+ specific coliphage. The bacterial population exhibited consistent values compared to bacteriophages over time which explains non-significant effect of seasonal variation, while the bacteriophage groups were not consistently isolated. Bacteriophages concentrations in the waste streams were inconsistent, varying from none to a high of 780,000/ 100 mL. F+ specific coliphage were rarely found in the waste water samples (table 5). Brion et al. (2002) also observed infrequent isolation of F+ coliphage from animal impacted surface waters. On the other hand, phages isolated on Salmonella typhimurium LT2 (RP1), which suppresses isolation of somatic salmonella phage and encourages the growth of

Table 5. Frequency of isolation of each phage group from each farm.

	Farm ID	n	Somatic Coliphage (pfu/100mL)	F+ Specific Coliphage (pfu/100mL)	Phage Isolated on S. typhimurium LT2 (RP1) (pfu/100mL)
Large-size	Farm 1	15	15	7	13
farms	Farm 2	15	14	6	13
	Farm 3	8	5	4	5
	Farm 4	8	7	1	5
Mid-size	Farm 5	8	7	3	6
farms	Farm 6	14	9	4	7
Small-size	Farm 7	8	6	3	5
farms	Farm 8	15	9	2	7

human sewage associated lipid phages such as PRD1, were found in more than 50% of the waste water samples indicating that animals may also be a source of these types of salmonella phage in the environment. Analysis of variance carried out for farm size indicated that there were no significant differences between bacteriophage numbers [F+ specific coliphage and phages isolated on *Salmonella typhimurium* LT2 (RP1)] for samples collected from different farms at the 5% level. However, coliform numbers were significantly affected by the farm size.

From this study, it is clear that the milk house waste water has a very high concentration of nutrients (nitrogen and phosphorus) and organic matter and that it should be treated before discharging. Nitrogen, phosphorus, and organic matter may be removed simultaneously if the different phases of the SBR system are cycled appropriately. For milk house waste water treatment, the appropriate sequencing batch reactor cycle will be anaerobic-aerobic-anoxic-aerobic. During the anaerobic phase, the phosphorus accumulating organisms will take up volatile fatty acids, storing them as polyhydroxyalkanoates (PHA) and the intracellular polyphosphate will be hydrolyzed to phosphate, which will then be released in to the liquid media (Smolders et al., 1994). During the aerobic phase, nitrification will occur and the phosphorus accumulating organisms will take up soluble phosphorus by utilizing nitrates or oxygen, as the final electron acceptor (Stevens et al., 1999). Phosphate and COD will also be removed during the anoxic phase, since stored PHA will be used simultaneously for denitrification and phosphate uptake. Different hydraulic retention times and sludge retention times will also be studied. In this study, the concentrations of chlorides, sulfates, and nitrates were low; however it will be important to determine how these levels affect the anaerobic-aerobic-anoxic-aerobic strategy. The effect of fat content on the efficiency of the SBR has not been studied to date and will be an important parameter when the treated waste water is analyzed

CONCLUSIONS

Presence of fecal coliform and coliphage indicate that the waste water from milk house is contaminated and should not be allowed to discharge into streams. The waste water varied significantly in nutrient content from farm to farm. Grouping the farms into large-, medium-, and small-sized farms also had a significant effect on nutrient content and the number of microorganisms in the waste water. Of all the parameters analyzed, fat, nitrate, and ortho-phosphate content exhibited

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