DAIRY WASTEWATER TREATMENT WITH EFFECTIVE MICROORGANISMS AND DUCKWEED FOR POLLUTANTS AND PATHOGEN CONTROL

M.T. Rashid and J. West
Department of Land Resource Science
University of Guelph, ON, N1G 2W1, Canada

Corresponding author: trashid@uoguelph.ca

Abstract:

Wastewater originated from dairy operations may harbor human pathogens including *Escherichia coli* (*EC*). Excess nutrients present in dairy wastewater can also pollute surface and ground waters. Effective microbes (EM) and duckweed have shown a great promise in wastewater treatment. The duckweed growth and EM applications were tested. Combined application of EM and duckweed growth significantly reduced the ammonium nitrogen, total phosphorus, total suspended solids and biological oxygen demand after three months and is a very efficient way of dairy wastewater treatment.

Keywords: Effective microbes, duckweed, total suspended solids, biological oxygen demand, *Escherichi coli.*

1. INTRODUCTION

In the Walkerton, Ontario, Canada, tragedy of May 2000, contamination of the municipal water system with *EC* O157:H7 and *Campylobacter jejuni* resulted in 2,300 people (out of 5,000) requiring medical attention, 7 of them died [1]. Investigation into the causes of the microbial contamination of the municipal well water indicated that the most likely cause was transport of manure bacteria to the aquifer by infiltrating water, although direct entry of surface runoff into the well could not be ruled out [2]. Wastewater originated from dairy operation may harbor different bacterial species including human pathogens such as enterohaemorrhagic *EC* [3, 4]. Cattle are considered a major reservoir of *EC* [5], this pathogen may potentially infect the drinking water supply from cattle wastewater [6]. Outbreaks are usually associated with consumption of contaminated food or drinking water exposed to pathogen laden animal manure or contaminated irrigation water [7].

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Discharging wastewater with high levels of phosphorus (P) and nitrogen (N) can result in eutrophication of receiving waters, particularly lakes and slow moving rivers. To prevent these conditions, regulatory agencies in many countries have imposed nutrient discharge limits for wastewater effluents. Recently restrictions on P discharge have become more stringent due to environmental problems. Dairy operations have been identified as a potentially significant source of nitrate [8] and phosphorus [9] contamination in groundwater.

Current mainstream technologies for wastewater treatment, such as the activated sludge process with N and P removal, are too costly to provide a satisfactory solution for dairy wastewater treatment specially in developing regions. Biological treatment processes are inexpensive and are known for their ability to achieve good removal of pathogens nutrients and organic pollutants. Duckweed-based pond system could be an attractive technology for wastewater treatment aiming at nutrient recovery and reuse [10].

Effective microorganism's technology (EM) was developed by Dr. Teuro Higa in 1970's at the University of Ryukyus, Okinawa, Japan. First solution contained over 80 microbial species from 10 genera, isolated from environments in Japan, however with time, the technology was refined to include only lactic acid bacteria, phototrophic bacteria, and yeast [11]. The innoculum includes high populations of lactic acid bacteria (*Lactobacillus* and *Pedicoccus*) at 1×10^8 cfu ml^{-1} suspension, yeast (*Saccharomyces*) at 2×10^6 cfu ml^{-1} suspension and phototrophic bacteria, 1×10^3 cfu ml^{-1} [12]. Application of EM in septic systems, lagoons, activated sludge systems, and other remediation projects has reduced water quality indicators such as biological oxygen demand and chemical oxygen demand [13]. Pig manure odor and coliform bacteria were drastically reduced when treated with EM [14].

In recent years, research has focused on duckweed and its role in wastewater treatment and potential for nutrient recovery [15]. The treatment of wastewater by duckweed reduces the wastewater contaminants either directly through the nutrient recovery or indirectly by release of oxygen in the water column [16]. Treatment systems with protein production using duckweed represent a comprehensive solution for wastewater treatment [17]. Duckweed wastewater systems have also been studied for dairy wastewater [18].

2. MATERIALS AND METHODS

A wastewater treatment pilot pond was constructed by dividing the existing dairy wastewater pond (16 × 25 m) into four equal portions at Dwany Dairy Farm, Ontario, Canada" (43° 7′ 60N, 80° 45′ 0W; 298 m above sea level). Farmer stores dairy wastewater in this pond and it is used to dilute the liquid dairy manure during hauling by pumping it back to the dairy barn. The experiment was conducted in 2004–2005 for two years during June to August; temperature varies between 25 and 32°C). The treatment system was in an open field exposed to weather conditions. Each of the 4 blocks of the main wastewater holding pond was assigned to following treatments. Block-1 was kept as control (untreated), Activated EM was applied to block-2 after every 2 weeks for three months at 1:100 ratio (1 part EM and 100 parts water; 6 applications). Duckweed (*Lemna gibba*) plants were grown in block-3 and block-4 received both treatments (EM application and duckweed growth). Duckweed plants were transferred into block 3 and 4 manually and acclimatized for a month (May) with wastewater. Wastewater samples were taken after every 15 days before the application of EM.

All samples were sent to a testing laboratory for total suspended solids (TSS), biological oxygen demand (BOD), NH_4-N, NO_3-N, total P, *EC*, and total coliform bacteria. Duckweed was also harvested after every 15 days and was incorporated in nearby manure piles. Analysis of variance for the data on TSS, BOD, NH_4-N, NO_3-N, total P, *EC*, and total coliform counts were performed by CoStat 6.3 statistical analysis program [19]. Data was collected for two years to: (1) investigate the performance of effective microbes and duckweed for dairy wastewater treatment and (2) to determine the effect of effective microbes and duckweed on reduction of nutrient and pathogenic bacteria from dairy wastewater.

3. RESULTS AND DISCUSSION

Chemical and biological properties of pretreated dairy farm wastewater are presented in Table 1. Chemical and biological analysis have not shown much differences between dairy wastewater samples taken in 2004–2005 in pH, TSS, BOD, NH_4-N, NO_3-N, Total P, *EC*, and total coliform counts. Obvious reason for this consistency could have been the same source, same time of the years and same dairy farming practices performed during the years.

3.1. Wastewater pH, TSS, and BOD

The pH in dairy wastewater ranged from 7.00 to 7.70 (initial values were 7.5 and 7.45 for 2004–2005) and was not drastically changed in all treatments during the course of the experiment (Table 2). Dairy farm wastewater pH values of all samples from all treatment ponds generally were near neutrality or basic with a very small variation (+1.33 to –6.67% decrease or increase). The application of EM (block-2) reduced the pH from 7.5 to 7.0 but it was still in the pH range where microbes exhibit optimal growth. Most microorganisms exhibit optimal growth at pH values between 6.0 and 8.0 and most can not tolerate pH levels above 9.5 or below 4.0 [20]. The declining pattern in TSS concentrations due to different dairy wastewater treatments is illustrated in Figure 1. Maximum temporal decrease in TSS contents was observed in block-4 where EM application to dairy wastewater was combined with duckweed growth followed by block-3 (duckweed alone), block-2 (EM alone) and block-1 (control), respectively. Total suspended solids in block-4 decreased from 380 to 65 mg l^{-1} at the end of experiment and an average TSS concentration reduction of 83% was observed in (Table 2) which was significantly higher (p >0.05) compared to EM application, duckweed growth alone and control treatments.

Table 1. Initial chemical and biological analysis before the start of experiment

Parameter	2004	2005
pH	7.50	7.45
TSS (mg l^{-1})	390	375
BOD (mg O_2 l^{-1})	680	670
NH_4-N (mg l^{-1})	72.00	68.00
NO_3-N (mg l^{-1})	6.00	6.70
Total P (mg l^{-1})	19.62	20.72
E. coli counts (cfu 100 ml^{-1})	5,000	5,200
Total coliforms (cfu 100 ml^{-1})	10,000	10,500

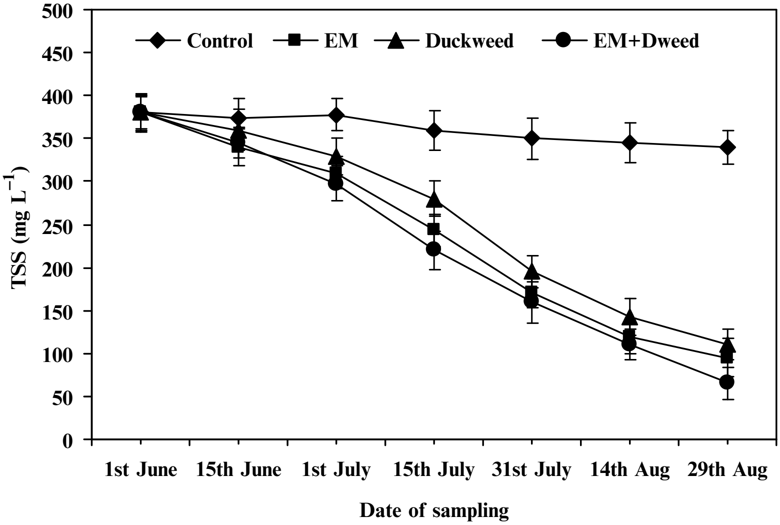

Figure 1. Reduction in TSS due to EM application and duckweed growth

The reduction in TSS in block-2 and block-3 was 75 and 71% respectively with no significant difference between these treatments. Least reduction in TSS contents (11%) was observed in control block. Duckweed removed 77% of TSS from a domestic wastewater pond [21]. Total suspended solids in duckweed ponds are mainly reduced by sedimentation process and biodegradation of organic matters, assimilation by duckweed roots and inhibition of algal growth [22].

Table 2. Effect on total TSS, BOD, NH_4-N, NO_3-N, total P contents, *EC*, and total coliform counts in water samples after the completion of the experiment

Chemical parameter	Control	EM application	Duck-weed growth	EM + Duck-weed	LSD
	(Percent reduction)				
pH	+1.33[a]	–6.67[b]	+1.33	–2.66	
TSS (mg l^{-1})	11 c	75 b	71 b	83 a	5
BOD (mg O_2 l^{-1})	20 c	94 a	77 b	95 a	7
NH_4-N (mg l^{-1})	16 d	44 c	72 b	86 a	7
NO_3-N (mg l^{-1})	37 b	43 b	57 a	67 a	11
Total P (mg l^{-1})	13 b	23 b	98 a	99 a	10
E. coli counts-cfu 100 ml^{-1}	29 b	71 a	75 a	75 a	6
Total coliforms-cfu 100 ml^{-1}	22 b	63 a	67 a	70 a	9

[a]Increase in pH of wastewater, [b]Decline in pH of wastewater

Duckweed plants in principle could also contribute to treatment process by direct assimilation of simple organic compounds, such as simple carbohydrates and various amino acids [23]. Largest reduction of TSS in this pond was probably due to transformation of complex organic molecules into simple organic compounds by yeast (facultative microorganisms) present in EM consortium [24] and then these compounds could have been assimilated by duckweed roots [16]. Biological oxygen demand (BOD) is defined as the amount of oxygen required to oxidize the organic content of wastewater and it is also the oxygen available to micro-organisms within the system. The patterns of a decline in BOD due to different dairy wastewater treatments are illustrated in Figure 2.

Maximum temporal reduction in BOD was observed in block-2 (94%) and block-4 (95%), where EM was applied alone or in combination with duckweed growth (Table 2). The reduction in BOD in both treatment blocks was statistically non significant, however, the reduction due to duckweed growth alone (77%) was significantly lower compared to EM application alone and EM application plus duckweed growth. However, reduction in BOD due to duckweed growth was significantly higher compared to control. The data regarding temporal reduction in BOD clearly show that application of EM was the most effective treatment for dairy wastewater treatment either alone or in combination of duckweed growth. Effective microbes are successfully being used in wastewater treatment in Japan and are becoming popular in wastewater treatment in many countries. The chemical oxygen demand (COD) and BOD of domestic wastewater were significantly reduced when treated with EM [25].

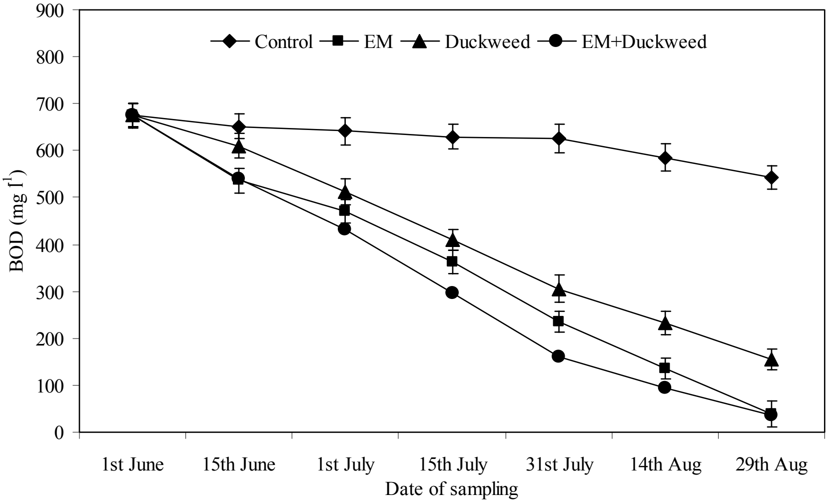

Figure 2. Reduction in BOD due to EM application and duckweed growth

Duckweed plants have been shown to release oxygen to wastewater at the rate of 3–4 g m^{-2} d^{-1} and this release of oxygen improves the oxygen supply. The wastewater with lower BOD always remains aerobic and duckweed can remove 70–96% of wastewater BOD [26]. Degradation of organic matter is enhanced by duckweed through addition of oxygen supply and additional surface for microorganisms responsible for organic matter decomposition [27]. Effective microbes might have been more active in block-4 due to the presence of duckweed plants as they provide more surfaces for the survival of microbes and this synergistic association might have accelerated the organic matter degradation.

3.2. *Escherichia coli* and Total Coliform Counts

The declining trends of *E. coli* and total coliforms in airy wastewater treatment blocks were illustrated in Figures 3 and 4, respectively. Maximum reduction in *EC* and total coliform counts in dairy wastewater initial counts were observed in block-4 (75 and 70%) where EM was applied in combination with duckweed growth. In this block the *EC* bacterial counts were reduced from 5,100 cfu 100 ml^{-1} to 1,300 cfu 100 ml^{-1}.

Coliform bacteria were reduced from 10,000 cfu 100 ml^{-1} to 3,000 cfu 100 ml^{-1}. Reduction in pathogenic bacteria in block-4 was not significantly different from blocks where EM was applied or duckweed was grown alone. These results show that duckweed and EM application were equally affective in the removal of pathogenic bacteria. The total coliform bacteria were reduced by 95% by growing duckweed for domestic wastewater treatment [28]. Pathogen removal is of utmost importance in case of dairy farm wastewater reuse for different purposes at the farm. Die-off of pathogenic bacteria is considered to be a complex phenomenon in waste stabilization ponds. Removal of *EC* and coliforms in duckweed ponds was probably through two main processes. First, the recovery of nutrients from the pond may have caused a deficiency in these nutrients required for microbial growth. Second, the adsorption of these bacteria to the duckweed followed by harvesting might have played a role in their removal [15].

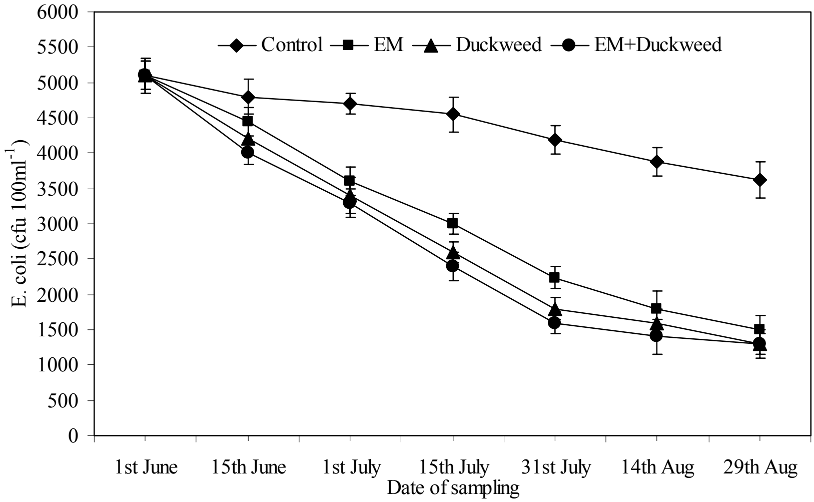

Figure 3. Reduction in *EC* counts due to EM application and duckweed growth

Effective microbe's innoculum have high populations of lactic acid bacteria (*Lactobacillus* and *Pedicoccus*), yeast (*Saccharomyces*) and phototrophic bacteria. Lactic acid bacteria present in EM produce lactic acid and other antimicrobial products as a result of carbohydrate metabolism [29]. During the biodegradation of organic particles in wastewater lactic acid bacteria might have produced antimicrobial products having antibacterial properties [30] and might have inhibited the growth of *EC* and total coliforms. Total and fecal coliforms in fish ponds receiving manure from EM-treated pigs were significantly lower than those from the control [14].

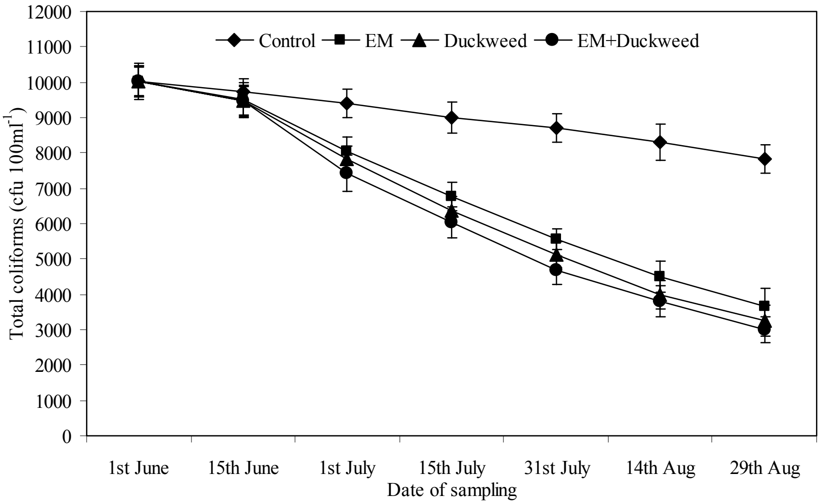

Figure 4. Total coliform counts due to EM application and duckweed growth

4. CONCLUSIONS

Combined application of EM and duckweed growth in dairy wastewater stabilization pond significantly reduced the NH_4-N, total P, and BOD compared to control treatment after three months. A threefold to fourfold reduction in total counts of *EC* and total coliforms also recorded after three months. As differences in the reduction of most of wastewater quality parameters due to EM application and duckweed growth were negligible, we suggest that any of the treatment option alone or in can be adapted for dairy wastewater treatment for its disposal to natural water streams or its reuse at the farm.

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