

Role of Bacterial Additives for Biological Treatment of Domestic Wastewater in Septic Tank

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Abstract: The main objective of this study was to evaluate the efficiency of bacterial additives for domestic wastewater treatment biologically in septic tanks. This research was carried out on two stages. The first stage use different doses of Biowash bacterial additives (100, 150, 200, 250 and 300 mg/L) in a bench scale system that consists of five chambers septic tanks to obtain the optimum dose. On the second stage the effluent wastewater treated with the optimum dose of Biowash bacterial additives would pass through four biofilters to improve the effluent treated wastewater quality in order to reuse it. The physico-chemical and bacteriological parameters for raw and treated effluent of DWW were analyzed. The obtained results revealed that the level of organic load represented by COD and BOD removal were reduced up to 98.3 and 96.6% from. Moreover, the fecal coliform count was reduced by about 5 log units. The quality of the treated wastewater was found to be within the permissible Egyptian standards. Thus, septic tank system with bacterial additives is a better technology for DWW treatment in small areas.

Key words: Domestic wastewater, septic tank, bacterial additives, coliform group, technology, treatment biologically

INTRODUCTION

Domestic wastewater treatment played a major challenge for civilizations during the last decades. Recently, it has been at the fore front of major concerns in most new and existing communities. The increase in population, rise in cost of treatment and through regulatory standards have made it necessary to analyze how we deal with our waste. Across the country, scientists working with communities are attempting to find alternative solutions to upgrade wastewater treatment processes focusing on increasing the removal efficiency of pollutant loads from municipal and industrial wastes. Wastewater contains a great variety of contaminants such as solids, organic and inorganic compounds present either in dissolved or suspended forms, heavy metals, adsorptive organic halogen compounds. These wastewater contaminants may be toxic or mutagenic. They may be biodegradable, difficult to biodegrade or non-biodegradable (Tang *et al.*, 2012).

Conventional biological treatment is employed to treat wastewater to meet the regulations requirements however, treatment technologies are usually too

expensive, therefore more attention should be given for innovative and alternative solutions (Bratby, 2006). Furthermore, septic tank system is considered as a most commonly known primary treatment method for onsite wastewater treatment. Where as, it can be remove the most settleable solids and function as an anaerobic bioreactor that promotes partial digestion of organic matter (Dawes and Goonetilleke, 2003). Also, it is a low cost and simple to operate and maintain although, sludge may cause an odor problem if left untreated for a long time (He *et al.*, 2012). In addition to primary treatment, the septic tank can reduce the sludge and foam volumes up to 40%. It also conditions the wastewater by hydrolyzing organic molecules for subsequent treatment in the soil or by other unit processes. Septic tanks are used in nearly all onsite systems regardless of the daily wastewater flow rate or strength. The tanks provide suspended solids removal, solids storage and digestion (US EPA., 2002). Three zones are present in a septic tank; a scum layer which forms a crust on the surface of the tank liquor; the wastewater from which solids deposit and a bottom sludge layer of deposited material.

The anaerobic digestion of organic matter depends on the tank size, frequency of cleaning and temperature. The conventional septic tank removes Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Kjeldhal Nitrogen (TKN) and helminthes eggs to a certain extent (Andreadakis and Christoulas, 1982). By integrating in-tank baffles a better contact can be achieved between the wastewater and the active biomass (sludge), leading to increased treatment efficiencies (Langenhoff *et al.*, 2000). Therefore, the main aim of this study was to evaluate the efficacy of bacterial additives for wastewater treatment biologically in septic tanks.

MATERIALS AND METHODS

Designed system for determining the optimum doses of bacterial additives: In order to study the optimum dose of different doses of Biowash (bacterial additives) for domestic wastewater treatment in septic tank, an anaerobic bench scale system was designed (Fig. 1). The system consists of five tanks each of 60 L capacity, 45 cm diameter and 95 cm height. Each tank was equipped with low speed motor for mixing the Biowash (bacterial additives) with domestic wastewater obtained from Zenien wastewater treatment plant. The Biowash was added to the septic tanks models by different doses as follows 100, 150, 200, 250 and 300 mg/L to determine the optimum doses of the Biowash (bacterial additives).

Designed system of multi stages biofilter: After determining the optimum dose of the Biowash (bacterial additive) a multi stages biofilters was used to enhance the effluent treated wastewater quality in order to reuse it. A continuous pilot scale system was designed as shown in Fig. 2. The domestic wastewater mixed with the optimum dose of the Biowash (bacterial additive) which obtained from the previous experiment in a model of septic tank of 100 L capacity, 45 cm diameter and 95 cm height. The contact time was 2 h and the flow rate was 0.5 L/min. The effluent treated wastewater was filtered through multi stages down flow biofilters. The multi stages biofilters consists of four tanks of 25 L capacity, 25 cm diameter and 40 cm height, filled with 15 cm gravel acting as a filter media. The size of gravels in tanks A and B was 5-6 mm while in tanks C and D was 1-2 mm. The experiment was repeated 4 times.

Sampling collection and procedure: At the first stage of the experiment samples were collected at the effluent of each tank of the bench scale septic tanks system. At the second stage samples were collected at the effluent of septic tank model and biofilters. Sample bottles were placed in a cooler containing ice and moved to the lab and kept in the fridge until tested. Tests were conducted within 24 h after samples were taken.

Physico-chemical characteristics: Physico-chemical parameters including pH, Chemical Oxygen Demand (COD) Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), ammonia, Total Nitrogen (TN), Total Phosphorus (TP) and oil and grease analysis were measured for all collected samples according to APHA (2012).

Bacteriological characteristics: Microbiological characteristics of the raw and treated effluents were investigated. Determination of Total Coliform (TC) and Fecal Coliform (FC) counts were carried out according to APHA (2012) using Most Probable Number (MPN) method.

Toxicity test for Biowash (bacterial additives) material: The determination of toxicity for 250 mg/L of Biowash was carried out using both cytotoxicity and Microtox analyzer 500.

Cytotoxicity test: HEP2 cells were grown in DMEM supplemented with 10% phosphate buffered saline, 10,000 U/mL of penicillin and 10 mg/mL of streptomycin. The culture was maintained at 37 C in a humid atmosphere at 5% of CO₂ and a half-open system and trypsinization was used for cell maintenance. For exposure to suspension of 250 mg/L of tested material, cells were seeded at a rate of 2×10^4 cells/well in a 96-well polystyrene microplate. After the cultures reached semiconfluence culture medium was replaced with the concentrated samples at different concentrations and the cells were exposed for 24 h.

Cells cultured in standard conditions were used as a negative control. All assays were repeated in quadruplicates in two independent experiments that had the same result profile.

Toxicity assay using microtox analyzer 500: Microtox analyzer Model 500 is the fully automated and temperature

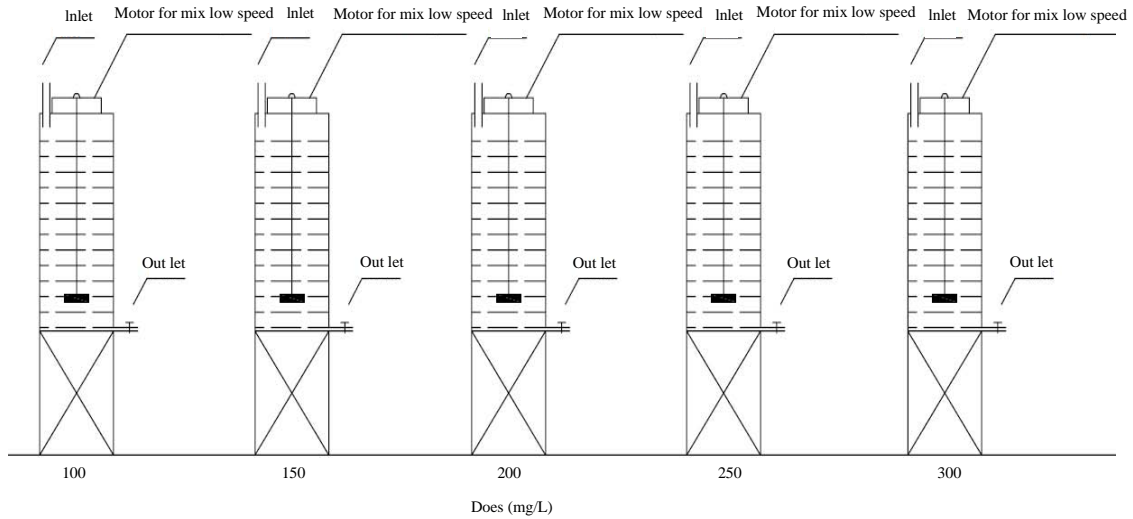


Fig. 1: Schematic diagram of bench scale of septic tanks with different (bacterial additives) doses

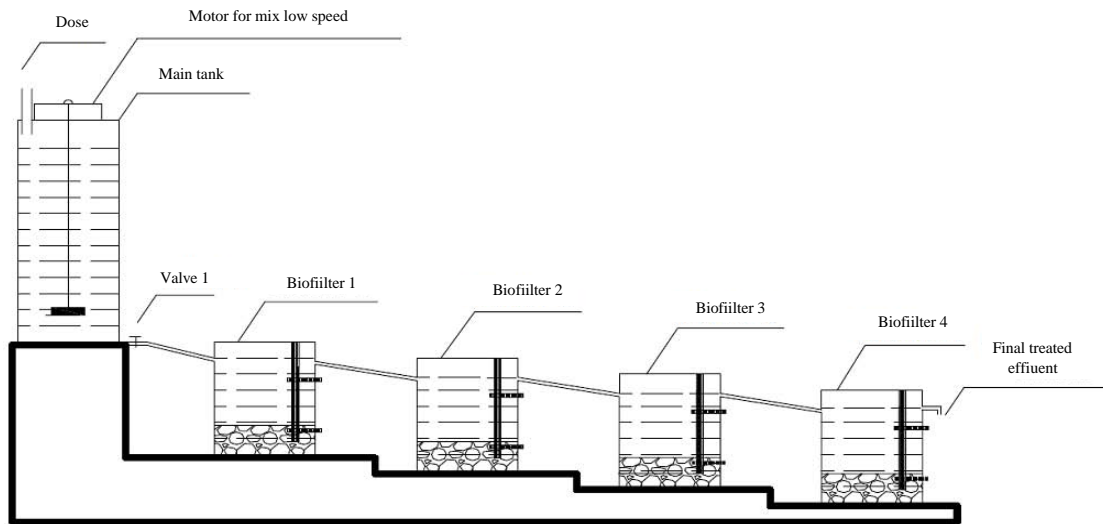


Fig. 2: A schematic diagram of the multi stages biofilter

controlled and needs no daily adjustment or calibration. A marine luminescent bacterium the *Vibrio fischeri* has been widely used for acute toxicity estimation the with several commercial tests.

Antibacterial effect of Biowash material: The suspension of different doses (100, 150, 200, 250 and 300 mg/L) of Biowash material was tested against different microbial strains including; *Listeria monocytogenes* ATCC25152, *Staphylococcus aureus* ATCC6538, *Pseudomonas aeruginosa* ATCC15442, *E. coli* ATCC13706 and *Candida albicans* ATCC10231 by using disc diffusion method.

RESULTS AND DISCUSSION

The characteristics of the domestic wastewater which initially inoculated to septic tank were shown in Table 1. This corresponds to high strength wastewater according to the classification by Metcalf and Eddy Inc. (2003). The ratio of COD: BOD for domestic wastewater was around 2.3:1; this provides a good indication that the wastewater can be treated biologically. These values are in agreement with Nasr *et al.* (2009) who stated that for domestic sewage which is known to be readily biodegradable and can be treated successfully worldwide using a variety of biological treatment methods, the

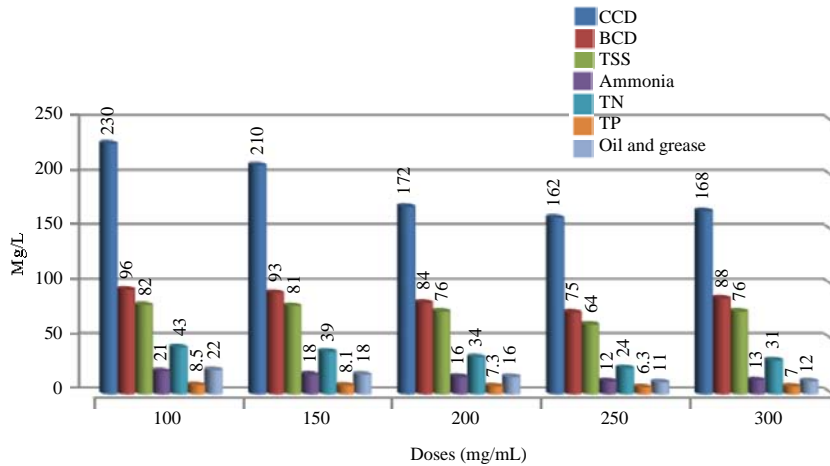


Fig. 3: The level of physico-chemical parameters after exposure to 100, 150, 200, 250 and 300 mg/L of Biowash

COD/BOD ratio varies from 1.5-2. With regard to the nutrient requirements of the wastewater micro-organisms, the average BOD/TKN/TP ratio was 100:16:1. Fecal coliform concentrations recorded an average value of 2.3×10^6 MPN-Index/100. These values are in agreement with results obtained (Nasr *et al.*, 2009).

The suspension of Biowash (250 mg/L) material had antimicrobial effect against tested microorganisms (Table 2). Regarding toxicity assay, the results for the suspension of Biowash (250 mg/L) material found that this material was nontoxic against cell culture.

Determining the optimum and effective dose of Biowash (bacterial additives): Different doses of Biowash (100, 150, 200, 250 and 300 mg/L) were studied to achieve the most effective dose. Results illustrated graphically in Fig. 3 and 4 found that 250 mg/L of Biowash was considered as the optimum dose for decreasing Physico-chemical (COD, BOD, TSS, ammonia, TN, TP and oil and grease). Also, results indicated that this dose able to kill the total and fecal coliform.

Regarding physico-chemical parameters results, it can be found that COD, BOD, TSS, ammonia, TN, TP and oil and grease for final effluent (after third chamber) with 100 mg/L (lowest dose) of Biowash were 230, 96, 82, 21, 43, 8.5 and 22 mg/L, respectively. While at 250 mg/L (highest dose) results were 162, 75, 64, 12, 24, 6.3 and 11 mg/L, respectively (Fig. 3).

In case of bacteriological analysis, results found that TC and FC at lowest dose were 2.1×10^3 and 1.0×10^3 MPN-Index/100 mL. At highest dose, results of TC and FC were 1.0×10^3 and 1.0×10^2 MPN-Index/100 mL (Fig. 4). This may be due to increase the dose of bacterial additive which can be able to reduce the organic load and inhibit the bacterial populations.

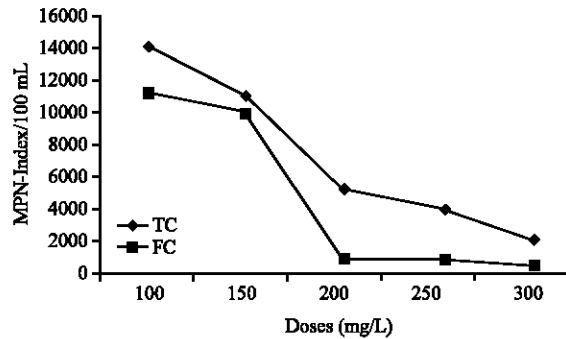


Fig. 4: The level of TC and FC after exposure to 100, 150, 200, 250 and 300 mg/L of Biowash

Table 1: Characterization of the feeding domestic wastewater to septic tank system

Parameters	Unit	Results
pH	-	7.4
COD	mgO ₂ /L	1372
BOD	mgO ₂ /L	257
TSS	mg/L	302
Ammonia	mg/L	21
NO ₂	mg/L	0.02
NO ₃	mg/L	0.30
Total Phosphorous (TP)	mg/L	1.3
Oil and grease	mg/L	50
Total coliform	MPN-Index/100 mL	6.8×10^6
Fecal coliform	MPN-Index/100 mL	2.3×10^6

The conventional septic tank removes Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Kjeldhal Nitrogen (TKN) and helminthes eggs to a certain extent (Andreidakis and Christoulas, 1982). By integrating in-tank baffles, a better contact can be achieved between the wastewater and the active biomass (sludge), leading to increased treatment efficiencies (Langenhoff *et al.*, 2000).

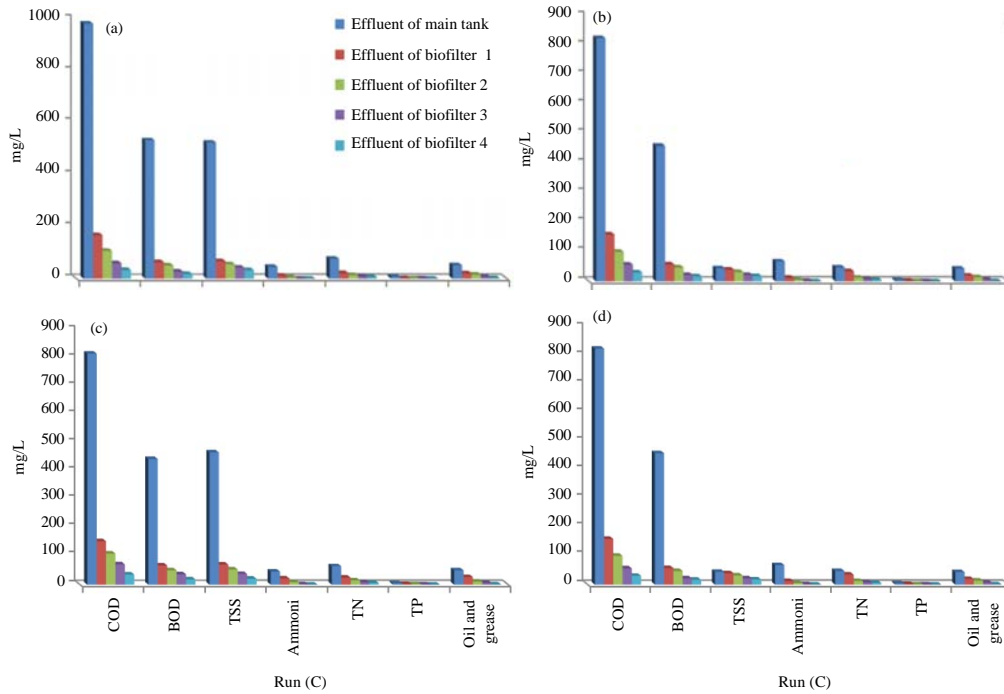


Fig. 5: Variation of physico-chemical parameters at different runs

Table 2: Antimicrobial effect for Biowash material

Biowash doses (mg/L)	<i>Listeria monocytogenes</i>	<i>Staph. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>Candida albicans</i>
100	+	+	+	+	+
150	++	+	++	+	+
200	++	++	++	+++	++
250	+++	+++	+++	+++	+++
300	+++	+++	+++	+++	+++

+: low effect; ++: moderate effect; +++: highly effect on microorganisms

Efficiency of biofilters using the optimum dose of Biowash

Effluent of run A: By subjecting the raw domestic wastewater to the chamber 1, the removal percentage of COD, BOD, TSS, ammonia, TN, TP and oil and grease was 98.3, 96.6, 93.7, 100, 90.9, 97.5 and 96.1%, respectively (Fig. 5). While, the level of TC and FC counts was 1.3×10^2 and 98 MPN-Index/100 mL (Fig. 6).

Effluent of run B: Regarding the results of the removal percentage of COD, BOD, TSS, ammonia, TN, TP and oil and grease was 96.2, 96.3, 95.9, 100, 87.3, 97.8 and 97.7%, respectively (Fig. 5). While, the level of TC and FC counts was 89 and 65 MPN-Index/100 mL (Fig. 6b).

Effluent of run C: Results of the removal percentage of final effluent for the COD, BOD, TSS, ammonia, TN, TP and oil and grease were 95.5, 95.7, 95.5, 100, 90.8, 97.9 and

98.0%, respectively (Fig. 5c). While, the level of TC and FC counts was 1.0×10^2 and 91 MPN-Index/100 mL (Fig. 6c).

Effluent of run D: Results of removal percentage for COD, BOD, TSS, ammonia, TN, TP and oil and grease for final effluent were 95.2, 94.2, 96.4, 100, 89.5, 100 and 95.4%, respectively (Fig. 5b). While, the level of TC and FC counts was 1.0×10^2 and 85 MPN-Index/100 mL (Fig. 6d).

These results agree with those obtained by Panswad and Komolmethee (1997) who used a full-scale conventional septic tank/anaerobic filter unit with a retention time varying from 22.5-90 h and achieved percentage removals of 52.1, 56 and 53.6% for COD, BOD and TSS at an average retention time of 22.5 h. Also, results of the present study are also in line with those obtained by Nguyen who obtained average removal efficiencies from 48-65 and 44-69% in terms of COD and TSS, respectively.

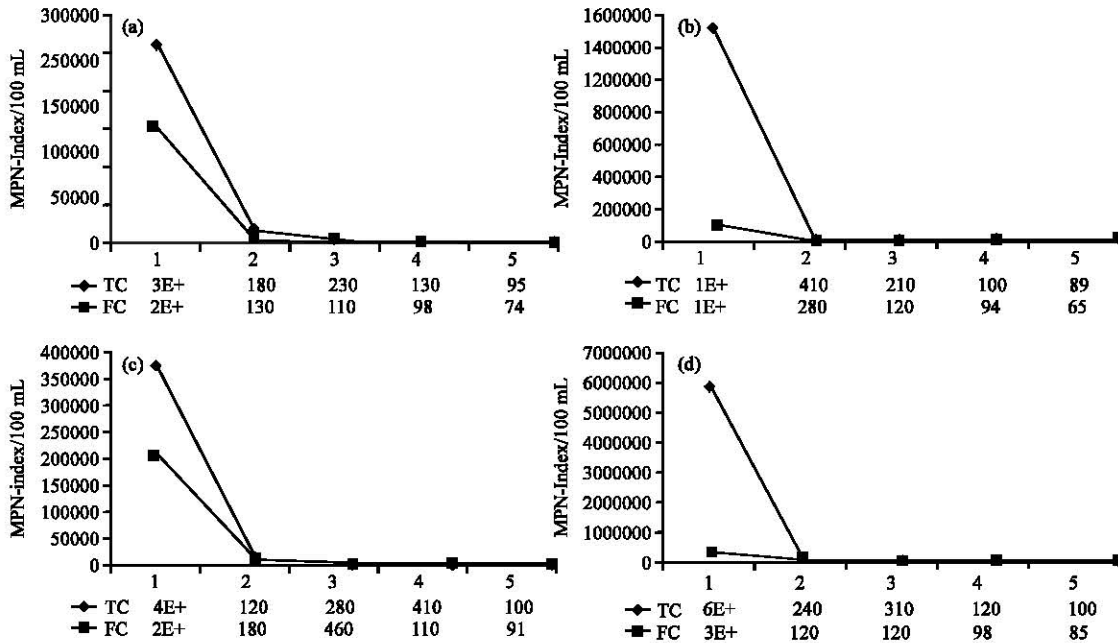


Fig. 6: Variation of TC and FC counts at different runs A, B, C and D

In case of Bacteriological examination of the two-baffle septic tank effluent revealed a removal efficiency of fecal coliforms of 95, 93 and 90% at HRTs of 72, 48 and 24 h, respectively with average residual values in the final effluent of 106 MPN/100 mL. These results agree with those obtained by Kamel and Hgazy (2006).

These results is compatible with Tang *et al.* (2012) who demonstrated that Bio-additives reduced oil and grease deposit formation and thus is helpful for alleviating potential sewer line blockages in wastewater collection systems. Bio-Amp also reduced COD, TN and TP in grease trap effluents which helps alleviate downstream treatment burdens. Bio-additives increased COD fractions which can potentially enhance Bio-P removal in subsequent wastewater treatment processes. Increased removal of unsaturated fatty acids fractions which are more difficult to biodegrade in treatments was also observed.

Biological Wastewater Treatment Systems (BWTSS) have found wide applications not only in developed countries like the Netherlands but also in developing countries such as China, due to increasing demands by the population for clean and safe water resources. BWTSS can efficiently remove Chemical Oxygen Demand/Biochemical Oxygen Demand (COD/BOD), N and P from wastewater and they are also relatively low in operational costs. Therefore, BWTSS have become the dominant systems for water pollution control. Despite considerable research efforts on BWTSS in recent decades it is too early to declare that the current systems are good enough in process design and sustainable

aspects. There are still significant knowledge gaps between our understanding of microbiological processes and the reality of engineering. One of the most critical knowledge gaps is related to endogenous processes in BWTSS (Keller *et al.*, 2002).

CONCLUSION

The 250 mg/L of Biowash (bacterial additives) is an efficient dose for domestic wastewater treatment using septic tank system. The septic tanks with bacterial additives are important treatment systems particularly for the decentralized areas. Employing the proper design of a septic tank as well as an efficient primary treatment system has improved the quality of the Domestic Wastewater (DWW) effluent. The biological treatment by using microorganisms is considered as a low cost, eco-friendly, easy to use technology especially in septic tank system. No problems with odor or insects occurred by employing the septic tank system. The treated DWW in septic tank system can be reused for irrigating the lumber forest trees. Improvement of treated wastewater is indeed an achievement towards the protection of the public health, the environment and the groundwater.

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