Thai Environmental Engineering Journal Vol. 36 No. 1 (2022) : 89-99
www.eeat.or.th

Bacterial Growth Patterns and COD Reduction in Agitated Vessels Treating Polluted Canal Water with Fermented Products Addition

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Abstract

Fermented Products (FPs) are mostly solution form compounds produced by the microorganism fermentation of sugar and/or lactose as carbon and energy sources. In this study, polluted water was taken from Kaja canal in Bangkok City. The experiment with this polluted water were divided in to two parts, that is, the one with sterilized polluted water and another one with non-sterilized polluted water. Both sterilized and non-sterilized polluted waters were experimented with five different types of commercial FPs. The FPs were added into polluted water (either sterilized or non-sterilized) with the ratio of 1:10,000 by volume as suggested by FP manufacturers. Whereas, the vessels without addition of FP were set up as the controls to receive scientific data to prove the benefit of FPs application, especially in treating polluted water. The mixtures (polluted water and FP) were then aerated through moderate agitation continuously for seven days of operation. Then, samples were taken on Days 0, 1, 3, 5 and 7 for bacterial count, while non-filtered COD (COD_T) analysis were monitored only in samples on Day 0 and 7. The growth patterns of total heterotrophs showed the enhancement of growth especially in sterilized pollute water in comparison to non-sterilized one. This was postulated to be the effect of autoclaving (high temperature and pressure) which made the organic matters be more easily degradable. Besides, the SPC, LAB and PNSB growth patterns were similar between no FP addition or different FPs addition, denoting no positive effect from the FP addition. For COD_T removals, the reduction of COD_T after seven-day operation did not show the substantial difference between FPs addition and the 'Control'. In brief, when both the bacterial count and COD_T reduction were considered, there was no clear benefit of the FP addition to the polluted canal water, regarding its water quality improvement.

Keywords : Effective Microorganism (EM); fermented products (FPs); purple non-sulfur bacteria (PNSB); lactic acid bacteria (LAB); wastewater treatment

Introduction

Fermented product (FP) or Effective Microorganisms (EM) is the technology developed by Professor Dr Teruo Higa, the University of the Ryukyu, Okinawa, Japan since 1970's. They have usually been made by composting food, vegetable or fruit wastes and natural microbial inoculums from the fertile soil from various sources. These are usually mixed with molasses to form FP liquid, while some are mixed with appropriate ratio of rice chaff, bran, sand, mold and clay and compressed to form a ball shape, known as EM balls. These fermented products are claimed to help improve polluted water quality [1-3]. The FP believers always explained that their FP solutions consisted of four important microorganisms which are lactic acid bacteria (LAB), photosynthetic bacteria (PB), yeast and actinomyces [4-7].

These microorganisms have been claimed to well treat wastewater especially those physical characteristics, e.g., color, turbidity, odor and debris, because of their acidic by-product. For instance, lactic acid is said to help eliminating odor by neutralization reaction [4]. EM or Effective Microorganism is the trade name of a main FP available in some parts of the world [8, 9]. There are many reasons of FP (or EM) solutions popularization in agriculture, livestock, fisheries and environmental management; e.g., their easy production, friendly operating and wide range applications.

For agricultural management, FP has been used for increasing crop yield, improving agricultural product and soil quality, and preventing the insect infestation [10-20]. For environmental management, FP has been applied for odor elimination of kitchen garbage, waste treatment plant, livestock farm, and also used for treating sludge from wastewater treatment plant and oily sludge from petroleum refinery plant in certain communities [1, 5, 21-25].

Some FPs, which were believed by some to be applicable to reduce organic matter in polluted water, were widely promoted due to its lower cost as well as ease of production and application. These FPs were exaggerated to improve polluted canal water quality during the Thailand severe flood situation in 2011 without reasonably scientific information. Although some researchers claimed that the microorganisms in FP had the ability to reduce the BOD, COD and eliminate odor [24-28], there still had been solid arguments against these claims by others. Moreover, the ability of the effective microorganism (EM) in inhibiting the growth of pathogenic bacteria was mentioned elsewhere [29].

Therefore, this study aimed to investigate the bacterial abundance in five fermented products (FPs) commercially sold in Thai market and their growth patterns. In addition, the treatability of five different FPs in polluted water were determined via the reduction of non-filtered COD (COD_T). Sterilized and non-sterilized polluted water were compared to distinguish the activities of indigenous microorganisms from the effects of FPs addition.

Methodology

Polluted water

This study was setup due to some certain FPs (EM liquid and EM ball) were claimed to reduce odor and turbidity of stagnant flood water during the severe flood in 2011. Some were even exaggerated that FPs can treat polluted water or any wastewater. However, such a polluted water in 2011 was not existed anymore. Hence, the authors chose a polluted canal in Bangkok area to imitate polluted floodwater. Kaja canal is selected to be a source of polluted water in this study because of its deteriorated water quality (like conventional polluted waters in Bangkok) and accessibility. Enough amount of polluted water was taken at once for all experiments to avoid different polluted water characteristics. Their characteristics (Temp, pH, DO, BOD, COD and SS) were analyzed before storage and application in the experiments.

This polluted water was divided into two portions, one of them was sterilized by autoclaving at 121˚C and 15 psi pressure for 15 minutes to destroy microorganisms in polluted water before it was experimented. The rest was directly used in the experiments as non-sterilized polluted water. Both sterilized and non-sterilized polluted water will be investigated to compare the effects of local microorganism in treating polluted water.

Fermented Product (FP)

Usually, the treatability of fermented products (FPs) has been explained that these FPs contain some important microorganisms capable to improve pollute water quality. Although the manufacturing of these FPs has never been standardized, any FPs suppliers always claim their similar effectiveness. Therefore, the authors selected five different brands of FPs from those commercially sold in Thai market for this study, namely FP1 to FP5. Each FP was added into polluted water both cases of sterilized and non-sterilized with the ratio of 1:10,000 by volume (as suggested by FP manufacturers). As previously mentioned, this study was attempted to find any scientific data to clearly discuss the treatability of FPs as their believers has been claimed. The authors tried to keep any application steps of FPs as similar as those instructed by their manufacturers/suppliers.

Experimental setup

Twenty-liter plastic tank equipped with mixer and sampling tube was used as an agitated vessel used in this study as shown in Figure 1. Slow speed mixing was applied to prevent settling during the experiment. For each portion of polluted water (sterilized and non-sterilized), six 20-L tanks were set up for the experiments with five FPs addition and the 'Control' (no FP addition). The addition of each FP was done only once at the beginning of the experiment. The mixture (polluted water and FP) were then gradually agitated to imitate natural aeration. The agitation was maintained for seven days of operation.

In order to obtain scientific data about the transition of microorganisms before and after applying FPs, water samples (about 500 mL) were taken from the vessels on day 0, 1, 3, 5 and 7 for bacterial count by standard plate count (SPC) [30]. Plate counting was focused on heterotrophic microorganisms (total microorganisms), anaerobic lactic acid bacteria (LAB) and photosynthetic bacteria (purple non-sulfur bacteria, PNSB). The media used for them were Tryptic soy medium, De Man-Rogosa-Sharoe medium (MRS) and Basal Agar medium, respectively. For treatability discussion, non-filtered BOD (BOD_T), non-filtered COD (COD_T) and SS were analyzed only on days 0 and 7. DO was also monitored to observe the degree of aeration. All parameters were analyzed according to the Standard Methods procedure [31]. Figure 2 showed the experimental setup for this study.

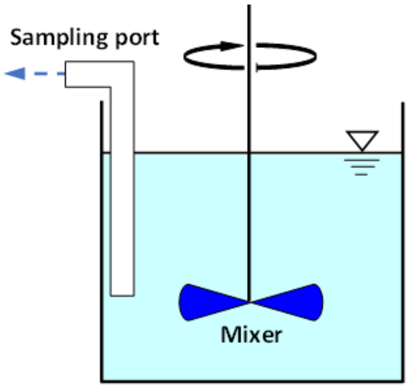

Figure 1 Agitated vessels

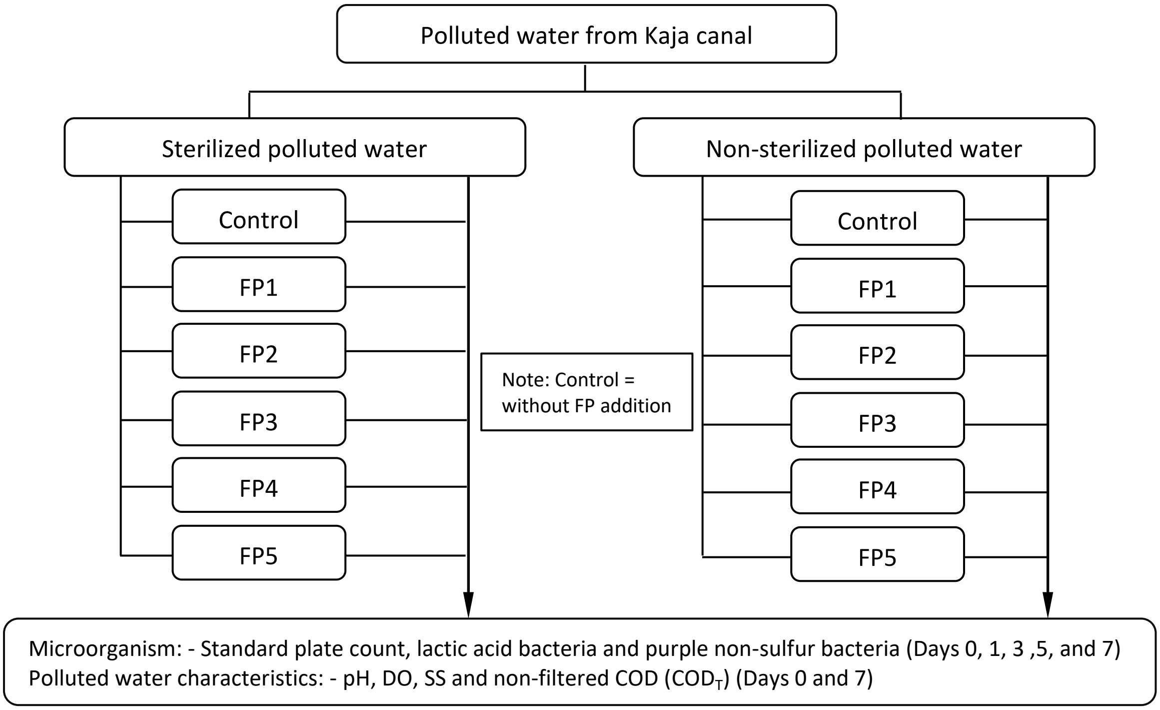

Figure 2 The experimental setup

Results and Discussions

Polluted water taken from Kaja canal were determined for their characteristics as shown in Table 1. Their low DO (0.61 mg/L) and substantial BOD value (22 mg/L) illustrated their 'polluted' situation. All five fermented products (FPs) used in this study were identified and bacterial counted for heterotrophic microorganisms before applied to the experiments.

Table 1 Characteristics of Kaja canal water

Parameters	**Kaja canal**
Temp (^oC)	30.2
pH	7.42
DO (mg/L)	0.61
Turbidity (NTU)	0.85
BOD (mg/L)	22.1
COD (mg/L)	80
SS (mg/L)	17

The result of bacterial counts as shown in Table 2 exhibited that each FP had different amount of total heterotrophic bacteria, especially FP5 that showed the significantly higher number of SPC and LAB than the others. In case of PNSB, similar amounts of PNSB (101–176 CFU/mL) were observed in four FPs, but the one as high as 600 CFU/mL was found in FP3. However, the number of heterotrophic microorganisms in each FP were not considerably high, the addition of FPs into polluted water with the ratio of 1:10,000 (the instructed ratio by manufacturers) should not help increase the initial amount of the microorganisms in the mixtures on Day 0 substantially.

Owing to the turbulence created by the agitation, the polluted water in a vessel became under aerobic condition. For sterilized polluted water vessels, DO levels were ranging from 3.0–3.8 mg/L on Day 0 to 4.0–4.9 mg/L on Day 7. For non-sterilized polluted water vessels, DO levels were in the ranges of 0.6–0.8 mg/L on Day 0 to 1.2–1.5 mg/L on Day 7 as shown in Figure 3. Admittedly, the higher

DO levels in sterilized polluted water would be due to any turbulence caused by water preparation (container transfer, autoclaving, etc.). Moreover, any DO consumption in non-sterilized polluted water could occur all the time due to the existing microorganisms were not destroyed. Obviously, increase of DO on Day 7 should be the result of continuous DO supply from agitation and slower DO consumption due to less organic matters.

Table 2 Amount of heterotroph microorganisms in each fermented product

Type of FPs	Total bacteria as SPC (CFU/mL)	Lactic acid bacteria (CFU/mL)	Purple non-sulfur bacteria(CFU/mL)
FP 1*	201,000±1,732	8,733±58	127±4.6
FP 2*	34,667±577	3,033±153	101±7.8
FP 3**	56,333±1,528	6,067±208	600±43.6
FP 4**	54,000±1,732	290±10	176±1.0
FP 5*	1,340,000±10,000	187,000±10,000	156±3.5

Note: *Company's product **Produced by local people; FP 4 was produced from fermented mushroo

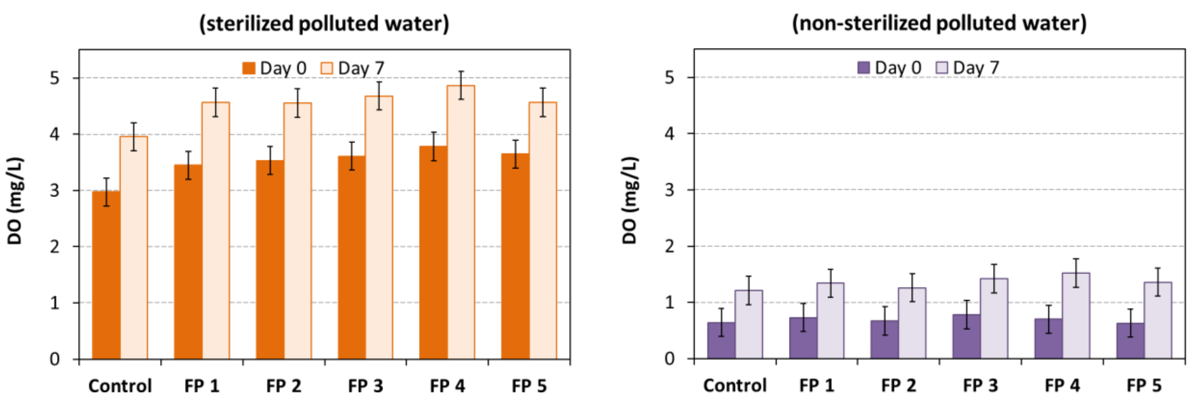

Figure 3 DO in agitated reactors with and without FPs addition

Heterotrophic microorganisms

From Figure 4a, it illustrated the growth patterns of heterotrophs, as counted by SPC method, in the experiment with sterilized polluted water. It is noteworthy that no colony of heterotrophs was found in the Control while the others (with FPs addition) were observed very small amount within the range of 6–680 CFU/mL. This assured that polluted water was all sterilized. When the operation went on, increases of heterotrophic microorganisms were observed in every vessel until Day 3. It can be seen that the Control (no FP) and five different FPs vessels with the sterilized polluted water, could produce similar increase patterns but different magnitudes, with FP5 giving the highest count of $63x10^6$ CFU/mL on Day 3. This was probably due to its higher SPC content in the FP5 in the first place (see Table 2). Interestingly, the Control vessel ranked the second high count of $53x10^6$ CFU/mL. Therefore, this evidently showed no beneficial result of the FP addition to the system, in terms of the SPC results.

In case of non-sterilized polluted water (Figure 4b), the initial counts of heterotrophic microorganisms (Day 0) were much higher than those of sterilized polluted water. That is, the Control (no FP) was observed the count of around $0.6x10^6$ CFU/mL, while the others (with FPs addition) were in the range of $0.7x10^6$–$0.9x10^6$ CFU/mL. However, the sterilized polluted water vessels (Figure 4a) could apparently induce the bacterial growth significantly despite local microorganisms in polluted water were all destroyed (by sterilization). The increase of bacterial count in case of sterilized polluted water could rise up to higher than 60×10^6 CFU/mL while those of non-sterilized pollute water was merely less than 5×10^6 CFU/mL (for FP5 scenario) as shown in Figure 4b.

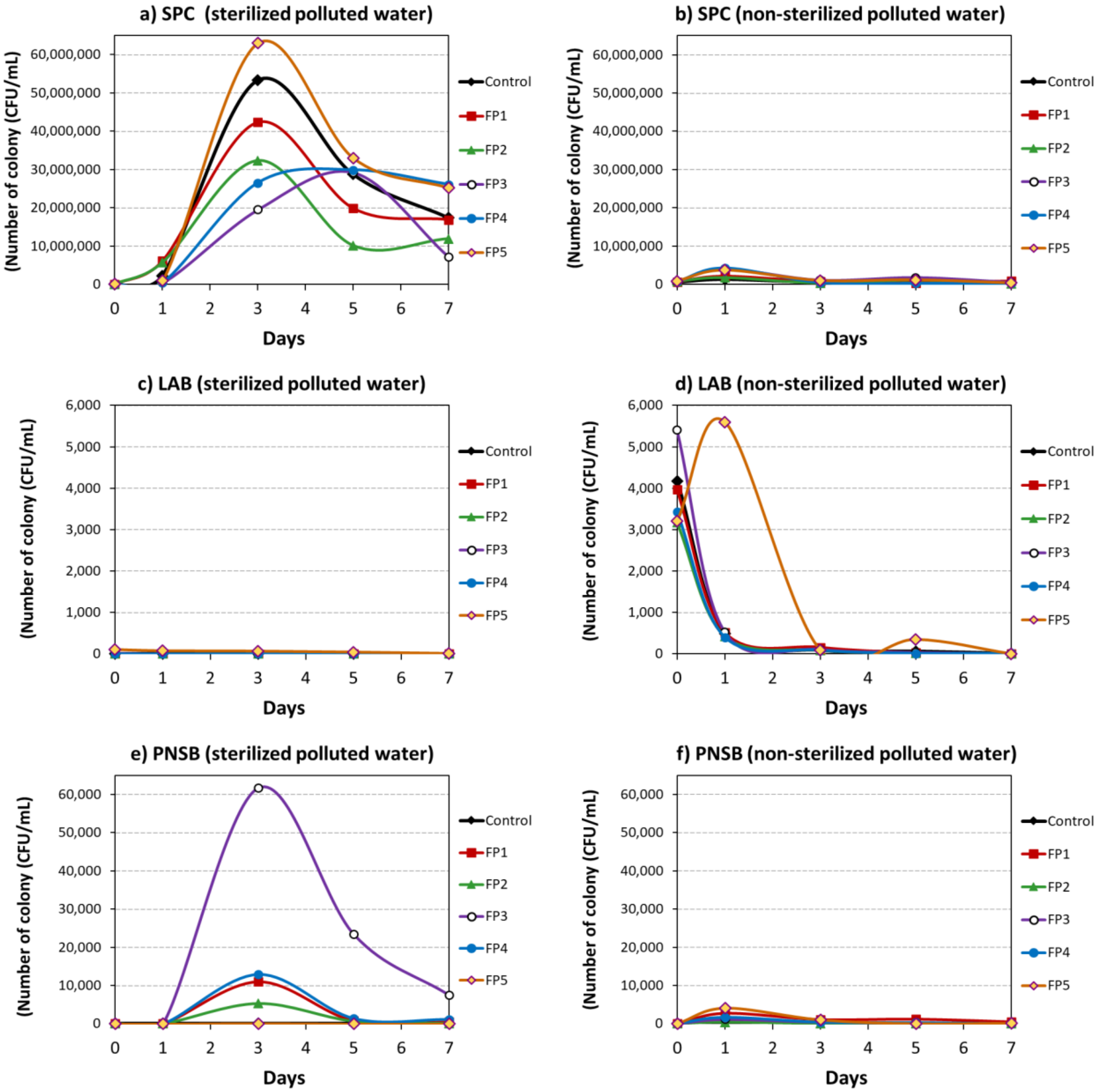

Figure 4 Growth patterns of total heterotrophs (expressed as SPC), lactic acid bacteria (LAB) and purple non-sulfur bacteria (PNSB)

Moreover, in comparison between sterilized and non-sterilized cases, the peak of bacterial count appeared different day. That is, the heterotrophic microorganisms grew and generally peaked on around Day 3 (or 5) in the sterilized vessels, while those in non-sterilized vessels increased to their peak on around Day 1. After their peaks, the number of heterotrophs declined. Two probable assumptions were 1) some organic matters were partly degraded under autoclaving, resulting in smaller and more easily biodegradable organics, as well as higher yield of bacterial growth; 2) certain growth inhibitors were present in the raw non-sterilized polluted water.

Lactic acid bacteria (LAB)

Figure 4c shows the increase patterns and counts of the lactic acid bacteria (LAB) in the sterilized case comprised of the Control and the test units with FP addition (FP1 to FP5). It is clear that they were very much alike, illustrating no clear benefit of the FPs addition. Especially, the initial numbers of LAB (Day 0) were extremely low, that is, no colony of LAB were found in the Control, FP2 and FP4 vessels. The others, namely FP1, FP3 and FP5, were observed the LABs counts of only 1, 13 and 99 CFU/mL, respectively. Nevertheless, LAB count did not show any increase during the 7-day operation. The presence of substantial DO in the sterilized vessels could possibly inhibit anaerobic growth of LAB.

In comparison, the non-sterilized case showed the higher initial number of LAB (Day 0) than sterilized case. That is, the number of LAB in the ‘Control’ (no FP) vessel was about 42×10^2 CFU/mL while those in the test units with FPs addition were in the range of 32×10^2–54×10^2 CFU/mL (Figure 4d). Obviously, the initial amount of LAB on (Day 0) in non-sterilized polluted water vessels were similar to each other. This could be said that LABs from FPs addition were insignificant, and most of them were local microorganisms in polluted water. But, after only two or three days of operation, the rapid decline of LAB occurred. This could be possibly due to some inhibition by the presence of DO (from agitation). Therefore, this such a decline of LAB did not support the exaggerated benefit in wastewater treatment, especially aerobic treatment.

Purple non-sulfur bacteria (PNSB)

Considering the results shown in Table 2, the amounts of PNSB originally available in FPs were in the range of 101–600 CFU/mL. This amount should have no considerable impact on the initial amount of PNSB in the mixtures on Day 0 due to the high dilution ratio of 1:10,000 (v/v). For sterilized polluted water vessels, negligible amount of PNSB (0 CFU/mL) was observed in all vessels on Day 0 as shown in Figure 4e. Surprisingly, PNSB in the sterilized system showed their increase to a certain number and declined after three days of operation (Figure 4e), especially with the addition of FP3 that contained the highest PNSB itself. The peak of PNSB count in FP3 vessel was up to $62\text{x}10^3$ CFU/mL. However, this increased PNSB were then declined after Day 5.

For non-sterilized polluted water vessels, little amount of PNSB were detected in the range of 58–84 CFU/mL as shown in Figure 4f. Obviously, FPs addition did not increase the number of PNSB in comparison to the Control (no FP). This illustrated that PNSB was a kind of indigenous microorganisms and already existed in this polluted water. The addition of FPs (with the ratio of 10:10,000 v/v) could not noticeably increase the amount of PNSB in the mixtures (of polluted water and FP). On the contrary to sterilized polluted water, PNSB in non-sterilized condition did not show any substantial growth during the experimental course. Slight increase of PNSB count was observed on Day 1, but this higher amount of PNSB did not last long throughout the seven days of operation. They eventually became less and undetectable at the end of the period. Probably, non-sterilized polluted water might contain some local microorganisms that competed against or hindered growth of PNSB.

Treatability of FP in polluted water

Batchwise experiments were further done to determine the reduction ability of non-filtered COD (COD_T) and non-filtered BOD (BOD_T) in the 'Control' (without FP) and the test units (FP 1 to FP 5). The non-filtered COD (COD_T) of the polluted water itself was about 83 mg/L and became 54 mg/L after being autoclaved. In the sterilized condition, addition of FP increased the initial COD_T concentration of the test units to the range of 67–79 mg/L. After seven days of operation, the COD_T of the vessels with FPs addition were in the range of 50–63 mg/L which were not much different from the 'Control' unit (60 mg/L) (Figure 5a). The 'Control' unit did not show any reduction of COD_T while there were some small COD_T reduction observed in the vessels with FP addition. However, this small COD_T reduction could not be said that the FP addition did help improve the COD_T reduction capability of the system.

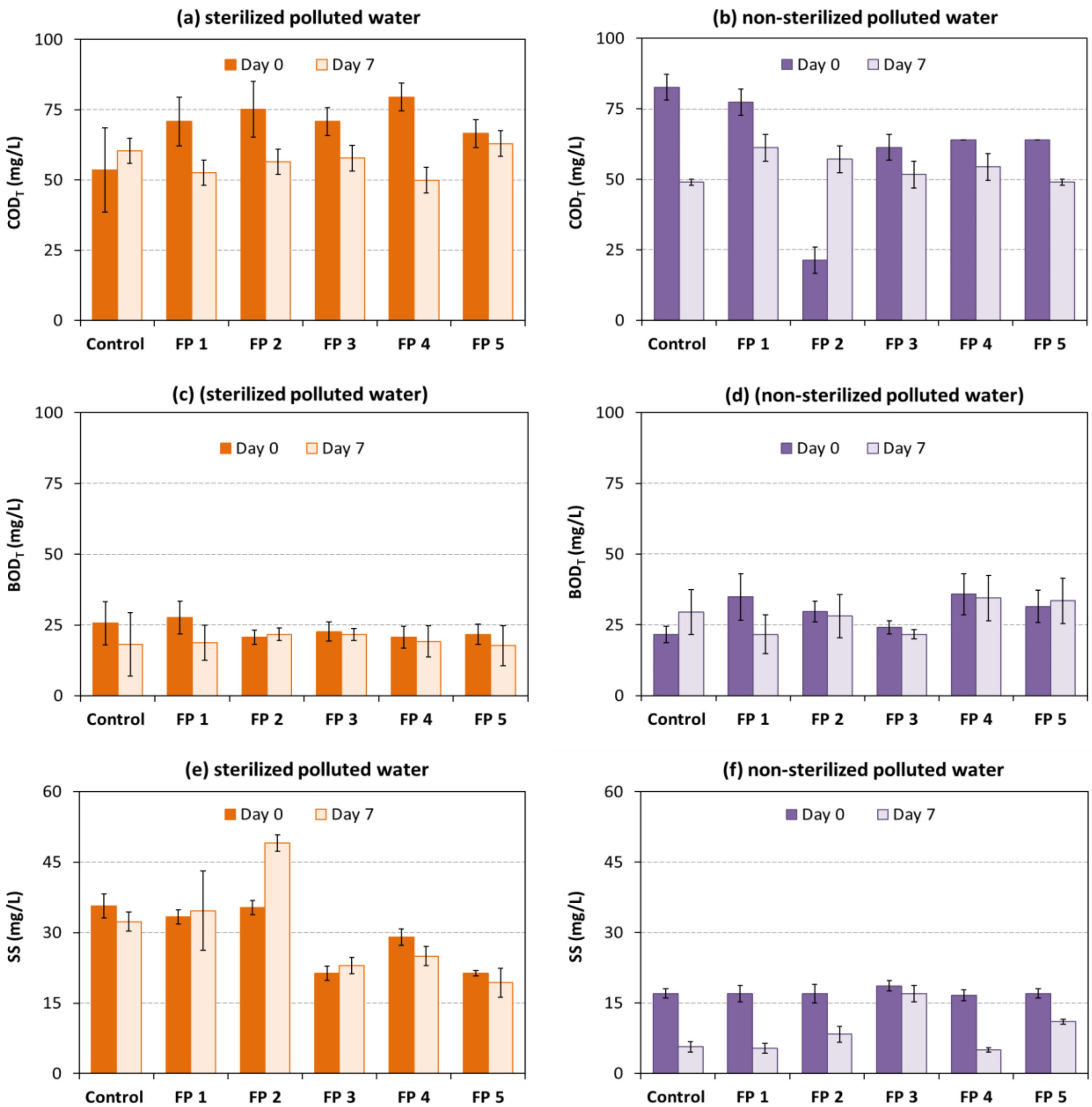

Figure 5 COD_T, BOD_T and SS reductions of agitated reactors with and without FPs addition

For the non-sterilized condition (Figure 5b), some minor change in the initial COD_T after FP addition was apparent, except the vessel with FP2 that very low COD_T (21 mg/L) was observed without reasonable explanation. However, at the end of seven-day operation, the COD_T of test units were in the range of 49–61 mg/L which were very similar to that of the 'Control' unit (49 mg/L). Therefore, it could be again concluded that FP addition in both sterilized and non-sterilized cases did not provide a positive effect on the COD_T reduction.

Figure 5c showed BOD_T change in sterilized polluted water vessels. The BOD_T values on Day 0 were in the range of 21–28 mg/L, while they were in the range of 18–22 mg/L after seven days of operation. Some slight reduction of BOD_T could be perceived, but difference between with and without FPs addition was again unclear. Similarly, BOD_T concentrations on Day 0 in non-sterilized polluted water vessels were in the range of 22–36 mg/L. But, BOD_T reduction after operation were indecisive as they were in the range of 22–34 mg/L (Figure 5d).

It is noted that these COD_T and BOD_T was the non-filtered type, then, the organic SS (or biomass) was counted as a part of analyzed COD_T or BOD_T. Likewise, SS did not show significant difference between the 'Control' and the ones with FPs addition (Figures 5e and 5f). The results from this study were inconsistent to some studies that concluded their achievement in wastewater treatment with FP addition [31–34].

Conclusions

For the sterilized samples, different FPs gave different heterotroph growth levels, with FP5 seemed to be the best growth promoter. Surprisingly, the 'Control' (no FP addition) ranked second in terms of SPC growth. This illustrated that there was no affirmative advantage of addition FPs to the system. Regarding the non-sterilized system, practically very low heterotroph growth (measured as SPC) was perceived. No obvious difference of SPC growth between with and without addition of FPs was scientifically noticed. This finding suggests that the FP addition did not enhance the treatment efficiency.

Considering the LAB and PNSB growth patterns and magnitudes, it was also apparent that FP addition did not increase their density in both sterilized and non-sterilized conditions, resulting in no advantage of adding the FPs to the system. Agitation should affect DO concentrations in the vessels, they were in the rages of 3.95–4.87 mg/L and 1.21–1.52 mg/L for sterilized and non-sterilized polluted water, respectively. Both LAB and PNSB microorganisms eventually decline to undetectable range after operation possibly due to the presence of DO.

In terms of treatability, FPs addition did not enhance the reduction of COD_T or BOD_T in comparison to the Control (no FP addition). Even suspended solid (SS) did not showed significant improvement by FPs addition. Therefore, it was concluded that no obvious benefit from FPs addition on canal polluted water treatment.

Acknowledgement

Financial support for this work was provided by the Research and Development Institute, Ramkhamhaeng University.

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