BP-P-08

The study of the biodiversity in local bio-fermented solution and the treatment of community wastewater at laboratory scale: wastewater from restaurants

Supatchayaporn Nitsuwat[1], Stefanus Edo Sanjaya[2], Suchawadee Wiratthikowit[3] and Viyada Kunathigan[4]*

ABSTRACT

Bio-fermented solution is a solution composed of a diverse variety of microbes coexisting together, aiding the metabolism of each other. Nowadays,bio-fermented solution is widely used for many purposes and is one of the popular usages for wastewater treatment. In this experiment, the biodiversity of the bio-fermented solution was determined by selective and non-selective enrichment media. For the treatment of restaurant wastewater (collected from local department store food court and steak restaurant), 0 (control), 0.25, 0.5 and 1 mL of bio-fermented solution were inoculated per liter of wastewater. In the treatment procedure, light (with light and without light), time (24 hours and 48 hours) and oxygen (with oxygen and without oxygen) were varied. After treatment, the wastewater sample's chemical and microbiological properties were tested. The chemical properties include total solid (TS), Biochemical oxygen demand (BOD), total suspended solid (TSS), total dissolved solid (TDS), pH and grease and oilcontent. The microbiological properties were measured by MPN method and total plate count method. The bio-fermented solution biodiversitieswere contained with *Bacillus* spp. 3.48×10^4 CFU.mL^{-1}, mold 3.63×10^3 CFU.mL^{-1}, lactic acid bacteria 4.35×10^4 CFU.mL^{-1}, actinomycetes 1.27×10^5 CFU.mL^{-1} and yeast 1.35×10^5 CFU.mL^{-1}. When using bio-fermented solution to treat restaurant wastewater, there were significant reduction of TS and grease and oil at 53.07% and 69.89% respectively. The best condition for restaurant wastewater treatment was 0.25 mL of bio-fermented solution per liter of wastewater without oxygen and light for 48 hours. However, the quality of treated wastewater was still above the standard required. Therefore, further experiment will be needed to improve the quality of water before discarding.

Keywords: Bio-fermented solution, biodiversity, TS, BOD, Grease and oil, MPN

INTRODUCTION

Water is essential for all living organisms. High amount of water is being used each day for many purposes ranging from drinking, cleaning to agricultural use. Therefore, this technique can be applied to numerous industries including food, agriculture, environment, energy, paper, construction materials and so forth. Main advantages of material characterization with electromagnetic fields and waves are the possibility of non intrusive and non destructive sensing, low operating costs as well as real time measurement. These activities resulted in high amount of discharged water contaminated chemically, biologically and microbiologically. Wastewater is defined as water that is contaminated, undesirable or unsuitable for consumption. The wastewater could contaminate and cause quality depletion of natural water resources. Restaurant wastewater is one type of community wastewaterswith the characteristics of greasy and oily, and contaminated with organic scraps and detergents from washing and cleaning (Pollution Control Department

[1] Department of Food Technology, School of Biotechnology, Assumption University, Huamak campus, Bangkapi, Bangkok 10240 Thailand

[2] Department of Food Technology, Soegijapranata Catholic University, Jl.PawiyatanLuhur IV/1 BendanDhuwur, Semarang 50234 Indonesia

[3] Department of Agro-Industry, School of Biotechnology, Assumption University, Huamak campus, Bangkapi, Bangkok 10240 Thailand

[4] Department of Food Biotechnology, School of Biotechnology, Assumption University, Huamak campus, Bangkapi, Bangkok 10240 Thailand

* Corresponding author: viyadaknt@au.ed

Thailand, 2002). According to the standards for the restaurant wastewatertreatment, the discharge must have pH of 5 to 9 with biochemical oxygen demand (BOD) of ≤ 200 mg.L^{-1}, total suspended solid (TSS) of ≤ 60 mg.L^{-1} and grease and oil content of ≤ 100 mg.L^{-1}. The wastewater must be treated before discharging and one of the methods to treat the wastewater is with bio-fermented solution (Ministry of Natural Resources and Environment, 2005). Bio-fermented solution is the product from fermentation of plants, fruits or animalswith sugarsor molasses also called as bio-extracts or effective microorganisms (EM). The microbes present will utilize the nutrients to increase its population and variety (Appropriated Technology Center, SakonNakhonRajabhat University, 2013). The microbes that can be found in the bio-fermented solution are *Bacillus spp.,* lactic acid bacteria*,* actinomycetes*,* purple non-sulphur bacteria, yeast and mold. Generally, the bio-fermented solution has the pH of 3.5 to 5.6 (Bunchoo, 2002). The most abundant microbes in the bio-fermented solution according to former research arelactic acid bacteria, yeast and mold (Tancho, 2006).Bio-fermented solution has gained much attention since its discovery. Its benefits have been widely studied and the usage of bio-fermented solution in wastewater treatment is one of them. In this research, the local bio-fermented solutionswere used tostudythe microbial biodiversityand their efficiency in treating restaurant wastewaters weredetermined in different physical conditions.

MATERIALS AND METHODS

Microbial Analysis of Local Bio-fermented Solution

The bio-fermented solution was provided by NAVA Social Enterprise, Bangkok, Thailand. It was contained in a plastic bottle and stored at room temperature away from direct sunlight. A new bottle of bio-fermented solution was used for each round of wastewater sample testing.

The local bio-fermented solution was examined for the presence of mold, yeast, lactic acid bacteria, actinomycetes and total viable cells using RBA (Rose-Bengal Agar), YM (Yeast Mold) agar, MRS (de Man, Rogosa andSharpe) agar, GYEA (Glycerol-Yeast Extract Agar) and PCA (Plate Count Agar),respectively as the enrichment media. The bio-fermented solution was analyzed by serial dilution and spread plate technique. All plates were incubated at room temperature for 24 hours. *Bacillus spp.* was analyzed by boiling the bio-fermented solution for 10 minutes before spread plating. The plates were also incubated at room temperature for 24 hours.

Restaurant Wastewater Treatment

The wastewater samples were collected from a western style restaurant and a food court in a department store. The wastewater samples were treated using factorial design. The samples were inoculated with the bio-fermented solution in the amounts of 0.25, 0.5 and 1.0 mL per liter of wastewater, respectively. The conditions of the treatments are varied between with exposure to light and without light as well as with oxygen and without oxygen. The samples were left under the varied conditions for 24 and 48 hours. The control was prepared by treating wastewater sample under the same conditions without inoculation of the bio-fermented solution.

Microbial Analysis of Treated Restaurant Wastewater

The total aerobic bacteria of the sample was determined by drop plate technique using nutrient agar (NA) as enrichment medium and the water quality was determined by using the 5 tubes Most Probable Number (MPN) procedure for total coliform, fecal coliform *and E.* coli bacteria (American Public Health Association *et al.*, 1999).

Chemical Analysis of Treated Restaurant Wastewater

The chemical analysis was implemented by measuring the total solid (TS), the total suspended solid (TSS), the total dissolved solid (TDS), the pH, the biological oxygen demand (BOD) and the grease and oil content. A multi-parameter meter (AMTEST EC-900) was used to measure TDS, DO and pH. As for TS and TSS drying method was used and the difference was determined. BOD_5 was measured by subtraction of BOD_0 and BOD_5 (AOAC, 1990) and liquid-liquid extraction was used to determine grease and oil content (Mohrig J.R. *et al.*, 2006). The results were analyzed with SAS 9.2 program using Tukey's multiple comparison test and Dunnett's multiple comparison test.

RESULT AND DISCUSSION

Microbial Analysis of Local Bio-fermented Solution

From analyzing the local bio-fermented solution, total aerobic bacteria counting in the local bio-fermented solution wasfoundthe number of survival cellsup to 1.22×10^{11} $CFU.mL^{-1}$ with most of it composing of actinomycetes and yeast as shown in table 1.

Table 1. The groups of the microorganisms found in the bio-fermented solution.

Description	Colony Forming Unit per mL (mean ± SD)
Total Viable Cell	$1.22 \times 10^{11} \pm 2.12 \times 10^{11}$
Actinomycetes	$1.26 \times 10^{5} \pm 6.86 \times 10^{4}$
Yeast	$1.35 \times 10^{5} \pm 2.82 \times 10^{3}$
Lactic Acid Bacteria	$4.34 \times 10^{4} \pm 5.01 \times 10^{4}$
Bacillus spp.	$3.48 \times 10^{4} \pm 4.50 \times 10^{4}$
Mold	$3.62 \times 10^{3} \pm 4.49 \times 10^{3}$

Note: The experiments were done for at least 2 replicates.

Microbial Analysis of Treated Restaurant Wastewater

The total aerobic bacteria in wastewater after treatment with the bio-fermented solution were analyzed. Total aerobic bacteria from the wastewater treatmentfor 24 or 48 hours were not significantly different (p>0.05) from the total aerobic bacteria of the untreated wastewater (raw) and the control of each condition (data not shown). However, the wastewater was treated for 48 hours, the total aerobic bacteriashowed an increase in number.

The treated wastewater was analyzed to determine its quality by detection of the indicator bacteria for poor sanitation. The total coliform and total fecal coliform bacteria of the samples were found to be over 16,000 MPN per

BP-P-08

100 mL (MPN/100mL) for all conditions. According to the surface water standards, the surface water should contain no more than 20,000 MPN/100 mL of total coliform. But due to the underestimation in the analyzed scale, further investigation will be needed to assess the total coliform bacteria in the sample. As for the total fecal coliform bacteria, the treated wastewater sample was over the standard limit of 4,000 MPN/100 mL (National Environment Board, 1994).

For the *E. coli*, the result (figure 1) indicated reduction of *E. coli* after treatment. This suggested that the local bio-fermented solution may have ability to reduce the *E. coli*. However, in these samples, the initial *E.coli* load were already within the standards of surface water which is within 406 *E. coli* per 100 mL of recreational water (New Hampshire Department of Environmental Services, 2011). The result showed that the control also had reduced *E. coli* load as well, this may be due to the nature of wastewater such as the presence of SDS (Sodium Dodecyl Sulfate) from detergents used in cleaning that caused destruction of the microbes' cell membrane.

Chemical Analysis of Treated Restaurant Wastewater

From figure 2, TS of restaurant wastewater treatmentfor 48 hours shows significant reduction when comparing with the untreated wastewater ($P < 0.05$). However, the control also showed significant reduction in TS, which may be due to the bacteriasthat were original strains in the wastewater. When considered the total number of aerobic bacteria in control that was increased after 48 hours.
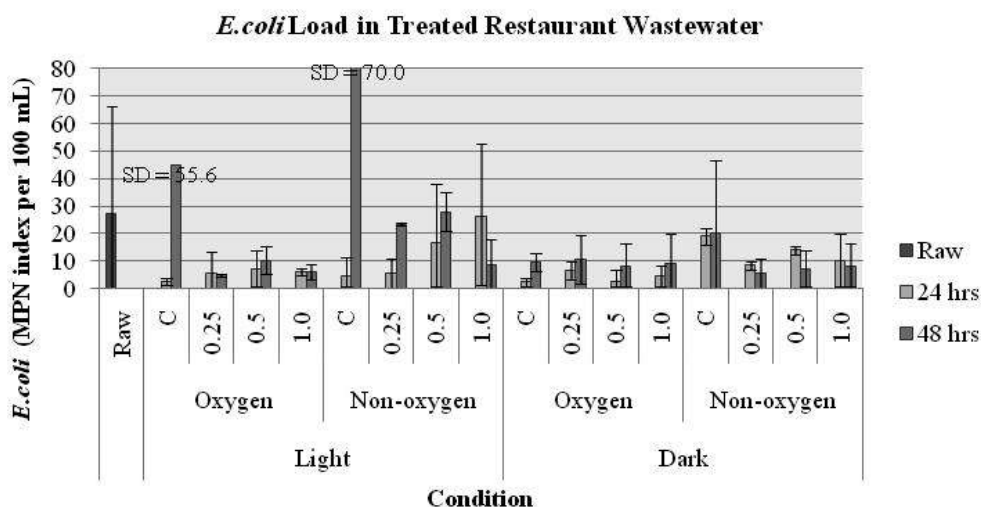

Figure 1 The *E.coli* load using MPN method in the restaurant wastewater after treatment with bio-fermented solution in different conditions.
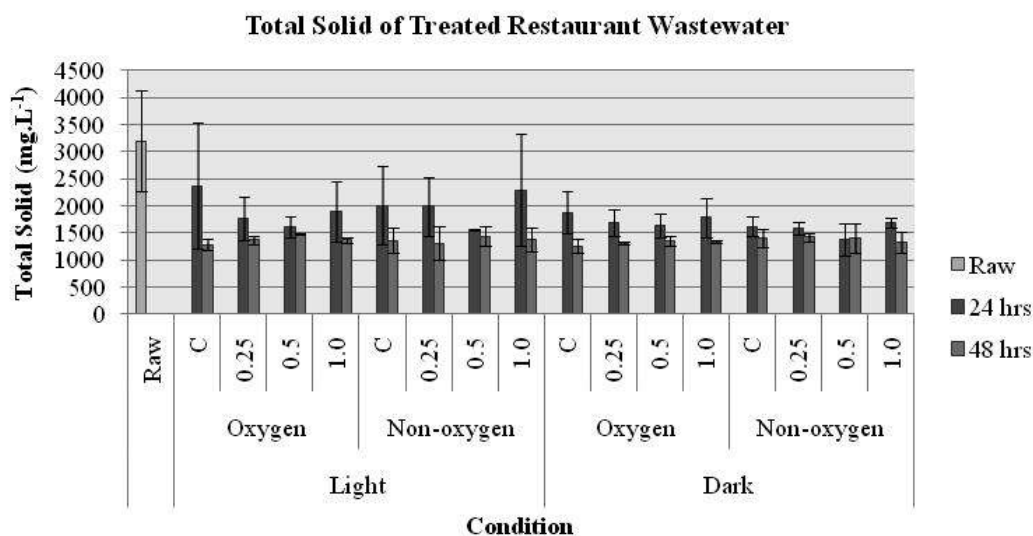
BP-P-08

Figure 2 The total solid of the restaurant wastewater after treatment with bio-fermented solution in different conditions.

From figure 3, grease and oil content were greatly reduced when treated for 48 hours in dark, non-oxygen condition with addition of bio-fermented solution. According to the results from a study of the biodiversity in this bio-fermented solution (Sanjaya and Kunathigan, work in progress) high percentage of lipid degrading bacteria were isolated, which could lead to the reduction of the grease and oil content. This reduction is also consistent with previous research that study the application of EM (Siripornadulsil and Labteephanao, 2008; Fadile *et al.*, 2011). The grease and oil content in 48 hours may also have decreased more significantly due to the lack of organic compounds that were degraded at 24 hours, causing microbes to utilize the grease and oil content as energy source instead.
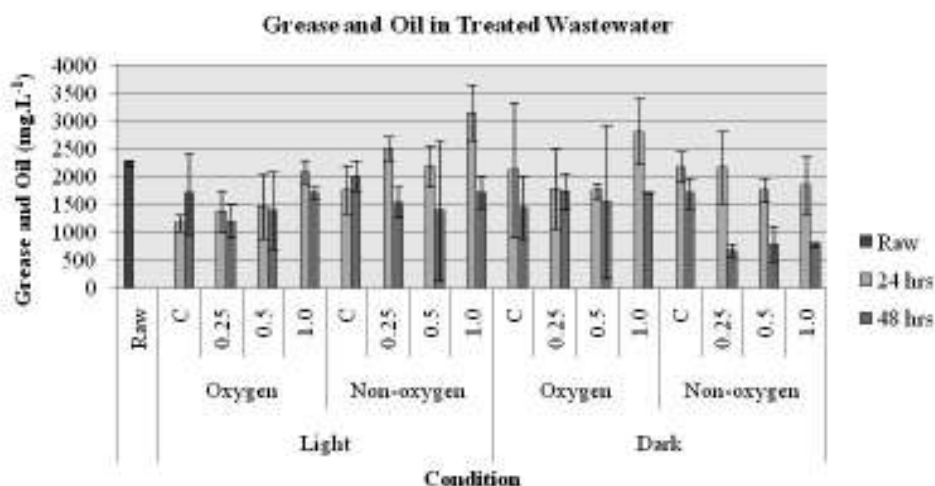

Figure 3 Grease and oil content in the restaurant wastewater after treatment with bio-fermented solution in different conditions.

BP-P-08

However, the grease and oil content were found to be over the standards of ≤ 100 mg.L^{-1} (Ministry of Natural Resources and Environment, 2005). Therefore, other methods should be used to primarily lower the grease and oil content before further treatments with the bio-fermented solution such as using a grease trap or increasing the treatment time.

The pH level was found withinthe range of approximately 5.3 to 6 and the BOD at approximately 1.8 to 3.2 mg.L^{-1}. Even though there were no significant changes from treating the wastewater with the bio-fermented solution, the initial values were already within the standards of 5 to 9 for pH and ≤ 200 mg.L^{-1} for BOD, causing the wastewater to be within the standards regarding pH and BOD (Ministry of Natural Resources and Environment, 2005).

From analyzing the results, the optimum treatment condition is treating with 0.25 mL.L^{-1} of wastewater for 48 hours without light and oxygen. This condition led to the reduction of TS and grease and oil were 53.07% and 69.89%, respectively. The *E. coli* load, total bacterial load, TSS, TDS, pH and BOD were at an acceptable level.

Further experiments willneed to control the quality of the local bio-fermented solution for consistence in every batch andcreated a custom made bio-fermented solution composed of the beneficial microbial strains that are suitable for certain types of wastewater treatments as well as how to effectively integrate the bio-ferment solution with other wastewater treatment methods.

CONCLUSION

The local bio-fermented solutionwere containedthe total survival cells up to 1.22×10^{11} CFU.mL^{-1} which mainly composedactinomycetes and yeast. The optimum treatment condition was the treatment with bio-fermented solution at 0.25 mL.L^{-1} of wastewater for 48 hours without light and oxygen. The treated wastewater from this condition showed the total solid reduction of 53.07% and the grease and oil reduction of 69.89% as well as thereduction of *E. coli* load. Detected duration is a major factor for influencing change in the wastewater more than light and oxygen.

ACKNOWLEDGEMENTS

LITERATURE CITED

Fadile, A., F.Z. El Hassani, H. Aissam, M. Merzouki and M. Benlemlih. 2011. Aerobic Treatmentof Lipid-rich Wastewater by a Bacterial Consortium. **Afr. J. Microbial. Res**. 5: 5333-5342.

American Public Health Association, American Water Works Association and Water Environment Federation. 1999. **Standard Methods for the Examination of Water and Wastewater**.

AOAC.1990. **AOAC Official Methods of Analysis**. 15th ed. Association of official Analytical Chemistry. Washington D.C.

Appropriated Technology Center, Sakon Nakhon Rajabhat University. 2013. Bio-extract. [Available from: http://atc.snru.ac.th/UserFiles/bioextract(1).pdf]. [Sourced: 14 July 2013].

BP-P-08

Bunchoo, M. 2002.**The Study of Microorganism Isolated From EM**. Bachelor Degree Thesis. Assumption University, Thailand.

Ministry of Natural Resources and Environment. 2005) Control Standard Specification: Wastewater Discharged from Buildings of Certain Size. **Royal Thai Government Gazette**. 122: 8-9.

Mohrig J.R., C.N. Hammond and P.F. Schatz. 2006. **Techniques in Organic Chemistry**. 2nd ed. pp. 75-99.

National Environment Board. 1994. Control Standard Specification: Quality of Water from Surface Water Sources. **Royal Thai Government Gazette**.111: 237.

New Hampshire Department of Environmental Services. 2011. Bacteria in Surface Waters.**Environmental Fact Sheet**.

Pollution Control Department Thailand. 2002. **Domestic Waste Water and Water Treatment System**.KhurusaphaLadprao Printing. Bangkok. 124 p.

Siripornadulsil, S. and W. Labteephanao. 2008. The Efficiency of Effective Microorganisms (EM) on Oil and Grease Treatment of Food Debris Wastewater. **KKU Science Journal**. 36: 27-35.

Tancho, A. 2006. Application of Natural Agriculture: Principle, Concept and Techniques for Implementation in Thailand. 1st ed. NSTDA Bookstore.Pathumthani.371 p.