## Processing of Bio-cleaner from Vegetable Wastes and Utilization in Treatment of Domestic Wastewater

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### Abstract

Wastes generated from fruits and vegetables are organic in nature and contribute a major share in soil and water pollution. Fruits and vegetable wastes (FVWs) are rich in moisture, carbohydrates and other compounds such as proteins, fats, antioxidants and other bioactive compounds. In this research, bio-cleaner was prepared from two different sources; wastes of tomatoes and peels of citrus fruits, and waste of cabbage and peels of citrus fruits through anaerobic fermentation. Brown sugar was added with the fruit waste substrates by 1:3; 1:4 and 1:5 (w/w). The characteristics of bio-cleaner such as pH, total solid, acidity and chemical oxygen demand (COD) were studied during the first 4 weeks and end of the 2<sup>nd</sup> month of fermentation. CFU/ml of bio-cleaner was also studied. Domestic wastewater was collected from ULB ward (about 68 families living), Kamayut Campus, University of Yangon and its characteristics such as pH, COD, Total dissolved solids, CFU/ml, ammonia nitrogen and phosphate were investigated. Two-month aged fermented broth (bio-cleaner) was used to treat collected domestic wastewater. Deodorization of wastewater was instantly achieved by all bio-cleaners. Aside from deodorization, distinct amount of total dissolved solids and COD of wastewater were removed.

key words: vegetable wastes, bio-cleaner, anaerobic fermentation, wastewater treatment

#### Introduction

Over half the world's rivers, lakes and coastal waters are seriously polluted by untreated domestic, industrial and agricultural wastewaters (United Nations Environment Programme, 2002: Beach, 2001), and they contain high numbers of faecal bacteria (Ceballos et al. 2003). For an environmental and human health, effective wastewater treatment needs to be recognized. Due to the increase of the worldwide population, the problem of sewage disposal and industrial waste management has become increasingly critical. Domestic wastewater is the water that contains all the materials added to the water during its use and contains human body wastes (faeces and urine) together with the water used for flushing toilets, and sullage, which is the wastewater resulting from personal washing, laundry, food preparation and water from the cleaning of kitchen. For domestic wastewater treatment, the removal of biological organic pollutants and nutrients is the main priority. Municipal wastewater typically consists of (50 - 90%) of domestic wastewater originating from residential sources, (5 - 30%) of commercial wastewater and (5 - 20%) of industrial wastewater (Tan, 2006). Although micro pollutants like endocrine disruptors, pharmaceuticals and acetaminophen are present in very low concentrations in domestic wastewater, they could ultimately react with disinfectants from water treatment and form hazardous products. Wastewater should therefore be treated before being discharged to receiving water bodies (Liu, 2009).

Agricultural waste became a threatened problem causing solid waste disposal. Wastes generated from fruits and vegetables are generally in solid or semi-solid forms except for the effluents generated from processing units. Various enzymes such as amylolytic enzymes, pectinolytic enzymes, invertase and lipases are present in fruits and vegetable wastes. Microbial processing has been applied for the products such as enzymes, organic acids, flavoring compounds, food colorants, bioethanol, and biomethane (Laufenberg et al., 2003).

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Microbial bio-processing of organic wastes is a potential tool to clean up the environment. Anaerobic digestion is a process by which organic waste can be biologically converted in the absence of oxygen. Mixed bacterial population degraded organic compounds and produces a valuable liquid termed as enzyme ionic plasma. Fruit and vegetable waste (FVW) is the most vital component of the organic portion of Municipal Solid Waste (MSW). Highly biodegradable FVW is a source of nuisance in municipal landfills because of its moisture and organic content. The FVW can cause several environmental and health hazards, affecting socio-economic growth of the area. The groundwater resources can be contaminated with fruit and vegetable waste causing diseases such as cholera, dysentery and typhoid (Misi & Forster, 2002; Liu et al., 2009). Garbage/citrus enzyme is used as a natural household cleaner; air purifier; deodorizer; insecticide; detergent; body care; car care; organic fertilizer, etc. It removes odour and dissolve toxic air released from smoking, car exhaust, chemical resides from household products, etc. Enzyme that flow underground will eventually purify the river and the sea. It reduces mosquitoes, flies, rats, cockroaches etc. (Pinang, 2012).

The objectives of the present study are as follows: to conduct anaerobic fermentation of vegetable waste as substrate with brown sugar; to study physical and chemical characteristics of liquid during fermentation; and to assess the quality of domestic wastewater and wastewater treated by fermented liquid (bio-cleaner).

## Materials and Methods

## Sample collection

Vegetable wastes were collected from the local vegetable market of Kamaryut Township, Yangon and brought to the laboratory of Department of Industrial Chemistry, University of Yangon.

## Preparation of bio-cleaner

The vegetables wastes were chopped to pieces as small as possible for experimentation. Bio-cleaner was prepared for; mixture of tomato wastes and peels of citrus fruits; mixture of cabbage waste and peels of citrus fruits with the ratio of 4:1 (w/w). Biomaterials were mixed with brown sugar at various ratios of 3:1, 4:1, and 5:1 (w/w). The mixture was then dissolved in 1000 ml of distilled water in a 2 L capacity of plastic containers. The plastic containers were covered tightly for anaerobic fermentation. The gases were released during the first three days of fermentations by flash opening the cover of the containers. The liquid was observed weekly for the first month of fermentation followed by monthly observation for another two months. The maximum fermentation period observed was limited to two months in this study.

## Assessment on characteristics of bio-cleaner during fermentation

## pH

pH of the liquid sample was measured using a pH meter (HANNA Waterproof pH tester). Before measurement of the liquid sample, the glass electrode was calibrated with pH 4 and pH 7 buffer solutions.

## Acidity

Acidity of the liquid sample as acetic acid was determined by titration method. 10 mL of filtered sample was taken into a 250 mL conical flask and 3 drops of phenolphthalein indicator was added. It was titrated with standardized NaOH solution until colourless to pink colour appeared. The titrant volume was recorded and calculated as follows:

Total Acidity (% 
$$\frac{v}{v}$$
) =  $\frac{mL \text{ of 0.1 N NaOH} \times \text{Equilvalent factor} \times 100}{mL \text{ of Sample taken}}$ 

# Chemical Oxygen Demand (Determination of $COD_{Cr}$ 5520 D (Closed reflux, Colorimetric method)

Potassium acid phthalate standard solution was diluted to 50 mL with distilled water. The standard solution was prepared for 2.5 mL, 5 mL, 10 mL, 20 mL, 30 mL, and 40 mL. The spectrophotometric absorbance of each standard and blank was measured at a wavelength of 420 nm. The calibration curves were plotted with the absorbance of each standard on the abscissa and mg/L of COD on the ordinate.

3.5~mL sulfuric acid and 1.5~ml  $K_2Cr_2O_7$  solution were added into Borosilicate tube. Then 2.0~mL of sample was added to the tube and carefully mixed by inverting to thoroughly mix the contents. The tube was placed in a block COD digester preheated to  $150 \pm 2^{\circ}C$  and refluxed for 2~hr. behind a protective shield and then cooled to room temperature. The absorbance of each sample was read and standardized against the blank at 600 nm using (APEL PD-303) Spectrophotometer. COD of the samples were calculated using the calibration curves.

### **Aerobic Plate Count**

Aerobic plate count (APC) of the liquid samples was determined using 3M petrifilm plates. Dilution samples were prepared as 10<sup>-1</sup> to 10<sup>-6</sup> and inoculated in pertifilm plates. The plates were incubated at 37°C for two days. The aerobic plate count was recorded as CFU/mL.

## Characterization of wastewater and treated Wastewater

## Sample collection of wastewater (domestic wastewater)

Wastewater was collected from the drainage channel of ULB ward yard, campus of UY. The treatment of wastewater was firstly carried out using diluted fermented liquid (bio-cleaner) of 5 %, 10%, 15%, 20% and 25%. Based on the results of primary treatment, 10% bio-cleaner was used for further treatment and the characteristics of treated wastewater were studied for treatment period of 5 days, 10 days, 20 days and 30 days. The procedures used in characterization of wastewater and treated wastewater followed the methods described in Murugesan & Rajakumari, (2005). pH, total dissolved solid, CFU/ml, ammonia nitrogen and phosphate of wastewater and treated wastewater were determined.

## RESULTS AND DISCUSSION

Bio-cleaner is a fermentation product based on vegetable-based kitchen waste such as fruit peels, vegetable trimmings, water and brown sugar. The pH profile of vegetable wastes liquids (bio-cleaner) during the two-month period are shown in Figures 1(a), (b) and (c). Acidic rage of pH was observed in all liquids of vegetable wastes. Abruptly decrease in pH occurred until the end of the first month fermentation period and cabbage fermented liquid showed distinct manner in a little increase in pH, however, within in acid range at the end of the 2<sup>nd</sup> month of fermentation for 3:1 only.

Low pH of liquid of vegetable wastes represented for high acidity as shown in Figures 2 (a), (b) and (c). Its converse was observed at the end of the fermentation period. Apparently, liquid of cabbage waste occurred low acidity in all ratio of substrates when compared to that of the liquids of tomato waste. The process of liquid enzyme is natural fermentation under anaerobic condition which produces alcohol and acetic acid. The acidic condition and fermentation process allow enzymes to be extracted from the waste materials into the solution.

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The high acid concentration and low pH are the main reasons for purposes of cleaning and odour removal (Whiteley and Lee, 2006).

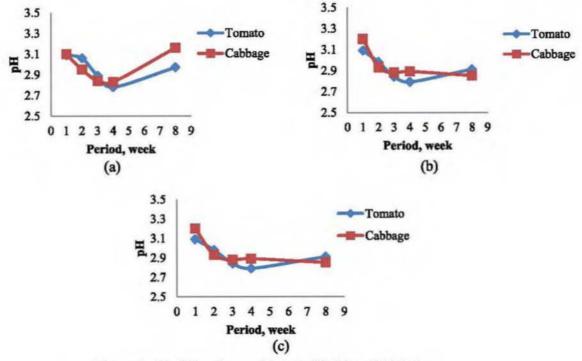


Figure 1 pH of bio-cleaner (a) 3:1 (b) 4:1 and (c) 5:1

Anaerobic digestion of VW was achieved by a series of biochemical reactions: liquefaction of particulate organic material of FVW such as cellulose, hemicellulose, pectin and lignin by extracellular enzymes before being taken up by acidogenic bacteria (Bouallagui et al., 2005). After that, soluble organic components including the products of hydrolysis were converted into organic acids, alcohols, H<sub>2</sub> and CO<sub>2</sub>. Finally, methane was produced by methanogenic bacteria from organic acids produced in earlier steps, H<sub>2</sub> and CO<sub>2</sub> gases as well as directly from other substrates such as formic acid and methanol, which were responsible for pH dynamics of anaerobic digestion of FVW (Veeken et al., 2000).

The profiles of COD in liquids are shown in Figures 4 (a), (b) and (c). Ever increase in COD was found in all bio-cleaners. High COD values were observed in liquid until the two-month fermentation was reached. The COD is an indicator of the biostabilization of the organic strength (Mahmood., et al., 2010). The COD test is used to determine the oxygen equivalent of the organic matter that can be oxidized by a strong chemical oxidizing agent (potassium dichromate) in an acid medium. The rise in COD concentration was supported by the acidogenic environment of the mixture.

Figures 5 (a), (b) and (c) present the bacterial count in liquids during two-month fermentation period. For the first three-week of anaerobic fermentation of VW, decrease in bacterial count was observed due to inhibitory effect of high substrate levels on the bacterial population. After one month, increase in bacterial count was found in all fermented liquids.

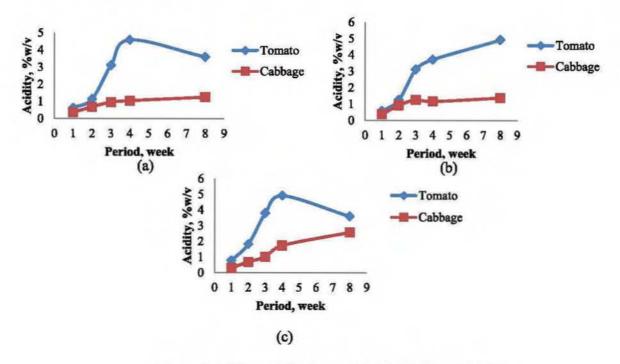


Figure 2 Acidity of Bio-cleaner (a) 3:1 (b) 4:1 and (c) 5:1

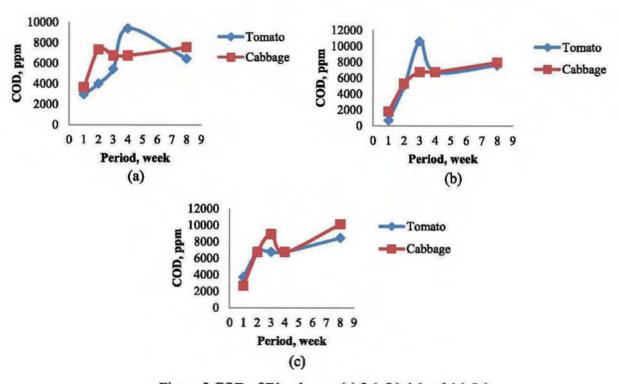


Figure 3 COD of Bio-cleaner (a) 3:1 (b) 4:1 and (c) 5:1

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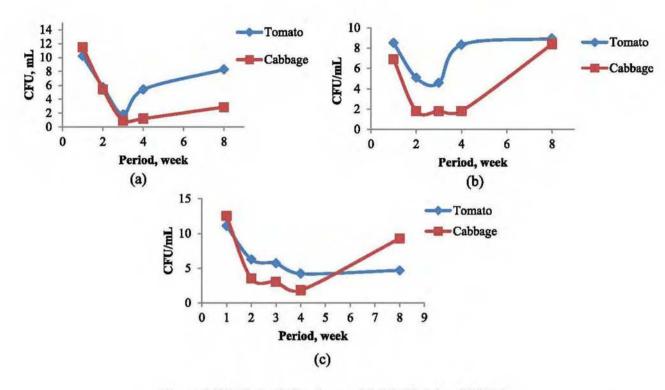


Figure 4 CFU/ml of Bio-cleaner (a) 3:1 (b) 4:1 and (c) 5:1

Domestic wastewater has the odor of kerosene or freshly turned earth. The characteristic of rotten egg odor of hydrogen sulfide and the mercaptan is indicative of septic sewage. It is gray in color and septic sewage is black. Primary treatment of wastewater by screening was carried out in this work. That removed insoluble matter such as grit, grease and scum from water. In addition, screening reduced the size of trash and large solids. This primary treatment also removed both settable and floatable solids.

As shown in Table 1, the characteristics of domestic wastewater collected from ULB ward indicated high concentration in contents of COD (4207.37 ppm), total dissolved solids (347 ppm), nitrogen (115 ppm) and phosphate (22 ppm). Their ranges fall between weak and medium concentration of untreated domestic wastewater cited by Mackenzie & Cornwell, 1998. Excessive amount of two nutrients such as nitrogen and phosphorus are pollutants that can lead to large growth of algae in receiving water. Some major sources of nutrients present in domestic wastewater are phosphorus based detergents, fertilizers and food-processing wastes.

Bhatia, (2013) also stated that organic nitrogen present in wastewater is converted to ammonium ion or nitrate and organic phosphorous to orthophosphate as secondary waste treatment. Ammonia in domestic wastewater is sourced from nitrogenous organic matter of sewage. The maximum permissible limit for phosphorous is 0.005 mg/l. The domestic sewage, agricultural run-off and synthetic detergents are the three major sources of phosphorous in water. Synthetic detergents contribute more to the inorganic phosphorous content in water. Inorganic phosphorous is released as a result of metabolic breakdown of proteins (in urine) and obviously sewage is rich in phosphorus. Detergent and other sources contribute significant amounts of phosphorous to domestic sewage and considerable phosphate ion remains in the effluent.

Table 1	Characteristics of	domestic wastewater and	d two-month aged bio-cleane	ers
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Characteristics	Wastewater	Bio-cleaners			
		Tomato	Banana	Cabbage	
pН	5.7	2.90	2.88	3.00	
TDS (ppm)	347	ş. <del></del>	-	-	
COD (ppm)	4207.37	7610.32	10919.12	10112.1	
Ammoniacal Nitrogen (ppm)	114.68	-	-	-	
Phosphate (ppm)	22		-	-	
CFU/mL	79 × 10 <sup>6</sup>	$3 \times 10^{6}$	3.1× 10 <sup>6</sup>	$3 \times 10^{6}$	

Based on preliminary results, 10% bio-cleaners of 3:1 ratio were chosen to treat domestic wastewater. Table 2 shows the characteristics of treated wastewater with 10% biocleaner (3:1) for 5, 10, 20 and 30 days. During 5-day treatment, it was observed that the contents of total dissolved solids, COD and nitrogen of wastewater decreased, specifically, ammoniacal nitrogen was extensively removed. It was observed that the ability of deodorization was instantly demonstrated by all bio-cleaners when treated to wastewater. pH of all bio-cleaners are in acidic range and the pH of wastewater increased to nearly pH 6. pH was still in acid range until the treatment period was 30 days. During 30 days treatment, total dissolved solids and COD contents in wastewater reduced to 45 % and 29 % of the original value by tomato waste bio-cleaner. Cabbage waste bio-cleaner showed its ability to reduce 41% and 48% of TDS and COD of wastewater. Moreover, all bio-cleaners revealed the effective removal of ammonia nitrogen and slight removal of phosphate from wastewater. 83% and 10 % removal of ammonia nitrogen and phosphate were resulted by tomato waste biocleaner. Likewise, cabbage waste bio-cleaner showed its removal of ammonia nitrogen and phosphate for 29% and 18% respectively. Generally, tomato waste bio-cleaner maybe effective agent to suppress ammonia nitrogen content in wastewater and all bio-cleaners could not be strongly recommended for effective removal of phosphate in wastewater. After 30 days treatment, the limitation of level of discharge was satisfactory in terms of COD, and TDS except for ammonia nitrogen and phosphate. Nitrification process in wastewater is more significant at pH greater than 6 and acetate ion present in bio-cleaner can be taken up by slow process of the phosphorus storing bacteria.

Table 2 Characteristics of domestic wastewater treated with 10% bio-cleaner (Tomato)

Characteristics	Wastewater	Treatment Period (Day)				
		5	10	20	30	
pН	5.7	2.38	3.23	4.44	5.73	
TDS (ppm)	347	324	309	235	189	
COD (ppm)	4207.37	3770.23	1658.52	3064.09	2990.11	
Ammoniacal Nitrogen (ppm)	114.68	36	30	20	20	
Phosphate (ppm)	22	22	21	20	20	
CFU/mL	$7.9 \times 10^{7}$	$8.8 \times 10^{7}$	$11.4 \times 10^{7}$	$17.1 \times 10^7$	$4.2 \times 10^{7}$	

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Characteristics	Wastewater	ter Treatment Period (Day)			
		5	10	20	30
pН	5.7	3.35	3.22	4.29	5.74
TDS (ppm)	347	340	326.8	286.5	205.9
COD (ppm)	4207.37	3662.63	2519.35	2337.77	2169.64
Ammoniacal Nitrogen (ppm)	114.68	104	98	90	81.74
Phosphate (ppm)	22	20	20	18	18
CFU/mL	$7.9 \times 10^{7}$	$14.3 \times 10^{7}$	$16 \times 10^{7}$	$42 \times 10^{7}$	18 × 10

Table 3 Characteristics of wastewater treated with 10% bio-cleaner (Cabbage)

Figure 4 shows the bacteria population in bio-cleaners. Low pH of all bio-cleaners suppresses the microbial population in wastewater. As shown in Figure 5, CFU of bio-cleaners declined with the aged of fermentation. The nutrient deficiency may hinder the growth of bacteria with aging.

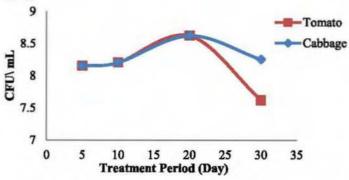


Figure 5 CFU in treated wastewater during 30-day period

### Conclusion

In this study, bio-cleaner can easily be prepared from fruits and vegetable wastes by natural fermentation under anaerobic conditions and unstabilized characteristics of bio-cleaners were found until 2—month aged of fermentation. Bio-cleaners were in acidic nature because of low pH and high concentration of COD. The quality of collected domestic wastewater falls between the low level in TDS and high concentration of COD, ammonia nitrogen and phosphate. The acidic nature of bio-cleaners can effectively remove malodor of wastewater instantly. Aside from deodorization of wastewater, effective removal of total dissolved solids and COD have been shown by bio-cleaners. Moderate removal of ammonia nitrogen and very slight removal of phosphorus was studied. Further study should be conducted on the removal of ammonia nitrogen and phosphate by bio-cleaners. Detailed study on characterization of bio-cleaners should also be explored.

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