INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–360/ABB/2010/12–2–194–198
http://www.fspublishers.org

Full Length Article

Biosynthesis of Enzyme Ionic Plasma for Wastewater Treatment using Fruit and Vegetable Waste

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ABSTRACT

Fruit and vegetable waste (FVW) is produced in large quantities in fruit markets and pose many environmental threats due to its high biodegradability. A possible way to dispose of this waste is its anaerobic digestion to biosynthesize enzyme ionic plasma (EIP) to digest FVW. The current study was focused on the biosynthesis of EIP using FVW and optimizing its process variables like substrate ratio, incubation time and temperature. Chopped FVW along with water and brown sugar was added to a plastic container of 2 L capacity under anaerobic conditions. The substrate ratio was optimized using different combinations of FVW and brown sugar at 25°C. The incubation time was varied from 10-20 days. After synthesis, the EIP was filtered for chemical and microbial analysis; microbial count, pH and chemical oxygen demand (COD). Best results were obtained when EIP was prepared from 1 L of water, 0.5 kg of FVW and 0.1 kg of brown sugar. The optimization studies showed best results of EIP biosynthesis after 20 days for 25°C. © 2010 Friends Science Publishers

Key Words: Vegetable waste; Anaerobic digestion; Enzyme ionic plasma; Wastewater

INTRODUCTION

Pakistan, being a developing country is more prone to the environmental problems of anthropogenic origin. An ever-increasing population growth rate, coupled with lack of resources, makes it difficult to cope with problems like diseases, drinking water and waste management. It has been estimated that Pakistan generates 55,000 tons of solid waste per day (JICA, 2005). Municipal solid waste (MSW) is disposed off into landfills in Pakistan, as a major source towards green house gasses (Batool & Chaudary, 2009). Proper management of these landfills is a difficult task due to lack of financial resources and skills (Batool *et al.*, 2008). This situation forces to adopt approaches of recycling and value-addition of solid waste. Thus, MSW management proves to be a challenging task for municipal authorities (Carneiro *et al.*, 2008).

Fruit and vegetable waste (FVW) is the most vital component of the organic portion of MSW. Highly biodegradable FVW produced in markets constitutes a source of nuisance in municipal landfills due to its moisture and organic content (Misi & Forster, 2002; Liu *et al*., 2009). The FVW can cause several environmental and health hazards, affecting socio-economic growth of the area. The FVW can contaminate groundwater resources causing diseases such as cholera, dysentery, typhoid etc. The FVW also attracts flies and rats spawn at these dump sites and which can spread plague, while the flies are a source of transmission for malaria and yellow fever (World Wildlife Fund-Pakistan, 2009).

Anaerobic digestion is a process by which almost any organic waste can be biologically converted in the absence of oxygen. Anaerobic digestion is a well established process for treating many types of organic waste, both solid and liquid. Anaerobic digestion requires specific environmental conditions and different bacterial populations. The main advantages of the anaerobic digestion are net production of energy, reduced CO_2 emissions and recycling benefits (Rizk *et al.*, 2007; Mohan *et al.*, 2009). Mixed bacterial population degrades organic compounds and produces a valuable liquid termed as enzyme ionic plasma (EIP). Microorganisms in EIP produce enzymes, which help to decompose organic substances and continue this decomposition via a chain reaction. EIP is the byproduct of anaerobic decomposition of FVW. It is an environment friendly product and can be used as a dishwashing liquid, toilet cleaner and plant fertilizer. It is also used to clean polluted water for color and odor removal (Paetkau, 2003).

The production of EIP is quite convenient, because of its easily available raw material and simple operations involved. Heaps of fruit and vegetable waste (FVW) can be converted to this useful versatile solution. It cannot only

To cite this paper: Mahmood, T., M.S.U. Rehman, A. Batool, I.U. Cheema and N. Bangash, 2010. Biosynthesis of enzyme ionic plasma for wastewater treatment using fruit and vegetable waste. *Int. J. Agric. Biol*., 12: 194–198

settle the waste disposal issues, but can also provide a universal solution with lots of applications in household and environmental cleaning activities. EIP biosynthesis involves some technical issues to be resolved to make it an appropriate environment friendly economical technology. EIP can be easily synthesized once its operating conditions are optimized.

The current study was focused to the biosynthesis of EIP using FVW and brown sugar as substrate. The optimization of process variables like substrate ratio, incubation time and temperature was carried out using pH, chemical oxygen demand (COD) and bacterial count as evaluating parameters.

MATERIALS AND METHODS

Sample collection: FVW was collected from the local vegetable market of Rawalpindi-Pakistan and brought to the laboratory of Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi-Pakistan. The FVW brought to the laboratory were chopped to particles as small as possible for experimentation. The surface area was increased by chopping the FVW. The FVW consisted of equal ratios of tomatoes, lemon, cauliflower and bananas.

Optimization of ratios of FVW and brown sugar: A series of laboratory experiments were conducted to optimize parameters for the preparation of EIP. Temperature incubation time and ratios of FVW to brown sugar were studied. EIP was prepared by using experimental conditions (Table I) at 25^{o}C. The pH, COD and colony forming units (CFU mL^{-1}) were measured. Experimental ratios used for to optimize FVW and brown sugar (in kg) in constant volume (1 L) of water were: 0.3:0.1, 0.3:0.2, 0.3:0.3, 0.4:0.1, 0.4:0.2, 0.4:0.3, 0.5:0.1, 0.5:0.2 and 0.5:0.3.

Optimization of temperature and time: Optimized FVW and sugar mass ratio in 1L of water were added to a 2 L plastic container and mixed well by shaking. The container was tightly sealed for anaerobic digestion of the mixture and stored at different temperatures (25, 35 & 45^{o}C) in an incubator. The incubation temperature was varied and the time was 10, 15 and 20 days. The pH, COD and CFU per mL were measured.

Analysis of samples: EIP was filtered out after its preparation and filtrate was subjected to chemical analysis. The chemical analysis of EIP included the measurement of pH, COD (APHA, 1998) and titrable acidity (Awan & Salim-Ur-Rehman, 2001). The pH of EIP was measured by pH meter (4500B; APHA, 2005). COD of EIP was determined through wet digestion by $K_2Cr_2O_7$ using closed reflux colorimetric method. A 3.7 mL COD reagent (add 500 mL distilled water, 10.22 g $K_2Cr_2O_7$,167 mL H_2SO_4, 33.3 g $HgSO_4$ & dilute to 1000 mL) was added to 2 mL of EIP. The solution was then incubated in block digester (Lovibond, Model ET 108, Germany) at 150ºC for 2 h and the absorbance was measured by COD analyzer (Lovibond PC checkit, Model SN 78602, Germany) at 600 nm (Himebaugh & Smith, 1979).

Measurement of bacterial count was done by serial dilution of the EIP. Nine test tubes containing 9 mL distilled water were sterilized, 10 fold serial dilutions were made by mixing 1 mL of EIP sample in first tube and then after mixing 1 mL of dilution was transferred to the second tube. This process was repeated up to the tube 12. A sterile tube was used to dispense 0.1 mL from each dilution on to the nutrient agar plate. Glass spreader was used for spreading on the plate. The plates were incubated at optimum temperature for the growth of bacteria for 24 h and then the next day, the colonies were counted. Colony forming unit per mL was calculated according to the following formula:

$$\text{CFU mL}^{-1} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Size of inoculum}}$$

Statistical analysis: The data obtained was tabulated and statistically analyzed. The 2 factor factorial CRD arrangement was applied. The means were compared by Least Significant Difference Test (LSD) at 0.05 confidence level.

RESULTS AND DISCUSSION

Optimization of ratios of FVW and brown sugar: The EIP was prepared by using different ratios of FVW and brown sugar. The samples were incubated at 25°C and different time intervals (15, 20, 25 days). Statistical analysis revealed that pH of EIP prepared by using different ratios of FVW and brown sugar at different time durations had non-significant difference (Fig. 1). The pH values were independent of substrate ratio and time of incubation. However quite acidic pH values were obtained as a result of microbial digestion, because the anaerobic digestion of FVW was accomplished by a series of biochemical reactions, which could be roughly separated into four metabolic stages. First metabolic stage involved liquefaction of particulate organic material of FVW like 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_2 and CO_2. Finally methane was produced by methanogenic bacteria from organic acids produced in earlier steps, H_2 and CO_2 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 COD was monitored as an indicator parameter of the biostabilization of the organic strength. It was observed that COD concentration increased as a function of feed ratio and incubation time (Fig. 2). The COD concentration increased when incubation time increased from 15 to 20 days but decreased afterwards. Statistical analysis showed highly significant difference ($P<0.05$) in treatment means

Table I: Experimental conditions used for the optimization of water, FVW and brown sugar ratio

Treatment	Code	Water (L)	FVW (kg)	Sugar (kg)
1	10:3:1	1	0.3	0.1
2	10:3:2	1	0.3	0.2
3	10:3:3	1	0.3	0.3
4	10:4:1	1	0.4	0.1
5	10:4:2	1	0.4	0.2
6	10:4:3	1	0.4	0.3
7	10:5:1	1	0.5	0.1
8	10:5:2	1	0.5	0.2
9	10:5:3	1	0.5	0.3

Fig. 1: The pH values of EIP prepared at different time durations and by using different ratios of FVW and brown sugar at 25°C

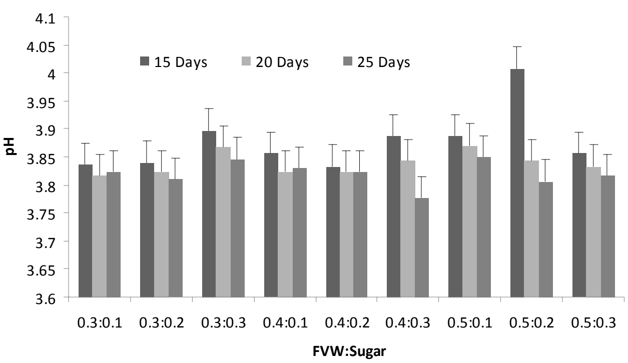
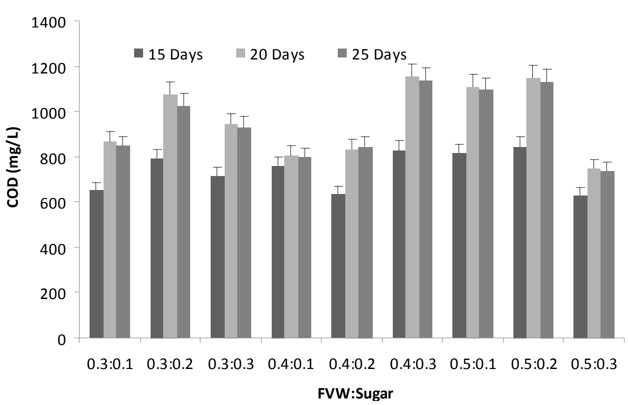


Fig. 2: The COD values of EIP prepared at different time durations and by using different ratios of FVW and brown sugar at 25°C



for COD values of EIP prepared at 25°C by using different ratios of FVW and brown sugar. The maximum COD value was obtained by FVW: sugar ratio of 0.5:0.2 followed by 0.4:0.3 and 0.5:0.1after 20 days of incubation. All these treatments had a higher ratio of FVW, providing more substrate to the bacteria resulting in their growth and increased COD values. Even FVW: sugar ratio of 0.5:0.3 had higher substrate ratio than 0.5:0.2 but could not attain the high COD values perhaps, because the high substrate resulted in reducing the bacterial activity and thus lowering COD. This can be supported by a decline in the bacterial count when the substrate was increased (Fig. 3).

It was shown (Fig. 3) that bacterial count increased with substrate ratio and incubation time (up to 20 days). The maximum bacterial count (4.35×10^{12}) was achieved by FVW: sugar ratio of 0.5:0.1 followed by 0.4:0.3>0.4:0.2>0.5:0.2>0.4:0.1>0.5:0.3>0.3:0.3>0.3:0.2> 0.3:0.1 after 20 days of incubation. The maximum growth was observed for 0.5:0.1 after which the bacterial count decreased. This decrease might be due to the inhibitory effect of high substrate levels on the bacterial population. Statistical analysis showed highly significant difference ($P<0.05$) in treatments for bacterial count of EIP prepared at 25°C by using different ratios of FVW and brown sugar.

From these results we could conclude that FVW: sugar ratio of 0.5:0.1 will be the better option for preparation of EIP as it showed better results and contained the highest ratio of FVW and lowest ratio of brown sugar. Thus the digestion would be economical as a large amount of FVW can be digested with the minimum amount of brown sugar. FVW: sugar ratios of 0.4:0.3 and 0.5:0.2 also showed good results but 0.5:0.1 is economical in comparison. Therefore, 0.5 kg of FVW and 0.1 kg of brown sugar was optimized for further study.

Optimization of incubation temperature and time: Optimization of temperature for the digestion of FVW showed lower pH values indicating the generation of acidic species. It was evident (Fig. 4) that the EIP produced was more acidic (pH 3.75) when anaerobic digestion took place at 35°C compared to 25°C and 45°C for any duration of time period. However pH values of EIP decreased when temperature was raised from 25°C to 35°C and increased thereafter, to its initial values at 45°C; almost similar to 25°C for different time duration. It was also noticed that pH decreased as function of time in all the cases implying the formation of organic acids over the period of digestion. Statistical comparison of treatment means showed that results of pH were highly significant ($P<0.05$). High temperature improved the anaerobic biodegradation of the complex organic matter (Bouallagui *et al.*, 2003). The thermophilic microflora has the capacity to use several sources of carbon than the mesophilic and psychrophilic mirocflora (Converti *et al.*, 1999).

The drop in pH was due to the formation of VFA with acetic acid and butyric acid being the main contributing organic acids when substrate was readily degraded by naturally existing enzymes in FVW (Bouallagui *et al.*, 2003). The lower pH value might also be due to degradation of FVW into CO_2 and water (Wills *et al.*, 1981). Higher COD concentration at 35°C (Fig. 5) also promoted generation and accumulation of volatile organic acids during digestion (Rizk *et al.*, 2007). Moreover several components of solid wastes like carbohydrate were reported to have the hydrogen potential. Lastella *et al.* (2002) showed that FVW has high carbohydrate content (about 75%) including a mixture of polymers such as cellulose, pectin, lignin and hemicellulose and carbohydrates were better precursor for

Fig. 3: Bacterial count of EIP prepared at different time durations and by using different ratios of FVW and brown sugar at 25°C

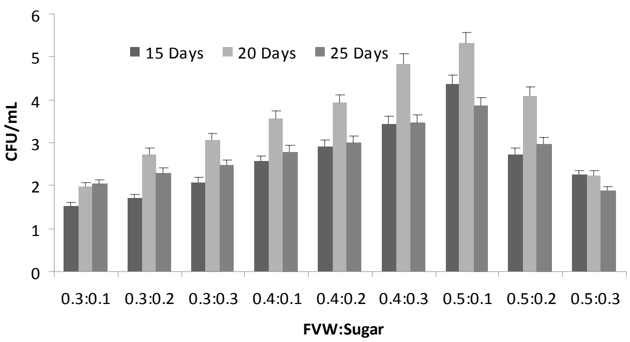
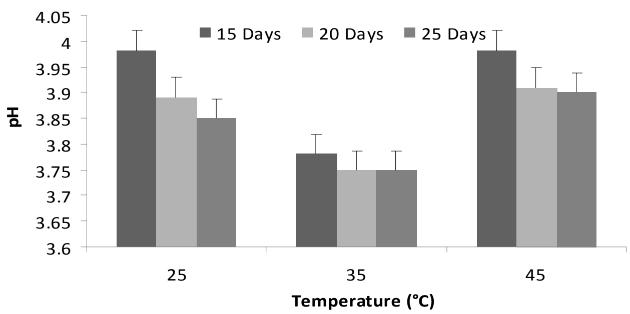


Fig. 4: The pH of EIP prepared at different temperatures and time durations



hydrogen coming from biological fermentation than lipids or proteins. This hydrogen contribution could be considered another possible reason for pH decrease during the anaerobic digestion. Moreover El-Jalil *et al.* (2008) reported that Salminen and Rintala (2002) found that lactic bacteria produced lactic acid during fermentation process and survived up to (pH 3-4.75).

The COD concentration of EIP observed a reverse trend when compared with pH values. COD concentration was doubled when temperature was raised from 25°C to 35°C and slightly decreased for 45°C. COD concentration at 35°C observed an increase by 33% when incubation time was increased from 15 to 20 days, while it remained constant afterwards till 25 days. However, COD concentration at 45°C observed about 40% of increase when incubation time was increased from 15 to 20 days, while it was halved by day 25th. The rise in COD concentration was supported by the acidogenic environment of the mixture. The high bacterial population at 35°C (Fig. 3) degraded FVW into dissolved and suspended organic matter, which resulted in the rise of COD concentration (Rizk *et al.,* 2007). Probability value of less than 0.05 (P=0.000) was obtained for all factors by ANOVA, which indicated that results for COD were highly significant due to different temperatures and time durations.

It was noticed that temperature of 35°C on day 20 of the experiment optimized with respect to bacterial count (Fig. 6). These results showed that anaerobic digestion of

Fig. 5: The COD of EIP prepared at different temperatures and time durations

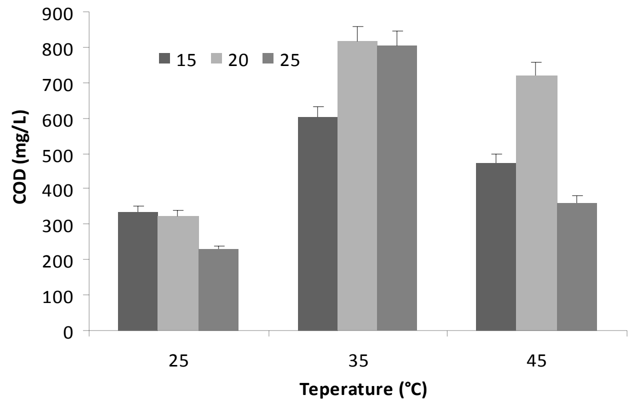
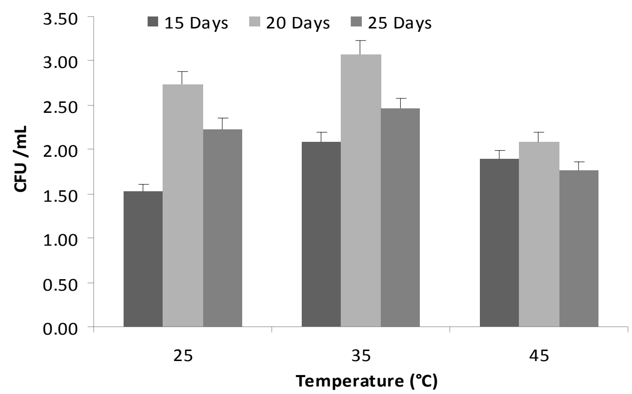


Fig. 6: The bacterial count of EIP prepared at different temperatures and time duration



FVW at the mesophilic (35°C) stage functioned properly (Bouallagui *et al.*, 2003; Khalaf & Meleigy, 2008). The increase in temperature allowed the fibrous vegetables substrate to liquefy (Bouallagui *et al.*, 2004). With increasinged temperature the organic part of the waste was split up into short chain fragments that were biologically well suited to microorganisms (Converti *et al*., 1999; Bonmati *et al.*, 2001). Comparison of treatment means showed that results of bacterial count were highly significant (P<0.05). It was obvious from the results of bacterial count that the bacterial population was high at 35°C after 20 days. However from technical viewpoint it is not economical to raise and maintain the temperature of this biochemical process up to 35°C. Thus the process was considered to be optimized at 25°C (Room temperature) in about 20 days, which was easy to implement.

CONCLUSION

Fruit and vegetable waste are major fraction of the MSW, which can be converted to many valuable products like EIP. EIP can be prepared by anaerobic digestion of FVW. Through process optimization, water, FRW and brown sugar in 5:1 ratio was selected. The optimum temperature for EIP preparation was 25°C that could be managed without any extra utilization of energy. EIP can be used for various purposes like waste water treatment. Further this strategy would help to reduce piling up of organic wastes and to clean up the environment.

Acknowledgement: Authors acknowledge Directorate of Planning and Research, PMAS Arid Agriculture University, Rawalpindi-Pakistan for providing partial funding for this project under Project NO. AAUR/DR-366, S.R. Rehman is thankful to EU for awarding EMQAL studentship.

REFERENCES

APHA-American Public Health Association, 1998. *Standard Methods for the Examination of Water and Waste Water,* 20th edition. Washington DC

Awan, J.A. and Salim-Ur-Rehman, 2001. *Food Analysis Manual*. Unitech Communications, Faisalabad, Pakistan

Batool, S.A., N. Chaudary and K. Majeed, 2008. Economical potential of recycling business in Lahore-Pakistan. *Waste Manag.,* 28: 294–298

Batool, S.A. and N. Chaudary, 2009. The impact of municipal solid waste treatment on greenhouse gas emissions in Lahore, Pakistan. *Waste Manag.,* 29: 63–69

Bonmati, A., X. Flotats, L. Mateu and E. Campos, 2001. Study of thermal hydrolysis as a pretreatment to mesophilic anaerobic digestion of pig slurry. *Water Sci. Technol.,* 44: 109–116

Bouallagui, H., M. Torrijos, J.J. Godon, R. Molette, R. Ben Cheikh, Y. Touhami, J.P. Delgenes and M. Hamdi, 2004. Two phases anaerobic digestion of fruit and vegetable wastes: Bioreactors performance. *Biochem. Eng. J*., 21: 193–197

Bouallagui, H., O. Haouari, Y. Touhami, R. Ben Cheikh, L. Marouani and M. Hamdi, 2003. Effect of temperature on the performance of an anaerobic tubular reactor treating fruit and vegetable waste. *Process Biochem*., 39: 2143–2148

Bouallagui, H., Y. Touhami, R.B. Cheikh and M. Hamdi, 2005. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. *Process Biochem.,* 40: 989–995

Carneiro, T.F., M. Pérez and L.I. Romero, 2008. Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste. *Bioresour. Technol.,* 99: 6994–7002

Converti, A., A. DelBorghi, M. Zilli, S. Arni and M. DelBorghi, 1999. Anaerobic digestion of the vegetable fraction of municipal refuses: mesophilic versus thermophilic conditions. *Bioprocess Eng.,* 21: 371–376

El-Jalila, M.H., A. Zinedine and M. Faid, 2008. Some microbiological and chemical properties of poultry waste manure after Lactic acid fermentation. *Int. J. Agric. Biol.*, 10: 405–411

Himebaugh, R.R. and M.J. Smith, 1979. Semi-micro tube method for chemical oxygen demand. *Anal. Chem.,* 51: 1085–1090

JICA, 2005. JICA (Japan International Cooperation Agency) and Pak-EPA (Pakistan Environmental Protection Agency), 2005. *Guidelines for Solid Waste Management*. Pak-EPA, Pakistan

Khalaf, M.A. and S.A. Meleigy, 2008. Reduction of free gossypol levels in cottonseed meal by microbial treatment. *Int. J. Agric. Biol.*, 10: 185–190

Lastella, G., C. Testa and G. Cornacchia, 2002. Anaerobic digestion of semi-solid organic waste: biogas production and its purification. *Ener. Conser. Manag*., 43: 63–75

Liu, G., R. Zhang, H.M. Al-Mashad and R. Dong, 2009. Effect of feed to inoculum ratios on biogas yields of fruit and green wastes. *Bioresour. Technol*., 100: 5103–5108

Misi, S.N. and C.F. Forster, 2002. Semi-continuous anaerobic co-digestion of agro-waste. *Enviorn. Technol*., 39: 39–48

Mohan, S.V., G. Mohanakrishna, R.K. Goud and P.N. Sarma, 2009. Acidogenic fermentation of vegetable based market waste to harness biohydrogen with simultaneous stabilization. *Bioresour. Technol.*, 100: 3061–3068

Paetkau, M., 2003. *Cleaning up Bangkok Water Ways.* Bangkok Post

Rizk, M.C., R. Bergamasco and C.R.G. Tavares, 2007. Anaerobic co-digestion of fruit and vegetable waste and sewage sludge. *Int. J. Chem. React. Eng*., 5: 1–10

Salminen, E. and J. Rintala, 2002. Anaerobic digestion of organic solid poultry slaughterhouse waste: -a review. *Bioresour. Technol.,* 83: 13–26

Veeken, B.A., S. Kalyuzhnyi, H. Sccharff and B. Hamelers, 2000. Effect of pH and VFA on hydrolysis of organic solid waste. *J. Enviorn. Eng*., 126: 1076–1081

Wills, R.H.H., T.H. Lee, D. Graham, W.B. McGlasson and E.G. Halls, 1981. *Post Harvest: an Introduction of the Physiology and Handling of Fruits and Vegetables*. AVI Publishing Co., West Port, Connecticut

World Wildlife Fund, Pakistan, 2009. *Municipal Solid Waste Factsheet*. http://www.wwfpak.org/factsheets_mswf.php

(Received 17 October 2009; Accepted 19 November 2009)