Investigation of Low-Cost Sugar Supplement for Lactic Acid Fermentation in Terra Preta Sanitation System

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Cite as: A. Yemaneh et al: Investigation of Low-Cost Sugar Supplement for Lactic Acid Fermentation in Terra Preta Sanitation System, in: Bettendorf, T.; Wendland, C.; Otterpohl, R. 2014. *Terra Preta Sanitation*.

Publisher: Deutsche Bundesstiftung Umwelt

ISBN 978-3-00-046586-4

http://www.terra-preta-sanitation.net

Abstract

Terra Preta Sanitation (TPS) is a sanitation system which combines biological treatment processes, lactic acid fermentation (LAF) and vermicomposting, for transforming human excreta to pathogen free humus rich in nutrients and organic matter. LAF is used during collection of human excreta in toilets for suppressing odour, pathogen reduction and conservation of nutrients and organic matter. For LAF process sugar content of the substrate is particularly crucial, as sugars constitute the growth limiting substrate for lactic acid bacteria (LAB). In this study the suitability of kitchen biowaste and molasses as sugar supplement for LAF of human faecal matter is investigated. Batch laboratory-scale experiments are conducted with the addition of sugar supplements and LAB inoculum to human faecal matter. It is found that with addition of 40-50% kitchen biowaste or 5-10% molasses as sugar supplement good LAF can be established achieving the desired effects of LAF application in sanitation. 50% kitchen biowaste and 10% molasses are required to get very effective LAF especially with respect to hygienization, which is important aspect in sanitation provision.

Keywords*:* terra preta sanitation, lactic acid fermentation, sugar supplement, lactic acid bacteria

INTRODUCTION

Availability of safe sanitation systems is still a major problem particularly in urban areas of developing countries. Very rapid increase in urban population makes it even increasingly challenging to improve the sanitation coverage and condition. The Millennium Development Goals (MDGs) with respect to sanitation is set to miss its target in most of the developing countries (WHO/UNICEF, 2013). New sustainable technologies and flexible service models in sanitation and waste management need to be explored to tackle the sanitation challenges in urban centers. Terra Preta Sanitation (TPS) has been developed as an alternative pathway in dry toilet sanitation and it is considered as more ecologically sound sanitation system suitable for both urban and rural settlement conditions. TPS is inspired by the discovery of the ancient anthropogenic Amazonian black soil called 'Terra Preta', which owed is formation from the accumulation and subsequent degradation of various organic residues including human excreta, biowaste, charcoal and etc (Glaser & Birk, 2011).

TPS is based on two combined natural biological treatment processes, application of lactic acid fermentation (LAF) in the toilet during collection, as used in food and silage preservation, and further treatment involving vermicomposting of the lacto-fermented excreta off-site. In TPS human excreta and other biowastes, with addition of biochar, are treated and transformed to pathogen free humus which is rich in nutrients and organic matter that can safely and sustainably be utilized in agriculture bringing long term positive effect on soil fertility and productivity (Otterpohl, 2012).

LAF is an anaerobic process in which lactic acid bacteria (LAB) and some fungi metabolize easily degradable carbohydrates, such as glucose, fructose, and sucrose to pyruvate via glycolysis. The pyruvate is then converted mainly to lactic acid and few other metabolic by-products depending on the type of lacto-fermenters involved. Two distinct pathways exist for carbohydrate metabolism by LAB, homolactic and heterolactic fermentation. Homofermentative LAB form only lactic acid as metabolic end product. For one mole of glucose, two moles of lactate are formed. Heterofermentative LAB, on the other hand, produces carbon dioxide and ethanol or acetate in equimolar quantities in addition to lactic acid. The common applications of LAF are the production of various fermented foods, food preservation, silage preservation, and in management and disinfection of various organic wastes (Hofvendahl & Hahn-Hägerdal, 2000).

For LAF process the sugar content of the substrate is particularly crucial, as sugars constitute the growth limiting substrate for LAB. Although, the composition of human excreta varies strongly with diet, climate and state of health of the persons, the amount of simple sugar excreted is negligible to support the growth of LAB for the fermentation process. From LAF research on other organic wastes it is established that in cases where there is not enough sugar in the original substrate adding sugar supplement is necessary to achieve good LAF conditions. Therefore, it is very important to find easily accessible low-cost sugar sources for the LAF phase of TPS for sustainable application of the sanitation system. Prior study at TUHH indicated that addition of simple sugar supplement is necessary to establish good LAF process in human faecal matter (Yemaneh et al., 2012). Kitchen biowaste can be one alternative sugar source for the LAF process in TPS system. It is a nutrient rich resource which is available in every household. In this study the suitability of kitchen biowaste and molasses as low-cost sugar supplements for LAF of human faecal matter is investigated using laboratory-scale experiments.

MATERIALS AND METHODS

Faecal Matter

The faecal matter used in the experiments is obtained from public toilets in Hamburg Central Station. The toilets used for collection are low-flush toilets which use 3.5 L water per flushing. The liquid fraction of the black water is separated from the solid fraction and is pumped back to the city sewerage system while the solid fraction, which is used in the experiments, is collected in an airtight container. The total solid content of the faecal matter is 17% (wet weight) and the volatile solid content is 65% of the dry matter.

Lactic Acid Bacterial Mix (LAB mix)

As per the findings of a prior study at TUHH mixed culture of three LAB strains, *Lactobacillus plantarum*, *Lactobacillus casei* and *Pediococcus acidilactici,* is used as inoculum for the fermentation experiments (Yemaneh et al., 2012). Inoculant is prepared by transferring frozen cultures to 100mL serum bottles containing 50ml of MRS medium. The flasks are incubated at 30°C for 20 h, the time needed for the bacteria to reach exponential growth phase. 10% (w/w) of the biomass solution is added to the fermentation reactors for the fermentation experiments.

Sugar Supplements

Kitchen biowaste and molasses are used as sugar supplements in the experiments. The kitchen biowaste used in the experiments was obtained from the TUHH Mensa. It was blended in food blender and is stored in fridge before added to the fermentation reactors. The molasses used was a commercial sugar cane molasses produced by "Emiko" and it contains 46% simple sugar as well as various other nutrients. The sugar content of the kitchen biowaste is used 9.6% (wet weight) and molasses contains 46% simple sugar (wet weight).

Experimental Design

Batch laboratory-scale fermentation experiments lasting three weeks are conducted in 1L glass fermentation reactors. Specific quantity of the excreta is transferred to the reactors and supplemented with additives (sugar supplement and microbial inoculant). Variations in the amount of sugar supplements added are considered in the experiments to determine the optimum level for achieving the desired effects LAF process. All experiments are conducted at 20°C in water bath under anaerobic condition with opening the reactors for sampling.

Analytical Methods

The LAF process is monitored by measuring pH, lactic acid (LA), volatile solids (VS), nitrogen (total nitrogen and ammonium nitrogen) and *E-Coli*. pH is measured directly with a microprocessor pH meter attached to pH electrodes. Total lactic acid (sum of D-and L-lactic acid) is determined reflectometrically using a lactic acid test strips from Merck after appropriate dilution of the samples. Volatile solids are determined following the DIN 38414 procedure. *E-coli* count is determined using ChromoCult Coliform Agar by spread plating technique after appropriate serial dilution of the samples. Total soluble nitrogen is measured by extraction with $CaCl_2$ solution and analysing the filtrate with TOC and TN analyser (Multi N/C 3000, Analytic Jena). Ammonium nitrogen is determined by steam distillation followed by back titration, following a method developed by TUHH central laboratory for chemical analysis. Odour in the fermentation reactors is evaluated by odour panel consisting of group of volunteer subjects asked to evaluate the odour using guidelines established for sensory evaluation mainly related to the type of smell observed and on the acceptability of the resulting smell.

RESULTS AND DISCUSSION

Fermentation Parameters

Different quantities of molasses and kitchen biowaste are added to determine the quantity of sugar supplement that needs to be added to establish proper LAF in the system which is considered to be achieved when pH is lowered to 4 or less and more than 2% lactic acid concentration is produced according to studies on other substrates (Jalil et al., 2008; Shirai et al.,2001; Kheratti et al., 1998; Zakaria et al., 1998). 0, 2, 5, 10, 15 and 20% molasses and 0, 20, 40, 50, 60, 80% kitchen biowaste by total wet weight of faecal matter are added to the fermentation reactors with LAB inoculum. The results of the pH and lactic acid concentration monitored over the fermentation period are shown in figures 1 and 2 below.

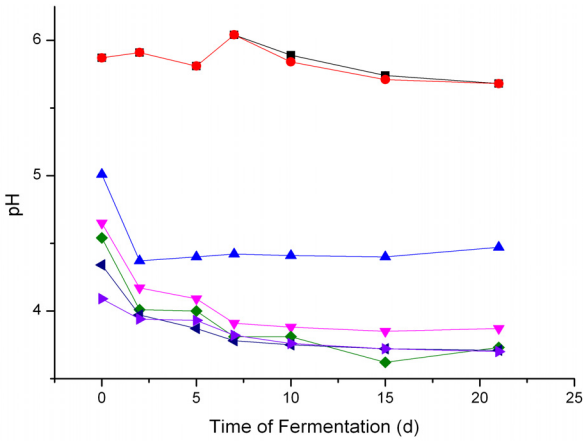

Figure 1: pH measurement for varying quantities of kitchen biowaste added. Control (■); LAB (●); LAB and 20% kitchen biowaste (▲); LAB and 40% kitchen biowaste (▼); LAB and 50% kitchen biowaste (♦); LAB and 60% kitchen biowaste (◄); LAB and 80% kitchen biowaste (►)

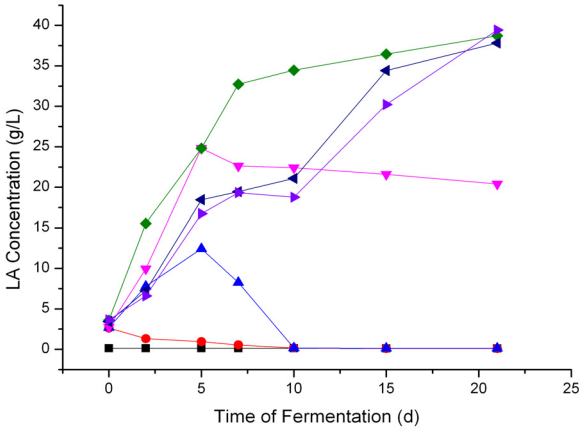

Figure 2: Lactic acid concentration for varying quantities of molasses added. Control (■); LAB (●); LAB and 2% molasses (▲); LAB and 5% molasses (▼); LAB and 10% molasses (♦); LAB and 15% molasses (◄); LAB and 20% molasses (►)

From the above figures it can be seen that addition of at least 5% molasses and 40% kitchen biowaste is necessary to achieve low final pH nearing 4 and lactic acid concentration above 2%. With 15 and 20% molasses additions the initial fermentation rate is slower than for the 10% addition, however the final pH and lactic acid concentrations are similar. Also, pH measurement for varying kitchen biowaste addition indicated similar pH profile for 50% and above kitchen biowaste addition. From the following figure 3 and 4 it can be further established that optimum LAF process can be established with addition of 10% molasses and 50% kitchen biowaste. The pH profile for these two supplements is similar although the lactic acid concentration for the 10% molasses addition is higher throughout the fermentation period. The similar fermentation pattern for 50% kitchen biowaste and 10% molasses additions can be attributed to the similar sugar concentrations in the fermentation system.

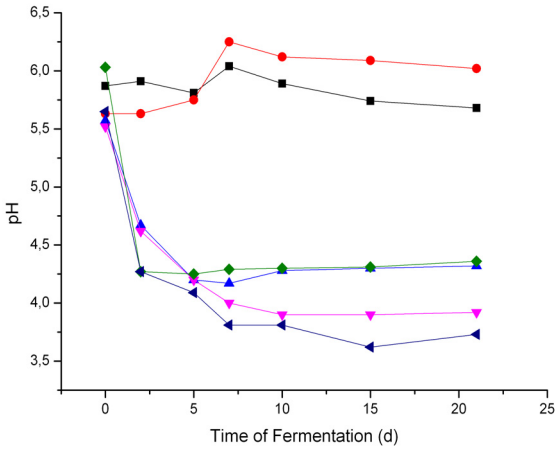

Figure 3: pH measurement for selected quantities of molasses and kitchen biowaste added. Control (■); LAB (●); LAB and 5% molasses (▲); LAB and 10% molasses (▼); LAB and 30% kitchen biowaste (♦); LAB and 50% kitchen biowaste (◄)

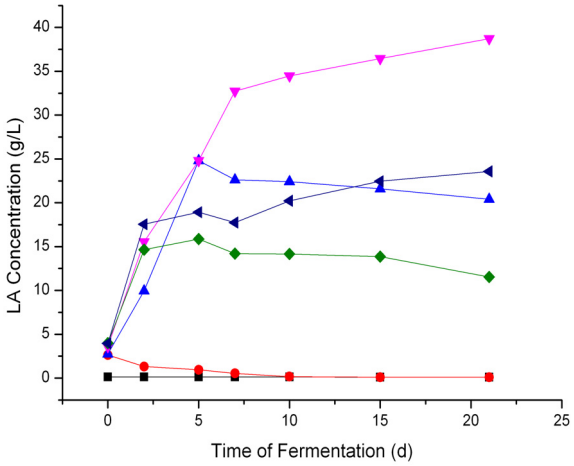

Figure 4: Lactic acid concentration for selected quantities of molasses and kitchen biowaste added. Control (■); LAB (●); LAB and 5% molasses (▲); LAB and 10% molasses (▼); LAB and 30% kitchen biowaste (♦); LAB and 50% kitchen biowaste (◄)

Different pre-treatments are conducted on kitchen biowaste, before added to the reactors, to see if the fermentation pattern can be improved by affecting the release of easily fermentable sugars so that they can be readily available to the LAB during fermentation. Grinding the kitchen biowaste followed by low-heat treatment (70°C) or pre-fermentation with LAB are conducted and compared with fermentation with direct addition of grinded kitchen biowaste. The results indicated that although pre-fermentation of the kitchen biowaste has accelerated the initial rate of fermentation, there is no significant difference on the fermentation parameters at the end of fermentation. The concentration of simple sugar in kitchen biowaste increased with low-heat treatment, however the fermentation pattern similar to the one with direct addition of grinded kitchen biowaste without pre-treatment. It can be concluded that pre-fermentation kitchen biowaste with LAB can help easy proliferation of LAB during fermentation and can enhance the desired effects of LAF in TPS. However, heat treatment doesn't seem to have significant effect on the fermentation process.

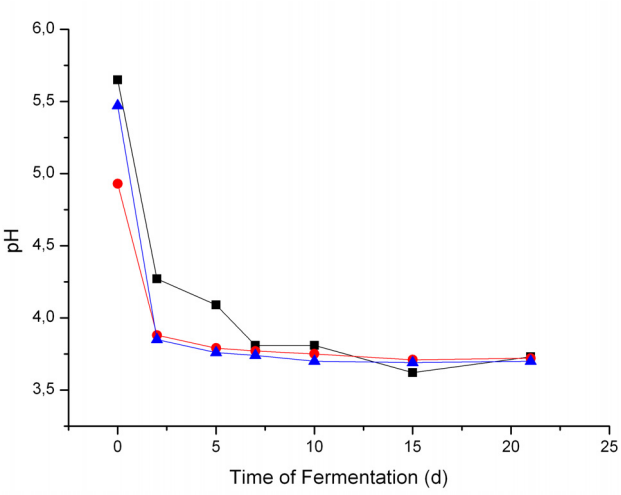

Figure 5: pH measurement for different pre-treatments of kitchen biowaste added. LAB and 50% kitchen biowaste without pre-treatment (■); LAB and 50% kitchen biowaste with pre-fermentation by LAB (●); LAB and 50% kitchen biowaste with low-heat pre-treatment (▲)

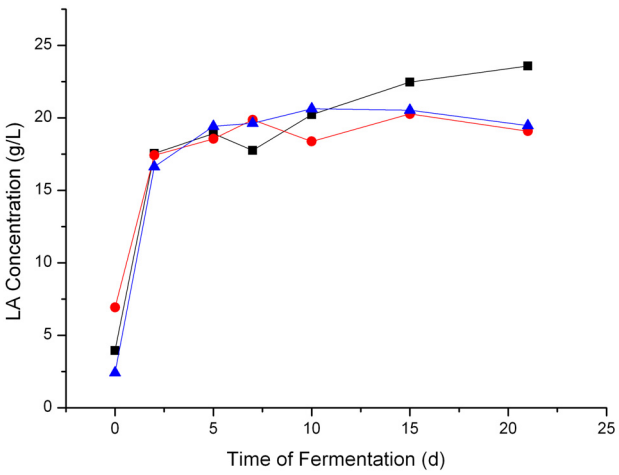

Figure 6: Lactic acid concentration for different pre-treatments of kitchen biowaste added. LAB and 50% kitchen biowaste without pre-treatment (■); LAB and 50% kitchen biowaste with pre-fermentation by LAB (●); LAB and 50% kitchen biowaste with low-heat pre-treatment (▲)

Volatile Solids

Organic matter conservation effect of the LAF process is investigated based on the determination of volatile solids (los on ignition). According to (Buzie, 2010) measurement of volatile solids can be good indicator for accessing organic matter decomposition in faecal matter. Generally it is observed that for fermentation with addition of LAB inoculum and sugar supplement only small relative decrease in VS content is observed indicating conservation effect of LAF process. More reduction in the treated reactors than the control is associated to the contribution of molasses to the VS content at the beginning and its transformation to lactic acid during the LAF process.

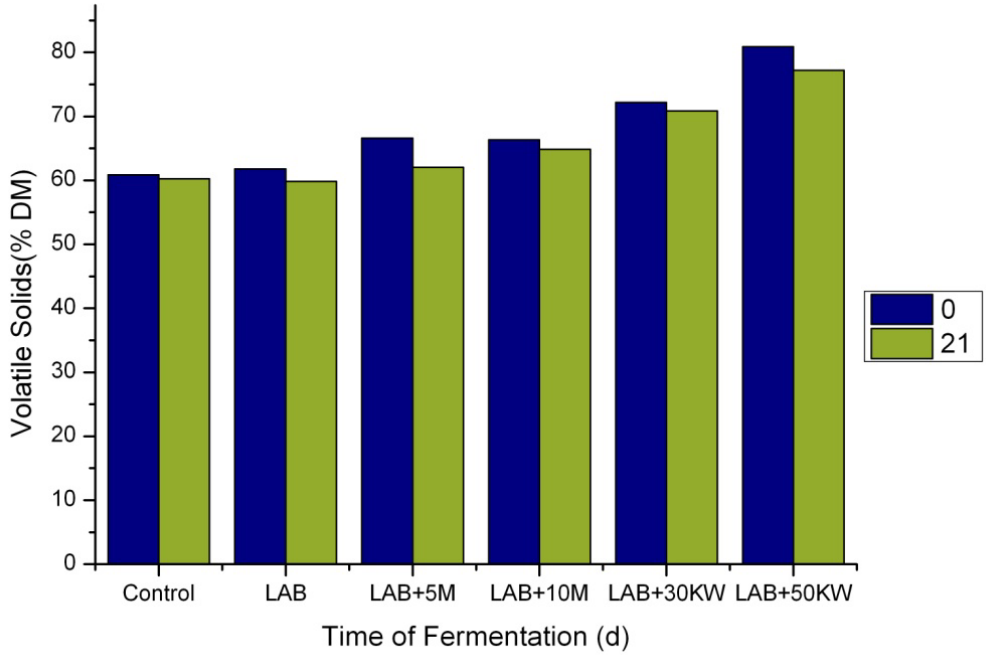

Figure 7: Volatile solid (VS) measurement at the beginning (0) and end (21) of fermentation (DM – dry matter, LAB – lactic acid bacteria, M – molasses, KW – kitchen biowaste)

Sanitation Indicator Bacteria

One of the effects of LAF process on faecal matter is the elimination of faecal pathogens and facilitating the safe recycling of organic matter and nutrients in agriculture. *E-coli* is monitored as sanitation indicator during the fermentation experiments. With addition of more than 5% molasses or more than 50% kitchen biowaste complete removal of *E-coli* is observed after three weeks.

Table 1: *E-coli* count (cfu/ml) for selected quantities of molasses and kitchen biowaste added

Reactors	Time of Fermentation (d)	
	0	21
Control	3,58E+06	5,80E+05
LAB	3,10E+06	1,35E+05
LAB+5%M	2,30E+06	0,00E+00
LAB+10%M	1,80E+06	0,00E+00
LAB+30%KW	1,73E+06	1,60E+03
LAB+50%KW	1,27E+06	0,00E+00

(LAB – lactic acid bacteria, M – molasses, KW – kitchen biowaste)

Ammonium Nitrogen and Total Nitrogen

In experiments with both molasses and kitchen biowaste addition there is a general decrease in ammonium nitrogen content at the end of the fermentation period. This can be attributed to the fact that LAB need inorganic nitrogenous compounds for their metabolic activity and it is likely that the ammonium nitrogen is consumed and transformed in to organic nitrogen in bacterial biomass. The utilisation of ammonium by LAB for their growth is also reported in literature (Mercier et al., 1992). With respect to total soluble nitrogen there is a general tendency of decrease in total soluble nitrogen at the beginning of fermentation with increasing quantity of molasses added due to dilution effect. Increase in the total nitrogen content is observed at the end of the fermentation period in all reactors with molasses and LAB addition, which can be explained by the mineralization of protein compounds in molasses. On the other hand reduction in total soluble nitrogen is observed for reactor with 50% kitchen biowaste and it can be associated with the complex composition of kitchen biowaste and the resulting bio-transformations.

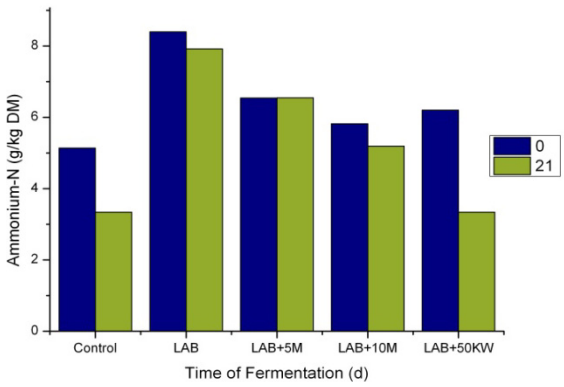

Figure 8: Ammonium nitrogen at the beginning (0) and end (21) of fermentation (DM – dry matter, LAB – lactic acid bacteria, M – molasses, KW – kitchen biowaste)

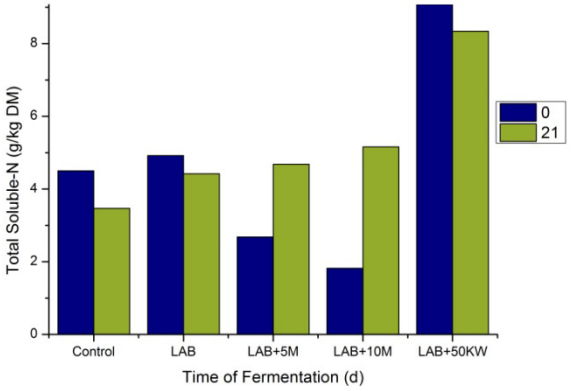

Figure 9: Total soluble nitrogen at the beginning (0) and end (21) of fermentation (DM – dry matter, LAB – lactic acid bacteria, M – molasses, KW – kitchen biowaste)

Odour Evaluation

Sensory evaluation of the fermentation process has indicated that for all treatments with 5% and higher molasses addition, and also with 50% kitchen biowaste addition, objectionable odour of the faecal matter is suppressed and is replaced by acidic smell. The reduction in smell is associated with destruction of microorganisms responsible for decompositions which may produce offensive odour. Also, formation of volatile fatty acids (VFAs), which constitute most of the objectionable odour in faecal matter, is inhibited by the LAF process. However, the resulting fermentation smell for kitchen biowaste addition varies depending on the composition of kitchen biowaste and sometimes it is strong. Therefore, it is recommended to add small quantity of charcoal or other natural smell adsorbents when kitchen biowaste is used as sugar supplement to remove residual fermentation smell.

Consideration for Practical Operation of TPS Toilet

For effective application of LAF process in TPS toilets availability of low-cost sugar supplement is crucial. From experimental studies it is demonstrated that molasses and kitchen biowaste can be used as a possible sugar supplement in TPS toilets. However, molasses may not be easily available for households and even if it is available it may have other competing uses. Kitchen biowaste on the other hand can be reliable source of sugar as it is available in every household. Considering per capita faecal matter production in the range of 100-400 g/d (Faechem et al. 1983), the quantity of kitchen biowaste generated in the household, which is in the range of 100 to 850 g/capita/day (Abel, 2007; Asomani-Boateng & Haight, 1999; Visvanathan & Tränkler, 2003), will be enough to supply the 40-50% kitchen biowaste (with respect to wet mass of faecal matter) that is needed to

establish effective LAF process. However the quantity of kitchen biowaste that need to be added is five times more than the quantity of molasses that is added to achieve the same effect and this aspect should be considered during designing volume of collection tank for TPS toilet.

CONCLUSIONS

The results of this experimental study indicate that LAF process can be very useful in dry toilet sanitation with respect to odour suppression, pathogen reduction and conserving nutrients and organic matter. It is demonstrated that kitchen biowaste can be low-cost sugar supplement for the LAF process. It is necessary to add 5-10% molasses or 40-50% kitchen biowaste with respect to the wet weight of faecal matter collected to get the desired effects of the LAF process. Combining collection of human excreta with organic kitchen biowaste will have further advantage of enabling the planning of more integrated and efficient waste management systems with the ultimate goal of sustainably improving soils. Rich soils help to ensure food security and play a key for reducing poverty and bringing overall sustainable development in a given region.

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