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Development of System for Waterless Collection of Human Excreta by Application of Lactic Acid Fermentation Process in Terra Preta Sanitation System

A. Yemaneh[1], M. Bulbo[1], H. Factura[1], C. Buzie[1], R. Otterpohl[1]

[1] Institute of Wastewater Management and Water Protection, Hamburg University of Technology, Eissendorfer Str. 42, 21073 Hamburg, Germany (E-mail:*yemaneh@tuhh.de*)

Abstract: The suitability of lactic acid fermentation (LAF) process as a method for collection and treatment of human excreta in dry toilets is investigated. Laboratory-scale batch LAF experiments lasting three weeks were conducted to select suitable microbial inoculants and also to determine the effect of different levels of sugar supplement and different modes of human excreta collection. The rate of pH decline, rate of lactic acid production and degree of odor suppression are monitored over the fermentation period as parameters for evaluating the efficiency of the LAF process. For the different lactic acid bacteria (LAB) inoculants with 10% (w/w) molasses addition as sugar supplement the pH of the fermentation is reduced to less than 4 in five days from an initial value of 5.2, the final concentration of lactic acid ranged from 20 to 38g/L and faecal odor is suppressed. For separately collected faecal matter and combined LAB inoculant, Escherichia coli monitored as sanitation indicator bacteria is completely eliminated after 5 and 21 days of fermentation for 10% (w/w) and 5% (w/w) molasses addition, respectively. The results of the study suggest that LAF can give new way in dry toilet sanitation for odorless collection of human excreta with high hygienization effect.

Keywords: terra preta sanitation, dry toilet, human excreta, lactic acid fermentation, lactic acid bacteria.

Introduction

In recent years there has been an increasing focus on technology development in developing sanitation systems which are safe and enabling more efficient and effective recycling of materials. Under ecological sanitation approach, dry toilet sanitation systems are considered as one alternative option for fast and sustainable improvement of sanitation coverage in developing countries, especially in water scarce regions. These systems have reduced water footprint and also enable easy nutrient recovery from undiluted sanitation products (faecal matter, urine and other organic biowastes). On the other hand, owing to the well documented environmental downsides of the existing conventional wastewater management practices, ecologically sound alternative technologies are sought in industrialized countries as well (Otterpohl et al., 1997).

Field observation of existing dry toilet sanitation systems in Ethiopia shows odor problem mainly associated with mixing of urine and faecal matter in urine diverting dry toilets and also toilet inlet clogging due to faulty use and poor operation and maintenance are the main reasons for failure of the systems. Unavailability of well developed low-cost toilets for easy and safe handling of human excreta suiting to all settlement conditions and absence of common and efficient treatment method during collection are other factors hindering the fast expansion of dry toilet sanitation systems in different regions of the world. Most dry sanitation systems need large amount of covering and absorbing materials, like wood ash or saw dust, to reduce odor and there is no mechanism for achieving fast and significant reduction of faecal pathogens at collection except the natural die-off and the temperature effect in composting toilets which also cannot be achieved even with the addition of large quantities of organic biowaste into the toilets. Technology development in collection system which ensures safe handling of human excreta for subsequent processing and recycling is suggested in different panels discussing dry toilet sanitation approaches.

Recently, 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 suiting for both urban and rural settings. 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 faeces, biowaste, charcoal and bones. Factura et al (2010) provides details on the link between Terra Preta and TPS.

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 impact on soil fertility and productivity (Factura et al., 2010).

LAF is a process that has been applied intensively in food preservation, silage preservation and in management of different biowastes, like kitchen waste and others (Hafid et al., 2010; Jalil et al., 2008; Yang et al., 2006; Danner et al., 2003; Wang et al., 2003; Shirai et al., 2001; Kheratti et al., 1998; Zakaria et al., 1998; Ohshimaa & McDonald, 1978). Lactic acid bacteria (LAB) metabolize easily degradable carbohydrates mainly to lactic acid and few other metabolic by-products. Lowering of pH due to lactic acid production, sterilizing nature of the lactic acid compound it self and production of antimicrobial compounds by LAB are the factors that inhibit the growth of pathogens and other undesirable microorganisms which are responsible for decompositions that can produce odorous compounds from organic wastes (Wang et al., 2003; Noike et al., 2002; Matsusaki et al., 1997). Few researches have been conducted on application of LAF process for human excreta collection (Factura et al., 2010; Scheinemann & Krüger, 2010). Factura et al. (2010) discussed elimination of odor during human excreta collection using sauerkraut (pickled sour cabbage) juice or effective microorganisms (EM) as inoculants. Scheinemann & Krüger (2010) assessed the sanitization effect of LAF using EM as inoculant.

In this study detailed investigation of LAF process is made using specific strains of LAB and optimizing the process in such way that it can be applied in toilets as treatment method for human excreta during collection. The specific focus in this study is to establish fermentation conditions where the pH in the fermentation system is reduced to the extent that the growth of microbes other than the LAB is inhibited, which usually is achieved when the pH is reduced to less than 4, and also to establish a method for odorless collection of human excreta. Based on the experience of applying LAF process in preservation of substrates which have low content of simple sugar sources, it is hypothesized that supplementing additional sugar is necessary for achieving low final pH during fermentation of human excreta, and thus optimizing the addition of sugar supplement is considered as one objective in the study.

Methods and Materials

2.1 Bacteria. Seven strains of homofermentative and facultatively heterofermentative LAB are identified after thorough review of literatures on the application of LAF process on range of waste materials and further screened by initial experiments (results are not shown in this paper). The following three LAB strains are selected for further experiments after the screening: lactobacillus Plantarum, lactobacillus Casei and Pediococcus Acidilactici. The bacteria are obtained from DSMZ (German Collection of Microorganisms and Cell Cultures) as freeze dried cultures and are grown utilizing MRS medium (De Man et al., 1960). The cultures are maintained in 25% glycerol solution at -80°C until used for the experiments. In addition to the selected LAB, EM is also used as microbial inoculant for comparison.

2.2 Substrate. The substrate used for the fermentation experiments is human excreta collected in an experimental toilet at Hamburg University of Technology, Institute of Wastewater Management and Water Protection.

2.3 Inoculant preparation. Inoculant is prepared by transferring the 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. For EM inoculant, EM-a solution purchased from the company triaterra is directly used.

2.4 Fermentation experiments. Batch laboratory-scale fermentation experiments lasting three weeks are conducted in 1L glass fermentation reactors (Figure 1). Specific quantity of the collected excreta is transferred to the reactors and supplemented with additives (molasses as simple sugar supplement and microbial inoculants). Variations in modes of human excreta collection are considered to simulate separate collection, combined collection and partially combined collection (1:10 faecal matter to urine ratio, assuming combined collection only during defecation and using urinals for separate urine collection when only urinating). Variations in the amount of molasses addition are considered in the experiments to determine the optimum level for achieving the desired effects LAF process. Moreover, effect of charcoal addition on the LAF and odor suppression is investigated. All experiments are conducted at room temperature in anaerobic condition with opening the reactors for sampling. Samples are withdrawn at defined time intervals for laboratory analysis.

Figure 1: Fermentation reactors for batch LAF experiments

2.5 Analytical methods. The LAF process is monitored by measuring pH, lactic acid (LA), volatile fatty acids (VFAs), total titrable acidity (TTA), dry matter (DM), volatile solids (VS), E-Coli and odor evaluation. 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. To determine total titrable acidity 5 g of sample was placed in a beaker and titrated with 0.1 M NaOH solution until pH of 8.3 is reached, a point which reflects the turning point of phenolphthalein indicator. VFAs are measured following the procedures outlined by central laboratory of TUHH (Wasserdampfflüchtige organischer Säure – Büchi). 5 g of sample is transferred to a buchi distillation tube and deionized water is added to make 50 mL final volume. 3 mL of H_3PO_4 was added to the sample and distillated buchi distiller. The distillate is then boiled for ten minutes and cooled down to 60ºC or below. Phenolphthalein indicator is added to the distillate and is titrated with 0.1M NaOH solution until pink color is observed. Dry matter and volatile solids are determined following the DIN 38414 procedures. E-Coli is determined using ChromoCult Coliform Agar by spread plating after appropriate serial dilution of the samples. Odor in the fermentation reactors is evaluated by odor 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 if it occurs in toilets.

Results and Discussion

During LAF of separately collected faecal matter, for all treatments with the different LAB inoculant variants and 10% (w/w) molasses addition as sugar supplement, a reduction in pH from an initial value of 5.2 to less than 4 was observed in the first five days of fermentation which stayed nearly constant for the rest of the fermentation period (Table 1). Also increase in LA concentration, increase in TTA concentration and decrease in VFAs production were observed during the fermentation period with not much difference among the LAB inoculant variants. No defined trend in DM and VS changes were observed during the three week fermentation period for the different treatments. Faecal odor is completely suppressed and is replaced by sour smell which is rated as acceptable according to an odor panel and no E. coli is detected after one week to the end of fermentation. E. coli is the sanitation indicator bacteria under investigation and the change in its viable cell count during the fermentation

period is considered to indicate the general sanitization effect of LAF process on human excreta.

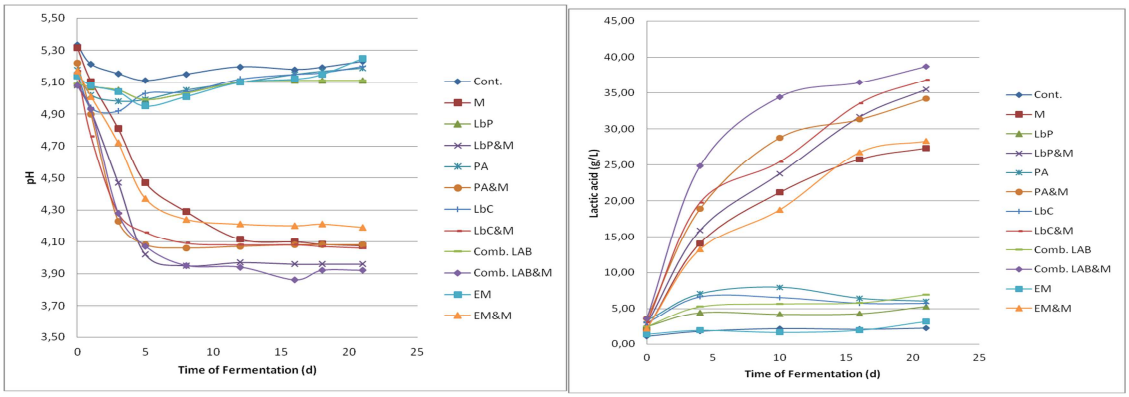
For treatment with only LAB without sugar supplement and for the control the pH did not change much during the whole fermentation period and only small increase lactic acid production is observed. Also, VFAs production show an increasing trend and faecal odor is not suppressed. Sato et al. (2001) & Moore et al. (1997) stated that VFAs are mainly (90%) responsible for the malodorous nature of faecal matter and thus VFAs can be used as index for monitoring odor in addition to other sensory evaluations. Here, less odor suppression effect in the control and for treatment with only microbial inoculants can be associated with relatively higher final VFAs concentration.

Table 1: Comparison of different parameters at the beginning and end of LAF experiments simulating separately collected faecal matter using different microbial inoculants

Treatment	pH		Lactic acid (g/L)		Total titrable acidity (g/L-lactic acid)		Volatile fatty acids (mmol/L)		Dry matter (% wet weight)		Volatile solids (% dry matter)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Cont.	5.33	5.23	1.07	2.24	12.24	17.53	83.15	289.07	16.15	15.71	86.82	86.16
M	5.32	4.08	2.05	27.35	11.91	59.12	118.84	353.23	21.72	21.80	86.34	85.83
LbP	5.09	5.11	2.47	6.98	14.86	17.30	198.53	232.35	14.17	13.66	85.94	84.00
LbP&M	5.08	3.96	2.78	35.50	15.09	50.73	117.54	114.37	17.81	18.03	85.09	85.22
PA	5.18	5.19	3.26	6.04	13.22	23.52	212.43	267.45	17.56	18.71	87.89	87.32
PA&M	5.22	4.08	3.55	34.23	14.78	43.88	124.97	69.15	19.54	17.73	86.76	87.05
LbC	5.16	5.20	2.97	5.74	13.05	25.16	227.68	257.61	17.25	16.90	87.19	87.11
LbC&M	5.17	4.06	3.01	36.86	13.24	61.37	136.50	146.42	19.68	19.22	86.53	87.58
Comb. LAB	5.12	5.15	3.24	5.89	13.54	23.62	212.42	241.14	14.21	13.58	87.39	87.73
Comb. LAB&M	5.08	3.92	3.38	38.73	14.09	51.23	154.33	121.34	18.92	17.53	86.49	87.35
EM	5.14	5.25	1.38	3.13	12.43	16.88	192.74	261.38	14.38	13.54	86.51	84.13
EM&M	5.17	4.19	2.12	28.33	13.11	58.55	160.98	339.63	18.09	16.90	86.02	85.97

(Cont. – control, M – 10% (w/w) molasses, LbP – lactobacillus Plantarum, LbP&M - lactobacillus Plantarum and 10% (w/w) molasses, PA - Pediococcus Acidilactici, PA&M - Pediococcus Acidilactici and 10% (w/w) molasses, LbC – lactobacillus Casei, LbC&M – lactobacillus Case and 10% (w/w) molasses, Comb. LAB – combination of LbP, PA and LbC, Comb. LAB&M – combination of LbP, PA and LbC with 10% (w/w) molasses, EM – effective microorganisms, EM&M – effective microorganisms & 10% (w/w) molasses. For all the treatments the amount of inoculant added is 10% (w/w)).

Combined LAB inoculant, which consists of all the three LABs used in the study, with 10% (w/w) molasses addition has slightly faster rate of pH reduction and lower final pH than the single strain LAB inoculants (Figure 2). Also, faster rate of lactic acid production and higher final lactic acid concentration are observed for combined LAB inoculant with sugar supplement (Figure 2). It is also observed that combined LAB inoculant and single strains of LAB inoculants are superior in terms of fast rate of lactic acid production, faster rate of pH reduction and odor suppression compared to the treatments with EM and 10% (w/w) molasses or for treatment with only 10% (w/w) molasses addition without microbial inoculants.



(a) (b)

Figure 2: (a) pH and (b) lactic acid concentration measurement for LAF experiments simulating separately collected faecal matter

(Cont. – control, M – 10% (w/w) molasses, LbP – lactobacillus Plantarum, LbP&M - lactobacillus Plantarum and 10% (w/w) molasses, PA - Pediococcus Acidilactici, PA&M - Pediococcus Acidilactici and 10% (w/w) molasses, LbC – lactobacillus Casei, LbC&M – lactobacillus Case and 10% (w/w) molasses, Comb. LAB – combination of LbP, PA and LbC, Comb. LAB&M – combination of LbP, PA and LbC with 10% (w/w) molasses, EM – effective microorganisms, EM&M – effective microorganisms & 10% (w/w) molasses. For all the treatments the amount of inoculant added is 10% (w/w)).

For LAF experiments simulating combined collection increase in pH is observed for control and for treatments with only microbial inoculants. Also, very strong odor is developed in the system. Treatments with microbial inoculants and 10 (w/w) molasses addition produced odor suppression effect, pH reduction to 4.3 and final lactic acid concentration of about 25 g/L (w/w) (Figure 3). Experiments simulating human excreta collection in partially combined mode showed comparable pH reduction to the experiments that simulate separate faecal matter collection (Figure 3). Also, increase in concentration of lactic acid and decreasing trend in concentration of VFAs are observed, which are again comparable to experiments that simulate separate faecal matter collection. Faecal odor is eliminated and replaced by sour smell which is rated as acceptable by the odor panel. Moreover, complete elimination of E-Coli is achieved after two weeks fermentation period.

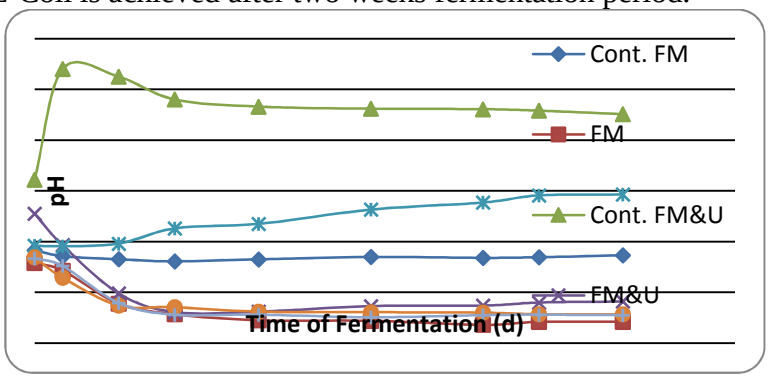


Figure 3: pH during LAF experiments simulating different modes of human excreta collection

(Cont. FM – separately collected faecal matter without any addition, FM - separately collected faecal matter with 10%(w/w) molasses and combined LAB addition, Cont FM&U – combined collection of faecal matter and urine without any addition, FM&U - combined collection of faecal matter and urine with10%(w/w) molasses and 10%(w/w) combined LAB addition, Cont. Part. Comb. – partially combined collection with faecal mater to urine ratio of 10:1 without any addition, Part. Comb. - partially combined collection with faecal mater to urine ratio of 10:1 with 10% molasses and 10%(w/w) combined LAB addition, FM&Charcoal - separately collected faecal matter with 10%(w/w) molasses, 10%(w/w) combined LAB, and 10%(w/w) charcoal addition).

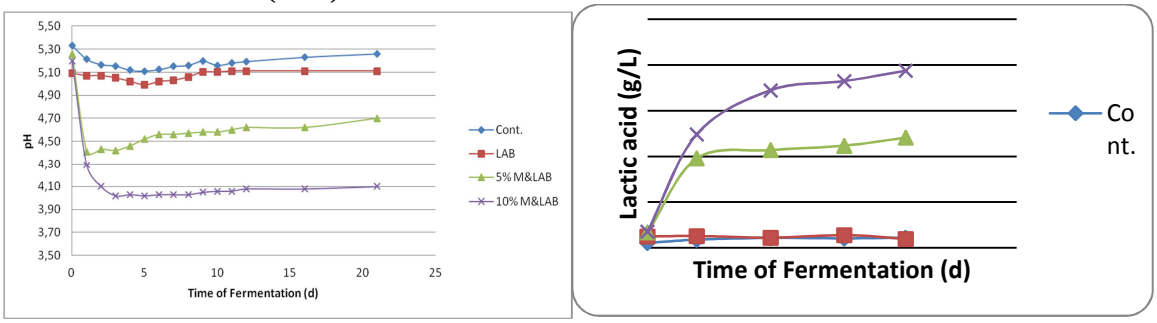
It is observed that adding 10% (w/w) charcoal at the beginning of fermentation has positive impact of removing the sour smell which resulted from the fermentation, without affecting other fermentation parameters except slight increase in the final pH due to the buffering effect of the charcoal (Table 2). Charcoal addition in the collection system may also have additional effect of conditioning the substrate in the toilets for further processing by vermi-composing. Initial observation on vermi-vermicomposting experiments showed that the substrate in which charcoal is added during fermentation has faster rate of stabilization compared to the substrate without charcoal (results not shown in this paper).

Table 2: pH and lactic acid concentration at the beginning and end of LAF experiments simulating different modes of human excreta collection

Treatment and mode of collection	pH		Lactic acid (g/L)	
	Initial	Final	Initial	Final
Cont. FM	5.33	5.23	1.07	2.24
FM	5.08	3.92	3.38	38.73
Cont. FM&U	6.72	8.01	0.01	0.00
FM&U	6.05	4.32	3.84	25.56
Cont. Part. Comb.	5.42	6.43	0.85	2.45
Part. Comb.	5.19	4.07	2.66	34.47
FM&Charcoal	5.17	4.05	2.34	34.35

(Cont. FM – separately collected faecal matter without any addition, FM - separately collected faecal matter with 10%(w/w) molasses and combined LAB addition, Cont FM&U – combined collection of faecal matter and urine without any addition, FM&U - combined collection of faecal matter and urine with10%(w/w) molasses and 10%(w/w) combined LAB addition, Cont. Part. Comb. – partially combined collection with faecal mater to urine ratio of 10:1 without any addition, Part. Comb. - partially combined collection with faecal mater to urine ratio of 10:1 with 10% molasses and 10%(w/w) combined LAB addition, FM&Charcoal - separately collected faecal matter with 10%(w/w) molasses, 10%(w/w) combined LAB, and 10%(w/w) charcoal addition).

To investigate the effect of different level of sugar supplement on LAF process, experiments are conducted for combined LAB inoculant with 5% (w/w) molasses addition as sugar supplement and results are compared with the 10% (w/w) molasses addition, without molasses addition and with the control (Figure 4). To all the treatments except the control combined LAB 10% (w/w) inoculant is added.



(a) (b)

Figure 4: Change in pH (a) and lactic acid concentration (b) for LAF experiments simulating separate collection of faecal matter for different level of sugar supplement

(Cont. – control, LAB – 10%(w/w) combined LAB addition, 5%molasses&LAB – 5% molasses and 10%(w/w) combined LAB addition, 10%M&LAB - 10% molasses and 10%(w/w) combined LAB addition).

The final pH for 5% (w/w) molasses addition is around 4.7 and odor suppression effect is observed but without complete elimination of faecal odor. Results of E-Coli monitoring indicate that there is a difference in viable cell count at different time scales during fermentation for the different levels of sugar supplement. At 10% (w/w) molasses addition

E-Coli is completely eliminated after 5 days, whereas for 5% (w/w) molasses addition elimination of E-Coli took longer time and no E-Coli is detected after the three week fermentation period is completed (Table 3). As the pH of 5% (w/w) molasses addition is quite higher than 4, the hygienization effect here may be attributed to sterilizing nature of lactic acid and its high concentration, which is about 2.5% (w/w).

Table 3: E-Coli count (cfu/g) for LAF experiments simulating separate collection of faecal matter for different level of sugar supplement

Treatment	Time of Fermentation (d)		
	0	5	21
Cont.	3.20E+06	2.60E+06	8.30E+04
LAB	3.10E+06	1.20E+06	6.50E+03
10% M	2.90E+06	3.70E+04	2.00E+02
5%M&LAB	2.30E+06	1.40E+02	Nil
10%M&LAB	1.80E+06	Nil	Nil

(Cont. – control, LAB – 10%(w/w) combined LAB addition, 5%molasses&LAB – 5% molasses and 10%(w/w) combined LAB addition, 10%M&LAB - 10% molasses and 10%(w/w) combined LAB addition).

Odor evaluation by the panel of observes indicate that for experiments simulating separate collection of feacal matter, faecal odor is not suppressed for controls and for treatments with only microbial inoculants. For treatments with microbial inoculants and 10% (w/w) molasses addition faecal odor is suppressed and is replaced by sour smell. The treatment with microbial inoculants and 5% (w/w) molasses the faecal odor is not fully suppressed. Treatment involving combined LAB microbial inoculant, 10% (w/w) molasses and 10% (w/w) charcoal showed complete odor removal during the fermentation period. Also, for experiments simulating partially combined collection and combined collection modes with combined microbial inoculant and 10% (w/w) molasses addition faecal odor is suppressed and acceptable final acidic smell is observed.

Observing the results of the different monitored parameters for LAF experiments it can be basically stated that LAF can be used as effective method for collecting human excreta for all the three modes of collections discussed using combined LAB inoculant consisting of the three LABs and adding 10% (w/w) molasses as sugar supplement. For operations in separate and partially combined collection modes, separately collected urine can also be collected applying LAF using the same inoculant and level of sugar supplement avoiding odour and nitrogen loss due to ammonia volatilization. For LAF experiments with LAB inoculants without sugar supplement, there are no much change in the monitored LAF parameters, pH and lactic acid, indicating that there is no sufficient simple sugar source in human excreta to be utilized by LABs. Therefore, for effective LAF process, 10% (w/w) molasses addition is necessary for all modes of collection. Other sugar sources can also be used, like kitchen waste after pre-treatment for hydrolysis of complex carbohydrates to simple sugars.

Conclusions

The results of the study show that LAF can be applied, in suitably designed toilets, for collection of human excreta with efficient suppression of odors. The LAF process will also achieve significant pathogen reduction during collection, while at the same time conserving nutrients and organic matter and preventing undesirable microbial decompositions. Possibility of application in combined and partially combined collection modes will allow

for implementation of toilets with only one inlet thus avoiding complexities associated with constructing and operating urine diversion dry toilets which are mostly considered as models for dry toilet sanitation approach.

Human excreta collection system with LAF is believed to provide a new way for dry toilets and would facilitate large scale applications of dry toilet sanitation in different regions of the world in varying settlement conditions with subsequent processing and recycling of human waste in agriculture. This will be a key to establishing sustainable provision of sanitation options and at the same time ensuring food security by providing organic fertilizer and humus for soil amendment. For effective implementation of the sanitation system there must be parallel activities to promote the sanitation system and create awareness in the societies to change perceptions on recycling human excreta. Future study will focus on investigating the whole TPS system with full mapping of nutrients, organic matter and pathogens with more detailed odour evaluation.

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