

**AN EVALUATION OF DIFFERENT COMMERCIAL
MICROBIAL OR MICROBIALY-DERIVED PRODUCTS
FOR THE TREATMENT OF ORGANIC WASTE IN
PIT LATRINES**

L Taljaard • A Venter • D Gorton

WRC Report No. 1377/1/05



Water Research Commission



An Evaluation of Different Commercial Microbial or Microbially-Derived Products for the Treatment of Organic Waste in Pit Latrines

Report to the
WATER RESEARCH COMMISSION

by

Leonie Taljaard, CSIR Food, Biological and Chemical Technologies (Bio/Chemtek)
Annette Venter, CSIR Water, Environment and Forestry Technology (Environmentek)
Dudley Gorton, Morgan Creek CC

WRC Report No 1377/1/05
ISBN No 1-77005-314-X

JULY 2005

Disclaimer

This report emanates from a project financed by the Water Research Commission (WRC) and is approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the WRC or the members of the project steering committee, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

EXECUTIVE SUMMARY

Background

Pit latrines operate on the principle of anaerobic decomposition. This process, however, is very slow, leading to organic waste build-up and subsequent system blockages. This results in odour production, and could pose health and environmental risks. There are claims that the use of microbial or microbially-derived products for the treatment of organic waste in pit latrines controls odour and also reduces the bulk of the organic material. Several of these products appear on the market, but there was no, or very little, reliable information available on the efficacy of these products. The objective of this project was, therefore, to compare the efficacy of these biological products by conducting laboratory scale analyses and on-site trials.

Project Aims and Approach

The first objective of this project was to conduct a market survey in order to determine what biological products are available in the South African market to treat pit latrines. The second aim was to compare the different products in terms of their mode of action and efficacy regarding organic waste digestion / reduction and odour control. The products were evaluated in small scale laboratory experiments, followed by on-site evaluation of two selected products.

Methodology

Market survey

Information on the different biological products was obtained from municipalities, enzyme companies and private traders by conducting telephonic conversations, correspondence by e-mail and internet searches. The products were surveyed on the basis of their claimed mode of action and dosage specifications, as well as price (as per specification sheets). Government departments and municipalities were also contacted to establish the current and past usage and application of specific products.

Obtaining selected products

One kilogram amounts of each product were obtained from enzyme companies or private traders.

Evaluation of products at laboratory scale

The products were compared with regard to their ability to digest organic material in small-scale laboratory trials. Two products were selected for evaluation in on-site trials.

Pit latrine field trials

Five kilogram amounts of selected products were obtained. The original plan was to evaluate the best three to four products in on-site trials, but due to limited testing facilities, only two products could be evaluated (against a control treatment). The pit latrines were treated (in triplicate) with the products, using equal dosages, and compared to the control latrines (no added product) with regard to volume / bulk reduction and the reduction of odour and flies. The pit latrines were monitored every week over a three month period, during which visual observations were made, and photographs taken. The reduction in organic waste volume was measured using long, marked poles. The observations and results were compiled and analysed.

Data compilation, analysis and report preparation

Collected data were compiled and analysed. The final report was compiled giving results and observations derived from this study as well as conclusions and recommendations.

Results and Discussion

Market survey

Information on the different biological products (claimed mode of action, dosage specifications, price and optimum conditions for different biological products) was obtained from enzyme companies and private traders by conducting telephonic conversations, correspondence by e-mail and by conducting internet searches. A total of 13 products were obtained from 9 suppliers. No information on current and past product usage could be obtained from government departments and municipalities. Some market information was obtained from

suppliers. Most of the companies, however, supply to traders and do not maintain records of actual usage. Most of the products are multi-purpose, and a small percentage of the sales go towards pit latrine treatments. Due to the difference in pit latrine sizes (from 400-2 000 L) and the uncertainty about the amount of people using a pit latrine per day or per month, it was difficult to make specific dosage recommendations for the use of most of these products. It was also not possible to determine usage or cost per capita. Suppliers seemed to rely significantly on prescribed dosages for septic tanks, adapting these dosages for treatment of pit latrines. An estimation of the annual costs of these products (based on prescribed dosages) showed a huge variation: from R13.86 up to R3 180 per pit latrine. Three new products were obtained at the end of this task and were included in the laboratory study.

Laboratory scale evaluation

Twelve products were selected from the 16 products (based on shelf-stability) and tested at laboratory scale to determine their efficacy in digesting organic waste. The aim of the study was to select the "top performing" products to be evaluated in field trials during the last phase of the project. The experimental procedure was simplified by studying the effect of the biological products on the biodegradation of faeces, the most important organic waste deposited in pit latrines. The relatively dry conditions in the pit latrines and the recommended dosage protocol of the biological products were simulated in the laboratory study as closely as possible. The treatment time in the field ranging from weeks to months, was reduced to five days in the laboratory study. Biodegradation of the faeces was monitored under aerobic conditions in terms of oxygen consumption, carbon dioxide production and removal of ammonia (NH_3), total Kjeldahl nitrogen (TKN), chemical oxygen demand (COD), total suspended solids (TSS) and volatile suspended solids (VSS). Based on data obtained from the experimental studies conducted and the manufacturer's recommended dosage specifications, the best results were obtained with Product M followed by Products F, P and B. When these values were adjusted to compensate for the inherent breakdown of the product itself, Product M still performed best, but Product B was determined to perform better than Product F. At this stage, the decision on the selection of products for the field studies had to be made and Products M and B were selected.

Pit latrine field trial

Pit latrines in the Magaliesburg area were selected for conducting the field studies. The Products (M and B) were applied to three pit latrines each, at equal dosages and compared with control pit latrines. The field trials were stopped after three months. The average results of the triplicate exposures in the field study indicated that relative to the control, Product B performed better than Product M, but no statistically significant differences could be determined due to the large variation between results within the different sets of triplicate exposures.

The main constraints during the field study were the compactness of the waste and the presence of non-biodegradable foreign objects in the latrines. These factors and the low temperatures ($8-10^\circ\text{C}$) during the three winter months may have caused the interaction between the microorganisms, enzymes and organic waste to slow down considerably. Visual observations showed that the layers of the treated pits started to liquefy. There were minimal changes in the pits treated with Product M and no changes in the control pits.

The odour and the population of flies in the treated latrines (especially with Product B) disappeared after the first dosages, whereas bad odours and flies persisted in the untreated latrines.

Conclusion

The general conclusion is that, based on the results obtained in this study, the use of biological products for the degradation of organic waste in pit latrines could be feasible.

Recommendations for Future Work

1. For the optimum field evaluation of biological agents for application in pit latrine, trials should be approached in a different manner. The trials should be started with newly dug pit latrines and monitored daily. Toilet paper or newspaper (or biodegradable material) must be provided. The prescribed optimum conditions and dosages must be adhered to as closely as practically possible. The planning of the experimental trial will have to make provision for enough replicates in each treatment. The use of these biological products must also be combined with an educational programme, perhaps through government health workers.

2. Future work could include a biological study into the claimed mode of action of these biological products. The products should be evaluated on the basis of the amount and type of microorganisms and enzymes present, and compared to the information and claims on the specification sheets.

ACKNOWLEDGEMENTS

The research in this report emanated from a project funded by the Water Research Commission and entitled:

"Evaluation of different commercial microbial or microbially-derived products for the treatment of organic waste in pit latrines"

The Steering Committee responsible for this project, consisted of the following persons:

Dr N P Mjoli	Water Research Commission (Chairperson)
Mr H M du Plessis	Water Research Commission (Chairperson)
Prof C Whiteley	Rhodes University
Mrs S A F Jackson	Ethekwini Municipality
Mrs L Boyd	Department Water Affairs and Forestry
Dr N Nuntsu	Border Technikon

The financing of the project by the Water Research Commission and the contribution of the members is gratefully acknowledged.

Inputs to the study by representatives from enzyme and biological treatment suppliers, traders and manufacturers, as well as government and municipal officials are also greatly appreciated.

The owners of the rose farm, the residents of the farm's staff compound, and the Magaliesburg Police, are also acknowledged for their assistance in the execution of the field trial component of this project.

TABLE OF CONTENTS

EXECUTIVE SUMMARY.....	i
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
1. INTRODUCTION.....	1
1.1 The Problem.....	1
1.2 The Solution.....	2
1.3 Project Objectives and Approach to the Study	3
2. BIOLOGICAL PRODUCTS AVAILABLE FOR THE TREATMENT OF PIT LATRINES: PRODUCTS AND MARKET INFORMATION.....	4
2.1 Approach.....	4
2.2 Results and Conclusions.....	4
3. LABORATORY SCALE EVALUATION OF DIFFERENT COMMERCIAL MICROBIAL OR MICROBIALLY DERIVED PRODUCTS FOR THE TREATMENT OF ORGANIC WASTE IN PIT LATRINES.....	9
3.1 Introduction	9
3.2 Materials, Equipment and Methods	9
3.2.1 Materials.....	9
3.2.2 Equipment.....	9
3.2.3 Methods.....	10
3.3 Results and Discussion.....	11
3.3.1 Biological products.....	11
3.3.2 Finalisation of experimental protocol.....	13
3.3.3 Biodegradation studies.....	15
3.4 Conclusions	19
3.5 Recommendations	20
4. FIELD TESTS OF TWO COMMERCIAL MICROBIAL OR MICROBIALLY-DERIVED PRODUCTS FOR THE TREATMENT OF ORGANIC WASTE IN PIT LATRINES.....	20
4.1 Introduction	20
4.2 Site Selection	20
4.3 Materials and Methods.....	22
4.3.1 Experimental set-up.....	22
4.3.2 Treatment sites.....	23
4.3.3 Application of biological products	24
4.4 Results.....	25
4.4.1 Observations made during evaluation intervals	25
4.4.2 Final observations.....	26
4.5 Discussion.....	27
4.6 Conclusions	27
4.7 Recommendations	27
5. SUMMATION OF STUDY CONCLUSIONS AND RECOMMENDATIONS.....	28
5.1 Biological Products Available for the Treatment of Pit Latrines: Products and Market Information.....	28
5.2 Laboratory Scale Evaluation of Different Commercial Microbial or Microbially Derived Products for the Treatment of Organic Waste in Pit Latrines	28
5.3 Field Tests of Two Commercial Microbial or Microbially-Derived Products for the Treatment of Organic Waste in Pit Latrines.....	28
5.4 General.....	29
References.....	30

LIST OF TABLES

Table 1 Different biological products for pit latrine treatments available in South Africa.....	5
Table 2 Estimated product cost per annum.....	8
Table 3 Preparation and dosage biological products	10
Table 4 Chemical and physical characterisation of biological products.....	12
Table 5 Inherent breakdown of biological product controls in terms of the removal of chemical and physical parameters monitored	12
Table 6 Inherent breakdown of biological products in terms of oxygen consumption and carbon dioxide production.....	13
Table 7 Chemical and physical characterisation of faeces used in experiments.....	15
Table 8 The effect of biological products on the breakdown of faeces in terms of oxygen consumption and carbon dioxide production.....	16
Table 9 The effect of biological products on the breakdown of faeces in terms of chemical and physical parameters monitored.....	17
Table 10 Breakdown of faeces minus the contribution of the inherent biological product breakdown	18
Table 11 Specific activity of biological products (per gram of product) measured in terms of their effect on faeces breakdown (removal of chemical and physical parameters monitored) minus the contribution of the inherent biological product breakdown	19
Table 12 Specific activity of biological products (per Rand of product cost) measured in terms of their effect on faeces breakdown (removal of chemical and physical parameters monitored) minus the contribution of the inherent biological product breakdown	19
Table 14 Final waste reduction results of field trial	26

LIST OF FIGURES

Figure 1 An example of the average pit latrine	2
Figure 2 Photograph of experimental vessels containing faeces and product	14
Figure 3 Faeces paste spread in a layer over bottom of flask – photo taken after termination of experiment – open patches indicate liquefaction of faeces.....	14
Figure 4 Biodegradation pattern of faeces controls	15
Figure 5 The selected site in Magaliesburg	21
Figure 6 Three pit latrines covered by corrugated iron roof.....	21
Figure 7 An example of the congested pit latrines	22
Figure 8 Pit latrine after clean-up.....	22
Figure 9 Poles with white markings were put into the pits to measure volume reduction.....	23
Figure 10 Blocks of 3 pit latrines for the treatments and control.....	24
Figure 11 Preparation of dry powders for application to pit latrines.....	25

1. INTRODUCTION

1.1 The Problem

It is no exaggeration to state that sanitation is a problem that has reached crisis proportions in many places around the world. The main reasons behind this are rapid population growth and an unsuitable technological response (www.lboro.ac.uk). If present trends continue, the majority of urban dwellers in the world will live in unplanned and unserviced informal settler areas, with water collected from a communal tap with erratic supply, no toilet or the use of pit latrines, and no garbage collection. Poor environmental conditions give rise to high rates of diarrhoeal diseases, especially in infants.

In South Africa, a statistical household study done in 1999 (Lehohla, 1999) showed that between 1995 and 2000 there has been a slight decrease in the proportion of households with access to flush or chemical toilets (56.9-55.8%). It was also shown that 51.7% of households living in traditional dwellings were using pit latrines, and 46.2% of households (traditional dwellings) were using the bush or a river as toilets. In general the percentage usage of the different types of toilet facilities in South Africa (all dwellings) is as follows:

Pit latrine	-	35.8%
Flush / chemical toilet	-	48.5%
Buckets	-	3.3%
None / other	-	12.4%

Pit latrines are also described as "drop-and-store" systems. They are simple, relatively low-cost, and easy to understand and operate. The drawbacks, however, are the odour, fly breeding, risk of pit collapse, and the short life-span. From time to time, new pits have to be dug, and this becomes difficult in crowded sites. Some areas are unsuitable for erecting pit latrines: rocky ground, where the water table is high, and in areas with frequent flooding (www.lboro.ac.uk/departments/cv/wedcv/garnet/ecosan.html).

Pit latrines are dry sanitation systems with a life span of 1-3 years, depending on the size of the pit and the frequency of use. When these latrines fill up they are either pumped empty or, in most cases, covered with soil. One would expect that only faeces, urine, toilet paper and newspaper are deposited in these latrines, but in fact these latrines are also used for the disposal of other wastes *e.g.* disposable nappies, sanitary towels, fetuses, dead animals, dishes, laundry, bath water, detergents, car oil, tins, plastic bags, *etc.* (Figure 1).

Sewage is rich in nutrients, which usually support the growth of natural microorganisms and the resultant breakdown of organic waste. Due to overpopulation, however, excessive demands are being placed on pit latrine systems, limiting the time for adequate waste breakdown. The high ratio of dry content to moisture in these latrines is not conducive to biological degradation of organic wastes, and the fact that wastes may be deposited that are not biodegradable by the microorganisms, reduces the breakdown of organic waste.

The high organic load (usually high fat content) and the use of chemicals (such as Jeyes Fluid) in pits further destroy the naturally occurring microorganisms. Often pit latrines are not constructed in soil types that allow for the broken down waste to be assimilated by the surrounding soil.

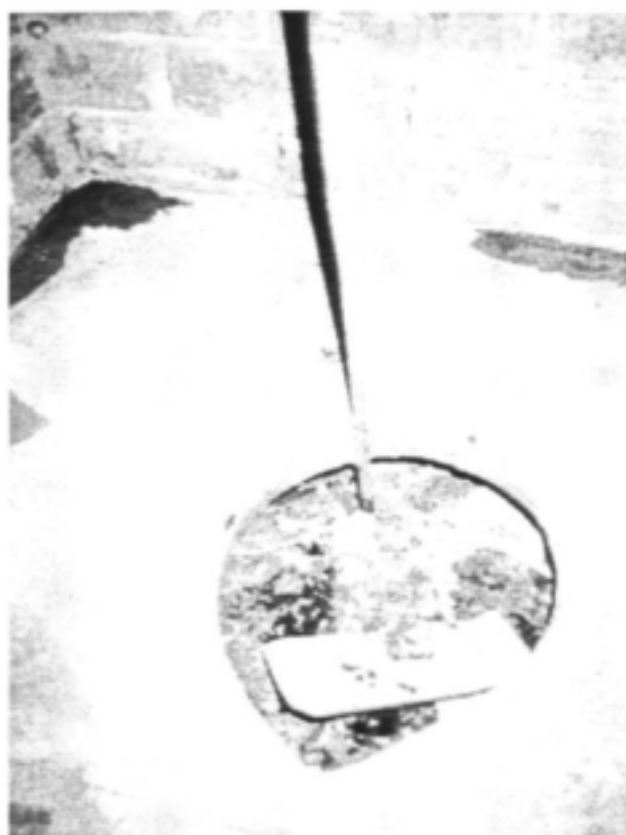


Figure 1. An example of the average pit latrine

1.2 The Solution

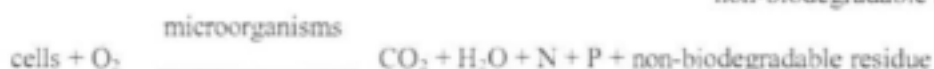
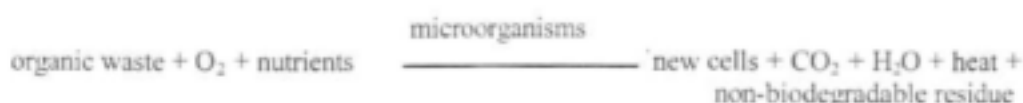
Suppliers of biological products claim that the addition of biological products (microbial or microbial enzymes-based) to the waste in pit latrines could enhance the breakdown process considerably and reduce the occupied volume in the pits (www.biosystemssa.co.za).

The addition of biological products, or "inoculum" containing microorganisms, could thus assist the natural breakdown process and enhance the degradation of organic waste in pits. When added to the organic material, the enzymes present in the product initiate the breakdown and solubilisation of the waste (complex carbon sources such as protein and fat), providing the nutrients for rapid bacterial growth and activity in the sewage. The microorganisms will grow, colonise the waste material and produce more enzymes. These enzymes will catalyse the further breakdown of proteins, lipids, cellulose, *etc.* present in the organic waste, thereby reducing the volume and the ammonia and other bad odours. The added microorganisms are selected to be fast growers and colonisers, and will compete for nutrients against pathogenic organisms, a mechanism called competitive exclusion. Jere *et al.* (1998) proved this concept by conducting a field trial in Harare, Zimbabwe, using a commercial biological product. The results showed that the pit contents, COD (chemical oxygen demand), BOD (biological oxygen demand) and K-nitrogen concentrations decreased significantly over a three-month period.

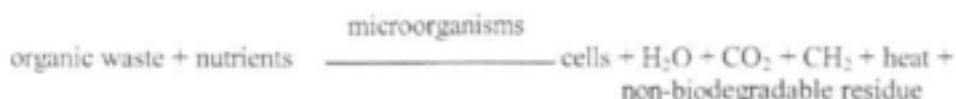
The microorganisms can be used under aerobic conditions for the biological treatment of an organic rich waste. During the exposure of the organic waste to the bacteria in the presence of oxygen, which would be the case in the top waste layer in the pit latrine, the following processes (or mode of action) occur:

- Oxygen is consumed by the organisms for their energy requirements, while new cell mass is synthesised; and
- The organisms simultaneously under progressive auto-oxidation reduce their cellular mass.

The reaction can be demonstrated by the following equations:



During anaerobic conditions the following reaction takes place:



For biological degradation, optimal conditions should prevail: pH (6-8), temperature (ambient), O₂ availability (for aerobic and facultative anaerobic bacteria), nutrients (PO₄-P and NH₃-N) and moisture (if applicable).

1.3 Project Objectives and Approach to the Study

Against this background, this project was executed. The first objective of the project was to conduct a market survey in order to determine what biological products are available in the South African market to treat pit latrines. The second objective was to compare the different biological products in terms of their efficacy regarding organic waste digestion / reduction and odour control.

Selected biological products were evaluated in small-scale laboratory experiments, followed by on-site testing of two selected products. The project tasks were as follows:

a) Products and market survey

Information on the different biological products was obtained from enzyme companies and private traders, and from government sources and municipalities.

b) Obtaining selected products

One kilogram amounts of each product were obtained from enzyme companies or private traders.

c) Evaluation of products at laboratory scale

Products were compared with regard to their ability to digest organic material in small-scale laboratory trials. Two products were selected for evaluation in on-site trials.

d) Pit latrine field trials

Pit latrines were treated with the products, using equal dosages and compared to the control latrines (no added product) with regard to volume / bulk reduction and the reduction of odour and flies. The pit latrines were monitored twice every week, over a three month period, during which visual observations were made and photographs were taken.

e) Data compilation, analysis and report preparation

Collected data were compiled and analysed, and the final report compiled.

2. BIOLOGICAL PRODUCTS AVAILABLE FOR THE TREATMENT OF PIT LATRINES: PRODUCTS AND MARKET INFORMATION

2.1 Approach

Information on the different biological products (claimed mode of action, dosage specifications, price, optimum conditions for different biological products, product sales and target market) was obtained from enzyme companies and private traders by conducting telephonic conversations, correspondence by e-mail and by conducting internet searches. Various municipalities were also contacted. A total of thirteen products were obtained from nine suppliers.

For reasons of confidentiality, product trade names were replaced by codes. The information was obtained during April and May 2002 and the product prices should be considered as being relevant to this time period.

2.2 Results and Conclusions

A total of thirteen products were obtained from nine suppliers and the product information (price, target market, uses, dosages, activities, etc.) is shown in Table 1. No information on current and past product usage could be obtained from government departments and municipalities. Some market information was obtained from suppliers. Most of the companies, however, supply to traders and they do not maintain records of actual usage. Most of the products are multi-purpose (e.g. grease and fat degradation applications) and a very small percentage of the sales go towards pit latrine treatments. Due to the difference in pit latrine sizes (from 400 – 2 000 L) and the uncertainty about the number of people using a pit latrine per day, or per month, it was difficult to make specific dosage recommendations for the use of most of these products. According to one of the suppliers, a pit size is about 1.2 m x 0.6 m x 0.6 m (about 400 L), but none of the other suppliers could estimate the "normal" size / volume of a pit latrine. It was also not possible to determine usage or cost per capita. Suppliers seemed to rely to a great extent on prescribed dosages for septic tanks, adapting these dosages for treatment of pit latrines. An estimation of the annual costs of these products (based on prescribed dosages) showed a huge variation, from R13.85 up to R3 180 per pit latrine. Three new products (Products P, Q and R) were obtained at the end of this task and were included in the laboratory study.

The actual size of the South African market for microbially-derived products for the treatment of organic waste in pit latrines has (at the time of compilation of this report) not been reported or recorded by a South African source.

It is estimated that about 6-10 tons of product is currently imported per annum. Some products are also "manufactured" locally, but this usually entails mixing and blending of concentrated imported products. Products are often sold to agents or traders for distribution in Southern Africa. The general sentiment is that the potential market is significant, but it is very often a difficult market to penetrate.

Table 2 shows the estimated annual product costs, based on the prescribed dosages and the assumption that the size of a pit latrine is 1000 L. It is clear that there are large differences in dosages and price caused by many variables: pit latrine size, product strength (ability to digest), the number of people using a pit latrine per month, uncertainty about actual pit latrine conditions (pH, temperature) and general knowledge of product performance.

Table 1. Different biological products for pit latrine treatments available in South Africa

Product designation	Target market	Main users or buyers	Price	Sales volume	Dosage	Bacteria present or bacterial count	Enzymes present	Conditions for product viability
Product A	Septic tank systems, pit toilets, mortality pits	Traders, hardware stores, co-operatives, municipalities	R135 / kg	No information given by supplier	100 g per pit latrine per month	<i>Bacillus subtilis</i> , <i>Cellulomonas</i> , <i>Aerobacter</i>	Lipases, proteases, amylases, hemicellulases, lactase	pH 4.5-9.5 Temp: 20-40°C
Product B	Septic tanks, pit latrines, grease traps, industrial effluent, agricultural waste, sewage plants	Traders (in general)	R93.60 / kg	No specific information given by supplier	100 g per week for 1 month, then 100 g per month for maintenance	Aerobes: 8 x 10 ⁸ cfu/g Anaerobes: 7.8 x 10 ⁸ cfu/g	Lipases, proteases, hemicellulases	pH 5-8.5 Temp: up to 50°C, Pre-treat with acid or lime
Product C	Pit latrines	Fruit farmers, taxi drivers, informal settlements	R1 198 / kg	Sales are seasonal, no further information	25 g per toilet at the beginning and then every three months	Total: 4 x 10 ⁹ cfu/g No further information	No information	No information
Product D	Waste water treatment, oxidation ponds, biofilters, lagoons, septic tanks, pit latrines, grease traps	Municipalities, restaurants, farmers	R110 / L	No information	100 ml. per 1 000 L. pit, thereafter 25 ml. per 1 000 L.	8.2 x 10 ⁹ cfu/L	Cellulases, lipases, proteases	pH 6-8.5 Temp: 5-49°C
Product E	Septic tanks, pit latrines	No information	R44 / L	No information	500 ml. per hole initially, thereafter 25 ml. per week	Aerobes: 9 x 10 ⁻⁷ cfu/mL Anaerobes: 5.3 x 10 ⁷ cfu/mL	Proteases, amylases, lipases, gluconases, cellulases, pectinases	pH 6-8.5 Temp: 5-49°C

Product designation	Target market	Main users or buyers	Price	Sales volume	Dosage	Bacteria present or bacterial count	Enzymes present	Conditions for product viability
Product F	Septic tanks, pit latrines, grease traps	No information	R150 / kg	Approx. 4 tons per month (2 – 2.5 tons per month for pit treatment)	100 g per treatment	Aerobes: 2×10^7 cfu/g Anaerobes: 2×10^9 cfu/g	Proteases, amylases, lipases	pH 6.8-7.2 Temp: 5-49°C
Product G	Septic tanks, drain pipes, pit latrines, waste treatment	Traders and distributors	R57.75 / kg	% sales towards pit latrines unknown, no direct sales to end users	1 kg per 50 kL or 20 g per 1 000 L	Aerobes: 1.2×10^4 cfu/g Anaerobes: 0.6×10^9 cfu/g	Several enzymes present	No information
Product H	Sinks, grease traps, drains	Traders	R50 / kg	% sales towards pit latrines unknown, no direct sales to end users	500 g per 5 000 L toilet initial, maintenance 100 g per 5 000 L every two weeks	Bacilli: 2.7×10^7 cfu/mL	Protease: 5 000 PV units Amylase: 400-700 DV units Lipase: 200 NF units	pH 5.5-8.5 Temp: up to 49°C, no activity under 5°C
Product I	Portable toilets, RV, marine holding tanks	Traders	R49.25 / 5 L or R238.75 / 25 L	% sales towards pit latrines unknown, no direct sales to end users	37 L per 5 000 L pit every 7-10 days	Bacilli: 2.7×10^7 cfu/mL	Lipase, protease, amylase, cellulase	pH 5.0-9.8 Temp: 3-63°C
Product J	Grease traps, septic tanks, carpets, automobiles, cat litter, urinals, deep freezers	Traders	R104.65 / 5 L or R515.75 / 25 L	% sales towards pit latrines unknown, no direct sales to end users	500 mL per 5 000 L pit, maintenance 100 mL every 7-10 days	Blend of microorganisms, no specific information	No information	No information

Product designation	Target market	Main users or buyers	Price	Sales volume	Dosage	Bacteria present or bacterial count	Enzymes present	Conditions for product viability
Product K	Bathroom maintenance, carpet and fabric care, laundry pre-spotter, waste treatment (pit latrines), grease traps, drains	Building companies, armies, police, municipalities, traders	R17 / L	Approx. 5 000 L are sold per month (sales of R65 000 per month), % towards pit latrines unknown	100 mL in 5 L water per pit, every two weeks	Total count: 1.2×10^6 cfu/mL	Lipases, proteases, amylases and cellulases present	pH 5-9 Temp: up to 45°C, no activity below 5°C
Product L	Septic tanks, drains, pit latrines	Agricultural co-operatives	R150 / L	200 – 300 L are sold per month, 1% towards pit latrines	500 mL per pit, 120 mL per pit per month maintenance	<i>Bacillus subtilis</i> , <i>B. amyloliquefaciens</i> , <i>B. licheniformis</i> , <i>Cellulomonas</i> sp., <i>C. biazotea</i> , <i>Pseudomonas stutzeri</i> , <i>P. denitrificans</i> , <i>Rhodopseudomonas palustris</i> Total count: 1×10^9 cfu/mL	Relevant enzymes present	pH 5-9 Temp: up to 40°C
Product M	Pit latrines, infested ponds, water treatment, organic waste treatment	Farmers	R400 / kg	Market not being actively pursued currently	1 Kg per toilet for four people for ten weeks (100 g per week)	5 strains of bacteria, 1 yeast. Approx. 6×10^6 cfu/g	Enzymes present	No information

Table 2. Estimated product cost per annum

Product	Price (R)	Dosage*	Initial dosage	Monthly dosage	Annual cost (per pit latrine) (R)
Product A	135 / kg	100 g per pit latrine per month	100 g	100 g	162
Product B	93,60 / kg	100 g per week for 1 month, then 100 g per month for maintenance	400 g	100 g	140,40
Product C	1 198 / kg	25 g per toilet at the beginning and then every three months	25 g	8,3 g	139,33
Product D	110 / L	100 mL per 1 000 L pit, thereafter 25 mL per 1 000 L	100 mL	25 mL	41,25
Product E	44 / L	500 mL hole initially, thereafter 25 mL per week	500 mL	100 mL	70,40
Product F	150 / kg	100 g per treatment	100 g	100 g	180
Product G	57,75 / kg	1 kg per 50 kL ; 20 g per 1 000 L, once a month	20 g	20 g	13,86
Product H	50 / kg	500 g per 5 000 L toilet, 100 g every two weeks	100 g	40 g	16
Product I	9,55 / L	37 L per 5 000 L pit every week	7,4 L	29,6 L	3 180,15
Product J	20,63 / L	500 mL per 5 000 L pit, then 100 mL per week	100 mL	400 mL	92,84
Product K	17 / L	100 mL in 5 L water per pit, every two weeks	200 mL	200 mL	40,80
Product L	150 / L	500 mL per pit, 120 mL per pit per month maintenance	500 mL	120 mL	273
Product M	400 / kg	1 kg per toilet for four people for ten weeks (100 g per week)	400 g	400 g	1 920

* Assuming a 1 000 L pit

3. LABORATORY SCALE EVALUATION OF DIFFERENT COMMERCIAL MICROBIAL OR MICROBIALLY DERIVED PRODUCTS FOR THE TREATMENT OF ORGANIC WASTE IN PIT LATRINES

3.1 Introduction

Based on shelf stability, 12 products were selected for this phase of the study from the total of 16 products obtained during the first phase of the project. The objective of this phase was to evaluate these products at laboratory scale for their efficacy in digesting organic waste, in order to select the "top performing" products. The selected products were evaluated in on-site trials during the last phase of the project.

3.2 Materials, Equipment and Methods

3.2.1 Materials

Biological products

The Products (A, B, C, F, G, H, K, L, M, P, Q, and R) were supplied in either a powder or liquid form. Product K was a cream suspension and Product L a yellow liquid with a precipitate at the bottom of the bottle. All the solid biological products were in the form of fine powders, except Product C which can be described as a blue-green coarse powder. Products F and H formed a suspension when diluted with water and formed bubbles in the solution. Product M also formed bubbles in the solution. The biological products were prepared as shown in Table 3.

Nutrient solution

A nutrient solution with the following composition was prepared: macro nutrients: 6.5% N, 2.7% P, 13% K, 7% Ca, 2.2% Mg and 7.5% S and micro nutrients: 0.15% Fe, 0.024% Mn, 0.024% B, 0.002% Cu and 0.001% Mo. Each of the replicate treatments received 2mg/L of the nutrient solution.

3.2.2 Equipment

Micro-Oxymax respirometer

The fully automated Micro-OxymaxTM respirometer (Columbus Instruments, Ohio, USA) is a closed circuit system, equipped with three gas sensors *i.e.* oxygen, carbon dioxide and methane, and ten temperature controlled test chambers that can be operated simultaneously. The gas concentration changes are monitored in the head space of the enclosed test chamber (250 mL Schott bottle) eliminating probe fouling experienced with dissolved oxygen probes coming into contact with test media. The refreshing frequency of the test chambers by outside air or bottled gas (nitrogen in case of anaerobic conditions) is pre-programmed. At pre-determined time intervals measurements are taken. For each measurement, the date and time, sample number, gas exchange rates, cumulative gas measurements at that time, incubation temperature, and the respiratory exchange ratio are calculated and recorded automatically for later processing.

Table 3. Preparation and dosage biological products

Product	Dosage specifications per manufacturer	Assumptions made regarding field application to calculate experimental dosage	Experimental dosage: 2.5 mL of:
A	100 g / 5 L lukewarm water, stir into pit, add more water and continue stirring with stick, treat once a month	25 g / 5 L / week	1 g / 200 mL
B	100 g / pit latrine / week for one month, then 100 g / month for maintenance (mix with water before application)	100 g / 5 L / week	4 g / 200 mL
C	12.5 g / pit latrine (1 m x 1 m x 700 mm pit) initially, then 15 g after two days, repeat every three months, add 15 g to bucket of water, stir and leave for 30 min, pour into pit followed by another bucket of water, after two days add 12.5 g (mixed with a bucket of water)	27.5 g / 20 L / week	0.3 g / 200 mL
F	100 g / treatment, soak required dosage in an equal amount of water and allow to stand for four hours, then apply	100 g / 100 mL / week	50 g / 50 mL
G	1 kg / 50 kL, thus approximately 1 g / 700 L pit latrine, no more information on application, but probably best to add water first	15 g / 5 L / week (no instructions from manufacturer – based on solubility of product)	0.6 g / 200 mL
H	Initial dose: 50 g / 5 000 L capacity (e.g. septic tank), maintenance: 100 g / 5 000 L, every 10-14 days, soak the required dose in an equal amount of water and allow to stand for four hours	50 g / 50 mL / week	50 g / 50 mL
K	Add 100 mL to 5 L water and pour into pit, every two weeks	50 mL / 5 L / week	2 mL / 200 mL
L	Initial dosage of 500 mL / pit, 120 mL / pit / month maintenance	30 mL / 5 L / week	1 mL / 200 mL
M	1 kg / toilet for four people for ten weeks (thus 100 g / pit latrine / week), add to lukewarm water and leave to stand for four hours, then apply	100 g / 100 mL / week (assumed soaking in equal amount of water to conform to dose prescriptions of F, H – samples were tested during same run)	50 g / 50 mL
P	10 g / pit initially, then 10 g a week later, followed by 10 g per month for maintenance. Mix with water	10 g / 5 L / week	0.4 g / 200 mL
Q	10 g / pit initially, then 10 g a week later, followed by 10 g per month for maintenance. Mix with water	10 g / 5 L / week	0.4 g / 200 mL
R	10 g / pit initially, then 10 g a week later, followed by 10 g / month for maintenance. Mix with water	10 g / 5 L / week	0.4 g / 200 mL

3.2.3 Methods

Chemical and physical analysis

Standard procedures were followed to determine the pH, total suspended solids (TSS) and volatile suspended solids (VSS), unfiltered chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and ammonia (NH₃) (APHA, 1992). The detection limits of the assays were as follows:

NH ₃	0.2 mg/L,
TKN	0.2 mg/L,
COD	<10 mg/L,
TSS	<20 mg/L, and
VSS	<20 mg/L.

Biodegradation studies

To simplify the experimental procedure, it was decided to only determine the efficiency of the different commercial microbial or microbially-derived products for the treatment of organic waste in pit latrines in terms of their efficiency in breaking down faeces, the most important organic waste deposited in pit latrines. A faeces stock (70 g faeces + 35 mL boiled tap water) was prepared fresh from the faeces supplied by one person for every experiment. For each experimental vessel 5 g of the stock (3.33 g faeces) was used. The faeces stock (can be described as a paste) was smeared evenly over the bottom of the vessel.

Sampling

To overcome the problem of taking a representative sample for chemical analysis from the relatively dry content of the reaction vessels, duplicates were prepared of the time zero mixtures (for analysis purposes) to which 150 mL deionised water was added. The contents were homogenised and a representative sample taken for analysis. At completion of the experiment, the experimental vessels were treated in the same way as the time zero vessels.

Experimental conditions

- Experiments were conducted at ambient temperature (22°C) under aerobic conditions (the biological products are applied to the top layer of the pit latrine, an aerobic zone). Singular control biological products and faeces experiments were performed due to a lack of space on the respirometer, but the faeces / biological product experiments were performed in duplicate.
- The rate of degradation of faecal matter was monitored in terms of oxygen consumption and carbon dioxide production rates under aerobic conditions. The cumulative gas consumption / production measurements and the chemical / physical analyses were used to calculate the effect the biological products had on the biodegradation of the faeces.
- *Control biological product conditions:* 2.5 mL of product dilution (Table 1) + 0.03 mL nutrients + 5 mL boiled tap water as replacement for faeces.
- *Control faeces conditions:* 5 g faeces stock + 0.03 mL nutrients + 2.5 mL boiled tap water as replacement for the biological product.
- *Experimental conditions* (in duplicate): 2.5 mL of the biological product dilution (Table 1) + 0.03 mL nutrients + 5 g faeces stock.
- *Time zero:* samples were taken for TSS, VSS, COD, TKN, ammonia and pH measurements from the control and experimental conditions.
- Commenced incubation for 5 days.
- *At termination of experiment:* samples were taken for TSS, VSS, COD, TKN, ammonia and pH measurements from all the control and experimental conditions.

3.3 Results and Discussion

3.3.1 Biological products

Chemical and physical analyses were performed on the biological product stock dilutions (Table 3) as shown in Table 4, in order to characterise these products in terms of the parameters selected to monitor biodegradation of the experimental material. Although there were large differences in the chemical and physical composition of these products, it was difficult to predict at that stage what effect the inherent breakdown of these products might have on the combined biodegradation results of the faeces / product mixture. The pH of Product C was far outside the optimum pH range of 6-8 for biological reactions. At the start of the experiment, however, the pH of the biological product control reaction mixtures were all in the biological optimum pH range as shown in Table 5.

Table 4. Chemical and physical characterisation of biological products

Product	NH ₃	TKN	COD	TSS	VSS	pH
	mg / g or mL of product					
A	0.7	15.5	485.3	1 826.8	690.7	6.4
B	0.6	10.1	432.8	1 661.1	585.0	8.1
C	5.3	10.5	70.6	1 628.6	33.7	9.4
F	9.6	51.6	2 423.0	109.1	17.3	7.6
G	12.0	65.4	166.2	1 787.4	90.0	7.8
H	22.4	125.1	1 558.7	464.5	18.6	8.0
K	2.7	6.7	87.2	123.3	1.8	8.3
L	3.7	4.5	3.4	185.0	2.7	8.7
M	33.6	224.1	5 493.3	267.0	13.5	7.5
P	8.4	42.0	53.6	2 954.7	103.7	8.0
Q	12.6	64.4	44.5	156.5	1.4	8.0
R	14.0	95.3	51.3	162.4	4.6	8.2

At the end of the experiment the pH of the reaction mixtures containing Products F, H, and M were all above 10. This can most likely be ascribed to the release of carbon dioxide gas during the biological product stock preparation (where bubbling was observed) as mentioned earlier, and shown in Table 6 in the case of Products F and H. The low oxygen consumption values achieved confirmed that the carbon dioxide production in these cases cannot be ascribed to biodegradation. Based on the results in Table 5, some of the biological products seemed to be more easily biodegradable than the faeces control. To determine whether the breakdown of the biological products by themselves might mask the breakdown of the faeces expressed in terms of oxygen consumption or carbon dioxide production, the gas production values obtained for the biological products were expressed as a percentage of the values obtained for the faeces control (Table 6). The carbon dioxide release by Products F and H exceeded the values of the faeces and could definitely have an effect on the interpretation of the gas exchange results of the faeces / biological product mixture.

Table 5. Inherent breakdown of biological product controls in terms of the removal of chemical and physical parameters monitored

Set No.	Product	pH begin	pH end	Percentage removal of				
				NH ₃	TKN	COD	TSS	VSS
Set 1	Control [†]	6.1	6.9	43	20	7	19	32
	A	6.5	6.7	52	20	71	23	35
	B	6.2	6.9	43	36	74	59	58
	K	6.3	6.9	52	40	23	20	64
Set 2	Control [†]	6.5	8.0	46	22	10	27	58
	C	7.2	7.0	0	3	20	30	67
	L	7.1	6.9	6	58	58	50	75
	P	7.1	6.8	0	0	87	48	63
Set 3	Control [†]	6.6	7.6	32	29	10	28	41
	G	6.7	7.4	0	20	45	62	27
	Q	6.7	7.6	10	32	59	60	34
	R	6.6	7.7	0	0	80	28	54
Set 4	Control [†]	7.6	7.0	31	27	20	16	20
	F	7.4	10.2	36	42	15	13	18
	H	7.4	10.2	47	41	18	13	32
	M	7.5	10.4	31	36	43	32	52

[†] faeces control

Table 6. Inherent breakdown of biological products in terms of oxygen consumption and carbon dioxide production

Set No.	Product	Total O ₂ consumed (mg)	Percentage of faeces control	Total CO ₂ produced (mg)	Percentage of faeces control
Set 1	faeces control	98	100	99	100
	A	10	10	8	8
	B	8	8	6	6
	K	3	3	3	3
Set 2	faeces control	71	100	75	100
	C	7	10	<0	0
	L	6	8	<0	0
	P	7	10	<0	0
Set 3	faeces control	79	100	99	100
	G	4	5	4	4
	Q	4	5	3	3
	R	4	5	4	4
Set 4	faeces control	58	100	86	100
	F	4	7	85	99
	H	<0	0	105	106
	M	3	3	3	3

3.3.2 Finalisation of experimental protocol

An adequate faeces sample size had to be selected to ensure that the quantities of gas used and produced during the biodegradation of the faeces, fell within the range of the gas sensors. To calculate the ratio of faeces to biological product to use in the reaction vessels, the following assumptions were made:

- 160 g of faeces / person / day.
- Life time of pit latrine used by four people: six months.
- Faeces deposited in the latrine during this period: 160 g x 4 x 180 days = 115 kg
- 115 kg is equivalent to 143.75 L, assuming that the specific gravity of faeces is 0.8 g/L.
- Urine drains away in the soil, therefore the pit latrine functions under relatively dry conditions.
- A bucket of water used to dilute many of these biological products was equivalent to 5L (for products where dilution volume was not specified, 5L was used). For some products the dilution volumes had to be increased to allow for a high absorbance of water.
- Monthly dosages of certain biological products were divided by 4 to provide the amount to be added per week.

The calculations based on the above assumptions, however, showed that microlitre quantities of certain biological products would have to be added to 2.5-5 g of faeces in a reaction vessel with a bottom surface of 28.27 cm². This would have resulted in the drying out of the sample and uneven contact between the faeces and the biological product. It was then decided to make the dilutions of the biological product in water, as prescribed, but to add the same volume (5 mL) of diluted biological product in all instances to 2.5 g of faeces (it was found that the gas exchange of 5 g of sample fell outside the range of the gas sensors) to prevent the drying out of the faeces. It was, however, noticed that the volume of biological product was still insufficient to prevent drying out of large portions of the sample as shown in Figure 2.

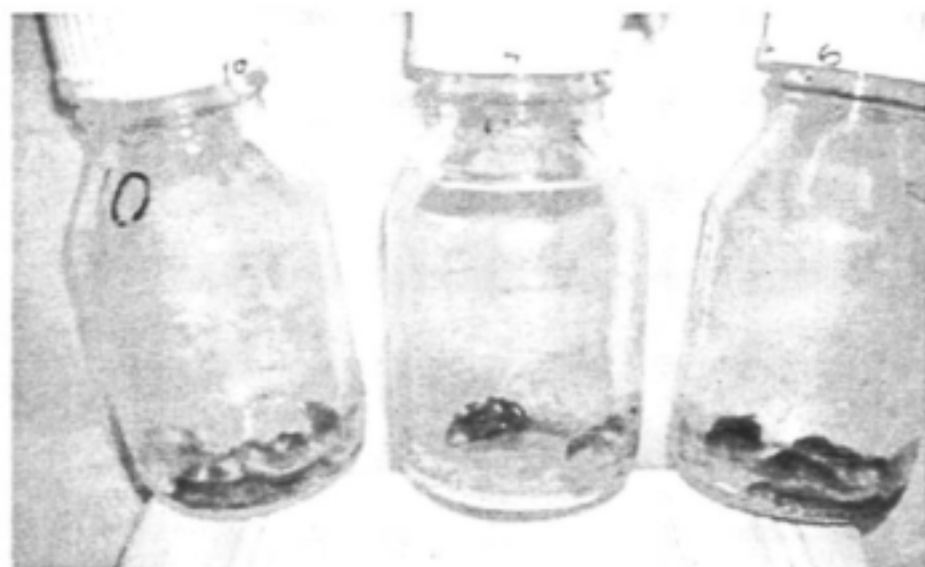


Figure 2. Photograph of experimental vessels containing faeces and product

In order to mimic as closely as possible the conditions in the pit latrine, it was decided not to increase the volume of the biological product. The "drying out" problem was solved by making a paste of the faeces with water (ratio 2:1) and spreading 5 g of this paste (it was also easier to get an even distribution of the sample between different vessels) evenly over the bottom of the experimental vessel to cover the whole bottom of the vessel in a layer of ± 0.5 cm, as shown in Figure 3.

The 2.5 mL of biological product was applied to the faeces paste with a pipette and drained into the faeces during the continuous shaking (recommended by the manufacturers of the respirometer for more accurate gas measurements) of the vessel for the duration of the experiment.



Figure 3. Faeces paste spread in a layer over bottom of flask – photo taken after termination of experiment – open patches indicate liquefaction of faeces

3.3.3 Biodegradation studies

Taking into consideration that the faeces samples used in the different experiments were in some cases collected weeks apart, and that the donor's diet might have changed during the time, the composition of the different batches was relatively consistent, as shown in Table 7. The faeces used for experiments in set No. 4 seemed to have been more granular judging from the high TSS values obtained. The biodegradation pattern for faeces used for experimental sets Nos. 2-4 were similar, as shown in Figure 4. It appears that the faeces used in set 1 were in a more advanced state of decomposition at the start of the experiment than for the other sets.

Table 7. Chemical and physical characterisation of faeces used in experiments

Set	NH ₃	TKN	COD	TSS	VSS	pH
	mg/g of faeces					
1	11.6	42.0	326.4	165.0	139.8	6.1
2	11.3	37.8	313.2	143.4	131.3	6.5
3	12.2	42.5	323.1	166.7	137.6	6.6
4	11.2	40.1	225.2	277.7	172.7	7.6
Average	11.6±	40.6±	297.0±	188.2±	145.4±	6.7±
	0.4	2.2	48.1	60.6	18.6	0.6

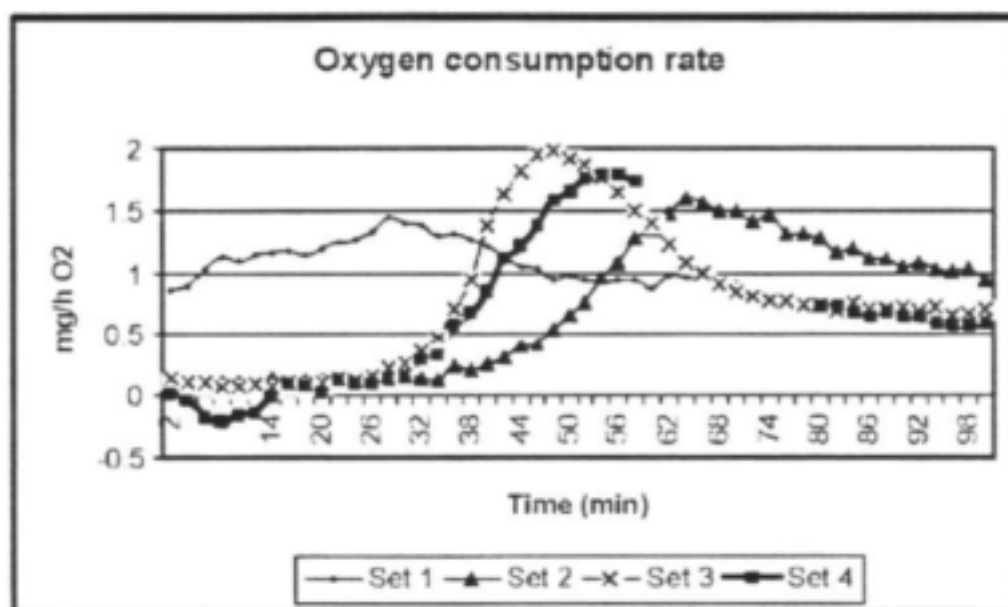


Figure 4. Biodegradation pattern of faeces controls

The addition of biological products did not seem to enhance the biodegradation of the faeces in terms of oxygen consumption and carbon dioxide production as shown in Table 8. Less oxygen was consumed in the presence of the biological products and in the case of products F, H, M, the carbon dioxide values exceeded those of the control because of the release of the gas by the biological products as previously mentioned.

Table 8. The effect of biological products on the breakdown of faeces in terms of oxygen consumption and carbon dioxide production

Set No.	Product	Total O ₂ consumed (mg)	Percentage of faeces control	Total CO ₂ produced (mg)	Percentage of faeces control
Set 1	faeces control	98	100	99	100
	+A	91 ± 1.4 CV: 1.6	93 ± 1.4 CV: 1.5	94 ± 0.8 CV: 0.8	94 ± 0.7 CV: 0.8
	+B	87 ± 4.2 CV: 4.8	89 ± 4.2 CV: 4.8	105 ± 8.5 CV: 8.1	106 ± 8.5 CV: 8.0
	+K	86 ± 0 CV: 0	88 ± 0 CV: 0	102 ± 0.7 CV: 0.7	103 ± 0.7 CV: 0.7
Set 2	faeces control	71	100	75	100
	+C	70 ± 9.2 CV: 13.1	98 ± 12.7 CV: 13.0	74 ± 10.6 CV: 14.4	93 ± 10.8 CV: 11.6
	+L	70 ± 15.6 CV: 22.2	99 ± 22.0 CV: 22.1	84 ± 5.0 CV: 5.9	112 ± 6.4 CV: 5.7
	+P	72 ± 7.1 CV: 9.8	101 ± 10.0 CV: 9.8	76 ± 9.2 CV: 12.1	100.5 ± 12.0 CV: 12.0
Set 3	faeces control	79	100	99	100
	+G	69 ± 4.3 CV: 6.2	88 ± 5.0 CV: 5.6	85 ± 6.4 CV: 7.5	86 ± 6.4 CV: 7.40
	+Q	70 ± 4.2 CV: 6.1	89 ± 5.0 CV: 5.6	90 ± 11.3 CV: 12.6	91 ± 11.3 CV: 12.4
	+R	71 ± 5.0 CV: 7.0	90 ± 6.4 CV: 7.1	87 ± 6.4 CV: 7.3	88 ± 6.4 CV: 7.2
Set 4	faeces control	58	100	86	100
	+F	<0 CV: -	0 CV: -	106 ± 5.0 CV: 4.7	123 ± 5.7 CV: 4.6
	+H	<0 CV: -	0 CV: -	103 ± 0 CV: 0	120 ± 0 CV: 0
	+M	65 ± 0.7 CV: 1.0	111 ± 1.4 CV: 1.3	91 ± 0.7 CV: 0.8	106 ± 0.7 CV: 0.7

When the effect of biological products on the biodegradation of faeces was measured in terms of ammonia, TKN, COD, TSS and VSS removal, it was found that more efficient removal of some of these parameters was found in the presence of biological products, as shown in Table 9. In the absence of sufficient replicate values for the control and experimental conditions to determine where the addition of a biological product significantly enhanced the biodegradation of faeces, it was decided to use 1.5 x the average CV ("cut-off" point) obtained for all the duplicate values applying to a specific parameter as a significant difference for that parameter.

Table 9. The effect of biological products on the breakdown of faeces in terms of chemical and physical parameters monitored

Set No.	Product	pH begin	pH end	Percentage removal of				
				NH ₃	TKN	COD	TSS	VSS
Set 1	Control	6.1	6.8	43	20	7	19	32
	A	6.4	6.9 ± 0	45 ± 0.7 CV(%): 1.6	20 ± 0 CV(%): 0	16 ± 1.4 CV(%): 8.8	18 ± 0 CV(%): 0	34 ± 2.1 CV(%): 6.2
	B	6.1	6.8 ± 0.1	46 ± 0.7 CV(%): 1.5	31 ± 0.71 CV(%): 2.29	45 ± 0.7 CV(%): 1.6	18 ± 1.4 CV(%): 8.0	7 ± 2.8 CV(%): 40.4
	K	6.5	6.8 ± 0	41 ± 1.4 CV(%): 3.4	11 ± 0 CV(%): 0	3 ± 2.1 CV(%): 70.7	14 ± 2.1 CV(%): 15.2	25 ± 1.4 CV(%): 5.6
Set 2	Control	6.5	8.0	46	22	10	27	58
	C	6.5	7.6 ± 0.3	21 ± 4.2 CV(%): 20.1	18 ± 0.7 CV(%): 3.94	21 ± 0.7 CV(%): 3.4	36 ± 1.4 CV(%): 4.0	61 ± 0.7 CV(%): 1.2
	L	6.6	7.6 ± 0	27 ± 5.6 CV(%): 20.9	37 ± 3.5 CV(%): 9.57	18 ± 0 CV(%): 0	34 ± 3.4 CV(%): 2.1	33 ± 2.2 CV(%): 2.2
	P	6.6	7.55 ± 0.1	21 ± 0 CV(%): 0	52 ± 0 CV(%): 0	22 ± 0.7 CV(%): 3.2	53 ± 1.4 CV(%): 2.7	52 ± 0.7 CV(%): 1.4
Set 3	Control	6.6	7.6	32	29	10	28	41
	G	6.5	7.8 ± 0	25 ± 1.4 CV(%): 5.6	28 ± 0.7 CV(%): 2.5	12 ± 11.3 CV(%): 94.2	17 ± 1.4 CV(%): 8.3	30 ± 0.7 CV(%): 2.4
	Q	6.6	8.0 ± 0.1	38 ± 1.4 CV(%): 3.7	36 ± 0.7 CV(%): 1.97	13 ± 7.2 CV(%): 5.2	26 ± 1.4 CV(%): 5.4	31 ± 0 CV(%): 0
	R	6.6	7.9 ± 0	29 ± 2.1 CV(%): 7.3	44 ± 0 CV(%): 0	36 ± 2.2 CV(%): 5.9	29 ± 0 CV(%): 0	37 ± 2.12 CV(%): 5.7
Set 4	Control	7.6	7.8	31	27	20	16	20
	F	6.8	10.0 ± 0	41 ± 0.7 CV(%): 1.7	31 ± 0.7 CV(%): 2.89	44 ± 17.0 CV(%): 38.6	37 ± 14.9 CV(%): 40.1	45 ± 3.5 CV(%): 7.8
	H	7.2	10.0 ± 0	40 ± 0 CV(%): 0	35 ± 1.4 CV(%): 4.03	3 ± 2.8 CV(%): 94.2	2.5 ± 2.1 CV(%): 84.8	4 ± 2.8 CV(%): 70.8
	M	7.7	10.5 ± 0.1	41 ± 2.8 CV(%): 7.0	49 ± 0.7 CV(%): 1.5	64 ± 0.7 CV(%): 1.1	78 ± 0.7 CV(%): 0.9	72 ± 0.7 CV(%): 1.0

The significant "cut-off" points obtained for the different parameters were as follows: ammonia (10%), TKN (10% - the calculated value was 5, but it was decided not to use a value lower than 10, based on experience in *in vitro* toxicity tests where the smallest "cut-off" point is normally 10%), COD (40%), TSS (15%) and VSS (15%). Using these derived "cut-off" points, it can be seen in Table 9 that at the experimental dosage used, Product M performed the best, followed by Products F, P and B. Knowing that under natural conditions in a pit latrine, the waste is broken down to smaller particles that can be assimilated by the surrounding soil where further breakdown will take place, TSS removal was considered an important determinant in the efficient breakdown of faeces. A high percentage removal of TSS (78%) was achieved in the presence of Product M.

During the visual observation of the experimental vessels after termination of the experiment, it was noticed that a high percentage of the contents of the Products F and M experimental vessels were in a liquid form, which is in agreement with the TSS results. The final pH values obtained for Products F, H and M fell outside their optimum pH range, which can probably be ascribed to the release of CO₂ during the experiment, as shown in Table 6. To determine whether inherent breakdown of the biological product could have had a major impact on the results obtained in Table 9, the values obtained for the biological product controls were deducted from the values obtained for the faeces / biological product mixture and the percentage removal recalculated and represented in Table 10.

Table 10. Breakdown of faeces minus the contribution of the inherent biological product breakdown

Set No.	Product	pH begin	pH end	Percentage removal of				
				NH ₃	TKN	COD	TSS	VSS
Set 1	Control	6.1	6.9	43	20	7	19	32
	A	6.5	6.7	52	36	71	23	35
	B	6.2	6.9	43	40	74	59	58
	K	6.3	6.9	52	57	23	20	64
Set 2	Control	6.5	8.0	46	22	10	27	58
	C	7.2	7.0	22	18	20	34	61
	L	7.1	6.9	28	31	21	34	33
	P	7.1	6.8	22	53	21	53	51
Set 3	Control	6.6	7.6	32	29	10	28	41
	G	6.7	7.4	26	27	12	14	30
	Q	6.7	7.6	36	33	14	24	32
	R	6.6	7.7	29	43	36	29	36
Set 4	Control	7.6	7.8	31	27	20	16	20
	F	7.4	10.3	40	41	47	29	47
	H	7.4	10.2	39	34	6	6	77
	M	7.5	10.4	42	51	73	71	59

Product M still performed best, but Product B now performed better than Product F. This can probably be ascribed to the fact that a more concentrated preparation of Product F was used, resulting in the larger impact of the inherent breakdown of the biological product on the combined faeces / biological product results.

To address the issue of the different amounts of biological products used in the experiments and the effect this might have had on the rate of breakdown of the faeces, the specific activity of the biological products per gram of product used of its economic efficiency per Rand of cost (both expressed in terms of faeces breakdown) was calculated. To relate the results obtained with the four different sets of faeces, controls at the start of the experiment presented in average values obtained for the faeces controls at the start of the experiment presented in Table 7, were used to perform these calculations. When efficiencies were compared in terms of experimental dosage, Products P, C and R performed best.

Table 11. Specific activity of biological products (per gram of product) measured in terms of their effect on faeces breakdown (removal of chemical and physical parameters monitored) minus the contribution of the inherent biological product breakdown

Product	Removal normalised in terms of results obtained with Product A (equated to 100%)					Average for all parameters used
	NH ₃	TKN	COD	TSS	VSS	
A	100.0	100.0	100.0	100.0	100.0	100.0
B	20.7	27.8	26.1	64.1	16.6	31.1
C	281.9	333.4	187.8	985.6	464.8	460.7
F	0.8	1.2	0.7	1.3	0.6	0.9
G	166.5	250.0	56.3	202.9	114.2	158.0
H	0.8	1.0	0.1	0.3	0.9	0.6
K	50.0	79.2	16.2	43.5	36.6	45.0
L	10.8	17.2	6.0	29.6	7.5	14.2
M	0.8	1.4	1.0	3.1	0.7	1.4
P	211.4	736.2	147.9	1152.2	291.4	507.8
Q	346.3	458.4	98.6	521.8	172.2	319.5
R	278.9	597.3	253.5	630.4	205.7	393.2

The economic efficiency of the biological products calculated per Rand of cost is shown in Table 12. The costs for some of the biological products were not available. Products G, K and A performed best in terms of economic efficiency.

Table 12: Specific activity of biological products (per Rand of product cost) measured in terms of their effect on faeces breakdown (removal of chemical and physical parameters monitored) minus the contribution of the inherent biological product breakdown

Product	Removal normalised in terms of results obtained with Product A (equated to 100%)					Average for all parameters used
	NH ₃	TKN	COD	TSS	VSS	
A	100.0	100.0	100.0	100.0	100.0	100.0
B	29.5	39.6	37.2	91.5	23.6	44.3
C	31.8	37.6	21.2	111.1	52.4	50.8
F	0.7	1.0	0.6	1.1	0.5	0.8
G	387.6	581.9	131.1	472.3	266.0	367.8
Product	Removal normalised in terms of results obtained with Product A (equated to 100%)					Average for all parameters used
	NH ₃	TKN	COD	TSS	VSS	
H	2.1	2.6	0.2	0.7	2.4	1.6
K	397.1	628.7	128.6	345.3	290.4	358.0
L	97.0	155.0	53.2	266.1	67.9	127.8
M	0.3	0.5	0.4	1.0	0.2	0.5
P	Cost of product not available					
Q						
R						

3.4 Conclusions

No conclusive differences were obtained based on the gas exchange studies and therefore, only results based on the changes observed in the chemical and physical parameters monitored could be used to compare the efficiencies of the biological products in terms of enhancement of faeces breakdown. Based on data directly obtained from the experimental studies (experimental dose) the best results were obtained with Products M, F, P and B. When the efficiencies were, however, compared in terms of their specific activities (extrapolated values), Products P, C and R were determined to perform best.

As far as cost is concerned, the laboratory study showed that the best results were obtained with the more expensive biological products at the tested concentrations. However, when comparing the economic efficiency of the biological products per Rand of biological product used (extrapolated values) (cost of Products P, W and R not available) Products G, K and A were determined to have performed best.

3.5 Recommendations

It is recommended that in future laboratory studies, the field conditions with respect to prescribed biological product dosage should not be taken into account. The attempt made in the current study to simulate field conditions in the laboratory complicated the execution of the experiments and processing of results. The experiment should run for a longer time period and the same biological product concentrations should be used. During the experiment, samples should be taken at shorter intervals to monitor progress. At least six replicates per treatment are required for statistical analysis.

4. FIELD TESTS OF TWO COMMERCIAL MICROBIAL OR MICROBIALLY-DERIVED PRODUCTS FOR THE TREATMENT OF ORGANIC WASTE IN PIT LATRINES

4.1 Introduction

Five kilogram amounts of selected products were obtained for the field trials. The original intent was to evaluate the best three or four products in on-site trials, but due to limited testing facilities, only two products could be evaluated (against a control treatment).

Based on data obtained from the experimental studies conducted, according to the manufacturer's recommended dosage specifications, compensation of these values for the inherent breakdown of the product itself, and time frame governing the execution of the project, Products M and B were selected for the field studies.

4.2 Site Selection

The initial challenge in starting the pit latrines field tests was to find a suitable site. For practical purposes the site had to be within an hour's drive of Randburg. A suitable venue was eventually identified in Hekpoort, in the district of Magaliesburg, approximately 45 minutes drive from Randburg.

The selected site was on a rose farm, in the area reserved for the accommodation of the farm labourers (Figure 5).



Figure 5. The selected site in Magaliesburg

A group of four pit latrines had been built to service the staff compound on the farm, approximately 200-250 people (depending on the season) and informal settlers who had settled in the compound. These latrine buildings were made of brick, divided into three pits per unit, and covered with corrugated iron for roofing (Figure 6). On inspection these pits were found to be totally congested and almost unable to serve their purpose, as they were filled to the brim (Figure 7). There were swarms of flies, and the odour was noticeable from a distance of approximately three metres.

The pits were originally mechanically dug to approximately 3 m long by 1.5 m wide and approximately 2.5 m deep, resulting in a total volume per pit of about 11.25 m³.



Figure 6. Three pit latrines covered by corrugated iron roof



Figure7. An example of the congested pit latrines

4.3 Materials and Methods

4.3.1 Experimental set-up

The first task was to sanitise and clean the area in order that the experiments could be initiated. The pits were not in optimal use at the time of the experiments, as the odour and flies prohibited the user from spending the required amount of time in that environment (Figure 8).

The experimental design also required that the residents and informal settlers not use the latrines once the experiments had been initiated, *viz.* once the odour and flies had been eradicated. The assistance of the Magaliesburg Police was obtained. The selected sites were taped off, indicating that these were police sites, and people were, therefore, prohibited from entering (Figure 6). Staff were also employed to, where possible, keep usage of the latrines to a minimum.



Figure 8. Pit latrine after clean-up



Figure 9. Poles with white markings were put into the pits to measure volume reduction

The progress of the biological treatment of the pits over time was monitored using iron poles of approximately 3 m in length, which were placed into the pits. Corrugated iron was removed from the roof of the latrine to facilitate the placement of iron poles into the pits. The poles were marked with white paint at 50 cm intervals. During observation visits, marks were placed at the line where the surface of the waste was observed (Figure 9).

Subsequent to obtaining permission to execute the field trial, the study pits were cleared of non-biodegradable matter.

4.3.2 Treatment sites

The latrine blocks were identified as per the products used. Product B (= "Block 2") had three pit facilities – left, centre and right. Block 3, identified as "M" for Product M had the same configuration as Block 2. The third block was identified as Block C which was the "Control Block" (Figure 10).



Figure 10. Blocks of 3 pit latrines for the treatments and control

4.3.3 Application of biological products

Two products were evaluated during the three month test period, namely, Product B and Product M.

The dosing regime was 100 g of dry biological product per pit, with three pits per block of latrines (three pit latrines per treatment and control). The specification for Product M required the powder to be dissolved in warm water and left overnight before application. It was decided, however, to treat Products B and M in the same manner, since the availability of warm water to the user is not guaranteed. The biological product treatment was, therefore, diluted in ± 25 L cold water, mixed well and the mixture then applied to the specific pits (Figure 11). The Control Block received no application of biological products, but 25 L of water was applied at the same intervals as biological treatment application to the other test sites.

Pits were routinely kept damp by watering on a daily basis or when necessary (all treatments and control). Water application was done, as in most working pits, urine keeps the pit content moist.

Subsequent to application of the biological products, sites were evaluated 3 times per week (Monday, Wednesday and Friday). The site visits also served as information sessions with the local residents.



Figure 11. Preparation of dry powders for application to pit latrines

The dosage regime and related activities are outlined in Table 13.

Table 13: Schedule for pit latrine field trial study

Activity	Date	Time interval from study initiation (days)
Permission to execute experiment	24 th April 2003	0
Facility clean up	5 th May 2003	11
First product application	26 th May 2003	32
Second product application	27 th June 2003	64
Interim dosing of 50 g of Product M was added to the three pits in Block 3, using warm water and an overnight standing period prior to application	11 th July 2004	78
Final doses applied - Block 2 received 100 g of Product B for each of the three latrines, diluted in cold water. Block 3 received 100 g of Product M per pit diluted in warm water and left to stand overnight prior to application	26 th July 2003	93
Site visitation	3 times / week	
Study termination	26 th August 2003	124

4.4 Results

4.4.1 Observations made during evaluation intervals

By the 2nd June 2003, a week after treatment was initiated, a reduction in odour and an absence of flies in Block 2 (Product B) were noticeable. There was, however, no noticeable reduction in waste volume. Block 3 (Product M) still had a noticeable odour, but the fly population was reduced. There was no change in odour and the fly population in the pit latrines of Block C (controls). These observations were independently verified on the 4th June 2003.

No noticeable reduction of the waste volumes was observed for both test sites during routine inspections in the interval leading to the 26th June 2003, but an absence in odour and flies in Block 2

(Product B) was recorded. There was, however, still a lingering odour from Block 3 (Product M), as well as the presence of flies.

Although a second product application was conducted on the 27th June 2004, by 7th July 2003 it became apparent that Product M in Block 3 was not performing to expectations. After consultation with the CSIR, an interim dosing to the pits in Block 3, using warm water and an overnight standing period prior to application, was done.

During subsequent visitations, it appeared that Product B in Block 2 was still working better (visually) than Product M in Block 3. The final product doses were applied on 26th July 2003. During the course of visitations from 29th July 2003 to the close of the study, it was still apparent that the biological product being applied in Block 2 was performing better than the biological product in Block 3.

4.4.2 Final observations

Overall observations for the field trial study were that the odour and flies were completely eradicated in Block 2 (Product B) within the first week of application. The pit latrines in Block 3 were, however, never completely rid of the odour or flies during the three-month field trial period. The Control Block, during the study, had notable odour and flies. No rain fell during the study period.

Table 14 indicates the final results relating to the waste reduction determination. For Block 2, the bulk of the waste was more loose and softer in appearance and showed signs of liquefaction. With Product M, however, the odour seemed to re-appear towards the end of the 30-day period, with little reduction of the waste in the pit. There was also some crusting on top of the waste.

Table 14. Final waste reduction results of field trial

Area and product	Latrine	Time Interval	Measurement
Block 2 (Product B)	Left hand latrine	Start Finish Loss	166 cm 153 cm 13 cm over the area Morning temperature 10°C
	Middle latrine	Start Finish Loss	100 cm 87 cm 13 cm over the area Morning temperature 9°C
	Right hand latrine	Start Finish Loss	197 cm 175 cm 22 cm over the area Morning temperature 9°C
Block 3 (Product M)	Left hand latrine	Start Finish Loss	198 cm 193 cm 5 cm over the area Morning temperature 10°C
	Middle latrine	Start Finish Loss	99 cm 96 cm 3 cm over the area Morning temperature 8°C
	Right hand latrine	Start Finish Loss	148 cm 138 cm 10 cm over the area Morning temperature 10°C
Block C (Control)	3 Latrines (ave)	Start Finish Loss	50 cm 50 cm 0 cm over the area Morning temperature 8-10°C

4.5 Discussion

As pit latrines are mostly dry sanitation systems, the contents should be mainly faeces, urine and paper used for the designated purposes. The selected pits had, however, been used as a disposal vehicle for numerous other non-degradable waste materials.

There is also a strong possibility that significant volumes of detergents (e.g. Jeyes Fluid) had been used over the years to control the odour of the toilets. Because of the ratio of foreign objects to faeces and urine (representing moisture), the biological products were, therefore, added to an environment not truly conducive to studying the maximum biological degradation, with the result that the products probably reacted more slowly than the norm. In addition, the cold temperatures experienced during the 3-month test period would also have adversely affected the rate of reactions of the biological products in the test environment.

The average results (volume reduction) indicated the Product B performed better than Product M in the field trials. The results are, however, not statistically validated since the coefficients of variation between the results for Products B and M were 32.5% and 60.1% respectively. One of the contributing factors to these results may have been differences in the composition of contents in the various pit latrines.

Various constraints were experienced during the execution of the field trials. These include:

- Difficulty in identifying a suitable site where new pits could be dug and where the experiment could be monitored. The alternative was to use "old" pits.
- The "old" pits were compacted and filled with, not only faeces and urine, but also newspaper, grass, plastic bags, tins and styrofoam containers. The pits had to be cleaned (non-degradable objects taken out) before experiments could be initiated.
- Due to the laboratory studies taking longer than expected, the field studies were executed during the coldest winter months. This would have slowed down the activities of the biological products, which would have manifested in reduced performance.
- The number of pit latrines available for testing. There were three buildings, each containing three pits, but the variation between the triplicate treatments was still too large for the detection of statistical differences.
- Difficulties in preventing people from using the "test" pits. The treated pits became much more user-friendly, because of a reduction in odours and flies.

4.6 Conclusions

Factors such as the original state of the latrines (foreign objects, overfull, compacted) and the low temperatures during the test period may have adversely impacted on the experiments and reduced the activities of the biological products in the test environment. The results derived from Product B are comparable to those obtained in the laboratory experiments, whereas the results from Product M did not validate those obtained in the laboratory study. The performance of biological products determined on small scale (laboratory experiments) could not be conclusively verified during these field tests. Based on the results of this study, however, the use of biological products for the degradation of organic waste in pit latrines may be feasible under optimum conditions.

4.7 Recommendations

For the determination of optimum performance of biological agents, an alternative approach may be considered for the execution of pit latrine field trials. The trials should be started with newly dug pit latrines, shallow in construction, as depth is not required if dosed on a regular basis.

In this case the daily monitoring of pit latrine usage to estimate the volume of waste-to-product consumed, will be important. Toilet paper or newspaper (biodegradable material) will also have to be provided to prevent the pits from being filled with non-biodegradable objects. If practically

possible, the planning of the experimental trial will have to make provision for sufficient replicates in each treatment (at least six), since large variations between results can be expected.

5. SUMMATION OF STUDY CONCLUSIONS AND RECOMMENDATIONS

5.1 Biological Products Available for the Treatment of Pit Latrines: Products and Market Information

A total of thirteen products, with accompanying product information, were obtained from nine suppliers. Some market information was obtained from suppliers, although most of the companies do not have records of actual usage. Most of the products are multi-purpose, with only a very small percentage of the sales going towards pit latrine treatments. No information on current and past product usage could be obtained from government departments or municipalities.

Due to the difference in pit latrine sizes and the uncertainty relating to the numbers of people periodically using a pit latrine, it was difficult to make specific dosage recommendations for the use of most of these products and also difficult to determine usage or cost per capita. An estimation of the annual costs of these products for potential application in a pit latrine showed a huge variation.

The size of the South African market for microbially-derived products for the treatment of organic waste in pit latrines has not been reported or recorded by a South African source. It is estimated that about 6-10 tons of product is currently imported per annum. Some products are also "manufactured" locally, but this usually entails mixing and blending of concentrated imported products. The general sentiment is that the potential market is substantial, but it is a difficult market to penetrate.

5.2 Laboratory Scale Evaluation of Different Commercial Microbial or Microbially Derived Products for the Treatment of Organic Waste in Pit Latrines

Twelve products were selected from the 16 products (based on shelf-stability) and tested at laboratory scale to determine their efficacy in digesting organic waste. The experimental procedure was simplified by studying the effect of the biological products on the biodegradation of faeces, the most important organic waste deposited in pit latrines.

The gas exchange studies were unable to provide evidence / data allowing product differentiation in terms of performance. Consequently, results, based on the changes observed in the chemical and physical parameters monitored, were used to compare the efficiencies of the biological products in terms of enhancement of faeces breakdown.

Based on data directly obtained from the experimental studies conducted, according to the manufacturer's recommended dosage specifications, the best results were obtained with Product M followed by Products F, P and B.

When these values were adjusted to compensate for the inherent breakdown of the product itself, Product M still performed best, but Product B was determined to have performed better than Product F. At this stage, a decision on the selection of products for the field studies had to be made, and Products M and B were selected.

5.3 Field Tests of Two Commercial Microbial or Microbially-Derived Products for the Treatment of Organic Waste in Pit Latrines

Volume reduction results indicated that Product B performed better than Product M in the field trials. Visual observations showed that the layers of the treated pits started to liquefy. There were minimal changes in the pits treated with Product M and no changes in the control pits. The odour and the population of flies in the treated latrines (especially with Product B) disappeared after the first dosages, whereas bad odours and flies persisted in the untreated latrines. The results, however, could not be statistically validated.

The main constraints during the field study were the compactness of the waste and the presence of non-biodegradable foreign objects in the latrines. This, and the low temperatures (8-10°C) during the three winter months caused the interaction between the microorganisms, enzymes and organic waste to slow down considerably.

The results derived from Product B are comparable to those obtained in the laboratory experiments, whereas the results from Product M did not validate results obtained in the laboratory study. The performance of biological products determined on small scale (laboratory experiments) could not be conclusively verified during these field tests. The results indicate, however, that the use of biological products for the degradation of organic waste in pit latrines may be feasible under optimum conditions.

For the determination of optimum performance of biological agents, an alternative approach may be considered for the execution of pit latrine field trials using biological products. The trials should be started with newly dug pit latrines, shallow in construction, with daily monitoring of pit latrine usage to estimate the volume of waste-to-product consumed. Prevention measures should be put in place to reduce instances of pits being filled with non-biodegradable objects. If practically possible, sufficient replicates of each treatment should be conducted so as to allow for statistical evaluation of the results.

5.4 General

The general conclusion is that, based on the results obtained in this study, the use of biological products for the degradation of organic waste in pit latrines could be feasible.

It is, however, recommended that if biological products are used to enhance the degradation of organic waste in pit latrines, they be used as early as possible after implementation of the pit latrine. The use of these biological products must also be combined with an educational programme, perhaps through government health workers. Aspects such as the negative effect of detergents and non-biodegradable objects in the pits on the activity of biological products should be brought to users' attention. The lack of toilet paper in schools for instance, has been noticed. The most important aspect, however, is the "cost" to the user, who will ultimately decide on the implementation of this system.

Future work could include a biological and biochemical study into the claimed mode of action of these biological products. The biological products should be evaluated on the basis of the amount and type of microorganisms and enzymes present and compared to the information and claims on the specification sheets.

The execution of this study allowed for development / enhancement of research capabilities in pollution and wastewater treatment amongst the project participants. With respect to biotechnological-derived treatments, the study emphasised the importance of understanding the environment, the factors that may influence the application of products, and the need to consider these aspects, in the application of existing products or in the development of new products.

6. REFERENCES

APHA (1992). Standard Methods for the Examination of Water and Wastewater, edited by M A H Franson, A.E. Greenberg, I.S. Clesceri and A.D. Eaton. American Public Health Association, American Water Works Association and Water Environment Federation Washington, USA.

Jere, M., Chadavaenzi, M., Nhandara, C. and Bradley, M. (1998). The effect of non-pathogenic bacteria on latrine sludge. Sanitation and Water for all. 24th WEDC Conference.

Lehohla, P. (1999). South Africa in transition. Selected findings from the October household survey of 1999 and changes that have occurred between 1995 and 1999. Statistics South Africa 2001.

Internet

www.lboro.ac.uk/departments/cv/wedc/garnet/ecosan.html; and
www.biosystemssa.co.za/new/how/pit_latrines.htm

Spanjers, H., van Rollengem, P.A., Olsson, G. and Dold, P.I. (1998). Respirometry in control of the activated sludge process. Principles. Scientific and Technical Report No. 7. International Association of Water Quality, London. pp 1-48.

TO ORDER: Contact Publications - Telephone No: 012 330 0340
Fax Number: 012 331 2565
E-mail: publications@wrc.org.za



177005314X

Water Research Commission

Private Bag X03, Gezina, 0031, South Africa

Tel: +27 12 330 0340, Fax: +27 12 331 2565

Web: <http://www.wrc.org.za>