PREDICTIVE ANALYSIS OF DEGRADATION INDICES AND MICROBIAL GROWTH DURING ENHANCED BIODEGRADATION OF MUNICIPAL SOLID WASTES IN UYO, NIGERIA

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ESSIEN[1] J. P., EKPO[1] M. A.,
BENSON[2] N. U., IKPE[1] D.,
UBOM[3] R. M. AND EBONG[4] G. A.
[1]Department of Microbiology, University of Uyo, Nigeria.
[2]Department of Chemistry, Covenant University, Ota, Nigeria.
[1]Department of Microbiology, University of Uyo, Nigeria.
[3]Department of Botany, University of Uyo, Nigeria
[4]Department of Chemistry, University of Uyo, Nigeria

ABSTRACT: The activity of bacteria, yeasts and fungi isolated from municipal solid waste (MSW) dumpsite on MSW-supplemented medium was screened to determine their waste utilization ability. Isolates (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Saccharomyces* sp.) with strong capability to degrade MSW as indicated by their high turbidity, high regenerability and or high growth rates were selected and constituted into bacteria (MSW-BB1) and fungi (MSW-BB2) consortia used for the degradation studies. The results indicate that treatment of MSW with MSW-BB1 and MSW-BB2 enhanced the rates of biodegradation of municipal wastes by the indigenous microbial load. This was revealed by the comparative increase in the bacterial densities of the treated wastes and the level of CO_2 released. The increased multiplication of bacterial cells resulted in increase in acidity of waste and rate of weight loss. Based on these degradation indices, the MSW-BB2 consortium performed better enhancing total bacterial build up of $8.67 \pm 0.2 \times 10^7$ cfu/g over 98 weeks. This resulted in the release of 12.80 ± 0.25 cm^3 of CO_2 per 100g MSW. However, statistical models show that changes in bacterial density are related positively to changes in CO_2 and weight loss in MSW but negatively with increase in acidity (pH). The relationships may not necessarily be of mutual dependence since the production of CO_2, changes in pH and weight loss of MSW may be affected by extraneous environmental factors (e.g. temperature) not measured in this study. While the trends in the degradation of MSW and evolution of CO_2 fitted the models, the negative significant relationship between bacterial density and pH reveals negative skewness, which resulted in significant utilization of MSW by microorganisms. The significant values of r determine the variability of values of the regressands, CO_2, pH and weight loss. The higher the absolute value of r the greater the quantity of MSW used up by the bacterial community in MSW. This enhances the line of best-fit regression line, since r is proportional to regression coefficient. Our findings have revealed that the relative ability of microorganisms to degrade organic wastes could be the main rate-limiting factor in the disposal of wastes in our urban cities. This notwithstanding the effective utilization of MSW by the fungal consortium MSW-BB2 comprising of *Aspergillus niger* and *Saccharomyces* sp is promising and a pointer to the success of the approach although increase in acidity will be a major hindrance.

INTRODUCTION

Waste management involves the generation, collection, processing and transport of waste as well as the minimization of the production of waste and the re-conceptualizing of waste as a resource. The public health impacts are influenced by the overall waste management strategy adopted locally, regionally and nationally. In proper waste management procedures, wastes are

mainly disposed through land filling, composting, incineration and recycling processes amongst other procedures. However, waste management in developing countries is usually equated with land disposal or discharge into bodies of water (Cilinskis and Zaloksnis, 1996). This method of waste management is unscientific and causes nuisance to the public, constituting pollution and health hazards. Improper disposal of untreated waste is not only harmful to human health but also constitutes a threat to the entire ecosystem.

When waste is dumped on land, soil microorganisms in the waste dump use the waste constituents as nutrients (Pavoni *et al*., 1975) through a process referred to as biodegradation. Microbial degradation involves chemical transformations mediated by soil microorganisms during which they satisfy their nutritional requirements, their energy requirements and detoxify their immediate environment (Ekpo, 2002). Soil microorganisms specifically fungi and bacteria are known to colonize the waste carrying out degradation and transformation of biodegradable (organic) materials in the waste (Stainer *et al*., 1996). According to Obire *et al* (2002), the major indigenous microbial genera involved in microbial degradation in the tropics among other bacteria are *Bacillus*, *Micrococcus*, *Pseudomonas Staphylococcus* and *Streptococcus* while the fungal genera include *Aspergillus*, *Saccharomyces*, *Penicillium* and *Mucor*. Algae are poor bio-degraders. These indigenous microorganisms in the waste dumpsite use the waste constituents as nutrients thus detoxifying the materials as their digestive processes breakdown complex organic molecules into simple, less toxic molecules (Pavoni *et al*., 1975). The degradation and stabilization of municipal waste are usually dominated by three groups of bacteria; namely the hydrolytic and fermentative bacteria, the actogens and the methanogens (Barlaz *et al*., 1990).

Biodegradation of waste is an essential process because it reduces the amount of waste in a dumpsite thus reducing cost of managing wastes. Enhanced biodegradation involves the additional treatment carried out on the natural processes for increased biological degradation. Enhancements that can be used to increase biological degradations in landfill bioreactors include leachate recirculation, moisture, temperature, nutrients, buffers; sludge addition, shredding and lift design (Kelley *et al*., 2002). Microorganisms require nutrients such as sulfur, calcium, magnesium, potassium, iron, zinc, copper, cobalt, molybdate, selenium, nitrogen and phosphorus to participate in anaerobic decomposition. In most cases, these nutrients are found in landfill but the heterogeneous and complex nature of the waste may limit their availability (Warith and Sharma, 1998). Obirie *et al*. (2002) and Kelly *et al*. (2002) have reported that well mixed and shredded refuse with small particle size are known to allow greater contact between substrate, microorganisms and moisture which could enhance biodegradation.

In Nigeria, dumping of waste is the commonest method of disposal of municipal waste as there is lack of sanitary landfill. Major urban towns including Uyo metropolis still indulges in the rudimentary form of waste management that involves the collection and dumping of wastes in various dumpsites scattered in the metropolis particularly in the Uyo Ravine. The health and environmental challenges associated with poor waste management practices cannot be over-emphasized. However, little or no work has been done on enhanced biodegradation of organic wastes in the tropics. In the present study, we evaluate the relations between degradation indices (such as pH, CO_2 evolution and weight loss) and microbial growth during enhanced biodegradation of municipal solid wastes (MSW) in Uyo metropolis by indigenous microorganisms using predictive statistical analytical approach.

METHODOLOGY

Source and Treatment of Municipal Solid Waste (MSW) Samples

The MSW samples used in this investigation were aseptically collected from Uyo Ravine waste dumpsite. The site is located within Uyo metropolis. During sampling, the surface debris at each sampling point was carefully removed, subsurface scooped at 5cm depth and transferred into sterile polyethylene bags. Prior to analysis, the MSW samples were measured out and air-

dried before segregation. Thereafter, the samples were sorted out or segregated to remove non-degradable components (such as stones, bottles, plastics, etc.) and the resultant biodegradable waste was aseptically milled and sieved with $0.5m^2$ wire mesh. The MSW powder was stored in airtight containers for microbiological, biodegradation and physicochemical studies

Microbiological Analysis of MSW

The heterotrophic bacteria and fungi counts in MSW were determined by the pour plate and spread plate techniques (Harrigan and McCance, 1976) using diluents prepared with sterile distilled water and cultured on Bacto nutrient agar (NA) and Sabouraud dextrose agar (SDA) respectively. The SDA was supplemented with 0.5mg of streptomycin per ml to inhibit bacterial contaminants. Inoculated NA and SDA plates were incubated at room temperature (28 ± 2^oC) for 48 and 120 hours respectively before colony enumeration with the aid of a Quebec colony counter.

The cultural characteristics of emerging colonies were observed after the incubation periods. Representative colonies of bacteria and fungi were isolated, purified by repeated sub-culturing on freshly prepared NA and SDA respectively. The resulting pure cultures were characterized using the taxonomic schemes of Cowan (1985) and Holt *et al*. (1994) for bacteria, while the taxonomic schemes of Domsch *et al*. (1990) and Barnet and Hunter (1987) were adopted for the identification of fungi. Yeast isolates were also subjected to series of biochemical tests as described by Barnett and Pankhurst (1974).

Determination of MSW Utilization Potential of the Microbial Isolates

The ability of microorganisms isolated from MSW to effectively utilize the waste as the sole source of carbon and energy was determined in *vitro* by the method previously adopted by Okpokwasili and Okorie (1988) and Itah and Essien (2005) using mineral salt medium (MSM) of Zajic and Supplisson (1972) plus 0.2g of the MSW powder. The MSW-supplemented medium was then sterilized by autoclaving at 121^oC for 15 minutes. Thereafter, 0.1ml (about 3.4×10^4 cells per ml) of nutrient and malt extract broth cultures of the bacteria and yeast isolates respectively was aseptically introduced into the MSW-supplemented MSM and then incubated undisturbed at 28^oC under static condition for 30 days. For filamentous fungi (moulds) 1g of wet weight of mycelium were inoculated. Un-inoculated tubes were included for each test inocula. The growth (cell division) as well as the biomass weight of moulds in MSW-MSM was determined every 10 days by cultural and analytical techniques as indices of their ability to utilize MSW for growth. Growth of the test organisms on the MSW based substrate was scored as abundant or high (+++), moderate (++) and minimal (+) depending on the degree of turbidity in the case of yeast and bacteria, and mycelium formation by moulds.

To determine the growth rate of moulds in MSW medium the mycelium of the cultures were harvested by filtration, washed with several changes of cold distilled water, dried to a constant weight in a draft oven at 60^oC and weighed in a chemical balance. Bacteria and yeasts growth were determined by viable cell counts on NA and SDA respectively using the standard spread plate technique (Harrigan and McCanes, 1990; Zuberer, 1994). Using the densities derived from viable counts, the number of generation (n), generation time (Gt) and growth rate (Gr) of the microbial consortia were estimated as described by Pelczer (1982) and Udofia *et al*. (2009).

Enhanced Biodegradation Studies

Microbial isolates with strong capability to utilize MSW for growth as indicated by the level of turbidity, ability to re-generate, growth rates and biomass weights were selected for the enhanced MSW degradation study. They were constituted into two microbial consortia designated as follows:

(i) MSW- BB-1 which comprises 4ml of *Bacillus* sp and 4ml of *Psueudomonas* sp.
(ii) MSW-BB-2 which comprises 4g of *Aspergillus* sp and 4ml of *Saccharomyces* sp.

To screen for the ability of the bacteria (MSW-BB-1) and fungi (MSW-BB-2) consortia to degrade MSW, the MSW-BB-1 and MSW-BB-2 consortia were seeded in 100ml of minimal basal salts medium (Johnson and Larsen, 1985 and Diaz, 2000) containing low levels (250µg/l) of peptone, yeast extract, and soluble starch, inoculated at room temperature (28 ± 2^oC) in a submerged culture microcosm test system (Essien *et al.*, 2005), and exposed to 100g of MSW. Un-inoculated MSW contained in flasks served as control for each consortium. The cultures were incubated on orbital Shaker (SGM-300, Gallenkamp, England) at 120 rev/min for 100 days. To achieve the goal of the study, seven replicates of each experiment were set up for destructive analytical process.

Plastic vials containing 1g BaO_2 in 10ml water were introduced into each microcosm to absorb the liberated CO_2. The flasks (microcosms) were incubated at 28^oC on a rotatory shaker (150rpm) for 98 days.

Estimation of MSW Degradation

The degree of degradation of MSW was measured using four indices:

(i) Determination of microbial growth rate
(ii) Determination of the changes in the pH levels of degrading MSW
(iii) Estimation of the total mineralization rate. Total mineralization was the sum of CO_2 evolved from each culture every 14 days during 98 days of incubation.
(iv) Determination of weight loss in MSW.

The MSW-BB-1, MSW-BB-2 growth were determined by viable cell counts on Bacto nutrient agar and Sabouraud dextrose agar respectively using the standard spread plate technique (Harrigan and McCance, 1990; Zuberer, 1994). The pH of the MSW prior to degradation and degrading MSW was determined by the method by Udo and Ogunwale (1986) using a calibrated pH electrode (Kenteil, 7020 Japlin). The volume of CO_2 liberated by the microbial isolates during growth on MSW was determined titrimetrically (Stotzky, 1965) and the CO_2 evolved calculated using the Stotzky's formula:

$$(B - V)\ NE.$$

Where B = Volume of acid of control experiment
V = volume of acid used in titration of test sample
N = Normality of the acid used
E = Equivalent weight of the acid

The percentage biodegradation rate of MSW was determined from the weight loss using the following relationship:

$$\%\,Degradation = \frac{[a-b]}{a} \times 100$$

where a = the weight of MSW (control)
b = the weight of MSW remaining.

Statistical Evaluation of Relations between the Degradation Indices

Comparative and continuous summary descriptive of the data were performed using the Analyze-It + 1.73 Statistical Software, with level of significance maintained at 95% for each test. The average, standard deviation, coefficient of variation, standardized skewness (central tendency) and standardized kurtosis (variability) of the degradation indices (growth rate, CO_2 evolution, pH and weight loss) were determined. Their predictive linear models were established using Statgraphics Centurion VI Statistical Software, with level of significance maintained at 95% for each test, and the Means Absolute Error (MAE) and order correlations estimated using the Durbin-Watson (DW) statistic tests (Durbin and Watson, 1950).

RESULTS AND DISCUSSION

The results of the MSW degradability test are presented in Table 1. The activity of the bacteria, yeasts and fungi isolated from waste dumpsite on MSW-supplemented medium was screened. Cultures with high turbidity, high regenerability and or high growth rates were selected. Among the isolates with strong capability to degrade MSW include *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Saccharomyces* sp. This implies that microbes with these growth attributes would readily colonize MSW adopted for the enhanced biodegradation studies. The MSW-grading isolates were therefore constituted into MSW-BB1 (comprising *Bacillus substilis* and *Pseudomonas aeruginosa)* and MSW-BB1 (comprising *Aspergillus niger* and *Saccharomyces* sp) consortia. The organic matter decomposition abilities of the selected isolates have previously been reported by Obire *et al* (2002).

Table 1: Screen Test for Municipal Waste Degradability of the Isolates

Isolate	Turbi-dity	Myce-lium Growth	B	B	N	Gt	Gr	Cumulative Biomass Weight (g)
Bacillus subtilis	+++	-	3.4×10^4	4.6×10^7	11	2	0.45	-
Bacillus megaterium	+++	-	3.4×10^4	3.4×10^6	7	3.4	0.28	-
Staphylococcus aureus	+	-	3.4×10^4	8.9×10^4	1	24	0.06	-
Peudomonas aeruginosa	+++	-	3.4×10^4	7.6×10^6	8	3	0.32	-
Micrococcus sp	++	-	3.4×10^4	6.7×10^5	4	6	0.18	-
Lactobacillus sp	++	-	3.4×10^4	6.4×10^4	0.9	24	0.04	-
Listeria sp	+		3.4×10^4	2.4×10^4	0.3	80	0.01	-
Agrobacterium sp	+	-	3.4×10^4	3.4×10^4	0	0	0	-
Streptococcus sp	+	-	3.4×10^4	4.9×10^5	4	6	0.16	-
Saccharomyces sp	++		2.2×10^3	9.4×10^5	9	8	0.06	-
Candida tropicalis	+	-	2.6×10^3	7.3×10^4	5	16	0.06	-
Candida pseudotropicalis	+	-	2.6×10^3	5.4×10^4	4	16.4	-	-
Aspergillus niger	-	+++	1g	-	-	-	-	1.98g
Aspergillus fumigatus	-	+++	1g	-	-	-	-	1.46g
Fusarium sp	-	++	1g	-	-	-	-	1.25g
Penicillium frequentans	-	++	1g	-	-	-	-	1.26g
Penicillium expansum	-	+	1g	-	-	-	-	0.28g
Cladosporium sp	-	++	1g	-	-	-	-	1.04g
Mucor sp	-	+++	1g	-	-	-	-	1.63g

B = Bacterial count at zero time, b = Bacterial count at the end of given period of time
n = Number of generations, Gt = Generation time
Gr = Generation rate, + = Minimal growth
++ = Moderate growth, +++ = High growth

Results presented in Table 2 indicate that treatment of MSW with MSW-BB1 and MSW-BB2 enhanced the rates of biodegradation of municipal wastes by the indigenous microbial load. This was revealed by the comparative increase in the bacterial densities of the treated wastes and the level of CO_2 released. The increased multiplication of bacterial cells resulted in increase in acidity of waste and rate of weight loss. Based on these degradation indices, the MSW-BB2 consortium performed better enhancing total bacterial build up of $8.67 \pm 0.2 \times 10^7$ cfu/g over 98 weeks. This resulted in the release of 12.80 ± 0.25 cm^3 of CO_2 per 100g MSW. The pH of MSW treated with MSW-BB2 was reduced from 6.88 ± 0.01 to 6.11 ± 0.25 (increased in acidity) over the same incubation period.

This resulted in 1.03 $\pm$ 0.15% of weight loss. On the other hand, the MSW-BB1 enhanced the growth of 8.60 $\pm$ 0.18 x 10^6 cfu/g of bacteria and a concomitant release of 6.80 $\pm$ 0.20 cm^3 of CO_2 per 100g of MSW. The substrate also became more acidic with a total weight loss of 0.97 $\pm$ 0.12% as against 0.33 $\pm$ 0.14% recorded for the control (un-enhanced waste treatment).

The general reduction in mass of the MSW samples degraded is illustrated in Fig. 1. As depicted, MSW degradation by MSW-BB2 exhibited remarkable capability to reduce (increase weight loss) the weight of MSW over time. Table 3 reports the summary descriptive of the relationships between the bacterial density and indices of degradation during enhanced biodegradation by the selected microbial consortia (MSW-BB1 and MSW-BB2) and indigenous microorganisms in MSW (unenhanced control). The result revealed a normal decomposition process in MSW treated with MSW-BB1. However, correlations between the indices of degradation (Table 4) revealed a significant negative relationship between CO_2 released and pH (r = -0.97) and between weight loss and pH (r = -0.92), while the relation between CO_2 evolution and weight loss was positively significant (r = 0.93). The simple regression analysis of the relations between bacterial density and CO_2 evolution (Table 5) shows a fitting linear model. The equation of the fitted model derived is: Bacterial density = 2.61941 + 0.798714* CO_2. This reveals a statistically significant (r = 0.88) positive relationship at 95% confidence level with a mean absolute error (MAE) of 0.36948. The predicted significant correlations based on the order in which they occur (as determined by the Durbin Watson (DW) statistic test) are illustrated in Fig. 2. Using a fitted linear model: bacterial density = - 2.61941 + 0.798714* CO_2 evolution, analysis of the relations between bacterial density and CO_2 evolution in the MSW-BB1 enhanced degradation (Table 5) revealed a significant (r = 0.88) relationship with a MAE of 0.32 (Fig. 2a). For relations between bacterial density and pH of treated MSW, the equation of the fitted model is: Bacterial density = 121.095 – 16.7607*pH, with r = -0.85. This indicates a negative but moderately strong relationship (Fig. 2b). On the other hand, the equation for the fitted model for relation between bacterial density and weight is: Bacterial density = 5.28575 + 3.38906* weight loss. The analysis revealed a relatively strong (r = 0.99) and positive relationship at MAE of 0.108739. The predicted order of variation is presented in Fig. 2c.

Table 2: Changes in the levels of degradation indices during enhanced biodegradation of MSW by MSW-BBI, MSW-BB2 and unenhanced biodegradation (control)

				MSW-BB1 Consortium				
Index	0	14	28	42	56	70	84	98
Bacterial Density (10^6cfu/g)	5.30 ± 0.10	6.10 ± 0.3	6.70 ± 0.30	6.50 ± 0.10	6.90 ± 0.08	6.80 ± 0.08	8.40 ± 0.02	8.60 ± 0.18
CO_2 evolution (cm^3/100g)	3.80 ± 0.20	4.10 ± 0.41	4.90± 0.10	4.60 ± 0.18	5.40 ± 0.10	6.60 ± 0.20	6.80 ± 0.15	6.80 ± 0.20
pH	6.88 ± 0.01	6.87 ± 0.02	6.86 ± 0.04	6.84 ± 0.10	6.79 ± 0.20	6.76 ± 0.16	6.76 ± 0.08	6.74 ± 0.12
				MSW-BB2 Consortium				
Index	0	14	28	42	56	70	84	98
Bacterial Density (10^7cfu/g)	0.53 ± 0.20	0.68 ± 0.16	3.70 ± 0.06	5.50 ± 0.04	5.90 ± 0.18	9.80 ± 0.16	8.89 ± 0.22	8.67 ± 0.20
CO_2 evolution (cm^3/100g)	3.80 ± 0.21	4.88 ± 0.20	9.50 ± 0.10	7.66 ± 0.15	7.94 ± 0.10	8.60 ± 0.10	10.80 ± 0.18	12.80 ± 0.25
pH	6.88 ± 0.01	6.87 ± 0.02	6.78 ± 0.20	6.74 ± 0.15	6.66 ± 0.20	6.67 ± 0.70	6.59 ± 0.05	6.11 ± 0.43
Weight loss (%)	0	0.28 ± 0.11	0.45 ± 0.05	0.48 ± 0.22	0.58 ± 0.18	0.66 ± 0.11	0.98 ± 0.15	1.03 ± 0.15
				Unenhanced (control)				
Index	0	14	28	42	56	70	84	98
Bacterial Density (10^5cfu/g)	6.93 ± 0.10	7.18 ± 0.11	7.7 ± 0.17	5.7 ± 0.47	6.9 ± 0.11	9.8 ± 0.12	9.8 ± 0.12	9.9 ± 0.11
CO_2 evolution (cm^3/100g)	3.80 ± 0.11	3.88 ± 0.10	3.50 ± 0.10	4.11 ± 0.13	4.54 ± 0.11	4.66 ± 0.21	4.80 ± 0.18	5.11 ± 0.15
pH	6.88 ± 0.01	6.87 ± 0.02	6.78 ± 0.20	6.74 ± 0.15	6.66 ± 0.20	6.67 ± 0.70	6.59 ± 0.05	6.11 ± 0.43
Weight loss (%)	0	0.11 ± 0.11	0.15 ± 0.15	0.18 ± 0.12	0.21 ± 0.11	0.24 ± 0.11	0.28 ± 0.13	0.33 ± 0.14

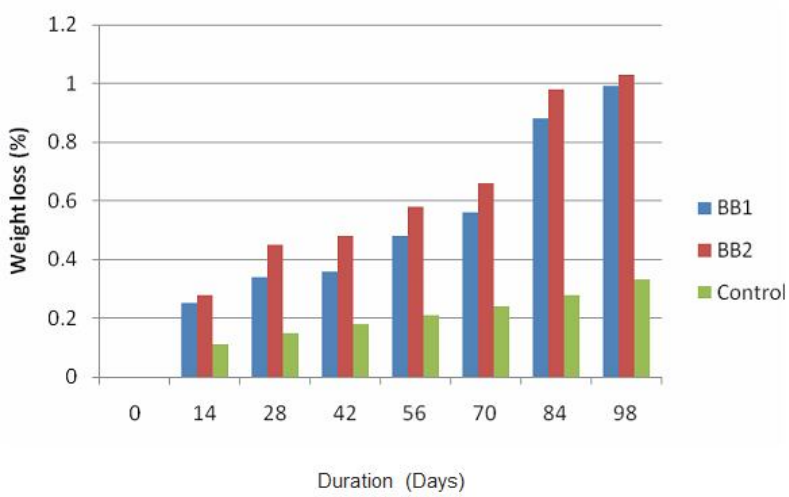

Fig.1: Weight loss in MSW during enhanced biodegradation by MSW-BB1 and MSW-BB2 consortia

Table 3: Summary descriptive of the indices of degradation during enhanced biodegradation of MSW by MSW-BBI, MSW-BB2 and unenhanced biodegradation (control).

		MSW-BB1 consortium		
		CO_2 evolution	pH	Weight loss
Ns		8	8	8
Average		5.38	6.81	0.48
SD		1.22	0.06	0.32
CV%		22.78	0.83	66.99
Minimum		3.80	6.74	0.0
Maximum		6.80	6.88	0.97
Range		3.00	0.14	0.97
Standard skewness		0.13	-0.07	0.36
Standard kurtosis		-1.09	-1.24	-0.24
		MSW-BB2 consortium		
		CO_2 evolution	pH	Weight loss
Ns		8	8	8
Average		8.25	6.66	0.55
SD		2.94	0.24	0.34
CV%		35.64	3.68	61.32
Minimum		3.80	6.11	0.0
Maximum		12.80	6.88	1.03
Range		9.00	0.77	1.03
Standard skewness		-0.12	-2.20	-0.08
Standard kurtosis		-0.19	2.51	-0.15
		Un-enhanced (control)		
	Bacterial density	CO_2 evolution	pH	Weight loss
Ns	8	8	8	8
Average	7.99	4.30	6.66	0.19
SD	1.62	0.56	0.24	0.10
CV%	20.35	13.02	3.68	55.11
Minimum	5.70	3.50	6.11	0.0
Maximum	9.90	5.11	6.88	0.33
Range	4.20	1.61	0.77	0.33
Standard skewness	0.19	-0.001	-2.20	-0.65
Standard kurtosis	-0.97	-0.78	2.51	0.24

ns = number of samples

Table 4: Correlations between degradation indices during enhanced biodegradation of MSW by MSW-BBI, MSW-BB2 and unenhanced biodegradation (control).

	MSW-BB1 consortium		
	CO_2 evolution	pH	Weight loss
CO_2 evolution	1	r = -0.9676 ns =8, P =0.0001	r = 0.9343 ns = 8, P = 0.0007
pH	r = -0.9676 ns = 8, P = 0.0001	1	r = -0.9185 ns = 8, P = 0.0013
Weight loss	r = 0.9343 ns=8, P =0.0007	r = -0.9185 ns =8, P =0.0013	1
	MSW-BB2 consortium		
	CO_2 evolution	pH	Weight loss
CO_2 evolution	1	r = -0.8447 ns =8, P =0.0083	r = 0.9358 ns = 8, P = 0.0006
pH	r = -0.8447 ns = 8, P = 0.0083	1	r = -0.8325 ns = 8, P = 0.0103
Weight loss	r = 0.9358 ns=8, P =0.0006	r = -0.8325 ns =8, P =0.0103	1
	Un-enhanced (control)		
	CO_2 evolution	pH	Weight loss
CO_2 evolution	1	r = -0.8180 ns =8, P =0.0131	r = 0.8484 ns = 8, P = 0.0078
pH	r = -0.8180 ns = 8, P = 0.0131	1	r = -0.8286 ns = 8, P = 0.0110
Weight loss	r = 0.8484 ns=8, P =0.0078	r = -0.8286 ns =8, P =0.0110	1

r = correlation coefficient, P = P-value, ns = number of samples

Table 5: Simple regression analytical notes on the relations between the microbial density and degradation indices

	MSW-BB1consortium		
Variables	Bacterial density & CO_2 evolution	Bacterial density & pH	Bacterial density & weight loss
Correlation coefficient (r)	0.88	0.85	0.99
R-squared %	78.32	72.78	97.25
R- squared (adjusted for d.f.) %	74.70	68.25	96.79
Standard error (SE)	0.55	0.62	0.19
Mean absolute error (MAE)	0.37	0.465801	0.108739
Durbin-Watson statistic	1.95 (P = 0.2822)	1.72 (P = 0.1689)	0.24 (P = 0.5897)
Lag residual autocorrelation	0.09	0.04	0.22
	MSW-BB2 consortium		
Variables	Fungal density & CO_2 evolution	Fungal density & pH	Fungal density & weight loss
Correlation coefficient (r)	0.81	0.69	0.89
R-squared %	66.36	47.18	78.95
R- squared (adjusted for d.f.) %	60.76	38.38	75.44
Standard error (SE)	2.26	2.84	1.79
Mean absolute error (MAE)	1.56	2.09	1.17
Durbin-Watson statistic	1.36 (P = 0.0739)	1.06 (P = 0.0259)	1.89 (P = 0.2580)
Lag residual autocorrelation	0.29	0.33	0.01
	Un-enhanced (control)		
Variables	Fungal density & CO_2 evolution	Fungal density & pH	Fungal density & weight loss
Correlation coefficient (r)	0.69	0.64	0.68
R-squared %	47.22	40.73	46.25
R- squared (adjusted for d.f.) %	38.43	30.85	37.29
Standard error (SE)	1.28	1.35	1.29
Mean absolute error (MAE)	0.88	0.91	0.88
Durbin-Watson statistic	2.00 (P = 0.3218)	1.64 (P = 0.1712)	1.41 (P = 0.0750)
Lag residual autocorrelation	0.01	0.17	0.24

For MSW treated with MSW-BB2 consortium (Table 3), of particular interest are the levels of the standardized skewness and standardized kurtosis, which can be used to determine whether the sample comes from a normal or unperturbed process. The values recorded for pH showed a departure from normality (outside the range of – 2 to +2). Negative relationships were established between pH and CO_2 evolution (r = - 0.84) and between pH and weight loss (r = - 0.83) although the relation between CO_2 evolution and weight loss was significantly (r = 0.94) positive (Table 4). Using a fitted linear model: bacterial density = - 2.79755 + 1.00107* CO_2 evolution, analysis of the relations between bacterial density and CO_2 evolution in the MSW-BB2 enhanced degradation (Table 5) revealed a significant (r = 0.81) relationship with a MAE of 1.56071. The predicted order of correlations is presented in Fig. 3a. Just like in MSW treated with MSW-BB1, the correlation between bacterial densities in the MSW-BB2 treated waste and pH (Table 5) using a fitted linear model: bacterial density = 72.8925 – 10.1214*pH revealed a moderately strong (r= -0.69) negative relationship and a wide predictive order of variation at MAE of 2.8905 as depicted in Fig. 3b. For weight loss, the application of the fitted linear model: bacterial density = 0.224338 + 9.38908* weight loss revealed a statistically significant (r = 0.89) positive relationship with bacterial density.

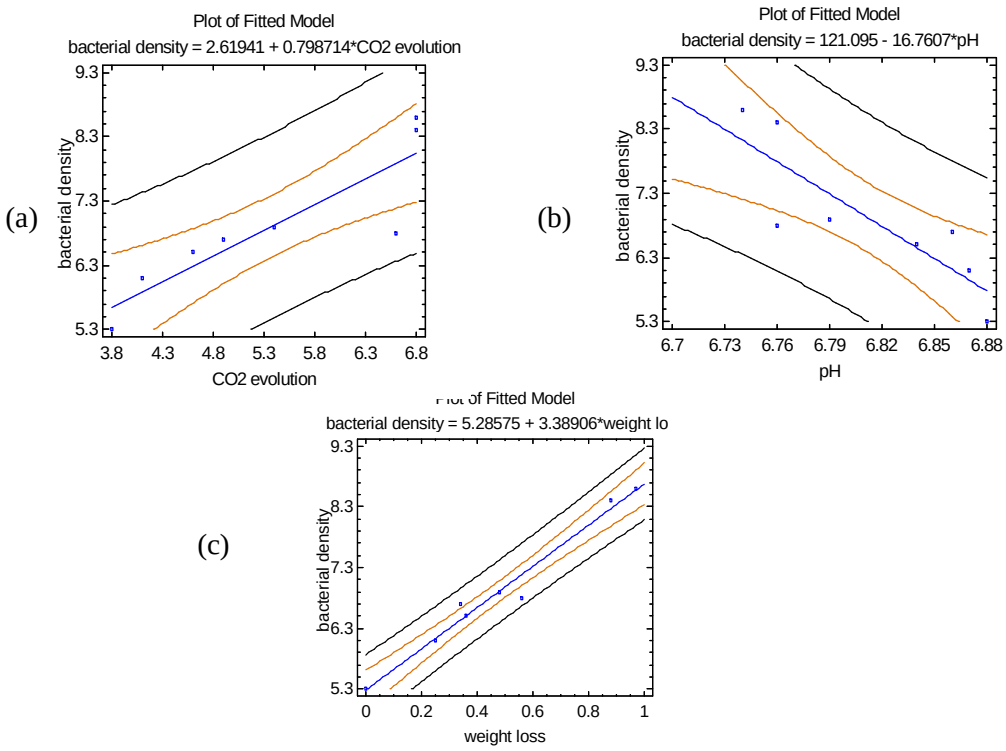

Fig. 2. Variations between degradation indices and microbial (MSW-BB1) growth rate during (a) CO_2 evolution and bacterial density (b) pH and bacterial density (c) Weight loss and bacterial density

The MAE established was 1.17086 and the predicted order of correlations presented in Fig. 3c. In the unenhanced treatment, the MSW decomposition by the indigenous microbial load was also perturbed by increase in substrate acidity (Table 3). Positive significant relationship was established between CO_2 and weight loss (r = 0.85) while the two variables correlated negatively with increase in waste acidity (Table 4). Predictive models have shown wide predictive variations in order of correlations between bacterial densities and CO_2 evolution (Fig. 4a), pH of substrate (Fig. 4b) and weight loss (Fig. 4c) with MAE of 0.884688, 0.906293 and 0.884492 respectively (Table 5).

The models show that changes in bacterial density are related positively to changes in CO_2 and weight loss in MSW but negatively with increase in acidity (pH). The relationships may not necessarily be of mutual dependence since the production of CO_2, changes in pH and weight loss of MSW may be affected by extraneous environmental factors not measured (e.g. temperature). The trends in the degradation of MSW and evolution of CO_2 fitted the models. The negative significant relationship between bacterial density and pH reveals negative skewness, which resulted in significant utilization of MSW by microorganisms. The significant values of r determine the variability of values of the regressands, CO_2, pH and weight loss. The higher the absolute value of r the greater the quantity of MSW used up by the bacterial community in MSW. This enhances the line of best-fit regression line, since r is proportional to regression coefficient.

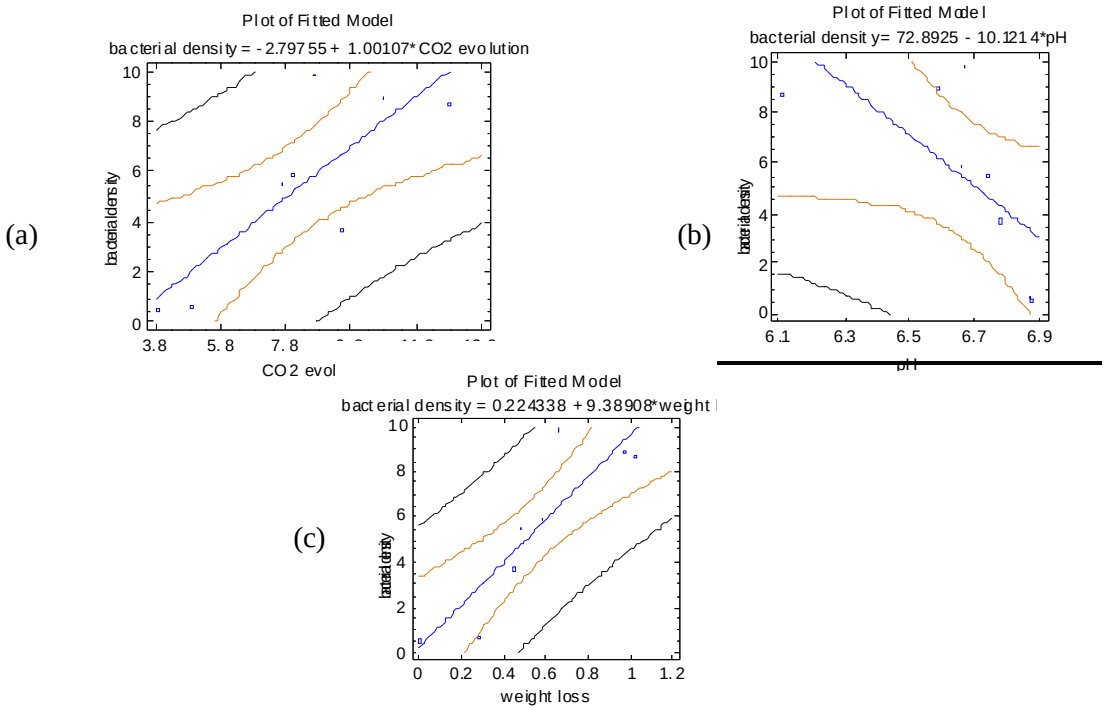

Fig. 3. Variations between degradation indices and microbial (MSW-BB2) growth rate during (a) CO_2 evolution and bacterial density (b) pH and bacterial density (c) Weight loss and bacterial density

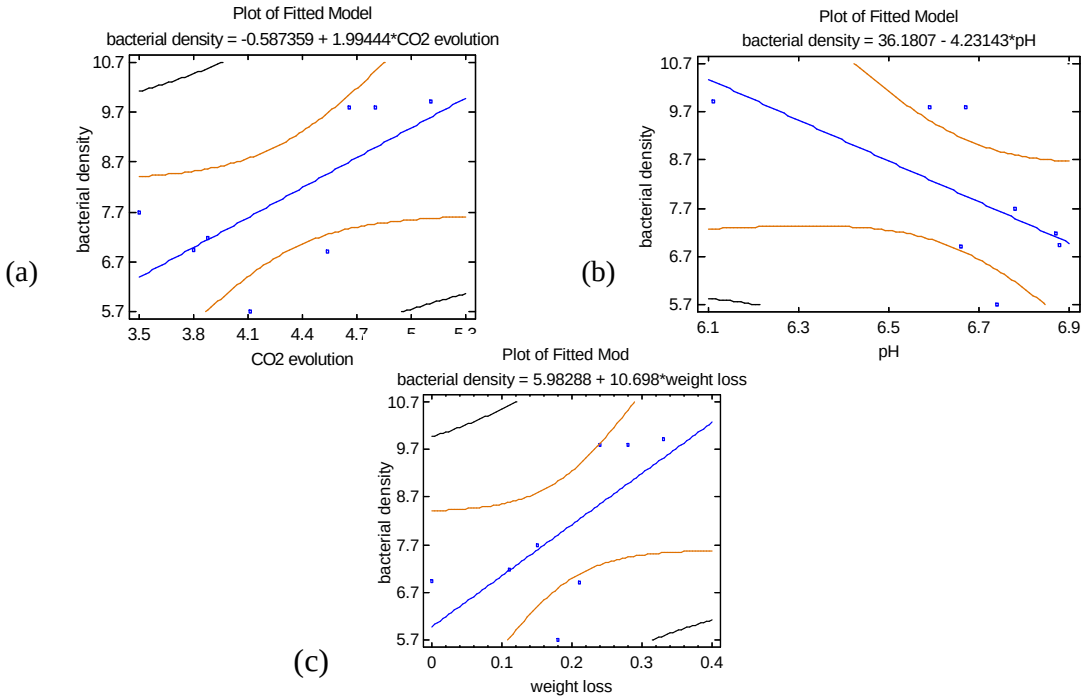

Fig. 4. Variations between degradation indices and microbial (Un-enhanced) growth rate during (a) CO_2 evolution and bacterial density (b) pH and bacterial density (c) Weight loss and bacterial density

The co-metabolism of complex organic wastes has been described for bacteria as well as for fungi (Cerniglia, 1981). It requires the elaboration of enzymes necessary for the breakdown of the recalcitrant components of organic wastes. The induction of this enzyme-system often

results in the catabolism of the complex wastes, with subsequent bond cleavage and degradation of cleavage to CO_2 (mineralization). This mechanism has previously been reported (Cerniglia, 1984; Bauer and Capone, 1988; Shiaris, 1989). In this study, the evolution rate of CO_2 from the control increased approximately more than one fold over the first 56 weeks following MSW mineralization, thereafter achieving steady increase. MSW enhanced degradation by MSW-BB1 and MSW-BB2 gave similar evolution rates until the 28th week, when there was a large increase in CO_2 evolution rate for the fungal consortium (MSW-BB2). The elevated values recorded for MSW-BB2 correlate with the enhanced-degradation potential of the consortium for the municipal solid wastes. Therefore MSW degradability was faster with MSW-BB2 consortium enhancement and the data shows that enhanced degradation by MSW-BB2 produced more CO_2 than the bacterial, MSW-BB1 consortium. Apart from its high ability to enhance biodegradation, the higher CO_2 evolution recorded for MSW-BB2 was consistent and may also be attributed to differences in cell mass, which essentially are not the same for bacterial and fungal cultures (Isinguzo and Odu, 1987). It is usually greater for fungi cell numbers not withstanding. The liberation of CO_2 from the un-enhanced treatment (control) was expected, and may be as a result of degradation by the indigenous microbes of MSW.

The relative ability of microorganisms to degrade organic wastes could be the main rate-limiting factor in the disposal of wastes in our urban cities. For example, the resistance of municipal solid wastes to microbial decomposition has been attributed mainly to the high content of recalcitrant organic compounds such as lignocelluloses complex (Tchobanoglous *et al*., 1993) and toxicants such as pesticides, paints etc. This research has revealed a not too impressive loss in weight in treated MSW. The minimal weight loss may be ascribed to the presence of certain catabolic compounds or nutrients, and harmful toxicants, which may suppress the degradation process. Fungi are known organic wastes decomposers and are generally capable of hydrolyzing cellulose as a major source of energy (Mandels and Weber, 1996). Plant wastes constitute a significant portion of municipal solid wastes (Tchobanoglous *et al*., 1993, Hulme and Shields, 1975). Their ability to elaborate thermo-stable enzymes is vital for MSW decomposition because of the thermogenesis of the process (Essien and Eduok, 2001; Tchobanoglous *et al.*, 1993, Moreira *et al*., 1981, Kane and Mullin, 1975). However, the temperature level of the decomposing MSW was not measured during the enhanced degradation process. This is because the experimental technique adopted and the amount of MSW used did not allow for temperature build up during degradation.

The pH of the MSW was affected by the biodegradation process. The utilization of MSW by the microbial consortia resulted in their growth and concomitant production of acid metabolic products. The acidic metabolites are responsible for the decrease in pH (increased acidity) of the MSW during enhanced biodegradation studies. The effect increases with time and varied between the two consortia investigated. The acidic level of MSW was more remarkably increased by the activities of the fungal (MSW-BB2) consortium. The constituent organisms enhance microbial growth and increase in acidity of the medium. The later was raised from pH 6.88 ± 0.01 to 5.9 ± 0.43 within 98 weeks of enhanced biodegradation. Increased in acidity of the decomposing MSW was expected and could be ascribed to differences in pH status of the organic components of the municipal solid wastes. During degradation, the organic acids that may have evolved from the process would depress the pH from the original levels (Sharma *et al*., 1997 and Jahnel *et al*., 1999). The pH values obtained in this study are in agreement with the levels reported by Baffa *et al*. (1996) and Deportes *et al*. (1998). The increase in acidity of degrading substrate would also favour the activities of fungi resulting in the release of simpler nutrients that enhances bacterial proliferation noticed in early states of enhanced degradation by MSW-BB2. However, only acid tolerant bacterial species can retain their competitive saprophytic potential in acid medium. This may be the reason for the decrease in bacterial activity as degradation progresses. Therefore, increase in substrate acidity would certainly influence the amount of nutrients generated from the process.

CONCLUSION

It is obvious that the problem of municipal solid waste disposal can be biologically tackled through the utilization of microorganisms with strong degrading capability. However, in order to achieve qualitative biodegradation of municipal solid wastes, it is important to know the chemical processes that occur during MSW decomposition as well as the enzymatic capability of the degraders. Enhanced biodegradation of MSW using indigenous microbes with strong MSW degrading capability promotes not only the proliferation of heterotrophs but also the effective reduction in waste weight which translates to waste stabilization (Reinhart and Townsend, 1998). It also creates an acidic condition that would enhance the migration of metals in soil and or formation of insoluble complexes that may not hamper biological activities in dumpsites and the contaminated environment. The differences in their biodegradability may be ascribed to variation in the physiological and nutritional requirements of microbial species, which is a genetically determined property (Smith and Berry, 1978; Essien *et al*., 2005). The effective utilization of MSW by the fungal consortium MSW-BB2 comprising of *Aspergillus niger* and *Saccharomyces* sp is promising and a pointer to the success of the approach although increase in acidity will be a major hindrance.

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