International Journal of Waste Resources
ISSN: 2252-5211

Kabbashi N, et al., Int J Waste Resources 2014, 4:4
DOI: 10.4172/2252-5211.1000169

Research Article Open Access

Kinetic Study for Compost Production by Isolated Fungal Strains

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Received date: October 25, 2014, **Accepted date:** December 19, 2014, **Published date:** December 22, 2014

Abstract

Organic wastes, food wastes and trimming yard (FW and YT) were composted using selected fungal strains (*Phanerochaete chrysosporium* (PC), *Lentinus tigrinus* (LT), *Aspergilus niger* (ASP) and *Penicillium Spp* (PEN)) in a solid state bioconversion process. Results obtained at $P \leq 0.05$ after ten harvests indicated the minimum value of germination index (GI) in the open system was 43 ± 105% while in the closed system it was 46 ± 132% respectively. The simplest zero and first order kinetic models described the microbial mineralization of carbon to nitrogen (C/N) relatively (R^2 range of 0.87-0.99), but the second order model explained the observed kinetics of the solid state bioconversion (SSB) better with R^2 range of 0.87–0.98 and a positive decay coefficient (k). The decay coefficient which indicates if all the components of the biomass decomposed at the same rate increases from -0.0584 to 2×10^{-4} for *Phanerochaete chrysosporium* stream & -0.0578 to 2×10^{-4} for *Lentinus tigrinus* stream in the open system across the zero, first and second order.

Keywords: Composting; Organic wastes; Fungal strains; Germination index (GI); Reaction rate

Introduction

Asia produces the largest amount of Urban Food Waste (UFW), which is expected to increase from 251 to 418 million tonnes (45% to 53% of total world UFW) from 1995 to 2025. Currently, the 17000 tonnes of waste generated per day in Kuala Lumpur [1] comprises of 57% food wastes, 17% mixed papers, 4.7% yard trimmings and others constitute the municipal solid wastes (MSW) generated and disposed [2,3].

Unlike submerged fermentation, growth generally occurs on the surface of water-insoluble substrates in the absence of free water or at reduced water levels [4]. Traditionally SSB, has been applied to composting of agricultural wastes for mushroom cultivation and production of organic acids. SSB has also been found to be an efficient process for enzyme production [5]. Although often used for soil studies, community level physiological profiles (CLPPs) have been rarely applied to compost, probably for the lack of standardized methodology. Recently, however, CLPPs have been proposed as a tool to assess the degree of maturity of compost. One of the major problems is that the rate of colour development is a non-linear process related to both time and inoculum density. The aim of author’s work was to investigate the suitability of data interpretation based on the kinetics of colour formation [6]. In another research carried by [7] for the degradation process was monitored, along with temperature, pH, total organic carbon, for the production of volatile fatty acids (VFAs) during the composting process of compost heaps in two different bioreactors (open and closed) at three different depth. Significant correlations were found between individual VFAs, as well as between VFA concentrations and organic carbon contents. Oxidizable carbon and mono- and oligosaccharides. Compost from vegetable residues is usually used as an organic amendment to soil; however, their thermal degradation characteristics show that it could be used as raw material in air gasification facilities. According to the obtained data by [8], hydrogen production is positively affected by composting, increasing hydrogen concentration. Using nth-order kinetic equations to describe component degradations, they have calculated a set of kinetic parameters which do not differ of the reported for other lignocellulosic materials.

In order to facilitate the operation and control of the composting process, however, there is a need for simple kinetic order of reaction that can accurately describe the dynamics of system. The rate of microbiological reaction comes from studies of the kinetics of microbial reduction of evaluation parameters such as C/N ratio, total organic matters (TOM) and others [9]. This paper presents results of an attempt undertaken to identify and understand the cycling of C/N ratio, GI and degree of degradation in the composting systems, there is need to describe the decomposition kinetics and the biodegradability of the substrate involved.

However, Malaysia waste treatment data revealed that 50% of these wastes are openly dumped, 30% land filled, 5% incinerated and only 10% composted [10]. This open dump of organically rich wastes could contributes significantly to the formation of leachate quality and quantity aside from the spread of disease vectors, odor, aesthetics and other environmental damages [11]. Leachates constitute major threat to underground water and the eco-system due to the presence of heavy metals. This organic content is beneficial for composting projects and not favorable for combustion or thermal technology as presently practiced [10]. Meanwhile, 89% of the entire waste generated are disposed while only 1% are converted to compost despite the suitability of the country climate for commercial compost production [12]. Therefore, composting of food waste and the institution yard waste with a bulking agent will solve the waste problem.

Materials and Methods

Experimental materials

The study is in two low technology adopted designs (open and close systems) in solid state bioconversion process experiment as indicated in Figure 1. The solid waste generated was sorted to remove the non-food components of the waste which includes plastics, papers and other non-organic components of the waste stream, while the yard trimming was collected separately as yard/lawn trimmings. The physicochemical properties of the comingled waste extract were analyzed and compared to the extract of major institutional wastes components food waste (FW), yard trimmings (YT) and soiled papers). All the substrates (food wastes, yard trimmings and sawdust) were characterized individually likewise the mixture, to determine the total organic carbon (TOC) content, total Kjeldahl nitrogen (TKN) contents, pH, moisture contents, ash content, hemicellulose, cellulose and lignin content among other parameters.

The fresh food waste collected was weighed and then dried in oven (MEMMERT GmbH Co. KG Germany) at a temperature of 105C. The dried substrates were then milled, grinded and sieved into smaller and uniform sizes of 1 to 2mm respectively to ensure homogeneity and faster degradation [13,14]. Yard trimming and flowers grown on the lawn across the land were regularly trimmed. These were collected and dried in the oven at 105C to remove the moisture content and ensure preservation. About 6-7 kilogrammes of fresh trimmings were raked on a 40m^2 area of the lawn. The dried samples were grinded using a Philip Twist home appliance (Model HR 1701, China), after which the electronic sieve (Model: AS 300, manufactured by Retsch GmbH Germany) was used to obtain 1-2mm sizes respectively as indicated for food wastes. The YT properties are determined while, the moisture content of the fresh trimmings before pretreatment was determined to be 69.88%. The sawdust (SD) used in the entire span of the experiment was collected from Forest Research Institute of Malaysia (FRIM) which are from chemically untreated log of woods (without preservative chemicals). The SD was dried as described above and sieve like the dried food and trimmings to obtain both 1mm and 2mm particle sizes.

These sources separated wastes were pretreated as substrates (food waste and yard trimmings) and composted with sawdust (SD) as the bulking agent after due characterization. Different ligninolytic fungi, *Phanerochaete chrysosporium, Lentinus tigrinus, Aspergilus niger* and *Penicillium spp.* were added at varying time interval to evaluate their effect using degradative indicators such as C/N, degradation degree (DD), and germination index (GI) coupled with the kinetics of the system.

Preparation of substrate mixture

All the substrates (food wastes, yard trimmings and sawdust) were characterized individually likewise the mixture, to determine the total organic carbon (TOC) content, total Kjeldahl nitrogen (TKN) contents, pH, moisture contents, ash content, hemicellulose, cellulose and lignin content among other parameters. The two main substrates (FW & YT) were mixed along with SD for the SSB process. The ratio of 1:1:0.5 (W/W) was used to mix FW (69.10% moisture, 48.85% TOC and 3.18% TKN) YT (69.88% moisture, 50.82% TOC and 1.46% TKN) and SD (19.32% moisture, 53.39% TOC and 0.27% TKN). The substrate to water ratio of 30:70 was used to maintain the optimum moisture content that is peculiar to SSB within the range of 50 – 60% [15-18]. Nutrients (gkg^{-1}) of K2HPO4 (0.3), NaCl (0.3) and $MgSO_4.7H_2O$ (0.3) were added to substrate as starter dose for activation of inoculated (*P. chrysosporium*) fungi Sawdust was added as a cheap source of carbon. In every container 400 grammes of substrates mixtures were used for the experiment. All substrates were autoclaved at 121C for 50 minutes after the addition of nutrients. Thereafter, the containers were inoculated with 6% fungal spores/mycelia out of the entire 70% water constituent (59% distilled water, 6% inoculum and 5% minerals) of each opened and closed system. The inoculum sizes used were 2.5 x 10^7 and 5.5 x 10^7 spores per ml for *P. chrysosporium* and *L. tigrinus* respectively; 84 x 10^6 and 92 x 10^6 CFU/g air dried inoculums for *A. niger* and *Penicillium spp.* respectively. Composting plastic containers bins were kept in the laboratory at room temperature with the open system uncovered and the holes created on the lids of the closed system were covered tightly with cotton wool as shown in Figure 2.

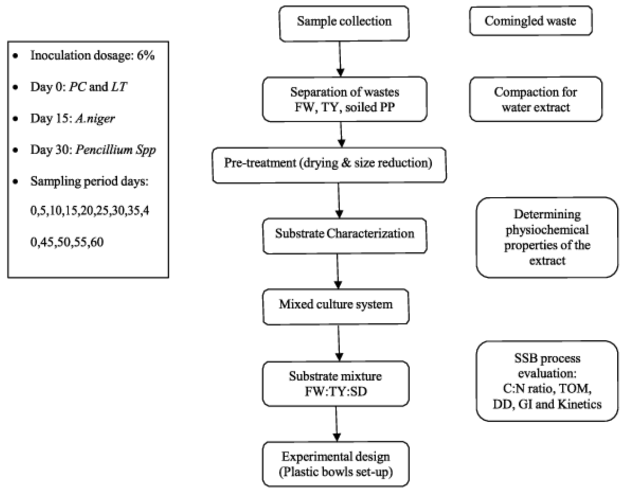

Figure 2: Closed (a) and Opened (b) Composting Systems.

The main characteristics of the mixture are thus : pH=5.68, Total Kjeldahl Nitrogen (TKN)=2.50%, Total Organic Carbon (TOC)=51.15%, Electrical Conductivity (EC)=5.62mS/dm (W.t), Salinity=5.62 0:00 (W.t) and Total Dissolved Solid (TDS)=2.77g/l. Likewise, the hemicellulose, cellulose, lignin and water soluble carbon content are 32.30%, 21.60%, 16.00% and 30.10% respectively.

Design of experiment

Two basic systems were deployed as Closed and Open Systems. Each of these systems comprises of the control (CR) and two treatment streams (as shown in Figure 2) to determine the optimal operating conditions, likewise, to compost the substrate mixture using

Phanerochaete chrysosporium (PC); *Lentinus tigrinus* (LT); *Aspergilus niger* (ASP) and *Penicillium spp* (PEN).

Results and Discussion

Isolation and identification of microorganism

Composting of the organic components of the institutional wastes (FW and YT) is a sustainable recycling process whereby organic matter are decomposed to shorter molecular chains, more stable, hygienic, humic rich and agriculturally useful product [19]. The selected fungi used in this study includes: *Phanerochaete chrysosporium* (PC); *Lentinus tigrinus* (LT); *Aspergilus niger* (ASP) and *Penicillium spp* (PEN). These selections were based on the physicochemical properties of the wastes or substrates involved. The weight is related to 69.1% and 69.88% moisture content of fresh FW and Yard Trimmings (YT) while sawdust has the lowest moisture content of 19.32%. Although all the substrates used for this experiment were dried at 105C for 24hrs to remove or reduce the moisture content. This is to ensure adequate preservation of the substrates throughout the experimentation process, thus most of the results are reported on dry matter basis (DMB). The initial pH of the substrate mixture is 5.68 a condition relatively good for aerobic composting using fungi, thus the pH of the system used was not adjusted. The TOC determined on a dry weight basis (DWB) was found to be 48.85% of FW (Table 1). Out of these, the water extractable carbon is 48.81% which justifies its total dissolved solids (TDS) concentration of 3.06 g/l. The Hemicellulose content of FW is 43.37% while the cellulose and lignin constitute 11.3% and 1.87% respectively (Table 1).

Substrates	FW	YT	SD	Mixture
pH	5.39	5.97	4.6	5.68
*TKN	3.18	1.46	0.27	2.5
*TOC	48.85	50.82	53.39	51.15
EC (mS/dm) w.t	3.04	4.13	0.32	5.62
Salinity(0/00)w.t	1.6	1.8	0.1	2.77
TDS (g/l) w.t	3.06	2.06	0.151	2.72
*Hemicellulose (%)	43.37	31.9	18.07	32.3
*Cellulose(%)	11.3	25.73	30.5	21.6
*Lignin(%)	1.87	21.53	45.93	16
**WSC (%)	43.46	20.84	5.5	30.1

Table 1: Characterization of substrates (**FW**=Food Waste; **YT**=Yard Trimmings; **SD**=Sawdust; *Dry weight basis; Wet basis (w.t); **Water Soluble Components).

Solid state bioconversion (SSB)

Since solid-state bioconversion (SSB) is considered as a hopeful novel, low- cost degradation of organic contaminants approach to control growth of microorganisms. The organic waste classification using simple waste separation technique shows that the organic component is 75% by weight and 18% by volume of the entire waste stream. This weight was related to 69.1% and 69.88% moisture content of fresh FW and Yard Trimmings (YT) while sawdust has the lowest moisture content of 19.32%. Since the systems are microbially induced, occurrence of rapid decomposition in the thin liquid films on the surface of the organic particles releases CO_2 and H_2O as end products [20]. Thus, lower moisture content (< 30%) inhibits microbial activities while the higher one (> 70%) results in slow decomposition, odor formation and nutrient leaching. The open system moisture contents are evenly distributed and fluctuate due to activity of the microbes as shown in Figure 3. In the closed system, beside the ambiguous drop (probably due to trans - evaporation and initial heat generated by the reactor) in the value of the control sample, waste reactor A closed (WRAc) and waste reactor B closed (WRBc) turned out to be relatively higher. This can be traced to the covering which disallowed the evaporation process to take place within the system. Meanwhile, the water holding capacity of *P. chrysosporium* and *A. niger* was higher in this system compared *to L. triganus* and *A. niger*. Subsequently after the inoculation of *Penicillium spp*. the moisture content of WRBc increased and decreased with a greater rate, thereafter stabilized at day 60. This could be related to anti pathogenic tendency of Penicillium spp. Statistically, the open and close system are significant (P<0.05), meanwhile, the system with *P. chrysosporium* (WRAc) and *L. triganus* (WRBc) indicates a Least Square Difference (LSD) values of 0.001 with respect to control (CRc) while *P. chrysosporium* (WRAo) *and L. triganus* (WRBo) are slightly significant with P=0.047. This implied that at 95% confidence it can be proved that the moisture content of the close system is significantly different. In every container 400 grams of substrates mixtures were used for the experiment.

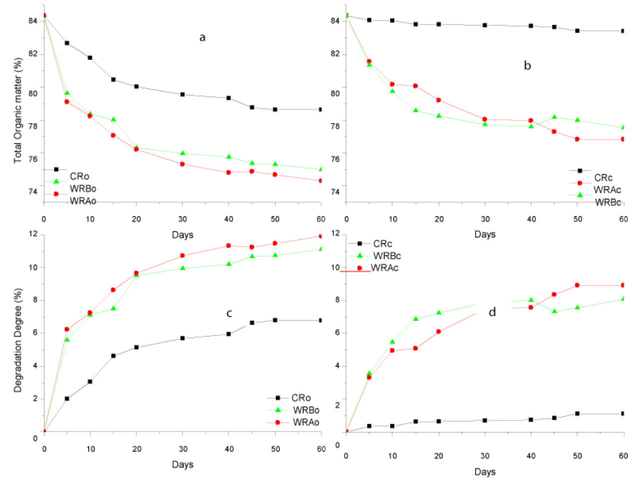

Figure 3: Degradation Degree (%) of (a) Open and (b) Close Systems.

Effect of Germination index (GI) on compost

GI of tomato seeds generally decreases gradually and significantly reaches the minimum at day 7 and 15 in the open and day 10 and 15 in the closed system respectively. This decrease might be due to the release of high concentration of ammonia and low molecular weight of organic acid [21]. The minimum value of GI in the open system was 53% and 55% while in the closed system it was 49 and 56% respectively. In all situations, the values of GI increased to about 90% above which correspond with the suggestion of Alberquerque (2006)

[22] that seed GI value above 50 is suitable for agriculture utilization while GI value above 80% indicates that the compost is mature [23,24]. The significant GI increase at day 15 through day 40 during the SSB process could be due to relief phytotoxins especially the ammonia volatilization, reduction of unstable organic acids and probably the anti-microbial strength of *Aspergilus* and *Penicillium spp.* At harvest the highest level of GI was 105 and 132 in open and closed system respectively. GI trend shows further increasing index even beyond the 60 day harvest period as shown in Table 2. This is a strong indication for the effectiveness of the anti-pathogenicity of *Penicilliium spp.* and the fitness of the product for use. Generally, the open system is significantly different at $P \leq 0.05$ level with value [$F(2, 27)=3.478$, $P=0.045$] with post–hoc Least Square Difference (LSD) values 0.047 and 0.023 for Waste Reactor Open with PC (WRAo) and Waste Reactor Open with LT (WRBo) respectively. While, the closed system is not significant ($P \geq 0.05$) with value [$F(2, 27) = 1.305$, $P=0.288$] and the post–hoc Least Square Difference (LSD) of 0.376 and 0.120 for Waste Reactor Closed with PC (WRAc) and Waste Reactor Closed with LT (WRBc) when compared with Close Control sample (CRc).

Test Parameters	Germany	Austria	USA	Produced Biofertilizer
C/N (%)	-	-	< 25	16.5-20
Salt ions (EC) mS/dm	2.5	2	2	2.72 ± 2.89
Germination Index (%)	> 90	80-90	< 80	105-132

Table 2: Characteristics of compost / biofertilizer produced with standards across countries.

Degree of degradation (DD) and ash content

The Degree of Degradation (DD) is inversely proportional to the TOC and the TOM (Figure 4a and 4b). *L. triganus* (WRBc) had a degradation degree of 3.02% on day 5 followed by *P. chrysosporium* (WRAo) with 2.80% while the control was 0.29% in the closed system. However in the open system degradation degree accelerated to be the highest for WRAo (5.29%) followed by WRBo (4.75%) and CRo (1.69%).

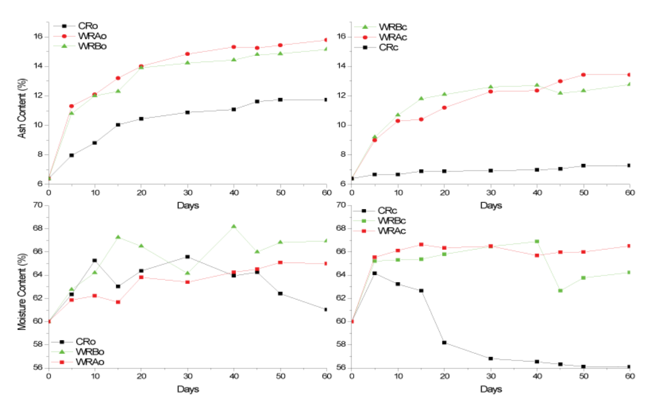

Figure 4: Ash Content (%) for (a) Close and (b) Open System

Generally the degradation degree of the open system were 10.12%, 9.44% and 5.76% for WRAo, WRBo and CRo respectively, while the closed system relative performance were 7.57%, 6.42% and 0.94% for WRAc, WRBc and CRc respectively. This can be related to high microbial activity, peak ammonia loss and / or reduced organic matter content during the composting process. The ash content of the waste mixture was 6% in day 0, it then increases from 6% to 11% and approximately 15% in the control and treated reactors (WRAo and WRBo) of the open system. In the closed system, ash content increases from 6% to 12% and 13% respectively for WRAc and WRBc, while CRc was almost null as shown in Figure 3. In the post-hoc test wherein WRAc and WRBc are significant to each other with equal value of P=0.001, while WRAo and WRBo are also significantly different with lower P values of 0.015 and0.017 respectively all at 95% confidence interval. This is a reflection of the diversity in microorganisms, a phenomenon that is common when two or more organic wastes are combined for composting. This trend indicates that inorganic ash content is released when the organic matter in the compost material was actively decomposed by microorganisms [25].

Kinetic study of compost

The rates of microbial metabolism are related to observed (or estimated) properties such as reactant concentration, microbial biomass and the thermodynamic favorability of the reaction. Since biochemical process is never the result of a single elementary reaction, rather a multistep process involving one or more enzymes. Fortunately, in many cases, it is possible to use elementary rate expressions to describe the dependence of an overall multistep reaction on the concentration of one or a few reactions that control the rate of the overall reaction. Thus, kinetic expressions for rates of microbial reaction are macroscopic descriptions of overall reactions which are derived by considering the carbon to nitrogen ratio concentration [C/N].

The rates of microbial reaction, often considered as macroscopic descriptions of overall reactions for the decomposition of organic matter was evaluated based on the reaction order that best fit the experimental degradation data. The zero and first order reactions have been widely fitted for biodegradation of many elemental compounds during composting [26-28]. Zero-order kinetics commonly describe homogeneous chemical reactions in which the concentration of a catalyst controls the rate of reaction as expressed in Equation (1) below and has been suitable in describing reaction rates in experiments where the period of observation is relatively low. The first-order kinetics indicates the dependency of the reaction rate to the concentration of the reactant in direct proportion as expressed in Equation (2).

The rate of microbiological reaction comes from studies of the kinetics of microbial reduction of evaluation parameters such as C/N ratio, TOM and others [9]. Thus, to understand the cycling of C/N ratio in the composting systems, there is need to describe the decomposition kinetics and the biodegradability of the substrate involved. The simplest zero and first order kinetic models described the microbial mineralization that is done through the decay coefficient (k) which indicates if all the components of the biomass decomposed as explained in the following equations

$$r_A = k[C/N]^n$$

$$r_A = \frac{d[C/N]}{dt} = k_0[C/N]^n$$

Since n =0, for zero order

$$\frac{d[OM]}{dt} = k_0$$

$$d[C/N] = k_0 dt$$

Integrating both sides, when

$$[C/N]_o at\ time(t) = 0, and [C/N]_t at\ time(t) = 0$$

$$\int_{[OM]_0}^{[OM]t} d[C/N] = \int_0^t k_0 dt$$

$$[C/N]\{(C/N_t) - (C/N_0)\} = k_0 t(t-0)$$

$$[C/N]_t - [C/N]_0 = k_0 t$$

$$[C/N]_t = k_0 t + [C/N]_0 \quad \text{..................(1)}$$

First-order Reaction Derivation

$$r_A = k[C/N]^n$$

That is

$$r_A = \frac{d[OM]}{dt} = k_1[C/N]^n$$

Since n=1 for first order

$$\frac{d[C/N]}{dt} = k_1[C/N]$$

Therefore,

$$\frac{d[C/N]}{[C/N]} = k_1 dt$$

Integrating both sides, when [C/N]o at time (t) =0, and [C/N]t at time (t) =0

$$\int_{[OM]_0}^{[OM]t} \frac{d[C/N]}{[C/N]} = \int_0^t k_1 dt$$

Thus,

$$In[C/N]\{(C/N_t) - (C/N_0)\} = k_0 t(t-0)$$

$$In[C/N]_t - [C/N]_0 = k_1 t$$

$$In[C/N]_t = k_1 t + In[C/N]_0 \quad \text{..................(2)}$$

The microbial effect can be determined based on the significant percentage decreased values of C/N ratios 5.37%, 5.23% and 7.60% in the WRAo stream coupled with 6.68%, 4.73% and 5.58% in WRBo stream on days 15, 40 and 60 respectively in the open system. Similarly, 2.32%, 1.74% and 4.54% of the WRAc stream and 5.01%, 5.32% and 3.05% of WRBc for days 15, 40 and 60 of the close system reflect that LT activity is higher in the two (close and open) streams or systems than PC. Moreover, the DD is higher in open system (11.92%) compared to closed system (8.93%) which indicates that the effect of other microbes in the SSB process is almost not significant. Similarly, the significance of the SSB reaction order indicates R^2 valuesof 0.984 and 0.981 for open systems while closed systems were 0.865 and 0.965 for WRA and WRB respectively. However, between the closed systems LT performance was relatively better than PC, which suggests why LT had the lowest OM, TOC and C/N values in the closed system at day 15. Consequently, the germination index of the open system was considerably low compared to those of the closed system. This could be as a result of the higher microbial activities in the open system compare to the close system where intrusions of microbes are restricted. The toxicity strength as expressed by the GI indicated that open system (43 ± 67%) produced a significantly toxic biomass compared to the close system (55 ± 80%) especially during the most active degradation period (day 15). Similarly, TOM is significant (P=0.001) in both open and close system while C/N ratio of the closed system is significant only between systems. Table 2 provides the summary of the biofertiizer properties compare with some countries standards.

The simplest zero and first order kinetic models described the microbial mineralization of C/N relatively (R^2 range of 0.87-0.99), but the second order model explained the observed kinetics of the SSB better with R^2 range of 0.87–0.98 and a positive decay coefficient. The decay coefficient (k) which indicates if all the components of the biomass decomposed at the same rate increases from -0.0584 to 2×10^{-4} for PC stream and -0.0578 to 2×10^{-4} for LT stream in the open system across the zero, first and second order. Likewise, the closed system follows the same trend with k values of -0.0232 to 6×10^{-5} for PC and -0.0448 to 1×10^{-4} for LT. The positive values of the second order justify its fitness for the degradation order (Table 3). Comparatively, LT stream (R^2=0.984) performed narrowly better than PC stream (R^2=0.9813) within the open system. The same trend was indicated in the closed system with R^2 values of 0.9646 and 0.8652 for LT and PC stream respectively.

Samples	Zero Order			First Order			Second Order		
	K_0	$[Conc]_0$	R^2	K_1	$[Conc]_0$	R^2	k_2	$[Conc]_0$	R^2
WCAo[C/N]	-0.058	20.39	0.99	0	20.44	0.99	2×10-4	20.49	0.98
WCBo[C/N]	-0.058	20.33	0.98	0	20.36	0.98	2×10-4	20.41	0.98
WCAc[C/N]	-0.058	20.3	0.87	0	20.36	0.87	6×10-5	20.33	0.87
WCBc[C/N]	-0.048	20.13	0.96	0	20.15	0.96	1×10-4	20.16	0.97

Table 3: Zero, first and second order kinetics.

Decay of the biomass decomposed on first & second-order kinetics

The decay coefficient (k) which indicates if all the components of the biomass decomposed at the same rate increases from -0.0584 to 2×10^{-4} for PC stream and -0.0578 to 2×10^{-4} for LT stream in the open system across the zero, first and second order. Likewise, the closed system follows the same trend with k values of -0.0232 to 6×10^{-5} for PC and -0.0448 to 1×10^{-4} for LT. The positive values of the second order justify its fitness for the degradation order (Table 3). Comparatively, LT stream (R^2=0.984) performed narrowly better than PC stream (R^2=0.9813) within the open system. The same trend was indicated in the closed system with R^2 values of 0.9646 and 0.8652 for LT and PC stream respectively. Generally between open and closed systems especially with respect to the R^2 values as expressed in the Table 3, open system performance is better but the closed system is better in terms of decay coefficient.

Conclusion

The feasibility and efficacy of SSB process for biodegradability of composted source separated FW and YT wastes were evaluated by examining twelve parameters at (ten harvests) 0, 5, 10, 15, 20, 30, 40, 45, 50 and 60 days after inoculation of the organic wastes. Considering basic composting evaluation indicators, open system performance is better than the closed systems. This is predicated in the significant percentage decrease in the C/N ratio (18.20% and 16.99% in PC and LT streams respectively) of the open system compared to the close system with 8.60% and 13.38% of PC and LT streams. Similarly, a reflection of the biomass mineralization as shown by DD results indicated that open systems (10.12% WRAo and 9.44% WRBo) performed better than the close systems (7.57% WRAc and 6.42% WRBc).

This submission was further proved by the positive correlation of the ash contents of open and close systems maintained by DD. Statistically the close systems are significantly different at $P \leq 0.01$ while the open systems 95% degree of confidence at $P \leq 0.05$ is also acceptable.

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