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International Journal
of Earth Sciences
and Engineering

www.cafetinnova.org

ISSN 0974-5904, Volume 09, No. 04

August 2016, P.P.1409-1420

Kinetics of Co-Composting of Organic Fraction of Municipal Solid Waste and Brewery Sludge

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Abstract: Enzyme kinetics approach using Michaelis-Menten equation is used to determine kinetic constants for evaluating the composting process.This study deals with the influence of levels of five factors namely A: the percentage of brewery sludge (20, 30), B: amendment type (cow dung, coconut pith), C: C/N ratio (15, 30), D: starting culture (without, with) and E: aeration rate (0.3 L/min/kg, 0.45 L/min/kg) at two levels and the interaction of factors, on the kinetic constants during the co-composting of organic fraction of municipal solid waste (OFMSW) and brewery sludge (BS). Taguchi's experimental design with an L_8 orthogonal array having 8 trials using an in-vessel batch-type composting reactor was used for conducting the experimental study. Temperature and organic matter content in the reactor were continuously monitored at regular intervals till the end of composting. The results exhibit comparable variations in the kinetic constants k_m and r_m under varying parameters during the entire composting process. S/N analysis is used for determining the relative importance of influencing factors on the reaction rate. Analysis of variance shows that the most significant factor influencing the kinetic constants is C/N ratio.

Keywords: *brewery sludge, co-composting, limiting velocity constant, Michaelis-Menten constant, OFMSW*

1. Introduction

Composting is the biological decomposition and stabilization of solid organic substrates, under conditions that allow development of thermophilic temperatures as a result of biologically produced heat, to produce a final product that is stable, low in moisture, free of pathogens & plant seeds and can be beneficially applied to land. Co-composting is the process of enhancing the composting by increasing the degradation rate and the quality of the compost, by modifications such as addition of biodegradable wastes (industrial and domestic waste, sludge etc.) to reach an optimum carbon-nitrogen (C/N) ratio. The rate of aerobic composting is influenced by many factors namely temperature, aeration rate, C/N ratio, pH, particle size, moisture content etc., that should be kept within an optimum range to achieve maximum efficiencies. The stabilization of an organic fraction depends on the composition of waste as well as some physicochemical factors. Temperature plays an important role for which it needs to be maintained within an optimum range. For evaluating the composting process system, determination of kinetic constants is required, since the rates of decomposition vary widely depending on the nature of compost substrate. In this study, the kinetic constants determined are Michaelis-Menten constant and limiting velocity constant.

2. Literature Review

2.1 Measuring Biodegradability

Substrate biodegradability is an important parameter that should be determined during design of any composting facility. A number of techniques are available in measuring or estimating substrate degradability. The equation determined by Chandler (Haug, 1980) to provide the best predictive model for substrate biodegradability is the same as the biodegradability coefficient, k_b.

$$B = 0.830 - (0.028)x \qquad (1)$$

Where B = biodegradable fraction of the volatile solids; x = lignin content, % of volatile solids (VS).

Mass balance approach is another method to determine the degradability of the mixed substrates. A limitation with this technique is that only the total biodegradability of all mixed materials are determined. In this method the two approaches used are total mass loss and conservation of ash. In the total mass loss approach the degradability coefficient for the mixture, k_b, can be defined as volatile solids lost from the process divided by the volatile solids input to the process. In the second approach the assumption made is that inerts entering the process should equal to inerts leaving the process in steady state. The ash content of the mixture and product are the same.

$$k_b = \frac{(VS_i\,\% - VS_f\,\%)\,100}{VS_i\,\%\,(100 - VS_f\,\%)} \qquad (2)$$

Where k_b is the biodegradability constant, VS_i and VS_f are the intial and final volatile solids content of the mixture respectively. Typical values of k_b for municipal refuse is 0.45 and for grass straws tend to range from 0.35 to 0.50 (Haug 1993). In the composting of sewage sludge using rotary compost reactor, a high value of 0.5887 was observed for C/N 30 (Ashish Kumar and Ajay, 2015).

2.2 Kinetic Analysis

Kinetics is the study of rates or velocities of reactions. From a fundamental engineering point of view, the kinetics of any reaction must be known in order to make a satisfactory design for a reaction system. Rates of decomposition vary widely depending on the nature of the organic substrate. Addition of food waste proves to be the best for enhancement in degradation of municipal solid waste (Sunil Kumar et. al. 2009). Microbe-substrate systems are generally divided into two distinct types, homogeneous and heterogeneous. In a homogeneous system, microbes are dispersed in an aqueous solution containing a soluble substrate. In the heterogeneous system, the substrate is insoluble and present in a particulate or solid form. Most composting substrates consist of solid organic matter with moisture limited to that bound with the substrate. Thus composting can be described as a heterogeneous system with solid substrate and limited moisture. The sequence of events involved in metabolizing solid substrate are hydrolysis of substrate molecules by hydrolytic enzymes, diffusion of solubilised substrate molecules to the cell, transport of oxygen through the voids between particles and diffusion through the liquid layers bound to the solid substrate, diffusion of oxygen in to the microbial cell and aerobic metabolism of the substrate and oxygen within the microbial cell (Haug, 1993).

2.3 Theoretical Considerations and Assumptions

Since composting is a biochemical process, enzyme kinetics approach can be adopted with some theoretical considerations. The organisms live on the base of substrate which is the source of reactive material as a nutritive medium and the moisture required for microbial growth is controlled by the solid organic substrate. It is assumed that each microbe in the reactor is uniformly dispersed in a solution of soluble organic matter to maintain homogeneity and under controlled aerobic conditions, the microbes are amended with organic matters and the moisture required for microbial growth is optimized with respect to readily available organic matter, and can be analyzed by Monod's kinetics to describe its process. As the substrate concentration is increased, the rate at first generally increases proportionally to the concentration and later reaches an asymptotic value. Such relationships between the rate of reaction and substrate concentration, which is the foundation of modern enzymatic kinetic theory, was first derived in 1902 by Victor Henri and later Michaelis and Menten established the enzyme-substrate complex model from their experimental data on hydrolysis of sucrose by yeast. Composting of organic ingredients or decomposition of organic matters are examples of an enzymatic related microbe systems (Agamuthu, 2000).

2.4 Michaelis-Menten Equation

Considering a hydrolytic enzyme that adsorbs to an active site on a solid substrate surface and in light of the mass law (the law of mass action can be valid in heterogeneous environments - Grima. R, 2006), enzyme concentration forms a complex with the substrate of enzyme substrate complex (EC). The decomposition of EC takes place in two ways as indicated in Eq.3

$$[E] + [C] \underset{k_2}{\overset{k_1}{\rightleftarrows}} [EC] \xrightarrow{k_3} [E] + [P] \tag{3}$$

Where E is the enzyme concentration (%); C is the limiting organic matter concentration (%); P is the by-product generated by endogenous reaction (%); k_1, k_2, k_3 are the specific reaction rates.

In equilibrium conditions,

$$k_1[E][C] = k_2[EC] + k_3[EC] \tag{4}$$

By solving, EC is given by Eq. (5)

$$[EC] = \frac{[E_T][C]}{[C] + \frac{(k_2+k_3)}{k_1}} \tag{5}$$

Where E_T is the total microbial concentration (%). On solving, we get

$$(r) = \frac{r_m[C]}{k_m + [C]} \tag{6}$$

Where $r_m = k_2\ [E_T]$, represented as maximum rate of enzymatic reaction (day^{-1}).

$$\frac{1}{r} = \frac{k_m}{r_m}\left(\frac{1}{C}\right) + \frac{1}{r_m} \tag{7}$$

This is the Michaelis–Menten equation in which k_m is the Michaelis–Menten constant (the dissociation constant) and r_m reaction rate constant (i.e., maximum or limiting velocity). The kinetic constants k_m and r_m can be graphically determined by a Lineweaver-Burke plot (Whang and Meenaghan, 1980) using Eq. 7 incorporating $1/r$ and $1/C$ data. It correlates the initial rate of reaction (consumption rate of substrate i.e., r) and the substrate concentration (C) as linear relationship. In the Lineweaver-Burke plot, the intercept on the y-axis gives the value of r_m whereas the value of k_m is obtained from the slope of the line. In the Michaelis–Menten equation, the value of k_m (i.e., dissociation constant) is the substrate concentration at which the reaction rate is at half-maximum and is inversely proportional to the chemical affinity of the enzyme for the utilization of organic matter. A small k_m indicates high affinity, meaning that the rate will approach r_m more quickly.

The value of k_m is dependent on both the enzyme and the substrate, as well as conditions such as temperature and pH. The value of r_m (i.e., maximum or limiting velocity) gives the extent of reaction rate which directly correlated with the operational parameters of the process such as temperature, moisture, aeration and chemical conditions. Higher the value of r_m, the faster is the rate of degradation of organic matter. In the composting of municipal solid waste, a value of 0.014 for maximum velocity constant and a value of 0.59 (gm of carbon/gm of ash) for Michaelis–Menten constant was reported by Sunil Kumar et.al. (2009).

2.5 Factors Affecting Microbial Reaction Rate during Composting

A number of factors can limit microbial reaction rate during composting. These include lack of degradable organics, very low or high process temperature, low moisture conditions, lack of free air space, low oxygen content, imbalanced pH conditions, lack of inorganic nutrients, lack of microbes (sterile substrate) and the presence of toxic substances.

3. Materials and Methods

Percentage of brewery sludge, amendment type, C/N ratio, starting culture presence and aeration rate are the five factors with interaction of two factors considered in the present study. Instead of using the conventional one -factor-at-a-time, statistically designed experiments - Taguchi's method is used. One-factor-at-a-time experiments are always less efficient than other methods based on a statistical approach and fails to consider any possible factor interactions.

Taguchi's method is a robust and multiparameter optimisation statistical technique which employs fewer numbers of experiments to identify and optimize parameters to achieve desired response. Taguchi design is a fractional factorial design using orthogonal array, allows the effects of many factors with two or more levels on a response to be studied in a relatively small number of runs.

In addition, the orthogonal array facilitates the analysis of the design. When used properly, Taguchi design may provide a powerful and efficient method to find an optimal combination of factor levels that may achieve optimum. Usually, with the aid of signal-to-noise (SN) ratio, the key factors that have significant effects on a response can be identified and the best factor levels for a given process can be determined from the pre-determined factor levels.

Taguchi method stresses the importance of studying the response variation using the S/N ratio, resulting in minimization of quality characteristic variation due to uncontrollable parameters. Here the kinetic constants k_m and r_m are the responses and SN analysis is done for them.

3.1 S/N Ratio Analysis

Taguchi method stresses the importance of studying the response variation using the signal–to-noise (S/N) ratio, resulting in minimization of quality characteristic variation due to uncontrollable parameters. After conducting the experiment for 8 trials, the responses are collected and they are analyzed by means of calculating the S/N ratio. Taguchi uses the S/N ratio analysis to measure the quality characteristic deviating from the desired value.

In S/N ratio, the term 'Signal' represents the desirable value (mean) for the output characteristic and the term 'Noise' represents the undesirable value for the output characteristic. In general, a better signal is obtained when the noise is smaller, so that a larger S/N ratio gives better final result. That means, the divergence of the final results become smaller. Depending upon the goal to be achieved for the responses, the goal options can be selected and the corresponding equations can be used for S/N analysis.

For the quality of processes or products when the responses are to be maximised use *larger is better* criteria; when it is to achieve a target value, use *nominal is best* option; on the other hand when the responses are to be minimised, use smaller is better criteria. The software Minitab 17 is equipped with facilities for doing the analysis for various quality criteria.

The quality options and S/N ratio formulae are presented in Table 1. Here in this work the responses kept are the kinetic constants k_m and r_m. k_m represents the chemical affinity of the enzyme for the utilization of organic matter. A small k_m indicates high affinity, meaning that the rate will approach r_m more quickly.

The value of r_m (i.e., maximum or limiting velocity) gives the extent of reaction rate which directly correlated with the operational parameters. Higher the value of r_m, the faster is the rate of degradation of organic matter. Therefore k_m is to be minimised, so smaller is better criterion; and r_m is to be maximised, so *larger is better* criterion is used.

3.2 Analysis of Variance

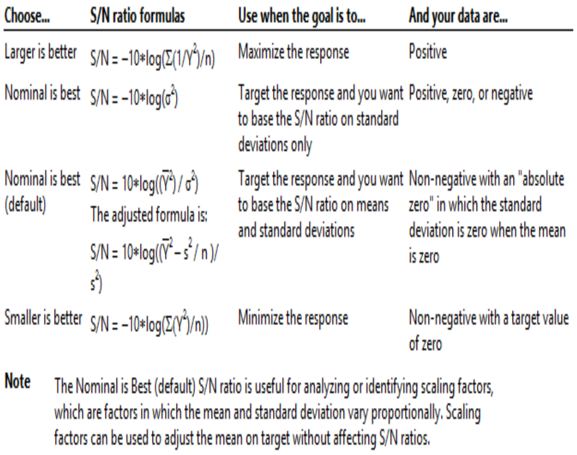
The relative contribution of the factors on responses is determined by comparing their variances by the process called analysis of variance (ANOVA). ANOVA is applied to Taguchi's statistical method to evaluate the relative significance of the individual factor and interaction effects on responses.

3.3 Fixing the Factors and Levels

Table 2 shows the factor notation, factor and factor levels.

The levels of brewery sludge and C/N ratio is fixed based on the micro composting study (Hema Nalini et. al., July 2015) and for the other factors based on the previous studies (Xueling Sun, 2006)

Table 1: *Quality options and S/N ratio equations*



Choose...	S/N ratio formulas	Use when the goal is to...	And your data are...
Larger is better	$S/N = -10*\log(\Sigma(1/Y^2)/n)$	Maximize the response	Positive
Nominal is best	$S/N = -10*\log(\sigma^2)$	Target the response and you want to base the S/N ratio on standard deviations only	Positive, zero, or negative
Nominal is best (default)	$S/N = 10*\log((\bar{Y}^2)/\sigma^2)$ The adjusted formula is: $S/N = 10*\log((\bar{Y}^2 - s^2/n)/s^2)$	Target the response and you want to base the S/N ratio on means and standard deviations	Non-negative with an "absolute zero" in which the standard deviation is zero when the mean is zero
Smaller is better	$S/N = -10*\log(\Sigma(Y^2)/n))$	Minimize the response	Non-negative with a target value of zero

Note The Nominal is Best (default) S/N ratio is useful for analyzing or identifying scaling factors, which are factors in which the mean and standard deviation vary proportionally. Scaling factors can be used to adjust the mean on target without affecting S/N ratios.

Where 'Y' is the response and 'n' is the number of tests in a trial. σ^2 = mean square deviation= $((Y_1-Y_0)^2+(Y_2-Y_0)^2+\ldots\ldots\ldots\ldots+(Y_N-Y_0)^2)/N$. Y_1, Y_2......Y_N are the responses and Y_0 is the target value.

Table 2: *Factor notation and levels*

Sl No	Factor Notation	Factor	Level 1	Level 2
1	A	Brewery sludge	20% of OFMSW	30% of OFMSW
2	B	Amendment	Cow manure	Coconut pith
3	C	C\N ratio	15	30
4	D	Starting culture	Without	With
5	E	Aeration rate	0.3 L\mt\Kg	0.45 L\mt\Kg

L_8 (2^7) orthogonal array as prescribed by Taguchi was adopted to carry out the experimental design with five 2 level factors with interaction among the factors as A x B and A x C. Minitab 17 software was used for creating the design and is presented in Table 3.

Table 3: *Taguchi orthogonal array design*

Trial	A	B	C	D	E
1	20	CD	15	Without	0.3
2	20	CD	30	With	0.45
3	20	CP	15	With	0.45
4	20	CP	30	Without	0.3
5	30	CD	15	Without	0.45
6	30	CD	30	With	0.3
7	30	CP	15	With	0.3
8	30	CP	30	Without	0.45

A: Brewery sludge (%), B: Amendment,
C: C/N ratio, D: Starting culture,
E: Aeration rate (L/min/kg)
CD: Cow dung, CP: Coconut pith

4. Experimental Set up

Reactor for in-vessel composting of 10 kg of substrate by wet weight with forced aeration system with accessories for purifying, humidifying, stabilizing and controlling the inlet air was designed with acrylic body. An air pump of variable speed type, electric motor with inverter fitting and an aeration rate range of 0-80 Lit/min was used for aeration. Two numbers of small ports were provided for inserting digital thermometers at one third and two third heights for temperature monitoring. At the top a large sized port for mixing the sample using hoe fork was also provided. Arrangement is provided at the extreme end of the exhaust for measuring the residual oxygen left after composting using a digital oxygen meter. The experimental setup is shown in Fig 1 (a) and (b).

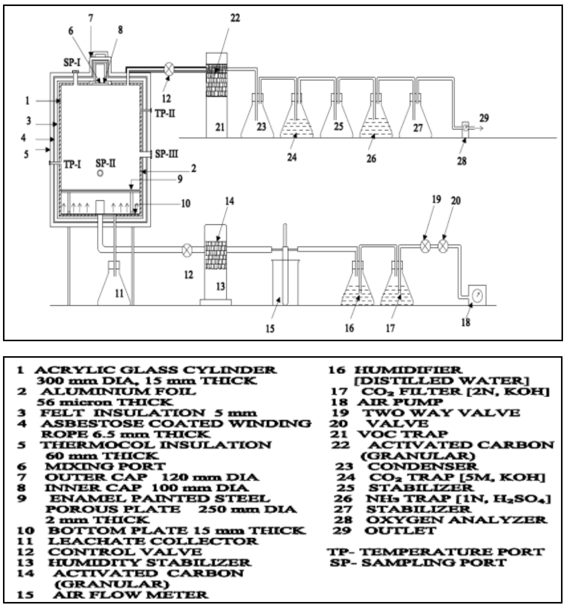


Fig 1 (a): *Diagram of experimental setup*

Fig 1 (b): *Photograph of experimental setup*

4.1 Preparation Substrate

The substrates used for the composting were synthetic Organic Fraction of Municipal Solid Waste (OFMSW) (Hema Nalini et. al., April 2015, 2) and Dewatered Brewery Sludge (Hema Nalini et. al., April 2015, 1). Use of synthetic waste in composting studies enables repeatability and reproducibility of the experiments. Simulated waste in experiments will give a true picture of the behaviour of the original waste. The sludge from the brew-house of United Breweries Ltd., Kanjikode, Kerala, was collected using the composite sampling technique. Compost recipe can be prepared for a given quantity of synthetic waste by knowing carbon, nitrogen and moisture content of each component in it. Once the carbon, nitrogen and moisture content of the components of substrates are known by choosing the right material and adjusting the weights, the compost recipe can be prepared for a given value of total weight, C/N ratio and moisture content, which can be done with the help of an Excel spread sheet. Table 4 shows the weights of raw materials in kg for the trials 1 to 8. The moisture content and the maximum particle size of the substrate were 70% and 5 mm respectively.

Table 4: *The weights of items in substrate for the experimental trials*

ITEM	**Trial**							
(kg)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Boiled rice	1.5	1.3	1.3	1.4	1.3	1.3	1.2	1.4
Pumpkin	0.8	1.8	0.75	1.8	1	1.7	0.3	1.3
Potato	0.6	0.2	0.75	0.3	0.5	0.15	0.75	0.3
Green Banana	0.8	0.2	0.9	0.2	0.5	0.15	0.75	0.3
papaya	2.2	0	2.2	0	2	0	2.1	0
Orange	0	2.3	0	2.2	0	1.8	0	2.1
Newspaper	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.4
Dry leaves	0	0.7	0	0.9	0	0.8	0	0.85
Grass clippings	0.5	0.3	0	0	0	0	0.5	0
Green leaves	0.8	0	1	0.4	1.05	0	0.4	0
Brewery sludge	1.9	1.8	1.8	1.9	2.85	2.7	2.7	2.85
Cow dung	0.5	0.5	0	0	0.5	0.5	0	0
Coconut pith	0	0	0.5	0.5	0	0	0.5	0.5
Unmatured Compost	0	0.5	0.5	0	0	0.5	0.5	0

4.2 Running Experiments

The random orderings for running the experiments is given by Robert. H.L. and Joseph. E.M in 200 different random combinations for 8 runs experiment. One among that random combination chosen here is 2, 7, 3, 6, 5, 8, 4, 1. The experiments were run as per this order. The contents in the reactor were mixed daily using a hoe fork.

4.3 Continuous Monitoring

Experiments were run for the 8 trials keeping the levels of the factors as represented in Table 2. The responses are the kinetic constants based on the degradation of organic matter. Therefore the percentage of organic matter remaining was determined at two days interval during the composting period. Organic matter content was measured by the ignition method (Black's method). A dried crucible (dried at 105 °C in the oven overnight to a constant weight) with a cover was first weighed ($W_{Crucible}$). About 5 g of compost sample was placed in the crucible and covered it to minimize evaporation and the weight was noted as $W_{Wet\text{-}Total}$ (fresh sample + crucible). The crucible with the sample was kept in the oven for drying at a temperature of 105 ± 5 °C for 4 hours. The sample with the crucible was then placed in a desiccators till it cools down to the room temperature and the weight was noted as $W_{Dry\ \text{-}Total}$ (dry sample + crucible). This dried sample ($W_{Dry\ \text{-}Total}$) was then ignited with the cover off in a muffle-furnace at 550 °C for about 4 hrs. Then it was cooled in desiccators to room temperature and the final weight was noted as W_{Final} (sample weight without organic part). The following equation was used to calculate the organic matter content:

$$Organic-matter\ Content = \frac{W_{Dry-Total} - W_{Final}}{W_{Wet-Total} - W_{Crucible}} \times 100\% \qquad (8)$$

In addition to this, temperature (inside the reactor and ambient) were monitored at 2 hrs interval for the first two days and 4 hrs interval for the remaining days. Temperature was measured using digital thermometers. Two temperature ports were provided at one third and two third heights of the reactor and thermometers were inserted into the ports for temperature measurement. The average of two temperature values was reported as the reactor temperature. Ambient temperature was measured each time using another digital thermometer. Composting is completed when the reactor temperature becomes equal to or less than the ambient temperature.

4.4 Biodegradability Coefficient

By knowing the initial and the final organic matter content and using equation (2) the biodegradability coefficient is determined.

4.5 Computation of Kinetic Constants

The percentage organic matter left is plotted with composting days for all the trials and is shown in Fig 4 to 11.The reaction rate r was determined by drawing the tangent to the resultant curve and *Origin 85* software was used for this purpose. The reciprocals of reaction rate ($1/r$) and organic matter percent ($1/c$) were computed and the results are summarized in Tables 5 to 12. The values of kinetic constants k_m and r_m of Eq.7 were determined graphically from the Lineweaver-Burke plots of $1/r$ versus $1/c$ data as shown in Fig. 12.

5. Result and Discussion

The temperature profile for reactor temperature (average of two readings), ambient temperature with composting time is plotted for various trials as shown in Fig 2 and Fig 3. Due to the presence of biodegradable organic matter in the substrate, the microbial activity quickly started, released heat and the temperature inside the reactor increased rapidly. During the initial 2 to 4 hours, mesophilic bacteria were highly active and temperature increased above 40^0C and thereafter thermophilic activity started and continued up to 76, 94, 78, 100, 84, 112, 88 and 82 hours for the trials 1 to 8 respectively. Maximum temperatures attained for trials 1 to 8 are respectively 61.5, 54.1, 53.3, 56.8, 59.1, 67.1, and 54.5 ^{0}C. After the easily degradable substrates were consumed during the active phase, cooling started. At the end of 312 to 480 hours the temperature reached ambient temperature indicating the stability of composting. The highest maximum temperature was for trial 7 and is 67.1^{0}C. From the temperature profiles it is clear that they maintain almost stable profiles at the final stages because of lack of biodegradable material showing the stability of compost.

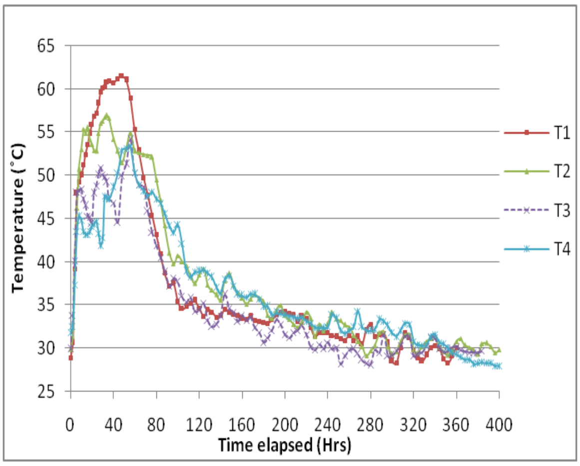


Fig 2: *Temperature variations with composting for trials 1to 4*

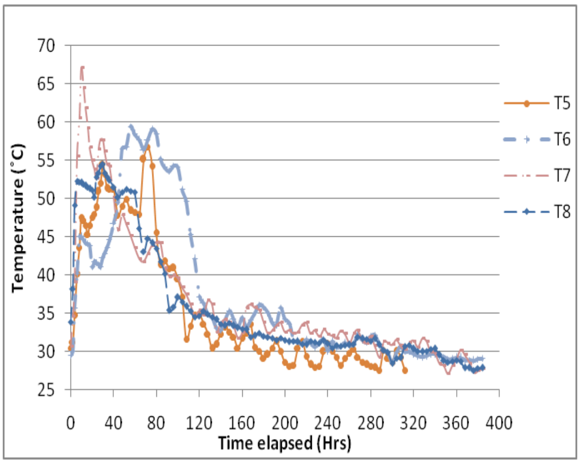


Fig 3: *Temperature variations with composting for trials 5to 8*

Fig 4 to Fig 11 represents the plot of organic matter content with composting time. From the graphs it can be seen that with composting the organic matter left is decreasing because of the degradation of organic matter.

The rate of decreasing of organic matter is more during the initial days and tends to decrease with composting. Slopes of the plotted points on the curve are used to find the reaction rate (r) on respective days. The reciprocal of the reaction rate (1/r) and reciprocal of organic matter (1/c) was used for plotting the Lineweaver-Burke plot.

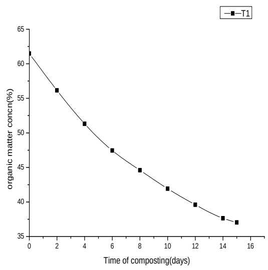


Fig 4: *Trial 1*

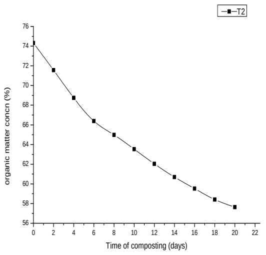


Fig 5: *Trial 2*

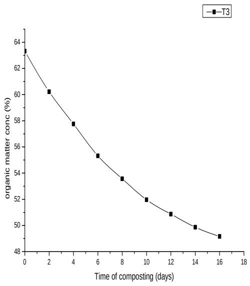


Fig 6: *Trial 3*

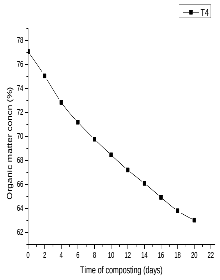


Fig 7: *Trial 4*

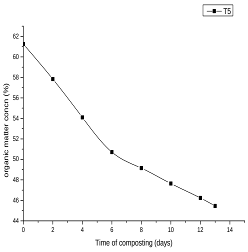


Fig 8:: *Trial 5*

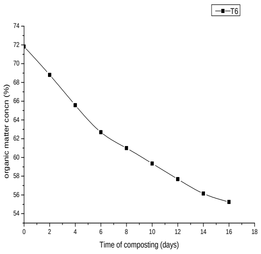


Fig 9: *Trial 6*

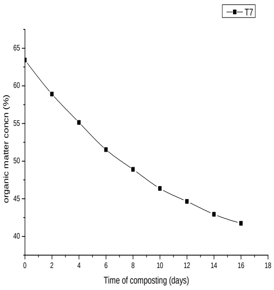


Fig 10: *Trial 7*

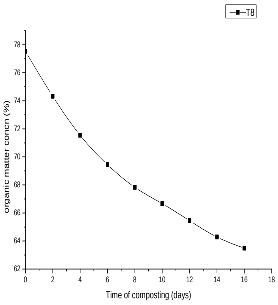


Fig 11: *Trial 8*

Fig 4 to 11: *Variation in organic matter content with composting time (hor. axis) for trials 1 to 8*

Table 5: *Organic matter content % and computed reaction rates with composting time for trial 1*

T	c	1/c	r (day^{-1})	1/r
0	61.49	0.016263	2.67	0.374532
2	56.15	0.017809	2.545	0.392927
4	51.31	0.019489	2.175	0.45977
6	47.45	0.021075	1.68	0.595238
8	44.59	0.022427	1.3825	0.723327
10	41.92	0.023855	1.2475	0.801603
12	39.6	0.025253	1.07	0.934579
14	37.64	0.026567	0.7942	1.259129
15	37.0316	0.027004	0.6084	1.643655

***Table 6:** Organic matter content percentage and computed reaction rates with composting time for trial 2*

T	c	1/c	r (day^{-1})	1/r
0	74.322	0.013455	1.381	0.724113
2	71.56	0.013974	1.3955	0.716589
4	68.74	0.014548	1.2925	0.773694
6	66.39	0.015063	0.94	1.06383
8	64.98	0.015389	0.715	1.398601
10	63.53	0.015741	0.735	1.360544
12	62.04	0.016119	0.7085	1.411433
14	60.696	0.016476	0.6285	1.59109
16	59.526	0.016799	0.57	1.754386
18	58.416	0.017119	0.4725	2.116402
20	57.636	0.01735	0.39	2.564103

***Table 7:** Organic matter content percentage and computed reaction rates with composting time for trial 3*

T	c	1/c	r (day^{-1})	1/r
0	63.32163	0.015792	1.5522	0.644247
2	60.2172	0.016607	1.39101	0.718902
4	57.7576	0.017314	1.2255	0.815993
6	55.3152	0.018078	1.0492	0.953107
8	53.5608	0.01867	0.8385	1.192606
10	51.9612	0.019245	0.67295	1.485995
12	50.869	0.019658	0.5246	1.906214
14	49.8628	0.020055	0.42828	2.334921
16	49.15588	0.020343	0.35346	2.829174

Table 5 to Table 12 represent the percentage of organic matter remaining (c), its reciprocal (1/c), reaction rate (r) and its reciprocal (1/ r) with composting days for trials 1 to 8.

***Table 8:** Organic matter content percentage and computed reaction rates with composting time for trial 4*

T	c	1/c	r (day^{-1})	1/r
0	77.0732	0.012975	1.0148	0.985416
2	75.0436	0.013326	1.0578	0.945358
4	72.842	0.013728	0.9632	1.038206
6	71.1908	0.014047	0.7654	1.306506
8	69.7804	0.014331	0.6794	1.471887
10	68.4732	0.014604	0.6407	1.560793
12	67.2176	0.014877	0.5934	1.685204
14	66.0996	0.015129	0.5719	1.748557
16	64.93	0.015401	0.5719	1.748557
18	63.812	0.015671	0.473	2.114165
20	63.038	0.015863	0.387	2.583979

***Table 9:** Organic matter content percentage and computed reaction rates with composting time for trial 5*

T	c	1/c	r (day^{-1})	1/r
0	61.2492	0.016327	1.707	0.585823
2	57.8352	0.017291	1.7885	0.559128
4	54.0952	0.018486	1.7825	0.56101
6	50.7052	0.019722	1.23775	0.807918
8	49.1442	0.020348	0.7644	1.308216
10	47.6396	0.020991	0.727825	1.373957
12	46.2329	0.02163	0.738325	1.354417
13	45.4596	0.021998	0.7733	1.293159

***Table 10:** Organic matter content percentage and computed reaction rates with composting time for trial 6*

T	c	1/c	r (day^{-1})	1/r
0	71.8272	0.013922	1.51	0.662252
2	68.8072	0.014533	1.565	0.638978
4	65.5672	0.015252	1.53	0.653595
6	62.6872	0.015952	1.145	0.873362
8	60.9872	0.016397	0.835	1.197605
10	59.3472	0.01685	0.825	1.212121
12	57.6872	0.017335	0.8	1.25
14	56.1472	0.01781	0.60891	1.642279
16	55.25156	0.018099	0.44782	2.23304

***Note**: In Table 5 to Table 12, T = Composting time in days, c = Organic matter % and r is the reaction rate.

***Table 11:** Organic matter content percentage and computed reaction rates with composting time for trial 7*

T	c	1/c	r (day^{-1})	1/r
0	63.4164	0.015769	2.255	0.443459
2	58.9064	0.016976	2.07	0.483092
4	55.1364	0.018137	1.8475	0.541272
6	51.5164	0.019411	1.5575	0.642055
8	48.9064	0.020447	1.29	0.775194
10	46.3564	0.021572	1.0675	0.936768
12	44.6364	0.022403	0.8575	1.166181
14	42.9264	0.023296	0.7275	1.37457
16	41.7272	0.023965	0.5996	1.667779

***Table 12:** Organic matter content percentage and computed reaction rates with composting time for trial 8*

T	c	1/c	r (day^{-1})	1/r
0	77.5376	0.012897	1.605	0.623053
2	74.3276	0.013454	1.4975	0.66778
4	71.5476	0.013977	1.22	0.819672
6	69.4476	0.014399	0.93	1.075269
8	67.8276	0.014743	0.695	1.438849
10	66.6676	0.015	0.595	1.680672
12	65.4476	0.015279	0.595	1.680672
14	64.2876	0.015555	0.4906	2.03832
16	63.4852	0.015752	0.4012	2.492522

Fig 12 represents the Lineweaver-Burke plot in which *1/r* is plotted on y-axis and *1/c* on x-axis for all trials. The plots are straight lines and linear regression equations are obtained for each line. The regression equation and coefficient of regression for the linear lines for each trial is presented in the Table 13. The y-intercept of the equations yield the reciprocal of the maximum or limiting velocity constant (r_m) and slope of the equations yield the ratio between Michaelis-Menten constant (k_m) and maximum or limiting velocity constant (r_m).

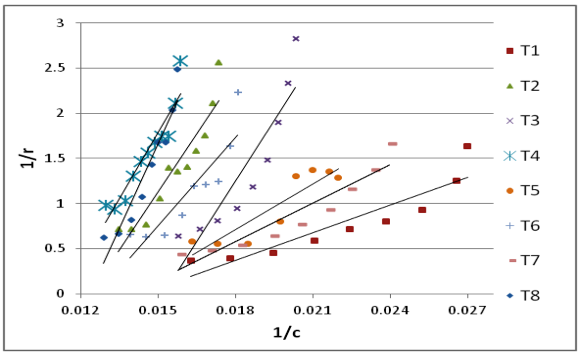


Fig 12: *Lineweaver-Burke plot for trials 1, 2, 3, 4, 5, 6, 7 and 8*

Table 13: *Regression eqation and coefficient for the Lineweaver-Burke plot for variou trials*

Kinetic analysis		
Trial	**Regression equation**	**Regression coefficient (R^2)**
1	101.53x – 1.4551	0.8341
2	430.4x – 5.325	0.885
3	443.1x – 6.73	0.827
4	495.2x – 5.638	0.895
5	168.9x – 2.331	0.815
6	327.0x – 4.159	0.80
7	141.7x – 1.973	0.888
8	634.1x – 7.843	0.903

Table 14 represents the Michaelis-Menten constant (k_m), maximum or limiting velocity constant (r_m) and biodegradability coefficient (k_b) and maximum temperature for different trials. Biodegradability coefficient estimation is a rough measure for evaluating the composting system. However it gives an idea about the biodegradability of the substrate used in the composting process. Trial 1 is having maximum biodegradability coefficient of 0.6317 and the lowest value is for trial 3 and is 0.4399. This may be due to the difference in the use of raw materials. For trial 3 the substrate contain amendment (coconut pith) and starting culture which have low biodegradability compared to the substrate of trial 1 which contain cow dung as amendment and free from starting culture. Also a positive correlation exist between temperature and biodegradability coefficient (r = 0.786 and p = 0.021). The Michaelis-Menten constant (k_m) is high for trial 4 which implies that the microorganisms did not decompose the organic matter efficiently. Maximum or limiting velocity constant (r_m) is more for trial 1 and is less for trial 8. A positive correlation exists between r_m and temperature (r = 0.748 and p = 0.033) and between r_m and k_b (r = 0.795 and p = 0.018).

Table 14: *Kinetic constants: Michaelis-Menten constant (k_m), maximum or limiting velocity constant (r_m) and biodegradability coefficient (k_b)*

	Kinetic constants			**Max. temp.**
Trial	**k_m**	**r_m**	**k_b**	**^{0}C**
1	69.775	0.6872	0.6317	61.5
2	80.826	0.1877	0.523	56.9
3	65.839	0.1485	0.4399	54.1
4	87.8325	0.1774	0.4927	53.3
5	72.458	0.429	0.4727	56.8
6	78.624	0.2404	0.5157	59.1
7	71.819	0.5068	0.5869	67.1
8	80.849	0.1275	0.4963	54.5

5.1 Influence of composting factors on k_m

SN analysis is done to know the relative importance of composting factors on k_m using Minitab 17 software. Table 15 represents the response table for Signal to Noise ratios for the response Michaelis Menten constant (k_m) *smaller is better* criterion. The table gives SN ratios for each factor at two levels and delta value, the difference between the SN ratios at two levels. The value of delta is used for assigning the rank of the influencing factor in minimising the k_m value. From Table 15 it can be seen that the order of influence of factors in minimising the value of k_m is C/N ratio, starting culture, aeration rate, amendment and brewery sludge. Fig 13 represents the main effects plot for the response k_m *smaller is better* criterion. From the main effect plot also it is clear that the most influencing factor is C/N with a level of 15. To check the interaction effects of factors, the two interactions considered are the interaction between brewery sludge and amendment as shown in Fig 14 and the interaction between brewery sludge and C/N ratio as shown in Fig 15. Since the interaction lines for both plots are almost parallel it is concluded that interaction is absent among the factors. To know the significance and the relative contribution of factors, analysis of variance is done. In the process of ANOVA to have a non zero error degrees of freedom the factors with least importance, brewery sludge, amendment and their interactions are pooled. The main effect plot for SN ratios of most influencing factor on the response is shown in the Fig 16 after pooling least influencing factors. Table 16 represents the analysis of variance table showing DF (degrees of freedom), Seq SS (sequential sum of squares), Adj. SS (adjusted sum of squares), Seq. MS (sequential mean squares), F (variance ratio) and P value. From the anova table for S/N ratios, C/N ratio (**0.006**) is the most significant factor at the 0.05 α-level to the response k_m. The remaining factors are not significantly related to the response. The last column in the anova table is representing the percentage contribution of the factors on responses and is diagrammatically represented by a pie diagram in Fig 17. From Fig 17 it is clear that C/N ratio is contributing 80.14% on the kinetic constant k_m. The factor levels that can yield a better enzyme substrate binding to reduce k_m is C1D2E2.

***Table 15:** Response Table for Signal to Noise Ratios for the response Michaelis Menten constant (k_m): smaller is better*

Level	Brewery sludge	Amend ment	C/N ratio	Starting culture	Aeration Rate
1	-37.57	-37.53	-36.89	-37.78	-37.70
2	-37.60	-37.63	-38.27	-37.39	-37.47
Delta	0.03	0.1	1.38	0.39	0.23
Rank	5	4	1	2	3

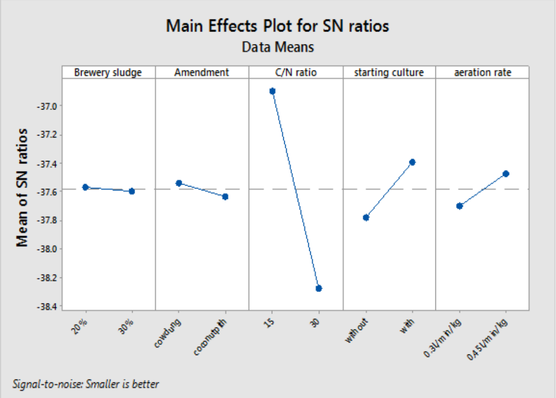


***Fig 13:** Main effects plot for SN ratios of the response k_m*

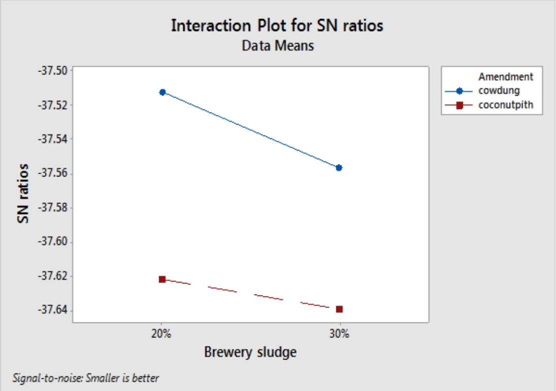


***Fig 14:** Interaction (A x B) plot for SN ratios of the response k_m*

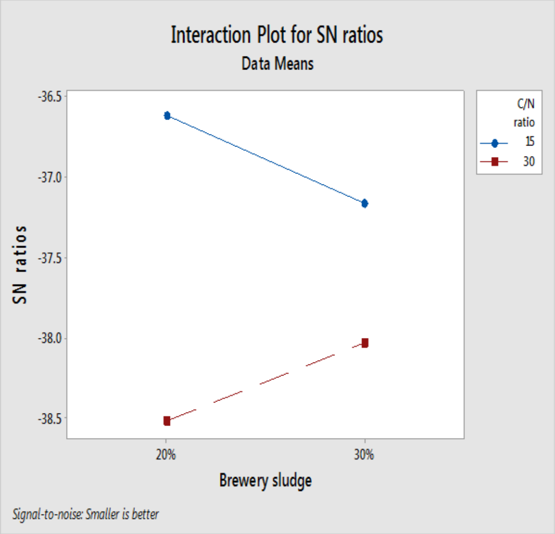


***Fig 15:** Interaction effect (AxC) plot for SN ratios of the response k_m*

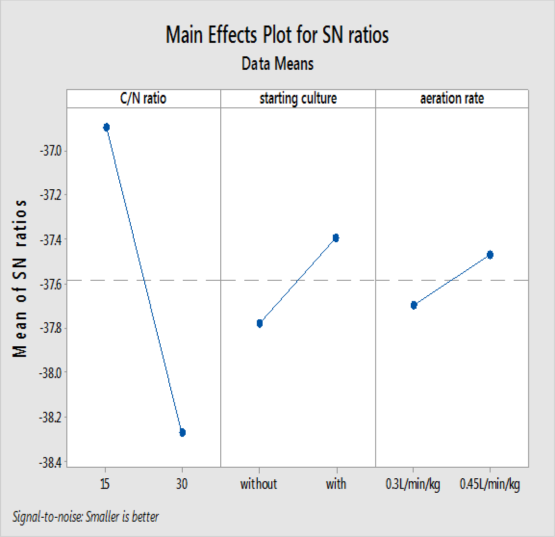


***Fig 16:** Main effects plot for SN ratios of the response k_m after pooling factors A, B, AxB, AxC*

***Table 16:** Analysis of Variance for SN ratios*

Source	DF	Seq SS	Adj SS	Adj MS	F	P	% Contribution
C/N ratio	1	3.8068	3.8068	3.8068	28.09	0.006	80.14
Start. culture	1	0.2987	0.2987	0.2987	2.20	0.212	6.29
Aer. rate	1	0.1029	0.1029	0.1029	0.76	0.433	2.17
Resid. error	2	0.5420	0.5420	0.1355			11.4
Total	7	4.7503					

5.2 Influence of composting factors on r_m

SN analysis is also done to know the relative importance of composting factors on r_m. Table 17 represents the response table for Signal to Noise ratios for the response maximum rate constant (r_m), *larger is better* criterion. From the table it can be seen that the order of influence of factors in maximising the value of r_m is C/N ratio, aeration rate, amendment, starting culture, and brewery sludge. Fig 18 represents the main effects plot for the response r_m larger is better criterion. From the main effect plot also it is clear that the most influencing factor is C/N with a level of 15. To check the interaction effects of factors the two interactions considered are the interaction between brewery sludge and amendment as shown in Fig 19 and the interaction between brewery sludge and C/N ratio as shown in Fig 20. Since the interaction lines for both plots are almost parallel it is concluded that interaction is absent among the factors. To know the significance and the relative contribution of factors, analysis of variance is done. In the process of ANOVA to have a non zero error degrees of freedom the interactions are pooled. Table 18 represents the analysis of variance for S/N ratios. From the table for C/N ratio, P = 0.090 is the most significant factor at the 0.10 □-level to the response r_m. The factors aeration rate and amendment are less significant and the least significant factors are starting culture and brewery sludge. The last column in the ANOVA table is representing the percentage contribution of factors on responses and is diagrammatically represented by a pie diagram in Fig 21. From the figure it is clear that C/N ratio is the most contributing on the kinetic constant r_m. The factor levels that can yield a higher maximum velocity constant is A2B1C1D1E1.This means that brewery sludge of 30%; cow dung as amendment (which contain more nutrient and seeding material compared to coconut pith); C/N ratio of 15 (which contain more nutrient compared to C/N 30); absence of starting culture and aeration rate of 0.3 L/min/kg are levels of composting factors required to increase limiting velocity constant.

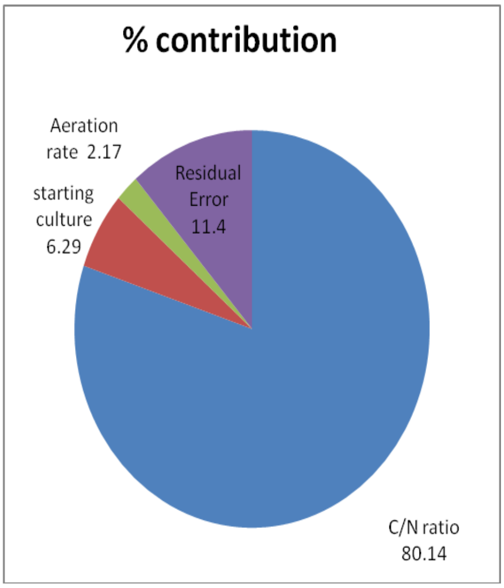


Fig 17. *Percentage contribution of factors on k_m*

Table 17: *Response Table for Signal to Noise Ratios of the response maximum or limiting velocity constant (r_m): Larger is better*

Level	Brewery Sludge	Amendment	C/N ratio	Starting culture	Aeration Rate
1	-12.344	-9.38	-8.269	-10.880	-9.141
2	-10.881	-13.845	-14.956	-12.345	-14.084
Delta	1.463	4.465	6.686	1.465	4.943
Rank	5	3	1	4	2

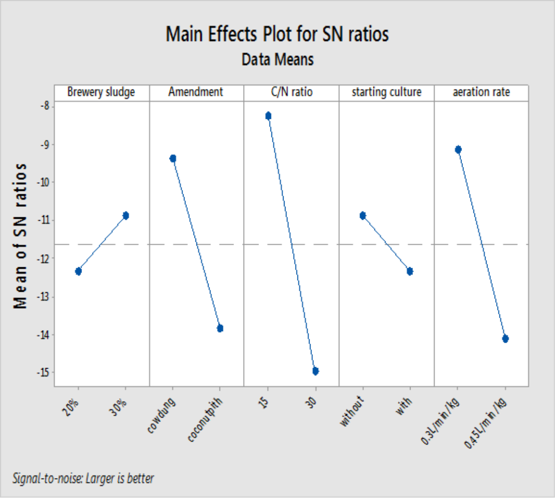


Fig 18: *Main effect plot for SN ratios of the response r_m*

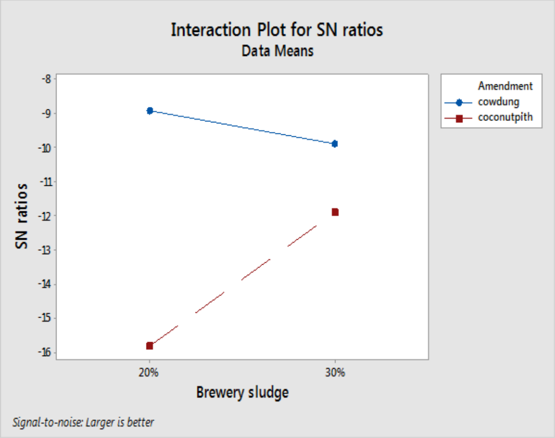


Fig 19: *Interaction plot (AxB) for SN ratios of the response r_m*

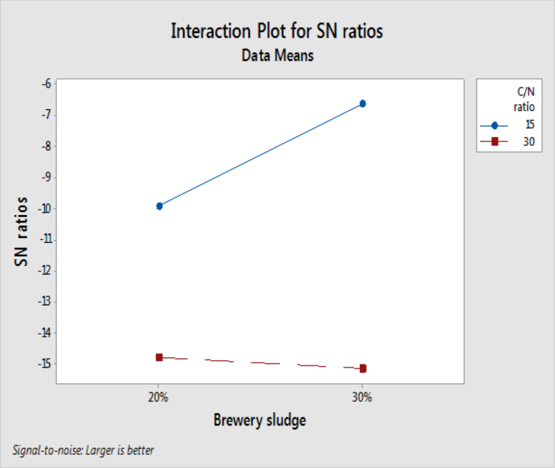


Fig 20: *Interaction plot (AxC) for SN ratios of the response r_m*

***Table 18:** Analysis of Variance for SN ratios of r_m after pooling interaction effects*

Source	DF	Seq SS	Adj SS	Adj MS	F	P	% contribution
BS	1	4.278	4.278	4.278	0.46	0.567	2.08
Amendment	1	39.865	39.865	39.865	4.31	0.173	19.43
C/N ratio	1	89.411	89.411	89.411	9.67	0.090	43.57
starting culture	1	4.294	4.294	4.294	0.46	0.566	2.09
Aer.rate	1	48.871	48.871	48.871	5.29	0.148	23.82
Res. Err.	2	18.491	18.491	18.491			9.01
Total	7	205.21					

6. Conclusion

Michaelis-Menten equation is used to determine kinetic constants for evaluating the composting process. Influence of five factors at 2 levels namely A: the percentage of brewery sludge (20, 30), B: amendment type (cow dung, coconut pith), C: C/N ratio (15, 30): D: starting culture (without, with) and E: aeration rate (0.3 L/min/kg, 0.45 L/min/kg) and the interaction of factors on kinetic contants were studied using Taguchi's experimental design for L_8 orthogonal array with 8 experimental trials.

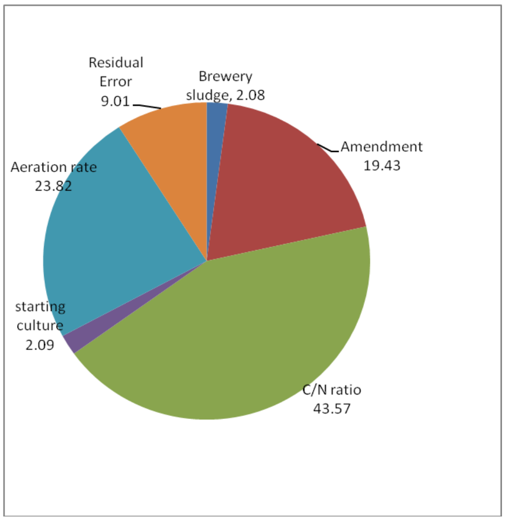


***Figure 21:** % contribution of factors on response r_m*

Trials were run in random order in a batch-scale in-vessel reactor with provision for monitoring temperature and organic matter remaining at regular intervals till the end of composting. Biodegradability coefficient, Michaelis Menten constant (k_m), and maximum or limiting velocity constant (r_m) were determined for all the trials. Out of three parameters biodegradability coefficient is a less important parameter and is not used as a response parameter for the analysis. The relative importance of factors on responses k_m and r_m were studied by SN analysis. Analysis of variance for SN ratio showed that the most significant factor influencing the kinetic constants is C/N ratio.

7. Acknowledgment

This study is funded by the Kerala State Council for Science, Technology and Environment of the Government of Kerala. The authors gratefully acknowledge the funding support.

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