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Biodegradation of Fruit Waste under Optimum Nutrient Conditions and Different Bacterial Inocula

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Abstract: Solid waste is becoming a major problem day-by-day. Fruit waste is a significant organic fraction of municipal solid waste and has high moisture content and high volatile solids concentration or organic content. Due to this, it easily degrades in the environment and releases nuisance gases like CH_4, H_2S, CO_2, NH_3, etc. A possible method for treating fruit waste is composting. The composting rate depends on the nature of the biodegradable materials, so the main objective of this paper was to characterize mixed fruit waste and determine the biodegradable fractions of this waste and the corresponding rate constants. The mixed fruit waste samples were biodegraded using different inocula such as soil bacteria, cow dung and sludge under aerobic conditions.

Five different fruit peels samples were used in this study: *Citrus limetta* (Mosambi), mango, banana, pineapple, pomegranate and the same were characterized for various physico-chemical and biological parameters. Batch biodegradation studies were conducted under aerobic conditions by incubating a mixture of all five types of fruit peels in equal proportions. In the first biodegradation study of fruit waste with soil bacteria as inocula, 73.12% removal of TSS and 70.12% removal of VSS were observed in 65 days. In the second batch biodegradation study of fruit waste with cow dung as inocula, TSS removals of 75.27% and VSS removals of 82.67% were observed in 65 days. In the third batch biodegradation study of fruit waste with sludge as inocula, TSS removals of 67.78% and VSS removals of 60.17% were observed. Fruit peels waste was found to degrade rapidly (within 8 to 21 days) after which very little change in TSS or VSS concentrations were observed.

Key words: Solid waste, C/N ratio, tannin-lignin, bacteria, reaction rate.

Introduction

A major environmental concern due to urbanization is solid waste management. Solid waste means any garbage, refuse, sludge from a wastewater treatment plant, water supply treatment plant, and other discarded materials resulting from residential, industrial, commercial, mining and agricultural operations. Major categories of wastes included in the term solid waste are municipal solid waste (MSW), agricultural wastes, industrial wastes, ash from thermal power plants and hazardous wastes.

Municipal solid waste in major Indian cities is estimated to have a biodegradable fraction of 42.5%, paper 9.63%, plastic and rubber 10.1%, metal (0.63%) and glass (0.96%) and inert material (17%) (Joshi and Ahmed, 2016). The moisture content of urban MSW is 47%, C/N ratio ranges between 20 and 30, and the average calorific value is 7.3 kJ/kg (1745 kcal/kg) (Annepu, 2012). Since a large fraction of MSW is biodegradable, it leads to greenhouse gas emissions and release of leachate during biodegradation. The leachate from these wastes contaminates groundwater and surface water. In addition, these illegally dumped wastes have adverse effects on human health and the environment. Proper management of municipal solid waste is necessary, otherwise it will occupy a major part of our residential and commercial areas

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in urban centres and lead to an increase in diseases. Segregation of waste at source into different fractions (biodegradable, recyclables and hazardous materials) is essential for better solid waste management. Biological processes like composting and biogas generation are attractive options for treating municipal solid waste that contains high moisture content and high organic content and require use of segregated organic waste. Design of biological processes requires knowledge of the extent and rate at which different organic fractions of municipal solid waste can be biodegraded.

India is the second largest producer of fruits and vegetables in the world (after China) with 221.431 million metric tons per year. The cumulative wastage is estimated to be 5.8 to 18% of the total produced fruits and vegetables (ICAR-CIPHET, 2015). The objective of this study was to determine the rate and extent of biodegradability of a significant organic fraction of municipal solid waste (OFMSW), i.e., fruit waste. This study was limited to IIT Kharagpur campus where 39.4 kg/d of fruit waste was estimated to be generated during a week-long survey conducted from 16 to 22 September 2017 (Rajput, 2018).

Materials and Methods

Sample Collection

For the study, five different fruit wastes were chosen: mango, banana, pineapple, *Citrus limetta* (mosambi) and pomegranate. Fruit peels and seeds are thrown away after juicing. These waste materials have high moisture content. Different fruit peels samples were collected from IIT Kharagpur Tech Market for detailed characterization. All samples were dried and shredded in a mixer grinder into powdered form. Shredding was performed to homogenize the sample for further analysis.

Sample Characterization

All fruit peels samples were characterized for the following parameters: moisture content, total solids, volatile solids, fixed solids, total organic carbon, total nitrogen, phosphate, tannin-lignin, calorific value and C/N ratio. Standard methods for water and waste water analysis (APHA et al., 2005) were used in all cases except where noted. All analytical methods are summarized in a subsequent Section.

Batch Biodegradation Studies

Three different batch biodegradation studies were conducted under aerobic conditions as shown in Figure 1 based on the procedure described here. Each study was conducted using different bacterial inocula.

Procedure for Each Batch Biodegradation Experiment

- Total of 55 autoclaved test tubes of 70 mL capacity were used in each batch biodegradation experiment. Triplicate sets of aerobic bioactive tubes and a single set of control test tubes were prepared for each experiment. The test tubes had vented caps that allow air to pass in and out of the test tubes to maintain aerobic conditions during incubation. The vents enforce a tortuous air path which prevent contamination from bacteria in the air outside the test tubes.
- 50 mg of mixed fruit peels samples were added to each 70 mL test tube along with 1 mL of aerobic bacterial seed and 50 mL mineral media to provide microbial seed for biodegradation and were capped loosely. For the control set, 50 mg of sample and 50 mL of mineral media, i.e., 1 g/L, without seed were added to 15 tubes and then autoclaved to ensure that there were no microbes in these tubes.
- All tubes were mounted in an incubator-shaker and incubated in the dark at a temperature of 35 °C with continuous rotation of 110 rpm as shown in Figure 1.
- Triplicate aerobic biotic tubes were sacrificed along with a control tube and analyzed on the following days: 0, 1, 2, 3, 5, 8, 13, 21, 34, 55, and 65 days. Each of the sacrificed tubes was analyzed for D.O., temperature, TSS, VSS, pH, conductivity, and HPC.

Dilution Water (Mineral Media)

Mineral media was added to each test tube to ensure that carbon from the solid waste samples was the only limiting nutrient and to provide a buffer during the incubation period. Amount of essential nutrients to be added were calculated based on standard BOD dilution water composition and the assumption that 100% of the sample added to each test tube would be biodegraded. In other words, inorganic nutrients were added in far greater amount than would be required for biodegradation (Method 5210B, APHA et al., 2005).

Mineral Media Preparation

Autoclaved double distilled water of 4 litres was taken in a large plastic beaker and the following nutrients were added: KH_2PO_4 – 0.32 g/4 L, K_2HPO_4 – 0.88 g/4 L, $Na_2HPO_4.7H_2O$ – 0.7 g/4 L, $MgSO_4.7H_2O$ – 1.6 g/ 4 L, NH_4Cl – 0.72 g/4 L, $CaCl_2$ – 0.12 g/4 L, $FeCl_3.6H_2O$ – 0.016 g/4 L and Peptone – 0.96 g/4L (for providing trace nutrients).

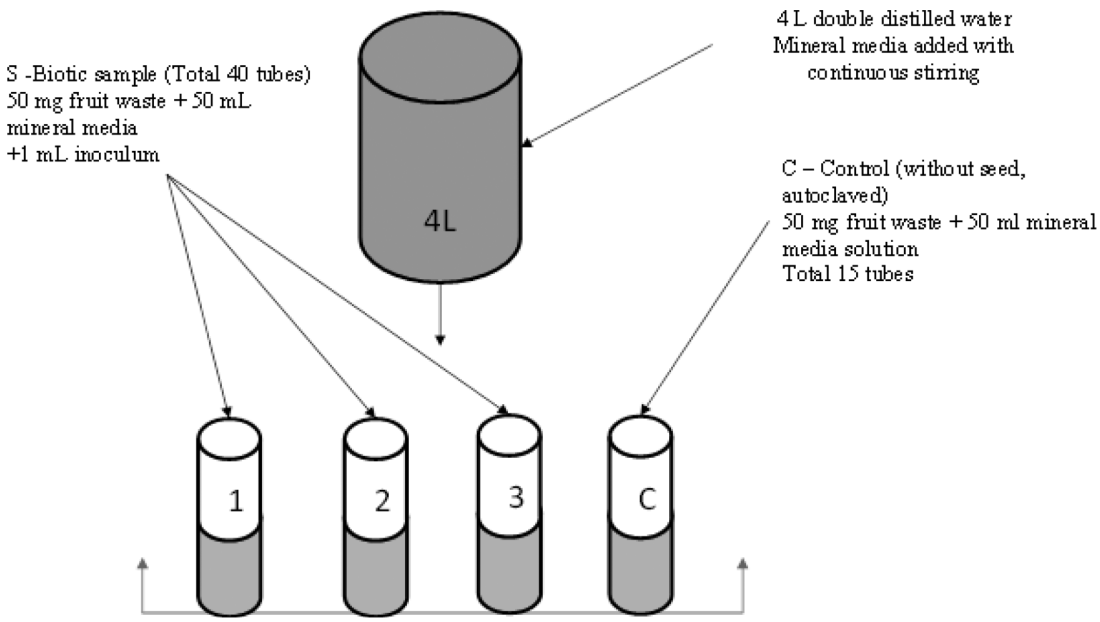

Figure 1: Procedure for setting up batch biodegradation experiments.

Preparation of Seed Material

First study: For microbial seed preparation, 1 g of soil was mixed with 10 mL of 17.2 mM phosphate buffer and was made to 100 mL. This sample was shaken for one hour and then allowed to settle for half an hour. 1 mL of supernatant solution was then added to each bioactive aerobic tube to provide microbial seed for biodegradation.

Second study: 1 g of cow dung was mixed with 10 mL of 17.2 mM phosphate buffer and was made to 100 mL using double distilled water. This sample was shaken for one hour and then allowed to settle for half an hour. Then 1 mL of supernatant solution was added to each 70 mL test tube along with 50 mg mix fruit peels sample and 50 mL mineral media to provide microbial seed for biodegradation.

Third study: For microbial seed preparation, 1 mL of sludge was mixed with 10 mL of 17.2 mM phosphate buffer and was made to 100 mL through double distilled water. This sample was shaken for one hour and then allowed to settle for half an hour. Then 1 mL of supernatant solution was added to each 70 mL test tube along with 50 mg mix fruit peels sample and 50 mL mineral media to provide microbial seed for biodegradation.

Analytical Methods

Moisture Content

Different fruit peels each of weight 25 g were taken in a separate crucible dish, and dried to a constant weight in an oven at 105 °C, and cooled to room temperature in a desiccator. After cooling, the dried weight was measured. Percentage weight loss of sample after drying gives the moisture content of the sample (Worrell & Vesilind, 2000).

Total Solids (TS), Volatile Solids (VS) and Fixed Solids (FS)

All fruit peels samples were characterized for TS, VS and FS. Each sample (25 g) was dried in an hot air oven at 105°C and cooled in a desiccator at room temperature. The weight of the dried sample is total solids (TS). For volatile solids (VS) and fixed solids (FS), 1 g of dried sample was taken and burned in a muffle furnace for 2 hours at 550°C. The weight of the residue is fixed solids (FS) and VS is calculated by subtracting FS from TS, i.e., VS = TS - FS.

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

TSS of the samples during the incubation period were analyzed based on Method 2540 D (APHA et al., 2005). TSS is defined as the residue left on a GF/A glass microfibre filter after sample filtration and evaporation at 103-105°C.

VSS of the samples were determined based on method 2540 E (APHA et al., 2005). It is defined as the weight of TSS lost during ignition at 550°C. VSS is a direct indicator of the combustible or organic matter contained in the samples.

Tannin-lignin

Tannin-lignin was determined in the lab using a spectrophotometer based on Method 5550 B (APHA et al., 2005). Each oven dried fruit peels sample (1 g) was digested on a hot plate with 50 mL (HNO_3 + $HClO_4$) acid for two hours and the samples were then filtered through Whatman filter paper (20 μ pore size, 934 AH glass fibre filter) and made to 100 ml with double-distilled water. Absorbance of each sample was measured at 700 nm.

Phosphate

Phosphate was determined in the lab using a spectrophotometer based on Method 4500-P: D (APHA et al., 2005). Each oven-dried fruit peels sample (1 g) was digested on a hot plate with 50 mL (HNO_3 + $HClO_4$) acid for two hours. Samples were then filtered through Whatman filter paper (20 μ, 934 AH glass fibre filter) and made to 100 mL with double-distilled water. Absorbance of each sample was measured at 690 nm.

Total Organic Carbon, Total Nitrogen, C/N Ratio

Total organic carbon, total nitrogen, and C/N ratio were determined with the help of a CHNS analyzer.

pH

pH was measured with the help of a digital pH meter by placing the pH electrode in each sample. The pH meter was initially calibrated with known standards of pH 4 and 7 before measuring pH of the sample.

Conductivity

Conductivity was measured with the help of a conductivity meter. It is a measure of the ability of an aqueous solution to conduct an electric current and depends on the concentration of ions. The instrument has to be calibrated with standard solutions of known conductivity of 2000 μS/cm or 5000 μS/cm before measuring conductivity of sacrificed samples.

Dissolved Oxygen

Dissolved oxygen was measured with the help of a D.O. meter. D.O. is the most important parameter for evaluating aerobic or anaerobic conditions in the reactor. When dissolved oxygen is 0 mg/L, it is considered to be anaerobic and above that aerobic conditions prevail. Initially, D.O. in distilled water was checked and found to be 7.53 mg/l at 30ºC. Dissolved oxygen of sacrificed samples was measured after that.

Heterotrophic Plate Counts (HPC)

Heterotrophic plate counts were determined on specified days for the samples during biodegradation. In a plate count, the numbers of colony forming units (cfu) are determined based on the assumption that each colony represents at least one cell. The initial sample is serially diluted in order to obtain a small but significant number of colonies on each plate. Sample volume added to each plate was 0.1 mL; the sample is poured and spread with the help of a spreader on nutrient agar filled in a petridish. The petridish plates were incubated upside down in an incubator at 35°C for 24 hours prior to counting of colonies. Only plates with 30 to 300 cfu were considered statistically significant results (APHA, AWWA and WEF, 2005). Since a single colony on a plate represents 10 cfu/mL, the detection limit of the method was 10 cfu/mL.

Results and Discussion

Characterization of Different Fruit Peels

The five different types of fruit peels collected were analyzed for various physical and chemical parameters.

Moisture Content

Moisture content of different fruit peels varied from 68.15% to 87.37%. Banana had the highest moisture content of 87.37% followed by *Citrus limetta* (mosambi) with 79.79%, mango peels 73.53%, pomegranate peels 73.15% and pineapple peels 68.5%.

Total Solids

Pineapple peels had the highest total solids content of 31.84% followed by pomegranate, mango, *Citrus limetta* (mosambi), and banana.

Fixed Solids

Fixed solids of different fruit peels varied from 31.40% to 4.40%. Highest fixed solids content was for *Citrus limetta* (mosambi) and lowest for pomegranate.

Volatile Solids

Volatile solids of different fruit peels varied from 95.60% to 68.60%. Highest volatile solids content was for pomegranate and lowest for *Citrus limetta* (mosambi).

Organic Carbon

Organic carbon in different fruit peels varied from 473.12 (mg/g) to 408.59 (mg/g). Highest organic carbon was found in pomegranate and lowest in banana.

Nitrogen and C/N Ratio

Nitrogen content of different fruit peels varied from 15.79 (mg/g) to 6.13 (mg/g). Highest nitrogen content was in banana and lowest in pineapple. Carbon to nitrogen ratio varied from 76.16 to 25.61. Highest C/N ratio was in pomegranate peels and lowest C/N ratio in banana peels. High C to N ratios are indicative of the low nitrogen content of the fruit waste samples.

Table 1: Characterization of different types of fruit peels

Parameter \ *Fruits*	*Citrus limetta (mosambi)*	*Banana*	*Mango*	*Pineapple*	*Pomegranate*	*Mixed fruit*
Moisture content (%)	75.79	87.37	73.53	68.15	73.15	75.6
Total solids (%)	24.2	12.62	26.46	31.84	26.84	24.4
Fixed solids (%)	31.4	13.74	7.12	5.4	4.4	12.4
Volatile solids (%)	68.6	86.26	92.88	94.6	95.6	87.6
Organic carbon (mg/g)	436.08	408.59	458.45	452.52	473.12	445.8
Nitrogen (mg/g)	8.82	15.79	11.64	6.13	6.27	9.7
Phosphate (mg/g)	0.043	0.039	0.02	0.02	0.048	0.034
Tannin lignin (mg/g)	19.23	24.67	32.65	37.51	29.87	28.8
Hydrogen (mg/g)	65.075	58.295	39.925	36.08	35.895	47.05
C/N ratio	49.71	25.61	39.42	73.88	76.16	52.96

Phosphate

Phosphate in different fruit peels ranged from 0.048 (mg/g) to 0.02 (mg/g). Highest phosphate content was in pomegranate peels and lowest in mango peels.

Tannin-lignin

Pineapple peels had the highest tannin-lignin content at 29.87 mg/g waste while *Citrus limetta* (mosambi) had the lowest tannin-lignin content at 19.23 mg/g of waste.

Batch Biodegradation Study

Three different batch biodegradation experiments were conducted with a mixture of fruit peels and different soil inocula. The first study was conducted with soil inoculum, second with cow dung and third with sludge and test tubes were incubated for 65 days. Various parameters were analyzed for each sampling day and the results for each parameter are discussed here.

Dissolved Oxygen

Average initial DO concentrations in experiments with soil inocula, cow dung and sludge were observed to be 6.5, 6.1 and 6.06 mg/L, respectively. It ranged from 5.5 to 6.86 mg/L for soil inocula, 5.56 to 6.23 mg/L for cow dung and 5.66 to 6.5 mg/L for sludge during the 65 day incubation period. The results indicate that completely aerobic conditions were maintained during incubation. Average DO on the final day was 5.67 mg/L in the biotic samples while it was 6.1 mg/L in the control indicating greater DO consumption in biotic samples as compared to controls.

pH

Variations in pH in each study are shown in Figure 3. Average initial pH in experiments with soil inocula, cow dung and sludge was observed to be 6.59, 6.54 and 6.77, respectively. It ranged from 4.95 to 7.34 for soil inocula, 5.54 to 7.63 for cow dung and 7.12 to 8.32 for sludge during the 65-day incubation period. Final pH for each study was 4.95 for soil inocula, 5.76 for cow dung and 7.37 for sludge. The low initial pH and subsequent decrease in pH after biodegradation indicates the greater acidity of fruit waste as compared to other organic fractions of MSW such as paper, leaf litter and floral waste (Kandakatla et al., 2013; Ranjan, 2016).

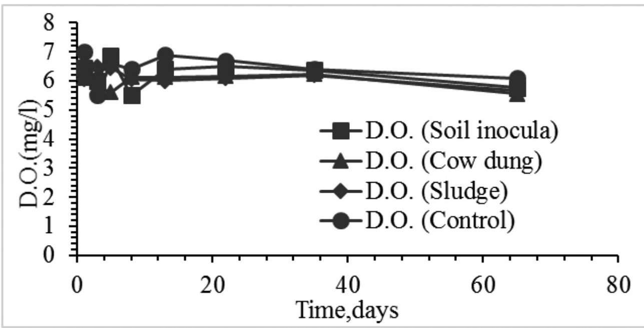

Figure 2: Variation in D.O. during incubation.

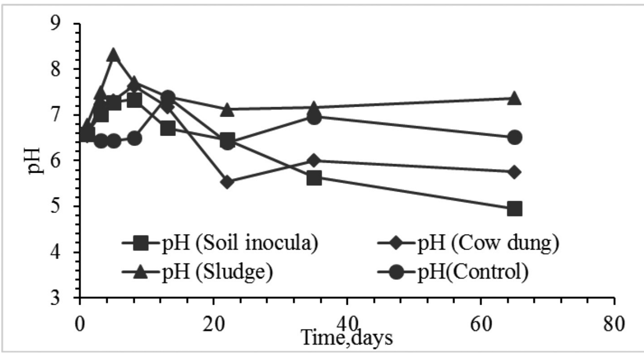

Figure 3: Variation in pH during incubation.

Conductivity

Average initial conductivity in experiments with soil inocula, cow dung and sludge was observed to be 1600, 1087.33 and 1119.66 µS/cm, respectively. Conductivity ranged from 553.66 to 1719 µS/cm for soil inocula, 856.96 to 1571 µS/cm for cow dung and 842.56 to 1545.67 µS/cm for sludge during the 65-day incubation period as shown in Figure 4. Final conductivity was 553.7 µS/cm for soil inocula, 857 µS/cm for cow dung, and 842 µS/cm for sludge.

Conductivity increased until day 4 for the study with soil inocula, until day 13 for cow dung and sludge and until day 21 for control followed by a steady decrease in all studies. Increase in conductivity during incubation is attributed to solubilization of solid waste material while the decrease in conductivity can be attributed to adsorption of ions to suspended solids or uptake of nutrients (as ions) by biomass.

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

Variations in TSS concentrations are shown in Figure 5. Average initial TSS concentrations in experiments with soil inocula, cow dung and sludge were 0.532, 0.809 and 0.655 g/l, respectively. TSS ranged from 0.143 to 0.532 g/l for soil inocula, 0.2 to 0.809 g/l for cow dung and 0.211 to 0.655 g/l for sludge during the 65-day incubation period with final concentrations of 0.143 mg/l, 0.2 mg/l and 0.211 mg/l for soil inocula, cow dung and sludge, respectively.

Variations in VSS concentrations are shown in Figure 6. Average initial VSS in experiments with soil inocula, cow dung and sludge were observed to be 0.498, 0.71 and 0.467 g/l respectively. VSS ranged from 0.148 to 0.498 g/l for soil inocula, 0.123 to 0.71 g/l for cow dung and 0.186 to 0.467 g/l for sludge during the 65-day incubation period. The lower initial TSS and VSS concentrations in the control samples were due to loss of suspended solids during autoclaving.

TSS removals were observed to be 73.12% while VSS removals were 70.12% after 65 days. TSS decreased up to day 21 while VSS decreased upto day 8 and was stable after that indicating that the organic fraction of fruit peels waste was rapidly biodegraded. In the control set, there was a slight decrease in TSS and VSS concentration due to solubilization of organic material present in the solution.

Heterotopic Plate Count (HPC)

Variations in bacterial concentrations are shown in Figure 7. Average initial HPC in experiments with soil inocula, cow dung and sludge were observed to be 2.57 × 10^9 cfu/mL, 2.48 × 10^9 cfu/mL and 2.18 × 10^9 cfu/mL, respectively. Maximum bacterial concentrations

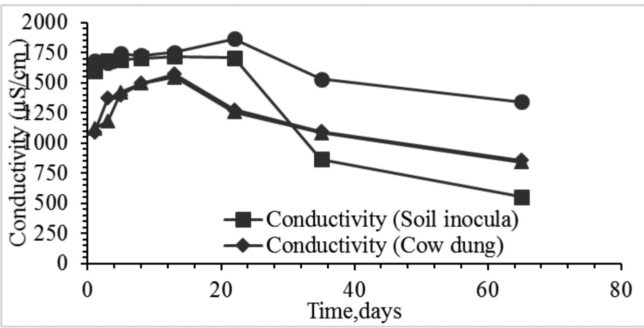

Figure 4: Variation in conductivity during incubation.

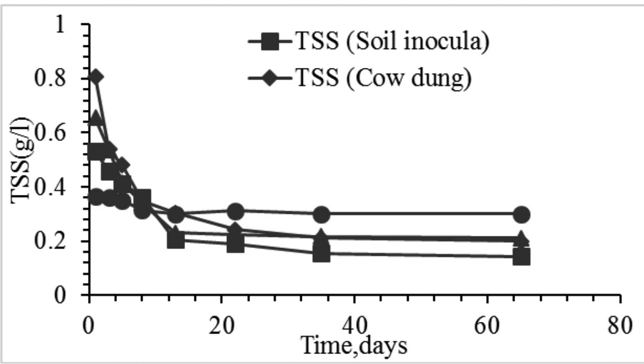

Figure 5: Variation in TSS during incubation.

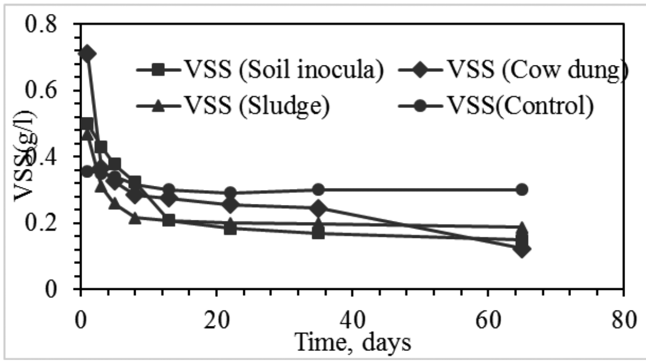

Figure 6: Variation in VSS during incubation.

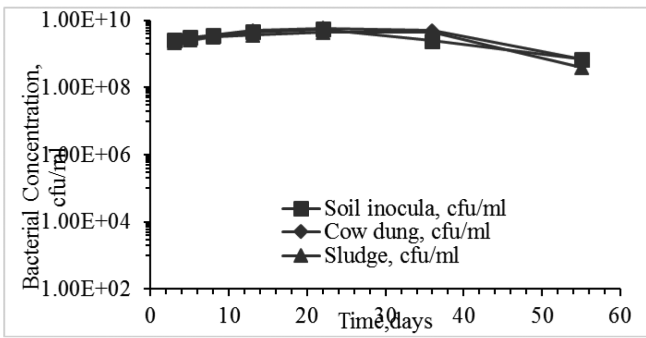

Figure 7: Variations of bacterial concentration during incubation.

in biotic samples were 5.55×10^9 cfu/mL, 5.92×10^9 cfu/mL, and 4.47×10^9 cfu/mL, respectively. No colonies were detected in the control samples indicating that degradation in these test tubes was due to abiotic processes only.

Determination of Reaction Rate Constants

Reaction rate constants were determined using the results obtained from batch experiments. The following differential equation was used to determine the order of reaction (n) and the reaction rate constant (K) for each study (Sengupta, 2014). The results are summarized in Table 2.

$$dC/dt = -KC^n$$

The linear form of the above equation was used to determine the reaction rate constant, K, and the order of reaction, n, as shown below.

$$\ln(-\Delta C/\Delta t) = \ln K + n \times \ln C$$

Graphs were plotted for $\ln(-\Delta C/\Delta t)$ versus $\ln C$ and the reaction order, reaction rate constant and regression coefficients (R^2) were determined for TSS and VSS concentrations in studies with different inocula. Results of the regression analysis are shown in Figures 8 to 10 for TSS and Figures 11 to 13 for VSS.

Table 2: Results of batch biodegradation of fruit waste with different inocula

	TSS			*VSS*		
	Soil inocula	*Cow dung*	*Sludge*	*Soil inocula*	*Cow dung*	*Sludge*
% removal efficiency	73.12	**75.27**	67.78	70.12	**82.67**	60.17
Order	3.39	3.973	5.14	3.673	4.5	6.712
K value	**2.45**	2.15	0.729	**1.506**	0.966	0.032
R square	0.9087	0.9161	0.8772	0.9355	0.8516	0.8282

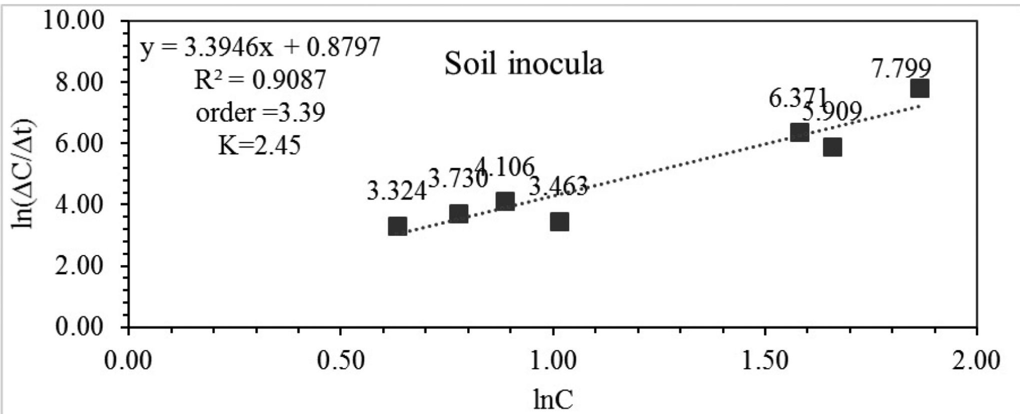

Figure 8: Regression analysis for degradation of TSS using soil inocula.

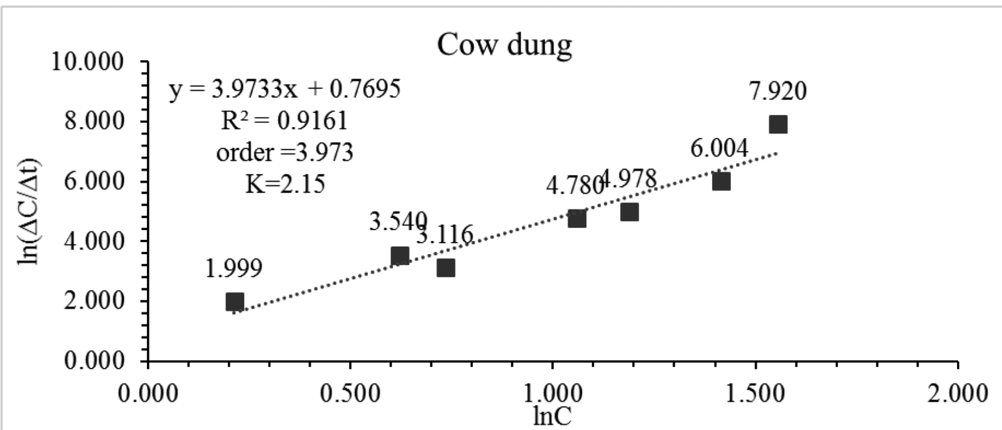

Figure 9: Regression analysis for degradation of TSS using cow dung inocula.

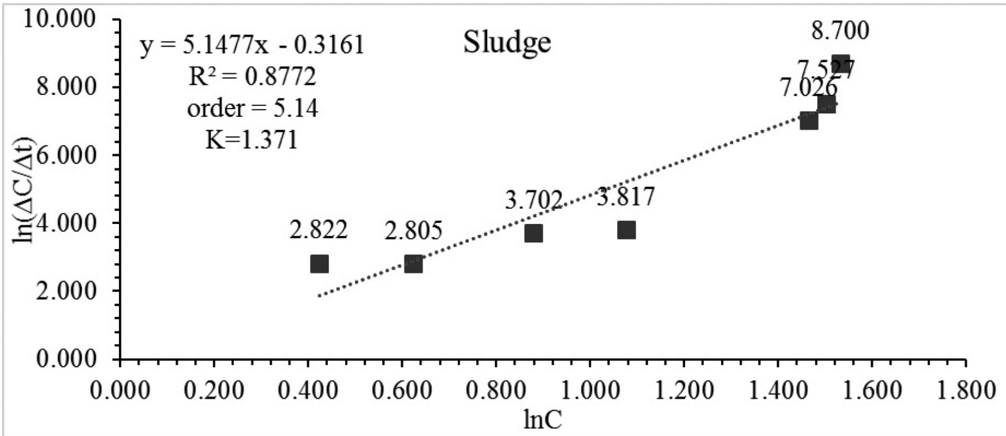

Figure 10: Regression analysis for degradation of TSS using sludge inocula.

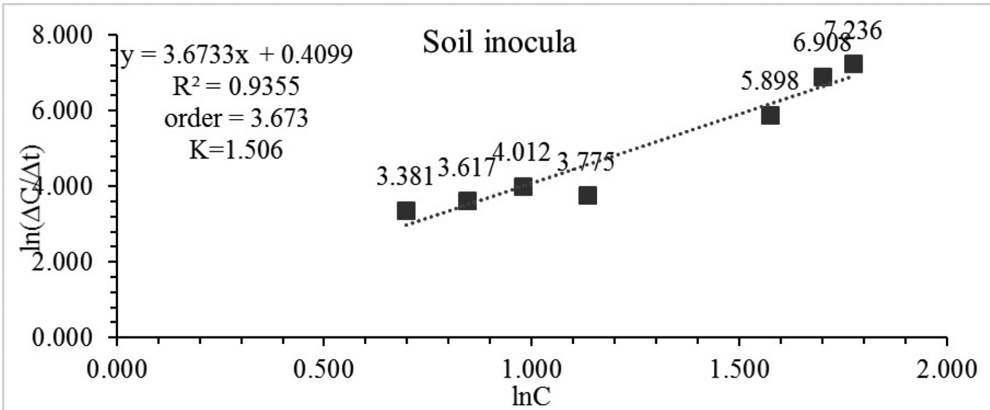

Figure 11: Regression analysis for degradation of VSS using soil inocula.

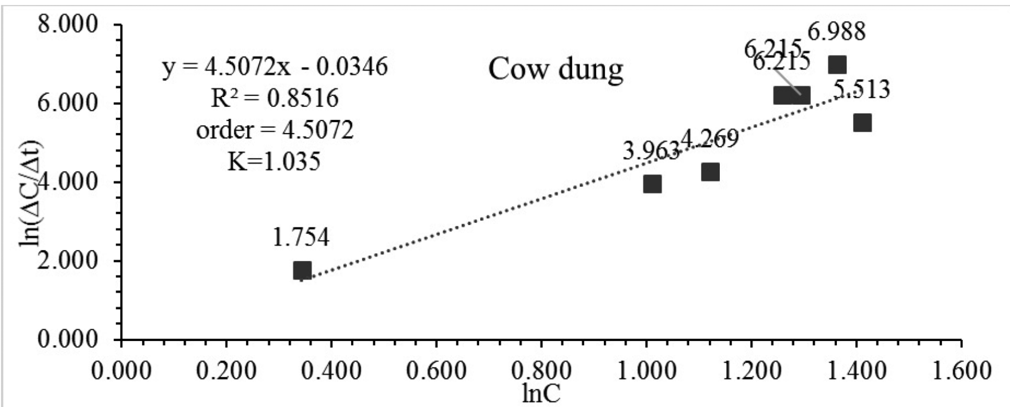

Figure 12: Regression analysis for degradation of VSS using cow dung.

Summary for the Batch Biodegradation Studies

Batch biodegradation studies with fruit waste and three different types of inocula were conducted and aerobic conditions were maintained throughout the 65-day incubation period. The results are summarized in Table 2.

- In general, conductivity increased up to 22 days and decreased after that in all cases. The first phase indicates solubilization of solid material and the second phase may be indicative of nutrient uptake or adsorption of dissolved ions.
- pH varied from 4.95 to 8.32 during the study indicating the release of acids from solid fruit waste during incubation. The final pH was much lower

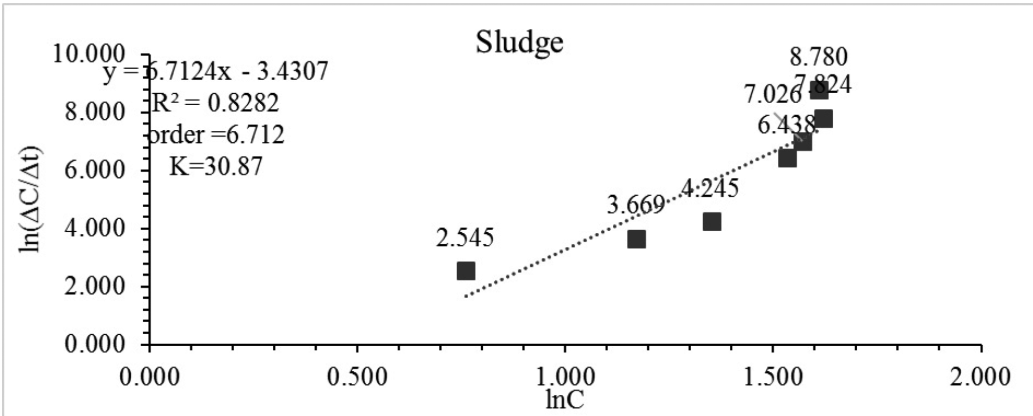

Figure 13: Regression analysis for degradation of VSS using sludge.

than the initial pH for soil inocula and cow dung but was higher than the initial pH for sludge.

- TSS was observed to be 75.27% removal and VSS was observed to be 82.67% removal after 65 days. TSS decreased up to day 21 and was stable after that. VSS decreased up to day 8 and was stable after that indicating that the organic fraction of fruit peels waste was biodegraded rapidly. In the control set, there was a small decrease in TSS and VSS concentrations during incubation due to solubilization of organic material present in the solution.
- Maximum bacterial concentrations were observed in all three studies indicating some increase in biomass due to biodegradation of fruit waste.
- Removal efficiencies for TSS and VSS were highest with cow dung inocula and lowest with sludge inocula. Rate constants for biodegradation of TSS and VSS were highest for soil inocula and lowest for sludge inocula. The reaction orders in all data sets ranged from 3.4 to 6.7 indicating the complexity of the degradation process.

Conclusions

Aerobic batch biodegradation studies were conducted with fruit waste under optimum nutrient conditions for a period of 65 days using soil, cow dung or wastewater sludge as inoculum. Major findings from these experiments were:

1. Mixed fruit was biodegraded rapidly with TSS stabilizing after 21 days and VSS after eight days.
2. Highest removal efficiencies (75.3% for TSS and 82.7% for VSS) were obtained with cow dung inocula while lowest were observed with sludge inocula.
3. Rate constants were highest for soil inocula and lowest for sludge inocula.

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