Global NEST Journal, Vol 25, No 9, pp 133-141

Experimental investigation of co-composting of gelatine industrial sludge combined with slaughter house waste and rice straw

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Received: 04/01/2023, Accepted: 18/09/2023, Available online: 24/09/2023
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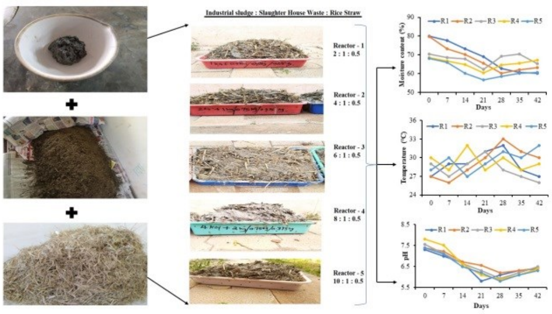
https://doi.org/10.30955/gnj.004725

Graphical abstract



Abstract

This work illustrates the co-composting of Gelatine Industry Sludge (GIS) combined with organic fraction of Slaughter House Waste (SHW) and Rice Straw (RS) employing 10% zeolite mixed with Enriched Nitrifying Bacteria Consortium (ENBC). Five piles of GIS will be prepared and mixed with SHW and RS at 2:1:0.5, 4:1:0.5, 6:1:0.5 and 8:1:0.5 and without GIS 0:1:0.5 (dry weight basis) served as control, while ENBC was inoculated in all piles and Windrow composted for 42 days. After completing the composting process to characterized to determine, Moisture content, Temperature pH, EC, TOC, TKN, P, K, C/N Ratio, Bacteria, Fungi, Actinomycetes as per the standard methods (Indian Standard). To find out the co-variations of the samples, and Least Significant Difference value calculated for each parameter. The reactor 4 with GIS, SHW and RS ratio 8:1:0.5 were reduced the nitrogen loss and co variations is very low. The co amendment of 8% Gelatine Industrial Sludge effectively buffered the pH between 6.5-8, while lower concentration of the Gelation Industrial Sludge was comparatively delayed the early decomposition. Therefore, our results suggested that suitable of initial sample ratio 8:1:0.5 as the best formulation for the composting of gelatine industrial sludge into value added stable product.

Keywords: Windrow compost, gelatine industrial sludge, slaughterhouse waste, enriched nitrifying bacterial consortium, co-variation

1. Introduction

In certain industrial effluents and municipal wastewater are often characterised by higher concentration of organic substance and nutrients such as nitrogen and phosphorus (Bhattacharya *et al.*, 2021; Hu *et al.*, 2021). Without proper treatment process, the disposal of wastewater into water bodies activating excessive bacterial growth and threating to aquatic ecosystem (Yan *et al.*, 2022). In general, the concentration of nitrogen and phosphorus are high in municipal wastewater. Especially amount of nitrogen species in wastewater plays a major role in treatment process (Wang *et al.*, 2021). Nitrogen is a more significant nutrient for all living organisms however, excessive amount of nitrogen discharge in water bodies can lead an abnormal growth of algae (called as eutrophication) and thus reduce the dissolved oxygen in water (Chaali *et al.*, 2018; Chen *et al.*, 2018)). In recent years, environmental laws and regulation are highly recommended to give more attention for efficient removal of nitrogenous species in wastewater before disposal into waterbodies (Estévez-Alonso *et al.*, 2021). Because, nitrogen is one of the primary elements causes the eutrophication and deterioration of water resources (Li *et al.*, 2022).

In recent days, several methods and technologies have been applied to reduce the amount of nitrogen transported into the aquatic environment. The conventional methods of biological process to treat the municipal wastewater are facing challenges like greenhouse gases emission (eg. Methane, Nitrous oxide and carbon dioxide), huge amount of activated sludge generation, high energy consumption and no energy recovery (Di *et al.*, 2013). Compared to physical and chemical methods, biologically removal of nitrogen is more efficient and are considered to be an eco-friendly wastewater treatment process. It is preferred to be removed biologically through the simultaneous nitrification and denitrification (SND) processes are performed in the same reactor, even if the two process requires two antipodal environmental conditions. SND is an efficient method to remove nitrogen in a single bioreactor under limited operating parameters such as carbon-nitrogen(C/N) ratio, dissolved oxygen concentration, hydraulic retention time (HRT), aeration pattern, pH, alkalinity and temperature (Bernet *et al.*, 2001). In recent days, many researchers were focused to understand the mechanisms and effects of various environmental conditions on SND and its combination with phosphorus removal (Bueno *et al.*, 2018). The removal process of nitrogen comprising autotrophic nitrification and heterotrophic denitrification have both significant and cost-effective advantages important to their extensive use around the world (Bagchi *et al.*, 2012).

The mechanism of SND to be determined by the function of autotropic nitrifiers and anoxic denitrifiers. The heterotrophic microorganisms are not prevailing for nitrogen removal in aerobic granular sludge (Zhao *et al.*, 2013). Consequently, recent investigation of the nitrogen removal mechanisms revealed that the high loading rate of nitrogen and COD in the heterotopic aerobic granular sludge helps to improve the nitrogen removal and efficiency of SND process. The aerobic nitrifying granular sludge in SND is the complex structure of autotropic ammonia oxidizing bacteria (AOB) on the outer layer and anoxic denitrifiers in the inner face (Chen *et al.*, 2015). In the complex structure, heterotrophic microorganisms were appearing and overlap with autotrophic microorganism (Duan *et al.*, 2015). Increase of COD-nitrogen concentration in influent increase the number of heterotrophic microorganisms and meanwhile decreases the AOB and nitrite oxidizing bacteria (NOB) (Duan *et al.*, 2022).

Heterotrophic nitrification process delivers a clear pathway for nitrogen removal differ from the autotropic nitrification process (Gupta *et al.*, 2022). The heterotrophic microorganisms convert ammonia into gaseous nitrogen and discharge it out of the bioreactor by using organic carbon source and it proven the simultaneous removal of COD and nitrogen in wastewater. In recent research divulged that, at high C-N ratio is primary reason for presence of high quantity of heterotrophic nitrifiers in aerobic granular sludge. During the nitrification process, alkalinity is consumed and produced during the denitrification process, accordingly neutral pH and less amount of alkalinity are most favourable condition for SND process (Di *et al.*, 2022). Also, amount of oxygen consumption may be reduced because NO3-N can be utilized as the alternate electron acceptor and very low dissolved oxygen (DO = 0.1 to 1.0 mg/L) is more sufficient for SND process (Ravishankar *et al.*, 2022). However, critical experimental and comparison studies of these techniques are needed to strengthen and identify the optimum value for each parameter in the SND process. The present research work is to fulfil the research gap identified in the previous research and objectives of the present work is to investigate the co-compositing process of gelatine industrial sludge combined with slaughter house waste and rice straw with different ratio.

2. Materials and methodology

2.1. Gelatine waste

Gelatine is most widely used food additives in food and non-food industries, it can be obtained from the denaturation of collagen. Gelatine used as water soluble compound to enhance the stability and consistency of food in food industry, soft and hard capsules, adsorbent pads and wound dressing in non-food industries like medical and pharmaceutical industries. Traditionally two methods have been employed to extract gelatine such as acid and alkali extraction (Arturi *et al.* 2019; Awasthi *et al.* 2016). In the present work, Gelatine extraction is carried out by acid extraction method. Extraction has to be carried out at optimum temperature. It has been reported that lower temperature results in fewer yields and higher temperature leads to lower gelatine quality (Figure 1). Gelatine industry sludge is considered to be one of the most abundant pollutants all over the world. So far there is only minimal number of studies associated with gelatin industry effluent treatment by composting. The composting is the novel technology consists of both aerobic and anaerobic treatment. The current study investigates the feasibility of employing composting to treat the gelatine industry sludge and to generate Fertilizer. Gelatine industry sludge used in this study was collected from gelatine production industry, located at Semmankuppam, Cuddalore District, Tamil Nadu, India. Slaughterhouse waste used in this study was collected from the Modern slaughterhouse commissioned by Department of Animal husbandry and welfare, Government of Tamil Nadu located at Kalanivasal, Karaikudi, Sivagangai District, Tamil Nadu, India. Rice straw used in this study was collected from Pudhuvayal, Sivagangai. The sample was collected as a grab sample and thus collected was preserved as stock solution in the refrigerator and used for the studies when required by diluting the stock solution to avoid fault results. (Table 1)

2.2. Slaughter house waste and rice straw

Slaughter house waste comprise of the indigestible part of animals derived from the food processing industries, meat production industries and other animal by products. The meat production industries produce huge amount of slaughter house waste due to the slaughtering of animals and cleaning of the slaughter house facilities. It is necessary to treat the slaughter house waste to maintain the sustainable environment. In the present study, slaughter house waste was used for co-compositing process and presented in Figure 2. Rice straw is another by-product of rice production during harvesting was used in the present study. It can be characteristically used to strengthen the soil properties, soil condition through composting and carbonization. In the present study, rice straw has been used as co-compositing material and it shown in Figure 3. The different ratio of gelatine industry sludge, slaughter house waste, rice straw is 2:1:0.5, 4:1:0.5, 6:1:0.5, 8:1:0.5 and 10:1:0.5 are carried out in the present study. The gelatine industry sludge, Slaughter House Waste, Rice straw collected was characterized to determine Free air space, moisture content, temperature pH, electrical conductivity, total organic carbon, phosphorus, potassium, C/N ratio, bacteria, fungi, actinomycetes as per the standard methods (Figures 4 and 5) recommended by Test methods for the examination of composting and compost (TMECC 2002), US department of Agriculture (USDA).

Figure 1. Gelatine Industry Sludge

Figure 2. Slaughter House Waste

Figure 3. Rice Straw

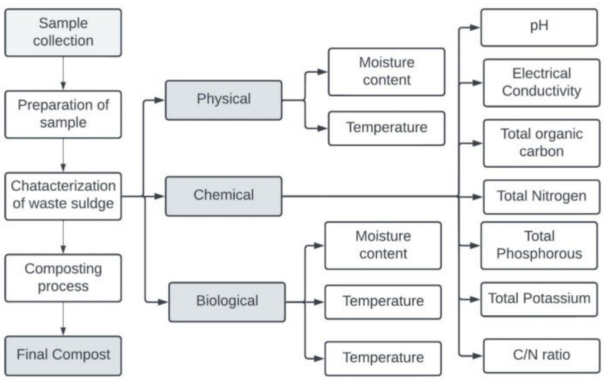


Figure 4. Methodology of the present research work

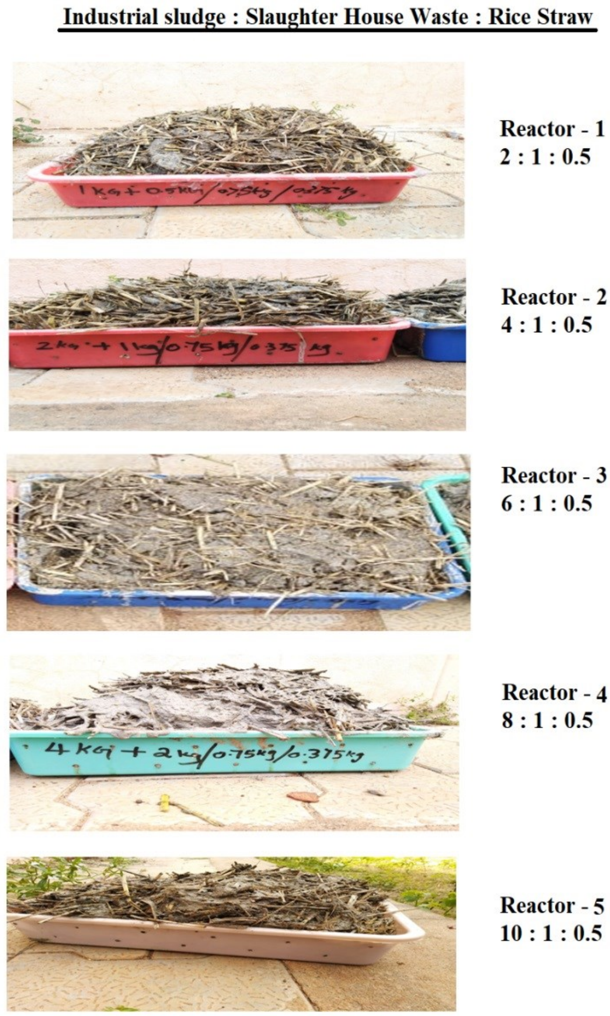


Figure 5. Reactor setup for compositing process

2.3. Co-variance of the testing results

In the present study, the testing results were analysed by co-variance methods. The covariance measures the variation relationship between the testing results. The positive value of the covariance indicates the standard deviations between analysed results.

$$CV = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{N-1} \quad (1)$$

Where, xi, yi are the results of the different variation of the mix, x and y are the mean of the xi and yi and N is the number of data values.

3. Result and discussion

3.1. Effect of moisture content (EoMC)

The moisture content is a most significant parameter to affect the process of decomposition in the reactor (Lone *et al.* 2019). The MC was decreased after day 7 and start to decomposition of material due to growth of microorganisms in the reactor. It has to maintain the optimum moisture level of 60-65% throughout the period of reaction. During decomposition process, it observed 40% of the moisture content at the end of 42 days (Figure 6). The EoMC results reveal that, high MC in the reactor decreases the activity of microorganism and simultaneously decreases the degradation process. The

MC covariation of each sample for entire period of analysis shows that reactor 4 (8:1:0.5) has the minimum and reactor 1 (2:1:0.5) has the maximum variation during the period of decomposition (Table 2).

Table 1. Characteristics of gelatine industry sludge, slaughter house waste and rice straw before treatment

S.No	Parameter	Unit	Gelatine Industry Sludge	Slaughter House Waste	Rice straw	Testing methods
1	Moisture Content	%	65.9	0.03	11.11	TMECC 2002
2	Temperature	°C	30	28	29	
3	pH	-	6.74	6.52	6.78	
4	Electric Conductivity	µS cm^{-1}	0.88	1.2	2.1	
5	Total Organic Carbon	%	29.84	49.8	57.72	
6	Total Kjeldhal Nitrogen	%	0.84	1.84	1.2	
7	C/N Ratio	-	35.52	27.06	48.1	
8	Total Phosphorous	%	2.82	0.34	0.36	
9	Total Potassium	%	0.72	0.81	0.89	
10	Bacteria	CFU g^{-1}	16.2 x10^{6}	10.2 x10^{6}	3.2 x10^{6}	-
11	Fungi	CFU g^{-1}	10.7 x10^{4}	5.7 x10^{4}	2.7 x10^{4}	
12	Actinomycetes	CFU g^{-1}	6.3 x 10^{2}	4.3 x 10^{2}	2.3 x 10^{2}	

Table 2. Effect of moisture content (%)

Reactor	Days							Co-Variation (%)
	0	7	14	21	28	35	42	
R-1	80.01	77.56	73.2	69.01	62.7	60.5	60.1	10.64
R-2	79.7	73.26	70.07	65.4	60.08	62.1	63.2	9.4
R-3	70.4	68.52	67.83	62.21	69.3	70.3	65.3	4.4
R-4	68.6	66.58	64.77	60.3	64.5	65.4	67.1	3.7
R-5	68.02	65.89	60.1	56.7	58.4	60.2	60.6	6.0

3.2. Effect of Temperature

Temperature is an essential parameter which affect the process of microbial activities in the reactor system (Sabzi *et al.* 2019). In the present investigation, temperature increases with decrease in MC and simultaneously decreases the microbial activity in the reactor. Beginning of the experiments, the temperature has increased rapidly due to biodegradation of organic matters in reactor. The rapid variation of temperature has been achieved at 28 days in all set of reactors, especially the peak temperature was recorded in reactor 3 and 4 during day 14 and 21 respectively (Figure 7). The results indicates that, presence of microorganism and nutrients in suspended solid could facilitate the biodegrading of organic matters and releases metabolic heat during composting process. In R3 and R4 reactors shows high variation of temperature during the experimental period compared with other reactors. It indicates that reactor supplies more nutrients for bacterial growth and explosion during compositing process. Awasthi *et al.* 2016 carried out the detail investigation in gelatine industry sludge mixed with slaughter house waste and rice straw and stated that temperature during composting process was increased more significantly during initial period of investigation. The temperature co-variation of all reactor shows that reactor-4 has minimum variation and reactor-2 has maximum variation during the period of investigation (Table 3).

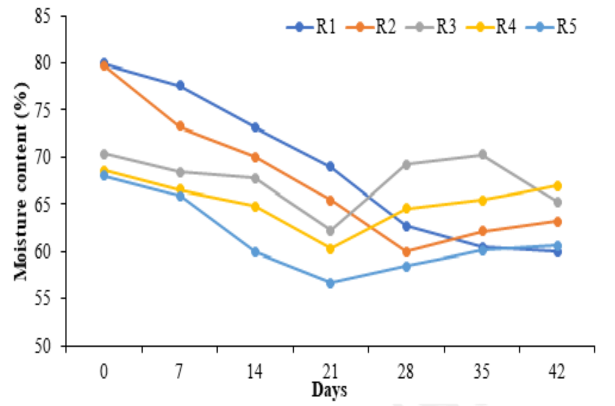


Figure 6. Moisture content variation during the period of investigation

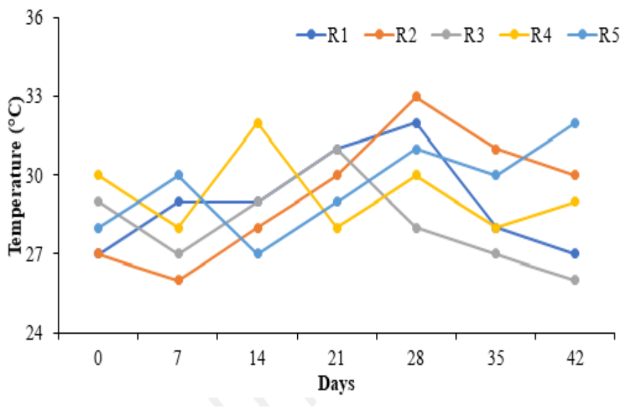


Figure 7. Temperature variation during the period of investigation

Table 3. Effect of Temperature (C)

Reactor	Days							Co-Variation (%)
	0	7	14	21	28	35	42	
R-1	27	29	29	31	32	28	27	6.1
R-2	27	26	28	30	33	31	30	7.6
R-3	29	27	29	31	28	27	26	5.5
R-4	30	28	32	28	30	28	29	4.7
R-5	28	30	27	29	31	30	32	5.37

Table 4. Effect of pH (no unit)

Reactor	Days							Co-Variation (%)
	0	7	14	21	28	35	42	
R-1	7.3	7	6.68	5.8	6.1	6.3	6.4	7.39
R-2	7.4	7.23	6.72	6.56	6.21	6.31	6.43	6.3
R-3	7.56	7.12	6.65	6.3	5.93	6.2	6.5	7.8
R-4	7.8	7.52	6.63	6.1	5.9	6.1	6.4	10.36
R-5	7.4	7.1	6.5	6.2	5.8	6.1	6.3	8.1

3.3. Effect of pH

pH is another important parameter that primary influenced the microbial activity and composting process (Lone *et al.* 2019). In the present investigation, gelatine industrial sludge also partial affect the pH of the reactor and it shows that 6.2 to 7.8 during the initial days. pH value of all rector has been decreased during the initial process (28 days) and increased gradually at the end of the period (Figure 8). It shows that, decreased value of pH indicates that decomposition of organic matters affects the reactor pH value and increase in growth of microorganisms might be stable the pH value of reactor. The co-variation of all reactor shows that, minimum and maximum variation was recorded in reactor 2 and 4 respectively (Table 4).

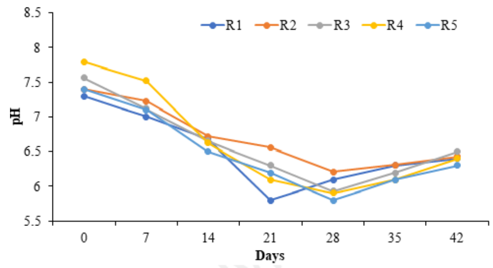


Figure 8. pH variation during the period of investigation

3.4. Effect of electric conductivity

The effect of electrical conductivity plays a vital role in microbial culture and reactor. In the present study, electrical conductivity of each reactor has been examined and the results shows that, the value was gradually increased with increase in time. It indicates that salinity of the reactor system has been increased with increase in biodegradation of the organic matter in gelatine industrial sludge (Figure 9). The observed covariance of EC value was minimum in reactor 1 and maximum in reactor 5. It divulged that increasing percentage of gelatine industrial sludge increases the electrical conductivity of the reactor (Table 5).

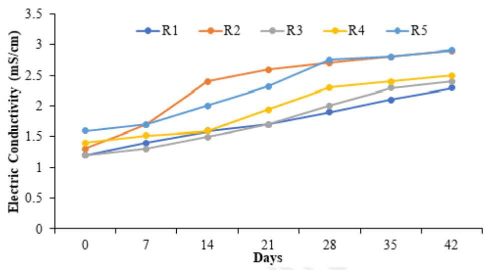


Figure 9. Electrical conductivity variation during the period of investigation

3.5. Effect of Potassium

The sludge from the gelatine industrial contains large amount of phosphorous, nitrogen, potassium, carbon and other organic matters (Tawfik *et al.* 2021). In present investigation, the amount of potassium was overserved very low during initial period (0 and 7 days). It remains constant until the 35 days and gradually increased at the end of the period (Figure 10). The results revealed that, potassium slightly affect the growth of microorganism and biodegradation process during composting. The overserved covariance of potassium was minimum and maximum in reactor 2 and 5 respectively (Table 6).

Table 5. Effect of Electric Conductivity (mS/cm)

Reactor	Days							Co-Variation (%)
	0	7	14	21	28	35	42	
R-1	1.2	1.4	1.6	1.71	1.9	2.1	2.3	20.5
R-2	1.3	1.7	2.4	2.6	2.71	2.8	2.9	24.06
R-3	1.2	1.3	1.5	1.7	2	2.3	2.4	24.8
R-4	1.4	1.52	1.6	1.94	2.31	2.4	2.5	21.59
R-5	1.6	1.7	2	2.32	2.75	2.8	2.91	21.8

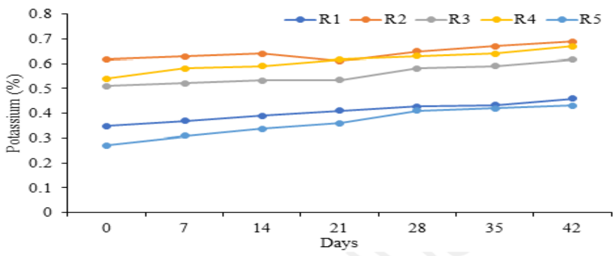


Figure 10. Potassium variation during the period of investigation

3.6. Effect of Phosphorous

In the present investigation, collected sludge from the gelatine industry contains larger amount of phosphorous. During the decomposition of organic matters in sludge, the amount of phosphorous was gradually increased at the end of the period. The results divulged that, increase in count of microorganisms increases the concentration of phosphorous in the system and it positively effect on the plant growth (Figure 11). All the reactor shows that gradual increase in amount of phosphorous with respect to time. The covariance of the phosphorous shows that minimum and maximum values were recorded in reactor 5 and 2 respectively (Table 7)

Table 6. Effect of Potassium (%)

Reactor	Days							Co-Variation (%)
	0	7	14	21	28	35	42	
R-1	0.35	0.37	0.39	0.41	0.43	0.433	0.46	8.7
R-2	0.62	0.63	0.64	0.61	0.65	0.67	0.689	3.3
R-3	0.51	0.52	0.532	0.534	0.58	0.59	0.62	6.9
R-4	0.54	0.58	0.59	0.62	0.631	0.64	0.67	6.5
R-5	0.27	0.31	0.34	0.36	0.41	0.42	0.432	15.5

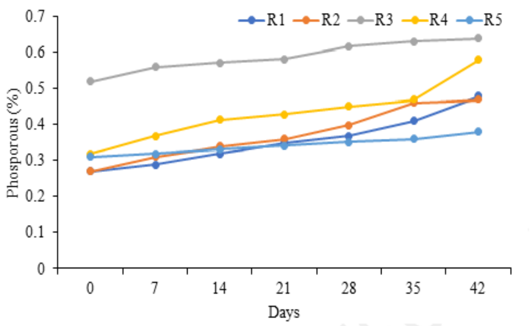


Figure 11. Phosporous variation during the period of investigation

3.7. Effect of total nitrogen

Total nitrogen is an important parameter in the microbial growth and decomposition of organic substance in the reactor (Yu *et al.* 2003). The total nitrogen content was highly dominated by the presence of ammonia content and the loss of weight of dry matters during decomposition process. In the present investigation, the total nitrogen content was increased by 48 to 79% in the reactor 1 to 5 respectively (Figure 12). It indicates that increase in amount of slaughter house waste increase the concentration of total nitrogen in residual material at the end of the biodegradation meanwhile it reduces the total mass. The covariance of total nitrogen shows that minimum and maximum variation was recorded in reactor 4 and reactor 5 respectively (Table 8).

Table 7. Effect of Phosphorous (%)

Reactor	Days							Co-Variation (%)
	0	7	14	21	28	35	42	
R-1	0.27	0.29	0.32	0.35	0.37	0.41	0.48	18.8
R-2	0.27	0.31	0.34	0.36	0.4	0.46	0.47	18.56
R-3	0.52	0.56	0.571	0.582	0.62	0.631	0.64	16.6
R-4	0.32	0.37	0.412	0.43	0.45	0.47	0.58	17.55
R-5	0.31	0.32	0.331	0.342	0.351	0.36	0.38	16.1

Table 8. Effect of total nitrogen (%)

Reactor	Days							Co-Variation (%)
	0	7	14	21	28	35	42	
R-1	0.7	0.83	0.91	1.1	1.12	1.2	1.25	18.6
R-2	0.75	0.78	0.84	0.96	1.11	1.21	1.3	20.2
R-3	0.81	0.84	0.87	0.871	0.95	1.25	1.32	19.5
R-4	0.91	0.95	0.98	1.121	1.32	1.34	1.35	15.9
R-5	0.62	0.691	0.751	0.78	0.81	0.95	1.31	25.11

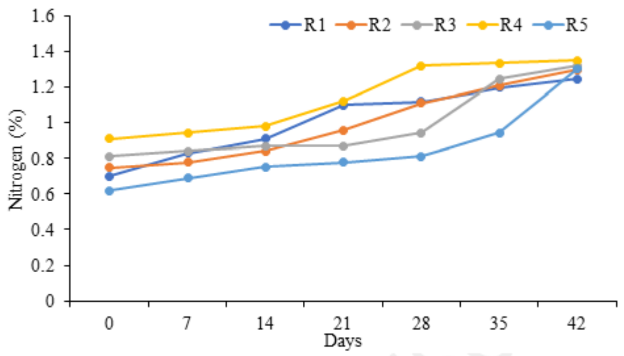


Figure 12. Nitrogen variation during the period of investigation

3.8. Effect of total organic carbon

Total organic carbon is most significant parameter in microbial growth and composting process. In present investigation, total organic carbon content gradually reduced with increase in slaughter house water proportion and time period. About 10-20% of the total carbon content was reduce during the period of compositing. It reduces the mass of waste material and help to improve solid waste management. In reactor 3 and 4 shows higher percentage of loss of organic matters and total carbon content during the period of experiment (Figure 13). The covariance of total carbon content was observed as minimum and maximum in reactor 3 and 1 respectively (Table 9).

3.9. Effect of C/N Ratio

The effect of C/N ratio plays a vital role in stabilization of solid and soluble phase during composting process. In present study, C/N ratio was reduced 42 to 56% in reactor 1 to 5 during the entire period of investigation (Table 10). The results show that, biodegradation of organic matters, reduce volume of waste, and reduce total carbon content during the period of 0 to 42 days (Figure 14). It helps to enhance the growth of plants and easy to handle the sludge after treatment. In previous research and studies stated that sludge is harmless and it can use as fertilizers for particular plants and partial replacement material in brick manufacturing process.

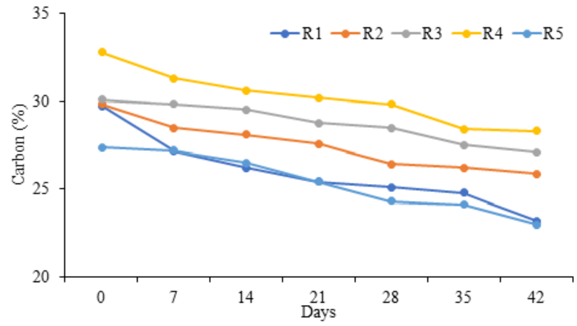


Figure 13. Carbon content variation during the period of investigation

Table 9. Effect of Carbon (%)

Reactor	Days							Co-Variation (%)
	0	7	14	21	28	35	42	
R-1	29.72	27.16	26.21	25.4	25.1	24.8	23.2	7.3
R-2	29.8	28.5	28.1	27.6	26.4	26.2	25.9	4.7
R-3	30.1	29.8	29.5	28.8	28.5	27.5	27.1	3.6
R-4	32.8	31.3	30.6	30.2	29.8	28.4	28.31	4.8
R-5	27.4	27.2	26.5	25.4	24.3	24.12	23	6.1

Table 10. Effect of C/N ratio (no unit)

Reactor	Days							Co-Variation (%)
	0	7	14	21	28	35	42	
R-1	42.5	32.7	28.8	23.1	22.4	20.7	18.6	28.8
R-2	39.7	36.5	33.5	28.8	23.8	21.7	20	29.4
R-3	37.2	35.5	33.9	33.1	30	22	20.5	20.12
R-4	36	32.9	31.2	26.9	22.6	21.8	20.97	20.14
R-5	44.2	39.4	35.3	32.6	30	25.4	17.55	25.5

3.10. Effect of Bacteria, Fungi, and Actinomycetes

The most important parameters are count of bacteria, fungi and actinomycetes in the co-composting process. In the present study, bacteria, fungi and actinomycetes count was increased during the beginning of the composting process and it has been decreased with respect to time increases (Figure 15a, b and c). It indicates that, the effect of moisture content, temperature and initial pH of each reactor are the major factor that dominating the process of compositing of gelatine sludge composition.

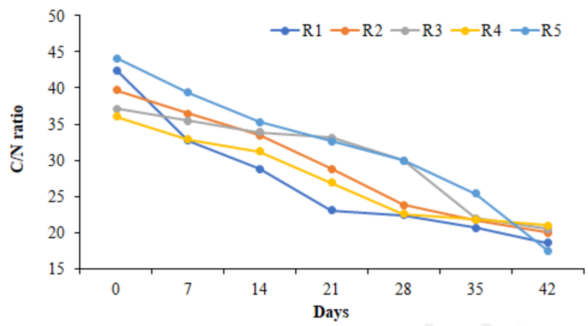


Figure 14. C/N ratio variation during the period of investigation

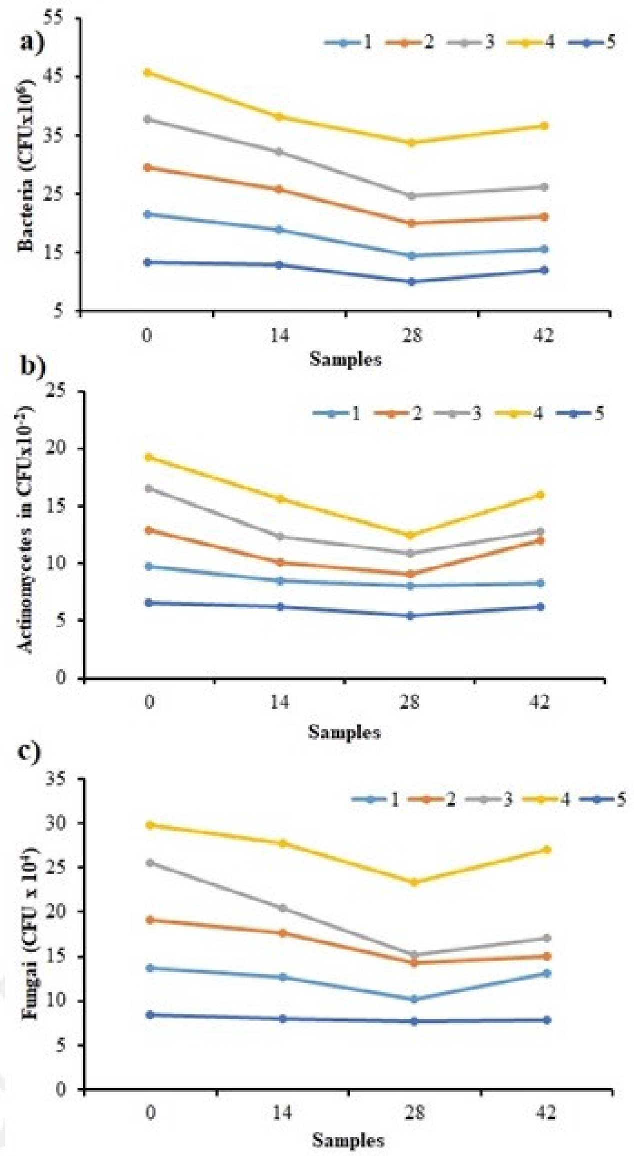


Figure 15. Effect of a) Bacteria, b) Fungi, and c) Actinomycetes

4. Conclusion

The initial characteristics of the gelatin industrial sludge, Slaughter house waste, Rice straw results given in this chapter. The results obtained while carrying out this work are discussed in detailed. Five piles of GIS will be prepared and mixed with Slaughter House Waste and Rice Straw at 2:1:0.5, 4:1:0.5, 6:1:0.5 and 8:1:0.5 and without GIS 0:1:0.5 (dry weight basis) served as control, while ENBC was inoculated in all piles and composted for 42 days. After complete the composting process to characterized to determine, Moisture content, Temperature pH, EC, TOC, TKN, C/N Ratio, P, K, Bacteria, Fungi, Actinomycetes as per the standard methods (Indian Standard). The sample 4 with Gelatin Industrial Sludge, Slaughterhouse Waste and Rice Straw ratio 8:1:0.5 were reduced the nitrogen loss. The co amendment of 8% gelatin industrial sludge effectively buffered the pH 6.5-8, while lower concentration of the gelatin industrial sludge was comparatively delayed the early decomposition. Therefore, our results suggested that suitable of initial sample ratio 8:1:0.5 as the best formulation for the composting of Gelatin industrial Sludge into value added stable product. The study recommended that co-composting process of gelatin industrial sludge is the most efficient and cost-effective technique. The limitations of the present research are odour, dust, reuse of final compost materials, long period of treatment process and preparation of site are the major factor that consider before co-composting process.

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