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Research paper

Laboratory Scale Modelling and the Treatment of Biomedical Wastes Through Herbaceuticals for Manure Production

E.D. Viswanath[1], N. Balasundaram*[2], T. Senthil Vadivel[3]

[1]*PG Student, Department of Civil Engineering, Karpagam Academy of Higher Education, Coimbatore – 641021. Tamilnadu.*
[2]*Professor & Head, Department of Civil Engineering, Karpagam Academy of Higher Education, Coimbatore – 641021. Tamilnadu.*
[3]*Professor & Head, Department of Civil Engineering, School of Engineering & Technology, Adamas University, Kolkata – 700126. West Bengal*
**Email: balasudaram49@gmail.com*

Abstract

Biomedical waste disposal practices are becoming important in both the developing and developed countries and need to be properly followed by the human being. An effective utilization of waste helps to have potent products like biofertilizer, bio-manure and also mineral resources like Ca, Mg, Fe, Co etc. The present study involves the treatment of biomedical waste by applying primary treatment using seaweeds (*Padina tetrastromatica* and *Amphiroa fragilissima*) and herboceuticals (turmeric & neem extracts). The experiments were designed to find out the optimum concentration of the substrate, incubation period and time in the treatment process. The secondary treatment process consisted of alkaline hydrolysis process with various concentrations to treat biomedical wastes. The bacterial load and count were analysed in all the treatment processes. The final characterization of the treated waste was analysed for the potential of bio-manure production. The result revealed that the bacterial reduction efficacy and residual mass was found to be 15000 CFU/mL and 0.94 g at the end of the 4th day, whereas the efficiencies optimized by RSM for bacterial count and residual mass were 16481.6 CFU/mL and 0.5426 g respectively. It was observed that bacterial count steadily decreased from 20000 to 5 CFU/mL of sample, when the temperature range varied from 30 to 75^0 C. The results of bio-manure production showed that nitrogen and phosphorous contents were enriched after the treatment procedures and the moisture content was reduced to < 2 %.

Key words: *Biomedical wastes, Herbaceuticals, seaweeds, bio-manure*

1. Introduction

The management of the biomedical waste is an integral part of traditional and contemporary system of health care (Rajan *et al.*, 2018). Proper biomedical waste management is an important factor in hospital cleanliness, hygiene, operational practices and maintenance activities. Effective BMW management (BMWM) is mandatory for maintaining a clean environment. (Datta *et al.*, 2018). According to Cointreau-Levine (1997), the decomposition of waste into constituent chemicals is a common source of local environmental pollution. Waste management is the actions required to manage waste from its inception to its final disposal (UNSD, 2017). It includes the collection, segregation, transport, treatment and disposal with proper monitoring under government regulation. Medical care system is essential for our life and health, but the wastes generated from the hospitals like tissues, fluids, organs etc., creates more problems to the environment. There is a huge amount of potentially infectious and hazardous wastes generated by the hospitals. The aim of the 3 R waste hierarchy is to utilize the maximum practical benefits in terms of biodegradable products to release the minimum amount of waste to the world (Albert and Raleigh, 2011). There should be an alternative method for the disposal of animal carcasses and slaughterhouse wastes, with the dual benefits of both eliminating waste material and producing energy (Insam *et al*., 2010). Most wastes are generated by the hospitals and medical clinics are non-hazardous general wastes from hospital organization activities such as human anatomical wastes, tissues, kitchen wastes, analytical laboratory wastes, office materials, workshop residuals, first aid packaging, used but emptied disposable bed liners, diapers and disposable masks. After the source segregation of recyclables, disposal is done typically by sanitary landfill. Biomedical general wastes of about 1.0 to 2.0 kg/bed/day and hazardous wastes of about 0.2 to 0.8 kg/bed/day are generated. The treatment of biomedical waste with viable technology is an urgent need eliminate the risks to humans and to environment. Zokaei *et al*., 2013.

The current disposal techniques adopted for the biomedical waste management are incineration and land fill. It provides uncontrollable process factors which may release toxic substances into the air. And keeping biomedical wastes in storage container for long period leads to adverse side effects like high virulence among pathogens. The present study focuses on the development of a compact biomedical digester for hospitals and health care industries as an effective tool. It has the application of in-house disposal system of biological fluids and liquids for managing biomedical wastes. It focuses on the effective management of biomedical wastes incorporating waste reduction and neutralization. Alkaline hydrolysis and the tissue digesters were originally developed for the disposal of the radioactive animal carcasses generated from biomedical and pharmaceutical research.

Plant species-mediated waste management will be the key idea of this current research work with cost effective strategy, when compared to conventional method. Seaweeds have good antimicrobial activity and bacteriostatic and bactericidal properties. *Padina tetrastomatica and Amphipora fragilissia* have good antibacterial activity (Sakthieswari and Srisudha, 2016).

Curcuma longa are commonly used as flavouring agents, preservatives and colouring agents and also in biomedical applications. The rhizome isolates of turmeric possess a wide range of biological activities (Tilak *et al*., 2004; Kumar *et al*., 2006), and antibacterial activity (Gupta and Sadhana 2005; Naz *et al*., 2010). Effective waste management consists of five steps such as monitoring, co-operation, collection, transportation and disposal of wastes.

Aim: The present study focuses on biomedical waste converted into manure with the seaweed.

2. Methodology

2.1 Material and Sampling Site

The biomedical wastes (Category 3 & 6) were collected from various hospitals in and around the Salem district of Tamil Nadu with prior permission. They were segregated based on different categories as per Biomedical Waste Management (BWM).

2.2. Collection of Seaweeds and Turmeric

Seaweeds such as *Amphiroa fragilissima* (KSR01) and *Padina tetrastromatica* (KSR02) were collected from Mandapam sea coast of Ramanathapuram, Tamil Nadu. It was authenticated by Rajendra Kumar, CSMCRI and voucher specimen was maintained at KSRCT culture collection centre, Namakkal, Tamil Nadu. Two varieties of turmeric samples (PTS10 and BSR) were collected from turmeric collection centre, Erode and were shade dried at 37º C for about 3 days. The samples were powdered and transferred to a sterile air tight container and stored at room temperature.

2.3. Optimization of Parameters to Reduce the Microbes

Response surface methodology is an effective investigational tool used for developing and optimizing the process parameters with a combination of strong mathematical and statistical techniques (Zokaei *et al.,* 2013). It can be used for evaluating the relative significance of different influencing factors, even in the presence of complex interactions.

2.4. Primary treatment

The primary treatment was designed with the aim of reducing bacterial population present in the biomedical wastes. Various substrates including seaweeds (*Padina tetrastromatica* and *Amphiroa fragilissima*) and turmeric were used to know their potential in waste management treatment process. All the experiments were conducted in 250 mL beaker having 100 mL of distilled water containing 50 gms of tissue waste. In this present study, three effective parameters (Substrate concentration, Agitation speed and Incubation time) were selected as variables to be applied for the design of the experiment (DOE) (Gerayeli *et al.*, 2013).

2.5. Secondary Treatment

After completion of the Primary treatment, the samples with minimal bacterial load were taken to the secondary treatment procedure in which the sample underwent alkaline hydrolysis using 5 % NaOH for 2 hrs. After this, the experiment was conducted to analyze the bacterial load and percentage of waste digested. During the experiment, an effort was made to understand the influence of temperature on the treatment by conducting the experiments at different temperatures, and the period of experiment was also calculated in order to optimize the efficient protocol for effective treatment. Then the digested fraction was monitored and analysed for various chemical and biological parameters.

2.6. The Influence of Temperature in the Treatment process

To study the influence of temperature in the treatment procedure, the experiment was done at different temperatures (30º C, 35º C, 40º C, 45º C, 50º C, 55º C, 60º C, 65º C, 70º C and 75º C). To the 250 mL beaker, 100 mL of sterile distilled water and 50 g of collected waste was added. Thereafter the preliminary treatment was carried out with the selected substrate at optimized process parameters. After 3 days of primary treatment, the secondary treatment was carried out for 2 hrs. During the experiment, pH of the sample was measured using a calibrated pH meter at every 6 h interval. At the end of the treatment, the supernatants were filtered using a 0.45 µm membrane and were analyzed looking for a reduction in the bacterial population using a culture sensitivity test.

2.7. Bio-manure Preparation

Neem leaf and turmeric were collected and kept shade dried for about two days. 10 gram of the sample were taken in 100 ml of water and the aqueous extracts were separated. The aqueous extract of Neem leaf (5 % w/v) was added to this sample to enhance the antibacterial activity of the final product. Finally, the sample was filtered and the residue air dried for 30 minutes and the powdered sample was analyzed for the presence of nutrients (NARES, 1999). Thereafter, pot analysis was done to check the efficiency of biomanure from treated tissue waste in the plant growth.

3. Results and Discussion

Table 1: Operational conditions for primary treatment procedure using different substrates

Variable	Tested conditions	Fixed parameters		
Substrate concentration	2.5, 5.0, 7.5, 10.0, 12.5, and 15 g/100 mL substrate	Agitation speed 100 rpm	Incubation time 48 hrs	Sample load 50 gms
Agitation speed	50, 100, 150, 200 and 250 rpm	Substrate concentration 10.0 g/ 100 mL	Incubation time 48 hrs	Sample load 50 gms
Incubation time	24, 48, 72, 96, and 120 hrs	Substrate concentration 10.0 g/ 100 mL	Agitation speed 150 rpm	Sample load 50 gms

The experiment of the optimized conditions obtained by the RSM based approach was investigated by performing an experiment in these optimized conditions. According to the experiments, the bacterial reduction efficacy and residual mass were found to be 15000 CFU/mL and 0.94 g at the end of the 4th day, whereas the efficiencies optimized by RSM for bacterial count and residual mass were 16481.6 CFU/mL and 0.5426 g respectively. The validation experiment and predicted values from fitted correlations were in close agreement with above 95 % confidence interval. The result indicated that the predicted and actual values were in close agreement at a 95 % confidence interval. Hence, the model was successful in predicting the effective treatment parameters. Figure 1. shows the beaker containing treatment samples initially and at the end of fourth day, where reduction in microbial population

happened when some amount tissue was digested at the end of the fourth day, compared to the sample at 0th day.

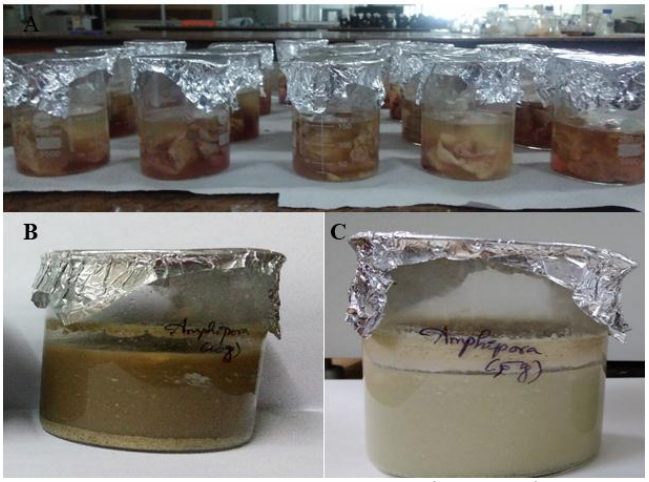

Figure 1: Samples at the end of primary treatment. A) 0th day, B) 2nd day and C) 4th day

Table 2: Validation of the RSM model

A (g/100mL)	B (rpm)	C (hr)	Bacterial count (CFU/mL)		Residual mass (g)	
			Predicted	Experimental	Predicted	Experimental
10.2	150.41	91.13	16481.6	15000	0.5426	0.94

At the end of the secondary treatment, where the tissue got completely digested, it was confirmed that the bacterial population got reduced at the end of the final treatment.

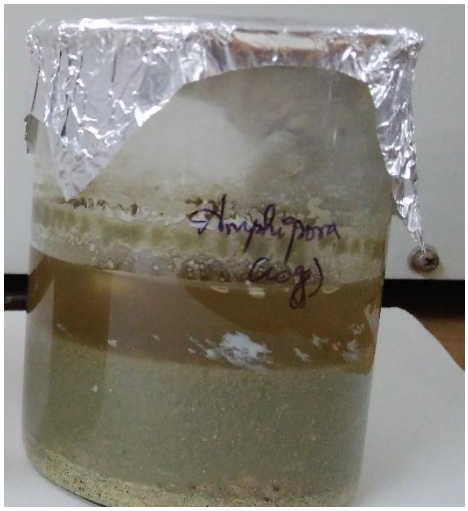

Figure 2: Sample at the end of secondary treatment

4. Influence of Temperature on Treatment Process

Alkaline hydrolysis method was done in order to inactivate the pathogens and it was carried out in the controlled environment where temperature was the major factor causing inactivation. To study the influence of temperature in the treatment procedure the experiment was done at different temperatures (30º C, 35º C, 40º C, 45º C, 50º C, 55º C, 60º C, 65º C, 70º C and 75º C). In this work, the influence of temperature was studied using 250 mL beaker which contained 100 mL of sterilized distilled water and 50 g of biomedical waste. The experiment was carried out with the optimized conditions of selected parameters (5 % NaOH, 5 % neem extract, and 150 rpm). After the treatment period of 1 hr., the sample was withdrawn and the bacterial population studied. It was observed that the bacterial count steadily decreased from 20000 to 5 CFU/mL of sample, when the temperature range varied from 30 to 75^0 C.

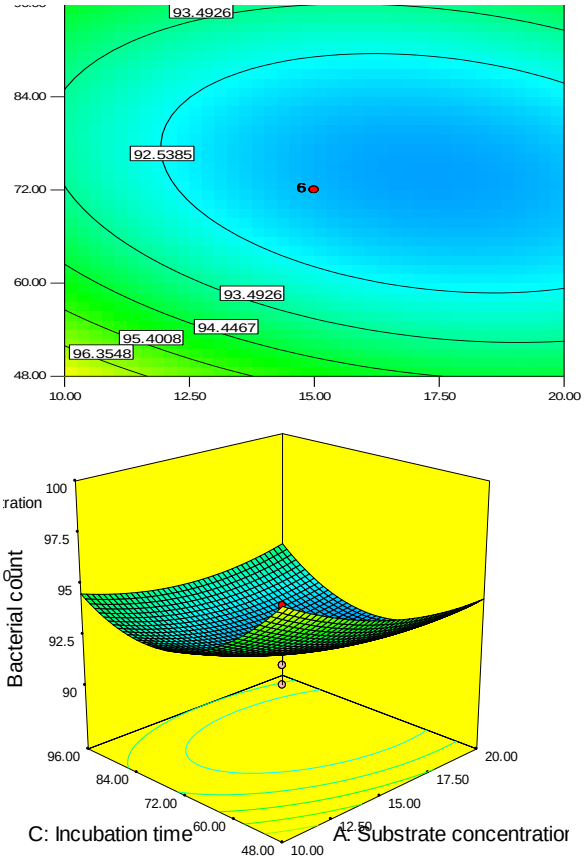

Figure 3: (a) contour and (b) surface plots for bacterial count with respect to Substrate concentration-Incubation time

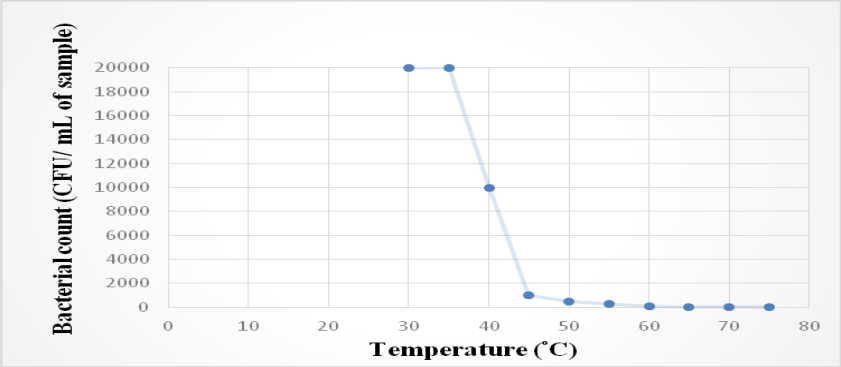

Figure 4: Influence of temperature on treatment process

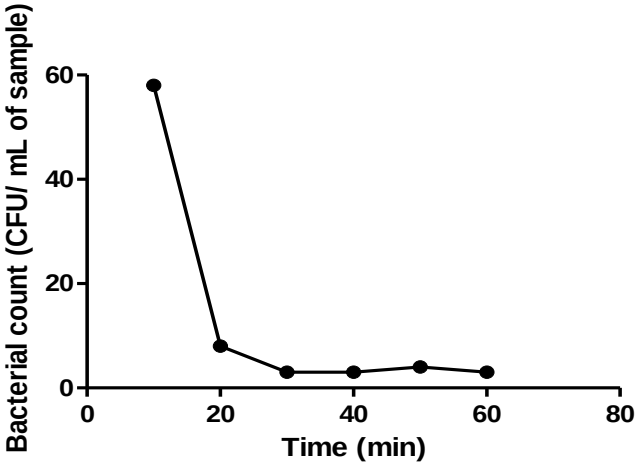

Figure 5: Effect of incubation time on treatment process

Table 3: Bacterial count at different concentrations of turmeric

Substrate concentration (g/L)	**2.5**	**5.0**	**7.5**	**10.0**	**12.5**	**15.0**
Bacterial Count (CFU/mL)	100000	100000	98000	96500	96000	95000

(At fixed values of Agitation speed, 150 rpm and Incubation time, 48 hrs)

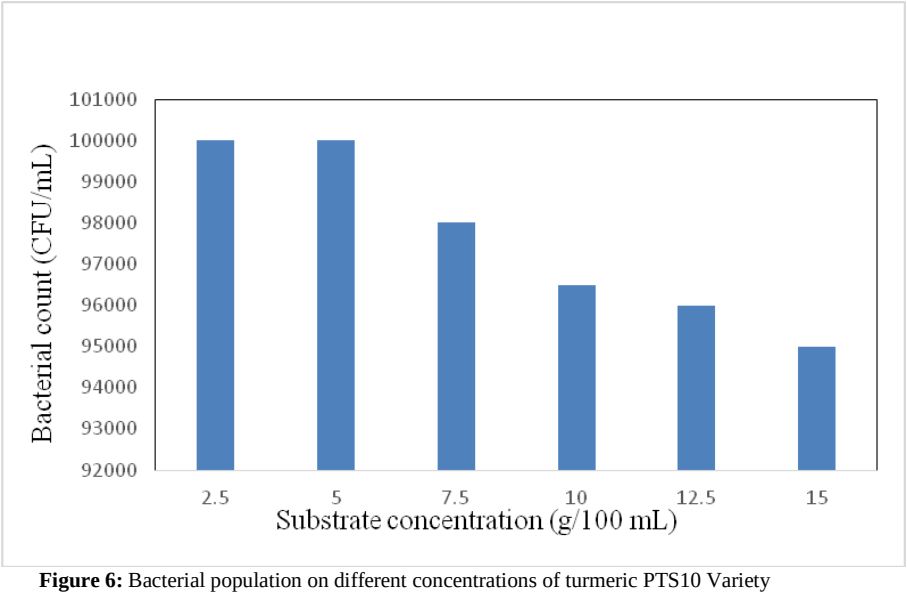

Figure 6: Bacterial population on different concentrations of turmeric PTS10 Variety

Table 4: Bacterial count at different agitation speed

Agitation speed (rpm)	**50**	**100**	**150**	**200**	**250**
Bacterial Count (CFU/mL)	98000	96000	95000	96000	95000

(At fixed values of Substrate concentration, 10 g/100mL and time, 48 hrs)

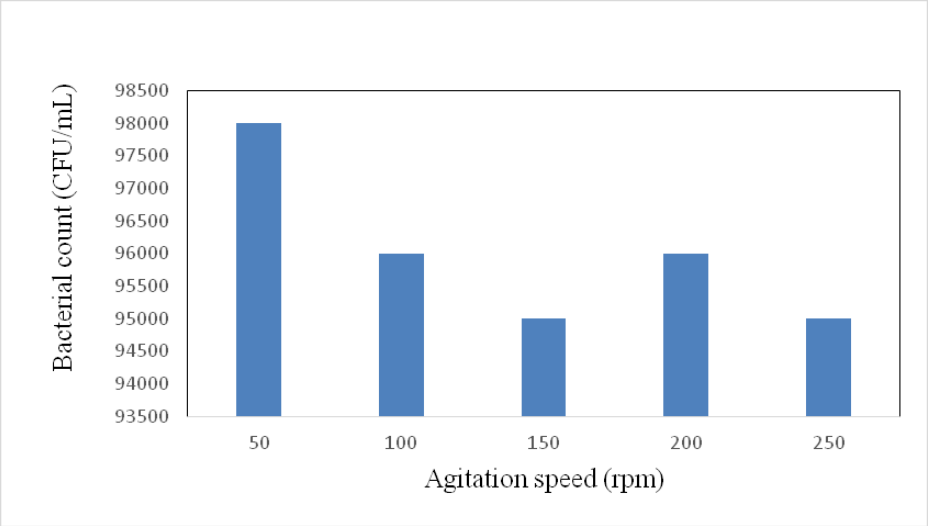

Figure 6: Bacterial populations on different agitation speed

Table 5: Bacterial count at different incubation period

Incubation time (hrs)	24	48	72	96	120
Bacterial Count (CFU/mL)	98000	95000	92000	92000	91000

(At fixed values of Substrate concentration, 10 g/100mL and agitation speed, 150 rpm)

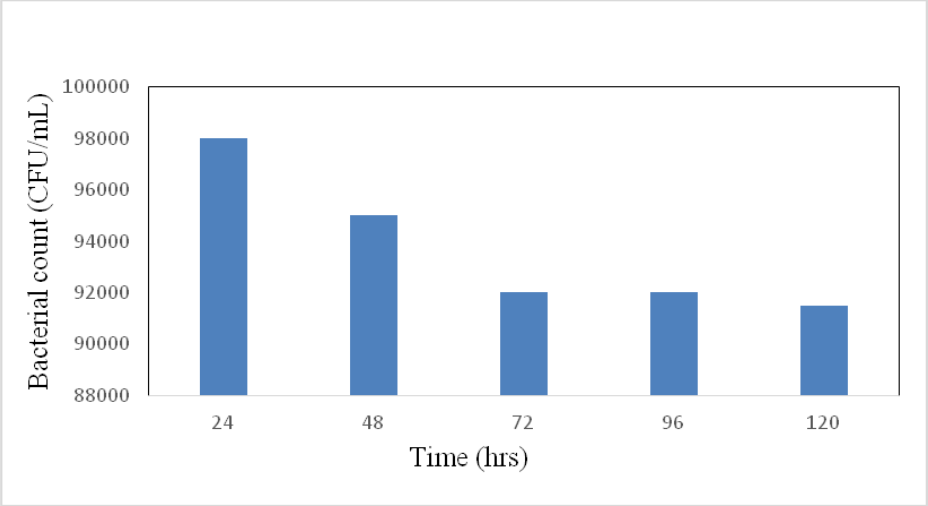

Figure 7: Bacterial population on different incubation period

4.1. Effect of incubation time on treatment process (in presence of optimized temperature)

Figure 5 shows the graphical representation of the bacterial count at different time intervals, which reveals that for an effective treatment of biomedical waste, after treatment using optimized conditions, it requires short period of incubation for the secondary treatment procedures. Efficiency of the bacterial reduction was obtained after the treatment of about 30 minutes. After that there was no change in the bacterial population which meant that the process had reached its saturation point.

4.2. Parameter Optimization Using Response Surface Methodology

In this study, three effective parameters (Substrate Concentration, Agitation Speed and Incubation Time) were selected as variables to be applied for the design of the experiment (DOE). Determining the central levels of the chosen parameters was important while implementing DOE. The central levels of the chosen parameters were determined from the results obtained by OVAT approaches. Since the optimization process was based on a 2-day period, the bacterial colony was determined at the end of the 2nd day. The minimum bacterial population acquired after 2 days was taken as central levels.

While varying substrate concentration (Figure 6) from 2.5 to 15 g/100mL with constant agitation speed (150 rpm), and Incubation Time (48 hrs), the minimum bacterial count of 95000 CFU/mL was obtained in the experiment conducted with the concentration of Substrate at 15 g/100mL. It indicated that 15 g/100mL of substrate concentration was appropriate for bacterial reduction. In the experiments conducted at different agitation speed ranging from 50-250 rpm while keeping constant parameters such as Substrate Concentration (15 g/100mL), and Incubation Time (48 hrs) 95000 CFU/mL was obtained as minimum in the experiment operated with 150 rpm. The occurrence of reduced efficiency beyond 190 r/min might be due to a mechanical stress among pulp density, which created adverse conditions to attain sufficient oxygen requirement for microorganism growth. OVAT tests on Incubation time ranging 24−96 hrs indicated that the experiment at 72 hrs showed an effective bacterial reduction to 92000 CFU/L by following constant parameters: substrate Concentration: 15 g/100mL, Agitation speed: 150 rpm. It is apparent that 15 g/L of substrate concentration, 150 rpm, and 72 hr incubation period could be used as central level of variables for DOE studies. Using this central level of independent parameter variables, further studies for design-matrix interaction and optimization were carried

out. The experimental response was done using central composite rotatable design (CCRD) for minimize bacterial count and residual mass and also the optimization of parameters (Chong *et al*., 2010).

4.3. Preparation of Bio-manure

Viability of the microorganisms was decreased. A complete destruction of the potentially infectious microbes took place after the primary and secondary treatments. As a result of the secondary treatment, digested sample contains the break down products of the tissues such as proteins, sugars, fats etc., The supernatant was removed and the residue kept under air to dry for 2 hrs. After that the produced bio-manure was characterized for use in the field.

4.4. Characterization of Bio-manure

The results are depicted in the Table 6. From the results it is understood that the nitrogen and phosphorous sources are enriched after the treatment procedures and the reduction in moisture content to < 2 % helps to overcome the microbial degradation when applied in the field (Bernhart and Fasina, 2009).

Table 6: Nutritional composition of bio-manure

Content	Range (%)
Total nitrogen	4.5
Phosphorous	1.8
Sodium	0.9
Moisture content	1.5

5. Conclusion

Plant species mediated biomedical waste management system was considered as the key idea of this current research work with cost effective strategy, when compared to conventional method like rendering, composting, anaerobic degradation and incineration. The current disposal techniques adopted for the biomedical waste management are incineration and land fill. It provides uncontrollable process factors which may release toxic substances into the air. The treatment of biomedical waste with viable technology is an urgent need to eliminate all the risks to humans and the environment. The biomedical wastes (Category 3 & 6) were collected and segregated. In this study, effective parameters such as Substrate concentration, Agitation speed and Incubation time were optimized to enhance the reduction of bacterial population during preliminary treatment process. It was attempted to check the efficacy of different substrates including *Amphiroa fragilissima*, *Padina tetrasomatica*, turmeric PTS10 variety and turmeric BSR variety in the treatment process. Central composite rotatable design (CCRD) was employed for minimizing the bacterial count and residual mass and also its optimization. It is apparent that 15 g/L of substrate concentration, 150 rpm and 72 hr incubation period could be used as central level of variables for DOE studies. The results showed that alkaline hydrolysis treatment carried out at a temperature range of 75º C for an incubation period of 30 min caused a tremendous decline in the bacterial count from 20000 to 5 CFU/mL. Seaweed and turmeric had good antibacterial activity against pathogens associated with biomedical wastes and they also caused tissue degradation to some extent. Animal tissues were digested completely and converted into a noninfectious effluent as a bio-manure. As a result, bio manure could be produced at the end of the primary and secondary treatment processes and its characterization was studied too.

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