Asian Journal of Biotechnology and Bioresource Technology

2(3): 1-7, 2017; Article no.AJB2T.38659
ISSN: 2457-0125

Degradation of Sawdust by Thermo Tolerant Microorganisms for Bio Fertilizer Synthesis

Christian O. Asadu[1*], Nebechukwu G. Aneke[1], Albert C. Agulanna[2] and C. N. Eze[3]

[1]Department of Chemical Engineering, Enugu State University of Science and Technology, P.M.B. 01660, Enugu State, Nigeria.
[2]Project Development Institute (PRODA), Emene Industrial Layout, Enugu, Nigeria.
[3]Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author COA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author NGA supervised the work and then managed the analyses of the study with author COA. Author ACA managed the literature searches. Author CNE provided the technical knowhow for microbial isolation. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJB2T/2017/38659
Editor(s):
(1) Vijai K. Agnihotri, Professor, CSIR-Institute of Himalayan Bioresource Technology, Himachal Pradesh, India.
(2) Pasquale Russo, Professor, Department of Science of Agriculture, Food and Environment, University of Foggia, Italy.
Reviewers:
(1) S. Sharmila, Bharath Institute of Higher Education and Research, India.
(2) Hanan E.-S. Ali, Egyptian Petroleum Research Institute, Egypt.
(3) Bhagwan Rekadwad, India.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/22712

Original Research Article

Received 5th October 2017
Accepted 5th January 2018
Published 12th January 2018

ABSTRACT

Sawdust has been neglected over the years in biofertilizer synthesis due to high lignin and cellulose content which are recalcitrant to biodegradation. This research has demonstrated ways of breaking down sawdust through the isolation of thermotolerant Actinomycetes. Six genera of Actinomycetes were isolated from landfill and compost extracts, three genera of the isolates was found to be *Streptomycetes spp*, while two genera was found to be *Rothia spp* and one *Actinomadura spp*. The potential of these organisms in degrading sawdust was examined. Results showed that all the organisms has a great potential of degrading sawdust with *Actinomadura spp* been the most effective degrading agent based on its high percentage degradation of cellulose (12.31%) followed by *Rothia spp* (9.90%). Results of the biodegradability analysis also showed that

**Corresponding author: E-mail: aasadu@yahoo.com;*

the organisms has the capacity to make sawdust amenable to biodegradation with up to 70.43% of sawdust been biodegradable after 35 days of incubation with *Actinomadura spp.* The results of these investigations have demonstrated that the consortium of these organisms has the capacity to degrade sawdust during composting.

Keywords: Biodegradability; sawdust; actinomycetes; degradation.

1. INTRODUCTION

Sawdust by definition is a by-product derived from wood processing, composed of fine particles of wood. It is a menace in manufacturing industries, considering its flammable nature. Secondly it is bulky in nature and occupies a lot of space causing a serious environmental pollution; burning sawdust produces thick black smoke that pollutes the environment. It has its usefulness in variety of ways, including serving as mulch, as an alternative to clay cat litter, as a fuel, or for the manufacture of particleboard. As an Agricultural wastes, it can be converted to biofertilizer by composting, but the problem with sawdust is that it is the least desirable source of organic matter for it ties up nitrogen as it decomposes. Raw sawdust takes an average of 180 days to decompose in the soil due to deficiency of nitrogen [1]. Longer period taken by sawdust to decay has been the major reasons it has been neglected over the years as a raw material for composting, therefore, any effort on how to reduce this wide range of time for the decay of sawdust is encouraged. Biofertilizer are substance that contains microorganisms which when applied to the soil, colonizes the rhizosphere of plants and increase growth by supplying the primary nutrients to the host plant [2].

Micro-organisms that decompose sawdust require nitrogen for metabolism, lack of nitrogen in sawdust makes it difficult for these microbes to increase and multiply. Sawdust has been reported to be resistant to biodegradation according to [1], this is as a result of high lignin and cellulose content of sawdust as reported by [3]. Lignin is protective shield over cellulose from microbial attack. Defiance nature of cellulose, lignin and hemicellulose and the importance of their biodegradation in the environment have attracted a great deal of interest for quite a number of years [4]. Cellulose has been known to be totally insoluble in water and has about 2000 – 10 000 glucose subunits and has a molecular weight ranging from 200 000 to about 2.4 million [5]. Cellulose fibre is an important raw material in textile industry, paper and etc due to its high tensile strength [4].

Degradation of sawdust by some organisms such as *Actinomadura keratinolytica Mycobacteriaceae, Actinomycetaceae, Streptomycetaceae and Actinoplanaceae* has been reported by [6]. [3] reported the cultivation of enzymes for the degradation of lignocellulosic materials. Actinomycetes and bacteria of the groups *Cytophaga, Erwinia*, *Pseudomonas,* Sp. *oroiytophaga, Xanthomonas* and *Streptomonas* have also been reported to degrade hemicelluloses [7]. This research is aiming to isolate thermotolerant microorganisms from compost extract and landfill and also screen the ability of the organisms in breaking down cellulose and lignin fraction of sawdust in order to make sawdust amenable to biodegradation for biofertilizer synthesis.

2. MATERIALS AND METHODS

2.1 Raw Material and Its Source

Fresh sawdust was collected from wood processing industry; timber shed Nsukka, Enugu State Eastern Provence of Nigeria.

Table 1. Physiochemical properties of raw sawdust

Parameters	Percentage composition
Moisture content	23.9±6.5
Total Organic carbon	38.6±0.5
Ash content	25.3±0.1
Nitrogen (N)	0.6±0.1
Phosphorus (P)	1.05±0.1
Potassium (K)	0.4±0.1
Lignin content	22.8±0.5
Cellulose content	54.7±0.5
pH	6.3±1.0
Organic matter	76±1.0

2.2 Physiochemical Characterization

The percentage composition of Organic matter, Ash content and moisture content was

determined by ASTM D2974-07 [8]. The nitrogen content was estimated by the Kjeldahl method [9]. Lining content was estimated by the method Ververis et al. [4]. The celluloses content was estimated by the method of Kulić and Radojičić [5]. Phosphorus and potassium was analysed using Atomic Absorption spectrophotometer (AAS) situated at Energy center University of Nigeria Nsukka model 2010.VGP manufacturer USA. pH was measured in the filtrate solution using pH-meter 340I/SET. Total organic carbon was determined by the method described by Schumacher [10].

2.3 Isolation of Thermotolerant Actinomycetes

2.3.1 Sample collection

50 g of soil sample mixed with an extract of agro-wastes compost from beneath Veterinary Teaching Hospital compost, University of Nigeria Nsukka, Enugu State.

2.3.1.1 Preparation of inorganic salt starch agar

Composition of the reagents: Agar- 20g, Soluble starch- 10 g, $CaCO_3$- 2.0 g, $(NH_4)_2SO_4$- 2.0 g, K_2HPO_4- 1.0 g, $MgSO_4.7H_2O$- 1.0 g, NaCl- 1.0 g, $FeSO_4.7H_2O$- 1.0 g, $MnCl_2.7H_2O$- 1.0 g, $ZnSO_4.7H_2O$- 1.0 g.

These reagents were dissolved in 1000ml distilled water and first homogenized in a water bath at 50°C, then sterilized using the autoclave at 121°C for 15-20 minutes. A 100 ml of the medium was dispensed into 250 ml conical flasks, sterilized and dispensed into 4 petri dishes.

2.3.2 Sample pre-treatment

Samples were filtered using a 2 mm filter to remove stones and large plant materials. Filtered sample was incubated in a conical flask at 50°C for 7 days to eliminate the mesophiles in the sample for isolation of thermotolerant bacteria.

2.3.3 Serial dilution and isolation

1.0 gram of the sample of compost extract was diluted with 10.0 ml of distilled water and homogenized. From this stock solution, a ten-fold dilution was carried out using sterile distilled water and test tubes. From each dilution 10^{-1} and 10^{-2}, 0.1 ml of the sample was pipetted aseptically into inorganic salt starch agar plates in duplicates using spread plate. Rifampicin and nystatin were incorporated into each media at concentrations 25 µg/l and 50 µg/l. Thus, four culture plates for the medium. The culture plates were incubated at 42°C for 5 days. Distinct colonies were picked after 5 days from each plate and streaked unto the Inorganic Salt Starch agar medium from where they were initially isolated. Thus, plates were labelled ISO1 (ISSA $10^{-1}{}_1$), ISO2 (ISSA$10^{-1}{}_2$), ISO3 (ISSA $10^{-2}{}_1$), ISO4 (ISSA $10^{-2}{}_4$), ISO5 (SCA $10^{-2}{}_1$), ISO6 (SCA $10^{-2}{}_2$) aseptically to obtain a pure culture of each isolate. Discrete colonies of the organisms were sub cultured and stock culture were prepared from the pure cultures was stored below the room temperature for further use.

2.3.4 Identification of the isolated microbes

Macroscopic and microscopic identification of the isolates was carried out together with the biochemical characterization Using standard microbiological procedures and Bergey's Manual of Determinative bacteriology.

2.4 Verification of the Isolates Potential in Breaking down Cellulose Content of Sawdust

3 g of fresh sawdust was introduced into six boiling tube each. 20 ml of distilled water was added into each tube and the content autoclaved at 121℃ for 20 mins. The tubes were numbered ISO1-ISO6 and control. The tubes containing samples of sawdust except the controls was inoculated with the isolates. The tubes and the contents were incubated for 35 days at 37℃. The liquid contents in the tubes were carefully decanted at the end of 35 days. The sawdust in each tube was examined for cellulose and lignin contents using the method [5] and [4].

3. RESULTS AND DISCUSSION

The bacterial isolates designated as ISO1, ISO2, ISO3, ISO4, ISO5 and ISO6 were obtained using standard microbiological procedures and Bergey's Manual of Determinative bacteriology, the bacterial isolates were identified as shown in Table 2.

Mesophilic actinomycetes have an optimum growth temperature of 25-30°C, thus the organisms were incubated at 42°C to obtain the thermotolerant ones. Serial dilution was carried out and initially dilutions 10^{-3} and 10^{-7} were plated on the isolation media but did not produce

any significant growth thus dilutions 10^{-1} and 10^{-2} were used for the isolation. Inorganic salt starch agar produced significant growth under the culture conditions. According to [11], antifungal antibiotics which have no effect on the actinomycetes such as nystatin used in this experiment should be used to prevent fungal contamination of the medium for isolation of bacteria. The isolates were identified using macroscopic and microscopic methods involving staining and slide culture as well as biochemical analysis. Out of the six isolates obtained during the investigation for bacteria, three were presumptively identified as *Streptomyces spp*., while others were identified as *Actinomadura spp*. and *Rothia spp* as shown in Table 2. Streptomyces spp. is known to be the largest genera of actinomycetes [12,6], and the presumptive identification of 3 isolates out of six isolates as Streptomyces spp is in line with this finding. One can also conclude that inorganic salt starch agar is good isolation media for thermotolerant bacteria which buttresses the report by [13,14].

Table 2. Bacteria isolated from the compost extracts

Isolates	Presumptive genera
ISO1	*Actinomadura spp*
ISO2	*Streptomyces spp*
ISO3	*Streptomyces spp*
ISO4	*Streptomyces spp*
ISO5	*Rothia spp*
ISO6	*Rothia spp*

Table 3 showed the results of the biochemical analysis carried out on the isolates. The results as indicated in the table showed that effective biodegradation of organic wastes, nutrients mineralization and nitrogen fixation can be achieved by the consortium of the organisms.

Nitrate Reduction test was performed on the isolates, it helps to determine the ability of an organism to reduce nitrate (NO_3) to nitrite (NO_2), ammonia, nitrous oxide and nitrogen using the enzyme nitrate reductase. It also tests the ability of an organism to perform nitrification on nitrate and nitrite to produce molecular nitrogen and the ability of the organisms to converts atmospheric nitrogen into nitrite. Plants need nitrogen inform of nitrites. Table 3 showed that all the bacteria isolates tested positive to nitrate reduction test apart from *Actinomadura spp.* This sign is an indication that the organisms has the capacity to fix nitrogen during composting and can be used in biofertilizer synthesis. This agrees with the report by [15].

Sugar fermentation tests are carried out to determine whether or not an organism can degrade a certain sugar/carbohydrate like sucrose, dextrose, mannitol, maltose and lactose. Fungi and Actinomyces are known to be very good in sugar fermentation according to [16]. Table 3 showed that *Actinomadura spp* tested positive to glucose, one Presumptive genera of *Streptomycets spp* tested positive to glucose, while another genus of *Strptomyces spp* tested positive to sucrose, the two *Rothia spp* genera tested positive to manitol, glucose, and sucrose, which showed that the consortium of these organisms can give the desired results for carbohydrates decomposition. The bacteria (actinomycetes) are known to decompose all variety of organic substances like cellulose, polysaccharides, protein, fats, organic acids e.t.c they are also responsible for further decomposition of humus (resistant material) in the soil as reported by [17,11].

Table 3. Results of biochemical analysis carried out on the isolates for bacteria identification

Identification parameter	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Gram reaction	+ve	+ve	+ve	+ve	+ve	+ve
Spore staining	+ve	+ve	+ve	+ve	+ve	+ve
Acid fast staining	-ve	-ve	-ve	-ve	-ve	-ve
Catalase test	-ve	+ve	+ve	+ve	+ve	-ve
Nitrate reduction test	-ve	+ve	+ve	+ve	+ve	+ve
Indole test	-ve	-ve	-ve	-ve	-ve	-ve
Urease test	-ve	-ve	+ve	+ve	-ve	-ve
Mannitol test	-ve	-ve	-ve	-ve	+ve	+ve
Glucose test	+ve	-ve	-ve	+ve	+ve	+ve
Sucrose test	-ve	+ve	-ve	-ve	+ve	+ve
Implicated organism	*A.spp*	*S. spp*	*S. spp*	*S. spp*	*R. spp*	*R. spp*

-ve = negative result; +ve = positive result; A. spp = Actinomadura spp.; S. spp = Streptomyces spp.; R. spp = Rothia spp.

Table 3 also showed that most of the organisms tested positive to catalase test. Catalase is an enzyme produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites e.g. H_2O_2 according to [17,12]. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects the organisms. Catalase mediates the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water. From the aforementioned analysis, one can conclude that the isolates can be used in bio-fertilizer synthesis.

3.1 Results of the Investigations on the Action of Microbes on Cellulose Contents of Sawdust

Samples of sawdust was incubated with the isolated microbes for 35 days and the effect of the microbes on the cellulose contents of sawdust presented as shown in Table 4.

The Presumptive genera of the bacteria isolates were shown in Table 2. The degradation of sawdust by the indigenous microorganisms isolated and their differences in utilization of the cellulose content of the sawdust after 35days incubation period has been demonstrated as shown in Table 4. Initial cellulose content of sawdust as shown in Table 1 was as high as 54.7% but the concentration of cellulose was observed to have reduced after treating sawdust with the isolates for 35 days as shown in Table 4. This indicates that the isolates are capable of utilizing sawdust as its source carbon and energy for growth. This finding buttresses the argument by [18,19] that bacteria degrade organic wastes such as sawdust. [20] Also reported the biodegradability of cellulose. This agrees with the findings of [3] when they reported the reduction of carbon content of sawdust and other agro-wastes when subjected to microbial degradation. Table 4 showed that *Actinomadura spp* has the highest percentage degradation of cellulose (12.31%) amongst the bacteria population isolated followed by *Rothia spp* (9.76%, 9.90%) and then the least *Streptomyces spp* (8.40%, 8.57%, 8.50). this indicates that *Actinomadura spp* has the highest capacity to secrete cellulase enzyme that degrade materials that contains cellulose. Sustainability of the degradation of cellulose by the bacteria isolates at 37℃ also proved that some of the bacteria isolates are thermo tolerant (Actinomyces). These reports have provided an insight into the possibility of breaking down agro wastes using indigenous microbes, thereby paving way for enhanced natural attenuation of agro wastes polluted sites.

3.2 Biodegradability of the Sawdust after Bio Treatment

The biodegradability of the sawdust in each boiling tube was calculated after 35 days incubation period by measuring the final lignin content, ash content and total volatile solid.

The empirical equation for the measurement of biodegradable fraction of organic wastes under anaerobic condition was developed by [[21] and is stated thus:

$$Biodegradabilty\ or\ Biodegradable\ fraction = 0.83 - 0.028 \times lignin_{\%Vs} \quad (1)$$

Where

$lignin_{\%Vs} = \frac{lignin_{\%}}{Vs_{\%}/_{100}}$ = lignin content as a percentage volatile solid.

$lignin_{\%}$ = The lignin content as a percentage total solid.

$Vs_{\%}$= Volatile solid as a percentage of total solid.

Evaluation of Volatile solid was done using the equation proposed by [22]

$$\%Vs = 100 - \%Ash \quad (2)$$

Table 4. Proportional degradation of cellulose in sawdust by bacteria

Isolates	Presumptive genera	Initial cellulose conc (%)	Cellulose conc (%) after incubation	Percentage difference
ISO1	*Actinomadura spp*	54.7	42.39	12.31
ISO2	*Streptomyces spp*	54.7	46.30	8.40
ISO3	*Streptomyces spp*	54.7	46.12	8.58
ISO4	*Streptomyces spp*	54.7	46.20	8.50
ISO5	*Rothia spp*	54.7	45.94	9.76
ISO6	*Rothia spp*	54.7	44.8	9.90
Control		54.7	54.54	0.16

Table 5. Action of the organisms on lignin content of sawdust after 35 days of incubation

Isolates	Presumptive genera	% Ash	% Vs	% lignin before incubation	% lignin after incubation	Biodegradability (%)
ISO1	*Actinomadura spp*	9.80	90.20	22.8	4.05	70.43
ISO2	*Streptomyces spp*	14.70	85.70	22.8	11.2	46.41
ISO3	*Streptomyces spp*	13.87	86.13	22.8	10.8	47.89
ISO4	*Streptomyces spp*	14.56	85.44	22.8	11.53	45.21
ISO5	*Rothia spp*	11.60	88.40	22.8	9.6	52.59
ISO6	*Rothia spp*	12.40	87.60	22.8	8.7	55.19
Control		21.50	78.50	22.8	21.73	5.49

The percentage lignin before incubation was taken from Table 1. Table 5 showed the effect of lignin content on biodegradability of sawdust. From Table 5, it was observed that 70.43% of the carbon content in sawdust is biodegradable after incubation with isolate 1, more than 40% of the carbon content of sawdust is biodegradable after incubation with Isolate 2, 3 and 4 while more than 50% of the carbon content of sawdust is biodegradable after incubation with isolate 5 and 6. Only 5.49% of the carbon content of sawdust is biodegradable in control after 35 days of incubation. Comparing the results in Table 5 with the control, one can conclude that the isolated microbes have the potential to breakdown complex carbohydrate in sawdust and make it amenable for biodegradation and enhance the quality of biofertilizer during composting. Increase in biodegradability would likely be even greater with a consortium of these organisms and extension of the incubation period from 35 days to 40 days as evident in Table 5.

4. CONCLUSION

Maximum Biodegradation of sawdust can be achieved with the combination of organisms such as *Actinomadura* spp, *Steptomycetes* spp, and *Rothia* spp.

ACKNOWLEDGEMENTS

The authors want to thank in a special way the institute of Energy research centre University of Nigeria Nsukka for making their equipments available for the work and also to the members of staff of microbiology department, University of Nigeria Nsukka for their immense contributions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Olayinka A, Adebayo A. Effect of pre-incubated sawdust based cow dung on the growth and nutrient uptake of *Zea mays* L. and on soil chemical properties. Biol. Fert. Soil. 1989;7:176-179.
2. Adeoye GO, Adeoluwa M, Oyekunle MKC. Sridhar EA, Olowake AA. Comparative evaluation of organomineral fertilizer (OMF) and mineral fertilizer (NPK) on the yield and quality of maize (*Zea mays* L.) Moench, Nigeria. J. Soil Sci. 2008;18:132-137.
3. Erikson KEL, Blanchette RA, Ander P. Microbial and Enzymatic Degradation of wood and wood components. Wood decomposition. Springerverlag, New York. 1990;41-62.
4. Ververis C, Georghiou K, Danielidis D, Hatzinikolaou D, Santas P, Santas R, Corleti V. Cellulose, Hemicelluloses, Lignin and Ash Content of Some Organic Materials and Their Suitability for Use as Paper Pulp Supplements. Bioresource Technology. 2007;98:296-301. Available:http://dx.doi.org/10.1016/j.biortech.2006.01.007
5. Kulić GJ, Radojičić VB. Analysis of cellulose content in stalks and leaves of large leaf tobacco. Journal of Agricultural Science. 2011;56:207-215.
6. Adeline SY, Ka LP. Indigenous actinomycetes from empty fruit bunch compost of oilpalm: Evaluation of enzymatic and antagonistic properties. Biocatalysis and Agricultural Biotechnology. 2014;3(4):310-315.
7. Durrant LR. Biodegradation of lignocellulosic materials by soil fungi isolated under anacrobic conditions. Int. Biodeteroration Biodegr. 1996;37:189-195.
8. ASTM D2974-07. Standard Test Methods for Moisture, Ash, and Organic Matter of

Peat and Other Organic Soils, ASTM International, West Conshohocken, PA; 2007.
Available:www.astm.org

9. Sobiecka E, van der Sloot H, Janssen E, Gawlik BM. Project HORIZONTAL validation report on Kjeldahl Nitrogen. Validation of a Horizontal Standard for The Determination of Kjeldahl Nitrogen in Soil Sludges and Treated Bio waste in a European Inter-comparison Exercise, (Luxembourg, European Commissions-Joint Research Centre-Institute for Environmental and Sustainability-European Communities); 2007.
10. Schumacher BA. Methods for the determination of total organic carbon (TOC) in soils and sediments. U.S. Environmental protection agency, Washington, DC, EPA/600/r-02/069 (Ntis PB2003-100822); 2002.
11. Porter JN, Wilhelm JJ, Tresner HD. Method for the preferential isolation of actinomycetes from soil. Applied Microbiology. 1960;8:174.
12. Antoinetta Tadei, et al. Isolation and identification of *Streptomyces* spp. from venezuelan soils: Morphological and biochemical studies. Microbiological Research. 2006;161:222-231.
13. Kuster E, Williams ST. Selection of media for isolation of streptomycetes. Nature, London. 1961;202:928.
14. Kumar N, Singh RK, Mishra SK, et al. Isolation and screening of soil actinomycetes as sources of antibiotics active against bacteria. International Journal of Microbiology Research. 2010;2: 12–16.
15. Bakulin MK, Grudtsyna AS, Pletneva A. Biological fixation of nitrogen and growth of bacteria of the genus Azotobacter in liquid media in the presence of Perfluoro carbons. Appl. Biochem. Microbiol. 2007; 4:399-402.
16. Kelsey RG, Shafizadeh F. Enhancement of cellulose accessibility and enzymatic hydrolysis by simultaneous wet milling. Biotechnol. Biengr. 1980;22:1025-1036.
17. Amina H, Ali L. Purification and biochemical characterization of a detergent-stable keratinase from a newly thermophilic actinomycete: *Actinomadura keratinolytica* strain isolated from poultry compost. Journal of Bioscience and Bioengineering. 2014;117(4):413-421.
18. Shide EG, Whyep PA, Nok AJ. Studies on the degradation of wood sawdust by *Lentinus squarrosulus* (Mont) singer. Afr. J. Biotechnol. 2004;3(8):395-398.
19. Shewale JG, Sadana JC. Enzymatic hydrolysis of cellulosic materials by sclerotium rolfisii, culture filtrates of sugar production. Can. J. Microbiol. 1978;25: 773-783.
20. Focher B, Marzetti A, Beltrame PL, Carnici P. Structural features of cellulose and cellulose derivative and their effects on enzymes hydrolysis. Biosynthesis and Biodegradation of cellulose. Marcel Dekker, New York, USA. 1991;293-310.
21. Chandler JA, Jewell WJ, Gossett JM, Van Soest PJ, Robertson JB. Predicting methane fermentation biodegradability. Biotechnology and Bioengineering. Symposium No. 10. 1980; 93-107.
22. Adams RC, MacLean FS, Dixon JK, Bennett FM, Martin GI, Lough RC. the utilization of organic wastes in N.Z.: Second interim report of the inter-departmental committee. New Zealand Engineering. 1951;15:396-424.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/22712