PHYSIOCHEMICAL ANALYSIS OF PRETREATED BIOMEDICAL WASTES

SREEREMYA S, RAJIV P*

Department of Biotechnology, Karpagam University, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India.

Email: rajivsmart15@gmail.com

Received: 30 June 2017, Revised and Accepted: 19 August 2017

INTRODUCTION

The hospital waste generally termed as biomedical waste which includes human anatomical waste, needle and sharp waste, cellulosic waste, and discarded medicines [1]. As a result of global economic development, burgeoning population growth, and urbanization, solid waste generation is a growing social and environmental concern [2]. Among the solid waste generated globally, nearly 12% of the waste produced is from the hospitals and laboratories [3]. An increased percentage of biomedical waste is released every day without proper processing [4]. Improper disposal of biomedical waste causes serious health issues to human beings, and efforts were made to reduce a load of biomedical waste by major methods such as incineration, which has side effects [5]. Adoption of certain pretreatment strategies to degrade the Category III biomedical waste was implemented [6].

Among the biomedical wastes, the release and percentage of cellulosic waste discharged is more [7]. In the past years, efforts have been made to reduce the cellulosic waste by different pretreatment methods [8]. Pretreatment of Category III biomedical waste is to reduce the percentage of cellulosic waste [9]. Pretreatment can be implemented in three ways: Physical, chemical, and biological methods (PCBs) [10]. There were studies carried out in physical pretreatment, where it is found that approximately 4-5% of the cellulose content can be reduced by physical pretreatment.

Ohkuma has reported the efficiency of a chemical method for the treatment of waste by acid and alkaline chemicals [11]. Tahoun and Elbhim have investigated the pretreatment of waste by physical method [12]. In the present study, biomedical waste was pretreated by physical, chemical, biological, and combination of each method, and to assess the physiochemical properties of before and after pretreated biomedical waste.

METHODS

Collection of biomedical wastes and chemicals

The biomedical wastes were collected aseptically from the hospitals and nearby laboratories of Palakkad (10.7867°N, 76.6548°E), Kerala. The collected biomedical wastes were segregated (Category III biomedical wastes), placed in aseptic containers, and transported to the laboratory for further analysis. The chemicals for the analysis were majorly purchased from Sigma Aldrich.

Treatment details

*Bacillus flexus* was isolated aseptically from Gobar Gas Digester, Palakkad, Kerala. The morphological, microscopic, biochemical, and the degradation of cellulose were assessed by screening techniques. The pretreatment methods such as PCB methods were incorporated in the study. About 5 kg of biomedical waste (Category III) were treated by autoclaving the substrates at different temperatures (121°C for 15 minutes, 60 minutes, and 120 minutes labeled as P1, P2, and P3, respectively). About 5 kg of the biomedical waste (Category III) were treated with 0.25 M HCl and 0.25 M NaOH (labeled as C1 and C2, respectively). After the chemical pretreatment, the substrates were dried in hot air oven at 60°C [13]. Culture of *B. flexus* in basal media broth (100 ml) was used for biological pretreatment. *B. flexus* was mixed with 5 kg of biomedical waste. The treatment details are B1: 5 kg of biomedical waste and 2×10³ colony-forming unit (CFU), B2: 5 kg of biomedical waste and 4×10⁴ CFU, B3: 5 kg of biomedical waste and 6×10⁵ CFU, B4: 5 kg of biomedical waste and 8×10⁶ CFU, B5: 5 kg of biomedical waste and 10×10⁵ CFU, and B6: 5 kg of biomedical waste and 12×10⁶ CFU and incubated for 12-24 hrs aseptically [14].

Analysis of physiochemical parameters

Determination of *pH*, electrical conductivity (EC), bulk density, and moisture content

The *pH* of before and after pretreated biomedical waste was checked using digital *pH* meter [2]. The EC was estimated by digital EC
mater [15]. The EC of before and after pretreated biomedical waste was assessed and expressed in dS m⁻¹. The bulk density was analyzed as described by Sun et al. [16]. The sample (0.5 g) was weighed with the glass crucible and placed in the air drying oven for 18 hrs at 105°C and cooled to room temperature in a desiccator and weighed [8]. The process was repeated until a constant weight was achieved, and thus making it free of moisture content [17].

Estimation of cellulose
The cellulose level of pretreated biomedical waste was evaluated [18]. A sample (0.5 g) was incubated to 100°C with nitric acid and acetic acid for 30 minutes. After centrifugation to 3000 g for 60 minutes at room temperature, a solution of 72% sulfuric acid was added. The spectrophotometric measuring was made against calibration curve of cellulose at 620 nm.

Estimation of hemicellulose
The hemicellulose content of the pretreated biomedical waste was analyzed [19]. Nearly 0.5 g of the sample was taken and mixed with 0.3 M NaOH was added. The mixture was boiled for 2 hrs in distilled water; then, it was filtered and washed until it becomes neutral pH and weighed initially. After weighing, the sample was dried at 105°C. The difference between the sample weight before and after the treatment was the hemicellulose content.

Estimation of carbohydrate
The carbohydrate content in the pretreated biomedical waste was analyzed by dinitrosalicylic acid method [20]. Anthrone dissolved in sulfuric acid may be used for the quantitative determination of different carbohydrates. The mixture of samples was estimated at 620 nm using the spectrophotometer. The concentration of total sugar was calculated using a standard curve prepared from glucose. The amount of cellulose present before and after the pretreatment.

RESULTS AND DISCUSSIONS
The pH of the raw biomedical waste was found to be acidic (6.9±0.15). The physically pretreated biomedical waste was moderately acidic (5.3±0.035) (Table 1). The chemically (NaOH) pretreated biomedical waste was alkaline in nature (8.0±0.09), and HCl-treated biomedical waste was acidic (5.1±0.95). The biologically pretreated samples were slightly acidic (7.1±0.26). The physically and chemically treated sample was slightly acidic (6.9±0.15). The physically, chemically, and biologically pretreated biomedical waste was neutral (7.0±0.296). The previous studies reported that the pH of physical pretreated cellulose mass was found to be 5.9 [22].

The EC of the raw biomedical waste was 0.33±0.17 dSm⁻¹. The EC of physical, chemical, biological, physical and chemical, and PCB pretreatments were performed, and it was found to be range between 0.34±0.23 and 0.59±0.23 dSm⁻¹ (Table 1). These results are in agreement with the previous study, the EC was low in physical pretreatment, and the EC was increased in the biological pretreatment [15].

The bulk density of raw biomedical waste was 0.59±0.22 when physical, chemical, biological, and combination of pretreatment such as physical, chemical, and PCB pretreatment was performed, and there is an increase in the bulk density 0.82±0.077 (Table 1). In the previous studies, while comparing the several factors before and after the pretreatment, there was a drastic increase in bulk density after pretreatment [6].

The moisture content of the raw biomedical waste before the pretreatment was 1.00 when PCB, and combination of pretreatment such as physical, chemical, and PCB pretreatment was performed, and there is a rapid increase in moisture content 44.2±4.05 (Table 1). Kushwaha et al. achieved a moisture content of 40±2.05 while assessing the moisture content of cotton waste [23].

The estimation of cellulose content was carried out to understand the amount of cellulose present before and after the pretreatment.

### Table 1: Determination of physical parameters (bulk density, EC, pH, and moisture content) before and after the pretreatment

| Treatment details | Before pretreatment | After pretreatment | Before pretreatment | After pretreatment | Before pretreatment | After pretreatment | Before pretreatment | After pretreatment |
|-------------------|---------------------|--------------------|---------------------|--------------------|---------------------|--------------------|---------------------|--------------------|
| Physical pretreatment |                      |                    |                      |                    |                      |                    |                      |                    |
| P1                | 0.59±0.22           | 0.67±0.028         | 0.33±0.17           | 0.19±0.12          | 6.9±0.087           | 5.2±0.035          | 1.00                | 29.2±0.60          |
| P2                | 0.59±0.22           | 0.68±0.007         | 0.33±0.17           | 0.18±0.26          | 6.9±0.087           | 5.3±0.035          | 1.00                | 29.7±0.25          |
| P3                | 0.59±0.22           | 0.69±0.034         | 0.33±0.17           | 0.17±0.31          | 6.9±0.087           | 5.3±0.035          | 1.00                | 31.3±0.87          |
| Chemical pretreatment |                   |                    |                      |                    |                      |                    |                      |                    |
| C1                | 0.59±0.26           | 0.60±0.007         | 0.33±0.17           | 0.21±0.044         | 6.9±0.087           | 8.0±0.09           | 1.00                | 14.40±0.66         |
| C2                | 0.59±0.26           | 0.62±0.007         | 0.33±0.17           | 0.34±0.045         | 6.9±0.087           | 5.1±0.95           | 1.00                | 19.11±1.66         |
| Biological pretreatment |                |                    |                      |                    |                      |                    |                      |                    |
| B1                | 0.59±0.22           | 0.71±0.014         | 0.33±0.17           | 0.38±0.24          | 6.9±0.087           | 7.8±0.268          | 1.00                | 19.5±0.41         |
| B2                | 0.59±0.22           | 0.73±0.042         | 0.33±0.17           | 0.41±0.03          | 6.9±0.087           | 7.6±0.127          | 1.00                | 21.0±0.35         |
| B3                | 0.59±0.22           | 0.74±0.049         | 0.33±0.17           | 0.44±0.01          | 6.9±0.087           | 7.6±0.127          | 1.00                | 23.9±0.30         |
| B4                | 0.59±0.23           | 0.76±0.063         | 0.33±0.07           | 0.47±0.007         | 6.9±0.087           | 7.4±0.014          | 1.00                | 27.3±2.10         |
| B5                | 0.59±0.23           | 0.76±0.063         | 0.33±0.17           | 0.51±0.03          | 6.9±0.087           | 7.2±0.155          | 1.00                | 27.6±2.31         |
| B6                | 0.59±0.23           | 0.79±0.070         | 0.33±0.17           | 0.54±0.05          | 6.9±0.087           | 7.1±0.226          | 1.00                | 26.7±1.67         |
| Physical and chemical pretreatment |            |                    |                      |                    |                      |                    |                      |                    |
| PCI               | 0.59±0.22           | 0.71±0.056         | 0.33±0.17           | 0.55±0.42          | 6.9±0.087           | 6.9±0.15           | 1.00                | 7.6±0.82          |
| PCB               | 0.59±0.23           | 0.82±0.077         | 0.33±0.17           | 0.59±0.23          | 6.9±0.087           | 7.0±0.296          | 1.00                | 44.2±4.05         |

P1, P2, P3 (121°C for 15 minutes, 121°C for 60 minutes, and 121°C for 120 minutes, respectively) - Physical pretreatment; C1, C2 (0.25M NaOH pretreatment and 0.25M HCl pretreatment, respectively) - Chemical pretreatment, B1 to B6 - Biological pretreatment (Volume of Bacillus flexus (CFU) - 2×10⁵, 4×10⁵, 6×10⁵, 8×10⁵, 10×10⁵, and 12×10⁵, respectively). PCI: Physical and chemical pretreatment, PCB1: Physical, chemical, and biological pretreatment, EC: Electrical conductivity, P: Physical, B: Biological, C: Chemical
The pretreated biomedical waste can achieve 32±3.5% reduction of hemicellulose while assessing the cellulose content in physical pretreatment (P), hemicelluloses content was reduced to 4% in biological pretreatment (B), and in chemical pretreatment (C), the cellulose content was reduced to 8%. In biological pretreatment (B), the cellulose content was reduced to 28-30%. The percentage of cellulose level reduction was found to be 35-40% in PCB method. In the subsequent study, the estimation of cellulose content in wood, paper, and pulp was carried out, and cellulose content after the biological pretreatment was 19% [24].

The present investigation proved that the pretreatment methods are effective in reducing the cellulosic content and changing the physiochemical parameters of the biomedical waste. Among the pretreatment methods, the combination of PCB methods are the best due to the presence of B. flexus. The pretreated biomedical waste can be used as a substrate for the production of vermicompost and bio gas because of the suitable physiochemical properties.

**CONCLUSION**

The present investigation proved that the pretreatment methods are effective in reducing the cellulosic content and changing the physiochemical parameters of the biomedical waste. Among the pretreatment methods, the combination of PCB methods are the best due to the presence of *B. flexus*. The pretreated biomedical waste can be used as a substrate for the production of vermicompost and biogas because of the suitable physiochemical properties.

**ACKNOWLEDGMENT**

The authors would like to thank the Management of Karppagam University, Karppagam Academy of Higher Education, Coimbatore, Tamil Nadu, India, for providing necessary facilities to carry out this work.

**REFERENCES**

1. Benedict RG, Carlson D. Aerobic heterotrophic bacteria in activated sludge. Water Res 1971;5:1023-30.
2. Li S, Xu S, Liu C. Fast pyrolysis of biomass in free-fall reactor for hydrogen-rich gas. Fuel Process Technol 2004;85(8-10):1201-11.
3. Harhay MO, Halpern SD, Harhey JS, Olliaro PL. Health care waste management a neglected and growing public problem worldwide. Trop Med Int Health 2009;58:17-23.
4. Bitton G. Wastewater Microbiology. 3<sup>rd</sup> ed. Hoboken, New Jersey: A John Wiley & Sons Inc.; 2005. p. 345-69.
5. Anitha J, Indira AJ. Isolation and identification of bacteria from biomedical waste (BMW). Int J Pharm Pharm Sci 2012;4(5):286-388.
7. Almuneef M, Memish ZA. Effective medical waste management: It can be done. Am J Infect Control 2003;31(3):188-92.
8. Ball AS, Betts WB, McCarthy AJ. Degradation of lignin-related compounds by actinomycetes. Appl Environ Microbiol 1989;55(6):1642-4.
9. Lee SM, Koo YM. Pilot-scale production of cellulose using *Trichoderma reesei* Rut C-30 in fed-batch mode. J Microbiol Biotechnol 2001;11(2):229-33.
10. Lynd LR, Wyman CE, Gerngross TU. Biocommodity engineering. Biotechnol Prog 1999;15(3):223-32.
11. Ohkuma M. Symbioses of flagellates and prokaryotes in the gut of lower termites. Trends Microbiol 2008;16:345-52.
12. Tahoun MK, Ibrahim AA. Conversion of natural cellulotic substrates into fermentable sugar by recombinant *Fungi* strain. J Environ Sci 1999;208:65-8.
13. Singh J, Batra N, Sobot RC. A highly thermostable alkaline CMCase produced by a newly isolated *Bacillus* sp. VG1. World J Microbiol Biotechnol 1998;17(8):761-5.
14. Mandels M, Anderotti R, Rochec C. Measurement of saccharifying cellulase. Biotechnol Bioeng 1976;95:391-414.
15. Jackson ML. Soil Chemical Analysis. New Delhi: Prentice Hall of India Pvt. Ltd.; 1973. p. 10-50.

16. Sun XF, Xu F, Sun RC, Fowler P, Baird MS. Characteristics of degraded cellulose obtained from steam-exploded wheat straw. Carbohydr Res 2005;340:97-106.
17. Balat M, Balat H, Oz C. Progress in bioethanol processing. Prog Energy Combust Sci 2008;34(5):551-73.
18. Updegraff DM. Semimicro determination of cellulose in biological materials. Anal Biochem 1969;32(3):420-4.
19. Blasi CD, Signorelli G, Di Russo, Rea G. Product distribution from pyrolysis of wood and agricultural residues. Ind Eng Chem Res 1990;38(6):2216-24.
20. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31(3):426-8.
21. Hansen J, Moller IB. Percolation of starch and soluble carbohydrates from plant tissues for quantitative determination with anthrone. Anal Biochem 1975;68(1):87-94.
22. Duff S, Murray JB. Bioconversion of forest products industry waste cellulosics to fuel ethanol: A review. Bioresour Technol 1996;55:1-33.
23. Kushwaha JP, Srivastava VC, Mall ID. An overview of various technologies for the treatment of dairy wastewaters. Crit Rev Food Sci Nutr 2011;51:442-52.
24. Yin L, Lin H, Xiao Z. Purification and characterization of cellulose from *Bacillus subtilis* YJI. J Marine Sci Technol 2010;18:466-71.