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Research Article

PHYSIOCHEMICAL ANALYSIS OF PRETREATED BIOMEDICAL WASTES

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ABSTRACT

Objectives: The main objective of the research is to pretreat the Category III biomedical waste using *Bacillus flexus* and to assess the physiochemical properties of pretreated biomedical waste.

Methods: Major pretreatment methods such as physical (P), chemical (C), biological (using *B. flexus*) (B), physical and chemical, and physical, chemical, and biological method (PCB) were adopted for this investigation. The nutritional status was analyzed before and after the pretreatment. The physiochemical parameters such as pH, electrical conductivity, moisture content, bulk density, cellulose content, and carbohydrate content were assessed.

Results: There was a significant difference between before and after the pretreatments of physiochemical properties. By treating, the biomedical waste with *B. flexus* has produced a drastic change in the cellulose level. By the assessment of each parameter before and after the pretreatment, the percentage of cellulose levels was found to be 35-40 in PCB method.

Conclusions: Hence, the study concludes that the pretreated biomedical waste contains suitable physiochemical properties, which may be used as a substrate for the production of vermicompost.

Keywords: Pretreatment, Biomedical waste, Biological treatment, Physiochemical.

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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 Ibrahim 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 2×10^5 colony-forming unit (CFU), B2: 5 kg of biomedical waste and 4×10^5 CFU, B3: 5 kg of biomedical waste and 6×10^5 CFU, B4: 5 kg of biomedical waste and 8×10^5 CFU, B5: 5 kg of biomedical waste and 10×10^5 CFU, and B6: 5 kg of biomedical waste and 12×10^5 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 meter [15]. The EC of before and after pretreated biomedical waste was assessed and expressed in dSm^{-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 non-reducing sugar presented in the biomedical waste sample was determined by subtracting of reducing sugars from total sugars [21].

RESULTS AND DISCUSSIONS

The pH of the raw biomedical was found to be acidic (6.9±0.087). 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 alkaline (7.1±0.226). 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 cellulosic mass was found to be 5.0 [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±0.234 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	Bulk density (mg/m)		EC (dSm^{-1})		pH		Moisture content (%)	
	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								
PC1	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 pretreatment								
PCB1	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) - 2×10^5, 4×10^5, 6×105, 8×10^5, 10×10^5, and 12×10^5, respectively). PC1: Physical and chemical pretreatment, PCB1: Physical, chemical, and biological pretreatment, EC: Electrical conductivity, P: Physical, B: Biological, C: Chemical

Table 2: Comparison of biochemical parameters (cellulose and carbohydrates) before and after the pretreatment

Treatment details	Cellulose content (%)		Estimation of carbohydrate (mg/g)					
	Before pretreatment	After pretreatment	Before pretreatment			After pretreatment		
			Total sugar	Reducing sugar	Non reducing sugar	Total sugar	Reducing sugar	Non reducing sugar
Physical pretreatment								
P1	53±0.2	51±1.4	144±0.02	137±0.01	7±0.02	115±0.36	111±0.30	4±1.64
P2	53±0.2	48±2.1	144±0.02	137±0.01	7±0.01	112±0.24	106±0.49	6±0.23
P3	53±0.2	47±2.8	144±0.02	137±0.01	7±0.01	108±0.58	99±0.45	9±1.88
Chemical pretreatment								
C1	53±0.2	50±1.4	144±0.01	137±0.01	7±0.03	106±0.35	94±0.60	12±0.70
C2	53±0.2	46±2.8	144±0.02	137±0.02	7±0.03	105±0.35	91±0.63	14±0.71
Biological pretreatment								
B1	53±0.2	43±2.1	144±0.02	137±0.03	7±0.01	102±4.00	88±0.75	14±3.18
B2	53±0.2	42±2.4	144±0.02	137±0.03	7±0.01	100±2.59	84±0.88	16±1.76
B3	53±0.2	41±0.7	144±0.02	137±0.02	7±0.02	98±1.18	80±0.81	18±0.35
B4	53±0.2	39±0.7	144±0.01	137±0.02	7±0.02	95±0.94	79±0.91	16±1.76
B5	53±0.2	37±2.1	144±0.02	137±0.01	7±0.04	92±3.06	72±1.66	20±1.06
B6	53±0.2	32±3.5	144±0.02	137±0.01	7±0.04	91±3.76	64±3.22	27±3.01
Physical and chemical pretreatment								
PC1	53±0.2	40±4.5	144±0.01	137±0.02	7±0.02	97±0.47	86±0.73	11±1.51
PCB pretreatment								
PCB1	53±0.2	32±3.5	144±0.01	137±0.01	7±0.03	83±6.42	59±4.31	24±3.88

P1, P2, P3 (121°C for 15 minutes, 121°C for 60 minutes, 121ºC for 120 minutes) - Physical pretreatment, C1, C2 (0.25 M NaOH pretreatment and 0.25 M HCl pretreatment, respectively) - Chemical pretreatment, B1 to B6 - Biological pretreatment (Volume of *Bacillus flexus* (CFU) - 2×10^5, 4×10^5, 6×10^5, 8×10^5, 10×10^5, and 12×10^5, respectively), PC1: Physical and chemical pretreatment, PCB1: Physical, chemical, and biological pretreatment, P: Physical, B: Biological, C: Chemical

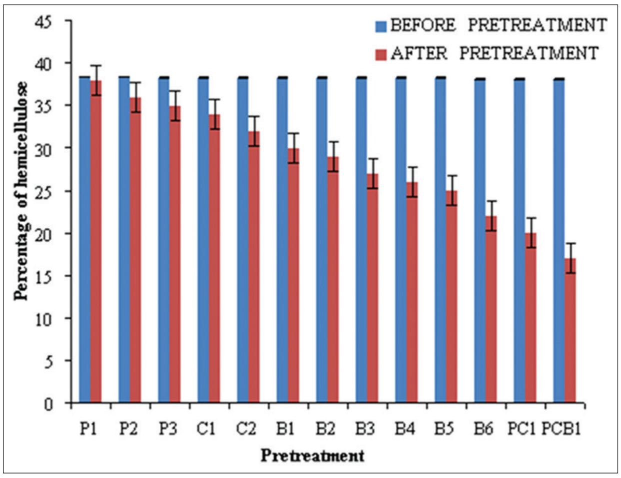

Fig. 1: Analysis of hemicellulose content in before and after pretreated biomedical wastes

When comparing the before and after pretreatments, the percentage of cellulose level was reduced drastically (Table 2). After the physical pretreatment (P), cellulose content was reduced to 4%, 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].

After the physical pretreatment (P), hemicelluloses content was reduced to 2% (Fig. 1). In chemical pretreatment (C), the cellulose content was reduced to 4%. The cellulose content was reduced to 7-9% in biological pretreatment. The percentage of hemicellulose level reduction was found to be 23-26% in PCB method. Blasi *et al*. achieved 18% reduction of hemicellulose while assessing the cellulose content in paper and pulp [19].

The Amount of carbohydrate content (total sugar, reducing sugar, and non-reducing sugar) before the pretreatment was found to be 144±0.02, 137±0.03, and 7±0.01 mg/g, respectively, and after the pretreatment process, there is a reduction in the total sugar content, reducing sugar, and non-reducing sugar (83±6.42, 59±4.31, and 24±3.88 mg/g, respectively) (Table 2). Lee *et al*. achieved the result that there was a rapid decrease in the percentage of sugar content during the pretreatment of cellulose [9].

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.

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