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Comparative efficiency of pretreatment methods on *Parthenium hysterophorus* L., as a potential feed stock

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Abstract- The efficiency of *Parthenium hysterophorus* L., as potential feed stock was investigated by using different pretreatment methods. *P.hysterophorus* L., being declared invasive weed, is an obnoxious flowering plant which is a big challenge to all attempts of control. Although several eradication measures have been undertaken in this regard for many years, not a single method is yet an option for the total eradication of *Parthenium*.Thus, the status of *Parthenium* management is visualized with respect to large-scale utilization. Therefore, in the present work, *Parthenium* is chosen to comparatively analyze its efficiency as a potential feed stock. Crop biomass mainly consists of cellulose, hemicelluloses and lignin, which act as limiting factor for being used as a potential feed stock. Thus, conversion of hemicelluloses present in the lignocellulosic biomass into xylose is one of the major steps in preparing the *Parthenium* as a potential feed stock. The objective of this work was to study the effect of pretreatments on the reduction of lignin and cellulose content which is an important factor when *Parthenium* is used as a potential feed stock. The experiments were carried out with four different pretreatment protocols which are acid, alkali, biological and water treatments at 4 g ml-1 concentration with uniform treatment period. A comparative graph obtained with total solids (TS), carbon (C), nitrogen (N), phosphorus (P), lignin and holocellulose parameters is explained in this paper.

Index Terms-Feedstock, Holocellulose, Lignin, *Partheniumhysterophorus* L, Pretreatment.

I. Introduction

Biomass has been defined to be "any material, excluding fossil fuel, which was a living organism that can be used as a fuel either directly or after conversion process" (ASTM 2002). The use of plant biomass as fuel for heat, light, food preparation has been a central to the evolution of our species (Gowlett, 2006). Although there are many forms of biomass derived fuels available today, it is still a challenge to meet the growing demand for sustainable energy systems. The plant biomass majorly consists polymers of cellulose, hemicellulose and lignin bound together in a complex structure which makes it difficult in being used as feedstock. This lignocellulosic biomass resists the bacterial degradation to a larger extent hence requiring a pretreatment process. The process by which the lignocellulosic biomass from its native form is converted into susceptible form that can be hydrolysed is referred as "pretreatment" in bioprocess engineering (Lynd et al., 2002). Thus the pretreatment protocol plays an important role in converting the plant biomass as a potential feed stock. In this present study *Parthenium hysterophorus*, an upright annual herb of 30-150 cm of family Asteraceae is considered to be a potential feedstock to meet the growing sustainable energy demand. The efficiency of *P.hysterophorus* as a potential feedstock is being investigated by using different pretreatment methods. The aim of this study is to comparatively analyze, the efficiency of pretreatment protocol on *P.hysterophorus* in making it a potential feedstock.

II. Materials and Methods

Processing of *Parthenium* for experiment:
Parthenium hysterophorus weed, used in this study was collected in Hesaraghatta, Bangalore. The plant when collected was in the flowering stage. The entire plant was carefully uprooted and was thoroughly washed under running tap water. Later the long stems along with other parts of the plant were cut into small pieces of 4-5inches in length. This was later Sun dried for 12 consecutive days. Then, the finally dried *Parthenium* material (DP) was milled to attain a final particle size of 3-5mm which was stored in closed container at room temperature until used.

Pretreatment 1: (PT1)
Dried *Parthenium* (DP) was pretreated with 6wt% NaOH solution at the rate of $4mlg^{-1}$ total solids (TS) at room temperature (RT) (28±2^{o}C) for 144hrs (6 days) with intermittent mixing. The initial pH at the beginning time of PT1 was found to be 10.5 which was then neutralized to pH 7.0 at the end of the treatment period. This pretreated sample was refrigerated for further analysis.

Pretreatment 2: (PT2)
DP was pretreated with 4wt% HCl solution at the rate of 4ml g^{-1} TS at RT (28±2^{o}C) for 144hrs (6 days) with intermittent mixing. The initial pH at the beginning time of PT2 was found to be 2.0 which was neutralized to pH 7.0 at the end of the treatment period. This pretreated sample was refrigerated for further analysis. For PT1 and PT2, Gunasheelan's (1995) method of pretreatment was slightly modified to suit the unsterilized *Parthenium* material.

Pretreatment 3: (PT3)
The DP was sterilized and then treated with sterile water at the rate of 4 ml g^{-1} TS at RT (28±2^{o}C) for 144 hrs (6 days) with intermittent mixing in an aseptic environment. The pH of this

treatment protocol had a static value of 6.8 throughout the treatment protocol.

Pretreatment 4: (PT4)
DP was pretreated with tap water at the rate of 4ml g^{-1} TS at RT (28±2°C) for 144 hrs (6 days) with intermittent mixing so as to allow the native microorganisms of the plant biomass to act upon it. The pH remained static at 6.8, before and after the treatment protocol, therefore neutralization was not required.

Analytical methods:
All compositional analysis was done in quadruplicate and data were corrected to a 100% dry matter (DM) basis. Since there was only a single sample of plant biomass, statistical analysis of the compositional data was not possible. The untreated *Parthenium* (UT) and pretreated samples (PT1, PT2, PT3 & PT4) were tested for Total Organic Carbon (TOC) by (USP – Pg 257), TS,N_2, C:N, C:P, Lignin, by APHA Standard methods, 1985 and Holocellulose by Roger M. Rowell, 2012.

III. Result and Discussion

Characteristics of UT and PT *Parthenium* biomass:
All the pretreatment procedures namely PT1, PT2, PT3 & PT4 led to changes in the physico-chemical characteristics of the plant biomass. The reported results are the mean of four replicates with standard deviation (mean±SD). The probability levels used for statistical significance were $P< 0.05$ for the tests. Statistical analysis of the data were carried out with one way analysis of variance (ANOVA).

Table 1: Effect of pretreatments on C:N & C:P of Parthenium plant biomass (Data presented is mean of determinations from the four replicates; mean±SD; n=4)

Treatments	TOC	N_2	C:N	P	C:P
UT	25.33±0.27	1.69±0.27	14.99	0.23±0.05	110.13
PT1	10.60±0.54	0.36±0.06	29.44	0.11±0.02	96.36
PT2	8.17±0.38	0.39±0.02	20.94	0.05±0.01	163.40
PT3	5.56±0.32	0.06±0.00	92.67	0.06±0.00	92.60
PT4	6.65±0.56	0.37±0.07	17.97	0.05±0.01	133.0

Table 2: Effect of pretreatments on Lignocellulosic content of Parthenium plant biomass(Data presented is mean of determinations from the four replicates; mean±SD; n=4)

Treatments	Lignin	Holocellulose
UT	13.31±0.35	19.23±0.22
PT1	6.64±0.03	10.70±0.27
PT2	9.66±0.21	3.21±0.05
PT3	6.23±0.02	11.90±0.38
PT4	5.98±0.06	6.92±0.14

C/N ratio:

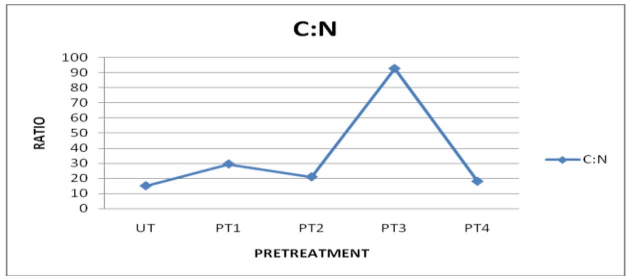

Fig: 1 Carbon Nitrogen ratio of PT *Parthenium* with that of UT *Parthenium*

Carbon and Nitrogen content present in the feedstock benefits by providing essential elements for the synthesis of Aminoacids, proteins and nucleic acid and also maintains neutral pH conditions essential for fermentative process by microorganisms. At the same time, presence of excess nitrogen in the feedstock might result in accumulation of toxic substance due to excess ammoia formation. The C/N ratio lower than 10:1 werefound to be inhibitory in various decomposition patterns (Fry 1975; NAS 1977; BORDA 1980; UNEP 1981; Kimchie 1984; Marchaim 1989). A C/N ratio of 30 is often cited as optimal (Uri Marchain, 1992). In this study, various pretreatment protocols were attempted in order to bring the C/N ratio to the optimum thus making the *Parthenium* substrate, a potential feed stock. From the data obtained, (Table 1; Fig:1) in comparison to that of the UT, all the PT have shown significant change in the C/N ratio ($P < 0.05$) which is confined to the relative optimum range of C:N 25-30:1(Dioha. I. J. et al., 2013) of *Parthenium* hydrolysis. Though the PT4 does not fall in the favourable range it is still seems to get reduced better than the UT *Parthenium*. Also there is an astoned increase in C/N ratio of PT3, whose efficiency of decomposition can be well established with further processing of the plant biomass.

C/P ratio:

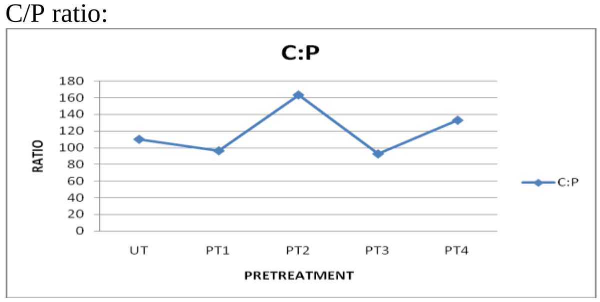

Fig: 2 Carbon Phosporus ratio of PT *Parthenium* with that of UT *Parthenium*

Nutritional value of C:N is essential for the bioprocess and metabolism, likewise C:P content is also important. During the decomposition, microorganisms assimilate and transiently store Phosphorus in their biomass, enabling active cycling of organic phosphate which is largely mediated by their metabolism. This net release of Phosphorus from decomposing depends on C/P ratio of the substrate. C/P ratio of plant tissue can vary subsequently. In this study the various pretreatments were monitored to determine the C/P ratio thus making the

Parthenium biomass a potential feedstock. From the tabulated C/P ratio (Table 1; Fig:2), all the pretreated *Parthenium* substrate including the untreated substrate fell in the optimum range for C/P ratio. Among the preliminary studies carried on the pretreatment protocols, PT2 is well capable of being used as feedstock for vermicomposting(Anoop. Y, et al., 2013) and all the other treatments were well suited for biofertilizer production, falling in between the optimum range of 75-150:1(Gregor. D. Z., et. al, 2012).

Lignin and Holocellulose:

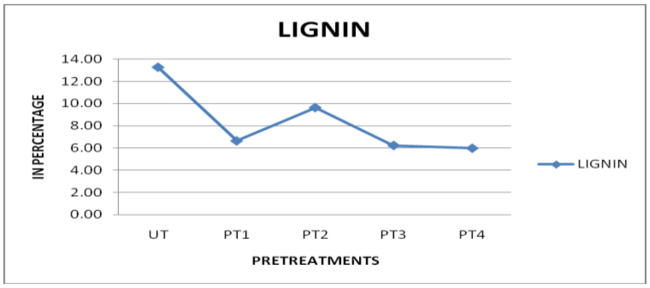

Fig: 3 Lignin (%) of PT *Parthenium* with that of UT *Parthenium*

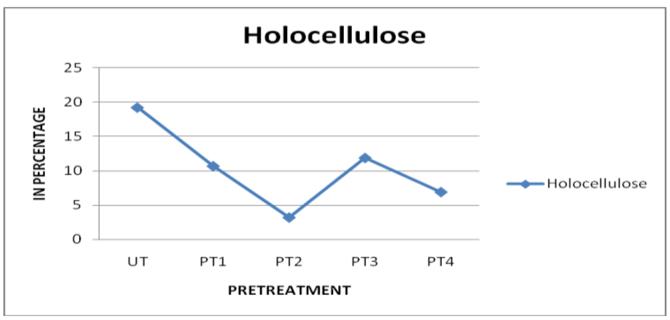

Fig: 4 Holocellulose (%) of PT *Parthenium* with that of UT *Parthenium*

Though there is abundance of organics in the weed biomass, much of it was not available to the microbes for better decomposition due to the presence of resistant and protective compounds like lignin and holocellulose. The breaking down of such compounds in original biomass by various pretreatment paves way for better utilization of the plant biomass as potential feedstock. In this study, there was a statistically significant ($P < 0.05$) reduction in the lignin and holocellulose percentage among the PT samples when compared to UT samples.

CONCLUSION

Parthenium hysterophorus a noxious weed has been a challenge for various prevention and eradication procedures, calling for an effective management method. Biomethanation being a sought after technique in weed management this paper has given an insight with various pretreatment protocols in making the *Parthenium* weed a potential feedstock. A conclusive single pretreatment protocol can be well established with further study on processing the biomass for the biomethanation process.

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