



Research Article

Enhancing the Absorbency of Bagasse through Enzymatic Delignification

V. Bhanu Rekha^{1*}, K. Ramachandralu¹ and T. Rasigha²

Abstract

Suitability of the Bagasse fibres to be used for the absorbent hygiene products were improved by enzymatic delignification process, which is the removal of the structural polymer lignin from plant tissue to increase the absorbency of the fiber which is the main requirement for sanitary napkins. Bagasse fibres were delignified in an eco-friendly method using the laccase enzyme and the delignification process was optimized using Box-Behnken experimental design. These delignified fibres were still stiff and may not be suitable for applications in hygiene products. So these fibres were converted into the pulp form in order to increase their suitability. Pulp stage was found to give a better performance than the fiber stage. The delignified bagasse fibres and pulp showed highest absorbency and lowest lignin content when compared to the raw and cleaned stages. This clearly shows that delignification using laccase enzyme can considerably improve the suitability of bagasse for the absorbent hygiene products and help in recycling bagasse to enhance its functionality.

Keywords

Bagasse; Delignification; Hygiene products; Box-behnken; Laccase enzyme

Introduction

Consumers are showing higher levels of environmental concerns and are displaying eco-friendly consumer behavior. This has increased the demand for improving the eco-friendly nature of apparel products [1] which is possible through recycling the waste products. Bagasse is a one such sugarcane by-product which could be recycled to improve its functionalities. The chemical composition of bagasse is approximately 50% cellulose, 25% hemicellulose, and 25% lignin. Bagasse is considered as a rich natural resource when compared to other agricultural residues because of its high yield and annual regeneration capacity [2]. In recent years, there has been an increasing trend towards more efficient utilization of agro waste products especially in sugarcane bagasse. The major constituent of all plant materials is cellulose which forms about half to one-third of plant tissues. One of the largest cellulosic agro-industrial by-products is sugarcane bagasse. It is a ligno-cellulosic residue of the sugar industry [3]. Generally this Cellulose is bonded with

Hemicellulose and Lignin. This increases the crystalline nature of the fibres and reduces the absorbency. So these hemicellulose and lignin has to be removed to convert the crystalline region into amorphous region. Delignification is the process of removal of lignin. This lignin removal can be done through various enzymatic methods which are commonly known as enzymatic delignification.

Lignin degradation is primarily an aerobic process, and in an anaerobic environment lignin can persist for very long periods [4]. Because lignin is the hardest component of the plant cell wall, the higher the proportion of lignin the lower the bioavailability of the substrate [5]. Some organisms can develop the necessary enzymes to break lignin separately [6]. These lignin biodegradation have been carried out mostly using white-rot fungi, which produce extracellular lignin modifying enzymes such as laccases and peroxidases (lignin peroxidases and manganese peroxidases) [7]. In this study the laccase enzyme were used for the delignification of bagasse fiber.

Along with the enhancement of absorbency, biodegradability also can be enhanced by pre-treating the lingo cellulosic fibres with alkali treatment [8]. In every pre-treatment step, a fraction of the lignocellulosic material will be removed from the solid bagasse and transferred to the hydrolysate.

It is found that the hemicellulose removal can be achieved by gradually increasing the NaOH concentrations in the alkali pre-treatments given to bagasse fibres, reaching an 88% maximum removal using a 2% NaOH solution [9]. Sodium hydroxide (NaOH) presents the greatest degradation of lignin when compared to other alkalis, such as sodium carbonate, ammonium hydroxide, calcium hydroxide and hydrogen peroxide [10,11]. So in this study the NaOH pre-treatments were given for bagasse fiber in order to reduce the hemicellulose and lignin content. Lower temperature pre-treatment gives coarser fibres whereas higher temperature pre-treatment removes more lignin. So in this study higher temperature of above 50°C was selected [12].

Material and Methods

Materials

Bagasse was collected from the sugarcane juice extraction vendors. After procurement of the fibres, they were cleaned with sapindus, an organic cleaning agent, in order to remove natural wax content and impurities. The hemicelluloses are chemically linked with cellulose molecules which reduces the absorbency of bagasse fiber. Hemicellulose removal was achieved using alkali pre-treatment using various NaOH concentrations. The removal of lignin that is delignification of fibres was done using laccase enzyme (Bactosal LAC powder) which was supplied by Clariant Chemicals Ltd, India for the removal of lignin. 15 samples of delignified bagasse fibres were prepared by varying the alkali percentage for the pre-treatment, enzyme concentration and temperature. Finally the delignification of the fibres was optimised using Box-Behnken experimental design software.

Methods

Cleaning of fibres with Sapindus (puchakai): For cleaning organic cleaning agents called (puchakai) were used. They were

*Corresponding author: V. Bhanu Rekha, Associate Professor, Kumaraguru College of Technology, Cbe-49, Coimbatore 641049, India, Tel: 984-333-9569; E-mail: rekha007_cbe@yahoo.com

Received: November 23, 2012 Accepted: January 15, 2013 Published: January 15, 2013

used as a traditional method of cleaning. Organic cleaning products have major health and environmental advantages [13]. The 10% concentration of puchakai solution was prepared to treat 10 grams of bagasse fiber with a M: L ratio of 1:0.1 till the boiling point.

Alkali treatment: Alkali treatment was given using various NaOH concentrations such as 2%, 4%, and 6% [9] at the pH of 12 at 100°C for 1 hr.

Delignification: Delignification is the removal of the structural polymer lignin from plant tissue. This Lignin reduces the absorbency of the fiber, so it has to be removed to convert the crystalline region into amorphous region in order to increase the absorbency of the fiber. The cleaned and alkali pre-treated fibres are treated with Bactosol (Laccase enzyme) for delignification with varying concentrations of 4%, 5%, 6% and varying the Temperature for 50°C, 60°C, and 70°C. This delignification process was optimised using Box-Behnken experimental design software by considering 3 variables for concentration of alkali and concentration of enzyme and temperature.

Box-Behnken experimental design software: A Box-Behnken statistical design with 3 factors, 3 levels, and 15 runs was selected for the optimization study. The experimental design consists of a set of points lying at the midpoint of each edge and the replicated center point of a multidimensional cube. 3 Independent variables with 3 levels of values each were defined as given in the Table 1. The independent variables selected were, alkali concentration in percentage, enzyme concentration in percentage and temperature in centigrade. A total of 15 runs with triplicate centre points as given in Figure 1 were generated. As per the generated runs fifteen batches were prepared and evaluated for determining the absorbency of Bagasse fibres. The optimization was done using the response factor analysis by giving the required absorbency target to be achieved. This will be generated depending on the maximum and minimum absorbency values which is obtained in the 15 runs.

Preparation of bagasse pulp from delignified bagasse fibres: As per the optimized parameters for the delignification process, cleaned fibers were delignified and the details are presented in the Table 2. The delignified samples A2E and A4E which were optimised from Box-Behnken experimental design were prepared and the corresponding pulp samples were also prepared. Samples R, C, 2A fibres have been taken for comparison along with their pulp forms. The fibers samples R, C, 2A, A2E, A4E as explained in the Table 2 have been selected to prepare the pulp. The processed bagasse fibers were still found to be crystalline and were not suitable enough to be prepared as a web in the carding machine. But due to its desirable absorbency percentage, these processed fibers were powdered to convert into pulp form to increase its suitability.

Evaluation of delignified fibres and prepared pulp: The following tests in Table 3 were done to analyse the characteristics of the processed bagasse fiber. The following tests in Table 4 were done to analyse the characteristics of the prepared bagasse pulp

Results and Discussions

Optimization of delignification of fibres with enzymes using the Box-Behnken experimental design

Based on runs generated in the below Box-Behnken Figure 1, the 15 delignified samples were processed and their absorbency was calculated for the response values as given in the Tables 5 and 6.

From the Figure 2 we can find that 2% and 4% alkali performed better than 6% alkali. Enzyme of about 5% shows good absorbency than 4% and 6% enzyme. The temperature of about 70°C performs better than 50°C and 60°C.

Optimisation for the absorbency target 1000

Absorbency was taken as the response factor in box-behnken experimental design and the delignification process of bagasse fibres was optimized. When the target was fixed as 1000 in the response

Table 1: Initial setting of independent variables and their 3 levels.

Independent Variables	Name	Units	Low Level	Medium Level	High Level
A	Alkali	Concentration in percentage	2%	4%	6%
B	Enzyme	Concentration in percentage	4%	5%	6%
C	Temperature	Centigrade	50°C	60°C	70°C

↓	C1	C2	C3	C4	C5	C6	C7
	StdOrder	RunOrder	PtType	Blocks	Alkali%	Enzyme%	Temp
1	14	1	0	1	4	5	60
2	7	2	2	1	2	5	70
3	3	3	2	1	2	6	60
4	8	4	2	1	6	5	70
5	6	5	2	1	6	5	50
6	11	6	2	1	4	4	70
7	9	7	2	1	4	4	50
8	10	8	2	1	4	6	50
9	13	9	0	1	4	5	60
10	1	10	2	1	2	4	60
11	2	11	2	1	6	4	60
12	4	12	2	1	6	6	60
13	5	13	2	1	2	5	50
14	15	14	0	1	4	5	60
15	12	15	2	1	4	6	70

Figure 1: Box-Behnken optimized 15 runs.

Table 2: Treatments given to the fiber samples.

Samples	Particulars of treatments given to fibers
R	Raw Bagasse
C	Cleaned Bagasse
2A	Fibers pre-treated with 2% Alkali
A2E	Fibers pre-treated with 2% Alkali & delignified with 5.5% Laccase Enzyme at 70°C
A4E	Fibers pre-treated with 4% Alkali & delignified with 5.5% Laccase Enzyme at 70°C

Table 3: Test conducted for Bagasse fiber.

Bagasse fiber	Testing standards
Absorbency	MA001-1-diapers-worldwide.com
Moisture content%	ASTM D 2495-01
Moisture Regain%	ASTM D 2495-01
Chemical composition test	AATCC Test Method 20A-2011

Table 4: Test conducted for Bagasse Pulp.

Bagasse Pulp	Testing standards
Absorbency	MA001-1-diapers-worldwide.com
Moisture content%	ASTM D 2495-01
Moisture Regain%	ASTM D 2495-01
Immersion time test method	US patent (5,419,955)
Fineness % with 90 micron diameter sieve	IS: 4031-P-I

Table 5: Parameters for the optimization of absorbency target 1000%.

Goal	Lower	Target	Upper
absorbency- Target	551%	1000%	1053%

Table 6: Parameters for the optimization of absorbency target 900%.

Goal	Lower	Target	Upper
absorbency- Target	551%	900%	1053%

optimizer in Box-Behnken, the global solution obtained was Alkali -2%, Enzyme -5.5% and Temperature -70°C. This is clearly represented pictorially in Table 5 and Figure 3.

Optimisation for the absorbency target 900

When the target was fixed as 900 in the response optimizer in Box-Behnken and the global solution obtained was Alkali% -2, Enzyme% -5.5 and Temperature -70°C. This is clearly represented pictorially in Table 6 and Figure 4.

Evaluation of prepared fibres and pulp

The Figure 5 shows the comparison of Absorbency of the processed Bagasse fibres and pulp. From the Figure 5 the bottom graph line which denotes the absorbency recorded by the fibres is much at a lower level. But definitely the various processes of cleaning (sample C), alkali pre-treatment (sample A2) and delignification (sample A3E & A4E) have showed an increase in the absorbency of the fibres. Alkali pre-treatment has removed the pith. The red line denoting the absorbency of the prepared bagasse pulp clearly denotes that the pulp stage can show a better absorbency than that of the fiber stage, owing to its increased surface area available of absorbency. This above stated increase has been found to be true with all the five samples R, C, A2, A2E and A4E. The maximum increase has been recorded by the samples C and A2E. The presence of more pith and the increase in the surface area in the pulp stage has obviously increased the absorbency in the case of sample C. The sample A2E has recorded the highest absorbency of 1597%. This clearly explains that 2% enzyme concentration is sufficient enough for delignifying the bagasse fiber. The sample A4E has recorded increase in absorbency in the fiber stage and the pulp stage but is lesser than the A2E sample. This can be explained with the chemical composition tests denoting the presence of lignin and cellulose in the samples given in Figure 6.

Lignin and cellulosic content of processed bagasse fiber

When compared to the lignin content of the raw fiber (sample R) in the Figure 6 the various processes have gradually helped in decreasing the lignin content in the samples, in the order of A2E, A2, A4E and C. When compared to the cellulosic content of the raw fiber (sample R) a gradual increase in cellulosic content in the samples are found, in the order of A4E, A2E, C and A2. The alkali pre-treatment and delignification process have considerably reduced the lignin from the fibres. When comparing between the delignified samples, A2E has the lowest lignin content than A4E, and this substantiates the better absorbency recorded by A2E in the pulp stage. The alkali pre-treated sample A2 has lower lignin when compared to the delignified sample A4E and this has helped in the higher absorbency of 1213%, when compared to that of 1063% of the A4E sample in the pulp stage.

Comparison in the moisture content% and moisture regain% between fiber and pulp stages

The above Figure 7 clearly shows a gradual increase in all the

parameters of Moisture content%, moisture regain%, in the order of R, C, A2, A2E and A4E.

Evaluation of immersion time (sec) and fineness% of prepared pulp

From the Figure 8 it is identified that a low immersion time has

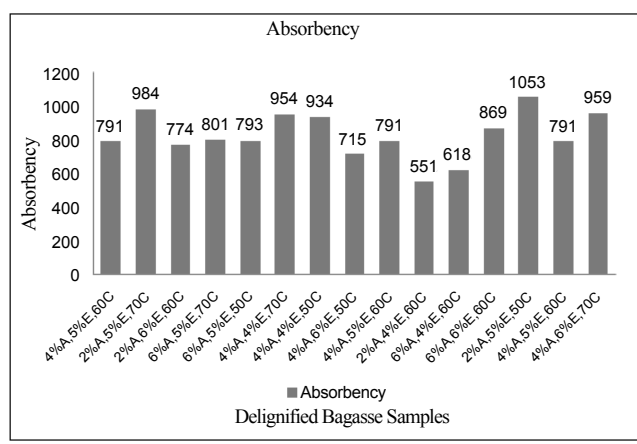
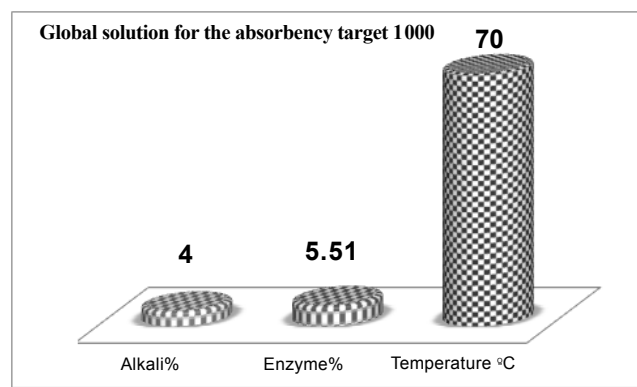
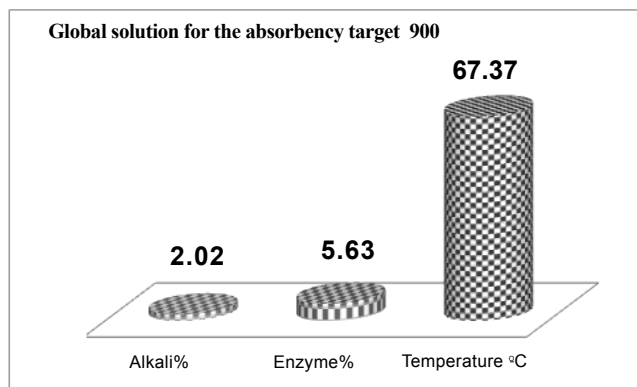


Figure 2: Absorbency for the optimized delignified samples.



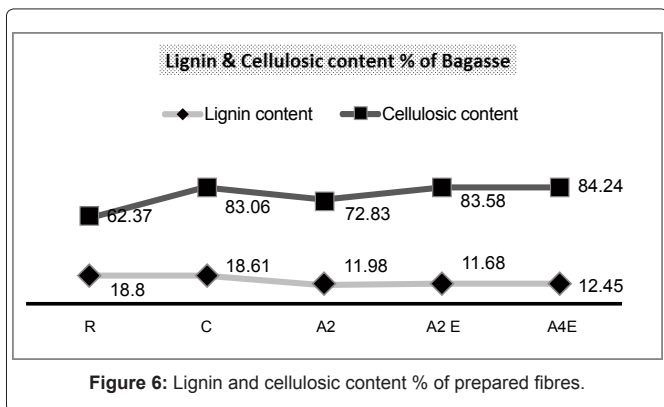
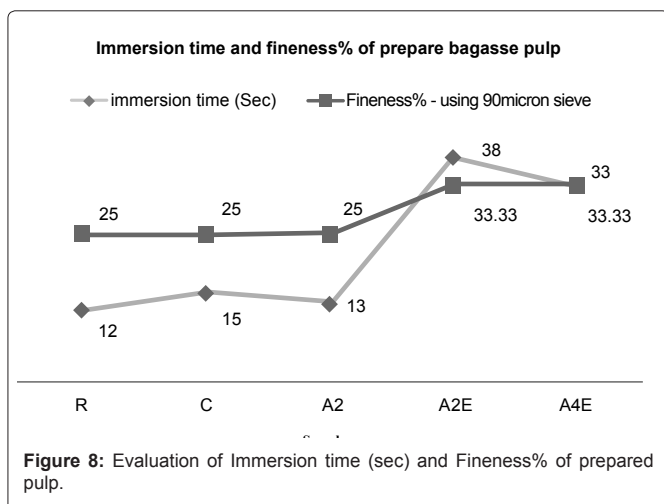
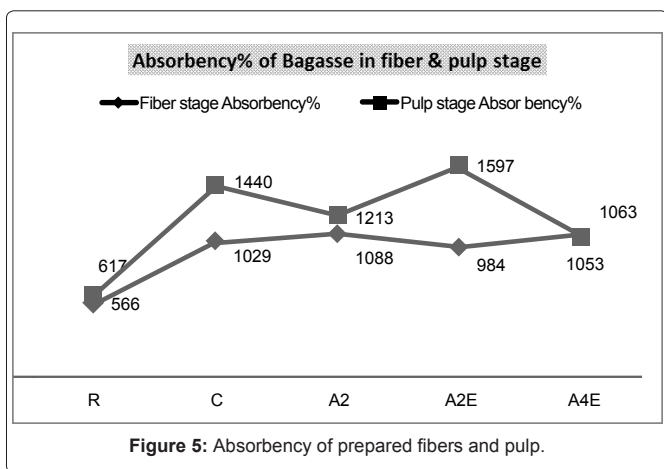
Parameters for optimising Delignification process - Box-Behnken

Figure 3: Optimization for absorbency target 1000.



Parameters for optimising Delignification process - Box-Behnken

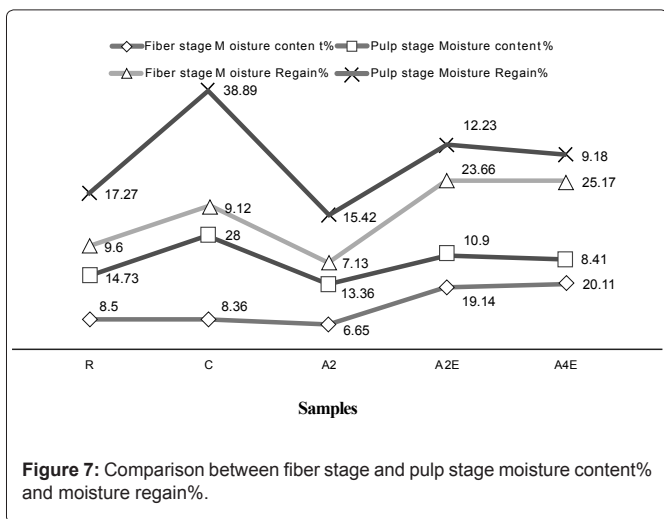
Figure 4: Optimization for absorbency target 900.



also increased. This is also marked by the presence of fibrous matter in the pulp found in the delignified samples of A2E and A4E.

Conclusion

Lower alkali concentrations used for pre-treatment of bagasse, gave a better impact on delignification of bagasse fibres, which is obvious through increased absorbency. Delignified fiber samples with 5% of enzyme concentration and with 70°C temperature gave better absorbency than other samples. 4% alkali, 5.5% of enzyme concentration and 70°C of temperature was found optimum to get an absorbency of about 1000%. 2% alkali, 5.5% of enzyme concentration and 70°C of temperature was found optimum to get an absorbency of about 900%.



been recorded in the case of R, C, A2 and it is due to the presence of pith and its faster rate of absorption in the these stages. In the case of delignified fibres, pulp is also removed along with the lignin, which increases the immersion time but increases absorbency due to the increase in cellulosic content and the capillarity of the cellulosic fibres. Higher lignin content in a fiber has higher crystallinity and makes the fibres more brittle and has led to pulp size being less than 25 microns. Removal of lignin has reduced the crystalline nature of the fibres and thereby the brittleness, so the size of the powder has

It is found that the pulp stage can show a better absorbency than that of the fiber stage, owing to its increased surface area available for absorbency. The maximum increase in absorbency has been recorded by the samples C and A2E. The presence of more pith and the increase in the surface area in the pulp stage has obviously increased the absorbency in the case of sample C. The sample A2E has recorded the highest absorbency of 1597%. This clearly explains that 2% alkali concentration is sufficient enough for delignifying the bagasse fiber.

Higher lignin content in a fiber has higher crystallinity and makes the fibres more brittle and has led to pulp size being less than 25 microns in the case of samples R, C and 2A. Removal of lignin has reduced the crystalline nature of the fibres and the brittleness, so the size of the powder has also increased to around 33.33 microns in both the delignified samples namely A2E and A4E. This is also marked by the presence of fibrous matter in the pulp found in the delignified samples of A2E and A4E. When comparing between the delignified samples, A2E has the lowest lignin content than A4E, and this substantiates the better absorbency recorded by A2E in the pulp stage.

Acknowledgement

The authors sincerely thank the Management, Department of Fashion Technology and TIFAC CORE of Kumaraguru College of Technology, Coimbatore, India for the support and infra-structure rendered for the successful conduct of the research. The authors also thank M/S Clariant Chemicals Ltd, India for supplying the laccase enzyme and rendering the technical support for the delignification process of bagasse fibres.

References

1. Kim H, Lee E-J, Hur W-M (2012) The Normative Social Influence on Eco-Friendly Consumer Behavior: The Moderating Effect of Environmental Marketing Claims. *Clothing and Textiles Research Journal*: 30: 4-18.
2. Xu Y, Wu Q, Lei Y, Yao F (2010) Creep behavior of bagasse fiber reinforced polymer composites. *Bioresour Technol* 101: 3280-3286.
3. Pandey A, Soccol C R, Nigam P, Soccol V T (2000) Biotechnological potential of agro- industrial residues. *Bioresource Technology* 74: 69-80.
4. Van Soest PJ (1994) *The Nutritional Ecology of the Ruminant*. (2nd edn), Cornell University Press, Ithaca, NY, USA.
5. Nur Husna, Baharuddin (2009) Delignification of banana stem waste in anaerobic conditions by using *Bacillus cereus*. EngD thesis, Universiti Malaysia Pahang.
6. Kirk TK, Farrell RL (1987) Enzymatic combustion, the microbial degradation of lignin. *Annu Rev Microbiol* 41: 465-505.
7. Georgieva N, Betcheva R, Yotova L, Hadzhiyska H (2007) Application of *Phanerochaete chrysosporium* 1038 – Enzyme Complex and Laccase in Biobleaching of Flax Fibers. *Bioautomation* 1: 154-161.
8. Jackson MG (1977) Review article: The alkali treatment of straws. *Animal Feed Science and Technology* 2: 105-130.
9. Rezende CA, de Lima MA, Maziero P, Deazevedo ER, Garcia W, et al. (2011) Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. *Biotechnol Biofuels* 28: 54.
10. Rodríguez-Vázquez R, Villanueva-Ventura G, Rios-Leal E (1992) Sugarcane bagasse pith dry pretreatment for single cell protein production. *Bioresour Technol* 39: 17-22.
11. Rodríguez-Vázquez R, Díaz-Cervantes D (1994) Effect of chemical solutions sprayed on sugarcane bagasse pith to produce single cell protein, physical and chemical analysis of pith. *Bioresour Technol* 47: 159-164.
12. Collier BJ, Arora M S (1996) Water Pretreatment and Alkaline Treatment for Extraction of Fibers from Sugar Cane Rind. *Clothing and Textiles Research Journal* 14: 1-6.
13. Saravanan K, Prakash C (2007) Bamboo fibers & their application in textiles. *The Indian textile journal*.

Author Affiliation

Top

¹Tejaa Shakthi Institute of Technology for Women, Cbe-659, Coimbatore, India
²Kumaraguru College of Technology, Cbe-49, Coimbatore, India

Submit your next manuscript and get advantages of SciTechnol submissions

- ❖ 50 Journals
- ❖ 21 Day rapid review process
- ❖ 1000 Editorial team
- ❖ 2 Million readers
- ❖ More than 5000 
- ❖ Publication immediately after acceptance
- ❖ Quality and quick editorial, review processing

Submit your next manuscript at • www.scitechnol.com/submission