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# Impact of anaerobic treatment on the chemical composition of cotton plant stalk

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**ABSTRACT :** Cotton plant stalk is a residual material generated by cotton cultivation. In contrast to other agricultural crop residues, cotton stalk is comparable to the most common species of hardwood in respect of cell wall composition and hence it can be used for the preparation of various value added products, one of them is pulp and paper. Standard conventional chemical processing of pulping involves cooking of cotton plant stalks in a digester under high temperature and pressure in the presence of chemicals. The effluents generated during this process causes environmental pollution. Biological processes offer an ideal and appropriate solution in this context. A large number of fungi and bacterial are found in nature, which can degrade lignin as well as other components of plant materials under aerobic as well as anaerobic conditions. Employing these microorganisms for softening of cotton plant stalks prior to chemical pulping can result in saving energy and chemicals as compared to the conventional chemical process.

**Key words:** Anaerobic treatment, chemical composition, cotton plant stalk

About 25 million tonnes of cotton stalk is generated in India every year (Shaikh *et al.*, 2010). A technology was developed to produce different grades of pulp and paper by conventional process. This however, involved cooking of raw material in alkali at very high temperature for a long time. The effluents that come out are also highly toxic. Therefore, there is a need to develop new more eco friendly process for preparation of pulp and paper. Referred to this a new bio pulping process has been developed.

Bio pulping is a relatively new concept, which in the early years employed *Basidiomycetous* fungi, *viz.*, *Phanerochete*, *Aureobasidium*, *Pleurotus*, etc. for breaking lignocellulosic bonds. The process developed by them involves the multiplication of each organism in pure culture. Besides demanding qualified and skilled personnel for inoculation, this process results in low pulp yield.

The process reported here involves *in situ* treatment of mild alkali treated cellulosic materials at room temperature for 7 days with mixed microbial consortium under anaerobic conditions. When lignocellulosic materials are subjected to anaerobic system containing a mixed microbial consortium, the organisms first try to attack the easily degradable materials. The study clearly indicated that it is possible to ascertain the susceptibility or otherwise of the lignocellulosic materials by subjecting them to a complex anaerobic system for varying periods and noting the changes occurring with regard to beta and gamma cellulose and lignin contents.

Lignin, which provides the structural support in wood, is a chemically resistant molecule, consisting of many benzene like structures, interconnected by carbon carbon bonds or carbon oxygen bonds. Anaerobic degradation of lignin molecules was not known before but recently some bacteria like sulfur reducing *Desulphovibrio* and some other bacteria are reported to degrade lignin in the absence of oxygen. Very little degradation of lignin was observed in a short incubation time of 7 days and at a lower concentration of alkali in the pretreatment. The present study was undertaken mainly with a view to biologically soften the cotton plant stalk *i.e.* to carryout chemical analysis of cotton plant stalks before and after anaerobic treatment and to process the alkali pre-treated raw materials anaerobically for various periods with a post alkali treatment.

## MATERIALS AND METHODS

The cotton plant stalk was procured from Central Institute for Cotton Research, Nagpur. All the chemicals used were of analytical reagent grade and the tests were done as per TAPPI standard methods.

**Biological softening Pre treatment :** The cotton stalk chips (100 g) was open boiled in an autoclave with 1 - 4 per cent (w/w) NaOH (1:10 bath ratio) for 30 min, cooled, washed with tap water and subjected to anaerobic treatment.

**Anaerobic treatment :** The pre treated raw material was transferred to 10 l capacity bottles. Six litres of tap water was added which included 100 ml of microbial consortium as inoculum. The mouth of the bottles were closed with one holed rubber cork through which an 'L' shaped glass tube was introduced. A rubber tube was connected to an air trap to prevent the entry of air. There was always a positive pressure in the digester due to the production of gas. Cotton stalk chips were subjected to 1, 2, 3 and 4 weeks of anaerobic treatment.

**Post alkali treatment :** The biologically treated cotton stalk chips were washed thoroughly with water and then given a post alkali treatment by boiling in an autoclave with 1 per cent NaOH (w/w) at a material to liquor ratio of 1:10 for 30 min and washed thoroughly with water. The materials collected after alkali pre treatment, after anaerobic treatment at different intervals and after post alkali treatment were used for chemical analysis.

**Chemical Composition :** Chemical composition of untreated and anaerobically treated samples for ash, lignin, alpha, hemicellulose were carried out as per TAPPI standard test method. Similarly holocellulose was determine.

## RESULTS AND DISCUSSION

**Table 1.** Chemical composition of cotton plant stalks

| Ash (%) | Lignin (%) | Alpha cellulose (%) | Hemicellulose (%) | Holocellulose (%) |
|---|---|---|---|---|
| 7.0 | 27.0 | 75.9 | 16.4 | 77.3 |

The chemical analysis data indicated that (Table 1) cotton plant stalks are rich in cellulose and are much similar to hardwood in respect of fibre dimensions and hence can be a potential raw material for manufacture of various value added products. Accordingly, technologies were developed at CIRCOT, Mumbai to utilise cotton plant stalks for the preparation of particle boards, fibre boards, various grades of pulps and paper, for growing edible mushrooms, etc. (Shaikh, 1990, Gurjar., 1994). A study on biological softening of cotton plant stalk for the preparation of hardboard was carried out at CIRCOT (Chaubal., *et al.*, 2011). The results indicated that there was not much change in the chemical composition after one day of anaerobic treatment, whereas after one week treatment slight changes in the chemical composition was observed. The process is eco friendly, economical and possible to scale up on a commercial scale.

**Effect of alkali pre treatment on chemical composition :** During sodium hydroxide pre treatment the alkali reacts with cellulose, hemicellulose, lignin and other chemical constituents and softens the fibre wall both within the middle lamella and within the secondary wall and assists in the fibrillation of the $S_1$ and $S_2$ layers. (Kirk *et. al.*, 1994) observed changes in chemical composition of lignocellulosic material during pre treatment. The changes in the chemical composition of cotton plant stalks brought about due to alkali pretreatment are given in Table 2. The data

clearly indicated a slight reduction in the percentage of hemicelluloses and lignin due to alkali pre treatment with increase in holocellulose percentage. However, the reduction was more pronounced with the increase in the concentration of alkali from (1 to 4%).

**Effect of anaerobic treatment on chemical composition**

**Degradation of hemicelluloses :** It is well known that when lignocellulosic materials are subjected to anaerobic treatment, the hemicelluloses are preferentially utilised. These are generally polymers and can be easily attacked by various microorganisms. The losses were not significant at lower concentrations of alkali pre treatment and in the initial stages of anaerobic treatment. However, there was a hemicellulose degradation observed with the increase in chemical concentration from 1 to 4 per cent as well as with the increase in the period of anaerobic treatment from 1 to 4 weeks (Table 3).

The hemicellulose content in cotton plant stalk was 16.4 per cent (Table 2). After 1 per cent alkali pretreatment followed by 1 week anaerobic treatment the loss of hemicelluloses was 9.1 per cent as compared to initial value (16.4%). However, the same was increased to 33.6 per cent when the period of anaerobic treatment was extended up to 4 weeks. Similarly, when 4 per cent alkali used in the pretreatment the loss of hemicellulose increased to 25 and 47.6 per cent with 1 and 4 weeks treatment, respectively.

**Effect of post alkali treatment on hemicelluloses**: When the anaerobically treated materials were given a post treatment with 1 per cent alkali, a drastic increase in the hemicellulose losses was noticed. The hemicellulose losses increased progressively with the increase in the concentration of alkali and period of anaerobic treatment. The loss of hemicelluloses with 1 per cent alkali varied from 19.5 to 39.0 per cent from 1 to 4 weeks. Similarly with 4 per cent alkali the loss of hemicelluloses varied from 35.4 to 60.9 per cent from 1 to 4 weeks (Table 3). The drastic changes in the loss of hemicelluloses due to alkali post treatment are very significant.

**Changes in alpha, beta and gamma cellulose due to anaerobic treatment** : When lignocellulosic materials were subjected to an anaerobic system containing a microbial consortium, the organisms tend to attack first the most easily degradable materials.

As given in Table 4, amongst the various chemical constituents, the gamma cellulose is the most easily susceptible constituent to microorganisms. Alpha cellulose increases from 75.9 to 76.8 per cent with 1 per cent alkali pre treatment. This increasing trend was observed with the increase in alkali concentration from 1 to 4 per cent as well as with the increase in the period of anaerobic treatments from 1 to 4 weeks. In the case of beta cellulose, there was no change observed with 1 per cent alkali pre treatment. The increasing trend was observed with the increase in alkali concentration from 2 to 4 per cent as well as with the increase in the period of anaerobic treatment from 1 to 4 weeks. Also with post alkali treatment there was further 1 per cent increase in the values of alpha and beta cellulose compared to corresponding anaerobic treatment.

**Degradation of gamma cellulose due to anaerobic treatment :** The degradation of gamma cellulose is quite low at initial stages of anaerobic treatment of alkali pretreated raw materials at 1 per cent concentration. However, an increasing trend was noticed in the gamma cellulose degradation with the increase in the concentration of alkali used in the pre treatment as well as with the increase in the period of anaerobic treatment. The degradation of gamma cellulose increased to 44.8 per cent from an initial level of 17.1 per cent with increase in the concentration of alkali and the period of anaerobic treatment. Maximum degradation of 74.3 per cent was achieved with 4 per cent alkali pre treatment and 4 weeks of anaerobic treatment. The loss of

**Table 2.** Chemical composition and Cellulose degrodatia of cotton plant stalks after anaerobic treatment

| Treatment | Holocellulose (%) | | Hemicellulose | | Lignin (%) | | Pre-alkali Treated | | | Post-alkali Treated* (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre treatment | Post treatment* | Pre treatment | Post treatment* | Pre treatment | Post treatment* | α-cellulose (%) | β-cellulose (%) | γ-cellulose (%) | α-cellulose (%) | β-cellulose (%) | γ-cellulose (%) |
| Control | 77.3 | | 16.4 | | 27 | | 75.9 | 1.1 | 21 | - | - | - |
| Alkali (1%) | 75.8 | | 15.4 | | 26.4 | | 76.8 | 1.1 | 20.1 | - | - | - |
| Alkali (1%)+ 1 week | 78.8 | 80.5 | 14.9 | 13.2 | 26.2 | 23.8 | 81.4 | 1.2 | 17.4 | 83.8 | 2 | 14.2 |
| Alkali (1%)+ 2 weeks | 80.1 | 81.2 | 13.8 | 12.5 | 26 | 22.4 | 83.3 | 2.2 | 14.5 | 85.1 | 2.7 | 12.2 |
| Alkali (1%)+ 3 weeks | 80.9 | 82.3 | 12.8 | 11.4 | 25.8 | 21.7 | 84 | 3.8 | 12.2 | 85.9 | 4.3 | 9.8 |
| Alkali (1%)+ 4 weeks | 82.1 | 83 | 10.9 | 10 | 25.5 | 20.2 | 84.3 | 4.1 | 11.6 | 85 | 5.5 | 9 |
| Alkali (2%) | 76.2 | | 14.9 | | 25.9 | | 78.2 | 1.4 | 20.4 | - | - | - |
| Alkali (2%)+ 1 week | 79.5 | 81.1 | 14.2 | 12.6 | 25.6 | 22.8 | 82.4 | 1.9 | 15.7 | 84 | 3 | 12.4 |
| Alkali (2%)+ 2 weeks | 80.9 | 82.5 | 12.8 | 11.2 | 24.9 | 21.2 | 84.3 | 2.8 | 12.9 | 85.8 | 4.1 | 10.1 |
| Alkali (2%)+ 3 weeks | 82.1 | 83.1 | 11.6 | 10.4 | 24.3 | 19.1 | 85.8 | 3.8 | 10.4 | 86.9 | 5 | 8.1 |
| Alkali (2%)+ 4 weeks | 83.5 | 84.1 | 10.2 | 9.2 | 24 | 18.2 | 86.6 | 4.9 | 8.5 | 86.5 | 6.6 | 6.9 |
| Alkali (3%) | 78.2 | | 13.8 | | 24.6 | | 78.4 | 1.9 | 19.7 | - | - | - |
| Alkali (3%)+ 1 week | 80.6 | 81.9 | 13.1 | 11.6 | 23.2 | 21 | 84 | 2.5 | 13.5 | 85.2 | 3.8 | 11 |
| Alkali (3%)+ 2 weeks | 81.8 | 83.2 | 11.9 | 10.5 | 22.9 | 19.8 | 85 | 3.9 | 11.1 | 86.8 | 4.4 | 8.8 |
| Alkali (3%)+ 3 weeks | 83.2 | 84.5 | 10.3 | 8.9 | 22.4 | 17.1 | 86.3 | 4.8 | 8.9 | 87.2 | 5.2 | 7.6 |
| Alkali (3%)+ 4 weeks | 84 | 85.1 | 9.2 | 7.7 | 22.1 | 14.9 | 87.5 | 5.5 | 7.5 | 87.5 | 5.8 | 6.7 |
| Alkali (4%) | 79.1 | | 13 | | 23.2 | | 79.1 | 2.3 | 18.9 | - | - | - |
| Alkali (4%) +1 week | 81.4 | 83.1 | 12.3 | 10.6 | 21.5 | 17.2 | 85 | 3.3 | 11.7 | 87.1 | 4.4 | 8.5 |
| Alkali (4%)+ 2 weeks | 83 | 83.2 | 10.7 | 9.8 | 21.2 | 15.9 | 87 | 4.3 | 8.7 | 87.8 | 4.9 | 7.1 |
| Alkali (4%)+ 3 weeks | 84 | 84.5 | 9.7 | 8.4 | 19.8 | 13.6 | 88.2 | 4.8 | 7 | 89 | 5.6 | 5.4 |
| Alkali (4%)+ 4 weeks | 84.8 | 85.1 | 8.6 | 6.4 | 19.4 | 11.7 | 89 | 5.6 | 5.4 | 90.1 | 6.1 | 3.8 |

* Materials were subjected to (1%) Post-Alkali treatment

**Table 3.** Loss of hemicellulose, gamma cellulose and delignification in cotton plant stalks due to anaerobic treatment

| NaOH (%) | Anaerobic treatment (Week) | Pre alkali treated | | | | | | Post alkali treated* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Based on original weight (%) | | | Based on weight after (%) | | | Based on original weight (%) | | | Based on weight after (%) | | |
| | | Hemi-cellulose | Gamma cellulose | Deligni-fication | Hemi-cellulose | Gamma cellulose | Deligni-fication | Hemi-cellulose | Gamma cellulose | Deligni-fication | Hemi-cellulose | Gamma cellulose | Deligni-fication |
| 1 | 1 | 9.1 | 17.1 | 3.0 | 3.2 | 13.4 | 0.8 | 19.5 | 32.4 | 11.8 | 14.3 | 29.4 | 9.8 |
| | 2 | 15.9 | 30.9 | 3.7 | 10.4 | 27.8 | 1.5 | 23.8 | 41.4 | 17.0 | 18.8 | 39.3 | 15.2 |
| | 3 | 22.0 | 41.9 | 4.4 | 16.9 | 39.3 | 2.3 | 30.5 | 53.3 | 19.6 | 26.0 | 51.2 | 17.8 |
| | 4 | 33.6 | 44.8 | 5.6 | 29.2 | 42.3 | 3.4 | 39.0 | 57.1 | 26.6 | 35.0 | 55.2 | 23.4 |
| 2 | 1 | 13.4 | 25.2 | 5.2 | 4.7 | 23.0 | 1.2 | 24.4 | 41.0 | 15.6 | 15.4 | 39.2 | 12.1 |
| | 2 | 22.0 | 40.2 | 7.7 | 13.6 | 36.8 | 3.9 | 31.7 | 52.4 | 21.5 | 24.8 | 50.4 | 18.3 |
| | 3 | 29.3 | 52.7 | 10.0 | 22.1 | 49.0 | 6.2 | 36.6 | 61.4 | 29.2 | 30.2 | 60.3 | 26.5 |
| | 4 | 37.8 | 59.5 | 11.1 | 31.5 | 58.3 | 7.4 | 43.9 | 67.1 | 32.6 | 38.2 | 66.2 | 30.0 |
| 3 | 1 | 20.1 | 35.7 | 14.1 | 5.1 | 31.5 | 5.7 | 29.3 | 47.6 | 22.2 | 16.0 | 44.2 | 14.8 |
| | 2 | 27.5 | 47.1 | 15.2 | 13.8 | 43.7 | 7.0 | 36.0 | 58.1 | 26.3 | 23.9 | 55.3 | 19.7 |
| | 3 | 37.2 | 57.6 | 17.0 | 25.4 | 54.8 | 9.0 | 45.8 | 63.8 | 36.6 | 35.5 | 61.4 | 30.8 |
| | 4 | 43.9 | 64.3 | 18.1 | 33.4 | 61.9 | 10.3 | 53.1 | 68.1 | 44.8 | 44.2 | 66.0 | 39.8 |
| 4 | 1 | 25.0 | 44.3 | 20.4 | 5.4 | 38.1 | 7.3 | 35.4 | 59.5 | 36.3 | 18.5 | 55.0 | 23.2 |
| | 2 | 34.8 | 58.6 | 21.5 | 17.7 | 54.0 | 8.6 | 40.3 | 66.2 | 41.1 | 24.6 | 62.4 | 31.5 |
| | 3 | 40.9 | 66.7 | 26.6 | 25.4 | 63.0 | 14.7 | 48.8 | 74.3 | 49.6 | 35.5 | 71.4 | 41.4 |
| | 4 | 47.6 | 74.3 | 28.1 | 33.9 | 71.4 | 16.4 | 60.9 | 81.9 | 56.6 | 50.8 | 79.9 | 49.6 |

* Materials were subjected to 1% Post-alkali treatment

gamma cellulose with respect to only anaerobic treatment was 13.4 per cent when 1 per cent alkali pretreated materials were subjected to 1 week anaerobic treatment as against 71.4 per cent with regard to 4 per cent alkali pre treatment and 4 weeks of biological treatment (Table 3).

**Effect of post alkali treatment on gamma cellulose :** When the anaerobically treated materials were given a post treatment with 1 per cent alkali, increase in the gamma cellulose losses was noticed. The gamma cellulose increased progressively with the increase in the concentration of alkali and period of anaerobic treatment. The loss varied from 32.4 to 57.1 per cent and from 59.5 to 81.9 per cent with increasing concentration of alkali and period of anaerobic treatment (Table 3).

The drastic change in the loss of gamma cellulose due to alkali post treatment is very significant. The gamma cellulose loss due to anaerobic treatment was 17.1 per cent, which increased to 32.4 per cent when anaerobically treated material was subjected to a post alkali treatment. The same was increased to 74.3 per cent when alkali concentration and the time of anaerobic treatment were increased to 4 per cent and 4 weeks, respectively. With post alkali treatment, the value further increased to 81.9 per cent.

**Delignification due to anaerobic treatment :** During anaerobic treatment, the degradation of lignocellulosic matrix takes place leading to the breakage of linkages between hemicelluloses and lignin. This results in the loosening of the lignocellulosic matrix, thereby opening up of the structure and fibres leading to enhanced extractability of lignin. (Vasudevan and Mahadevan, 1991) reported that *Acinetobacter* sp was found to degrade lignin model compounds and teakwood lignin observed that there is a degradation of lignin and other components of wood due to anaerobic treatment.

The data on degradation of lignin due to anaerobic treatment clearly indicates a marginal reduction in lignin content in the initial stages of treatment, when lower concentration of alkali was used in the pre treatment. This was mainly due to the hard nature of the lignocellulosic matrix, which defies microbial attack at lower concentration of alkali. However, at higher concentration of alkali the structure gets swollen and allows better penetration of chemicals and microorganisms. Therefore, a gradual decrease in the lignin content was

**Table 4.** Statistical analysis of pre and post alkali anaerobically treated samples

| Particular | Constituents | Mean difference | d.f | *'t'* - value | Table value for 't' |
|---|---|---|---|---|---|
| **Chemical composition** | Holocellulose | 1.106* | 15 | 9.046 | 2.602 |
| Hemi cellulose | -1.412* | 15 | -17.10 | 2.602 | |
| Lignin | -4.63* | 15 | -11.30 | 2.602 | |
| **Cellulose degradation** | Alpha cellulose | 1.225* | 15 | 6.92 | 2.602 |
| Beta cellulose | 0.875* | 15 | 8.20 | 2.602 | |
| Gamma Cellulose | -2.21* | 15 | -12.04 | 2.602 | |
| **Loss in hemicellulose** | Based on original weight | 8.68* | 15 | 16.68 | 2.602 |
| Based on weight after pre treatment | 10.00* | 15 | 14.97 | 2.602 | |
| **Impact on gamma cellulose** | Based on original weight | 7.86* | 15 | 9.41 | 2.602 |
| Based on weight after pre treatment | 11.15* | 15 | 12.29 | 2.602 | |
| **Delignification** | Based on original weight | 17.23* | 15 | 11.18 | 2.602 |
| Based on weight after pre treatment | 18.63* | 15 | 10.32 | 2.602 | |

*significant at one per cent level of significance
(- sign indicating the loss in hemicllulose, lignin and gamma cellulose)

noticed with increasing concentration of alkali from 1 to 4 per cent followed by anaerobic treatment from 1 to 4 weeks.

As shown in Table 6, the delignification per centage increase from 3.0 per cent (1per cent NaOH with 1 week) to 5.6 per cent (1 per cent NaOH with 4 weeks) and from 20.4 per cent (4 per cent NaOH with 1 week) to 28.1 per cent when the concentration of alkali in the pre treatment was raised to 4 per cent followed by 4 weeks of anaerobic treatment.

Delignification after post alkali treatment The data clearly indicates a significant reduction in lignin content due to post alkali treatment. The lignin content showed a gradual reduction with increase in the concentration of alkali and time of anaerobic treatment.

The delignification increased from 3.0 per cent (1 per cent NaOH with 1 week) to 11.8 per cent after post treatment with 1 per cent NaOH. The delignification per cent after post alkali treatment showed a gradual improvement with the increase in the period of treatment from 1 to 4 weeks. Further, there was a gradual increase in the delignification when the concentration of alkali in the pre treatment was increased from 1 to 4 per cent and the period of anaerobic treatment from 1 to 4 weeks (Table 6).

At 1 per cent pre treatment followed by 1 week anaerobic treatment maximum lignin removal due to post alkali treatment was 11.8 per cent. While at 4 per cent alkali pre treatment and 4 week anaerobic treatment followed by post alkali treatment, maximum delignification was 56.6 per cent.

As can be seen from Table 7, it is clear that all the parameters are found to be significant at one per cent level.

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