

# Effects of a Bacterial Treatment on the Brightness and Strength Properties of Kraft Bagasse Pulp

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## ABSTRACT

Kraft bagasse pulp was treated with several bacterial strains, including *Pseudomonas fluorescens* (Pf1 & Pf2); and *Bacillus subtilis* (B) for 10, 20, 30 and 40 days. Strength and brightness properties of hand sheets made from bacterial treated pulp were examined. The characteristics of bacterial treated pulp using scanning electron microscope were carried out. The relative changes in the properties of hand sheets made from treated pulp with *Pseudomonas fluorescens* and *Bacillus subtilis* followed by hydrogen peroxide bleaching were studied. The effect of the degree of polymerization of bleached bacterial treated pulp on the incubation time was carried out.

**Keywords:** Bacterial treatment; Kraft bagasse pulp; Hydrogen peroxide bleaching; Papermaking; Scanning electron microscope

## INTRODUCTION

The use of agricultural residues for pulp and paper production has been steadily increased in recent years. Bagasse is readily available and easily accessible in many tropical and subtropical countries in the world and is often abundant in countries without supplies of wood resources (1). The development and use of biotechnology in the pulp and paper industry started in the early 1970 and depends mainly on the production of inexpensive biocatalysts in enormous volumes and weights for industrial production (2). Some biotechnical processes have already been implemented in the industry such as bio-bleaching with xylanases, pitch reduction with lipases and enzymatic deinking. Other biotechnical techniques are close to implementation in the pulp and paper industry such as bio-pulping, bio-bleaching with laccase/ mediator system and bio-filtering of white water to remove organic materials. Many possibilities exist for implementing biotechnology in industry (3, 4). Lignocellulosic materials, bio-pulping, bio-bleaching and pulp modifications represent the bio-technical applications with the greatest potential in terms of cost reduction, process improvement and decrease environmental impacts (5-9). Uses of enzymes in bleaching of pulp and treatment of wood substrates (tissue and fibers) by fungi and bacteria have already been commercialized

(10). It is widely accepted that bacteria are not as effective as fungi in wood lignin and lignin compounds (11, 12). The potential advantages of using bacteria instead of fungi are possibly shorter treatment times due to higher growth rates, more convenient inoculation and more economic inoculum preparation techniques (12). The first report of using bacteria in wood chip treatments was made with southern yellow pine using strain of *Pseudomonas fluorescens* (13). Moreover, *P. fluorescens*, *P. marginalis* have been reported to produce extracellular enzymes e.g. lipases, which are responsible for degrading the wood extractives (14, 15). Research in these topics is focused on the search for pretreatments which improve the strength properties of the paper. The residual lignin in unbleached kraft bagasse pulp was removed to afford fully bleached pulp strength a multistage bleaching process using a combination of chlorination and alkaline extraction stage. But the effluent from such a bleaching process contains numerous chlorinated organic substances including mutagenic chlorinated phenols and dioxins (16, 17). There is a great interest therefore, in eliminating or at least reducing the use of chlorine- based chemicals in bleaching. However, these bacteria were selected not for pulp bleaching but for lignin degradation in lignocellulosic materials. A hydrogen peroxide dependent lignin peroxidase has been implicated in lignin degradation (18,

19). Hydrogen peroxide can be used as a bleaching agent particularly for brightening of mechanical and chemical pulps (20). On the other hand, hydrogen peroxide is unstable in alkaline conditions and readily decomposes, particularly in the presence of certain transition metals (21). This metal- catalyzed decomposition of hydrogen peroxide is generally considered undesirable in the bleaching operation, therefore, stabilizing agent including sodium silicate which has a buffering effect causing stabilization of hydrogen peroxide (22- 24), magnesium sulphate (Epsom Salt) (25) and complexing agents as DTPA (diethylene triamine pentaacetic acid and EDTA (ethylene diamine tetraacetic acid) are commonly used to limit the decomposition of hydrogen peroxide. This paper presents additional information on the effect of *Bacillus subtilis* and *Pseudomonas fluorescens* on the bleachability and strength properties of kraft bagasse pulp. The characteristic of bacterial treated kraft bagasse pulp using scanning electron microscope has been studied.

## EXPERIMENTAL

Unbleached kraft bagasse pulp was provided by Edfo Mill, Egypt and has the following analysis according to Tappi standard methods: - cellulose is 64.0%, pentosane is 27.5%, and lignin is 4.8% and kappa no. 34.5. Pure chemicals of laboratory grade were used.

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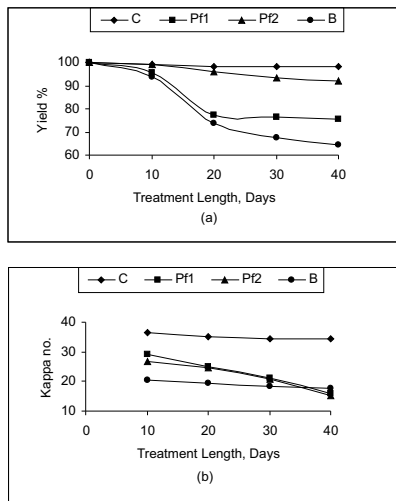


Fig. 1 (a,b): The effect of treatment duration on pulp yield % and kappa number

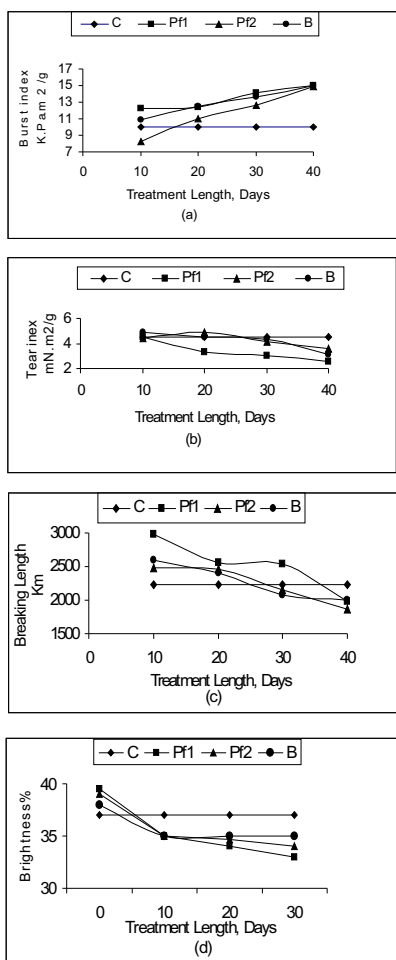


Fig.2 (a, b, c, d): The effect of treatment duration on strength and brightness properties of hand sheets made from bacterial treated kraft bagasse pulp

## Materials and methods

Strains of bacteria (*Pseudomonas fluorescens* Pf1 & Pf2 and *Bacillus subtilis* B) were evaluated for their aid in bleaching and strength properties of kraft bagasse pulp. These strains obtained from plant pathology Dept.,

National Research Centre, Egypt. The used strains were cultivated under aseptic conditions. Bacterial growth was prepared by growing each tested strain into conical flasks (250 ml) contain 100 ml of autoclaved Nutrient Broth Medium (NBM). NBM is a specific media for abundant growth of bacteria. The inoculated flasks were incubated at  $30 \pm 2^\circ\text{C}$  for 48 hour using incubator shaker. The bacterial inoculum was adjusted as  $6 \times 10^6$  CFU. CFU is colony for unit; it is used as a rat for determine microbial count in different substances.

## Bleaching sequence

Each of the bacterial treated and untreated [control] pulp samples were chelated before bleaching with hydrogen peroxide. The conditions for chelation were: start pH of 5.5, 3% consistency, 1% EDTA (ethylene diammine tetraacetic acid) at  $50^\circ\text{C}$  for 30 minute. After chelation, the pulp received a water wash at 1% consistency and the fines were passed through twice. The peroxide bleaching of the bacterial treated and untreated kraft bagasse pulp was carried out in sealed polyethylene bag at a 10% consistency, 2%  $\text{H}_2\text{O}_2$ , 1%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 2%  $\text{NaOH}$  and 3%  $\text{Na}_2\text{SiO}_3$  for 2 hrs at  $80^\circ\text{C}$ . All bleached pulps were thoroughly washed after filtration with distilled water till neutrality, then, the peroxide bleaching is repeated. Degree of polymerization of the pulp was determined (26).

Pulp and Paper testing: The kraft bagasse pulp was disintegrated to SR degree of about 40 according to SCAN-C 19: 65. Each of bacterial treated; untreated (control) and bleached pulps were used to make five physical hand sheets for the determination of the burst index, tear index, breaking length and brightness. Tests were conducted according to Tappi Standard Methods.

## Scanning electron microscope

Dehydration, critical point drying, mounting of dried pulp and 60-g/m<sup>2</sup> sheet specimens on aluminum stubs, and vacuum coating with approximately 100 angstroms of gold. Samples were examined with a Philips XL30 scanning electron microscope.

## RESULTS AND DISCUSSION

### Bacterial treatment of the pulp

The effects of different treatment durations were tested using Pf1, Pf2 and B for 10, 20, 30 and 40 days on the kraft bagasse pulp. Figure 1(a, b) illustrated the results of yield% and kappa no. after bacterial treatment. The kappa no. and yield% were decreased with increasing bacterial treatment time, for example, the kappa no. was 20.42 after 10 days of B treatment, while the value was 17.53 after 40 days of B treatment of kraft bagasse pulp. This was mainly due to the delignifying function of bacterial strain (27).

The relative changes in the properties of hand sheets made from unbleached kraft bagasse pulp treated by Pf1, Pf2 and B for 10, 20, 30 and 40 days are shown in Figure 2(a, b, c, d). The improvement in burst index and breaking length are (22.6- 49.5%) and (7.9- 33.4%) respectively, but tear index show a marginal change after 10 and 20 days and decrease after 30 and 40 days. Whereas, brightness increased after 10 days and decreased after 20, 30 and 40 days. As observed from the same figure, maximum improvements in burst index for sheets produced from unbleached kraft bagasse pulp at about 30 and 40 days. However, the maximum brightness and breaking length changes occurred within 10 days. It was reported that a bacterial treatment of unbleached kraft Bagasse pulp increased the strength properties of resulting pulp (8). It was postulated that bacterial treatment loosened the fibers resulting in easier refining and a longer average fiber length distribution (28). So, cellulose to cellulose bonding may have enhanced the hand sheet strength properties in a manner proportional to the extent of delignification. Also, an increase in carboxyl and hydroxyl groups of lignin was considered as one reason for the increased strength observed in bacterial treated pulp (29). Other reasons may be the bacterial treatment causes the fibers to stick together (5). In addition enzymes that attack cellulose and xylan often have portions (domains) of protein that bind to cellulose, which are called Cellulose Binding Domains (CBD). Deletion of the CBD of xylanases from *Pseudomonas fluorescens* and *Cellulomonas fimi* did not affect the ability of the enzymes to attack pulp xylan but had variable effects on reducing the lignin content and bleaching (31). *Pseudomonas fluorescens* enzyme has the same effect on bleaching with or without CBD, whereas *C. fimi* enzyme was

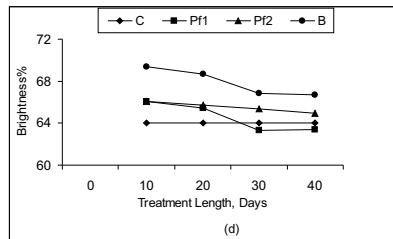
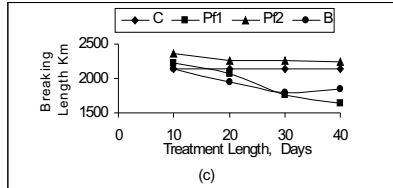
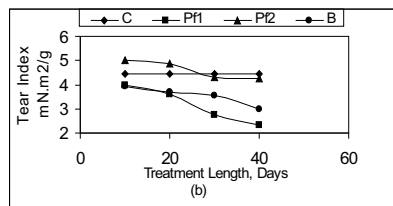
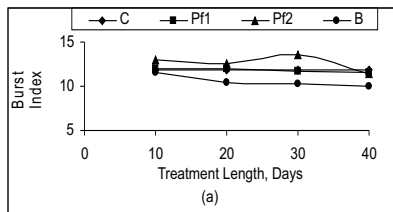


Fig. 5(a, b, c, d): The effect of treatment duration on strength and brightness properties of hand sheets made from bleached bacterial treated kraft bagasse pulp

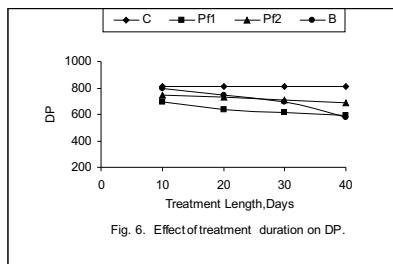


Fig. 6: Effect of treatment duration on DP of bleached bacterial treated kraft bagasse pulp

marginally better with CBD. As shown from figure 2 (d), bacterial treatment reduced or marginally increased brightness after 10 days significantly, but brightness was restored to the level of bleached control with additional hydrogen peroxide (31). Although brightness is an important aspect of paper quality; bacterial treatment results in darkening of pulp as shown in figure 2(d). Pellinen et al. (29) suggested that this discoloration was due to the formation of melanin, in addition discoloration might be caused by a process similar to the Bavendomm reaction, whereby polyphenol oxidases (laccase and tyrosinase) turn various phenolic, such as gallic and tannic and

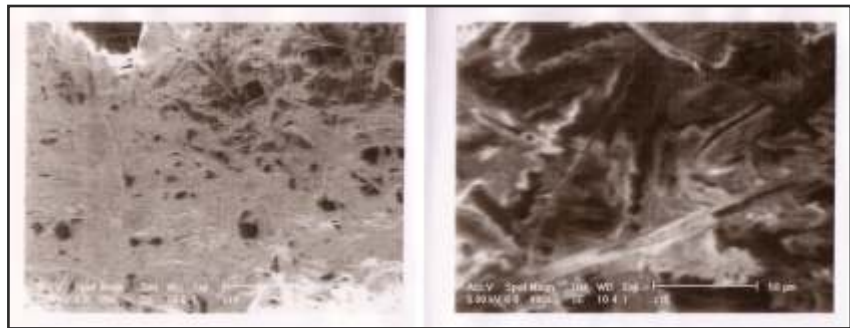


Fig. (3): SEM micrograph for untreated kraft bagasse pulp at two levels of magnifications

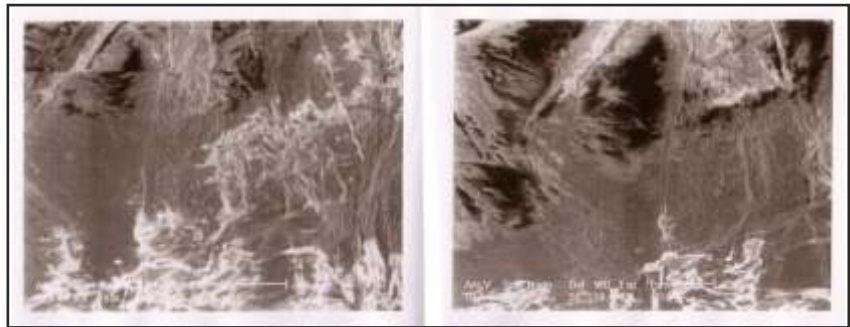


Fig. (4): SEM micrograph for treated kraft bagasse pulp by Pf1 after 10 days at two levels of magnifications

perhaps lignin, brown. So, an effort to bleach the bacterial treated kraft bagasse pulp was partially successful.

### Scanning- electron microscope study

The characteristics of bacterial treated kraft bagasse pulp using scanning electron microscope show that; the bacterial treated fibers (figure 4) were woolly and loose; these fibers were of more uniform length and have more abundant fibrillation than the untreated kraft bagasse pulp (figure 3.) The bacterial treated hand sheets fibers exhibit excellent compressibility and conformability, contributing to good bonding but in a kraft hand sheets, fibers collapse well. When bacterial treated pulp fibrillized, it emerged woollier, looser, more regular, more flexible and had more uniform fiber length than pulps produced with conventional method. Consequently, hand sheets from bacterial treated pulp were more conformable and had greater strength properties than hand sheets from kraft pulps (32).

### Bleaching studies

Figure 5(a, b, c, d) shows the relative changes in the brightness and strength properties of hand sheets made from bleached bacterial treated kraft bagasse pulp whereas, treatment was carried out by using Pf1, Pf2 and B for 10, 20, 30 and 40 days followed by hydrogen

peroxide bleaching process. The strength properties were increased after 10, 20 and 30 days and then, decreased or the same as control results. This decrease in strength properties may be due to the extensive degradation of the carbohydrate moiety (33). Figure 5(d) show that, the improvement in the brightness of the bleached bacterial treated kraft bagasse pulp was (72.9-86.5%) compared with control sample. As shown from brightness results, hemicelluloses produced by *B. Subtilis* were less effective on kraft pulps. The brightness of the pulps was increased by the hemicellulase treatments in accordance with the decreasing kappa no. The modest effect of *B. Subtilis* hemicellulase treatment on kraft pulp can be explained by absence of both  $\beta$ -xylosidase and arabinosidase activities (34).

Figure 6 shows the effect of treatment duration on DP of bleached bacterial treated kraft bagasse pulp, the degree of polymerization of bacterial treated pulp by Pf1 and Pf2 were decreased markedly after 10 and 20 days, whereas after 30 and 40 days, the degree of polymerization shows marginal decrease. But, the treatment of pulp by *B. subtilis* followed by peroxide bleaching results in decrease in the DP with increasing the incubation time, consequently, the cellulose chains were degraded.

### CONCLUSION

The effects of different treatment durations were tested using Pf1, Pf2 and B from (10- 40) days on the kraft bagasse pulp. The yield% and kappa no. were decreased with increasing bacterial treatment time. The burst index and breaking length were increased markedly, whereas tear index show a marginal improvement after 10 and 20 days in addition, a decrease in tear index and brightness after 30 and 40 days. The scanning electron microscope shows that; in a kraft bagasse hand sheet, fibers collapse well, bacterial treated hand sheet fibers emerged more woolly, looser, more regular and had more uniform fiber length than pulps produced with conventional method. The relative changes in the properties of hand sheet made from bacterial treated pulp followed by hydrogen peroxide bleaching were investigated. The results show that, an improvement in brightness takes place compared with control sample. The degree of polymerization of bacterial treated pulp was decreased with increasing the incubation time.

## REFERENCES

1. Bustamant, P., Ramos, J., Zuniga, V., Sabharwal, H. S. and Young, R.A. *Tappi J.* 82(6)123(1999).
2. Petit- Conil, M., Semar, S., Niku- Paavola, M. L., Sigoillot, J. C., Asther, M., Anke, H. and Viikari, L. *Biotechnology in the Pulp and Paper Industry* 102, 193,(2002).
3. Eriksson, K. E. L. *Tappi Pulping / process and Product Quality Conference, Boston, Proc.Session* 5(2000).
4. Moran, R. A. *Paper Age.* 114(4)10(1998).
5. Setliff, E. C., Marton, R., Granzow, S. G. and Eriksson, K. E. L. *Tappi J.* 73(8)141(1990).
6. Akhtar, M., Attridge, M. C., Myers, G. C. and Blanchette, R. A. *Holzforchung* 47, 36(1993).
7. Akhtar, M. *Holzforchung* 48, 199(1994),
8. Messner, K. and Serebotnik, E. *FEMS Microbiol. Rev.* 13(1994).
9. Esteghlalian, A. R., Srivastava, V., Gilkes, N. R., Kilburn, D. G., Warren, R. A. J. and Saddler, J. N. *Appl. Biochem. Biotechnol.* 93(3)575(2001).
10. Prasad, D. Y., Rao, N.R.M., Rajan, K.S., Praburaj, T.T. and Joyce, T. W. *Tappi J.* 9(8),133(1996).
11. Vicuna, R. *Microb. Technol.*, 10,646(1988).
12. Zimmermann, W. J. *Biotechnol.* 13,119(1990).
13. Burnes, T. A., Blanchette, R. A., Farrell, R. L. *Appl. Environ. Microbiol. J.* 66, 5201 (2000).
14. Al- Saleh, A. A., Zahran, A. S. *Microbiol.* 16, 149(1999).
15. Kallioinen, A., Vaari, A., Ratto, M., Konn, J., Siika- aho, M. and Viikari, L. *J. Biotechnol.*, 103, 67(2003).
16. Kringstad, K. P. and Lindstron, K. *Envir. Sci. Technol.* 18,236(1984).
17. Rappe, C., Swanson, S., Glas, B., Kringstad, K. P., De Sousa, F., Johansson, L. and Abe, Z. *Pulp Paper Can.* 90, 273(1989).
18. Tien, M. and Kirk, T. K. *Science J.* 221,661(1983).
19. Glenn, J. K., Morgan, M. A., Mayfield, M. B., Kuwahara, M. and Gold, M. H. *Biochem. Biophys. Res. Commun.* 114,1077(1983).
20. El- Saied, H., Nada, A. M. A., and Adel, A. M. *Research and Industry,* 40,217(1995).
21. Colodette, J. L., Rothenberg, S. and Dence, C.W. *J. Pulp and Paper Sci.*, 14(6)126(1988).
22. Burton, J. L., Compball, L. L. and Donnini, G. R. *Pulp Paper Can.* 88(6)144(1987).
23. Kutney, G. W. and Omori, S. *Pulp Paper Can.*, 86(12)182(1985).
24. Colodette, J. L., Rothenberg, S. and Dence, C. W. *J. Pulp Paper Sci.*, 15(2)45(1989).
25. Lachenal, D., Dubreuil, M. and Bourson, L. *Tappi J.*, 75,195(1990).
26. Glockner, G., Linov, K. J. and Philipp, B. F. *Faserforsch Textiltech.*, 19(3)120(1968).
27. Henriques, J. A. P., Andretta, C.W.S., Rosa, R.M., Tondo, E.C. and Gylard, C.C. *Chemosphere,* 55, 631(2004).
28. Myers, G. C., Leatham, G. F., Wegner, T. H. and Blanchette, R. A. *Tappi J.*, 71(5), 105(1988).
29. Pellinen, J., Abuhasan, J., Joyce, T.W. and Chang H.M., *J. Biotech.* 10,161(1989).
30. Rixon, J. E. , Clarke, J. H., Hazlewood, G. P., Hoyland, R. W., Mc Carthy, A. J. and Gilbert, H. J. *J. Appl. Microbiol. Biotechnol.* 46, 514(1996).
31. Akhtar, M., Rosses., Eric G.H., Grey C.M., Grey M.S., Mike J.L. *And Marguerite S.S International Mechanical Pulping Conference Tappi Proceedings Biomechanical Pulping: Amill- scal evaluation,* 1- 10(1999).
32. Sachs, I. B., Leatham, G. F., Myers, G. C. and Wegner, T. H. *Biotechnology Tappi J.*, 26,249(1990).
33. Ed de Jong, Chandra, R. P. and Saddler, J. N. *Bioresource Technology,* 61, 61(1997).
34. Viikari, L., Ranua, M., Kantelinen, A., Linko, M. and Sundquist, J. *Fourth International Symposium on Wood and Pulping Chemistry,* 27(1987)