Degradation of Black Liquor, A Pulp Mill Effluent, By Aeromonas Formicans In Batch And Continuous Processes

Jain Neeraj, Srivastava S.K. and Shrivastava A.K.

ABSTRACT

Studies have been conducted on the degradation of black liquor from a kraft pulp and paper mill by a bacterial strain Aeromonas formicans in batch and continuous processes. The results revealed that the strain was able to remove 70 to 78% of chemical oxygen demand (COD) and lignin, while the colour removal efficiency was around 86% in 10 days of retention time in batch process. The removal efficiencies in the case of continuous treatment were almost in the same range (72-86%) for all the three parameters even up to 35th days of the experiment.

KEYWORDS: *Black liquor, biodegradable, lignin, kraft process, cultures, enzymatic, hydrolase.*

INTRODUCTION

Pulp and paper mills, using the kraft process and agricultural residues such as bagasse, wheat and rice straw as raw materials produces significant amount of brown coloured effluent called black liquor from the digestors. In case of large mills generally black liquor recovery is practised However in small mills (Less than 30 Tonnes per day) the recovery is not possible and the black liquor from the pulping section joins the effluent. This black liquor has characteristically high biochemical oxygen demand, COD and suspended solids along with some slowly biodegradable lignin compounds and their derivatives. These compounds, besides imparting colour, cause aesthetic pollution as these are not readily biodegradable. Discharge of untreated waste water, into water-courses, damages the water quality and colour persists for long distances. Several workers, have tried aerobic treatment of black liquor by various bacterial strains.

Woodard et al. (1) reported the degradation of lignin in black liquor upto 90% and COD removal up to 80% with the bacteria Leptothrix ochracea, Pseudomonas multistreata and . Flavobacterium ochracea isolated from activated sludge. Deschamps et al. (2) isolated Aeromonas sp. which was found to grow on industrial kraft lignin (1.0g litre^{-1}) as sole carbon source, degrading it to the extent of 98% in five days of incubation.

Bharati et al. (3) investigated the role of the cyanobacteria viz. Chrococcus mintus and phormidium ambiguum in the removal of lignin, from the waste water of two pulp and paper mills. In one case the lignin level droped, on the fifth day of incubation, from 93.0 to 25.0 and 25.5 mg litre^{-1} by C. mintus and by P. ambiguum respectively, while in the other case the fall in lignin concentration, during the same period, was from 11.0 to 8.8 and 6.5 mg litre^{-1} by the two strains mentioned above.

Srivastava et al. (4) conducted batch studies on the degradation of black liquor by a strain of

Department of Civil Engineering
University of Roorkee
ROORKEE-247667 (U.P.) INDIA

bacteria, Pseudomonas putida for the removal of COD, colour and lignin. The maximum removal efficiency obtained for COD, colour and lignin in 10 days was found to be 73, 89, and 80% respectively. Degradation of black liquor by two bacterial strains Pseudomonas putida and Acinetobacter calcoaceticus have also been reported by Jain et al. (5). The study revealed that the two strains were able to remove 70 to 80% of COD and lignin, while the colour removal efficiency was around 85% in a retention time of 8 days.

Investigations reported in this paper deal with the attempt made to degrade black liquor by Aeromonas formicans in batch and continuous processes.

MATERIALS AND METHODS

Organism

Cultures of the bacterium A. formicans were obtained from the National Chemical Laboratory, Poona (India). Stock cultures of the strain were stored on slants of nutrient agar at 4°C and periodically subcultured.

Effluent Characteristics

A sample of effluent was collected from the pulping section of a kraft paper mill (based on agricultural residues eg, bagasse, wheat and rice straw as raw material) at the first washing stage. After filtration through a 0.45 μm membrane filter, the effluent was analysed as described in APHA (6). The main characteristics of the effluent were : pH= 11.36, COD=5389 mg litre^{-1}, Colour=7640 Co-pt units, Lignin=2.18 g litre^{-1}, glucose=Nil, Total nitrogen=3.13 mg litre^{-1} and phosphorus=0.06 mg litre^{-1}. The dark brown coloured, turbid effluent was stored at 4°C and used within three months. Filtered samples (0.45μm) were used throughout the study.

Media and Culture Conditions

Initially bacteria were grown in basal medium having the following composition (g litre^{-1}):- $(NH_4)_2SO_4$, 2.6; K_2HPO_4, 1.0; KH_2PO_4, 0.1; $MgSO_4$, 0.2; $CaCl_2$, 0.01; $FeSO_4$, 0.001; yeast extract, 0.1 and 2.0 glucose as a carbon source. The pH of the medium was adjusted to 7.2 (using HCl/NaOH) before autoclaving. Portions (150) ml of inoculated medium were incubated in 500 ml flasks on a rotary shaker at 30°C for 3 days.

Preparation of Inocula

Bacteria were collected by centrigugation in a R23 REMI research centrifuge at 1000 g for 15 minutes from each culture flask, washed with sterile water and resuspended in 150 ml of sterile water to give a cell suspension of 10^2 - 10^4 cells/ml. This inoculum was used for the degradation of black liquor in batch and continuous processes.

Determination of COD

The chemical oxygen demand was determined by dichromate method as given in APHA (6).

Determination of Colour Units

The pH of each aliquot was measured and adjusted to 7.6 with 2M NaOH. Colour Units were determined as Cobalt-Platinum (Co-pt) units, as given in APHA (6) on spectronic-20 (Bausch and Lomb) at 465 nm.

Determination of Lignin

To estimate the amount of lignins or total aromatic compounds present in the waste, lignins were precipitated, filtered and purified by an organic extraction method (7) from black liquor. A standard solution of purified lignin was prepared in IM NaOH and used to determine the extinction coefficient (absorptivity) by method of Roy et al. (8). Once the extinction coefficient was obtained, the estimation of lignin from the sample was done by the following expression given in Tappi standard (9):

$$B = \frac{A}{19.4} \times D$$

where A is absorbance at 280 nm, B the lignin contents in g litre^{-1} and D is dilution factor (if the absorbance of the sample is high, the sample is diluted) expressed as V_D/V_o, where V_D is the volume of diluted sample and V_o is the volume of original sample taken and 19.4 lg^{-1} cm^{-1} is the extinction coefficient.

Batch Process

Batch experiments were carried out in 500 ml Erlenmeyer flasks containing 200 ml of sterilized black liquor supplemented with nutrients at optimum concentration and pH for A. formicans (10). Glucose as extra carbon source and NH_4Cl as additional nitrogen source were added in the effluent at a

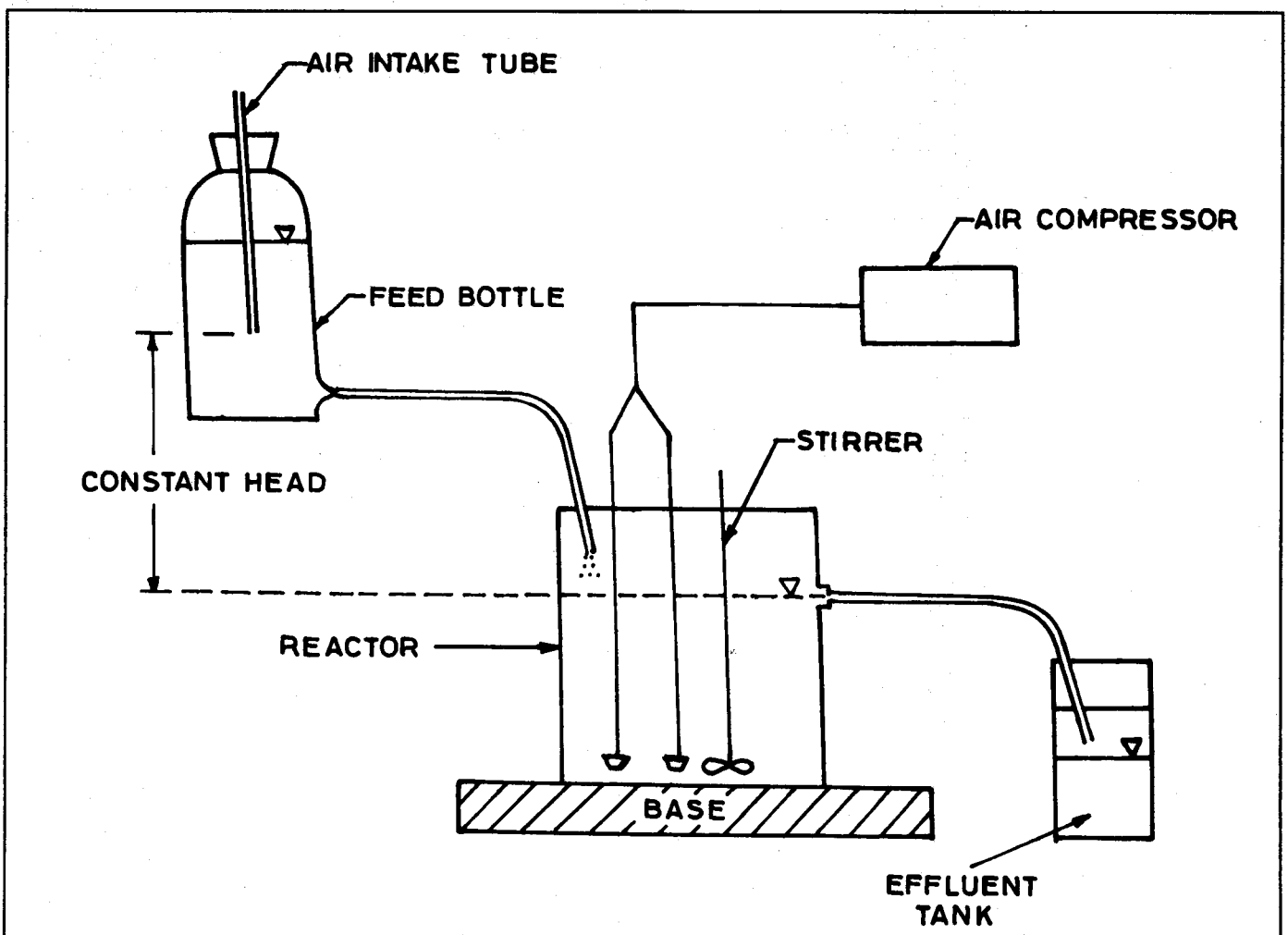

Fig.1 Continuos reactor experimental set-up

concentration of 0.4% and 0.12% respectively. The pH of the effluent was kept at 8.0. Portions of cell suspension (20ml) were used to inoculate 200 ml aliquot of black liquor. Experiments were carried out in duplicate. All flasks were kept in a water bath at 30°C and the level of dissolved oxygen was maintained at 1-1.5 mg litre^{-1} in each flask by compressed air and checked throughout the experiment. Contents of each flask were analyzed for COD, colour and lignin at time intervals of 0 to 6, 8, 10, 15 and 20 days. Non-inoculated controls were run side by side with inoculated flasks at optimum pH, with nutrients and without nutrients. All conditions were kept the same as in the case of inoculated flasks.

Continuous Process

. To study the continuous degradation of black liquor on the laboratory scale, completely mixed, continuous flow aerated lagoon reactors were used. The reactors were fabricated using plexiglas having a capacity of 6 litres and the working volume was 4 litres. Compressed air was introduced by air diffuser pipes with nozzles and the concentration of dissolved oxygen, measured by DO probe, was not allowed to fall below 1.0 mg litre^{-1}, to maintain aerobic conditions. A through mixing of the black liquor in the reactor was maintained by stirring. The substrate feed was allowed to flow under gravity by a constant head arrangement from a reservoir (capacity 8 litres) through a rubber tubing arrangement. All experiments were conducted at room temperature (25-30°C). A schematic representation of the experimental set-up is shown in **Figure-1.**

Experiments were conducted in lab models of lagoons having four litres black liquor which was separately inoculated with 400 ml of cell suspension

of A. formicans containing 10^2-14^4 cells/ml. Black liquor was supplemented with an optimum dose of nutrients (C and N) as in batch process at pH 8.0. The lagoons were in batch mode for 8 days to achieve a mature state. The efficiency of removal of COD, colour and lignin was determined on the 8th day and thereafter 500 ml of black liquor was fed every day (starting from the 8th day) drop by drop continuously from reservoir at a rate of about 0.3 ml per minute to give a detention time of around 8 days. The influent, which had a constant composition, was supplemented with nitrogen as before. Further addition of glucose was not required and no recycling of sludge was done. The efficiency of treatment was measured after 1-6, 8-10, 15, 20, 25 and 35 days of incubation by measuring reactor effluent COD, colour and lignin concentration.

RESULTS AND DISCUSSION

Batch Process

Results of batch study on removal efficiency of COD, colour and lignin by bacterial strain A. formicans are depicted in **Figure-2.** The percent removal efficiencies increase with time from 0 to 10th day, and thereafter very little change is observed (figure-2). Most of the removal of COD, colour and lignin takes place in the first 6 days. The maximum removal of COD, colour and lignin as achieved in 10 days is about 71, 86 and 78% respectively. A. formicans, shows continued activity until about the end of the 20th day of the experiment, although the removal of COD, colour and lignin becomes fairly constant after 10 days.

No perceptible changes in COD, colour and lignin content of black liquor were observed in controls without nutrients. In controls having nutrients, the change in COD and colour were negligibly small (almost ~ 10 percent) in eight days. This may be attributed to chemical oxidation of lignin due to aeration and agitation (11). Chemical oxidation affects some functional groups of the kraft lignin, but does not modify the absorbance at 280 nm which is normally used to evaluate its concentration.

Kinetics of the COD, Colour and Lignin Removal

Data corresponding to figure-2 exhibits a first order kinetics for the removal of each parameter. Values of rate constant having been evaluated in each case with the help of following expression :

$$Y_t = Y_{max} (1-10^{-kt})$$

where, Y_t =Percent removal of COD, colour or lignin at time t

Y_{max}=Maximum attainable values of COD, colour and lignin removal (%)

k =Kinetic coefficient

t =Time in days

Values of Y_{max} and k are calculated with the help of the experimental data **(Fig.2)** using a computer programme and are shown in **Table-1.**

Table-1

Values of Kinetic Parameters for COD, Colour and Lignin Removal by A. Formicans.

Parameters	K (d^{-1})	Y_{max} (%)
COD	0.43	71.84
Colour	0.60	84.11
Lignin	0.55	77.42

Continuous Process

The results of the contimuous degradation of black liquor on laboratory scale by A. formicans are presented in **figure-3.**

A perusal of figure-3 indicates that the removal efficiency of COD, color and lignin increases with time from Ist to 8th day of incubation during batch phase and it is in the range of 70 to 82% for all the three parameters. Further analysis of the curves revealed that when black liquor is continuously fed in the reactor from the 8th day, a negligible increase in efficiency is observed for the first two days. But after this time, a slow enhancement in the removal efficiency of COD, colour and lignin is observed upto the 15th day and then becomes fairly constant. The negligible changes in efficiency of removal in the first two days is due to shock loading. The maximum efficiency of removal at the end of experiment (35th day) is found to be around 73, 88 and 77% for COD colour and lignin respectively.

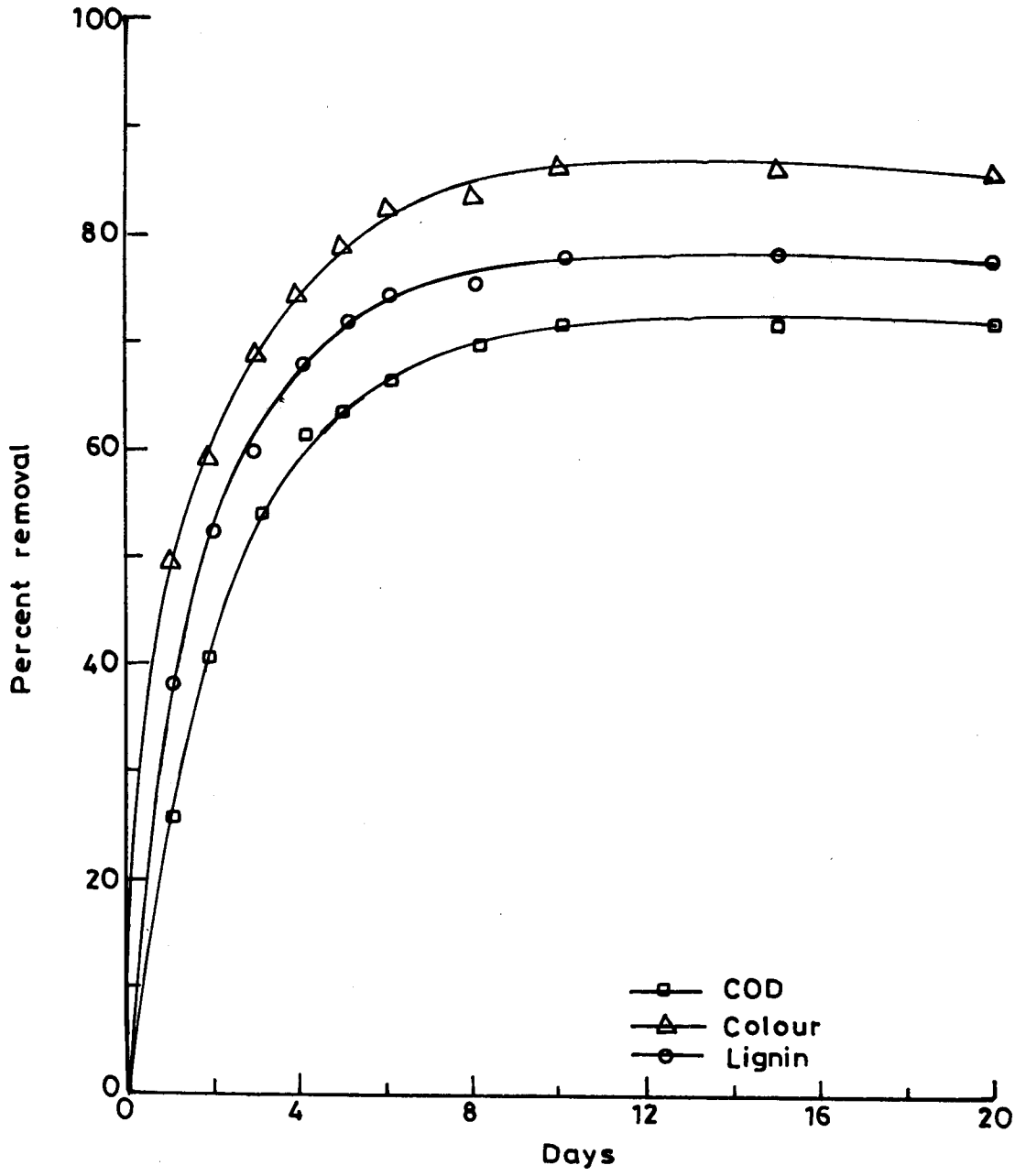

Fig. 2 Removal of COD, colour and lignin from black liquor as a function of time (batch mode)

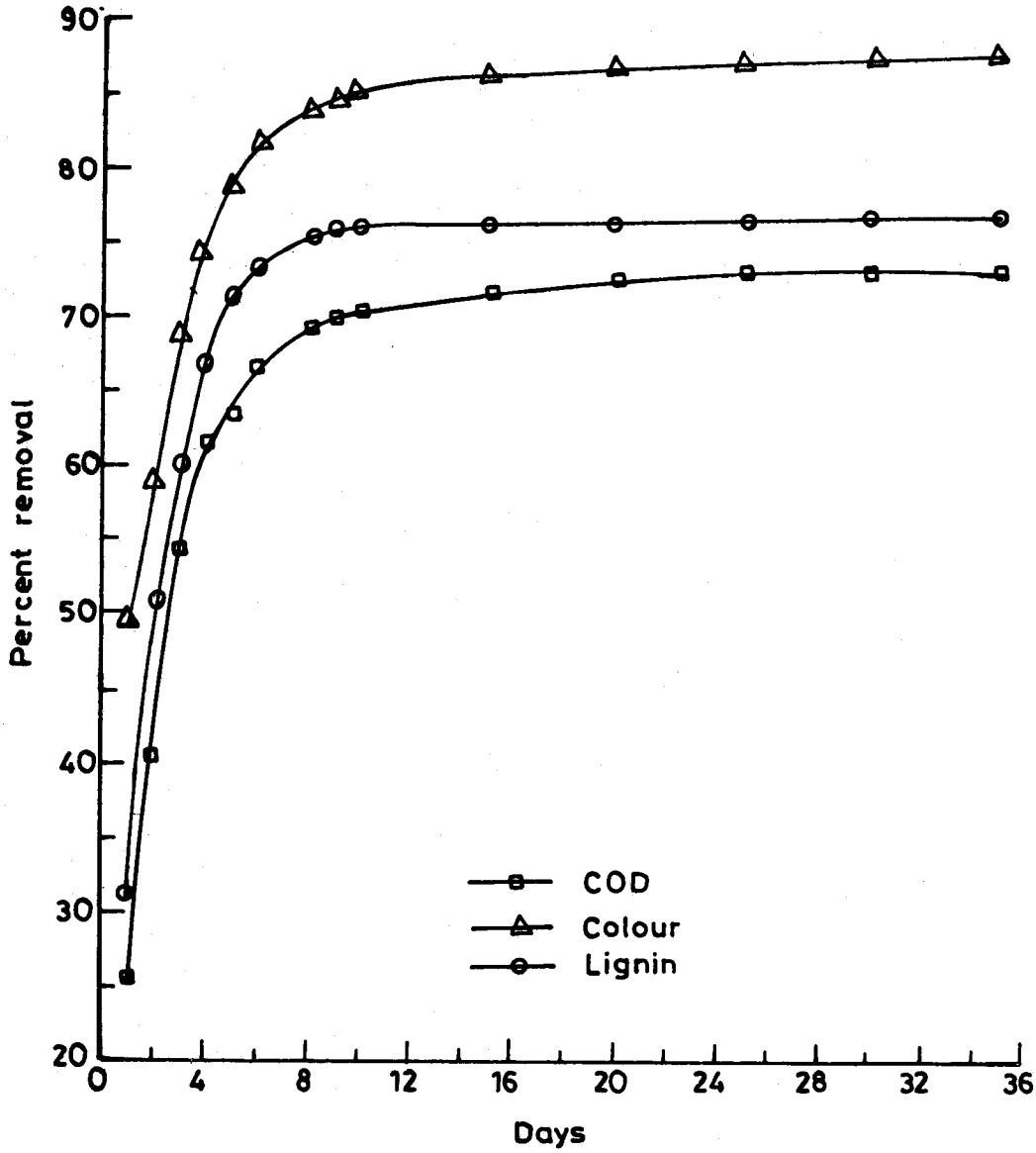

Fig. 3 Removal of COD colour and lignin from black liquor as a function of time (continuous mode)

Two mechanisms have been proposed by Borrough (12) for the removal of colloidal organic matter (lignin) from wastewater (black liquor). The principle behind the adsorption/absorption theory of organic removal is, that the wastewater interacts with the culture of microbial mass surface. At the same time a large portion of the soluble organic matter is rapidly absorbed or diffused into the cells. Subsequently the adsorbed portion is hydrolysed, released back into the solution and diffuses back inside the cell for metabolism.

In the enzyme theory of organic removal, it is believed that organic matter is transported across a bacterial cell wall by a specific set of enzyme theory of organic removal, it is believed that organic matter is transported across a bacterial cell wall by a specific set of enzymes called permeases and once inside the cell, the enzymatic reactions in metabolic pathways are completed for synthesis of new protoplasm and production of new cells. In this theory, it is also proposed that extracellular hydrolase enzymes are secreted to hydrolyse long chain, high molecular weight substances into smaller units in order that these may be transported into cells by permeases.

CONCLUSIONS

It is concluded from the lab studies that the strain A. formicans exhibits promising results when applied for the degradation of black liquor. This strain has the potential of being tried on a pilot plant scale before applying it on a large scale for the treatment of pulp mill effluent.

REFERENCES

1. F.J. Woodard, O.J. Sproul, and P.F. Atkins, The biological degradation of lignin from pulp mill black liquor, J. Water Poll. Control Fed. 36 (1964), 1401 ff.

2. A.M. Deschamps, G. Mohoudean, and J.M. Labeqult, Fast degradation of kraft lignin by bacteria, Eur. J. Appl. Microbiol. Biotech. 9 (1980), 45 ff.

3. S.G. Bharati, A.S. Salanki, T.C. Taranath, and M.V.R.N. Acharyulu, Role of cyanobacteria in removal of lignin from the paper mill waste waters, Bull. Environ. Contam. Toxicol. 49 (1992), 738 ff.

4. S.K. Shrivastava, A.K. Shrivastava and N. Jain, Degradation of black liquor, a pulp mill effluent by bacterial strain Pseudomonas putida, Ind. J. Exp. Biol. 33 (1995), 962 ff.

5. N. Jain, A.K. Shrivastava, and S.K. Srivastava, Treatment of black liquor by Pseudomonas putida and Acinetobacter calcoaceticus in a continuous reactor, Environmental Technol. 17 (1996), 903 ff.

6. American Public Health Association (1985). Standard Methods for the Examination of Water and Waste Water. APHA, AWWA and WPCE, 16th Am. Public Health Assoc. Byrd Progress, Springfield, New York.

7. K. Lundquist, and T.K. Kirk, Fracticnation-purification of an industrial kraft lignin, Tappi J. 63 (1980), 80 ff.

8. T.K. Roy, N. Marwan, S.K. Kapoor, R. Pant, and A. Panda, Utilization of lignin from agricultural residues in the manufacture of dispersants, IPPTA Seminar (1989), 1 ff.

9. Tappi Standard (1976). Acid soluble lignin in wood and pulp, Tappi Useful Method-250.

10. N. Jain, S.K. Shrivastava, and A.K. Shrivastava, optimization of culture parameters for degradation of black liquor by Aeromonas formicans, Fresenius Environ. Bull. 5 (1996), 213 ff.

11. L. Larrea, C.F. Forster, and D. Mele, kraft lignin behaviour in diffused aeration of kraft effluents, Water Sci. Tech. 21 (1989), 241 ff.

12. Borrough, Improved activated sludge process, Chemistry and Industry 36 (1967), 1507 ff.