Bio-Technical Aspects of Various Bagasse Storage Systems

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Nature is composed of organic and inorganic material. The organic chemist, the physical chemist, the mathematician and astronomer all comprehend the inorganic nature in terms of the property of matter. However, the organic nature and especially those recognised as the 'life' are organised into systems that are more complex than those of atoms and molecules. The most significant attribute of all living organisms is their ability of 'self' duplicating'. The property of self recreation is imparted by the presence of 'nucleic acids'.

Micro-organisms are intricately interconnected with our day-to-day life. The foulding of vegetables and meat products, curdling of milk, role of yeast in fermentation etc are only a few to mention. The groups virusus, bacteria, actinomycetes and fungi play an important role in this process.

At the outset there is a distinct and important nutritional distinction between different kinds of living organisms. Some of them synthesize their own food material from inorganic material—Autotrophs while yet others depend on autotrophs as they cannot synthesize their own food material—Heterotrophs.

Micro-organisms have significant impact on the paper industry. The deleterious effect of these organisms on the standing pulpwood crop, e.g. Cylindrocladium blight and pink disease on eucalyptus, dothistroma blight of pines in the log yard cause loss of valuable raw material. The bacteria, actinomycetes and fungi contribute to this deterioration. Most of the members being heterotrophs cannot synthesize their own food material and hence depend on plant tissue for their nutrition. They breakdown the cellwall components and utilise them as the sole carbon source.

Many of the aerobic and anaerobic bacteria can, breakdown cellulose, hemicellulose, pectin and partly lignin. The plasmodial actinomycetes cause soft-rots in wood. The fungal basidiomycetes cause white-rot and brown-rot of both standing and felled material. These can preferentially degrade either the lignin or the polysaccharide components.

Bio-technology has immence scope in the paper industry. This includes isolation and identificotion of organisms causing decay and discolouration of bagasse during storage, preferential treatment of hardwoods with isolates which can remove middle lamella lignin resulting in defibration and in consequence reduction in energy inputs during pulping, utilisation of bacteria and fungi for waste water treatment, decolourisation and reduction in BOD and COD loads and in slime control.

This paper, however, confines itself to deliberations on biotechnology in its application to bagasse storage and preservation systems.

PROBLEMS IN THE STORAGE OF BAGASSE

Bagasse is a material with a very low bulk density. This low bulk density makes it necessary that the material be either baled in dense bales or piled in high dense stack in order to facilitate storage. Excessive fermentation and heat build-up during storage degrade the quality of the fibre. Therefore, a need for a better and less costly method of storage has become apparent.

Bagasse deteriorates during storage due to the action of undesirable micro-organisms. Considerable amount of bagasse is rendered unsuitable for pulping due to biodegradation. The residual sugar content of bagasse, the heterogeneity of its tissues, the vast exposed surface and the conginial tropical environmental conditions

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facilitate the growth and colonisation by micro-organisms. Technically as well as economically efficient utilisation of bagasse for pulping process depends on the way of storing the bagasse during the off-season periods.

The type of damage to bagasse in storage can be classified into two categories viz. chemical degradation and discoloration. The chemical degradation is the consequence of bio-chemical alterations and depends on temperature, pH, amount of nutrients and the degree of aerobicity. This process affects yields and properties of pulp. The discoloration reaction is strongly related in its origin to chemical degradation reactions. However, in aerobic environment by the action of heat and light the darkening effect is accelerated.

BIO-CHEMICAL REACTIONS (1)

The bagasse obtained from sugar mill contains 2.5 to 3.0% residual sugar. The bio-chemical reactions involved in bagasse storage as well as corresponding heat generation are given in Table—1. In actual practice, all the mentioned reactions take place at once, the rate of reactions depends on many factors, some of them are given below :

TABLE 1

CHEMICAL REACTIONS AND HEAT GENERATION (1)

		Heat Generation Cal / Gr Mol of Surcose
1.	$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2(C_6H_{12}O_6)$ Sucrose → Gulcose & Fructose	+ 8 32
2.	$2(C_6H_{12}O_6) \longrightarrow 4(C_2H_5OH) + 4CO_2$ Glucose & Fructose → Ethanol	+ 33 16
3.	$C_{12}H_{22}O_{11} + 12O_2 \longrightarrow 12(CO_2) + 11(H_2O)$ Sucrose	+ 1348 20
4.	$2(C_6H_{12}O_6) + 6O_2 \longrightarrow 6CO_2 + 6H_2O$ Glucose & Fructose	+ 1339.88
5.	$2(C_6H_{12}O_6) \longrightarrow 4(CH_3CH(OH)COOH)$ Glucose & Fructose → Lactic Acid	+ 32.68
6.	$4(C_2H_5OH) + 4O_2 \rightarrow 4(CH_3COOH) + 4H_2O$ Ethanol → Acetic Aaid	+ 470.64

The micro-organisms that show he highest activtiy in the initial stages of the storing process are the yeasts (Saccharomyces) that split glucose and fructose into alcohol and carbon-di-oxide (Reaction 2).

The same micro-organisms hydrolize the sucrose to six atoms sugar (Reaction 1). These micro-organisms do not attack cellulose. In the above reactions only a fraction of the energy liberated is utilised for the manufacture of micro-organisms life such as bio-synthesis and assimilation, while major part appear in the form of heat.

In the presence of atmospheric oxygen the dissimilation process are accelerated and more heat is released. These process may oxidize completely glucose, fructose and sucrose to carbon-di-oxide rather than ethanol and carbon-di-oxide (Reactions 3 and 4).

The increase of temperature inhibits the growth of saccharomyces. Other types of organisms start developing in favourable conditions. For example, Lactobacilli may proliferate at a temperature of about 40°C and produce lactic acid (Reaction 5). However, unless the environment is at a low pH, other harmful thermophilic organisms may also develop.

In general, the predominent bio-chemical reactions are the spiliting of sugars into ethanol and CO_2 and in the presence of atmospheric oxygen some degree of oxidation of sugars to H_2O and CO_2 and of ethanol to acetic acid (Reaction 6).

The optimum conservation of the cellulosic contents of bagasse can be achieved by controlling the unfavourble bio-chemical reactions and restricting them to react with only soluble organic matter.

ACTIVITIES OF MICRO-ORGANISM IN STORED BAGASSE

In order to understand the succession and activity of micro-organisms in stored bagasse, Schmidt and Walter (2) analysed the microbial flora at different times of storage. As many as 2×10^9 living micro-organism per gram of dry bagasse has been reported. In rotten bagasse, the microbial population builds upto 5×10^8. Four hundred isolates of microbes have been reported from stored bagasse. The microbial flora consists of

74% bacteria, 6% actinomycetes, 13% yeasts and 7% fungi. The succession of microbial utilisation of the bagasse components during storage shows that the yeast dominate in early fermentation, followed by bacteria and then by actinomycetes and fungi. At first, the residual sugar is consumed by the yeast. Then the bacteria degrade the pectin, the hemicellulose and cellulose. Later, the actinomycetes and the fungi attack the hemicellulose, the cellulose and lignin within the bagasse fiber.

The temperature within the bagasse pile appears to be the most influential factor in determining the growth of the various strains of micro-organisms since nutrients are present in abundance. The evalution of microbial life in a bagasse pile involves a succession of microbial types, each depends on the gradual change of the micro-environment.

The effect of temperature of bagasse pile on enzyme activities was reported by Dr Cusi (1). Activities of micro-organisms in pile with respect to temperature can be classified into four evolutionary stages.

Stage 1

At ambient temperature (18—20°C) the most active micro-organisms are fungi and yeast which live on soluble sugars. In aerobic conditions, the sugars are oxidised to water and CO_2. As the environment becomes increasingly anaerobic because of accumulation of new bagasse, the hydrolysed sugar split into ethanal and CO_2. This causes no damage to the fibrous structure of bagasse.

Stage 2

When temperature increases over the optimum for yeasts (22°C), their activity decreases and other mesophilic micro-organisms that use sugar as primary nutrient appear. Some cellulose attacking micro-organisms are also generated at this stage.

Stage 3

At about 40°C the microbial population that predominated at lower temperature becomes inactive and slowly other types of bacteria, the 'Thermophiles' takes over. The attack on cellulose is intensified by the celluloytic micro-organisms at this stage.

Stage 4

Due to the development of thermophilic micro-organisms the pile temperature increases to the maximum level of their tolerance or to the level at which the enzymes fail to be active. The micro-organisms attack on cellulose continues at a lower rate.

These micro-organisms are potentially capable of utilizing all the major cell wall components namely, cellulose, hemicellulose, pectin and lignin for their growth and activity. A direct consequence is the loss of strength properties of the fibre. If enough soluble nutrients are available and if temperature and other environmental factors are favourable, the predominent thermophilic organisms will be lactic acid producing bacilli. This retards the growth of cellulose attacking micro-organisms.

CONSERVATION OF STORED BAGASSE

Literature review shows that the bulk storage is advantageous over classical bale storage methods. Biological pretreatment is favoured in many mills because of the very low fibre losses and the high uniform quality of fibre recovered from storage.

Techniques involved for bagasse conservation are formulated to prevent or hinder the proliferation of cellulolitic micro-organisms by the use of bactericides such as SO_2, Formaldehyde, Sodium Carbonrte etc. in a bagasse pile. However, the economic consideration makes these techniques unacceptable. As an alternative, the promotion of growth of non-cellulolitic micro-organisms that may retard or prevent the proliferation of those that damage the fibres has been suggested. In this method, the non-cellulolitic micro-organisms are promoted by enhancing the predominance of acid producing bacteria (Thermophilic) or by promoting the predominance of mesophilic micro-organisms.

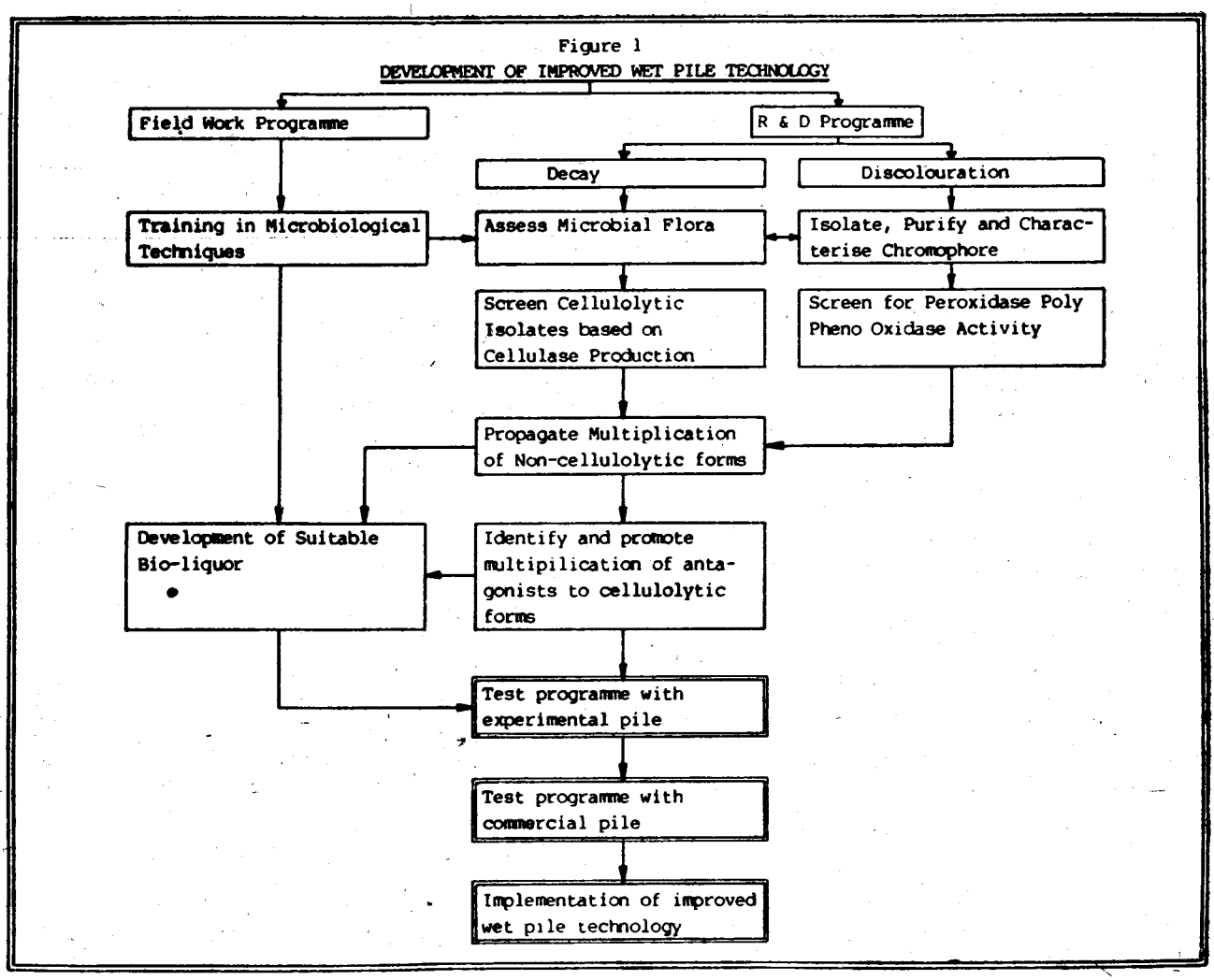
Bagasse conservation method by promoting the growth of acid producing bacteria uses the natural evolution of fermentation in a closed system in the presence of fermentable sugars and absence of air. Under these conditions, the Lactic acid producing bacteria prevail over other micro-organisms and retards the degradation of fibre by slowing down the growth rate of cellulose attacking micro-organisms. Dr Ritter's process of bio-liquor treotment is based on this concept (3).

Bagasse conservation method by promoting the mesophilic non-cellulolitic micro-organisms provides a process for preserving bagasse by controlling the flow of air through the pile to create a controlled temperature environment for mesophilic bacteria like yeast to grow and consume soluble sugars. The growth of other micro-organisms that degrade the fibres is retarted by reducing the moisture content of the material below 28%. Dr Cusi's process of conservation of bagasse by ventilation is based on this concept (4).

DEVELOPMENT OF IMPROVED WET-PILE TECHNOLOGY

Establishment of a wet-pile storage system from the data gathered through literature, is difficult due to lack of information in major areas. Sufficient informations are not available in the preparation of suitable biological liquor for storing bagasse. Bagasse pile formation, maintenance and management need assistance to improve the storage techniques. Strategies for solving the prolems in the implementation of wet pile technology involve understanding the causes of bagasse deterioration in storage and the techniques involved in the wet bulk storage of bagasse. Hence, a study programme to develop the wet pile technology was started at the Tamil Nadu Newsprint and Papers Limited (TNPL), India. Our approach to solve the problem consists of a field-work programme and a research and development programme (Fig 1).

Figure 1
DEVELOPMENT OF IMPROVED WET PILE TECHNOLOGY



Field work programme

The scope of the field work programme includes :

— Development of a suitable bio-liquor

— Training of personnel in micro-biological methods

— Selection of appropriate storage conditions for bagasse.

— Evaluation of physical and chemical characteristics of bagasse before and after storage

Bio-liquor Preparation

The initial seed lactic culture was obtained from National Chemical Laboratory, Pune, India. This was further sub-cultured for seeds in distilled water mixed with several nutrient ingradients such as glucose, yeast etc. under the temperature of 35—37°C for three days. The nutrients were first sterilised in an autoclave at 120°C for 15 minutes. Sub-culturing of the bacteria was carried out in the inoculation chamber. After inoculation, the test tubes were kept in an incubator at standard temperature for known period for the bacterial growth. This process was repeated and multiplied generation by generation in the laboratory. The seed bacteria tubes were stored in the refrigerator at 4°C for bio-liquor preparation.

10 ml of the seed bacteria was sub-cultured with 100 ml of nutrients. This 100 ml of the cultured liquid in each flask was propagated to two sterilized culture flask of 2000 ml each filled with distilled water containing 2.5% molasses. These are kept in the incubator for three days at 35°C for the growth of the lactic cultures. The growth of the bacteria in the bio-liquor was checked by measuring the population density. After ensuring the satisfactory growth, the bio-liquor was introduced into the pile.

Experimental piles

A field work programme to study the wet bulk storage of bagasse in order to understand the unknown areas in fibre storage technique was launched. The programme consisted of building up of four experimental piles with and without bio-liquor in the site area. Performance evaluation of the piles was made through various laboratory test programmes. The results concluded that the effect of bio-liquor is found to be more pronounced in case of depithed bagasse compared to undepithed bagasse since the former has less residual sugar. The pulping studies on bagasse stored with bio-liquor showed lower chemical consumption and higher yield compared to the bagasse stored without bio-liquor. Visual inspection of the pilot piles indicates better preservation of fibre and colour for bagasse stored with bio-liquor.

R & D Programme

The R & D programme envisages the understanding of the decay process and discolouration encountered during storage. Preliminary studies were initiated to identify the microbial flora of the bagasse at the mill site. The results are presented in Table—2. 8 isolates of bacteria, 2 actinomycetes and 14 fungi were found constantly associated with the decayed bagasse.

TABLE 2

MICRO-ORGANISMS AND THEIR FREQUENCY OF OCCURRENCE IN STORED BAGASSE

Sl. No.	Micro-Organisms	Occurrence
1.	**Bacteria**	
	— Rods	++++
	— Bacilli	++++
	— Cocci	++++
2.	**Actinomycetes**	+
3.	**Fungi**	
	— Mucor Sp	++
	— Rhizopus Sp	++
	— Saccharomyces Sp	++
	— Penicillium Sp	++
	— Paecelomyces Sp	++
	— Trichoderma Harzianum	+++
	— Aspergillus Terreus	++++
	— Dreschlera Australiensis	+
	— Sporothrix Sp	+
	— Agaric 1	+
	— Agaric 2	+
	— Mycclia Sterilla	+
	— Aphyllophorales 1	++
	— Aphyllophorales 2	++

++++ Abundant
+++ Frequent
++ Present
+ Scarce

To gain an insight into the influence of these organisms during storage fresh and decayed bagasse samples were collected and pulped under laboratory conditions. The proximate analysis is presented in Table—3. In the decayed bagasse 63.6% of the pentosons, 14% hollocellulose and 6.3% of klason lignin were found to be depleted while the hot water, 1% NaOH and alcohol : benzene extractives increased by 67.8%, 53.5% and 358.8% respectively over the fresh samples.

TABLE 3

PROXIMATE ANALYSIS

		Fresh	Decayed
1.	Ash	2.6	4.7
2.	Hot water extractive	5.9	9.9
3.	1% NaOH extractive	31.6	48.5
4.	Alcohol/Benzene extractive	1.7	7.8
5.	Klason Lignin	26.9	25.2
6.	Pentosans	28.9	10.8
7.	Holocellulose	66.1	56.8

The pulp and pulp evaluation data are presented in Table—4. A 35% drop in the yield of pulp from decayed bagasse was recorded inspite of the fact that there Kappa numbers were ten times as higher as those fresh ones. The freeness of the pulp dropped by 74% indicating poor drainability. The breaking length and tear factor decreased 45% and 85% respectively.

The pulp from decayed bagasse indicated a 54% drop in the brightness. This corroborates well with the earlier observation that the 1% NaOH extractives were higher in the decayed samples. UV spectra of the extractives indicated characteristic peaks in the 280—320 mm range indicating the occurance of aromatic hydroxyl group containing compounds like phenolics.

In a number of host pathogen interactions, it has been well demonstrated (5, 6) that O-Dihydroxy Phenols occuring bound to the plant cell get released due to the activity of cell lases. These are further acted upon by the peroxidases and polyphenol oxidases of microbial origin and get converted into complex coloured quinone like compounds. The probable involvement of a similar mechanism in stored bagasse cannot be ruled out.

TABLE 4

CHEMICAL PULPING OF BAGASSE

Cooking Schedule				
—	Bath ratio		1:4	
—	Chemical as Na_2O		14%	
—	Cooking temperature		170°C	
—	Cooking time		20 minutes	
Pulp Data				
		Unit	**Fresh**	**Decayed**
—	Total yield	%	57.9	39.0
—	Kappa number		11.4	101.4
—	Brightness	%ISO	38.9	17.9
Pulp Evaluation				
—	Freeness	ml/csf	585	150
—	Breaking length	m	6730	3690
—	Tear factor		70.0	11.0
—	Burst factor		38.0	14.0
—	Brightness	%ISO	38.1	18.8
—	Scattering coefficient	m^2/kg	33.8	20.2

Studies are underway to isolate, purify and characterise these chromophores and to identify the microbial role in the formation of these compounds. Means to overcome the same will be pursued.

CONCLUSIONS

Many techniques have been suggested to improve storage methods and to reduce handling costs and losses in storage as is evidenced by the literature survey. Greater uniformity in the quality of bagasee is highly desirable.

Pulp quality can be substantially improved by a selective biological pretreatment in the pile. The gen-

cral principles of the process involves in creating an acidic and anaerobic environment in the pile which in turn retards the proliferation of undesirable micro-organisms. Our field trials with Lactobacilli are in conformity with the above.

We have already initiated a detailed systematic study on the microbial flora which contributes to the decay and discolouration during storage. Pile management techniques to overcome these problems are being studied.

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