# THE MICROBIOLOGY OF THE BAGASSE OF SUGARCANE

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## ABSTRACT

The contamination of the sugarcane starts during its growing stage especially when the microflower is rapidly developed after the harvest and during the warehousing of the bagasse. The types of microorganisms also unite during this process. Firstly, the bacteria and the yeasts predominate and the fungi are few. The bacteria continue to predominate during the warehousing of the humid mass of the bagasse, producing acidic conditions, more in great quantities when there is a rapid growth of fungi and other execrence, causing spontaneous heating at high temperatures of 50° C. The final microflower is characteristically fond of warm place and contains organisms that present risks to the health of the workers who manipulate the warehousing of the bagasse. These risks include infections or allergies provoked by *Arpergillus fumigatus* and other species of *Arpergillus*, y bagazosis, a form of cavities caused by spores of thermofile fungus *Thermoactinomyces sacchari*. Some fungi and other infections also produce cellulitic enzymes that attack the fibers depreciating the quality of the bagasse.

The prevention of the mold is made by treating the bagasse partially dry with at least 0.67% p/p with propionic acid; the treatment should be uniform in order to prevent the growth of fungi capable of metabolizing the acid.

## INTRODUCTION

Bagasse is the fibrous residue remaining after sugarcane stalk has been crushed and the juice removed. As it leaves the mill, the bagasse contains about 50% water and 3-6% of sugar and other soluble materials. It thus forms a rich substrate for microbial growth. This is indicated by the speed with which heating can occur as a result of respiration by rapidly growing microorganisms. However, before considering colonization of bagasse and the development of the microflora under different storage conditions, it is necessary to consider its structure and chemical composition and what can be utilized as food sources by microorganisms.

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*The structure and composition of bagasse*

Sugarcane *(Saccharum officinarum)* is a member of the grass family *(Gramineae)* and has a stem structure resembling other monocotyledonous plants. However, it differs from many grasses because its stem is not hollow (Fig. 1a). The outermost single layer of cells is the epidermis. The cells are either elongated and rectangular or short and rectangular, trapezoidal or triangular. The long cells have thick, pitted walls and a thick cuticle and alternate with pairs of short cells, one of which is suberized and known as a 'cork' cell and the other is a thick-walled 'silica' cell. Inside the epidermis is a layer of small thick-walled lignified cells, then a narrow cortex of thin-walled parenchyma cells. These merge into the parenchymatous pith that fills the central area and contains most of the sugar at harvest. The vascular bundles are scattered irregularly through the pith, but increase in density towards the periphery while decreasing in size until near the cortex they are so close-packed as to form an almost continuous ring.

The structure of each vascular bundle is similar, consisting of two large xylem vessels with several smaller ones between, a group of phloem vessels with their companion cells and groups of thick-walled, lignified sclerenchyma cells (Fig. 1b). At the periphery the development of sclerenchyma behind the xylem is more marked than in the central bundles. These sclerenchyma bundles constitute the fiber of the stem, the cells having length: diameter ratios of about 70, in contrast to ratios of about 5 for the parenchyma tissue of the pith.
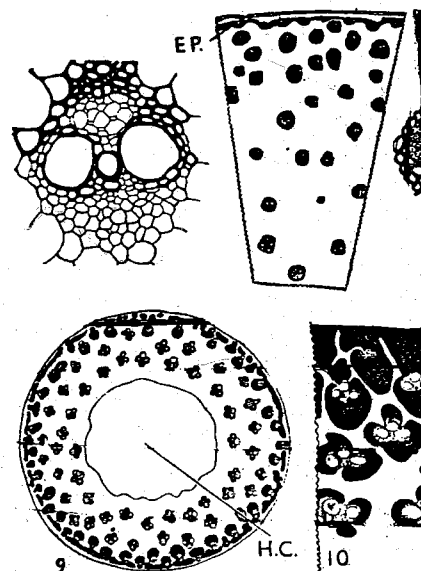


**FIGURE 1a & 1b.** The structure and composition of bagasse

Chemically, the cell walls of the bagasse are made up of cellulose, hemi-cellulose, pentosans and lignin. The relative proportions of these components are shown in Table 1. Cellulose forms the fundamental structure of the cell wall and is a polymer of about 2,000 to 3,000 glucose units. As the plant ages, it becomes encrusted with other materials such as lignin. The hemicelluloses and 'pentosans' are complex polysaccharoses found in cell walls particularly when they are lignified. They are made up of sugar residues, including long polysaccharose chains, but con-tain also other residues, which may sometimes be sugar derivatives such as uronic acid. The pentosans are strictly polysaccharoses formed of pentose units only. Sometimes hemicelluloses rich in pentoses such as xylose or arabinose have also been referred to as pentosans, but these also contain uronic acid. Lignin is a com-plex compound made up of benzene rings with some free and many more methy-lated phenolic groups, but the structure has not been fully elucidated. It is closely associated with cellulose and hemicellulose in the plant, making up the hard thick walls of the fiber cells and protecting the cellulose from breakdown.

**TABLE 1.** Proportions of main cell wall constituents in bagasse

|               | Percentage of dry weight |
| ------------- | ------------------------ |
| Cellulose     | 26 – 43                  |
| Hemicellulose | 17 – 23                  |
| Pentosan      | 20 – 33                  |
| Lignin        | 13 – 22                  |

*Microorganisms and their growth requirements*

A wide range of microorganisms may colonize on the bagasse but their num-bers and types will depend on the conditions of storage. The wet conditions of the Ritter process will favor bacteria while the drier conditions in baled bagasse favor growth of yeasts, fungi and actinomycetes.

The bacteria are the simplest of these microorganisms, consisting of single cells which multiply by division. The cells contain cytoplasm and nuclear material, but this is not retained by a membrane. Some types also produce resistant spores within the cells (endospores) which are extremely resistant to heat and adverse con-ditions. Actinomycetes have a very similar cellular structure and physiology to bacteria, but the cells are at some stage in the life cycle, in many species persistent-ly, joined into a thread-like mycelium which can ramify through the substrate. Some species multiply by disintegration of the mycelium into individual cells re-sembling bacteria, while in others, only specialized branches divide into spores with thickened walls that can easily become airborne when disturbed. In one genus *Thermoactinomyces,* which includes species implicated in bagassosis, the spores are formed singly in a similar manner and having a similar structure to bacterial endo-spores. They are also similarly resistant to adverse conditions.

The fungi also usually consist of a much-branched filamentous mycelium, the individual branches of which are known as hyphae. The hyphae may be divided by cross-walls into cells containing cytoplasm and one or more discrete nuclei, each enclosed in a nuclear membrane. However, some fungi, such as *Mucor* and *Rhizopus* species and their relatives form few or no cross-walls. Reproduction is usually by spores carried on sporophores of varying complexity or in other specialized fruiting bodies characteristic of the species. Yeasts differ from this general description in that mycelial development may be much reduced or in many species, absent. The cells then multiply by budding and division. Spores may also be formed in some species.

Neither bacteria nor fungi possess chlorophyll and therefore cannot synthesize carbohydrates from carbon dioxide and water in the presence of sunlight. Instead they must be supplied with ready-made food by growing parasitically on living plants or animals, or saprophytically on dead organic matter like bagasse. They produce enzymes that can break down complex sugars or other carbohydrates and proteins to provide substances useful for growth. However, not all species possess the same complement of enzymes so that they occur in different phases of an ecological succession. In bagasse, for instance, the species that would be able to utilize sugar would be among the early colonizers, followed by those able to utilize more resistant nutrients such as hemicellulose and cellulose and finally those able to degrade lignin. Tissues may also differ in their susceptibility to breakdown. For example, bagasse pith consisting mainly of cellulose would decompose more readily than fibers protected by lignin. Besides nutrient, microorganisms need water, oxygen, and a suitable temperature and pH. Few fungi will grow on substrates in equilibrium with a relative humidity less than 75% while some species of fungi and most bacteria will require equilibrium with air close to saturation or even free water on the substrate before they can grow. Bagasse would require a water content of about 13% to give equilibrium with air of 75% relative humidity, or about 35% or more to give saturated air. Above 35% water content, some free water would also be present.

The temperature requirements for growth vary with species of microorganisms. Most species can grow at normal ambient temperatures, with limits between 10 and 40°C and with optima between 20 and 30°C, but there are thermophilic fungi, bacteria and actinomycetes that have optima above 40°C and limits between 25 and 65°C. By contrast, the fungus *Cladosporium herbarum* can germinate and grow at 6°C. A few fungi can grow over a very wide temperature range. For instance, *Aspergillus fumigatus* has a minimum temperature for growth of about 12°C and a maximum of about 52°C. The high temperatures required by thermophilic microorganisms may be generated by microbial activity, so-called spontaneous heating, rather than originating from external sources of heat. In substrates like hay and bagasse, respiration of plant cells and the first microorganisms to colonize the material produces heat faster than it can be dissipated. As the temperature increases, microbial activity proceeds at a faster rate and conditions become suitable for those species with higher temperature requirements. This pro-

cess continues until the temperature is high enough and other conditions are suitable for chemical oxidation processes to start. This leads to spontaneous ignition, or until drying of the substrate prevents further heating. Spontaneous heating has been studied most closely in hay where the maximum temperature attained is closely related to water content, and these factors together determine which organisms predominate (Figs. 2, 3). Storage of hay at 25% water content results in heating to 31°C maximum and the *Aspergillus glaucus* group predominate; at about 28% water content, the maximum temperature is about 34°C with *Aspergillus versicolor* and *Scopulariopsis brevicaulis* predominating; with 31% water and 40°C, *Aspergillus nidulans, Absidia* spp. and *Streptomyces albus* flourish, but with 40% water and 63°C, these are replaced by *Aspergillus fumigatus, Mucor pusillus* and the thermophilic actinomycetes that cause farmer's lung (Festenstein, Lacey, Skinner, Jenkins and Pepys, 1965).
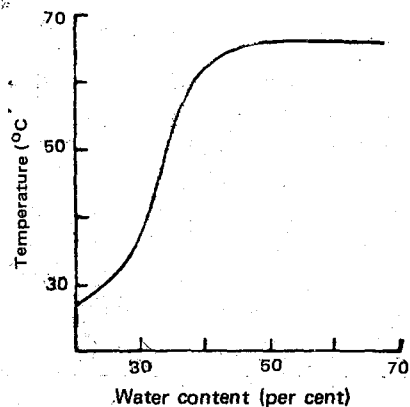


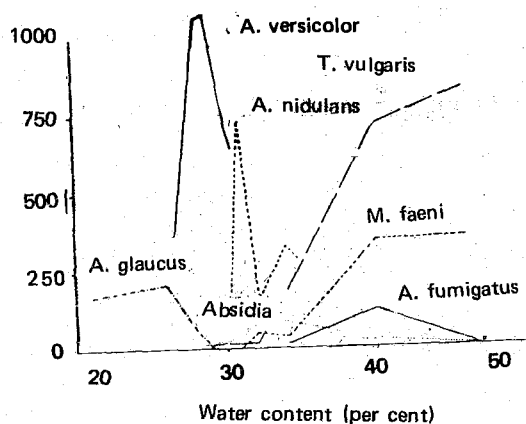FIGURE 2. Relationship of water content and maximum temperature in hay



FIGURE 3. Relationship between water content and occurrence of some fungi and
           actinomycetes in hay.
           A = Aspergillus
           T = Thermoactinomyces
           M = Micropolyspora

Some bacteria and yeasts can grow in atmosphere containing little or no oxygen. However, most fungi and actinomycetes are strictly aerobic. They require oxygen for their growth and are inhibited by high levels (10%) of carbon dioxide. The pH of the substrate is probably most critical for actinomycetes. Although most bacteria are inhibited by a pH of 4, most actinomycetes can only grow in neutral or alkaline conditions. Fungi can grow over a wide pH range from less than pH 3 to pH 10 or more. Spore production is often inhibited by less unfavorable conditions than growth.

*The significance of microbial growth on bagasse*

The growth of microorganisms has important consequences for the storage and subsequent utilization of bagasse. It can result in loss of fiber, variable quality, spontaneous combustion and hazards to the health of workers, but not all the effects are deleterious. For example, in the Ritter process, bacterial growth is intently encouraged to convert sugar into organic acids in order to preserve the bagasse in a similar manner to grass silage. Even in the storage of baled bagasse, fermentation of the residual sugar by yeasts may be useful in decreasing the risk of foaming in paper-making and of moulding of boards made from the bagasse. However, in normal storage the process does not stop there, and other microorganisms develop in association with spontaneous heating that increase pH, break down cellular material and form health hazards.

Loss of weight of fiber resulting from microbial growth may amount to 30% under poor storage conditions (FAO, 1955). This occurs because fungi and actinomycetes utilize cellulose as pointed out previously. The cellulolytic activity of a number of organisms isolated from bagasse is shown in Table 2. Of these, *Talaromyces emersoni, Allescheria terrestris, Chrysosporium pruinosum, Thermoascus aurantiacus* and *Sporotrichum thermophilum* have also been shown to be  important causes of weight loss from wood chip piles (Ofosu-Asiedu and Smith, 1973; Lundstrom, 1972). *Chrysosporium pruinosum* is one of the most common and fastest-growing organisms isolated from stored bagasse. Of the organisms tested, only one actinomycete, *Streptomyces thermoviolaceus,* showed cellulolytic ability.

In addition to weight losses from decomposition of cell wall material, losses may also occur because the bagasse shreds more easily after fungi have decomposed the middle lamella that holds the cells together. This results in a greater proportion of fines on screening the bagasse. Fresh bagasse will give 5-10% retained on the 4-mesh screen with most retained on 8 to 12 mesh screens. After deterioration, more will be retained on 20 and 24 mesh screens and some may even pass the last of these.

Lignin is most resistant to fungal attack and may only be decomposed where basidiomycetes (toadstool fungi) can grow in the outer layers of stacks which are subject to constant weathering by rain.

Subsequent utilization of the bagasse is made difficult because deterioration

is rarely uniform. It will often occur only in pockets, since it depends on the presence of propagules of the microorganisms the degree of aeration and spontaneous heating, water content and rate of drying, and other factors. Apart from discarding the outermost layers of the stacks, it would be an impossible task to eliminate other variability before processing. Another deleterious effect of microbial growth is discoloration of the fiber, so that more bleaching agents are required during paper making which themselves decrease fiber strength. The low pH of bagasse stored wet by the Ritter process is favorable for paper pulp production but it is unfavorable for particle board manufacture or in bagasse to be used for cattle feed. For both purposes the pH should be greater than 4 with an optimum of 6 for resins used in board production.

**TABLE 2.** Cellulolytic activity of bagasse microorganisms assessed from clearing to cellulose agar after 10 days incubation

| (a) Strongly cellulolytic | (c) No cellulolytic activity |
|---|---|
| *Allescheria terrestris* | *Aspergillus fumigatus* |
| *Chrysosporium pruinosum* | *Candida krusei* |
| *Sporotrichum aureum* | *Cladosporium sp.* |
| *S. thermophile* | *Kluyveromyces fragilis* |
| *Talaromyces emersonii* | *K. marxianus* |
| | *Monilla sitophila* * |
| | *Mucos pusilus* |
| **(b) Moderately cellulolytic** | *Paecilomyces varioti* |
| *Acremonium thermophilum* | *Pseudonocardia sp.* |
| *Penicillium janthinellum* | *Talaromyces vermiculatus* |
| *Streptomyces thermoviolaceus* | *Thermoactinomyces sacchari* |
| *Talaromyces spiculisporus* | *Thermoascus aurantiacus* |
| | *T. crustaceus* |

*Very slight clearing after 4 wk incubation*

Apart from the deterioration of the fiber, some of the fungi and actinomycetes colonizing stored bagasse can present health hazards to workers handling the material subsequently and to farm animals that might be exposed to it. Fungi and actinomycetes can cause disease in three ways, by infection, by allergy or by producing toxic metabolites in the substrate (Table 3). Most important to the worker handling bagasse are the first two, but livestock may be subject to all.

*Absidia* spp., *Aspergillus fumigatus* and other *Aspergillus* spp. occurred in more than 25% of bagasse samples examined. *A. fumigatus* is particularly well known as a cause of respiratory disease, and mouldy bagasse has been implicated

TABLE 3. Health hazards from bagasse micro-organisms

| Type of hazard | Name of disease | Cause |
|---|---|---|
| Infection | Aspergillosis | *Aspergillus fumigatus* and other *Aspergillus* species |
| | Mycotic abortion of cattle | *Aspergillus fumigatus Absidia* spp. |
| | Phycomycosis | *Mucor pusillus*, other *Mucor, Rhizopus, Absidia* spp. |
| Allergy | Rhinitis, Asthma | *Aspergillus* spp. *Penicillium* spp. *Cladosporium* spp. |
| | Bagassosis (hyper-sensitivity pneumonitis) | *Thermoactinomyces sacchari T. vulgaris* |
| Mycotoxicosis | Aflatoxin poisoning Ochratoxin poisoning Citrinin poisoning etc. of livestock | *Aspergillus* spp. *Penicillium* spp. |

as a source of infection in outbreaks of aspergillosis among poultry (Morales-Otero and Koppisch, 1933; Hutson, 1966). In man, it may colonize old tuberculosus cavities or grow in airways, while in cattle, it is important as a cause of mycotic abortion, together with *Absidia* spp.

Allergy has been defined as an acquired, specific, altered capacity to react (Von Pirquet, 1906). It is acquired by exposure to the allergen, is specific to that allergen and results in an altered reaction, the allergic reaction, on subsequent exposure to the allergen. The allergic reaction may present in two forms depending on the immunological reactivity of the subject. It may appear as an immediate reaction in the upper airways, giving hay feverlike symptoms (rhinitis) and asthma within minutes of exposure to the allergen, or as a delayed reaction affecting the gas exchange tissue of the alveoli several hours after exposure. Immediate allergic reactions predominate in the 10% of the population described as atopic, who are constitutionally predisposed to allergy, easily sensitized by small numbers of spores (usually fewer than $10^6/m^3$ air) and often sensitive to several different allergens such as grass pollen, ragweed pollen, house dust, cat and dog dander and some fungus spores e.g. *Cladosporium*. Delayed type allergy occurs mainly in non-atopic individuals exposed to large numbers of spores (usually more than $10^6$ and often up to $10^9$ spores/$m^3$ air) or for long periods, often in occupational situations. Hay fever is the characteristic form of immediate allergy while farmer's lung is the most

studied form of delayed allergy or hypersensitivity pneumonitis. Bagassosis is a disease clinically similar to farmer's lung but caused by different actinomycetes. Most important in farmer's lung are *Micropolyspora faeni* and *Thermoactinomyces vulgaris* from mouldy hay while bagassosis may be caused by *Thermoactinomyces sacchari* (Lacey, 1971).

No bagasse has been examined for mycotoxins although many of the species reported from stored bagasse are known to have toxin producing strains including *Aspergillus flavas, A. ochraceus, A. terreus, A. versicolor, Paecilomyces varioti, Penicillium islandicum* and other *Penicillium* spp. These toxins are probably of little consequence in the industrial utilization of bagasse, but could be important where it is incorporated into cattle feed or used as poultry litter.

### Microbiology of sugarcane before harvest

Sugarcane standing in the field before harvest will carry a small but varied microflora of microorganisms growing on the stems and leaves or their propagules, deposited from the air or splashed up from the soil. Some may grow as epiphytes on the plant surface, perhaps utilizing cell exudates but having no pathogenic effect on the plant while others cause diseases and still others grow only as saprophytes on the senescent and dying herbage. Over 400 species of actinomycetes, bacteria and fungi were listed by Stevenson and Rands (1938) associated with sugarcane and its products. A few were only recorded from sugar and molasses and others were root pathogens, but the majority were isolated from leaf and stem material. They included an unidentified actinomycete, 26 bacteria and about 50 sugarcane pathogens. Of the remainder, most of the fungi were characteristically 'field' species that would not develop further on stored bagasse, but some were 'storage' species providing an inoculum to develop further after milling. These included 14 species of *Aspergillus* and 8 species of *Penicillium*. Characteristically these storage species are only present in very small numbers before harvest and easily overlooked.

The occurrence of microorganisms in green cane in Queensland and the effects of burning and chopping were studied by Bevan and Bond (1971). Green cane carried about 50 species of microorganisms with species of bacteria common in the soil prevalent. *Leuconostoc* occurred under leaf sheaths and in growth cracks with yeasts from the genus *Saccharomyces* and lactic acid-producing bacteria. These growth cracks provided excellent sites for microbial growth and enabled organisms to survive during burning. Fungi, including a dextran-utilizing *Penicillium* sp. and actinomycetes, including an acid-producing *Streptomyces* sp., were also common. All organisms could utilize sugar; some, including *Leuconostoc mesenteroides*, produced polysaccharide and many were heat resistant, growing well at temperatures of 50-55°C. Storage-organisms were not identified by Bevan and Bond (1971) but Lacey (1971) reported the occurrence of *Thermoactinomyces sacchari* on stem pieces, particularly at the nodes.

Only ten minutes after burning it was still possible to isolate bacteria from the canes, especially from the growth cracks where *Leuconostoc* spp. survived to-

gether with heat-resistant yeasts. Some of the bacteria formed highly heat-resistant spores. After 24 h, fungi such as *Penicillium*, *Rhizopus* and *Aspergillus* became common, together with yeasts from the genera *'Torula'*, *Rhodotorula* and *Candida*. The bacteria and yeasts spread rapidly to the interior of the stems along cutting cracks when the cane was chopped. Organisms were often abundant 15 cm from the chopped and 1½ h after harvest (Bevan and Bond, 1971). The small number of storage organisms present might be expected to become similarly redistributed during harvesting and milling.

## Microorganisms in the mill

After shredding and hammermilling, sugarcane was found to be heavily contaminated with the organisms present before harvest (Bevan and Bond, 1971). These included yeasts, *Leuconostoc*, *Brevibacterium* (especially *B. imperiale*) and some fungi and actinomycetes, including the thermophilic *Thermoactinomyces thalpophilus*. These thoroughly dispersed through the cane, utilized and degraded sucrose, producing organic acids and dextran-like material. *Leuconostoc* predominated in the milling train but yeasts were also common. These organisms also predominate in the mixed juice where they may form tapioca-like grains composed of *Leuconostoc mesenterioides*, a yeast (either a *Pichia* sp. or a *Hansenula* sp.) and an unidentified rod-shaped bacterium. It is thought that these may form a triple symbiosis with the yeasts supplying the *Leuconostoc* with growth factors and the rod utilizing lactic acid produced by the *Leuconostoc* to maintain a favorable environment for all three (Bevan and Bond, 1971).

Extremely thermophilic bacteria and actinomycetes were found in other parts of sugar mills even at temperatures close to 100°C. These included a large rod-shaped sporing organism, resembling *Bacillus megatherium* but with an optimum temperature for growth of 80-85°C and a minimum of 50°C, another resembling *Leuconostoc*, and others capable of utilizing cellulose.

## Microbiology of wet-bulk-storage of bagasse

The principle of wet-bulk-storage of bagasse is claimed to be analogous to ensilage of grass. When fresh-cut or slightly wilted grass, containing 70 to 80% water is piled to give anaerobic conditions, bacterial fermentation of the carbohydrates by *Lactobacillus* spp. leads to the formation of lactic acid and a pH of 4 to 4.5 that inhibits undesirable organisms.

The Ritter Process of bagasse storage to the flumes in which bagasse is washed to the storage area where it is piled up to 25 m high (Mobius, 1965). The biological liquor consists of lactic acid bacteria which Morgan et al (1974) describe as a mixture of *Streptococcus lactis* and *Lactobacillus delbrueckii*. These are grown separately in a dilute molasses solution then mixed before adding to the slipstream used to transport and condition the bagasse. Fermentation of the residual sugar in the molasses by these microorganisms produces lactic acid and decreases the pH to 4 to 4.5.

Conditions in the storage pile are most constant in the middle (Mobius, 1965; Morgan et al., 1974; Dalabar and Maza, 1973).Here the water content decreases slowly over the storage period from about 85% to about 75% after 10-15 months. The pH remains more or less constant within the range 3.5-5 and the temperature at about 30° to 40°C. Nearer the surface the water content may fluctuate widely, depending on prevailing weather conditions, and the pH increases to from 6.5 to more than 7. According to Morgan et al. (1974) this pH rise is caused by the leaching of organic acids by rainfall, but the higher temperatures in this region, often exceeding 50°C, suggest microbial activity perhaps including aerobic fungal growth which could also serve to raise the pH (Gregory et al, 1963). Although lactic acid was present in the original biological liquor, Morgan et al (1974) were surprisingly unable to detect this in their experimental piles. They concluded that acetic and butyric acids were the most important organic acids in the bagasse causing the low pH, but propionic acid was also present in significant amounts. Weight losses were estimated at between 5 and 10% during storage after Ritter Treatment. Morgan et al (1974) found losses of about 5% from test samples placed in the pile, indicating loss of little more than fermentable material during 20 weeks storage. The difference may be from acid hydrolysis of pentosans. By contrast, Dalabar and Haza (1973) found weight losses of 10%, 7% due to loss of solubles and 3% to loss of fiber on the storage slab and from the discarded, deteriorated and discolored outer layer of the pile.

The significance of the biological liquor added to the bagasse is difficult to assess, for Morgan et al (1974) showed that similar results could be obtained both by Backwater treatment, using liquid downstream of the factory digester with a pH of 9.5 to 10, and 'organic acid' treatment, using formic acid and sulphuric acid to decrease the pH of the circulating system to 4.3 to 4.5. With the Backwater Treatment the pH of the liquid fell rapidly to 5.1 to 5.4 on mixing with the bagasse. Other bacteria have also been used. Hsieh et al 1973), for instance, used unidentified bacteria isolated from stored bagasse, water channels and soil, selected by odor of the culture solution, pH and effects on exposed bagasse.

Information on the microbiology of wet bulk storage is limited to that of Morgan et al (1974). Bacteria were numerous, supporting active anaerobic fermentation, but populations varied according to factors within the piles, such as oxygen content, available carbohydrates, weather and pH. Numbers tended to be smallest at the top of the piles, greatest at the bottom (Table 4). Trends with time were inconsistent, depending on the treatment and depth in the pile. Counts following Backwater Treatment were greatest after 2 weeks storage and following Organic Acid Treatment after 8 weeks. Only one set of results for Ritter Treatment were obtained when numbers were smaller than for both other treatments (Table 4).

The species of bacteria present were identified as Clostridium botulinum, C. fallax, Bacillus subtilis, B. strearothermophilus, B. Licheniformis and B. coagulans.

**TABLE 4.** Mean bacterial counts after 8 weeks storage following different treatments (Morgan *et al* 1974).

| Treatment Position in pile | Ritter | Backwater (No. bacteria/g fresh wt bagasse)* | Organic Acid |
|---|---|---|---|
| Top | 100 | 0 | 600 |
| Middle | 300 | 300 | 135,000 |
| Bottom | 31,000 | 137,000 | 100,000 |

These were present in all piles but neither *S. lactis, L. delbrueckii* nor any other lactic acid bacteria could be found in any of the piles. High organic acid contents mainly coincided with high bacterial counts, and acetic acid is known to be produced by both *Clostridium* and *Bacillus* species, but butyric only by *Clostridium*.

The conclusion that must be drawn from Morgan *et al* (1974) is that the addition of bacterial cultures has no overriding effect on the microflora of wet bulk stored bagasse. The inoculum already carried by the bagasse is sufficient to produce a fermentation governed by conditions in the pile, leading to production of volatile fatty acids and low pH.

*The microbiology of bagasse stored in bales*

Fresh bagasse leaving the sugar mill contains about 50% water and 3-5% sugar. Following depithing, if this is carried out, the bagasse is compressed into bales about 46 x 56 x 81 cm in size, weighing about 115 kg (Paturau, 1969). Stacks about 37 x 20 x 9 cm are constructed from the bales ensuring adequate air channels to allow heat and moisture to escape. The finished stacks must then be protected from rain to prevent rotting of the exposed bagasse and minimize losses.

There have been few studies of the microflora of baled bagasse and most of those that have been published have failed to recognize the significance of heating to the microflora. Consequently, only the most recent studies used incubation temperatures that would allow the isolation of thermophilic fungi. It was only in 1946 that Hunter and Perry recognized that the dust from bagasse was largely composed of fungus spores, finding about $240 \times 10^6$ spores g. They isolated 20 species of fungi including species of *Paecilomyces, Aspergillus, Penicillium, Monilia, Mucor,* and *Rhizopus*. Viswanathan *et al* (1963). Nicholson (1968), Gallego (1969) and Seabury *et al* (1968) added other species to this list. Most of these species were also isolated in our studies of bagasse, mostly from the West Indies, which, with other species we found made a total of nearly 80 fungi (Lacey, 1974). Thermophilic actinomycetes were first reported by Nicholson (1968), Seabury *et al* (1968) and Lacey (1971), and we have now identified some 17 species (Lacey, 1974). The frequency of isolation of the most common species from bagasse baled wet, after partial drying or after propionic acid treatment are shown in Table 5.

**TABLE 5.** Frequency of isolation in fungi and actinomycetes from bagasse

| No. samples examined | Bagasse baled wet | Bagasse dried before baling | Bagasse treated with propionic acid |
|---|---|---|---|
| | 90 | 17 | 50 |

| | % samples yielding colonies | | |
|---|---|---|---|
| **Fungi** | | | |
| *Absidia* spp. | 33(1)* | 24 | 30 |
| *Allescheria terrestris* | 22(4) | 6 | — |
| *Aspergillus flavus* | 20(1) | 29 | 4 |
| *A. fumigatus* | 58(19) | 76(12) | 40(2) |
| *A. glaucus* group | 18(1) | 12 | 32 |
| *A. niger* | 24 | 41(29) | 18 |
| *A. terreus* | 23(4) | 18(12) | — |
| *A. versicolor* | 13(1) | 24 | 4 |
| *Cladosporium* spp. | 40(9) | 53(24) | 50(16) |
| *Humicola lanuginosa* | 27(12) | 6 | 24 |
| *Mucor pusillus* | 7 | 24(18) | — |
| *Paecilomyces* type | 84(42) | 59(35) | 54(2) |
| *Penicillium* spp. | 88(34) | 71(6) | 48 |
| *Sporotrichum pruinosum* | 54(6) | 24 | 50 |
| *Synephalastrum racemosum* | 9 | 29(12) | 4 |
| *Thermoascus aurantiacus* | 20 | — | 18(4) |
| Yeasts | 51(24) | 53(6) | 46(6) |
| **Actinomycetes** | | | |
| *Pseudonocardia* sp. | 34(26) | 12 | 12(4) |
| *Streptomyces albus* | 23(2) | 4 | 18 |
| *S. griseus* | 34(6) | 59(6) | 12 |
| Grey *Streptomyces* spp. | 49(8) | 71 | 44(10) |
| *Thermoactinomyces sacchari* | 41(29) | 24 | 32(16) |
| *T. vulgaris* | 79(8) | 94 | 80(10) |
| White *Thermonospora* spp. | 10 | 18 | 2 |
| *T. viridis* | 18 | 29 | 8 |

*Figures in parentheses indicate % samples containing*

Thermophilic and thermotolerant fungi and actinomycetes were usually present and sometimes abundant in bagasse baled wet. Of these the most common fungi were *Allescheria terrestris, Aspergillus fumigatus, A. terreus, Humicola lanuginosa, Mucor pusillus* and *Paecilomyces varioti,* while *Saccharopolyspora hirsuta, Pseudonocardia* sp., *Streptomyces albus, Thermoactinomyces sacchari* and *T. vulgaris* were the most common actinomycetes. Several of the fungi are known to be potential pathogens and the *Thermoactinomyces* species have been implicated in bagassosis (Hargreave *et al.,* 1968; Lacey, 1971). *T. sacchari* was found in 40% of samples originating from Trinidad, Jamaica, Puerto Rico, India, Mauritius, the United States and from South Africa. Of these, 80% yielded more than $10^5$ colonies/ g with a maximum of $5 \times 10^6$ colonies/g. By contrast, *T. vulgaris* occurred in 80% of samples but only 10% yielded more than $10^5$ colonies/g. Mesophilic fungi included numerous *Aspergillus, Penicillium, Absidia* and *Cladosporium* species, yeasts and *Syncephalastrum racemosum.*

In our study, besides isolating and growing colonies of the different microorganisms for identification, the wind-blown dust from bagasse was examined microscopically and the different spore types were classified and counted. Bagasse baled wet and allowed to heat and mould naturally, contained up to $14 \times 10^8$ spores/g dry wt (Table 5). Over 70% of samples contained more than $2 \times 10^6$ fungus spores or actinomycete spores − bacteria/g (Table 7), although both groups were seldom abundant in the same sample, greatest numbers of fungi were found in samples from the weathered superficial layers of the bales, while actinomycetes were slightly deeper although still in the outer layers.

Drying the bagasse to about 25% water content decreased the total spore content to about a half. However, 'actinomycetes + bacteria' were affected much more than fungi. Fungus spores declined only by 25% and the species composition changed while 'actinomycetes + bacteria' decreased by 80% Thermophilic fungi were few, while *Aspergillus flavus, A. niger, Mucor cusillus, Syncephalastrum racemosum* and *Paecilomyces varioti* increased in number. Of the actinomycetes, only *Streptomyces griseus* was common.

Experiments in Trinidad showed differences in the patterns of colonization, heating and biochemical changes between bagasse baled wet or after partial drying. When baled at 50% water content, the bagasse heated rapidly to 54°C within 3 days (Fig. 4) then remained between 40 and 50°C for 45 days, by which time the water content had decreased to 20%. Within 4 days, nearly 90% of the residual sucrose had disappeared, and the remainder was utilized in 18 to 27 days. Invert sugar showed an initial increase before declining slowly until it disappeared 39 days after baling. Dried bagasse heated only to 45°C after 4 days storage and had cooled below 40°C after 9 days. Sucrose persisted for about 50 days while there was usually about 1% of invert sugar. They showed wider variation in the dried bagasse than that baled wet. Numbers of fungi were similar in bagasse baled both wet and dry, but 'actinomycetes + bacteria' were fewer in the dried bagasse (Fig. 5). The

bagasse was colonized initially by yeasts and *Paecilomyces varioti,* but with heating, these were replaced by thermophilic fungi and actinomycetes including *Sporotrichum thermophilum, Humicola lanuginosa, Allescheria terrestris, Talaromyces emersonii, Thermoactinomyces sacchari* and *Pseudonocardia* sp. The predominant organisms in the dried bagasse were *P. varioti, Aspergillus niger, Penicillium* spp., *Mucor nusillus* and *Syncephalastrum racemosum.* Few actinomycetes were isolated.
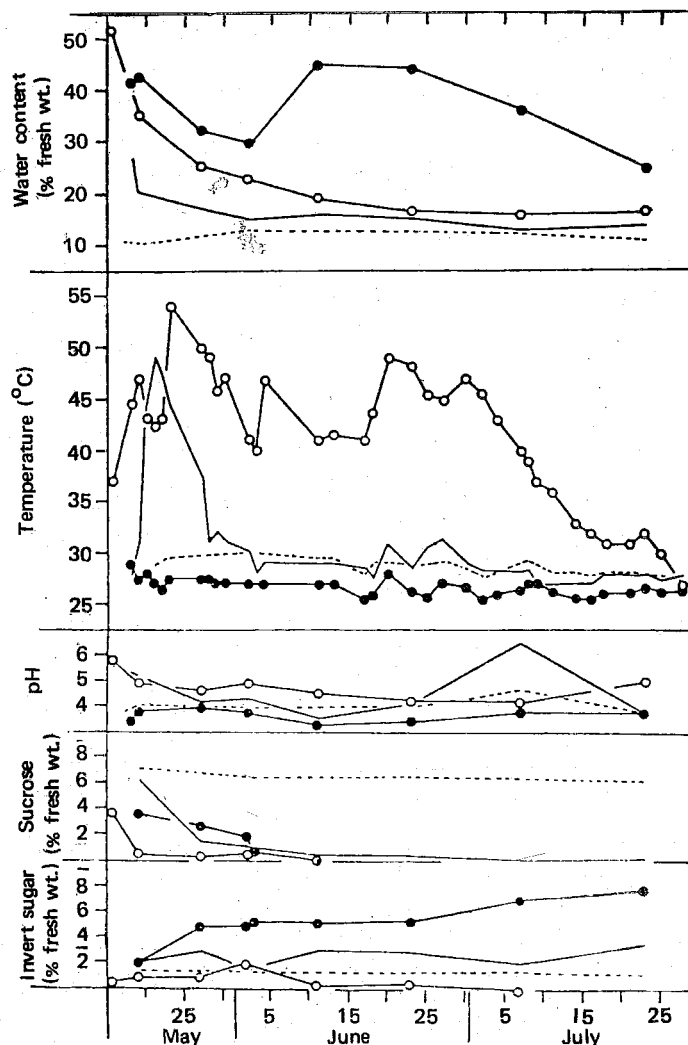


**FIGURE 4.**   Changes in baled bagasse stacked in Trinidad, — O —, baled fresh containing about 50% water; —, baled after drying to 27% water content; — O —, baled wet, treated with 2% of undiluted propionic acid; ----, mean results from bales dried, hammermilled and treated with either 2% of undiluted propionic acid or with 2% propionic acid applied as a 50% aqueous solution.

**TABLE 6.** The spore content of bagasse after storage

| Bagasse | Spore content | Range | | Mean | S.E. |
|---|---|---|---|---|---|
| Bagasse stored wet | Actinomycetes + bacteria | <0.1 − 106.1 | | 11.7 | |
| Bagasse stored wet | Actinomycetes + bacteria | <0.1 − 106.1 | | 11.72 | ± 1.87 |
| | Fungi | <0.1 − 113.2 | | 12.42 | ± 1.89 |
| Bagasse part-dried before storage | Actinomycetes + bacterial | <0.4 − | 8.0 | 2.45 | ± 0.58 |
| | Fungi | <0.1 − | 61.8 | 8.60 | ± 3.80 |
| Bagasse treated with propionic acid | Actinomycetes + bacteria | <0.1 − | 37.3 | 4.87 | ± 1.26 |
| | Fungi | <0.1 − | 9.6 | 3.41 | ± 1.33 |

**TABLE 7.** Occurrence of different spore types in bagasse samples

| | Bagasse baled wet | Bagasse dried before baling (25-27% water) | Bagasse treated with propionic acid |
|---|---|---|---|
| No. samples examined ... | 90 | 17 | 50 |
| Spore type | % samples containing spores | | |
| Actinomyces + bacteria | 99(70)* | 100(35) | 100(34) |
| Fungi | 99(70) | 88(53) | 80(18) |
| Aspergillus glaucus group/ A. flavus | 21 | 35(6) | 8 |
| A. riger | 3 | 35(12) | 4 |
| Other Aspergillus spp. | 91(39) | 71(24) | 70(10) |
| Cladosporium | 20 | 24 | 12 |
| Mucor type | 26(7) | 55(6) | 12 |
| Municola lanuginosa | 51(9) | 12 | 20(2) |
| Prown ascospores | 31(7) | 6 | − |
| Paecilomyces | 47(11) | 47(29) | 18 |
| Hyaline ascospores | 34(11) | 12 | 14 |
| Sporotrichum | 26(10) | − | − |
| Yeasts | 29(14) | 18(12) | 26(4) |
| Myxomycete | 1 | 12(6) | 4 |

*Figures in parentheses indicate % samples containing $> 2 \times 10^6$ spores/g dry wt.

*Prevention of moulding of baled bagasse*

Lathrop and Moore (1934) suggested moulding and loss of fiber could be decreased by encouraging heating, which they claimed pasteurized the bales, and by treating the outermost bales with boric acid. Losses of fiber were claimed to be decreased from 30% to 6% by these two treatments. However, neither temperature records nor microbiological assessments were given and the importance of thermophilic micro-organisms was not then appreciated. There is therefore still the risk that thermophilic fungi and actinomycetes could have grown, giving rise to health hazards.

More recently, propionic acid has been used to prevent moulding of grain and we have tested its use to prevent moulding of bagasse (Lacey, 1974). The effects of spraying bagasse with undiluted propionic acid to give a final concentration of 2% w/w in one experiment are shown in Figs. 4 and 5 and the microflora of samples receiving 0.6 to 4.25 w/w of propionic acid is shown in Table 5 to 7. In experimental stacks of both fresh-milled bagasse or partially-dried hammer-milled bagasse treated with propionic acid, there was no evidence of heating and tempera-
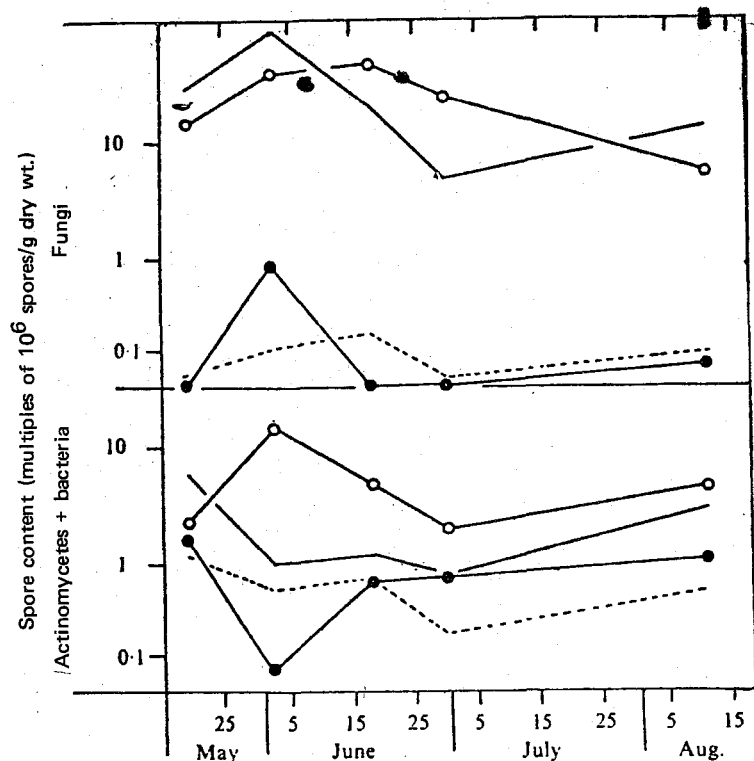


**FIGURE 5.** Spore content of baled bagasse stacked in Trinidad (For key, see Fig. 4)

tures remained close to ambient, below 51°C. Lack of microbial activity was indicated by high sucrose contents, greater than, in the drier bagasse. However, sucrose disappeared from the wet bagasse within 17 days but this was probably inverted in the maint, acid conditions as concentrations of invert sugar reached 5.1 to 7.9 cer ave with 1.1 to 1.5 in the drier material. It was found preferable to treat the, rise after drying to about 25% and hammermilling, as mixing was easier and the treated bagasse remained white and free from dust, contrasting with brown, dusty, untreated bagasse.

The microflora was much decreased when 0.6 to 4.25% w/w of propionic acid was mixed uniformly with the bagasse. The spore content was usually less than $4 \times 10^6$ spores/g dry wt. Most were 'actinomycetes + bacteria' but few actinomycetes were isolated, suggesting a predominance of bacteria. No fungal species predominated and only the occasional colony grew on isolation plates inoculated from propionic acid-treated bagasse (Plate 1).
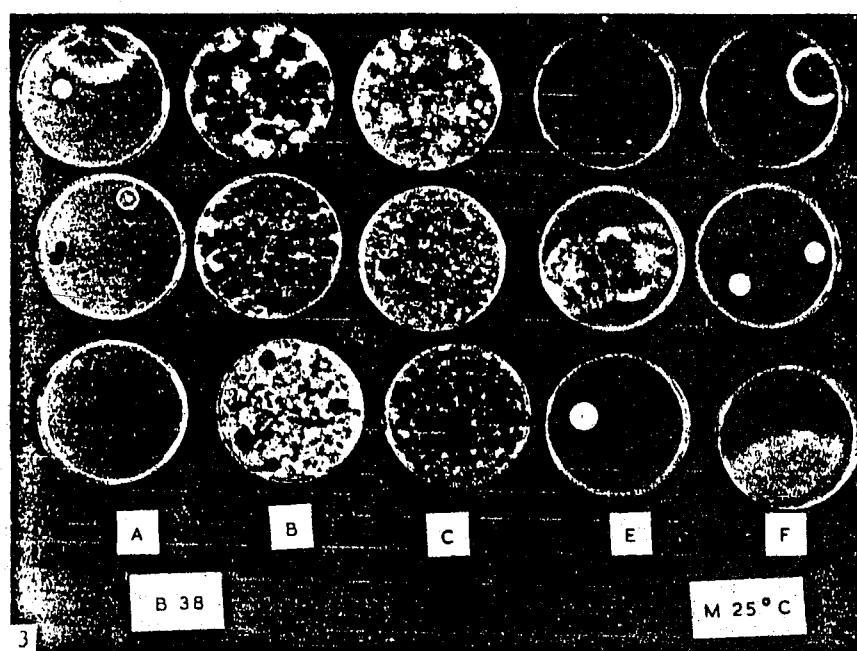


PLATE 1.

A = Wet treated

B = Wet untreated

C = Dry untreated

E,F = Dry, treated

When the propionic acid was not uniformly incorporated into the bagasse, moulding could occur, resulting in up to $47 \times 10^6$ spores/g dry wt, with species composition similar to that of untreated bagasse. Possibly growth of *Paecilomyces varioti* initiated this colonization, as it is tolerant of greater concentrations of propionic acid than most fungi and can degrade it (Lacey, Lord, Manlove and Charlick, 1976).

Well-treated bagasse remained free of mould for long periods. For example, samples treated with 1.2 to 1.6% of propionic acid were still clean after 18 months storage. When this was used to make particle board, much dust was produced as the mattress was formed. However, only $2.6 \times 10^6$ spores/m$^3$ could be found nearby, rising to $15.4 \times 10^6$ spores/m$^3$ near to the source of dust. No *Thermoactinomyces sacchari* could be isolated from the bagasse and a bagassosis patient, sensitive to this actinomycete, failed to react. *Paecilomyces varioti* was common in the air but no other fungus.

## CONCLUSION

For a long time sugarcane bagasse has been considered a waste product (the term 'bagasse' was originally applied to olive waste, but eventually came to mean anything worthless). Now it is finding increasing use and value as a replacement for timber in the manufacture of paper, fibers and particle boards, as cattle feed and for futural production. Previously, heating and moulding were of little consequence so long as spontaneous ignition did not occur. Now they are important as they can lead to unwanted variability in the raw material for processing, losses, discoloration and deterioration of the fibers, and to health hazards to workers handling the bagasse.

Steps to decrease moulding and heating will decrease all the undesirable factors listed above, but it is necessary to design the process to the subsequent use for the bagasse. Wet-bulk storage may be satisfactory for paper but not for particle board and while propionic acid may preserve bagasse well for particle board, the final pH may be too low for satisfactory curing of resins. However, alternative chemicals are available, such as ammonium propionate and 'ammonium bis-propanoate' (BP Chemicals), which are almost as effective as propionic acid in preventing moulding of hay, but which would not give quite such a low pH in the bagasse. Further trials are necessary, however, to assess their suitability and effectiveness in this substrate.

In the meantime, bagasse needs to be handled with care to avoid creating unnecessary dust and consequent health hazards to workers. Where there is a risk of dust, there should be ventilation and filtration to remove it from the working environment and to trap it, whilst workers should have respiratory protection available and regular medical surveillance.

# LA MICROBIOLOGIA DEL BAGAZO DE CAÑA DE AZUCAR

J. Lacey

## RESUMEN

La contaminación de la caña de azúcar inicia durante sucrescimiento mas la microflora se desarrolla rapidamente después de la cosecha y durante el almacenamiento del bagazo. Los tipos de microrganismos también cambian durante este proceso. Primeramente, las bacterias y las levaduras predominan y los hongos son pocos. Las bacterias continuan a predominar durante el almacenamiento de la masa humeda de bagazo, produciendo condiciones acidas, mas en grandes cantidades hay un rapido crescimiento de hongos y actinomicetos, causando calentamiento expontaneo a temperaturas superiores a 50°C. La microflora final es caracteristicamente — termofilica y contiene organismos que presentan riesgo a la salud de los trabajadores que manipulan el bagazo almacenado. Estos riesgos incluyen infecciones o alergias provocadas por *Aspergillus fumigatus,* y otras species de *Aspergillus,* y bagazosis, una forma de alveolite causada por inhalación de esporos de actinomiceto termofilico *Thermoactinomyces sacchari.* Algunos hongos e actinomicetos también producen enzimas celuloticas que atacan las fibras despreciando la calidad del bagazo.