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A Novel Method of Stabilising Hevea Latex

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Fresh field latex which coagulates within a few hours of tapping can be kept fluid for about 12 h if ammoniated to pH 9.0 and indefinitely by maintaining this pH by continuous addition of ammonia until a stage is reached when no further additions are required. Alternatively, this can be achieved by urea on its own, or in combination with ammonia. Latex thus stabilised still contains large numbers of bacteria, but they are incapable of producing acids. Stabilisation of field latex kept for a long period could offer considerable advantages in general rubber factory practices.

Latex within the *Hevea* tree is sterile. However, after tapping, the latex, when it flows along the tapping cut into the cup and when subsequently collected and bulked in the factory becomes heavily contaminated with bacteria and yeasts (TAYSUM, 1957; JOHN AND TAYSUM, 1963) which produce acids from the non-rubber substances in it (ARCHER *et al.* 1963, JOHN, 1966 a and b). The acids thus produced cause the latex to coagulate. To keep the latex fluid, small quantities of anticoagulants such as ammonia, formaldehyde or sodium sulphite are added. The concentration of the anticoagulants depends on the period for which the latex has to be kept fluid. For 6 h either 0.05% ammonia, 0.06% formaldehyde or 0.1% sodium sulphite is required; for 24 h either 0.15%, 0.2% or 1.0% of the respective acids is required (COOK, 1960). This paper describes a simple method of keeping field latex fluid for an indefinite period.

METERIALS AND METHODS

Latices

Fresh latex was collected either from the field or from the factory bulk about 5 h after tapping. Ammoniated latex was prepared by adding 25% solution of ammonia in water to the fresh latex, based on weight. Sterile latex was collected aseptically from trees of clone RRIM 501 using an extractor (MCMULLEN, 1949); the latex obtained was always checked for sterility before use.

Growth Medium

Molasses — yeast-extract agar consisting of 0.5% molasses, 0.5% yeast extract, 0.005% bromocresol purple and 1.25% Oxoid agar No. 3 was used in the enumeration, streaking and passaging of bacteria (JOHN, 1968).

Enumeration of Bacteria

Bacteria were enumerated by surface-plating ten-fold serial dilution of the test materials in solid medium, incubating at 30°C for three days and counting the colonies by the method of JOHN AND TAYSUM (1963). All bacterial counts are expressed in log per millilitre.

Passaging of Cultures

Bacterial colonies after three days of incubation at 30°C were picked off from a convenient surface plate, streaked on to a fresh plate and incubated at 30°C for three days. The operation was repeated.

Fermentation of Latex

A fermentation unit was set up with 30 litres of factory bulk latex with the pH maintained at 9.0 by addition of ammonia solution. Every day a certain quantity of

fermented latex was withdrawn and an equal quantity of fresh factory-bulk latex added, the pH of which had been adjusted to 9.0 to keep constant the volume and pH of latex in the fermenter. When required, the quantity of the sample withdrawn (output) and of the addition (input) were simultaneously varied to give a retaining time ranging from two to ten days. The input and output samples were then coagulated.

Coagulation of Latex

Latex was coagulated in two ways: (a) with 2% formic acid at pH 5.2 and (b) by steam coagulation in which latex was poured into aluminium pans and autoclaved at 1.0 kg/cm2 pressure for 15 min (JOHN AND SIN, 1974).

RESULTS

Degradation of Fresh Latex

The bacterial population of fresh latex obtained from factory bulk and kept at room temperature, increased from log 7.12 to 8.96 per millilitre in 9 h, followed by a stationary phase *(Figure 1)*. The pH dropped progressively from 6.5, and by the fourth hour, the latex began to thicken. With time, the pH dropped further and the latex partially coagulated. Coagulation remained incomplete even after 24 h, with a substantial amount of rubber remaining in the milky serum.

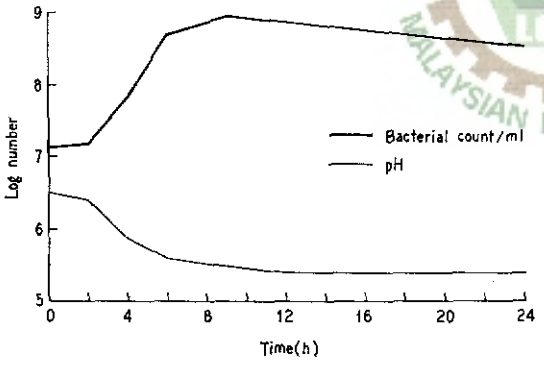

Figure 1. Changes in bacterial population and pH of fresh latex on storage.

Degradation of Ammoniated Latex

The bacterial population of fresh latex, when ammoniated to pH 9.0 and kept in a closed container, increased after a lag-phase of 8 h, by about 100 times in 24 h *(Figure 2)*. The pH dropped markedly during the first 12 h and the latex began to thicken and partially coagulate. With the onset of coagulation, further pH decrement was arrested. After 24 h a good part of the rubber had coagulated leaving a milky serum of pH 6.5. Thus, latex initially ammoniated to pH 9.0 also deteriorated as a result of bacterial metabolic activities and underwent partial coagulation within 24 hours.

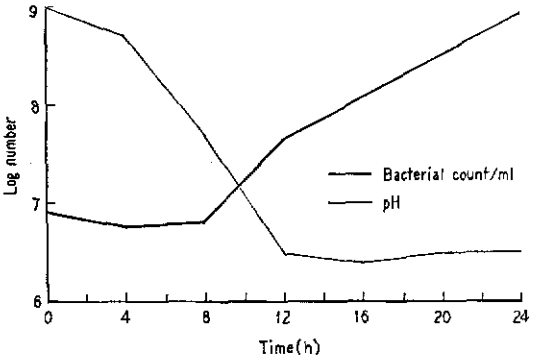

Figure 2. Changes in bacterial population and pH of ammoniated latex on storage.

Stabilisation of Latex

Ammonia. Factory-bulk latex, the pH of which was initially adjusted to 9.0 with ammonia solution, remained fluid indefinitely on further continuous addition of ammonia *(Table 1)*. At the start, ammonia had to be more frequently added to make up for the downward drift in pH, but progressively less so with time; the latex became 'stable' in a week without having to add any more ammonia.

TABLE 1. QUANTITY OF AMMONIA ADDED DAILY TO 30 LITRES OF FRESH LATEX TO MAINTAIN IT AT pH 9.0

Period of storage (day)	Cumulative quantity of 25% ammonia added (ml)		
	Experiment 1	Experiment 2	Mean
0	100	60	80.0
1	185	160	172.5
2	245	260	252.5
3	300	260	280.0
4	360	360	360.0
5	420	440	430.0
6	440	440	440.0
7	440	440	440.0

Ammonia and urea. Factory-bulk latex ammoniated to pH 9.0 and treated with various levels of urea kept the latex fluid; the period of fluidity depends on the concentration of urea *(Figure 3)*. In the absence of urea, the latex began to thicken within

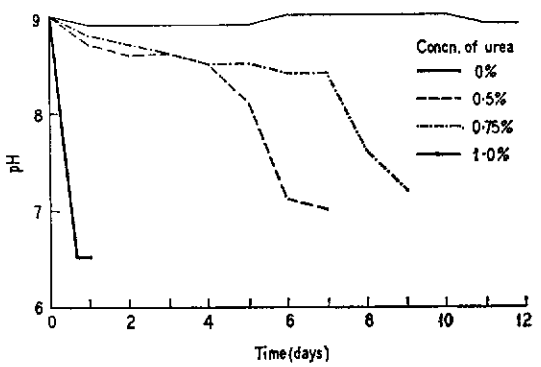

Figure 3. Effect of urea on the fluidity of latex ammoniated to pH 9.0.

12 h, the pH dropped to about 6.5 in 24 h, with partial coagulation. But in the presence of 0.5% additional urea the latex remained fluid for about four days, with 0.75% for seven days and with 1.0% indefinitely. The pH of the systems using 0.5% and 0.75% additional urea dropped on keeping, leading to partial or complete coagulation, whereas 1% urea maintained the high pH indefinitely.

Urea. Latex can be kept fluid indefinitely in the sole presence of urea, provided the concentration is not less than 2.0% *(Figure 4)*. With 1.0% urea, the pH rose to 8.5 in two

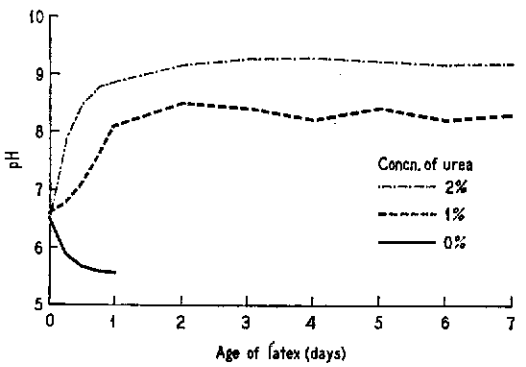

Figure 4. Effect of urea on the pH of latex.

days and remained steady for a week, the latex thickened slightly, and partially coagulated when kept longer.

With urea and urease. When fresh latex was treated with 1% or 2% of urea and 0.01% urease, the pH rose to about 8.0 within 1 h and 9.0 in 4 h; this level was thereafter maintained indefinitely *(Figure 5)*. Of the two systems, 2.0% urea with urease gave not only a more immediate increase

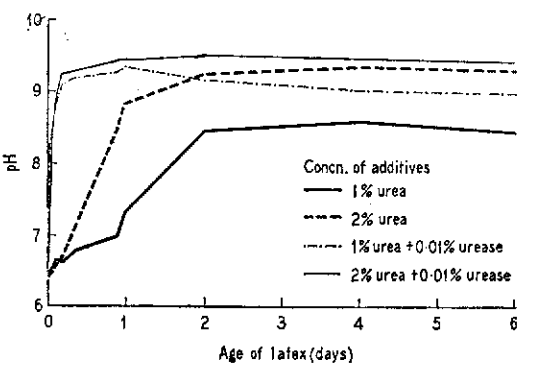

Figure 5. Effect of urea and urease on the pH of field latex.

in pH but also sustained it at the high level, in comparison with 1.0% urea with urease.

Urea/urease in sterile latex. When sterile latex was treated with filter-sterilised urea or with urea/urease, the pH substantially increased with time *(Table 2)*.

When treated with 1% urea, the pH increased from 6.5 to nearly 9.0 in a day but in the presence of additional urease the pH began to increase almost immediately reaching a similar level in a few hours, while the control sample hardly showed any change.

TABLE 2. EFFECT OF UREA/UREASE SYSTEM ON pH OF STERILE LATEX

Age of latex (day)	Sterile latex	Sterile latex + 1% urea	Sterile latex + 1% urea + 0.01% urease
0	6.50	6.50	6.60
1	6.50	8.75	9.05
4	6.65	8.95	9.00
8	6.70[a]	8,80[b]	8.80[b]

[a]Coagulated
[b]Latex thickening slightly

All samples remained fluid up to the fourth day but the control coagulated when kept longer. The two treated samples remained fluid despite slight thickening. All samples remained sterile throughout the experiment.

Bacteriological Changes

The bacterial population of latex stabilised with continuous addition of ammonia continued to have a high population *(Figure 6)*. Even so, the latex kept fluid without any thickening. The great diversity of bacterial populations, characteristics of *Hevea* latex, drastically changed in about a week, the flora reduced to one or two types of pin point colonies, often *Streptococcus* spp. *(Figure 7)*.

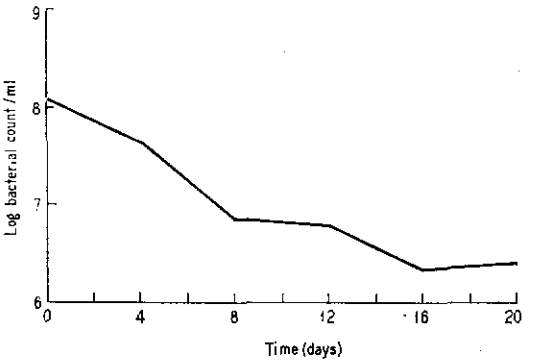

Figure 6. Bacterial population of latex stabilised with ammonia.

Most of the colonies isolated in the initial stage of stabilisation were strong acid producers. However, with time they diminished, disappearing completely in about a week. The *Streptococcus* spp., surviving in the stabilised latex, did not produce any acid. However, when they were transferred to molasses yeast-extract agar and repeatedly passaged, their capacity for acid production was gradually restored.

The Streptococcal culture isolated from the stabilised latex gave large colonies with unusually large cells lacking their typical round to oval shape. Chain formation, characteristic of this genus, was also lost, giving rise to clusters of deformed cells scattered in an irregular manner. However, when these colonies were repeatedly passaged through fresh medium the colonies became gradually smaller with the cells reverting to the original round to oval shape and frequent short-chain formation.

The bacteriological properties of latex stabilised with ammonia and urea were similar to those that were obtained from latex preserved with ammonia alone; in twenty days the population dropped by about two orders of magnitude — from log 8.85 to 6.83 per millilitre — with the flora reduced to a few types of non-acid producing colonies. However, on passaging the acid production was restored. The bacterial

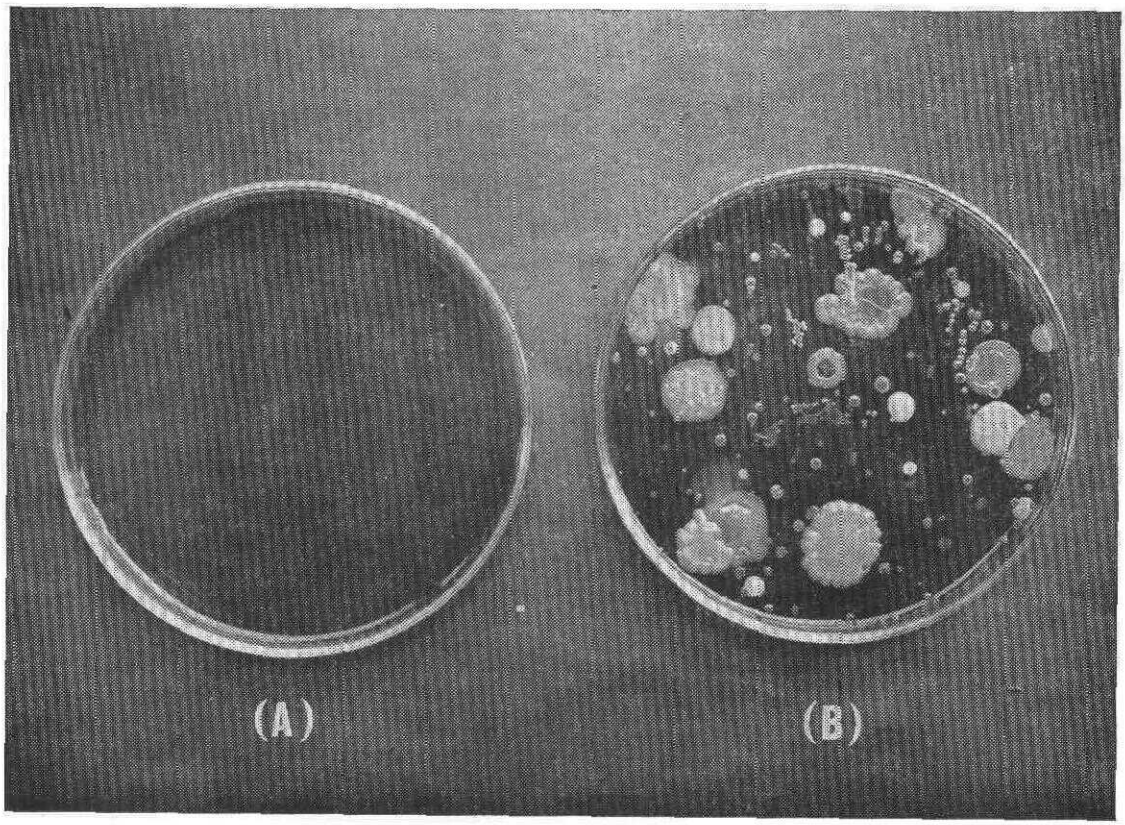

Figure 7. Microbial flora on 10^{-5} dilution surface plates after six days incubation at 30°C. (A) *Stabilised latex.* (B) *Fresh field latex.*

properties of the 1.0% urea-urease system was slightly different, in that it steadily increased from log 7.30 to 8.36 per millilitre in twenty days. Nevertheless, the latex was fluid and without any objectionable odour. The biochemical behaviours of the bacterial colonies were similar to those observed in the system preserved in the sole presence of ammonia.

Coagulation of Stabilised Latex

Latex stabilised with ammonia, urea, urea and ammonia, or urea and urease and kept for more than four days gave incomplete coagulation when treated with formic acid. On the other hand, when there was a daily or alternate daily withdrawal of fermented latex and addition of an equal volume of fresh latex the efficiency of coagulation improved. The extent of improvement was dependent on the retention time of latex in the holding tank. When a retention time of up to six days was provided, with a daily withdrawal and simultaneous addition of 5 litres in a 30-litre tank, the efficiency of coagulation was as good as that expected from fresh latex. However, when retention time was increased to eight days, with a daily withdrawal and addition of 3.75 litres, coagulation was less efficient. On further increasing the retention time to ten days, with a daily withdrawal and addition of 3 litres, coagulation was only half complete. However, when the latex was steam-coa-

gulated, a complete coagulation was obtained, irrespective of the retention time of the latex in the fermentor.

DISCUSSION

Fresh latex putrifies as a result of the microbial metabolic activities and coagulates within a few hours. If ammoniated to pH 9.0, it can be kept fluid for at least 12 h because of the bactericidal and alkaline properties of ammonia. On the other hand, it can be kept fluid indefinitely by maintaining its pH at this level. Thus, when the pH of the field latex is raised from its natural level of about 6.5 to 9.0 by the addition of ammonia solution and continuously maintained at this level by further additions of ammonia, a stage is reached when no further additions are required to keep it fluid indefinitely.

Urea can be used in place of ammonia to maintain the pH. It is broken down either by the enzyme urease produced by the naturally occurring bacterial population, or by hydrolysis, or by both, liberating ammonia which then makes up the downward drift in pH. Latex treated with 1% urea takes at least 24 h to reach pH 8, in the meantime undergoing slight thickening and partial coagulation. This can be overcome either by raising the concentration of urea to 2%, or still better, by raising the initial pH to 9.0 with ammonia and then treating it with 1.0% urea. Alternately, 1.0% urea can be supplemented with 0.01% urease to raise the initial pH to 9.0, but this may not prove economical as urease is prohibitively expensive. The practical thing therefore is to use ammonia to obtain the initial high pH and add 1.0% urea to retain the pH level. Latex thus stabilised can be kept fluid indefinitely.

The large numbers of bacteria (about log 6.0 per millilitre) still present in latex and thus 'stabilised' show that its stability is due to the drastic alteration in the metabolic activities of the organisms in such a way that acid-producing bacteria lose their ability to produce the acids that normally destabilise the latex. This was further evident when it was observed that these bacteria when cultivated under optimum conditions of growth regained the ability to produce acid.

In solid rubber production, latex is consumed mostly within 8 – 12 h of its collection and certainly within 24 h, preventing any possibility of mixing it with the latex of the following day. In latex concentrate production, on the other hand, latex is preserved for a longer period and is frequently a mixture of various ages and from various sources. Consequently, latex concentrate is less variant in technological properties, in comparison to the solid rubber.

Stabilising field latex for long periods could therefore be advantageous. It would help in bulking latex of varying ages and from wider sources than are currently possible, thereby offering the possibility of reducing wide variations in technological properties. Further, it would greatly facilitate the setting up of large centralised processing factories receiving latex from diverse sources. Stabilisation of the field latex also increases the scope for continuous coagulation of latex in which a constant feed from a bulk store is continuously coagulated and directly fed into the next set of machines (JOHN AND NEWSAM, 1969).

Continuous preservation of field latex will also provide uninterrupted procesing as latex can be drawn out of a bulking tank. Presently, especially during rainy season, processing can be seriously interrupted as it depends on the daily availability of fresh latex from the field. By taking advantage of the continuous stabilisation system, latex can be bulked in enormously large bulking tanks with daily withdrawal of the bulked latex and addition of fresh latex making available a ready, reliable and less variable supply of latex. The quantities withdrawn and added can be so adjusted to suit the

processing capacity of the factory and the daily supply of fresh latex. If there is no fresh supply on a day, factory processing can still be continued by drawing from the bulk and topping up the bulk at the earliest opportunity with fresh latex. This will of course cause a slight change in the retention time of the bulk but it should not affect the efficiency of coagulation.

Latex thus bulked and stabilised does not give any difficulty in acid coagulation as long as the retention time of the bulk does not exceed six days. If it exceeds six days, acid coagulation becomes less efficient. However, a complete coagulation is still possible with steam.

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