

With regard to the copper temperature of the wort he had recorded a temperature of 219° F. at a gravity of 30 lb. and 213°-214° F. at a gravity of 7 to 8 lb. These temperatures were obtained under a pressure of 1½ lb.

He thought he had been slightly misunderstood regarding the use of wooden vessels.

He was not in favour of them for the present day brewing of light ales, but he wished to state that wooden vessels imparted a better flavour to heavy beers than that obtained in a metal-lined vessel.

A vote of thanks was accorded the author for his interesting paper.

COMMUNICATION.

BIO-CHEMICAL CONTROL OF THE VINEGAR INDUSTRY.

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IN an article on "Micro-Organisms and Some of their Industrial Uses" (Journ. Roy. Soc. Arts, vol. 69, p. 604), A. Chaston Chapman states that :—

"Notwithstanding the great advances made in many directions in industrial Zymo-Technology as a result of improved methods of bacteriological technique and in consequence of the greater amount of attention devoted, during recent years to this important subject, the position of the vinegar industry has almost remained stationary. Apart from the crude apparatus they employ, vinegar-makers rarely, if ever, know anything of the precise character of the all important organism (or organisms) they are using, nor, except in a very rough way, do they know the most suitable conditions for obtaining—through its agency—the maximum yield of acid." Again, Lafar, an authority on technical mycology, developing the theme on almost similar lines, says : "This highly necessitous industry has more perhaps than any other, to struggle against a variety of difficulties; the actual losses of alcohol are enormous, and no one is able to offer any reliable explanation of their cause. The introduction and intelligent use of pure culture ferments would be a great boon."

The above two quotations will suffice to show how much remains to be done to put this ancient and interesting industry on a really satisfactory bio-chemical basis and are a sufficient plea to indicate in this article recent lines of development of the subject in the laboratories of the Indian Institute of Science. The author does not lay any claim to have perfected a process for the manufacture of vinegar, but enough has been done on the simple bacterial conversion of alcohol into acetic acid, which in turn has opened up increasing possibilities of application.

Several attempts have been made in the past on the same lines; but they have resulted in failures owing to the fact that the organism or

organisms employed were not systematically studied with regard to their nutrition, growth and functional activity. It is this significant fact, which has not received much attention, not only in the hands of vinegar makers, but also in those of bacteriological chemists, that has shrouded the whole subject in mystery and made the manufacturers "trust to luck."

The conversion of alcohol into acetic acid is one of the simplest bacterial oxidations and a large number of organisms have been found to affect it. How far all of these could be looked upon as different species in a biological sense it is difficult to decide; but apart from morphological considerations, the bio-chemist looks upon the organism as an effective chemical agent and his efforts are directed towards obtaining the greatest concentration of enzymic activity. The organism that has been employed in these laboratories is one of a numerous species *B. Aceti*, Hansen, which was obtained from a medium composed of suitable proportions of alcohol, acetic acid (vinegar) and mineral phosphates of potassium, ammonium and magnesium. On exposure to air in an incubator at 95° F., they grow very easily and purification for experimental purposes has been accomplished by combining plating and dilution methods. After purification the cultures were inoculated into larger and larger volumes of medium, and in this way considerable quantities of the organism were built up as a fine grey deposit at the bottom of the culture flask. By slowly increasing the concentration of acetic acid in the medium the acid-sensitive bacteria were eliminated and the acid resistant ones, which are the most important for acetification purposes, were favoured.

In the course of preparing the cultures used in the work a number of interesting observations were made as to the most suitable nutritive material for the bacteria. It should be understood that the requirements vary as the object in view is to produce growth in bulk or to encourage functional (acetifying) activity. The former condition obtains when preparing large masses prior to the production of acetic acid, the latter as soon as acetification begins. Mineral phosphates, such as those of magnesium and ammonium, in presence of suitable quantities of glucose, have been found to increase the growth activity at the expense of acetification. Accordingly these phosphates have been omitted in conducting acetification experiments, and the proportions of ammonium phosphate and glucose have been reduced to a minimum to prevent starvation.

Having obtained pure cultures of the right organism in quantity, experiments were carried out with dilute alcohol of specific strength 4 per cent. in the absence of artificial aëration. Good results have been obtained, and the influence of catalysts like ethyl acetate and manganese sulphate, the former extending the acetification to a greater maximum acidity and the latter quickening the period of acetification has been very beneficial. Carrying on the experiments further, with increased volumes of inoculant and acetifying liquid the necessity for artificial aëration became increasingly marked, due to lack of insufficient oxygen. It was also evident from observations of sundry cultures kept without regard to aeration that if large quantities of inoculant were to be used it would be necessary to introduce air artificially to prevent the putrefaction of the bacterial masses. The conditions of maximum efficiency to be aimed at would, therefore, be the employment of the highest quantity of inoculant which would be kept by adequate food supply, and sufficient oxidation to prevent putrefaction, or even the production of intermediate products, but insufficient to encourage super-oxidation to carbon dioxide. Experiments were carried out on these lines, using a bottle with an outside temperature 89.6° F., and with air supply from a filter pump. Further alcohol was added as the initial alcohol became oxidised, and working this way under sterile conditions, an acidity of 10 to 12 per cent. of acetic acid was obtained.

The experiments were then translated to a larger scale using an inverted bell-jar and with

air from a gas-holder. The exact translation has not been, however, possible, but working thus an eventual acidity of 9 to 10 per cent. was obtained.

Attempts were now made to approach actual conditions of working on a large scale, in order to anticipate some of the difficulties involved therein, chiefly by way of infection from outside. For these experiments a 10-gallon tub was used. To avoid blowing much air and at the same time to ensure thorough mixing of the bacterial masses the method of paddle-wheel aëration was adopted with electric motor and stirrer. For a detailed account of the apparatus the reader is referred to the author's paper on "Studies in Intensive Bacterial Oxidation" (Journ., Indian Institute of Science, Vol. VI, Part viii). The temperature of the liquid was kept at 77° F. to approach factory conditions. The acid formation developed up to 6.9 per cent., but suddenly at this point slimy growths of mucous bacteria made their appearance, attaching themselves to the sides and internal projections in the barrel. These soon developed at the expense of the right organism, and the result was a diminution in acid content. Later experiments showed that this growth tended to oxidise the acetic acid to carbon dioxide. It is interesting here to note that this very often becomes the pest of vinegar factories. Once they get these growths in vinegar, makers have to throw out a whole series of barrels and start the plant afresh. The highest developed, most evolved, and hence much feared of these growths is the so-called *B. Xylinum*. It has been found that the remedy for these things lies in prevention rather than cure. If the temperature of the fermentation liquor is kept in the neighbourhood of 89.6° F. from the outset, those bacteria which grow best at that temperature and which again are the most active acetifiers are favoured at the expense of the mucous formations. This whole question of large-scale control boils down to encouraging the growth and activity of the right type of organism in the life struggle for predominance which is incessantly going on between the various other types which come in from infection in large scale work.

An experiment with the barrel, using a heating coil (nichrome wire wound round two mica plates, these again being sandwiched between two plain mica plates, the whole being held in a wooden frame) giving a temperature of 89.6 F., resulted in the production of nearly 9 per cent.

acid. The process was then made continuous by withdrawing small quantities of the liquid and refilling with alcohol of the same strength. In this way an intensive oxidation effect has been obtained, and the barrel worked to its maximum capacity.

Incidentally several interesting observations have been made as to the toxicity of certain salts on the acetification process. These have important theoretical bearings and for an account of work on the subject, the reader is again referred to the author's paper mentioned above.

To conclude, if earnest attempts are made on

the part of vinegar-makers to bring to bear on to large scale work these conditions regarding purity of culture, air-supply, temperature-control and nutrient material with beneficial results the author's labours will have been amply rewarded.

The work outlined in this paper was carried on in collaboration with Dr. G. J. Fowler, Professor of Bio-chemistry in the laboratories of the Indian Institute of Science, Bangalore, India.

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ABSTRACTS.

I. MATERIALS AND ANALYSIS.

The Gout Fly of Barley. A. D. IMMS (*Journ. Ministry Agric.*, 1925, 31, 1137-1140).—Reference is made to the recent work at Rothamsted by J. G. H. Frew on the Gout Fly, a well-known pest on barley in many parts of the country. The flies lay their eggs, which are visible to the unaided eye, singly on the upper side of the leaves of young shoots at the end of May, and the fly is liberated in August. The second generation lays its eggs mainly on couch grass, the larvæ remain dormant during winter, and the flies appear once more in May. Shoots attacked by winter larvæ at the beginning of December, are stunted and thickened and die without further growth in late February. The distortion produced on summer shoots is more variable, but in general the ear does not escape from its sheathing leaf to ripen. Since the larvæ always migrate downward in the shoot, any borne on leaves below the ear soon die for lack of food. For this reason leaves above the ear are termed "critical" and those below "non-critical;" any arising half-way up the ear are "half-critical." There seems to be no selective instinct on the part of the parent fly when laying. The degree of infestation is found to be largely controlled by the manurial treatment, any manures which stimulate the plant so as to raise the position of the ear—thus reducing the ratio of critical to non-critical leaves—being beneficial. Superphosphate, farmyard manure and complete minerals gave satisfactory results in this

direction. Heavy dressings of nitrogenous fertilizers were deleterious, but used in smaller amount in conjunction with superphosphate or complete minerals were markedly beneficial. Sowing at the earliest possible date is recommended, and the value of preventive measures such as these and the uselessness of remedial measures with an infested crop is emphasised.

A. A. D. C.

Stripe Rust (*Puccinia Glumarum* of Cereals and Grasses in the United States). H. B. HUMPHREY, C. W. HUNGERFORD and A. G. JOHNSON (*Journ. Agric. Res.*, 1924, 29, 209-227).—Stripe rust was first reported from Europe by Schmidt in 1827 who described it as *Uredo glumarum*. It was later transferred by other workers to the genus *puccinia* and is now known as *puccinia glumarum*. It is widely distributed throughout Europe chiefly to the northern countries. In America it has not so far extended eastward beyond 103° West longitude in spite of the fact that in addition to the cultivated hosts, wheat, barley and rye it occurs naturally on at least 34 wild grasses, and it is not improbable that under optimum conditions its ravages might prove a serious menace to wheat culture on this continent. In most varieties of wheat, stripe rust infection is confined chiefly to the leaves. Certain varieties of wheat are apparently much more subject to glume and kernel infection than others, and all susceptible varieties show general leaf infection. An aerial host for *P. glumarum* has no