

Short Communication

Pak. j. sci. ind. res., vol. 32, no. 2, February 1989

**CULTIVATION OF SYMBIOTIC MICRO-ORGANISMS UNSUCCESSFUL AS DUE
TO PEROXIDES FORMED BY SMEARED TISSUE**

S. Mahdihassan

SD-34, Block A, North Nazimabad, Karachi-33

(Received January 3, 1989)

Tissues contain fats and when smeared give rise to peroxides. These are antibacterial and also injurious to healthy body cells. Tissues containing symbiotic germs, when smeared, likewise produce antibacterial peroxides and prevent isolation of germs. But leaving a piece of intestine of insects, in the midst of smears of tissue with germs, serves as reducing centres and counteracts the action of peroxides. Tissue smears with pieces of intestines left in the culture plates enable successful isolation of symbiotic germs. The role of peroxides in human pathology has been properly expounded in Bradford Research Institute in California.

Keywords: Germs, Antibacterial, Peroxides.

Buchner [1] has published a classic on symbiosis mostly between insects and micro-organisms like yeasts and bacteria. That symbiosis is a widely spread phenomenon in nature appears as the most impressive feature of this work. However, the role of symbiosis is poorly indicated since the isolation of symbiotes had not been carried out except in a couple of solitary cases. What however has greatly hampered the isolation of the symbiotes is the fact that cellular degradation products or cellular debris have been mistaken for real germs, while these were overlooked as components of normal tissues. Nevertheless in some insects, like species of Lecanium, which is a scale insect or coccid, the symbiote is a yeast-like microorganism. Even Prof. P. Linder of Berlin, who was a specialist of yeasts and an author of a handbook on that subject, could not isolate the symbiote of a Lecanium. I had the honour of knowing him personally and I can report what had been his actual experience. Now the lac insect, again a Coccid, also contains symbiotic yeasts. I tried to grow them but at first with negative results. Believing that the symbiotic germ would prefer an extract of lac insects, since it grew within their bodies, a special media was prepared. Even with such an additive to the culture media attempts to grow the symbiotic germ were negative.

Consulting literature I came across the work of Meyer [2]. He found bacteria in the nephredia of some land molluscs. Trying to isolate them he mostly got negative results. He writes (on p. 72) that "it was expected that the smearing of crushed concretions, teeming with bacteria, on suitable medium would produce a confluent growth. Instead however sterile plates or a few scattered colonies have been obtained."

Now the germs which were isolated as exception belonged to the common soil bacterium *Pseudomonas fluorescens*. That it should not grow well from tissue containing the same could not be explained. Here was a parallel case to what was observed while isolating the symbiotic yeast of the lac insect.

After much persistent efforts, I did succeed in isolating the symbiotic yeast of the lac insect and also *P. fluorescens* from *Cyclostoma elegans* and two other molluscs. What enabled me to succeed can be stated what has been mentioned before [3]. On p. 172 I wrote "When a tissue infected with a germ is teased out and exposed to air it acquires bactericidal properties specific to that germ. I have come to imagine that the bacteria produce fatty acids and they give rise to specific peroxides which are highly bactericidal. The one trick I resort to in culturing symbiotic germs was to leave a bit of intestine as a centre where reduction is assured and peroxide formation avoided. The lac yeasts are embedded in fatty tissue so that the insect fat itself can be a potent source of producing peroxides or toxins. It may be realized that the symbiotic germ does not grow at random within the host and is kept within control. What then is the mechanism by which the germ is kept in check by the host."

Realizing that a tissue can produce a metabolite which is antibiotic, along with a colleague, Dr. Bakshi, I prepared liver extracts. What was water soluble enabled bacteria to grow well but the fraction which was also alcohol soluble was antibiotic (Mahdihassan and Bakshi, [4]. Further it was found that Stein [5] published an illuminating article on antibacterial substances produced by tissues. And we have to assure that peroxides, acting upon tissue metabolites,

these would function as antibiotic substances. My interest arose again when I got a copy of the publication, through my son, Dr. Mohsin Ali Hassan, "Oxidology" by Drs. R.W. Bradford, H.W. Allen and M.L. Culbert [6]. They have shown how peroxides can explain many a degenerative disease. What I had also found before was that liver extract can contain antibacterial metabolites. Such a substance is not reported in the above work, "oxidology". But it does appear that no one before had emphasized the wide occurrence of peroxides causing degeneration of tissues.

REFERENCES

1. P. Buchner, *Endosymbiose der Tiere mit planzlichen Mikroorganismen* (1953), 2nd ed.
2. K.F. Meyer, *J. Inf. Dis.*, 36, 1 (1925).
3. S. Mahdihassan, *Zool. Anz.*, 167, 170 (1961).
4. S. Mahdihassan and V.M. Bakshi *Curr. Sci.*, April (1950), pp. 131.
5. H. Stein, *Beit. Klin. Tuberk.*, 118, 314 (1958).
6. R.W. Bradford, H.W. Allen and M. L. Culbert, *Oxidology* (Los Altos, California, 1985).

INTRODUCTION

Although the importance of the downcomer, as a contributor to the mass transfer in a distillation unit was pointed out as early as [1], but very limited data is reported in the literature and that too is very much contradictory. It has been stated by Thomas and Campbell [2] that the behaviour of the tray plus downcomer, as a unit may be very important in certain circumstances. Due to paucity of data the design of downcomer is still mainly based on empirical equations.

The present study was carried out to establish the overall performance of a downcomer.

Downcomer theory. The mechanism of bubble and froth formation in a downcomer is unexplored. Small bubbles of almost uniform sizes are collected at the base of the downcomer. As we pass upwards these bubbles grow and a gradual transition to froth occurs. A large deal depend upon the mode of entry of the liquid from the tray above.

The earlier workers [1, 3, 4] were of the opinion that column would flood when the retained liquid reaches the top of the exit weir. Thomas and Shah [5] has shown that the most important design factor is clear liquid height, the froth height contributes little to the tendency of flooding. The clear liquid height in the downcomer can be calculated from the following equation.

$$\Delta L = P + L_s + (0.4V_s^2)^{0.5} \dots (1)$$

The liquid flow over the weir is no longer considered to be a limiting factor in tray designing [2].

Relative foam density values in the downcomer are not available in the literature. Since specific foam density varies approximately from ρ_f at the bottom of the downcomer to ρ_c at the foam vapor interface. Therefore, a conservative

average value of the relative of the density $\phi = 0.2$ is widely used in the design of the downcomers [3].
A certain residence time of liquid in the downcomer is necessary in order to allow collapse of foam. It is common practice to base this residence time on the total downcomer volume. The minimum allowable residence time should be based on the foamability of the system.

A true residence time of the retained mass in the downcomer is given as [3].

$$t = 0.083 \frac{A \Delta L}{\phi Q} = 0.083 \frac{A \Delta L}{\phi Q} \dots (2)$$

Where ΔL , $\phi = \Delta \rho$ and $A \Delta L$ is equivalent to the clear liquid volume. ρ is the liquid flow rate. Then t as given in the above equation 2 represent to "Plug flow".

A value of 2 seconds for plug flow in the downcomer is suggested by Davis [1]. Observations made by Thomas et al [2] certainly leads one to expect anything but a plug flow in the downcomer.

EXPERIMENTAL

Since the circular downcomer provides very low downflow area and poor vapor disengaging space and usually constitute the first bottle neck to column capacity, therefore a segmental downcomer was selected for the present studies. The downcomer was 12.5 cm deep, 30.5 cm wide and 60 cm long with the provision of measuring points.

The pilot plant [2] was operated, as under the normal conditions, using O_2 desorption from aqueous glyceric (20% by wt) solution. Froth height and clear liquid heights were measured visually, as the downcomer was constructed from transparent material. For mass transfer efficiency studies, liquid samples were withdrawn at inlet, and outlet