

### INFLUENCE OF YEASTS ON PROTOZOAL ACTIVITY IN SEWAGE

IN the course of our studies on the fundamental role of protozoa in sewage purification,<sup>1-8</sup> some interesting observations have been made in regard to the influence of yeasts, when introduced along with the protozoa in different combinations into sterilised sewage, on the purification process.

We have carried out a number of experiments by inoculating cultures of *Saccharomyces cerevisiae* (one of the most commonly occurring species in certain trade effluents and sewage) together with *Epistylis* sp. (the indicator organism for well conditioned sludge) into sterilised sewage and bubbling air through the suspensions. One set of these experiments is briefly described below.

Samples of actively fermenting yeasts were treated as follows before they were inoculated

along with the protozoa into sewage:— (i) the yeast inoculum consisted of both the yeast cells and the fermented liquor, in the form of mixed suspension (100 c.c.); (ii) the inoculum was only the yeast cells (about 2.5 g. in the wet condition, contained in 100 c.c. of the mixed suspension) filtered and carefully washed with ammonia-free distilled water in order to make the cells free of acid; (iii) 100 c.c. of the fermented liquor without the yeast cells (the filtrate from the previous preparation) was employed; (iv) the washed cells (acid-free, about 2.5 g. in the wet state) were autoclaved at 15 lbs. pressure for 30 minutes, and the cooked material as such was used; and (v) the water extract of the autoclaved material (100 c.c.) was the inoculum. For each of these yeast preparations two litres of heat-sterilised sewage was employed, so that the added yeast suspensions formed about 5 per cent. by volume of the sewage; such a proportion of domestic sewage to trade effluent (as represented by yeast additions) obtains at certain industrial centres. The protozoan inoculum for these experiments consisted of 20 c.c. of fresh *Epistylis* sp., washed well in ammonia-free distilled water. The progress of purification of the sewage samples in the different cases was determined by examining the sludges and the supernatants at frequent intervals during a period of 96 hours. The results of chemical analyses of the supernatants and microscopic examination of the protozoa during the first 24 hours after the treatments are given in Table I.

TABLE I

Treatments of sewage	One hour after the treatments					After 24 hrs. of aeration			
	Supernatants				Microscopical observations on the protozoa	Supernatants		Condition of the protozoa	
	Oxygen absorbed from potassium permanganate in 3 mins., 4 hrs.	Free and saline ammonia (N)	Albuminoid nitrogen (N)	pH		Oxygen absorbed from potassium permanganate in 4 hours	pH		
Protozoa alone ( <i>Epistylis</i> sp.) control series	1.75	4.08	1.40	0.40	7.6	Extremely active	0.96	7.6	Active
Protozoa & yeast cells ( <i>Saccharomyces cerevisiae</i> ) together with the fermented liquor	19.60	46.00	3.12	1.02	6.0	Inactive	50.40	6.1	Dead
Protozoa & washed yeast cells	1.84	4.08	1.25	0.62	7.6	Very active	1.12	7.5	Active
Protozoa & yeast liquor with the cells	17.20	46.00	3.57	1.02	6.0	Inactive	50.40	6.1	Dead
Protozoa & yeast cells washed and autoclaved	1.92	4.24	1.40	0.89	7.5	Very active	1.04	7.4	Active
Protozoa & water extract of the autoclaved yeast material	1.76	4.08	1.56	0.62	7.6	Very active	0.96	7.6	Active

The results of chemical analyses of the supernatants are expressed as parts per 100,000.

The results of analyses of samples of the supernatants taken during the subsequent periods of observation consistently showed that the purification of sewage was adversely affected in presence of the actively fermenting yeasts and was traceable to the products of yeast fermentation (the acids, alcohols and related products) rather than to the yeast cells themselves. Addition of lime to the yeast liquor (neutralising the acidity) was found efficacious in the prevention of these adverse effects.

The sensitiveness of the protozoa to reaction and other conditions is, therefore, of great technical importance and emphasises the need for trade effluents to be discharged into the sewers in equable flows so that the whole sewage is not affected.

The above observations would explain the cause of a somewhat mysterious breakdown in one of the earlier Activated Sludge installations in England where flushes of brewery waste were liable to enter the sewer.<sup>9</sup>

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