CXXII. THE GRAVIMETRIC ESTIMATION OF BACTERIA AND YEAST.

By HERBERT ISAAC COOMBS and MARJORY STEPHENSON.

From the Biochemical Laboratory, Cambridge.

(*Received August 9th, 1926.*)

Since the micro-balance has reached its present state of perfection it has been possible to weigh to 0·01 mg. or even 0·001 mg. with accuracy and ease. It seemed likely that the micro-balance might provide a comparatively quick and easy method of estimating the growth in a bacterial or yeast culture where a knowledge of the total amount of cells (living plus dead) produced was required. In the present communication the technique which was found suitable for the estimation of *B. coli communis* growing in broth and also of *Saccharomyces cerevisiae* growing in glucose broth is given.

The estimation of *B. coli communis*.

The medium was a tryptic digest of caseinogen, described by Cole and Onslow [1916], the nitrogen content of which was equivalent to 4 mg. of nitrogen per cc. It was found, however, that when 10 cc. of this broth was run through a Pregl filtering tube a considerable amount of material was held by the asbestos of the filter. Moreover, the dry weight was not constant but varied between 0·15 and 0·35 mg. It was thought that this must be due to adsorption of the larger colloidal particles on the asbestos and to remove these the broth was subjected to a preliminary treatment with charcoal (Merck's). 500 cc. of the ordinary broth, after being adjusted to p_H 7·6, was mixed with 10 g. of charcoal, steamed for 1 hour and filtered. This process was repeated with a result that the medium was then almost colourless and gave a small and constant blank of about 0·12 mg. (incidentally, it was noticed that the medium gave no glyoxylic reaction).

By means of a pipette 10 cc. of the broth was measured into each of 50 test-tubes which had previously been thoroughly cleaned out with chromic acid and distilled water; these were then plugged and autoclaved. A cotton wool was chosen which did not tend to drop particles into the tubes.

Previous to inoculation the tubes were incubated for some hours so that the cells were sown into a medium of the same temperature (37°) at which the incubation was to proceed, thus reducing the lag due to a cold medium. Each tube was inoculated from an 11 hours' culture in broth identical with that used for the experiment. As the inoculation took a considerable time

an allowance of time had to be made for the later tubes, otherwise an appreciable error would be introduced into the estimation, which would be apparent on the steep part of the curve. At suitable intervals tubes were withdrawn and treated with 1 cc. of $N/10$ NaOH to coagulate the growth of bacteria and were well mixed. This method worked well with *B. coli* but for other organisms coagulation would probably occur at a different p_H and the method would need modification.

The contents of the tube were now filtered exactly as described by Pregl [1924] in the filters prepared for the filtration of silver chloride. The residue was washed with 200 cc. of 1 % acetic acid to remove any calcium phosphate, etc. which might be present, dried at 120°–125° by the usual method of Pregl and weighed.

Table I. *Dry weight of* B. coli *obtained from* 10 *cc. of medium.*

Time hours	Weight mg.	Weight due to medium mg.	Weight due to bacteria mg.	Average mg.
0	0·10 0·16 0·14 0·15 0·11 0·12 0·08	0·12 (average)	—	—
2	0·14	0·12	0·02	0·02
4	0·24 0·12	0·12	0·12 0·00	0·06
6	0·43 0·41 0·40	0·12	0·31 0·29 0·28	0·29
8	0·99 1·02 1·07	0·12	0·87 0·90 0·95	0·91
9	1·40 1·69	0·12	1·28 1·57	1·42
10	1·93 2·02	0·12	1·81 1·90	1·85
12 and later—average from Table II				1·91

The results showing the growth of *B. coli* are given in Table I and the average weight obtained at various hours is plotted in Fig. 1. It will be seen that the maximum error is most likely to occur when the growth is rising most rapidly and particular care must be taken in these estimations that no errors are introduced owing to slight differences in the time of sowing.

The remarkable feature of the results is the sharp flattening of the curve which occurs at the tenth hour (which has been confirmed in many experiments) and also the close agreement in the weight obtained at the twelfth hour and later (Table II). It will be noticed that in 13 estimations taken from different experiments there is an average difference from the mean of only 3·5 %. This close agreement has led us to place considerable confidence in the value of the method.

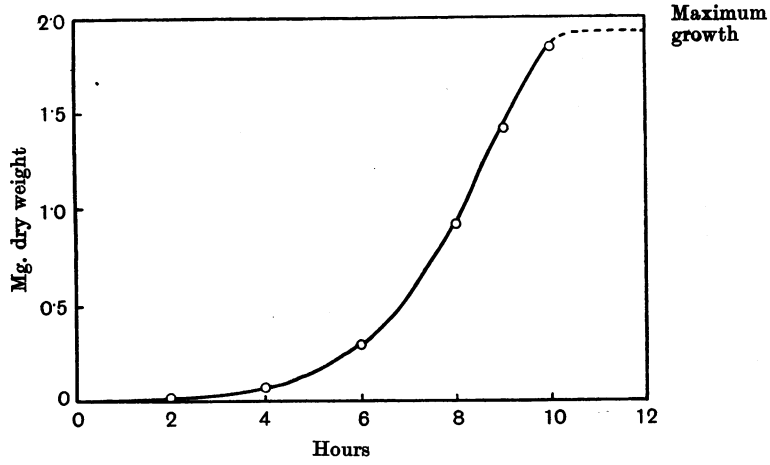

Fig. 1. Growth of *B. coli* on tryptic broth.

Table II. *Dry weight of maximum growth of* B. coli (12 *hours and after*).

No. of experiment	Weight of bacteria mg.	Difference from average %
1	1·87	− 2
2	2·04	+ 6
3	2·11	+10
4	1·90	− 0
5	1·83	− 4
6	2·06	+ 7
7	1·84	− 3
8	1·88	− 1
9	1·86	− 2
10	1·83	− 4
11	1·87	− 2
12	1·90	− 0
13	1·81	− 5
Average	1·91	3·5 %

THE ESTIMATION OF *SACCHAROMYCES CEREVISIAE* (NATIONAL TYPE COLLECTION 815).

The procedure adopted was very similar to that given above, but owing to the greater weight and size of the cells the estimation was considerably easier. The medium was a tryptic digest of one-quarter the strength used in the previous experiment and containing 1·5 % of glucose, the p_H being adjusted to 6·0. Incubation was carried out at 23°.

The inoculation was from a 48 hours' culture of yeast on the same medium; 0·2 cc. of the inoculating culture was introduced into each tube by means of a sterile graduated pipette. Unlike *B. coli* the weight of the inoculating cells was not negligible but amounted to 0·09 mg. It was found that this figure agreed remarkably well with that obtained in the experiment which followed where after 48 hours a weight of 4·01 mg. (Table III) of yeast was present in 10 cc. of medium. This gives a value of 0·08 mg. for 0·2 cc. corresponding with the 0·09 mg. actually found immediately after inoculation.

Table III. *Dry weight of yeast* (Saccharomyces cerevisiae) *obtained from* 10 *cc. of medium.*

Time hours	Weight mg.	Weight due to medium mg.	Weight due to yeast mg.	Average mg.
0 (medium)	0·08	0·10	—	—
	0·10	(average)		
	0·09			
	0·07			
	0·11			
	0·14			
0 (after sowing)	0·19	0·10	0·09	0·09
	0·20		0·10	
	0·18		0·08	
24	1·18	0·10	1·08	0·99
	1·00		0·90	
	1·08		0·98	
31	2·10	0·10	2·00	2·13
	2·26		2·16	
	2·33		2·23	
48	4·10	0·10	4·00	4·01
	4·12		4·02	
73	5·88	0·10	5·78	5·93
	6·19		6·09	
80	6·09	0·10	5·99	6·02
	6·16		6·06	
96	6·25	0·10	6·15	6·15
	6·25		6·15	
100	6·21	0·10	6·11	6·17
	6·34		6·24	

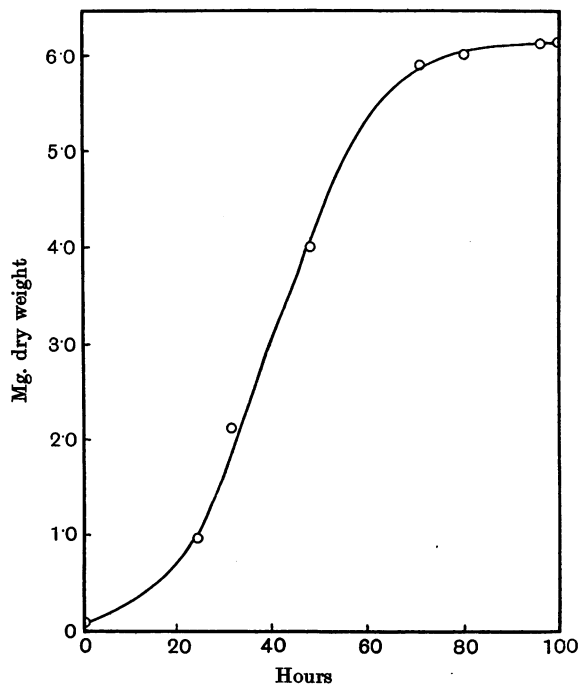

Fig. 2. Growth of *Saccharomyces cerevisiae* on glucose (1·5 %) tryptic broth.

The only notable difference in technique was that no coagulating treatment was necessary, because of the large size of the cells, and 200 cc. of water was used for washing in place of the acetic acid. Moreover, because of the much slower growth samples were taken off at longer intervals.

The results are given in Table III and the averages at the various hours have been plotted in Fig. 2.

In order to ascertain whether all the cells had been held back by the filtering asbestos one filtration was carried out with sterile precautions; 1 cc. of the filtrate was then withdrawn by means of a sterile pipette and inoculated into a test-tube of fresh medium and incubated. No growth took place.

One of us (H. I. C.) was in receipt of a personal grant from the Department of Scientific and Industrial Research and the other (M. S.) held a grant from the Medical Research Council. We also have to acknowledge a grant from the Royal Society and would like to thank Sir F. Hopkins for the interest he has shown in this work.

REFERENCES.

Cole and Onslow (1916). *Lancet*, ii, 9.

Pregl (1924). Quantitative Organic Microanalysis. Translated by E. Fyleman. (Churchill.)