ENUMERATION OF PROTISTS AND SMALL METAZOANS IN ACTIVATED SLUDGE

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INTRODUCTION

Augustin et al. (2) standardized a method for enumerating active protists and small metazoans in activated sludge. This direct counting method recovers an average of 85 % ciliates (n = 165 countings), 95 % nematodes (n = 10), 84 % rotators (n = 10), and 78 % testate amoebae (n = 10). The recovery rate is correlated to the size of the organisms (Fig. 2) and is not influenced by sludge structure. This simple method seems to be an acceptable compromise between time spent and degree of accuracy. Hiller (4) described another simple method for estimating individual numbers of organisms and particles; however, this technique requires that objects are non-motile.

PROTOCOL

1. Place 10 µl fresh, well-mixed activated sludge on one of both central areas of a Thoma counting chamber (Fig. 1).
   Remarks: Use a rather large-bored pipette (c. 0,5-1 mm), e. g. "Assipette-digital". Activated sludge containing very many organisms should be diluted (for example 1:1) with liquid obtained by centrifugation of the sludge investigated. It is recommended to get acquainted with the respective species inventory beforehand to restrict time-consuming identification during enumeration.
2. Cover droplet with coverslip (c. 18 x 18 mm).
   Remarks: The 10 µl sludge used fill exactly the hatched area of the chamber (Fig. 1).
3. Count organisms using a compound microscope and a magnification of 100:1.
   Remarks: Count the complete area of 14,5 x 7,0 mm (see explanation to Fig. 1). Counting 5 x 10 µl activated sludge needs about 2 hours. Very small protists, e. g. heterotrophic flagellates and most naked amoebae, must be counted with the oil immersion objective at a magnification of 1000:1. For such organisms it is sufficient to count 5 nl of sludge which is obtained by appropriate dilution (1).
4. Repeat steps 1 to 3 of protocol 4 times.
   Remarks: A statistical evaluation is possible because 5 separate subsamples are counted. The dispersion of the organisms usually follows a Poisson distribution which, however, should be tested with the dispersion index (4). A computer program is available (5) for the calculation of the dispersion index, the arithmetic mean with confidence interval, the coefficient of variation, the standard deviation, the number of individuals per milliliter extrapolated from arithmetic mean, and the percentage confidence limits.

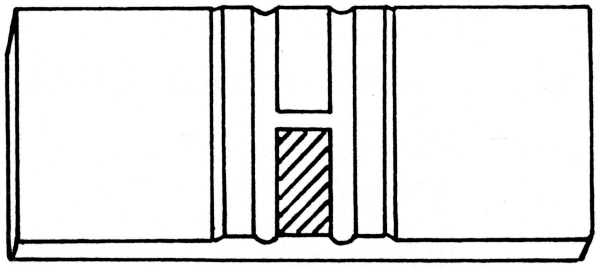

Fig. 1. Thoma counting chamber (from [2]). Unlike its normal use, the whole hatched area (not only the grid) carrying a capacity of 10 µl is counted.

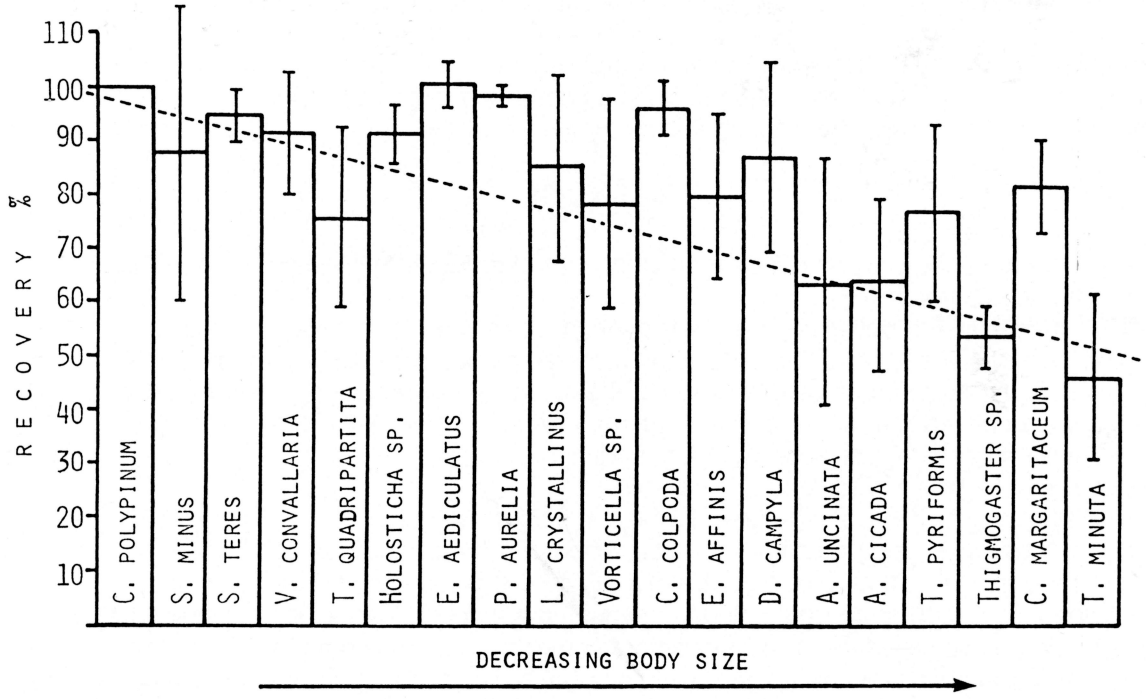

Fig. 2. Percentage recovery with standard deviation (vertical bars) for some ciliate species and correlation between body size and recovery rate (dotted line) in activated sludge (from [2]). *Colpidium colpoda*: number (n) of experiments = 20; *Cinetochilum margaritaceum*, *Euplotes aediculatus*, *Vorticella convallaria*: n = 15; *Dexiostoma campyla*, *Euplotes affinis*, *Litonotus crystallinus*, *Tetrahymena pyriformis*, *Vorticella* sp.: n = 10; *Acineria uncinata*, *Aspidisca cicada*, *Carchesium polypinum*, *Holosticha* sp., *Paramecium aurelia*, *Spirostomum minus*, *S. teres*, *Thigmogaster* sp., *Tokophrya quadripartita*, *Trochilia minuta*: n = 5.

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