

## COMMUNICATION

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### ON THE EVALUATION OF MICROSCOPIC METHODS FOR ACTIVATED SLUDGE ANALYSIS

#### 1. INTRODUCTION

The lack of uniform biological criterion for estimating the course of wastewater treatment with activated sludge does not allow its full characterization. The fact that the biologists apply different methods makes it difficult to compare the results obtained. The values of these results do not correspond to technological parameters by which the process is evaluated. Consequently, despite numerous investigations, there are no biological indicators and "measurable" parameters which would be understandable for technologists. The purpose of the present paper is to compare and evaluate the results obtained from the most frequently applied methods of microscopic analysis and to propose a new method comparable with the methods used to determine physical parameters of activated sludge.

#### 2. MICROSCOPIC METHODS OF ACTIVATED SLUDGE ANALYSIS AND THE ASSUMED METHOD OF THEIR EVALUATION

Biological evaluation of activated sludge is performed by one of the following methods:

##### 2.1. DILUTION OF ACTIVATED SLUDGE TAKEN DIRECTLY FROM THE AERATION TANK

According to this most frequently applied method the qualitative analysis of the sample before its microscopic analysis is recommended. To this end 1-2 cm<sup>3</sup> of activated sludge is poured on a Petri dish of the diameter of 5 cm and diluted in tap water to obtain besides the flocs also the individuals examined. Those individuals should be distinctly seen when observed under different magnification in a stereoscopic microscope. To identify the separate specimens, they can be easily transferred on a micro slide and then determined using an adequate magnification. When the systematics of the organisms is established, the sample can be analysed quantitatively.

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Assuming that the activated sludge is usually too much condensed, it should be diluted in tap water in a 1:10 ratio before the count of individuals is started. To this end 0.1 cm<sup>3</sup> of activated, thoroughly mixed sludge is poured into a Sedwick-Rafter type of cell made of plexiglass, 0.9 cm<sup>3</sup> of tap water is added and mixed thoroughly with the activated sludge. The bottom of the cell is divided into 1000 squares. When we count the individuals appearing in mass, we analyse 10–20 squares of the cell and calculate the average for 1 cm<sup>3</sup> of activated sludge. If, however, the individuals are not so numerous or are of larger sizes, then the average is obtained from a greater number of squares or even from the whole cell.

The results obtained are presented as mean values of the number of individuals of the given species in 1 cm<sup>3</sup> of activated sludge, classified according to four-grade scale denoted by + signs.

## 2.2. MICROSCOPIC ANALYSIS OF NON-DILUTED ACTIVATED SLUDGE TAKEN DIRECTLY FROM THE AERATION TANK

This method is not so frequently applied being recommended when the activated sludge is to be analysed in early stages of raw wastewater treatment in aeration tank. The procedure of counting and determining the species is similar to that discussed in the previous method.

## 2.3. SLUDGE THICKENING METHOD

The new method proposed in this paper, contrary to those presented above, consists of the microscopic analysis of condensed activated sludge. The material to be examined is taken directly from the aeration tank and mixed thoroughly. 10 cm<sup>3</sup> of the mixture is poured to a measuring cylinder and left for 30 minute sedimentation. During this time the way of the settling of sediment suspension is observed. Thereupon the amount of sediment is recorded and the supernatant decanted. After mixing thoroughly the sediment, a 0.05 cm<sup>3</sup> sample is taken and analysed in microscope using a suitable magnification. If the results obtained give any objections, the observations may be many times repeated.

This method is still improved in order to present the results in a form corresponding to a technological estimation.

The efficiencies of the method discussed have been compared by applying them to the analysis of the same activated sludges. The results obtained are recorded on photographs.

The microscopic analysis was performed on two activated sludges operating parallelly under laboratory conditions.

Activated sludge I was used in the treatment of sanitary sewage and is presented on photographs 1a, 1b, 1c, 2a, 2b and 3.

Activated sludge II was used in the treatment of the same sanitary sewage but mixed with wastewater from the wool industry in a 1:1 ratio and is presented on photographs 4a, 4b, 5 and 6.

Both activated sludges gave a high percent reduction of basic indices of the organic type, and their biological development observed for one month did not show any essential differences. The photographs of two sludges were taken 29 III 1979, using a C. Zeiss microscope of ergaval type with a camera for photomicrographs and, applying the 20× and 40× magnifications (photos 1a, 2a, 4a and 1b, 1c, 2b, 3, 5, 6, respectively). Before the pictures were made, the whole volume of 0.05 cm<sup>3</sup> was thoroughly mixed and surveyed under the given magnification to select the most representative field of vision.

Since several aspects of a microscopic picture had to be underlined simultaneously, it was taken repeatedly using a greater magnification. This allowed a better presentation of flocs, the way in which they have been populated by activated sludge organisms and the spaces without flocs. For these reasons the pictures presented show an apparent bad quality.

### 3. DISCUSSION

Microscopic pictures of sludges, illustrating the methods discussed above, are presented below, starting with the third method.

For the activated sludge I, the method of sludge after 30 minute condensation is illustrated by the following photographs:

Photograph 1a (magn.  $20\times$ ) represents a general microscopic picture including flocs of sludge, their agglomeration and interfloccular spaces with numerous individuals of *Amoeba* species.

Photograph 1b (magn.  $40\times$ ) represents a smaller field of vision of the same sludge, underlying the character of flocs, their fluffing and the way they are populated by sedentary colonies of *Ciliata*.

Photograph 1c (magn.  $40\times$ ) emphasises free interfloccular spaces, including the population density of *Amoeba* sp.

The method of a direct analysis of a sludge from aeration tank is illustrated by a following photograph:

Photograph 2a (magn.  $20\times$ ) represents flocs of the sediment, their agglomeration and free spaces. This picture should closely correspond to photograph 1a. When, however, two fields of vision are compared, it seems that the sludges examined differed in numbers of flocs, their agglomeration ability and the degree of the development of *Amoeba* sp. individuals.

Photograph 2b (magn.  $40\times$ ) represents a smaller field of vision for the same sludge which should correspond to photographs 1b and 1c. In this case the differences discussed above are also observed, while comparing the pictures, except for the organisms populating the flocs, as in this sludge a mass development of *Opercularia* took place.

A method of tenfold dilution is illustrated by the following photographs:

Photograph 3 (magn.  $40\times$ ) should be comparable with the photographs 1b, 1c, and 2b. Although this method was applied to the same activated sludge, nevertheless, the obtained microscopic picture shows a sludge whose flocs are small, poorly populated by *Opercularia* and there are no organisms in the interfloccular spaces. Thus, these features are quite opposite to those observed in previous methods.

Activated sludge II was analysed in a similar way. In view of a strong resemblance between the two sludges both in the character of flocs, their agglomeration and the organisms populating the flocs, chief attention has been paid to the differences, thus to the character of interfloccular spaces.

For the activated sludge II the method of sludge thickening after 30 minute is illustrated by the following photographs:

Photograph 4a (magn.  $20\times$ ) and photograph 4b (magn.  $40\times$ ) have been focussed on interfloccular spaces which on the two plates show the same character, being free of organisms, and visually seem to be almost ideally void of impurities.

The method of a direct analysis of sludge from the aeration tank is illustrated by the following photographs:

Photograph 5 (magn.  $40\times$ ), where similarly clean interfloccular spaces are observed. The appearance of those spaces corresponds to that stated in the method of sludge thickening after 30 minute.

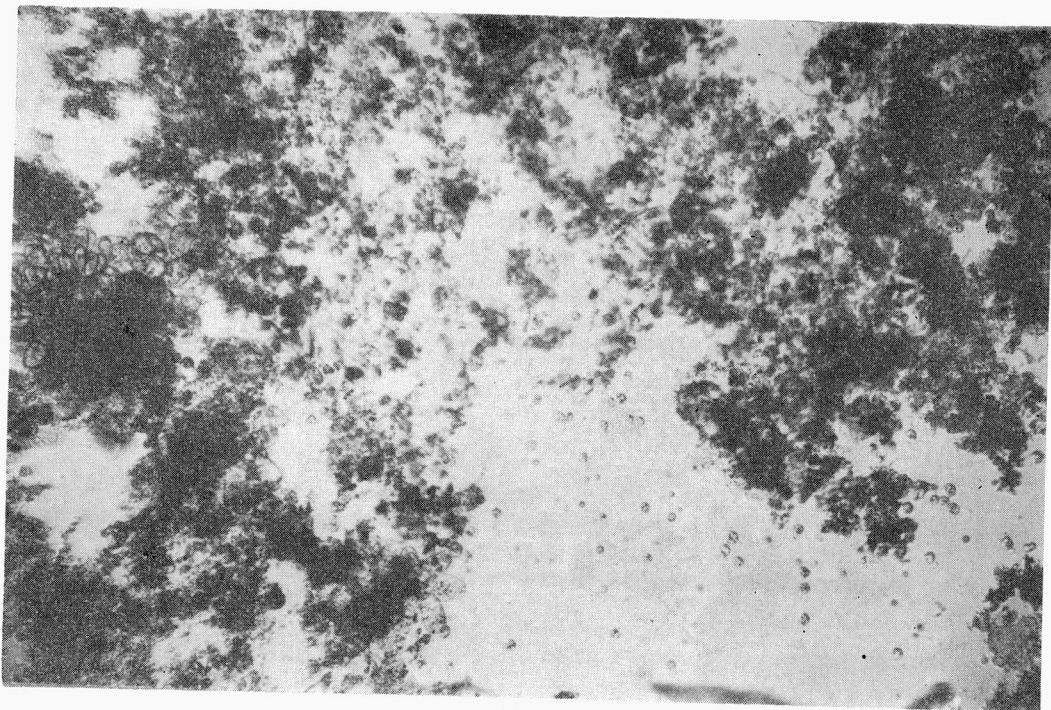
The method of tenfold dilution is illustrated by the following photographs:

Photograph 6 (magn.  $40\times$ ) also represents a different picture of the sludge. Some impurities present in interfloccular spaces have not been observed in the previous methods. These impurities are due to fragmentation of flocs occurring during the necessary mixing or to rapid change of medium. The sludge was diluted in tap water in a 1:10 ratio.

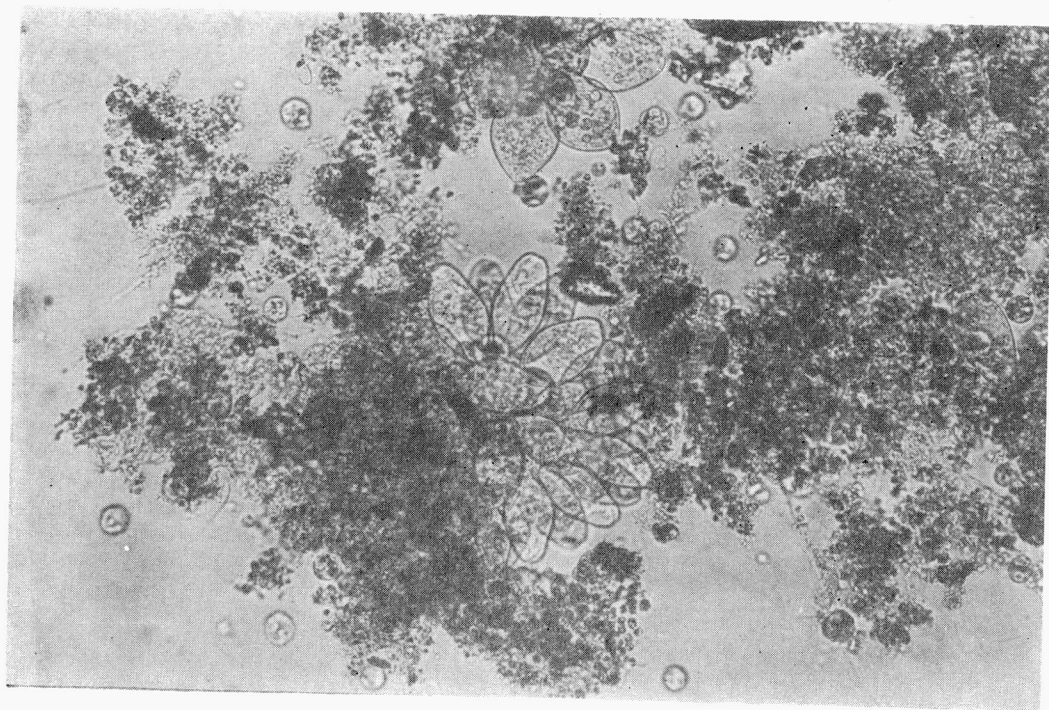
### 4. CONCLUSIONS

The assessment of usability of the methods discussed in microscopic analysis led to the following conclusions:

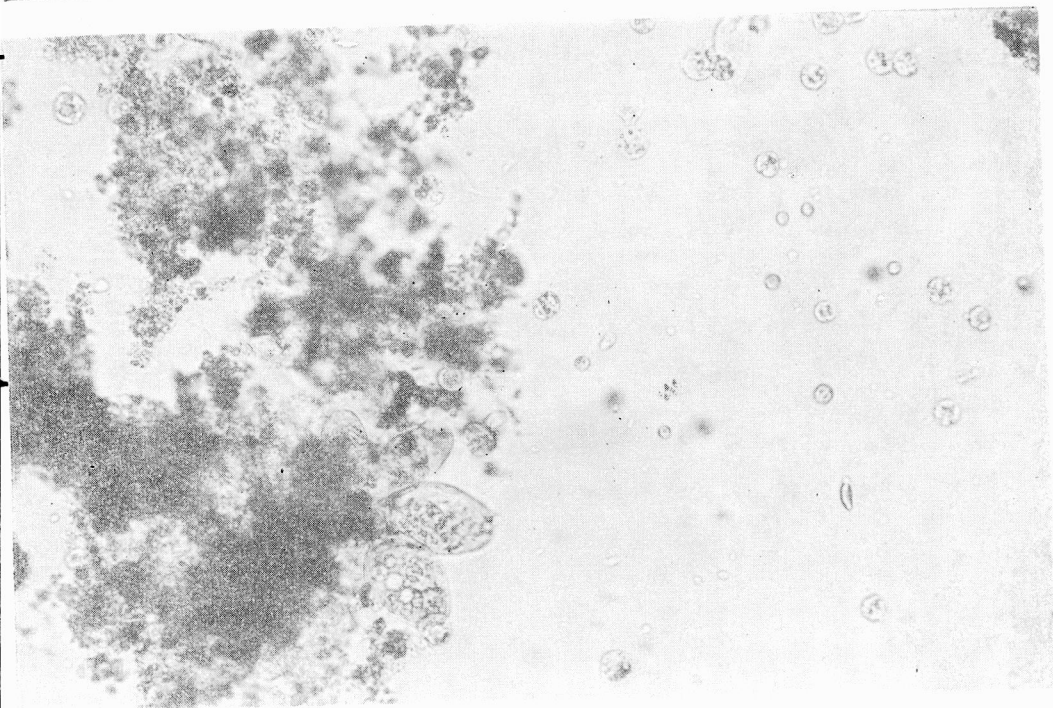
Dilution of the activated sludge does not render its true character.



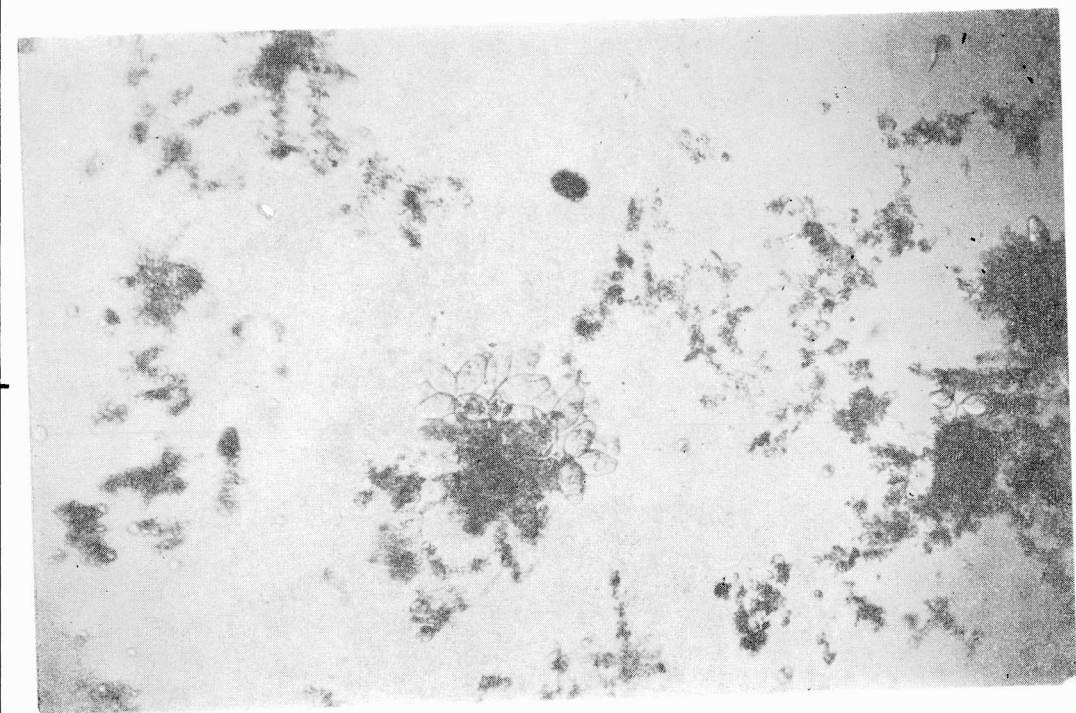
Photograph 1a  
Zdjęcie 1a



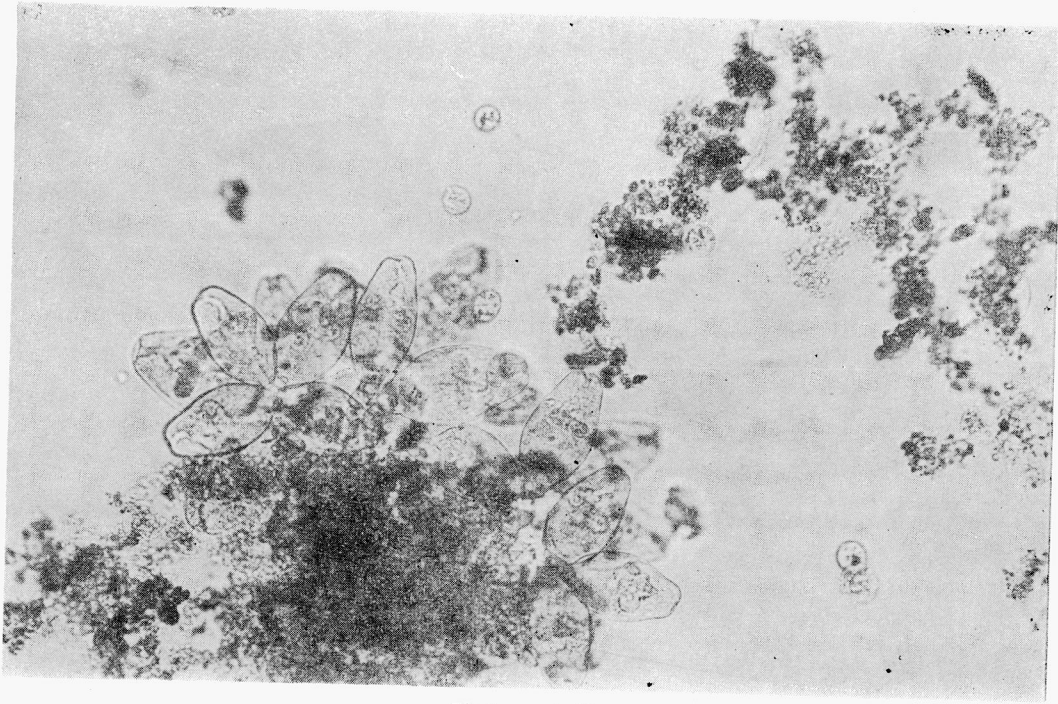
Photograph 1b  
Zdjęcie 1b



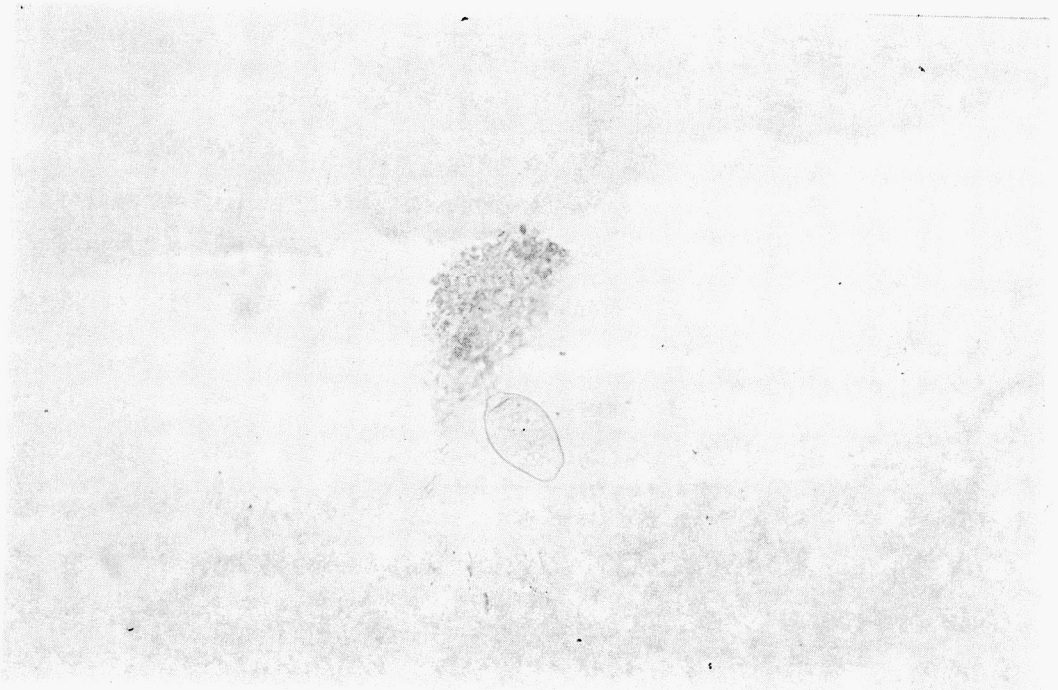
Photograph 1c  
Zdjęcie 1c



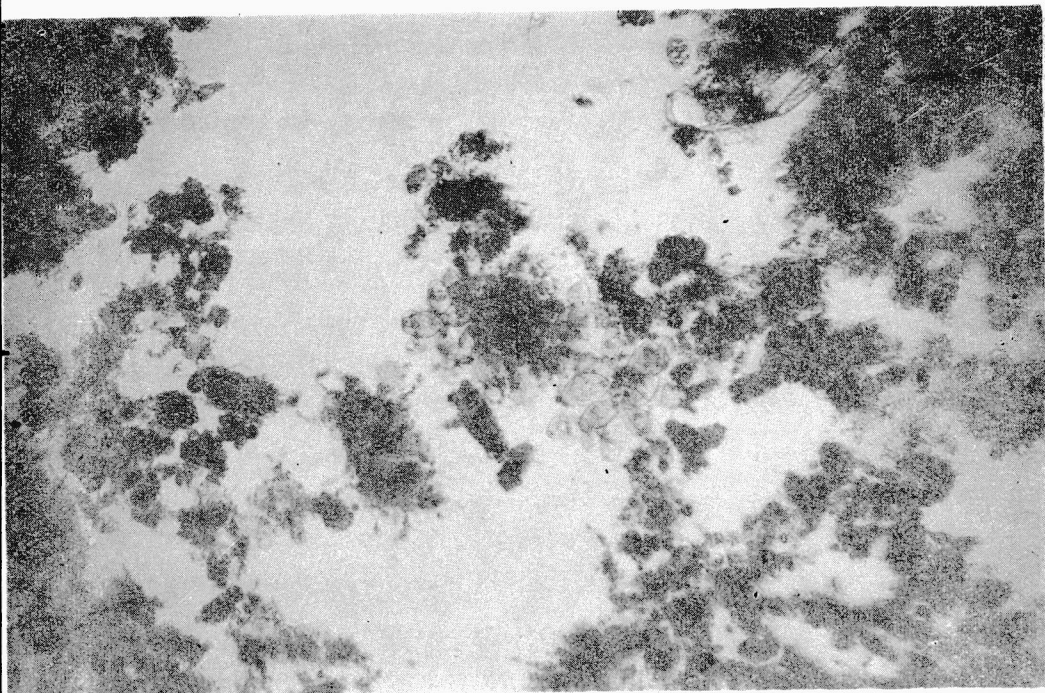
Photograph 2a  
Zdjęcie 2a



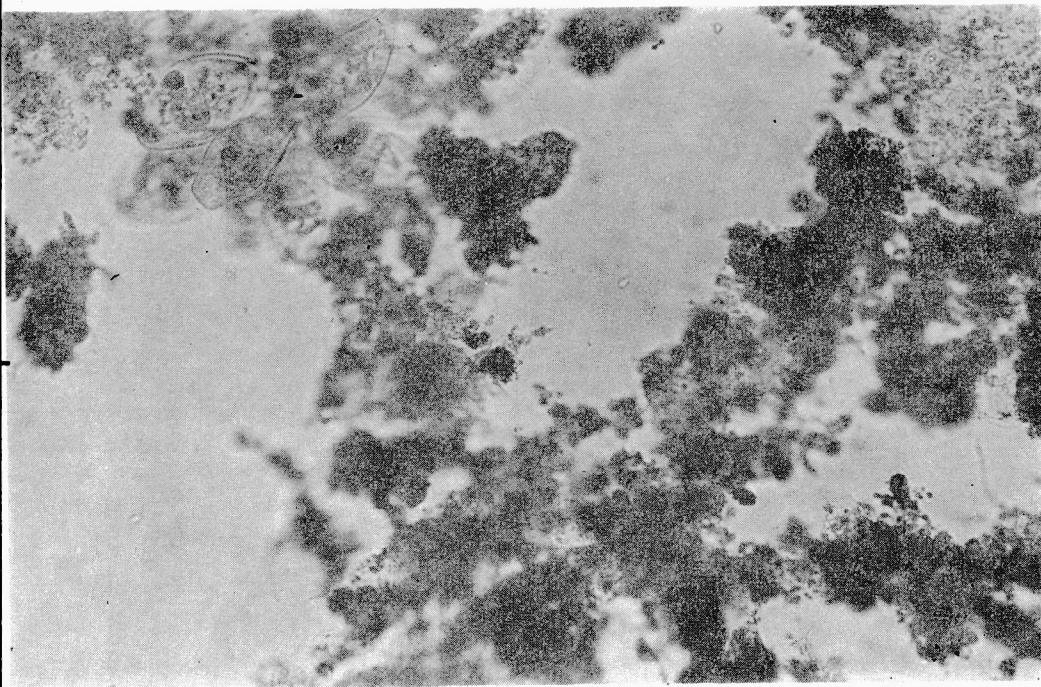
Photograph 2b  
Zdjęcie 2b



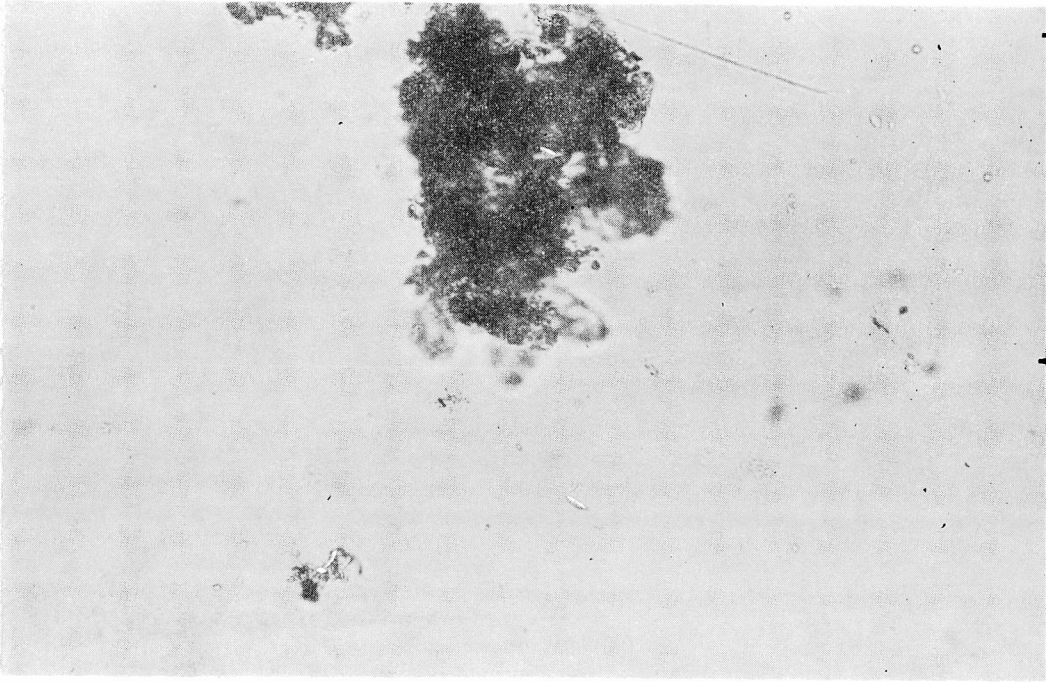
Photograph 3  
Zdjęcie 3



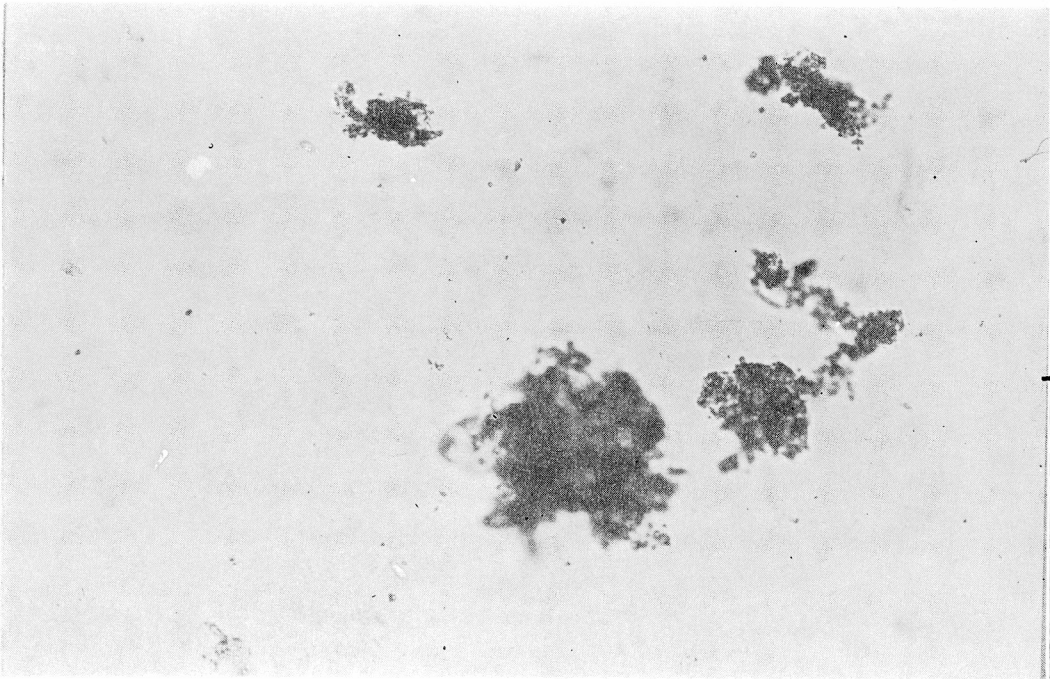
Photograph 4a  
Zdjęcie 4a



Photograph 4b  
Zdjęcie 4b



Photograph 5  
Zdjęcie 5



Photograph 6  
Zdjęcie 6



Biological assessment of identical sludges analysed by dilution and condensation methods is not comparable.

In the method of direct analysis of sludge from the aeration tank there occurs a randomness in numbers flocs sampled, and the microscopic pictures do not allow the giving of quite approximate estimation the sludge.

In the method of activated sludge after a 30 minute condensation, proposed in this paper, the randomness of sampling is avoided. This method allows also observation of the physical properties of the sludge well as of the separate components of activated sludge biocenosis. Its application gives results correlating with a control technological parameter, i.e. a 30 minute sludge index.

#### REFERENCES

- CURDS C. R., COCKBURN A., *Protozoa in biological sewage-treatment processes. II. Protozoa as indicators in the activated-sludge process*, Wat. Res., Vol. 4, Pergamon Press, 1970.
- KLIMOWICZ H., *Mikrofauna osadu czynnego o różnym obciążeniu w urządzeniach laboratoryjnych i pół-technicznych*, Gaz, Woda i Techn. Sanit., Vol. 10 (1970).
- KLIMOWICZ H., *Znaczenie mikrofauny przy oczyszczaniu ścieków osadem czynnym*, Wydawnictwo Katalogów i Cenników, Warszawa 1977.
- PAWLACZYK-SZPIŁOWA M., *Biologia sanitarna*, p. II, Politechnika Wroclawska, Wrocław 1976.
- PIKE E., CURDS C. R., *The microbial ecology of the activated sludge process*, *Microbial Aspects of Pollution*, Acad. Press, London and New York 1971.