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INTERECTION OF FLUOROGENIC SUBSTANCES WITH VIABLE AND HEAT-KILLED CELLS OF MICROORGANISMS*

A simple method is presented for an effective estimation of biomass of microorganisms in their natural environment on the basis of a fluorimetric measurement of fluorescein diacetate hydrolysis by living cells contained in a sample.

The question "How many microorganisms are in a given sample?" is easy to answer only in a case of pure culture. With mixed communities, which is the usual case, however, the problem becomes exceedingly complex. This is because microorganisms are extremely diverse, and the methods used to enumerate one group of microorganism may be inappropriate for enumeration of another group [1].

It has been found by R o t m a n and P a p e r m a s t e r [2] that diacetate of fluoresceine (DF), which is not fluorescent, was hydrolyzed in living mammalian cells to give fluorescein, which accumulated intracellularly and was readily detected by its fluorescence.

Such a behaviour characterize fluorogenic substances [3].

The aim of the present paper was to examine if DF can be used in determinations of the viable biomass of microorganisms.

In the paper the following systems with microorganisms:

1) unicellular alga - monoculture of Chlorella pyrenoidosa,

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2) filamentous alga - monoculture of Nostoc sp.,

3) microorganisms of surfice reservoirs - samples of pond water,

4) soil microorganisms - water extracts from the soil, and

5) microorganisms of activated sludge - samples from sewage purification plant; have been investigated.

The effect of environmental conditions: pH of the sample, ionic strength of the buffor and substrate concentration, as well as the reaction time, on the intracellular hydrolysis of DF for the above mentioned systems has been studied.

Results

Figure 1 presents an example of the dependence of the fluorescence intensity (registered at 525 nm) of the intracellularly released fluoresceine on the relative concentration of viable biomass. The same linear dependences have been observed for ot-

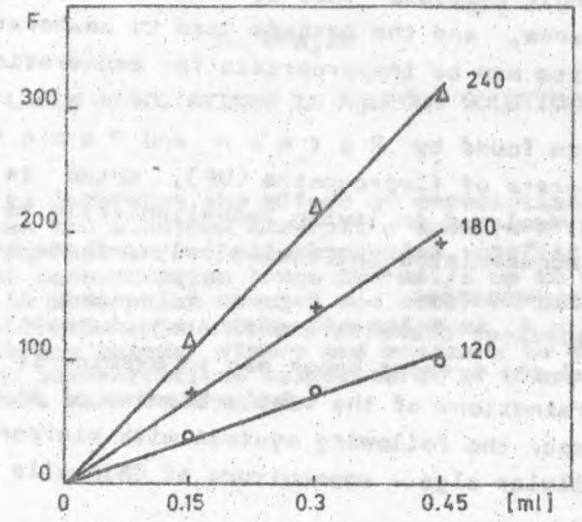

Fig. 1. The relation between the fluoresceine fluorescence (the product of the intracellular hydrolysis of diacetate of fluoresceine) and the relative biomass concentration of soil microorganisms

Związek pomiędzy fluorescencją fluoresceiny (produktu wewnątrzkomórkowej hydrolizy dioctanu fluoresceiny) a względnym stężeniem biomasy mikroorganizmów glebowych

her systems studied, provided the concentration of DF was kept to be in excess to the concentration of intracellular esterases (that are responsible for the hydrolysis of DF).

Such a linear dependence was revealed for the biomass concentration in the region from a fraction to several hundreds of μg/ml.

Heat-killed cells of microorganisms did not show, practically, any signal of fluoresceine fluorescence.

The observed in experiments time dependence of the fluoresceine fluorescence signal is examplefied by Fig. 2.

Environmental conditions, especially, pH of solution, influenced the efficiency of intracellular hydrolysis of DF very much (see Fig. 2).

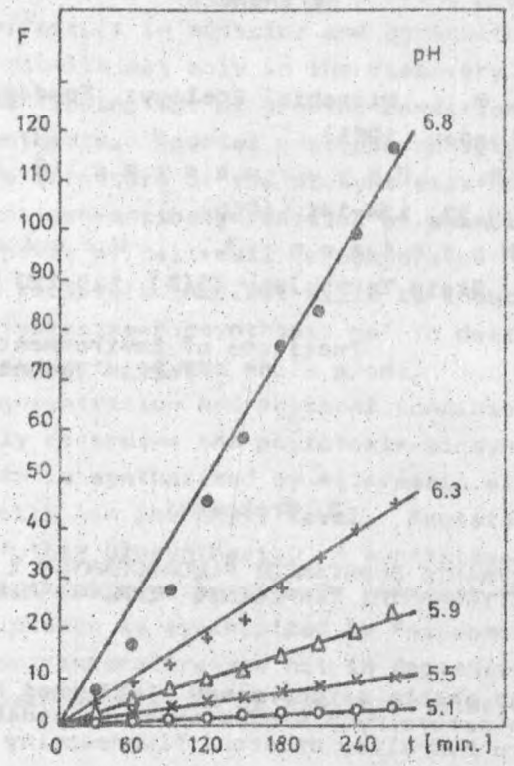

Fig. 2. Time dependences, at various pH's, of the registered signal of fluorescence in a case of pond water samples

Zależność czasowa rejestrowanego sygnału fluorescencji w różnych wartościach pH, w przypadku próbek wody stawowej

For pH above 7.0 some spontaneous (without the participation of esterases) hydrolysis of DF have been observed.

Conclusions

On the ground of the obtained results it seems that diacetate of fluoresceine offers an effective and simple method for rutine determinations (or monitoring) of the viable biomass of microorganisms in their natural conditions (ponds, lakes, rivers or biochemical reactors).

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ODDZIAŁYWANIE SUBSTANCJI FLUOROGENNYCH Z ZYWYMI I INAKTYWOWANYMI TERMICZNIE MIKROORGANIZMAMI

Przedstawiono prostą metodę oceny efektywnej biomasy mikroorganizmów w ich naturalnych środowiskach na podstawie fluorometrycznego pomiaru hydrolizy dioctanu fluoresceiny przez żywe komórki zawarte w próbce.