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A calorimetric method for the rapid evaluation of toxic substances using *Tetrahymena pyriformis*

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Abstract

Calorimetric measurements are performed to study cultures of *Tetrahymena pyriformis*. These protozoa produce heat by metabolism and movement. The addition of toxic substances results in a reduction of the heat production. The concentration and the chemical structures of the toxic substances have an influence upon the toxicity measured by the heat production of *T. pyriformis*. The calorimetric measurements enable a rapid and accurate determination of toxic effects against *T. pyriformis*.

Keywords: Calorimetric measurements; Toxic substances; *Tetrahymena pyriformis*

1. Introduction

For the determination of the toxicity of substances in water, sewage and sludge the german standard methods are mostly used. These methods, which require a duration between 7 and 72 h, are summarized in Table 1.

The organisms used in these methods – with the exception of the *Leucisus idus melanotus* and *Daphnia magna* – are simple organisms. They are all present in one part of the entire ecosystem. To detect the toxicity of substances in water, sewage and sludge the populations of these organisms have to be observed over a given period of time in the presence of these substances. Mostly turbidometric measurements are used. Before starting the measurement exact requirements have to be fulfilled as e.g. a defined population density. Depending on the toxicity of the substance, the population density of the cultures will be reduced leading to alterations of the turbidity. Often these methods are not very sensitive due to relatively high experimental errors. Thus, e.g. not all dead organisms have sedimented or substances and metabolism products or reaction products of both have precipitated. As a result, the accuracy of the turbidometric results is reduced. On the other hand, the metabolism of the organism used in the german standard methods differs from the metabolism of human organism. Therefore it is difficult to predict the toxicity of substances with regard to human organism.

Another possibility to determine the populations of an organism results from calorimetric measurements. Toxic substances influence the heat produced by metabolism and sometimes the movement of the organism. The calorimetric observations of organisms and their reactions on substances or mixtures of substances are part of biochemistry methods for several

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Table 1
Different methods for the judgement of the toxicity of chemical substances

Method	Organism	Representative organisms for	Duration (h)
Pseudomonas cell multiplication inhibition test [1]	*Pseudomonas putida*	Heterotrophic microorganism in freshwater	16±1
Scenedesmus cell multiplication inhibition test [2]	*Scenedesmus subspicatus*	Primary producer in plankton in freshwater	72
Photobacterium phosphoreum cell multiplication inhibition test [3]	*Photobacterium phosphoreum*	Primary producer	7±1
Fish test [4]	*Leuciscus idus melanotus*	Higher organism	48
Daphnia test [5]	*Daphnia magna*	Consumers of lower order	24

years. Already in 1931, Wilson and Peterson have measured the free energy of metabolism processes of bacteria with the assistance of a calorimeter [6]. The use of calorimetry in the study of microbiological and biological systems has already been described in detail [7–10]. Recently calorimetric studies have been reported using *Locusta migratoria*, *Manduca sexta* [11] and fish from freshwater [12].

The *Erlanger Ciliatentest* [13] is well known in dermatology and hygiene since several years. Using *Tetrahymena pyriformis* (*T. pyriformis*) as organism this test allows tendentious statements about the toxicity of substances with regard to human organism. *T. pyriformis* shows similar sensitivity as human cell cultures. Furthermore, this organism also possesses an organelle, which enables this organism to incorporate substances as particles or dispersions. Also *T. pyriformis* can be easily cultivated and the results are well reproducible. The *Erlanger Ciliatentest* is performed using turbidometric measurements. We will report first the results of calorimetric measurements with *T. pyriformis*.

2. Experimental

2.1. Chemicals

The organism *T. pyriformis* is cultivated at 28°C in autoclaved ATTC-Medium, consisting of 5.0 g trypton (pancreatic digest of casein especially rich in tryptophane), 5.0 g proteose pepton No. 3 (enzymatic digest of protein) (both from Difco), 0.2 g di-potassium-hydrogenphosphate with a purity >99% (Fluka) dissolved in demineralized water. The pH-value of 7.2 is adjusted by the addition of 0.1 mol/l hypochloric acid. As test substances are used: dodecylsulfate sodium salt (SDS, Merck), nonylphenol with 15 ethoxy groups (NP 15, Bayer), nonylphenol with 1.5 ethoxy groups in average (NP 1.5, Stockhausen), chlorobenzene (Merck) and *p*-phenylene diamine (Merck). All substances are of the highest purity commercially available. They are used without further purification.

A Tronac Model 450 calorimeter is used to measure the heat production from cultures of *T. pyriformis*. Most measurements are performed using cultures which are at least 48 h old. The measured heat Q is the sum of the heat of metabolism and movement Q_{met} of *T. pyriformis*, the heat of stirring Q_{stirr} and the heat exchanged with the surroundings Q_{surr}:

$$Q = Q_{met} + Q_{stirr} + Q_{surr}. \quad (1)$$

Under the experimental conditions no heat of dilution is observed. The substances used to test their toxicity are dissolved in demineralized water (0.36–0.01 mol/l) and are titrated with a constant burette rate (0.33423 ml/min) into 40 ml containing the culture of *T. pyriformis*. The addition of toxic substances to a culture of *T. pyriformis* results in a reduction of the heat produced. A schematic measurement of this is presented in Fig. 1. Normally all measurements with toxic substances are completed within 1 h.

2.2. Treatment of the experimental data

In Fig. 1a calorimetric measurement of a culture of *T. pyriformis* is shown schematically. At the beginning of the measurement the temperature increases linearly (a). As soon as the addition of a toxic substance starts (b), the metabolism and movement of *T. pyriformis* is influenced and the heat production decreases. Depending on the toxicity of the added substance the heat

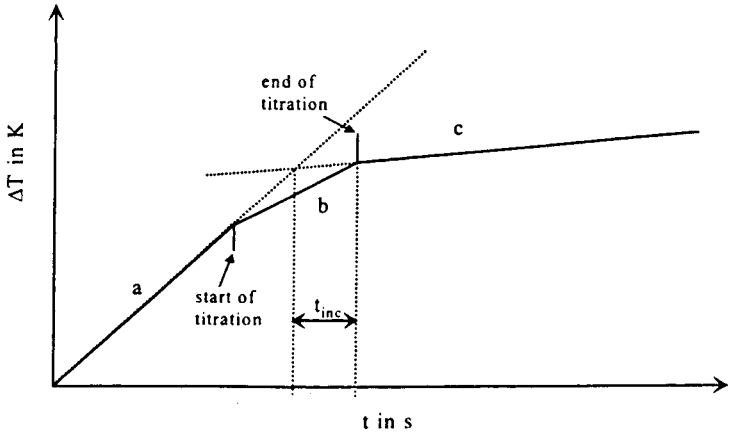

Fig. 1. A schematic calorimetric measurement of a culture from *T. pyriformis* (temperature change caused by: (a) the heat of stirring, metabolism and movement, (b) the heat of stirring and a reduced heat of metabolism and movement, and (c) the heat of stirring).

production by metabolism and movement will come to an end. At this time the increase in temperature observed is only caused by the heat of stirring and the heat exchanged with the surroundings (c).

If no toxic substances are added to the culture of *T. pyriformis* the increase of the temperature would go on linearly (a) as shown in Fig. 1 by the dotted line. Without a living culture of *T. pyriformis* the heat production corresponds to curve (c). The point of intersection between both curves characterizes the moment when the heat production from the culture of *T. pyriformis* ends. At the end of the titration period the concentration of the toxic substance into the culture of *T. pyriformis* has reached its final value. The difference between the point of intersection and the end of titration is defined by us as the incubation time t_{inc} which should be related to the toxicity of the substance added.

3. Results and discussion

The heat produced by a culture of *T. pyriformis* depends on the population density and therefore on the age of population. In Fig. 2 the heat production of cultures of *T. pyriformis* at different population densities are shown. Demineralized water contains no organisms of *T. pyriformis* and hence the increase of temperature only results from the heat of stirring and the heat exchanged with the surrounding. After a longer time interval both contributions cancel each other. As a result the temperature reaches a constant value. A culture of *T. pyriformis* with an age of zero hours contains only a few organisms. So, the heat production of this culture is equal to the curve observed for pure water during 5 h. After this period the culture reaches its exponential phase of growth resulting in an increase of temperature. As shown in Fig. 2 the temperature change per hour increases with the age of the culture because of the higher population density. All temperature curves in Fig. 2 reach a limiting value because the heat production and heat loss are equal. This effect can be neglected for the measurements performed in the presence of toxic substances because the duration of these measure-

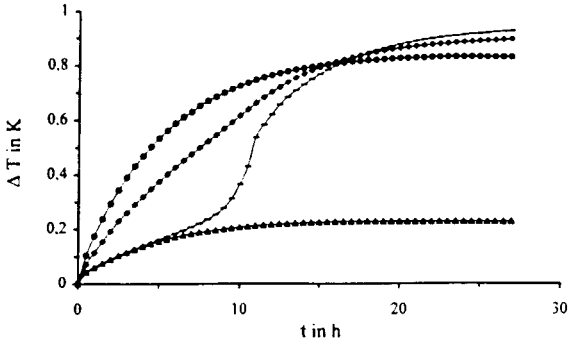

Fig. 2. Measurement of the heat production of cultures of *T. pyriformis* of different population densities (▲: demineralized water, cultures of *T. pyriformis* at different ages: (—) 0 h; (◆) 24 h; (●) 48 h).

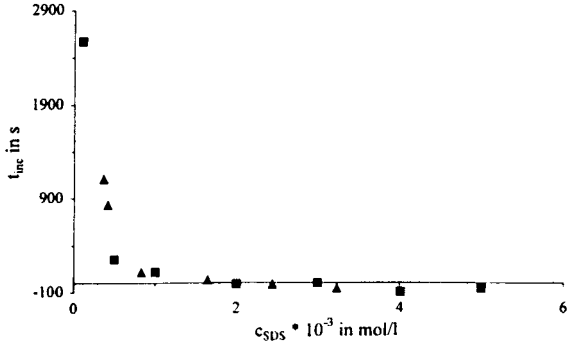

Fig. 3. The incubation time depending on the concentration of SDS (▲: variation of the SDS concentration by changing the time of titration; ■: variation of the SDS concentration by changing the concentration of the solution being titrated).

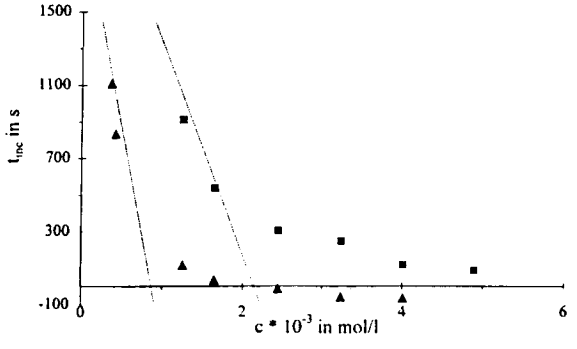

Fig. 4. The incubation time depending on the concentration of SDS and NP 15 (▲: SDS; ■: NP 15).

ments is shorter than 1 h. Under this condition the temperature change is linear with respect to time.

As shown in Fig. 3 the incubation time depends upon the concentration of the toxic substance added to the culture. SDS is used because the toxicity of SDS against *T. pyriformis* is well known. In principle two different possibilities exist to reach a certain concentration of SDS in the culture. To realize a certain concentration of SDS the experimental setup used allows two different possibilities. Thus it is possible to vary the concentration of SDS in the solution titrated into the culture or to vary the titration time. The results presented in Fig. 3 clearly demonstrate that the incubation time is not influenced by the way the final concentration of SDS in the culture is achieved.

The incubation time as an indicator of the toxicity of a substance depends upon the chemical structure of the substances as shown in Fig. 4. Both surfactants show, in principle, the same dependence of t_{inc} on the concentration of the surfactants. The curve for NP 15 is moved to higher concentrations compared with the curve for SDS. As a result, SDS possesses a higher toxicity against *T. pyriformis* than NP 15. So for the quantification of the toxicity of substances examined is not possible. Thus, the initial slope indicated by the dotted line in Fig. 4 or its intercept with the concentration axis may be helpful. Further measurement with other substances are necessary to answer this question.

The incubation times of some ecologically relevant substances are summarized in Table 2. NP 1.5 is very interesting because this substance acts in the environment like oestrogen. In addition, the substance is added to the culture of *T. pyriformis* as a dispersion. Even under these circumstances a toxicological effect is observed. Also chlorobenzene and phenylene diamine are of interest for testing. Even at low concentration an influence upon the heat of metabolism and the heat of movement is found. Also for these substances the measured incubation times depend upon their concentration.

Table 2
Incubation times of ecologically relevant substances

Substance	c (10^{-3} mol/l)	t_{inc} (s)
NP 1.5	0.40 (dispersion)	606
Chlorobenzene	1.91×10^{-2}	495
	9.55×10^{-3}	609
p-Phenylene diamine	8.36	33
	4.18	570
	2.45	1374

The calorimetric measurement of the influence of toxic substances upon *T. pyriformis* show some advantages compared to the turbidometry. The problems arising with incomplete sedimentation have no influence upon the calorimetric measurement. Also the changes in optical density are much smaller compared with the observed heat effects which can be measured very accurately. Another advantage of this method is the relative short duration from about 30 min to a maximum of 1 h. Furthermore, the use of *T. pyriformis* allows estimates bout the toxicity of substances with regard to human organism.

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