

UDC 582.282.23 : 577.472/502.55 = 20

SACCHAROMYCES CEREVISIAE TEST (YTT) AS
THE WATER TOXICITY DETERMINATION
METHOD

(Short Communication)

BOŽIDAR STILINOVIĆ

(Department of Botany, Faculty of Science, University of Zagreb)

Received December 10, 1980

Introduction

In testing toxic properties of sewage various bio-assays are used for the determination of inhibiting effect of water on particular test organisms. So far, several bio-assays have been proposed using bacteria, single celled algae, protozoans and some other invertebrates as well as some higher plants and fishes as test organisms. This paper presents a simple new technique for sewage toxicity determination, with yeast *Saccharomyces cerevisiae* as test organism (Habdija, Stilinović, Primc, 1980).

Material and Methods

The yeast toxicity test (YTT) was carried out in two steps, A and B, as shown in Fig. 1. The *Saccharomyces cerevisiae* culture has been maintained on a nutrient medium consisting of:

Saccharose (Difco Bacto)	2,4 g
Yeast extract (Difco Bacto)	0,7 g
Agar-agar (Difco Bacto)	1,8 g
Tap water	100 ml

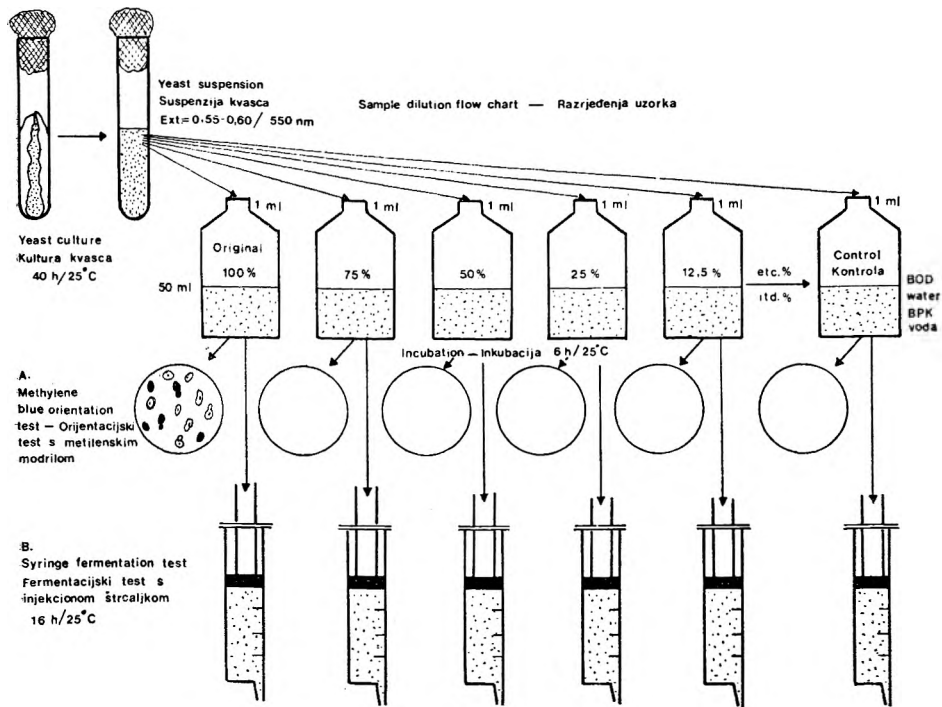


Fig. 1. Yeast toxicity test (YTT)

A. Methylene blue Orientation Test

After 40-hour incubation at 25°C, the yeast culture was removed from the nutrient medium and suspended in BOD water to a density corresponding to an extinction (optical density) of 0.55 to 0.60 at 550 nm. After vigorous shaking, 1 ml of the suspension was added into each bottle containing 50 ml of the original sample, into the series of BOD water dilutions of the original sample as well as into pure BOD water (control). Bottles prepared in this way were incubated for 6 hours at 25°C. Then, the yeasts in suspension were stained by methylene blue solution and microscopically examined. One drop of suspension shaken was placed on a glass slide and mixed with one drop of 0.4% methylene blue water solution; then the cover glass was set. Two minutes later, the uncolored (physiologically active) and blue colored (dead, old or physiologically weakened) yeast cells were counted (Fig. 2) (Jerotijević, 1973). A minimum of 200 yeast cells were counted. On this basis the percent of uncolored cells in all dilutions was calculated; the percentage of uncolored cells in all dilutions related to the control (taken as 100%) was calculated separately. Table 1 shows the method used for the estimation of active cells percentage, used as the basis for plotting and estimation of LD₅₀ value (lethal dose 50) (Fig 4a).

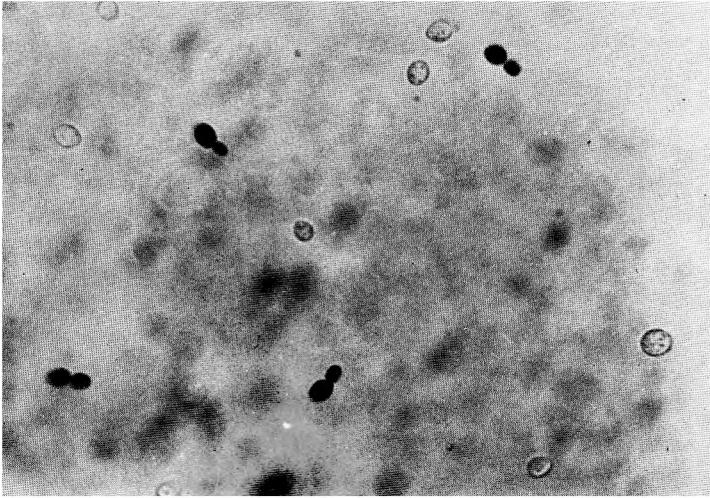


Fig. 2. Uncolored and colored nyeast cells (Methylene blue test)

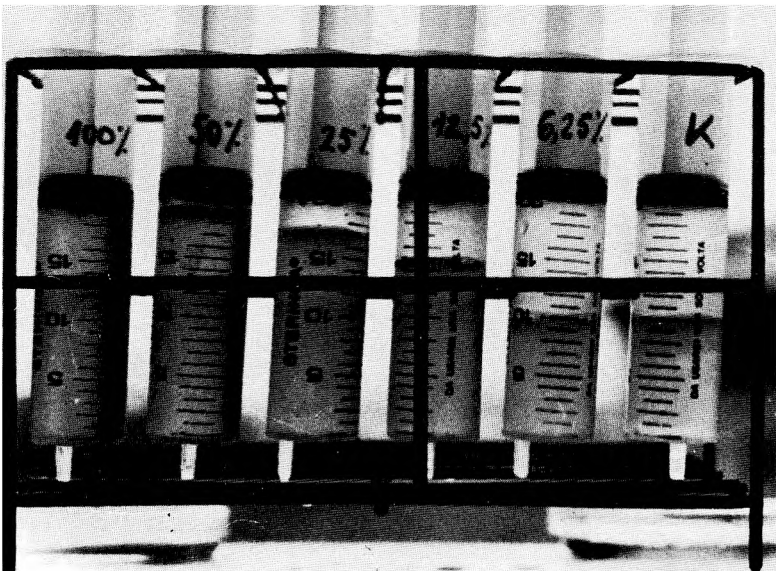


Fig. 3. Syringe fermentation test

Table 1. Percentage estimation of uncolored yeast cells by methylene blue orientation test.

	Sample dilutions					
	100 ^o / _o	75 ^o / _o	50 ^o / _o	25 ^o / _o	12,5 ^o / _o	6,25 ^o / _o
% of uncolored cells in control	92,0					
% of uncolored cells in sample dilutions	0	0	0	30,3	46,1	80,4
% of uncolored cells related to control	0	0	0	33,0	50,1	87,3

B. Syringe Fermentation Test

The toxicity-positive samples from methylene blue orientation test could immediately be examined more precisely by means of a biochemical test, where the gas volume produced in various sample dilutions by yeast-induced saccharose fermentation was measured. For this purpose syringe was used as a simple means for determination of gas volume produced (Fig. 1). The method was accomplished the following way: 1.2 g saccharose and 0.35 g of yeast extract was added into each bottle containing various sample dilutions of the water examined and the yeast suspension incubated 6 hours at 25° C. After these substances were complete dissolved the bottles were shaken and their content poured into 20 ccm syringes, up to their full volume, and then incubated for 16 hours at 25° C. When the incubation period had passed, the volume of gas produced in

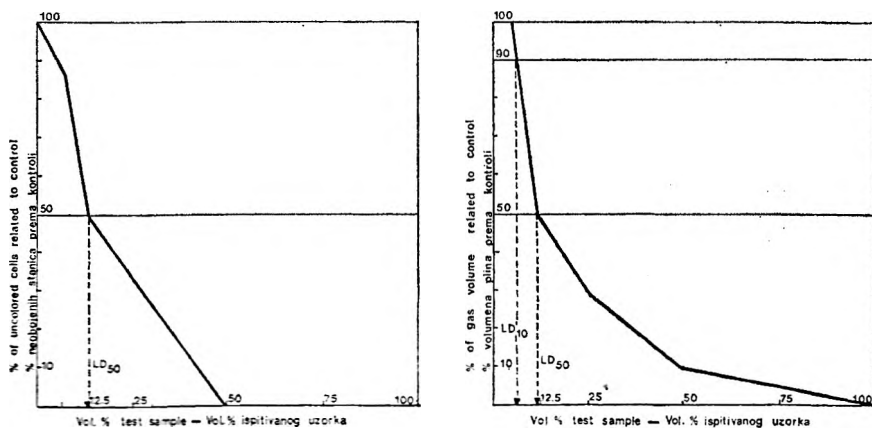


Fig. 4. a — Graphical determination of LD_{50} value by means of methylene blue orientation test
 b — Graphical determination of LD_{50} and LD_{10} values by means of syringe fermentation test

each syringe was read (Fig. 3). Here, the gas volume produced in the control syringe was regarded as 100%, and the gas volumes produced in the test syringes containing the original sample and its various dilutions were taken as percentages of control. At least, two parallel series of test syringes should be set; the gas volumes in these parallel series should be summed-up. In this way the LD_{50} and to certain extent the LD_{10} (as the minimal lethal dose) can be graphically estimated (see Fig. 4b).

Discussion

Especially pronounced toxic properties have been determined by means of yeast toxicity test (step "A" and step "B") for leather and chemical industry sewage as well as for water polluted by synthetic detergents. It was often noticed in the course of this bio-assay (especially in the testing of polluted river water) that more gas was produced in diluted samples than in the control, due to the presence of nutritive substances in these waters, which stimulate the growth of yeasts, especially during the first six hours of inoculum incubation in the original water. Concerning the fermentation test, the gas production in parallel syringes may be different.

Summary

Water toxicity determination method by means of yeast *Saccharomyces cerevisiae* requires little time and simple procedure without special equipment. After the incubation of yeast in toxic water with saccharose, the gas volume could be measured directly in a syringe particularly adjusted for this purpose. Although a relatively crude method, the yeast toxicity test is undoubtedly practicable, especially with regard to LD_{50} value determination.

References

- Habdija, I., Stilinović, B. *Primc.*, 1980: Utvrđivanje stupnja toksičnosti otpadnih voda efluenata grada Zagreba na temelju biotestova i prijedlog metoda za njihovu primjenu (elaborat). Zavod za zaštitu zdravlja grada Zagreba, Zagreb.
- Jerotijević, H., 1973: Praktikum za mikrobiologiju. Privredni pregled, Beograd.

S A Ž E T A K

SACCHAROMYCES CEREVISIAE TEST (YTT) KAO METODA ZA ODREĐIVANJE TOKSIČNOSTI VODE

Božidar Stilinović

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

U radu se iznosi vlastita metoda za određivanje toksičnosti otpadnih voda s pomoću biotesta s kvascem *Saccharomyces cerevisiae*. Biotest se odvija u dvije faze (sl. 1). U prvoj fazi (A) boje se stanice kvasaca 0,4%-tnom vodenom otopinom metilenskog modrila nakon prethodnog držanja inokuluma kvasca u ispitivanoj vodi i njezinim razrjeđenjima, te BPK vodi kao kontroli. Broje se nakon 2—3 minute nebojene (»žive«) i plavo obojene (mrtve, stare, fiziološki oslabljene) stanice (sl. 2). Prema postocima nebojenih stanica kvasaca u seriji razrjeđenja otpadne vode a u odnosu na kontrolu koja se uzima kao 100% (tablica 1), može se grafički orijentaciono izračunati LD₅₀ (letalna doza 50), te izvršiti selekciju uzoraka za dalju obradu (slika 4a). U drugoj fazi (B), koju predstavlja fermentacijski test s injekcijskim štrcaljkama dodaje se u originalni uzorak i njegova razrjeđenja, te kontrolu sa suspendiranim kvascima poslije 6 sati inkubacije na 25° C po 1,2 g saharoze i 0,35 g kvašćeva ekstrakta. Nakon potpunog otapanja tih dodataka i energičnog mućkanja, napune se suspenzijom injekcione štrcaljke od 20 ml do pune zapremine izbjegavajući pritom mjehuriće zraka. Nakon inkubacije od 16 sati na 25° C očitava se u štrcaljkama volumena plina nastalog fermentacijom saharoze s pomoću kvasaca. Usporedbom postotaka volumena plina u pojedinim štrcaljkama (sl. 3) s obzirom na kontrolu koja se uzima kao 100% može se grafički odrediti LD₅₀, a u određenoj mjeri i LD₁₀ (minimalna letalna doza) (sl. 4b).

Metoda bojanja stanica kvasaca metilenskim modrilom služi u prvom redu kao orijentacija u vezi s toksičnošću otpadnih voda, a može se izvesti brzo i izravno na terenu. Fermentacijski test s injekcijskom štrcaljkom usprkos određenim nepreciznostima metode može pružiti upotrebljive podatke o toksičnom stupnju vode, posebno određivanju LD₅₀. Prednost tog biotesta je da ne traži specijalne aparature, jednostavno se može izvesti u svakom laboratoriju, a podaci se mogu dobiti u roku od 24 sata.

U dosad provedenim ispitivanjima kvasci su bili jako inhibirani otpadnim vodama tvornice kože, kemijske industrije i sintetičkim detergentima.

Prof. dr. Božidar Stilinović
Botanički zavod
Prirodoslovno-matematičkog
fakulteta Sveučilišta u Zagrebu
Rooseveltoy trg 6
YU-41000 Zagreb (Jugoslavija)