The Applying of Yeast Saccharomyces Cerevisiae for Rapid Estimation of Surface-Active Substances’ Toxicity

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Abstract. To assess the toxicity of several surface-active substances the foaming activity of the yeast Saccharomyces cerevisiae is proposed to use as biotest. The criterion of toxicity in this test was the suppression of foaming in the suspension of yeast with glucose. The proposed test reaction showed a higher sensitivity to surface-active substances compared with the survival rate and the growth function of yeast. The results of the toxicologic testing are collated with data obtained using conventional techniques. The benefits of a new test reaction are technical simplicity, rate, low material costs, lack of need for constant maintenance in the active state of the test-culture, good reproducibility.

1. Introduction

Recommendations of the European Council with the requirements to reduce the number of vertebrate animals used in toxicological tests were published. It was suggested to replace them with invertebrate animals, plants, organs, tissues or cell cultures [1]. The yeast Saccharomyces cerevisiae as single-celled organisms allow avoiding ethical and economic problems and security issues associated with the use of more complex eukaryotic organisms. S. cerevisiae possess such important advantages for their use in toxicological experiments, as lack of pathogenicity, high growth rate, and effortless reproducibility in the laboratory. The results obtained with yeast can be extrapolated to animals with a certain degree of caution [2]. The yeast is used to assess the overall toxicity and toxico-genetic effects of pollutants, including surface-active substances, heavy metals, pesticides [3, 4, 5, 6].

However most known of biotests with the use of S. cerevisiae are complex and require expensive hardware support. It is known that S. cerevisiae as top-fermentation yeast intensively foaming on the surface of the fermentation liquid [7]. The biotest based on the ability of baker's yeast, which are distinguished by rapidity, methodological simplicity, good reproducibility, was proposed by us earlier [8].
The aim of this research is to verify the possibility of using a new test reaction with the use of S. cerevisiae for the comparative evaluation of the toxicity of several surface-active substances, including flocculants and commercial detergents.

2. Materials and equipment
The test objects were yeast-powder «SAF-Moment» (LLC «SAF-Neva» in Russia), the culture of S. cerevisiae extracted from it, infusoria Paramecium caudatum Ehrenberg, the seeds of radish Raphanus sativus (variety «Red giant», CJSC «Irkutsk seeds»).

For this research were taken following surface-active substances: sodium dodecyl sulfate, tween-85, flotation reagents – oxal T-92 and butyl potassium xanthogenate, synthetic detergents – washing powder «Myth» (LLC «Procter & gamble – Novomoskovsk», Russia; ingredients: 5-15 % anionic surface-active substances, phosphates, < 5 % nonionic surface-active substances, cationic surface-active substances, polycarboxylates, enzymes, optical brightener, perfume), dishwashing liquid «AOS» (JSC «Nafis cosmetics», Russia; ingredients: 5-15 % anionic surface-active substances, less than 5 % ampholytic surface-active substances, conservation agent (lactic acid), perfumes).

The toxicity of the investigated pollutants was measured using the developed bioassay of change foaming activity of S. cerevisiae [8]. For achieving this 1.36 g of yeast-powder «SAF-Moment» were added to 20 ml aqueous-based solution of surface-active substance, were thoroughly mixed and added 0.4 g of glucose. Prepared reaction mixture was poured into 10 ml volumetric flasks per 3 ml in each and incubated for 15 min at +20 °C. Then the volume of the foam was determined. The suspension of yeast with glucose without any testing toxicants served for verification. The influence of surface-active substances on the growth of S. cerevisiae was determined during cultivation in liquid medium IEPD with following composition (g/l): glucose – 20, peptone – 10, yeast autolysate – 5. Surface-active substances in amounts of 0.0001 – 10 g/l were added to the medium. The media were seeded with suspension of yeast in the amount of 1 % (of the total). The IEPD without adding surface-active substances served for verification. The cultures were cultivated for 24 hours at +30 C. The number of yeast cells in media was determined by the Koch’s method.

The effects of surface-active substances on the survival of S. cerevisiae were evaluated. To accomplish this we prepared aqueous solutions of the test substances with a concentration of 0.0001 – 10 g/l. To the flask contained 30 ml of a solution of a certain concentration was introduced 0.3 ml of a suspension of 2 daily culture of S. cerevisiae (number of yeast cells ~10⁶). Flasks with solutions were incubated for 3 hours on a shaker (160 rpm). The suspension of yeast without adding surface-active substances served for verification. Then the number of viable yeast cells in the test solutions and the reference solution was determined, using the Koch’s method.

For a comparative assessment of the toxicity of the test substances the biotests of the survival rate of P. caudatum and germination of seeds of R. sativus were used [9].

All experiments were carried out at least in 3 independent tests with 3-6 parallel measurements each. For statistical processing of obtained data was performed using the software package Microsoft Excel. The conclusions were made up with a probability of a correct forecast equaled R ≥ 0.95. The veracity of the difference of the results was determined using the Student’s criterion.

3. Results and discussions
Testing of surface-active compounds on the basis of the proposed reaction of foaming revealed the following. The sodium dodecyl sulfate inhibited the process of foaming in yeast suspension at the concentration of 0.0001 g/L. The dishwashing liquid «AOS», washing powder «Myth» and tween-85 showed toxic activity against S. cerevisiae at higher content of 0.001 g/L. Floation reagents oxal T-92 and butyl potassium xanthogenate began to depress the foaming process in the yeast suspension at the content of 0.01 and 0.1 g/l, respectively (Figure 1).
The bioassay of survival rate of the yeast was less susceptible to the tested surface-active substances than foaming activity of S. cerevisiae. After 3-hour incubation of yeast in solutions of testing surface-active substances sodium dodecyl sulfate caused cell death at the concentration of 0.01 g/L. Meanwhile the number of viable cells of S. cerevisiae decreased in 1.8 times in comparison with standard solution. The toxic effect of washing powder «Myth» was shown at the concentration of 0.1 g/L, «AOS» – 1 g/L. In the first case, the number of viable yeast cells was reduced by more than an order of magnitude (from 7.4±1.9×10⁶ to 4.3±1.0×10⁵ CFU/ml), the second – order of magnitude (from 8.5±1.5×10⁶ to 9.8±1.0×10⁵ CFU/ml). The exposure of yeast in solutions containing of 0.0001 – 0.1 g/l of tween-85, did not lead to a significant decrease in the number of viable cells (Figure 2).
Growth function of S. cerevisiae has also been found less sensitive to the test surface-active substances in comparison with the reaction of the yeast foaming. The growth inhibition of S. cerevisiae in the IEPD medium occurred with the presence of 0.1 g/l of sodium dodecyl sulphate and flotation reagent oxal T-92. Butyl potassium xanthogenate inhibited the growth of S. cerevisiae at the concentration of 1 g/L. The least negative impact on the growth of the yeast had a tween-85. It began to inhibit the growth of S. cerevisiae when the content was about 10 g/l (Figure 3).

**Figure 3.** The influence of the studied surface-active substances on the growth of *S. cerevisiae*.

The test results of surface-active substances with the use of the foaming reaction of S. cerevisiae were compared with data obtained by using the methods included in the state register of toxicological control. All of the researched surface-active substances: butyl potassium xanthogenate, sodium dodecyl sulfate, tween-85, dishwashing detergent «AOS» and washing powder «Myth» had a toxic effect on infusoria *P. caudatum* at the concentration of 0,00001 g/l (Figure 4).

**Figure 4.** The influence of the studied surface-active substances on survival of infusoria *P. caudatum*. 
The test on germination of seeds of radish R. sativus showed low sensitivity to the testing surface-active substances. Tween-85 was discovered toxic effect of this test parameter at the concentration of 0.1 g/l of washing powder «Myth» and butyl potassium xanthogenate – 1 g/l. Sodium dodecyl sulfate and synthetic detergent «AOS» at the content of from 0.00001 to 1 g/l did not influence germination of seeds of R. sativus (tab. 1).

Table 1. The influence of testing surface-active substances on seed germination of radish R. sativus.

| The concentration of toxicant, g/l | Sodium dodecyl sulfate | Tween-85 | «Myth» | «AOS» | Butyl potassium xanthogenate |
|----------------------------------|------------------------|----------|--------|-------|-----------------------------|
| 0,00001                          | 89,0±5,4               | 92,5±5,3 | 97,3±5,1 | 95,6±3,1 | 95,0±3,5                   |
| 0,0001                           | 89,0±5,4               | 90,0±5,0 | 99,0±3,5 | 95,3±4,7 | 85,0±5,7                   |
| 0,001                            | 95,6±3,1               | 87,5±5,7 | 97,3±3,4 | 95,0±5,4 | 88,0±5,4                   |
| 0,01                             | 95,6±2,4               | 82,5±5,1 | 95,6±1,2 | 90,6±2,4 | 80,0±4,1                   |
| 0,1                              | 99,0±5,4               | 72,5±5,4 | 94,0±4,3 | 80,6±6,2 | 86,7±3,2                   |
| 1                                | 85,6±1,2               | -        | 75,6±4,3 | 89,0±4,1 | 73,4±4,8                   |
| Check sample                     | 95,6±8,5               | 92,5±5,1 | 95,6±8,5 | 95,6±8,5 | 92,5±2,4                   |

A significant decrease of the length of the stems of seedlings of R. sativus seeds compared to the check sample was observed in the presence of 0.001 of sodium dodecyl sulfate, 1 g/l of butyl potassium xanthogenate, detergents «Myth» and «AOS». Tween-85 over the range from 0.00001 to 1 g/l did not exert significant influence on the length of germs (tab. 2).

Table 2. The influence of the testing surface-active substances on the length of seedlings of radish R. sativus.

| The concentration of toxicant, g/l | Sodium dodecyl sulfate | Tween-85 | «Myth» | «AOS» | Butyl potassium xanthogenate |
|----------------------------------|------------------------|----------|--------|-------|-----------------------------|
| 0,00001                          | 0,5±0,05               | 0,8±0,09 | 0,7±0,05 | 0,7±0,06 | 0,7±0,05                   |
| 0,0001                           | 0,5±0,05               | 0,7±0,06 | 0,5±0,04 | 0,6±0,08 | 0,6±0,05                   |
| 0,001                            | 0,4±0,06               | 0,7±0,05 | 0,7±0,07 | 0,7±0,06 | 0,8±0,05                   |
| 0,01                             | 0,4±0,06               | 0,6±0,05 | 0,5±0,06 | 0,5±0,05 | 0,6±0,05                   |
| 0,1                              | 0,4±0,03               | 0,6±0,06 | 0,5±0,04 | 0,5±0,05 | 0,6±0,05                   |
| 1                                | 0,2±0,02               | 0,7±0,04 | 0,4±0,04 | 0,3±0,03 | 0,4±0,05                   |
| Check sample                     | 0,6±0,07               | 0,7±0,08 | 0,6±0,07 | 0,6±0,07 | 0,7±0,09                   |

Thus, the testing surface-active substances due to toxic effect on test parameters using in this research form following series (on reducing of toxicity): survival rate of P. caudatum – butyl potassium xanthogenate > sodium dodecyl sulfate > tween-85 > washing powder «Myth» > synthetic detergent «AOS»; foaming activity of S. cerevisiae – sodium dodecyl sulfate > synthetic detergent «AOS» > tween-85 > washing powder «Myth» >浮選剤オキサル酸二丁アシル; survival rate of S. cerevisiae – sodium dodecyl sulfate > washing powder «Myth» > synthetic detergent «AOS» > tween-85; growth of S. cerevisiae – sodium dodecyl sulfate >floatation reagent oxal T-92 > butyl potassium xanthogenate; survival rate of radish R. sativus – tween-85 > washing powder «Myth», butyl potassium xanthogenate > sodium dodecyl sulfate > synthetic detergent «AOS»; the length of seedlings of R. sativus – sodium dodecyl sulfate > butyl potassium xanthogenate > washing powder «Myth», synthetic detergent «AOS» > tween-85.

On the basis of comparative assessment of susceptibility tested biotests to surface-active substances can be built following series (on reducing of test’s susceptibility): survival rate of P. caudatum >
foaming activity of *S. cerevisiae* > survival rate of *S. cerevisiae* > growth of *S. cerevisiae* > seed germination of *R. sativus*, the length of seedlings of *R. sativus*. It should be noted that the test reaction based on the foaming activity of *S. cerevisiae* differs from other studied bioassays. It has greatest rapidity (time of test response is 15 min), technical simplicity, minimal material cost, lack of need to maintain the culture in an active state. This biotest might be applied both in laboratory and in field conditions.

4. References

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