The influence of surfactants of different classes on the toxicity of diesel fuel for *Saccharomycetes*

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Abstract. The effect on the survival of the yeast *S. cerevisiae* of mixtures of diesel fuel (summer variety) with four representatives of different classes of surfactants – cationic (cetyltrimethylammonium bromide), anionic (sodium dodecyl sulfate), nonionic (Tween-80) and polymeric (polyvinyl alcohol) – was studied. It was shown that diesel fuel and two representatives of the classes of anionic and cationic surfactants (sodium dodecyl sulfate and cetyltrimethylammonium bromide) in the mixture exhibited higher toxicity than when exposed individually. At the same time, the toxicity of diesel fuel mixed with a nonionic surfactant (Tween-80) and a polymer surfactant (polyvinyl alcohol) was comparable to their individual toxicity.

1 Introduction

Surfactants are widely used in the oil industry. They are used for hydrophobization of the bottomhole formation zone in order to increase oil recovery, used for cleaning aquifers, included in drilling fluids, oil-repellent, anti-paraffin and paraffin cleaning agents; wetting, anti-corrosion, emulsifying and viscosity-reducing agents, detergents, antistatic agents, etc. [1-2]. In addition, surfactants are used for remediation of oil-contaminated areas [3-6]. All this leads to the fact that detergents almost always accompany oil pollution. Considering that with multicomponent pollution, the toxic effects of pollutants can differ significantly from their individual effects (both weakened and enhanced), the study of the combined effects of petroleum hydrocarbons with surfactants is of significant interest.

In this regard, in this work we assessed the effect of surfactants of different classes on the toxicity of diesel fuel. The baker's yeast culture *Saccharomyces cerevisiae* was chosen as a test object for biotesting mixtures of surfactants and petroleum products.

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2 Materials and methods

The test object in the work was the culture of *S. cerevisiae* isolated from the preparation of dry baker's yeast "Saf-Moment" (LLC "Saf-Neva", Russia). Yeast cultivation was carried out on solid and liquid YEPD media of the following composition (g/l): glucose – 20, peptone – 10, yeast extract – 5, bacteriological agar (for solid medium) – 23.

The work studied the effect of the following compounds on *S. cerevisiae*:

- Diesel fuel (summer grade) (GOST 305-82)
- Surfactants belonging to 4 different classes (Table 1).

Table 1. Main characteristics of surfactants used in work.

Name	Chemical nature of the compound	Surfactant class
Sodium dodecyl sulfate (sodium lauryl sulfate) (chemically pure)	$C_{12}H_{25}SO_4Na$ sodium salt of lauryl sulfuric acid	anionic surfactant
Tween-80 (Tween-80, polysorbate 80) (import)	$C_{64}H_{26}O_{124}$ polyoxyethylene, derived from sorbitan and oleic acid	nonionic surfactant
Cetyltrimethylammonium bromide (CTAB) (import)	$C_{19}H_{42}BrN$ quaternary ammonium surfactant	cationic surfactant
Polyvinyl alcohol (grade 11/2) (import)	$(C_2H_4O)_x$ water-soluble, thermoplastic polymer based on polyvinyl acetate	polymer surfactant

0.5 ml of a 2-day culture of yeast *S. cerevisiae* was added to aqueous solutions/emulsions of the test substances (or mixtures thereof) with a volume of 50 ml and kept for 3 hours on a BIOSAN OS-20 orbital shaker (Biosan, Latvia), stirring speed was 60 rpm. After this, the number of viable cells was recorded using the Koch method. Yeast colonies were counted on the 2nd day of cultivation.

Based on a preliminary assessment of the individual effect of the studied surfactants and diesel fuel on the survival of the yeast culture, the following concentrations of the tested toxicants were selected:

- Diesel fuel – 100 ml/l.
- Tween-80 – 50 g/l; 100 g/l.
- Cetyltrimethylammonium bromide – 0.1 mg/l.
- Sodium dodecyl sulfate – 1 mg/l; 10 mg/l.

At these concentrations, the tested pollutants did not lead to complete death of the yeast culture, but caused a decrease (~ 10-100 times) in the number of viable *S. cerevisiae* cells.

Polyvinyl alcohol 11/2 was used in concentrations that did not affect the viability of Saccharomyces – 10 and 50 g/l.

All experiments were carried out in at least 3 independent experiments with 3 parallel measurements. For statistical processing of the obtained data, the Microsoft Excel software package was used. The significance of the differences in results was determined using the Student's test. The conclusions were made with the probability of an error-free prediction $P \geq 0.95$.

3 Results of research

The yeast *S. cerevisiae* turned out to be sensitive to diesel fuel when its content in an aqueous emulsion was 100 g/l. At the same time, the number of viable cells after a 3-hour incubation decreased 17 times compared to the control ($(3.9 \pm 0.06) \times 10^5$ and $(6.8 \pm 0.07) \times 10^6$ CFU/ml, respectively). The nonionic surfactant Tween-80 at a concentration of 50–100 g/l also had a toxic effect on the culture of *S. cerevisiae*. After a 3-hour exposure to this surfactant, the number of yeast cells was lower than in the control by 18.9 and 12.8 times ($(3.9\pm0.06)\times10^5$; $(5.3\pm0.07)\times10^5$ and $(6.8\pm0.07)\times10^6$ CFU/ml, respectively).

Tween-80 in the concentration range of 50-100 g/l had no effect on the toxicity of diesel fuel to *S. cerevisiae*. After 3-hour treatment of the *S. cerevisiae* culture with mixtures: 1) Tween-80 (50 g/l) + diesel fuel (100 ml/l) and 2) Tween-80 (100 g/l) + diesel fuel (100 ml/l) k) the number of cells was almost the same in both versions of the experiment – $(3.5\pm0.06)\times10^5$ and $(3.3\pm0.03)\times10^5$ CFU/ml, respectively. At the same time, the indicators of the number of yeast cells did not differ significantly from the results obtained after keeping *S. cerevisiae* separately: in a diesel fuel emulsion (100 ml/l) and Tween-80 solutions (50 and 100 g/l) - $(3.9\pm 0.06)\times10^5$; $(3.6\pm0.05)\times10^5$ and $(5.3\pm0.07)\times10^5$ CFU/ml, respectively.

Table 2. Effect of nonionic surfactant Tween-80 (50 and 100 g/l), diesel fuel (100 ml/l) and their mixtures on the survival of *S. cerevisiae* (time of treatment of yeast with toxicants - 3 hours).

Experience Option	Number of viable cells, CFU/ml
diesel fuel 100 ml/l	$(3.9\pm0.06)\times10^5$
Tween-80 50 g/l	$(3.6\pm0.05)\times10^5$
Tween-80 100 g/l	$(5.3\pm0.07)\times10^5$
diesel fuel 100 ml/l + Tween-80 50 g/l	$(3.5\pm0.06)\times10^5$
diesel fuel 100 ml/l + Tween-80 100 g/l	$(3.3\pm0.03)\times10^5$
control	$(6.8\pm0.07)\times10^6$

Ionic surfactants (sodium dodecyl sulfate, cetyltrimethylammonium bromide) had (in mg/l terms) a stronger toxic effect on the yeast *S. cerevisiae* than Tween-80. Thus, after a 3-hour exposure in a solution with 1 mg/l sodium dodecyl sulfate (SDS), the number of viable yeast cells decreased by more than an order of magnitude compared to the control, and in a solution with 0.1 mg/l cetyltrimethylammonium bromide (CTAB) – by almost two orders of magnitude.

To a greater extent, these surfactants also increased the toxicity of diesel fuel. Thus, the number of yeast cells in mixtures of SDS with diesel fuel was an order of magnitude lower than with individual contamination with these pollutants. For example, in a sample containing 100 ml/l of diesel fuel, after a 3-hour exposure, the number of cells was $(4.6 \pm 0.06) \times 10^5$ CFU/ml, in an SDS solution with a concentration of 10 mg/l - $(1.9 \pm0.02)\times10^5$ CFU/ml. And with the simultaneous presence of SDS and diesel fuel in the solution at the indicated concentrations, the number of viable cells was $(2.0\pm0.01)\times10^4$ CFU/ml. A similar picture was observed at a lower concentration of SDS – 1 mg/l (Table 3).

Table 3. Effect of anionic surfactant sodium dodecyl sulfate (1 and 10 mg/l), diesel fuel (100 ml/l) and their mixtures on the survival of *S. cerevisiae* (time of treatment of yeast with toxicants – 3 hours).

Experience Option	Number of viable cells, CFU/ml
diesel fuel 100 ml/l	$(4.6\pm0.06)\times10^5$
SDS 1 mg/l	$(4.2\pm0.01)\times10^5$
SDS 10 mg/l	$(1.9\pm0.02)\times10^5$
diesel fuel 100 ml/l + SDS 1 mg/l	$(1.6\pm0.02)\times10^4$
diesel fuel 100 ml/l + SDS 10 mg/l	$(2.0\pm0.01)\times10^4$
control	$(6.8\pm0.07)\times10^6$

Mixtures of diesel fuel with the cationic surfactant cetyltrimethylammonium bromide had an even more pronounced toxic effect. In this case, in the mixture of diesel fuel (100 ml/l) + CTAB (0.1 mg/l), complete death of yeast cells occurred, at least they could not be detected by the Koch method. At the same time, in an individual CTAB solution of 0.1

mg/l, the number of viable cells was (9.7±0.3)×10^4 CFU/ml, and in a diesel fuel emulsion (100 ml/l) – (4.6± 0.07)×10^5 CFU/ml (Table 4).

Table 4. The influence of the cationic surfactant cetyltrimethylammonium bromide (0.1 mg/l), diesel fuel (100 ml/l) and their mixtures on the survival of *S. cerevisiae* (time of treatment of yeast with toxicants – 3 hours).

Experience Option	Number of viable cells, CFU/ml
diesel fuel 100 ml/l	(4.6±0.07)×10^5
CTAB 0.1 mg/l	(9.7±0.3)×10^4
diesel fuel 100 ml/l + CTAB 0.1 mg/l	0
control	(6.8±0.07)×10^6

The polymer surfactant polyvinyl alcohol (PVA), like the nonionic Tween-80, did not change the toxicity of diesel fuel (Table 5).

Table 5. The influence of polymer surfactant polyvinyl alcohol grade 11/2 (10 and 50 g/l), diesel fuel (100 ml/l) and their mixtures on the survival of *S. cerevisiae* (time of treatment of yeast with toxicants - 3 hours).

Experience Option	Number of viable cells, CFU/ml
diesel fuel 100 ml/l	(5.7±0.07)×10^5
PVA 10 g/l	(1.0±0.05)×10^6
PVA 50 g/l	(1.2±0.07)×10^6
diesel fuel 100 ml/l + PVA 10 g/l	(6.5±0.02)×10^5
diesel fuel 100 ml/l + PVA 50 g/l	(1.5±0.04)×10^5
control	(1.2±0.03)×10^6

Thus, it was revealed that in relation to *S. cerevisiae*, ionic surfactants had a stronger toxic effect compared to nonionic ones: SDS at a concentration of 1 mg/l led to a decrease in the number of viable yeast cells by more than an order of magnitude compared to the control, and CTAB (0.1 mg/l) – by almost two orders of magnitude. This ratio of toxicity of the tested ionic and nonionic surfactants was demonstrated by us earlier when assessing their effect on the enzyme system of luminous bacteria (NAD(P)H:FMN oxidoreductase + luciferase) [7] and on the release of ions from the cells of the aquatic plant *Elodea canadensis* [8], on the survival of the Baikal oligochaete *Mesenchytraeus bungei* Michaelsen, 1901 [9]. Other authors also demonstrated higher toxicity of ionic surfactants compared to nonionic surfactants [10-14].

The results of toxicometry suggest increased toxicity in mixtures of diesel fuel and two representatives of the classes of anionic and cationic surfactants (sodium dodecyl sulfate and cetyltrimethylammonium bromide) in relation to *S. cerevisiae*. When biotesting mixtures of diesel fuel with representatives of polymer and nonionic surfactants (polyvinyl alcohol and Tween-80), no increase in the toxic effect of the tested pollutants was observed. Their toxicity was comparable to individual effects. It is likely that the increased toxicity is associated with an increase in the bioavailability of diesel fuel under the influence of cationic and anionic surfactants, which occurs due to an increase in the solubility (solubilization) of hydrocarbons in the presence of surfactants [15-17]. At the same time, the observed effect of CTAB and SDS may be associated with their weakening of the barrier properties of cell membranes [18-20]. This makes it easier for diesel fuel to enter the cell, thereby increasing its toxicity. In connection with these assumptions, in order to clarify the reasons for the observed effects, in subsequent studies it is planned to study the processes of emulsification and dispersion of diesel fuel with the tested detergents, as well as their effect on the hydrophobicity of the surface of yeast cells and their permeability.

4 Conclusion

Thus, ionic surfactants had a stronger toxic effect on yeast compared to nonionic ones: SDS at a concentration of 1 mg/l led to a decrease in the number of viable yeast cells by more than an order of magnitude compared to the control, CTAB 0.1 mg/l – by almost two orders of magnitude. These surfactants increased the toxicity of diesel fuel to *S. cerevisiae*, especially CTAB, which led to the complete death of the yeast culture after a 3-hour exposure.

The nonionic surfactant Tween-80 exhibited a toxic effect on the studied yeast culture at a fairly high concentration of 50–100 g/l. The polymer surfactant polyvinyl alcohol (PVA) had a similar mild effect, which in the concentration range of 10-50 g/l did not have a negative effect on the viability of yeast cells. Tween-80 and PVA at the indicated concentrations did not affect the enhancement of the toxic effect of diesel fuel against the studied microorganism.

The data obtained can be used for biotesting using *S. cerevisiae* in aquatic environments contaminated with diesel fuel, as well as for assessing the toxicity of mixtures of petroleum products with surfactants used in the elimination of oil pollution, and predicting situations in the event of environmental pollution with hydrocarbons and surfactants.

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