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Use of Prokaryotic and Eukaryotic Biotests to Assess Toxicity of Wastewater from Pharmaceutical Sources

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Abstract

The yeast toxicity test (YTT), based on the inhibition of saccharose fermentation by the yeast *Saccharomyces cerevisiae*, was evaluated using standard toxicants (copper sulphate, formaldehyde, sodium nitrite, sodium sulphite, phenol and zinc sulphate). Repeated measurements of YTT accuracy with the standard toxicants showed EC_{50} values characterized by low standard deviation and coefficient of variation (7.62%). Moreover, the YTT was compared to standard prokaryotic biotests by estimating the toxicity of wastewater from pharmaceutical sources. The toxicity of wastewaters measured by YTT agrees sufficiently with those measured by standard methods of the determination of toxicity such as inhibition of bioluminescence, TTC-dehydrogenase activity, aerobic bacterial growth and anaerobic sludge biogas production. The YTT is a rapid, simple, reliable, sensitive and cost-effective method for presumptive estimations of the toxicity of wastewaters, which could be performed in laboratory as well as in field conditions.

Key words: biotest, pharmaceutical wastewater, *Saccharomyces cerevisiae*, yeast, toxicity test.

Introduction

Wastewaters from pharmaceutical sources, due to their complex and specific composition, present a potential risk for the environment. Traditionally, the quality control has been based on the chemical control of wastewaters, but actually there is a consensus on the use of bioassays. The main advantage of the bioassays is their ability to account for the biological effects of the pollutants. Therefore, there is a need to develop and validate toxicity bioassays as useful tool for the assessment of the wastewater pollution. A range of toxicity bioassays has been developed to establish the toxicity level of wastewater against different organisms, such as bacteria, algae, plants, invertebrates and fish.[1] A lot of effort has been made to develop the toxicity methods applying bacterial tests based on measuring the inhibition of oxygen uptake or aerobic growth of heterotrophic bacteria, inhibition of bioluminescence and inhibition of dehydrogenase activity.[1] If the toxicity tests are to be used in routine examinations of industrial or urban effluents, they should preferably not be time- and space-consuming or need specialised equipment nor operators with adequate skills, but they have to give reproducible results.[2]

One of such tests could be the yeast toxicity test (YTT), which is based on the saccharose fermentation accompanied by the production of gas by *Saccharomyces cerevisiae*.[2,3] If the toxicity is present, the carbon dioxide production will be suppressed in comparison with the control. Eukaryotic cell structure of the yeasts resembles that of higher organisms.[4] The use of yeasts avoids the ethical problems and problems of variability found with more complex organisms, thus providing advantages for toxicity assessments. There are indications[2,3] that it could be a rapid, simple, sensitive and cost-effective method, which is suitable for testing in the field. The proposed YTT in its previous form (syringe method) has been studied as a rough test,[2,3] and it may be expected to give information on toxicity levels comparable to those found with other microbial assays. However, there is a need to optimise the procedure (broth bottle syringe method) for routine use with a good reproducibility, and to see how they compare to the results of other standardised toxicity tests.

The goal of this study was to assess precision of the YTT broth bottle syringe method using standard toxicants (copper sulphate, formaldehyde, sodium nitrite, sodium sulphite, phenol and zinc sulphate) prior to testing on real systems. The standard toxicants were tested by the YTT procedure and observed data were compared to the literature data. The YTT was compared with standard microorganism toxicity tests such as the inhibition of bioluminescence, TTC-dehy-

Table 1. Average chemical composition of tested wastewaters (these results are values of raw wastewaters before treatments and treated effluents are in accordance with the regulations).

Type of wastewater	COD	BOD_5	Kjeldahl nitrogen	Sulphite	Sulphate	Chloride	Phosphate	pH
	Concentration (mg/L)							
W1	136490	20256	15200	N.D.	43500	53700	N.D.	2.5
W2	15139	4900	850	N.D.	29100	33110	N.D.	8.5
W3	35600	3035	3695	N.D.	9000	1040	1155	7.5
W4	10410	–	37	N.D.	108	4895	3	6.2
W5	15360	1100	1838	N.D.	< 1	5782	1629	7.1
W6	88525	26000	84	N.D.	160	13580	N.D.	9.9
W7	79600	3280	4149	58744	3897	3900	N.D.	4.1
W8	6300	40	16147	N.D.	22	30	53	8.2
W9	40690	–	1170	N.D.	N.D.	5460	N.D.	2.7
W10	44613	14800	3080	N.D.	6930	1190	23	5.6

COD = chemical oxygen demand N.D. Not detected
BOD_5 = biochemical oxygen demand – Not measured

drogenase activity (DHase), aerobic bacterial growth (ABG) and anaerobic sludge biogas production (ASBP) by estimating the potential toxicity of wastewater from pharmaceutical sources.

Experimental

Test microorganisms

A yeast strain *S. cerevisiae* ATCC 64252 was used in this study. The strain of luminescent bacteria *Vibrio fischeri* was obtained from the supply company Dr. Lange, Berlin, Germany. Aerobic activated sludge was obtained from the aerobic municipal wastewater treatment plant in Velika Gorica, Croatia. Anaerobic sludge originated from the anaerobic reactor of the municipal wastewater treatment facility of PLIVA Company, Savski Marof, Croatia.

Standard toxicants

Four inorganic ($CuSO_4$ x $5H_2O$, $NaNO_2$, Na_2SO_3 and $ZnSO_4$ x $7H_2O$) and two organics (formaldehyde and phenol) compounds of analytical grade (Kemika, Croatia) were used as toxic chemicals. Different concentration intervals were used for the tested chemicals depending on the expected EC_{50} values. The tested concentrations of chemicals were (in mg/L of distilled water): for $CuSO_4$ 10–500; $NaNO_2$ 100–10000; Na_2SO_3 100–10000; $ZnSO_4$ 5–100; formaldehyde 10–500; phenol 25–1000. The pH of solutions was adjusted to 6.5 with 1 M NaOH or 1 M HCl.

Wastewater samples

Samples of 10 different wastewaters from pharmaceutical sources were tested. The following abbreviations were used for wastewaters originating from the industrial production processes:

W1 = azalide antibiotic, raw water;
W2 = azalide antibiotic, pre-treated water;
W3 = antibiotic for local application, raw water;
W4 = broad spectrum tetracycline antibiotic, raw water;
W5 = medicament for the improvement of the general condition of human health, raw water;
W6 = medicament that acts as an antidepressant, raw water;
W7 = medicament acting on gastrointestinal tract, raw water;
W8 = diuretic production, raw water;
W9 = disinfectant chlorhexidine-dihydrochloride, raw water;
W10 = molasses slops, raw water.

Chemical composition of wastewaters (Table 1) was determined according to the Standard Methods for the Examination of Water and Wastewater.[5] The original wastewater samples were diluted with tap water to achieve a range of concentrations as volume percent from 100, 75, 50, 25, 13, 6 to 3% or lower, depending on the wastewater toxicity. The pH of the wastewaters was adjusted between 6.8 and 7.5 with 1 or 4 M NaOH or 5 M H_2SO_4 before experiments.

Experimental methods

YTT broth bottle syringe method

This method is based on the fact that the yeast *S. cerevisiae* is able to ferment saccharose to carbon dioxide. The fermentation of saccharose takes place in a closed bottle containing a liquid medium. During the fermentation process gas is produced, which presses out to the open syringe the equivalent volume of liquid. If saccharose is combined with some other matter that influences the yeast and thwarts the fermentation, the amount of created carbon dioxide is reduced or it is not formed at all. By measuring the volume of liquid pressed out, the amount of gas produced during the

fermentation and the intensity of fermentation could be estimated indirectly. The used broth bottle syringe method is an improvement of the previously presented[2,3] syringe method.

The liquid medium for YTT contained (in g/100 mL of distilled water): saccharose 4.0, peptone 2.0, yeast extract 1.7, pH=7.0±0.2. A volume of 4 mL of the medium was dispensed in 24 mL glass bottles and sealed with rubber bungs and aluminium caps prior to the autoclaving at 121 °C for 15 min. The strain of *S. cerevisiae* was pregrown on YM agar (Difco 0712) at 30.0±0.1 °C for 10 h to obtain a log-phase culture. The biomass was resuspended in distilled water and the density of the cell suspension was adjusted to an absorbance of 3.0 at 550 nm, against the distilled water as blank. Broth bottles were inoculated using the sterile syringe (2 mL) and needle (18G, 5 cm length) with 0.5 mL of cell suspension. Then, the bottles were filled up with range of concentrations of chemicals, using 20 mL syringe and employing the additional sterile needle for exhausting air (Figure 1). Control bottle was filled up with distilled water. An 18G needle was stuck to its end through the rubber bung into the liquid medium in each bottle. Finally, the open syringe (10 mL) was stuck on the needle. Inoculated bottles were incubated in the dark at 28.0±0.1 °C for 16 h. After the period of incubation the volume of liquid, which is equivalent to the volume of gas produced during the fermentation of saccharose, pressed out to the open syringe was measured (Figure 2).

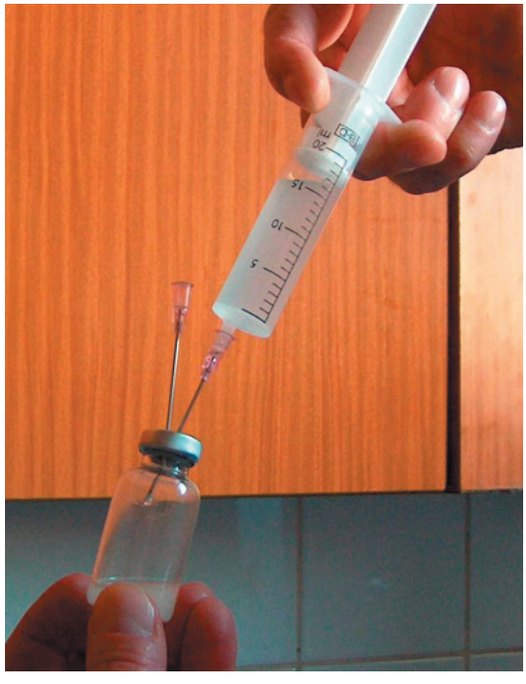

Figure 1. Broth bottle syringe yeast toxicity test – sample inoculation employing two needles.

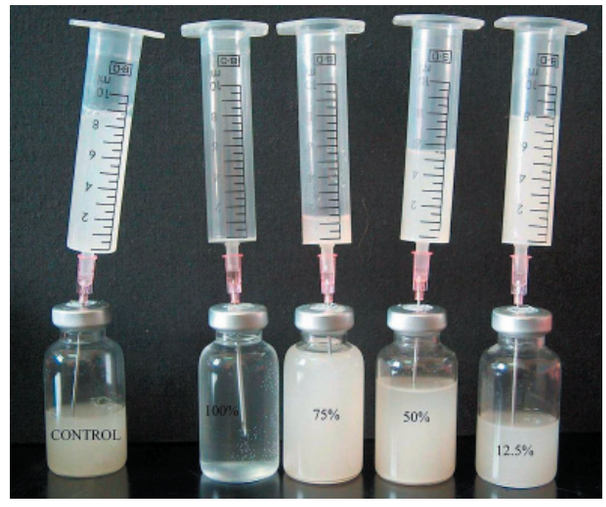

Figure 2. Yeast toxicity test – gas production in control bottle and in bottles with different dilutions (100, 75, 50, 12.5%) of original wastewater.

Each standard chemical and wastewater sample was tested at least in triplicate in series of wastewater dilutions and control bottles. The volume of gas produced in control bottles of YTT containing the distilled water varied between 6.5 and 7.6 mL with 5.71% accuracy. In order to simplify the method and prepare the dilutions of wastewaters with tap water, the control bottles containing the tap water were set up. The volume of gas produced in control bottles of YTT containing the tap water varied between 6.7 and 9.0 mL with 9.88% accuracy.

Bioluminescent method

This method is based on the estimation of the decrease of physiological activity of pure culture of *V. fischeri* in the presence of toxic matter. Natural bioluminescence is used as a measure of physiological activity of those bacteria, which was measured in spectrophotometer. If dissolved matter is toxic to bacteria and influences its activity, the bacteria will create less amount of light or nothing at all. The toxicity test based on the inhibition of bioluminescence of bacteria *V. fischeri* was performed according to Bulich[6], and the intensity of bioluminescence was measured after 45 min of exposure to the concentration range of wastewater.

TTC-dehydrogenase activity inhibition method (DHase)

2,3,5-triphenyltetrazolium chloride (TTC) is used as an indicator of the activity of dehydrogenase in live bacterial cells. When it is oxidised that compound is colourless and when reduces by bacterial dehydrogenase in aerobic activated sludge it becomes red (formazane). Formazane is extracted by ethanol and the intensity of

red extract is measured in the spectrophotometer to measure the level of ecotoxicity of wastewater. In the toxicity test based on the inhibition of dehydrogenase activity of aerobic activated sludge,[7,8] the absorbance of formazane evolved from TTC was measured at 482 nm after 180 min of exposure to the concentration range of wastewater.

Aerobic bacterial growth inhibition method (ABG)

This test is based on the measurement of turbidity. With the increase of the intensity of turbidity, the biomass of microorganisms in the wastewater also increases. If the toxic matter is dissolved in wastewater it will inhibit the growth of microorganisms of the activated sludge and the suspension will remain transparent. Aerobic activated sludge is used as inoculum and it originates from the aerobic part of the municipal wastewater treatment plant. The sludge was kept in active condition in laboratory reactor. The aerobic bacterial growth inhibition test[9] was performed by exposing the supernatant of the filtered aerobic activated sludge to the concentration range of wastewater for 16 h. The turbidity of the samples was measured spectrophotometrically at 550 nm before and after incubation.

Inhibition of anaerobic sludge biogas production method (ASBP)

Anaerobic ecotoxicity is defined as a harmful influence of some matter on the methanogenic culture in anaerobic sludge. This method is based on the comparison of biogas production in the examined and control samples during 5 or 10 days. Anaerobic sludge originating from the anaerobic reactor of the municipal wastewater treatment facility was used as inoculum and grown in continuous laboratory reactor. Anaerobic sludge toxicity test[10,11] was performed by sludge exposure to the concentration range of wastewater in a hermetically closed bottles. The evolved biogas was measured during 5 days of incubation. The produced biogas was measured[10,11] using a needle pierced through the rubber seal at the bottle, connected to a graduated syringe. The piston of the syringe slides until the pressure in the bottle becomes equivalent to the atmospheric pressure. The shift of the piston in the syringe is equivalent to the volume of produced biogas.

Data analysis

The statistical analyses were done using the computer program Statistica.[12] The volume of gas produced in the control bottles of YTT was set as maximum (100%), hence the lower values indicated the inhibition of fermentation process by *S. cerevisiae*. The percentage of the inhibition of gas production during fermentation in series of standard chemicals or wastewater dilutions was calculated, taking the inhibition in the corresponding control bottles as 0%. According to the percentage of gas volume produced in the bottles with wastewater dilutions, EC_{50} values, giving 50% of inhibition, were calculated. As the percentage of EC_{50} values decreased, the toxicity of the wastewater increased. In order to compare the results of YTT and other already standardized toxicity tests, results were set up as EC_{50} values estimated by YTT versus EC_{50} values estimated by other four tests. The correlation between variables was estimated using the Pearson linear correlation. Results were taken to be significant at the 5% level ($p=0.05$).

Results and discussion

Standard toxicants

Repeated measurements of YTT accuracy with the standard toxicants showed EC_{50} values characterized by low standard deviation and coefficient of variation (Table 2). The coefficient of variation averaged 7.62%, which is satisfactory for a biological method. The EC_{50} toxicity values estimated by YTT for different toxic chemicals (Table 2) show that formaldehyde is found to be more toxic than the other chemicals investigated. Intermediate values were obtained for $CuSO_4$, $ZnSO_4$ and phenol. The $NaNO_2$ and Na_2SO_3 exhibit very low toxicity values. It should be emphasized that the standard toxicants were not tested by all previously mentioned biotests, but they were only data from the conducted YTT procedure compared to the available literature data. The toxicity data for the corresponding chemicals reported in the literature (Table 3) show that the toxicity for the same chemical is characterized by a high variability range. This indicates that prokaryotic and eukaryotic organisms are different in response to the same toxic compound. The YTT appeared to be less sensitive than bioluminescence or DHase biotests, but more sensitive than the activated sludge respiration inhibition method. The YTT EC_{50} values can be classified as the closest to the macrodilution broth method[14] and fermentation test[2] which also use the *S. cerevisiae* as test organism.

Table 2. Mean EC_{50} values estimated for different standard toxicants by yeast toxicity test (YTT). SD=standard deviation; CV=coefficient of variation (%).

Toxicant	EC_{50} (mg/L)	SD	CV
$CuSO_4$	83.97	5.47	6.51
Formaldehyde	21.87	0.47	2.15
$NaNO_2$	3081.67	178.44	5.79
Na_2SO_3	2917.00	430.61	15.84
Phenol	461.60	40.58	8.79
$ZnSO_4$	52.13	3.46	6.64

Table 3. The EC_{50} values reported in the literature for the standard toxicants tested by yeast toxicity test (YTT).

Toxicant	EC_{50} (mg/L)	Biotest, reference
$CuSO_4$	1.1	Bioluminescence[13]
	2.5	Dehydrogenase activity[13]
	140*	Macrodilution broth method[14]
Formaldehyde	8.1	Bioluminescence[15]
	62.69	Respirometry[15]
	92.5*	Macrodilution broth method[14]
$NaNO_2$	3750*	Macrodilution broth method[14]
Na_2SO_3	5000*	Macrodilution broth method[14]
Phenol	7.99	Bioluminescence[1]
	13–26	Bioluminescence[13]
	22–26	Bioluminescence[16]
	177–210	Dehydrogenase activity[13]
	480	Fermentation test[2]
$ZnSO_4$	1.6–3.1	Dehydrogenase activity[13]
	1–5	Bioluminescence[13]
	9–11	Bioluminescence[16]

* = minimal inhibitory concentration (MIC).

Wastewaters from production of antibiotics (W1, W2, W3, W4)

The raw wastewater from azalide antibiotic production (W1, Table 4) showed the most toxic effect (EC_{50}=0.0001–3.07%) among all examined waters in all performed tests. This wastewater originated from the production of an antibiotic of wide antibacterial spectrum. For this antibiotic, it is not reported to be toxic against yeasts. Therefore, it could be speculated that except for the residual antibiotic in the wastewater (which inhibits test bacteria), the other chemicals and their combinations would be responsible for the average acute toxicity against yeasts.

The pre-treated wastewater from azalide antibiotic production (W2, Table 4) showed lower acute toxicity than the raw one (W1) in YTT (EC_{50}=71.93%), DHase (EC_{50}=51.83%), ABG (EC_{50}=21.53%) and ASBP (EC_{50}=21.42%) tests. Therefore, it is necessary to conduct a pre-treatment of the wastewater from the production of this azalide type of antibiotic, in order to reduce its toxicity against microorganisms in the anaerobic and aerobic activated sludge treatment plants. High average acute toxicity (EC_{50}=3.99–10.70%) against all test microorganisms of wastewater from the production of antibiotic for local application (W3, Table 4) could be due to the presence of various compounds formed during the process of its production by prokaryotes. For the wastewater originated from the production of tetracycline antibiotic (W4, Table 4) EC_{50} values were much higher when estimated by YTT (72.35%), since when estimated by ABG or ASBP they were low (13.43 and 0.37%, respectively). This could be explained by the presence of a residual tetracycline derivate, which does not inhibit the yeast growth while it inhibits the growth of bacteria.[17]

Wastewaters from production of medical drugs (W5, W6, W7, W8)

The wastewater from the production of medicine for the improvement of general condition of human health (W5, Table 4) showed higher toxicity in YTT (EC_{50}=11.84%) than in other performed toxicity tests (EC_{50}=52.67–90.33%). The specificity of this medicine for eukaryotic cells probably possessed a more pronounced detrimental effect on yeast, than on bacterial cells. The wastewater from the production of drugs used as antidepressants (W6, Table 4) showed a similar toxicity in YTT (EC_{50}=45.34%) with those obtained in DHase (EC_{50}=52.68%) and ABG (EC_{50}=33.10%), since the toxicity against anaerobic sludge was very high (EC_{50}=0.98%). The wastewater originating from the production of medicine acting on gastrointestinal tract (W7, Table 4) possessed high toxicity against *S. cerevisiae*, *V. fischeri,* and microorganisms of aerobic as well as anaerobic activated sludge (EC_{50}=0.03–2.12%). This is probably due to the presence of high concentration of sulphite ions in the wastewater. It has been reported[14] that sulphite is more toxic for bacteria (MIC 22.5 mg/L) than for *S. cerevisiae* (MIC 5000 mg/L). Moreover, the high sulphite content in the wastewater can affect the oxygen levels because it removes it quickly, affecting toxicity values. This mechanism could affect the toxicity in the examined bioluminescence, DHase and ABG toxicity tests. The YTT is based on the fermentation of saccharose by yeast as a facultative anaerobic microorganism. Therefore, the oxygen levels do not have a detrimental effect on the results. The same situation is with the ASBP test, which includes obligate anaerobic bacteria. For the wastewater from the production of diuretics (W8, Table 4), similar toxicity was obtained by YTT and DHase (EC_{50}=12.19 and 11.97%, respectively), since by bioluminescence and ASBP tests the toxicity was significantly higher (EC_{50}=1.32 and 2.43%, respectively) and by ABG the toxicity was lower (EC_{50}=30.00%).

Wastewaters from production of disinfectants (W9)

For the wastewater originating from the production of bacterial disinfectant chlorhexidine-dihydrochloride (W9, Table 4) the highest toxicity was estimated against anaerobic activated sludge (EC_{50}=0.05%), followed by yeast and aerobic bacteria (EC_{50}=5.07 and 6.69%, respectively), and then the inhibition of DHase (EC_{50}=22.22%).

Molasses slops (W10)

According to the chemical analysis, it was expected that *S. cerevisiae* would be able to use the molasses

Table 4. Mean EC_{50} (volume %) values estimated for different wastewaters by yeast toxicity test (YTT), toxicity tests based on the inhibition of bioluminescence (Biolumin), TTC-dehydrogenase activity (DHase), aerobic bacterial growth (ABG) and anaerobic sludge biogas production (ASBP). SD=standard deviation; CV=coefficient of variation (%); – Not measured.

Toxicity test		Type of wastewater									
		W1	W2	W3	W4	W5	W6	W7	W8	W9	W10
YTT	EC_{50}	1.11	71.93	3.99	72.35	11.84	45.34	1.91	12.19	5.07	27.14
	SD	0.31	3.22	1.11	5.83	2.74	11.14	0.95	2.04	0.67	4.42
	CV	27.93	4.48	27.82	8.06	23.14	24.57	49.74	16.74	13.21	16.29
Biolumin	EC_{50}	–	–	–	–	38.12	–	3.42	1.32	–	–
	SD	–	–	–	–	8.33	–	0.86	0.25	–	–
	CV	–	–	–	–	21.85	–	25.15	18.94	–	–
DHase	EC_{50}	3.07	51.83	–	–	90.33	52.68	1.47	11.97	22.22	26.78
	SD	0.94	4.31	–	–	19.86	16.64	0.33	2.08	2.94	3.44
	CV	30.62	8.16	–	–	21.99	31.59	22.45	17.38	13.23	12.85
ABG	EC_{50}	0.012	21.53	5.70	13.43	72.67	33.10	2.12	30.00	6.69	45.84
	SD	0.007	1.66	2.38	4.91	12.01	11.41	0.64	4.58	3.58	16.66
	CV	60.08	7.71	41.75	36.56	16.53	34.47	30.19	15.67	53.51	36.34
ASBP	EC_{50}	0.0001	21.42	10.70	0.37	52.67	0.98	0.03	2.43	0.05	20.78
	SD	0.0000	2.66	3.37	0.19	11.37	0.18	0.02	0.61	0.02	11.70
	CV	55.28	12.43	31.50	51.35	21.59	18.76	75.75	25.10	38.02	56.30

slopes (W10, Table 4) from the production of baker´s yeast as a carbon source and produce a higher volume of gas than in control bottles. The molasses slops themselves lacked in specific potentially toxic components, but appeared toxic in YTT and other preformed tests (EC_{50}=20.78–45.84%). This could be the result of the accumulation of detrimental yeast metabolites formed during the production of molasses. However, all types of investigated wastewaters produced in pharmaceutical factory are treated in biological wastewater treatment plant according to the regulations prior to their discharging into the sewage system.

Our investigations (unpublished data) with municipal wastewaters and leachate samples sometimes showed that the gas production was higher in bottles with tested wastewater than in the control bottle. This could be due to the presence of various inorganic and organic nutrients in the wastewater which stimulate the yeast activity better than the test medium. In such cases good biodegradability of the tested wastewater was concluded. While testing the wastewaters from pharmaceutical sources, such cases were not observed.

Summarising the results for all examined wastewaters, EC_{50} toxicity values estimated by YTT positively correlated (r=0.222, p=0.050) with those obtained by other four standardized toxicity tests. The highest correlation of EC_{50} values estimated by YTT was observed with those obtained by ABG (r=0.787, p=0.020), followed by DHase (r=0.548, p=0.160), ASBP (r=0.476, p=0.234) and bioluminescence (r=0.405, p=0.319) inhibition tests. For different wastewaters tested, the coefficient of variation for EC_{50} values estimated by YTT averaged 21.20%. This indicates a low variation and satisfactory reproducibility considering the average coefficient of variation for bioluminescence (21.98%), DHase (19.78%), ABG (33.28%) and ASBP (38.61%) tests.

Among five toxicity tests performed, one appeared more sensitive than the others did to the particular wastewater of pharmaceutical origin. Each wastewater type from pharmaceutical source possessed a specific inhibitory influence against single test organism, without the rule if prokaryotes or eukaryotes were employed. These support the recommended use of a batch of different test species[1] in order to estimate the overall toxicity of wastewater. For the assessment of the wastewater toxicity on microorganisms in biological wastewater treatment it is necessary to perform a toxicity test by applying the relevant culture of microorganisms, in order to prevent underestimation of wastewater toxicity on biological treatment process. On the basis of our investigations, the YTT as a biotest using the eukaryotic microorganism could be included in the everyday praxis of toxicity determination of wastewater from pharmaceutical sources.

Conclusions

The evaluated YTT is a rapid (16 h of incubation), simple, reliable, sensitive and cost-effective method for presumptive estimations of the toxicity of wastewater, which could be performed in laboratory as well as in field conditions. The toxicity of wastewater from pharmaceutical sources obtained by YTT agrees sufficiently with those obtained by standard methods of toxicity determination such as the inhibition of bioluminescence, DHase, ABG and ASBP.

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Povzetek

Strupenostni test s kvasovkami *Saccharomyces cerevisiae* (YTT), ki temelji na merjenju zaviranja fermentacije saharoze, je bil ovrednoten z uporabo standardnih strupenih snovi (bakrov sulfat, formaldehid, natrijev nitrit, natrijev sulfid, fenol in zinkov sulfat). Večkratne meritve so pokazale majhno standardno deviacijo in koeficient variacije za EC_{50}. S tem testom smo ovrednotili tudi strupenost farmacevtske odpadne vode in jo primerjali s standardnim biološkim testom s prokarionti. Dobljeni rezultati so bili primerljivi z rezultati standardnih metod, kot je na primer merjenje zaviranja bioluminescence, merjenje zaviranja aktivnosti TTC dehidrogenaz, merjenje zaviranja rasti aerobnih bakterij in anaerobne proizvodnje bioplina. Uporabljani YTT test je hitra, enostavna, zanesljiva, občutljiva ter poceni metoda za grobo oceno strupenosti odpadnih vod, ki jo lahko izvedemo v laboratoriju in tudi na terenu.