Decontamination of Nitrophenolic Compounds by Yeast Suspensions

Statistical study

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Since the bioremediation which involves microorganisms in the removal of various pollutants is a promising, relatively efficient, and cost-effective technology, we proposed here the use of Saccharomyces cerevisiae as a nitrophenols biodegradating and bioaccumulating material, due to easy availability and low cost of yeast suspensions. The biodegradation process of the investigated compounds was followed by germination wheat test. In addition, statistical test applied on germination experiments demonstrate that yeast can be used in a decreasing of nitrophenol compounds toxicity.

Keywords: dinitrophenols compound; statistic, germination test, bioremediation, yeast

Numerous hazardous chemicals from various industrial sources enter the environment daily [1, 2]. Moreover, some others can be used both as drugs and poisons. Many of these compounds, including dinitrophenols, such as: 2,4-dinitrophenol (DNP), 4,6-dinitrocresol (DNOC) or Karathane (Krt.) are widely used pesticides that persist in certain contaminated soils [3-5]. They have been found in ground-waters, causing health and environmental hazards, being subjects of forensic toxicology. Dinitrophenols have multiple biological effects acting as metabolic inhibitors. DNP cannot be easily removed from natural bodies of water through chemical reactions [6, 7]. However, actions of some microorganisms in water might be the most important processes of DNP removal from water. Fortunately, they can be degraded by anaerobic microorganisms, such as *Pseudomonas sp.* and *Alcaligenes sp.* strains, which may use these noxious compounds as nitrogen source [5]. Indeed, DNP in soil is mainly destroyed by microorganisms, so that it may take 4 to 80 days for the level of DNP in soil to halve [8].

An anaerobic consortium of different bacterial species is able to completely degrade dinitro-derivatives. The results suggest that yeast (*Saccharomyces cerevisiae*) could be used as a genuine biodegradation agent in the contaminated environments with dinitrophenol pesticides and related compounds [7, 9]. The yeast-associated microbiological degradation can be applied in the case of uncoupling dinitrophenols, whereas phytoremediation may be recommended to other pollutants [10].

Our main goal for this paper was to study the efficiency of dinitrophenols compounds decontamination by yeast *Saccharomyces cerevisiae* using one-way Anova statistical tests, applied on the germination wheat test [11].

Experimental part

Reagents. The used reagents were of analytical grade, and the aqueous solutions were prepared with double distilled high purity water (R = 18.2 Ω). All reagents were purchased from Merck (Darmstadt, Germany) and used without further purification. Dinitrophenols were acquired from Sigma-Aldrich (SUA). Dinocap (Karathane-Krt), which is a mixture of 2,4-dinitro-6-octylphenyl crotonates and 2,6-dinitro-4-octylphenyl crotonates, octyl here being a mixture of the methylheptyl-1-ethylhexyl-and 1-propylpentyl-isomers, was purchased from a crop protection shop in Iasi. DNG and other dinitrophenols and derivatives have been prepared both under classical conditions and under microwaves [12]. Four investigated compounds were selected: DNP (2,4-dinitrophenol), DNOC (2-methyl-4,6-dinitrophenol), DNG (3-(2,4-dinitrophenyl)propane-1,2-diol), and Krt. (*RS*)-2,6-dinitro-4-octylphenyl crotonates and (*RS*)-2,4-dinitro-6-octylphenyl crotonates (fig. 1).

Biological materials. Baker's yeast was purchased weekly from SC Rompak srl Pascani (Romania), and kept in a humidor at 4°C. Wheat seeds (*Triticum aestivum*), Gasparom variety, were obtained from Suceava Agricultural Research Station.

Procedure. Yeast suspensions (5 $g \cdot L^{-1}$ and 0.40 $g \cdot L^{-1}$ KH_2PO_4) with or without 5 $g \cdot L^{-1}$ glucose, and the corresponding investigated compounds (10^{-3}M) were introduced, in three replicates, in 300 mL Erlenmeyer flasks. The biodegradation process occurred for 7 days on an IKA-KS 4000 ic control orbital stirrer at 25°C and 50 rpm. The supernatants were used in germination experiments [7, 9] (fig. 2).

Biological experiments: The germination parameters were measured according to ISTA recommendations [13], however we worked also with lots of 50 seeds, grown at 20°C which were laid to germinate on filter paper, in Petri dishes, in three repetitions. After 7 days (germination rate, GR) the germinated, abnormal and dead seeds as well as the resulting plantlets were counted. The treatment lasted for an hour, and then the seeds were laid as uniformly as possible in Petri dishes, on double filter paper, together with the treatment solution. The seeds with a visible root were considered germinated. The seeds were watered daily with

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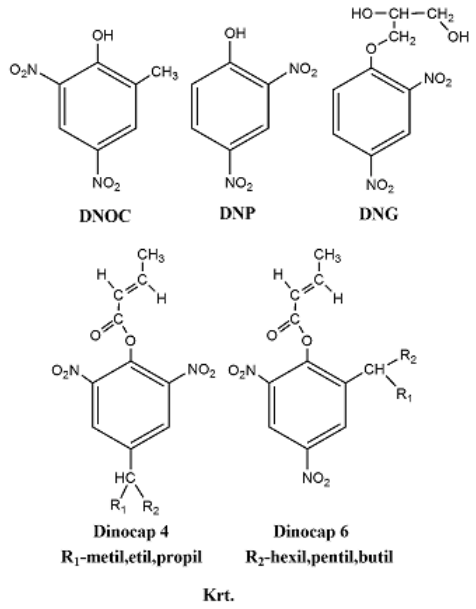

Fig. 1. The chemical structure of investigated nitrophenolic pesticides. DNP (2,4-dinitrophenol), DNOC (2-methyl-4,6-dinitrophenol), DNG (3-(2,4-dinitrophenyl)propane-1,2-diol), and Krt. (*RS*)-2,6-dinitro-4-octylphenyl crotonates and (*RS*)-2,4-dinitro-6-octylphenyl crotonates

5 mL of bidistilled water. The plantlets were cut at the level of the seeds 7 days after, measured and weighed (height, H, in cm and mass, m, in grams).

Statistics: All findings were validated using the ANOVA test in IBM SPSS Statistics 21 Program [14]

Results and discussions

The biodegradation of nitrophenol compounds with *Saccharomyces cerevisiae* can be easily demonstrated on the color changes of nitrophenols in contact yeast suspensions (fig. 2). It is known from literature that biodegradation process of nitrophenols derivatives formed amino derivatives (red color) [15]

Wheat germination and growth is completely inhibited by nitrophenol compounds solution (10^{-3} M), whereas the solution resulted from yeast decontamination was proved to be non-toxic to wheat seeds and seedlings (table 1). Besides, the resulted solution after nitrophenols decontamination using *Saccharomyces cerevisiae*, had a slightly stimulatory effect on wheat development within the germination experiments when compared with control treatment (distilled water). Generally, yeast suspensions

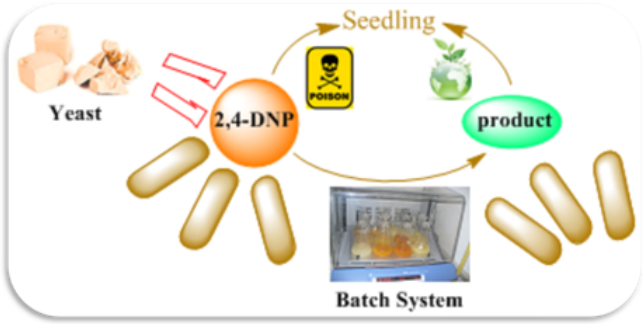

Fig. 2. Schematic representation of nitrophenol decontamination by yeast.

containing glucose proved to be less effective in decreasing pesticide dinitrophenol toxicity than those without glucose [7, 9].

The graphics comparison of the indicators average included in the study, on the variance analysis

The indicators monitor in this study, named in text *variables* or *variables-factor* are: 1) number of plantlets; 2) number of germinated seeds; 3) number of dead seeds; 4) height of plantlets (cm); 5) weight of plantlets (g).

The difference between treatments and lists the variable names was determined by one-way Anova. The F-statistic (*Fischer-Snedecor*) distribution, was calculated on IBM SPSS Statistics 21 Program, F(60, 106.8) = 8.910, at the p = 0.05 level of significance. The levels of significance were comparable between the types of treatments.

For number of plantlets variable (fig.3), the largest averages shall be recorded for the DNOC+Yeast+Glucose and DNOC+Yeast treatments, the both exceeding the average value of the control. The lowest average was recording for DNP 10^{-3}M and DNG 10^{-3}M toxic treatments.

In figure 4 is presented the means (from three individual experiments) for germinated seeds variable. In this case, the highest averages it's recording for the DNG +Yeast+Glucose and DNOC, the both exceeding considerable the average value of the control. The lowest averages were for DNP 10^{-3}M and DNG 10^{-3}M treatments.

The difference between treatments and number of dead seeds variable recorded that the highest average was in DNP 10^{-3}M and DNG 10^{-3}M treatment case, the both toxically treatments killed all of wheat seeds (fig. 5). The nontoxic effect on wheat germinated seeds (dead seeds) it has been met on DNOC+Yeast+Glucose and DNOC+Yeast treatment case, this treatment had a slightly stimulatory effect compared with control.

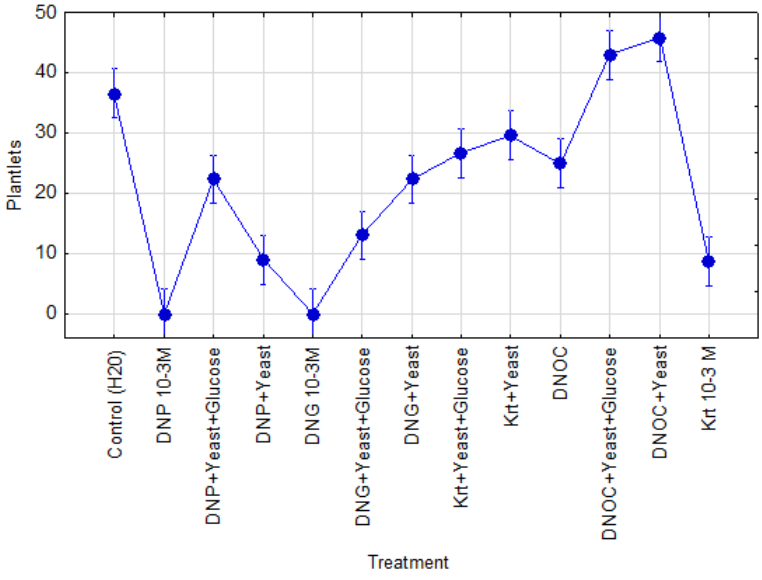

Fig. 3. Confidence Intervals of *Plantlets* variable with individual 95% confidence intervals for all treatments applied

Table 1

THE EFFECT OF DIFFERENT NITROAROMATIC COMPOUNDS ON THE GERMINATION IN CONTACT WITH YEAST SUPENSIONS

Treatment*)	Plantlets in 50-seed lots	Germinated seeds in 50-seed lots	Dead seeds in 50-seed lots	Heights of plantlets in 50-seed lots (cm)	Plantlet weight 50-seed lots (g)
Control, H_2O	40±2	5±1	5.0±0.1	270.5±11.5	0.95±0.04
DNP 10^{-3} M	0	0	0	0	0
DNP+Yeast+ Glucose	22±3	2±1	25.7±1.8	61.7±0.7	0.83±0.25
DNP+Yeast	9±2	3	38±3	13.3±3.9	0.13±0.04
DNG 10^{-3} M	0	0	0	0	0
DNG+Yeast+ Glucose	13±1	6±1	30.1±0.1	17.7±1.7	0.18±0.02
DNG+Yeast	22±1	2.3±0.3	25.3±0.9	41.0±4.7	0.18±0.02
Krt. 10^{-3} M	8.66±1	1±0,1	40.33±2	23.16±3.5	0.22±0.03
Krt.+Yeast+ Glucose	7±1	2.3±0.3	21.7±2.3	65.3±10.7	0.65±0.08
Krt.+Yeast	30±1	2	18.3±0.9	82.3±3.08	0.76±0.04
DNOC 10^{-3} M	25±5	4.0±0.2	21.0±0.1	117.2±12.5	0.73±0.33
DNOC+Yeast	46±1	2.0±0.1	2.0±0.1	383.9±15.8	2.12±0.14
DNOC+Yeast+ Glucose	43±2	1.0±0.1	6.0±0.1	306.9±24.4	1.73±0.14

*) Mean of three individual experiment

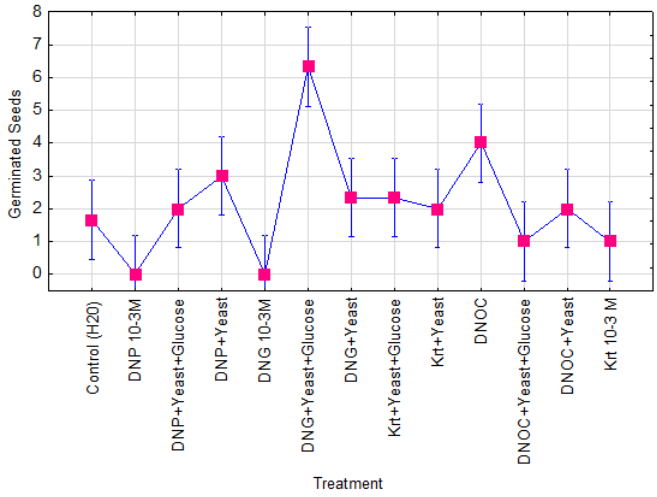

Fig. 4. Confidence Intervals of *Germinated seeds* variable with individual 95% confidence intervals for all treatments applied

For height of plantlets (cm) variable (fig. 6), the largest averages shall be recorded for the DNOC + Yeast + Glucose and DNOC + Yeast treatments, the both exceeding the average value of the control. The lowest average was recording for DNP 10^{-3}M and DNG 10^{-3}M toxic treatments. The values obtained for height of plantlets (cm) was similar with the number of plantlets value.

Partial eta-squared approached somewhat of the significance of the square of the coefficient of correlation, being an indicator of *Quality Assurance* that variables of *predict'/anticipate* better than other variables dependent variance of the system. By definition, *partial eta-squared* is the ratio between the amount of the range for the variable investigated (here *Height of plantlets (cm)*) and the sum of the range for the variable investigated plus the error range amount, provided by the ANOVA procedure (table 2). It is the most net separation between lowest and highest average values of all the variables studied. In this case, the treatment effect on the Height of plantlets (cm) does not take into account the treatment effect on the other variables from the study.

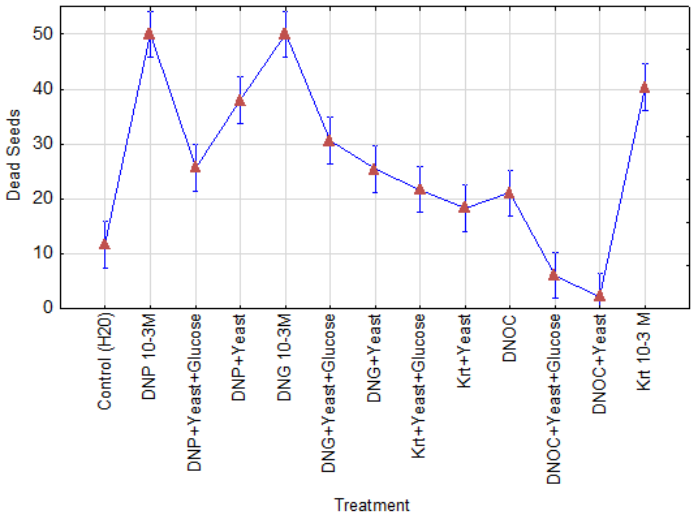

Fig. 5. Confidence Intervals of *Dead seeds* variable with individual 95% confidence intervals for all treatments applied

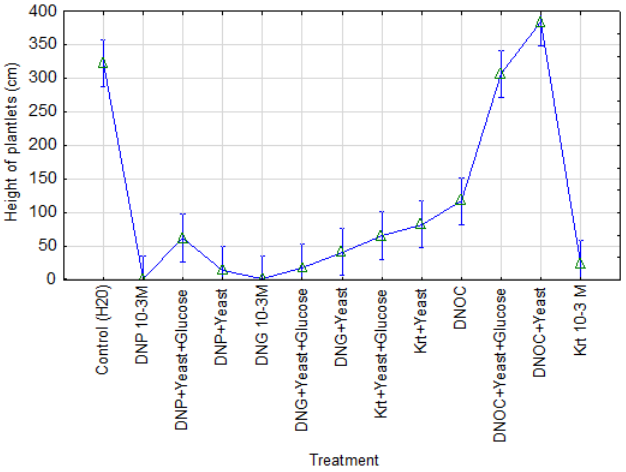

Fig. 6. Confidence Intervals of *Height of plantlets (cm)* variable with individual 95% confidence intervals for all treatments applied

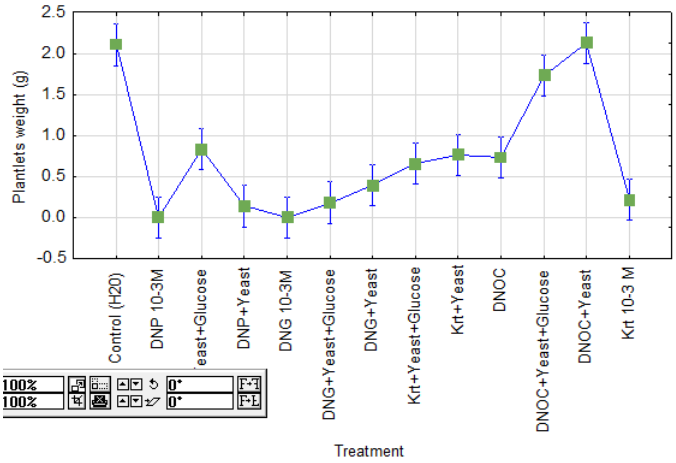

Fig. 7. Confidence Intervals of *Weight of plantlets (g)* variable with individual 95% confidence intervals for all treatments applied

Table 2

THE RESULTS *PARTIAL ETA-SQUARED* OBTAINED in SPSS STATISCS FOR *HEIGHT OF PLANTLETS (cm)* VARIABLE

Parameter	Test	Value	F	Effect – df	Error – df	p	Partial eta-squared	Non-Centrality
Intercept	Wilks	0.000	190842.2	5	22.000	0.05	0.999	954211.1
Treatment	Wilks	0.000	**8.91**	60	106.795	0.05	0.833	534.7

The same highest average was obtained for Weight of plantlets (g) like as a number of plantlets and height of plantlets (cm) for DNOC+Yeast+Glucose and DNOC+Yeast treatments, the both not exceeding the control (with distilled water) average value (the highest value of any average selected in this variable reaches the control average value). The lowest averages were recorded for DNP 10^{-3}M and DNG 10^{-3}M treatment, the both and also the all of the average obtained in this case being smaller than the control average value.

Conclusions

Following the measurements, we concluded that the investigated microorganisms (*Saccharomyces cerevisiae*) can be a useful candidate in the biodegradation of nitrophenols pesticides and related compounds. The microbial biodegradation can be applied in the case of uncoupling dinitrophenols, whereas phytoremediation may be recommended to the others.

A lower toxicity of the supernatants resulted from dinitrophenol derivatives treatment with yeast suspensions was found in germination experiments using wheat seeds. The germination parameters of all treatments statistically vary significantly between them ($F = 8.91$, $p < 0.05$). Also, DNP 10^{-3} M and DNG 10^{-3} compounds exhibit most pronounced influence/toxicity, significant statistically, on germination parameters. Further research is needed to understand the fate of this compound over time under the microbiologic conditions investigated here

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