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Effect of a static magnetic field on *Saccharomyces cerevisiae* growth in wastewater containing phenol and p-chlorophenol

Anna Rutkowska-Narożniak, Elżbieta Pajor

Warsaw University of Technology, Faculty of Building Services, Hydro and Environmental Engineering

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Abstract: The effect of a static magnetic field (MF) of 7 mT with phenol (P) or p-chlorophenol (p-chP) concentrations of 100 mg·dm^{-3} on the proliferation of *Saccharomyces cerevisiae* yeast was investigated. The abundance of the microorganism was determined under static culture conditions on a YPG medium with or without the addition of P or p-chP and exposed or unexposed to the MF over 48 h of the experiment. A static MF of 7 mT was shown to have a stimulating effect on *S. cerevisiae* cell proliferation after 24 h. It was proved that P and p-chP were used as an additional carbon source by yeasts. The greatest stimulation of the growth of the studied microorganisms was observed under the simultaneous effect of an MF and in presence of either P or p-chP. It was generally about 2 times higher at the time of the study than in the control. Statistical analysis of the results was carried out using, among other things, analysis of variance (ANOVA). A statistically significant difference in the growth of the tested microorganisms was observed. The study results indicate the possibility of applying an MF of 7 mT to enhance the process of phenol and p-chlorophenol removal from industrial wastewater.

Keywords: p-chlorophenol, phenol, proliferation, *Saccharomyces cerevisiae*, static magnetic field, wastewater

INTRODUCTION

As one of the physical factors, static magnetic fields have an effect on organisms. Knowledge of the nature of this effect is essential in view of the exposure caused by the stronger intensities of fields generated during the operation of the various types of electrical equipment used in households, industry and medicine, compared to that of the Earth. Exposure to magnetic field (MF) is thus greater nowadays than ever before. As exposure to MF has increased, consideration has begun to be given to their effects on the functioning of living organisms, mostly on microorganisms.

An MF can alter or regulate bacterial activity. A study by KHOKHLOVA and VAINSHTEIN [2017] showed that the application of a static MF of up to 173 mT did not affect amylase activity in *Rhodospirillum rubrum* over a 3-h exposure, while application of a 25 mT alternating MF affected such activity over a 2-h exposure.

The effects of static MF at 40, 80, 120 and 160 mT on the ultrastructure of *Escherichia coli* type 1 cells were investigated by KAMEL *et al.* [2018]. It was shown that exposure to an MF caused changes in the activity of arginine dihydrolase, gelatinase and the ability to use citrate as the sole carbon source. Furthermore, changes in colony morphology on MacConkey agar were also observed.

Current research often focuses on the use of an MF to inhibit pathogens. BRKOVIC *et al.* [2015] showed that a one-day application of a 60 mT static MF reduced the number of plaque microorganisms *in vitro*. QUIÑONES-PEÑA *et al.* [2017] investigated the effect of an MF on the pathogenic *E. coli* strain (EPEC) E2348/69. They found that a 5 min exposure to an MF of either 53 or 100 mT reduced autoaggregation (by 28 or 50%, respectively), while increasing the exposure time to 30 min (in a 100 mT MF) further modified the adhesion pattern. These effects were probably due to changes in the expression of the gene encoding the adhesion factor located on the plasmid. BODNARIUC [2017] indicated that exposure of *E. coli* to a 19.5 mT MF can increase its sensitivity to ampicillin and streptomycin.

To explain the action of weak MF on microorganisms, it is necessary to examine the field of quantum physics. The effects of

MF on organisms can also be regarded as paradoxical phenomena [Binhi 2002], as they do not change linearly with increasing induction. This trend may be due, among other factors, to the window effect. The effect of static MF on bacteria is determined not only by the magnitude of magnetic induction and exposure time, but by the influence of many biotic and abiotic factors. Kohno *et al.* [2000] showed that the presence or absence of oxygen can influence the effect of weak MF on bacteria. The available data on the effects of MF on bacteria are often inconclusive. We can assume that the problems hindering the study of the response of bacteria to applied MF may result from fluctuations in the geomagnetic field (the so-called magnetic background) during long-term experiments or different responses of bacteria during different growth phases [Khokhlova, Veinshtein 2017]. They may also be caused, for example, by the presence (in *Rhodospirillum rubrum* VKM B-1621) of intracellular magnetosensitive inclusions containing cobalt or chromium [Ariskina *et al.* 2004].

The effect of MF with different magnetic induction values (45, 450, 1200, 1800 or 3500 mT) on the survivability of *E. coli* 10032 has been investigated by Ji *et al.* [2009]. They found that this was highest at 450 mT. Moreover, the bacterial counts decreased at higher temperatures and longer exposure times. The effect of a static MF on *E. coli* was also studied by Haghi *et al.* [2012], who showed that an MF of 1.6 mT increased the duration of the logarithmic phase at 4-h exposure and decreased it at 16 and 18 h of exposure. Al-Barzenji *et al.* [2010] found that an MF greater than 15 mT had an inhibitory effect on the growth rate of *Streptococcus mitis* and *S. salivarius*, while at a magnetic induction value of up to 5 mT, the field had no clear effect on the growth rate of these bacteria. Bajpai *et al.* [2012] showed an inhibitory effect of a 100 mT static MF on the adhesion and growth of Gram-positive (*Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*) bacteria on a hydroxyapatite surface. Mhamdi *et al.* [2016] investigated the differences in the adhesion of *E. coli* bacteria to a glass surface under a 0.5 T MF. A decrease in adhesion in the MF was observed. Khaled and Abdullah [2015] studied the effect of a weak static MF on the growth of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* bacteria. After 24 h of exposure in an MF (30, 50 or 80 mT), the bacterial proliferation rate was found to depend on the species and on the MF induction value. The greatest decrease in bacterial counts of *E. coli* and *S. aureus* and the greatest increase in *B. subtilis* were observed for higher MF intensities.

Kthiri *et al.* [2019] investigated the antioxidant enzyme activities of *Saccharomyces cerevisiae* after 9 h of exposure in a 250 mT MF and reported increased superoxide dismutase and catalase activity and a decrease in glutathione peroxidase activity in the cultures tested, suggesting oxidative stress.

Genotoxicity studies have shown that exposure to strong MF can disrupt cellular defense mechanisms. Cells may become less resistant to other potentially harmful agents. When *E. coli* was treated simultaneously with a 2 or 5 T MF and one of ten chemical mutagens (e.g. ethyl ethanesulfonate), the mutation rate increased well above the level seen when using the mutagen on its own [Ikehata *et al.* 1999].

No mutagenic effect was observed (using the Ames test) for the *E. coli* strains tested when the MF exposure took place in a 1.5 T or 7.2 T field. Furthermore, no synergistic effect of exposure to the MF in combination with known genotoxic chemicals was observed [Teichmann *et al.* 2000]. In our studies (Rutkowska-Narożniak [1997] and Łebkowska *et al.* [2011; 2013]), we found that a MF of 7 mT has a positive effect on the biodegradation of refractory organic compounds and an increase in the abundance and biomass of microorganisms was observed. The exposure to the aforementioned MF reduced the adaptation time of activated sludge microorganisms to the degradation of refractory compounds in the MF and increased the growth rate of the yeast *Candida boidinii* during the adaptation phase and exponential phases [Rutkowska-Narożniak 1997]. Peña-Guzmán *et al.* [2019] also demonstrated stimulation of the growth of activated sludge microorganisms exposed to an MF. This group found that following 60-min exposure in a 10 mT MF and 30-min exposure at 20 mT, there was an increase in bacterial proliferation of nearly 68%, while for fungi at all exposure times (30, 60 or 120 min), the greatest increase (50%) was observed in a 5 mT MF.

Supporting the treatment of industrial wastewater containing refractory organic compounds by means of weak MF may be an alternative technology for hard-to-treat industrial wastewater. A static MF can interact with microorganisms and may be used to assist in the removal of refractory organic pollutants from wastewater by biological means.

Parachlorophenol (p-chP) is toxic, mutagenic and dangerous for both humans and animals. It has uses, among others, in the chemical and pharmaceutical industries. It is easily absorbed through the respiratory tract, skin and digestive tract. This compound's main toxic effect is on the nervous system. It can enter the human body by ingesting chlorinated water and by inhalation [Dutkiewicz 2008]. Sithole and Williams [1986] studied 40 Canadian water treatment plants and showed the presence of p-chP formed during water chlorination. The presence of this compound was found in 1 to 12 stations depending on the study cycle. It was also proved that p-chP is toxic to aquatic organisms.

Phenol (carbolic acid) is highly toxic, corrosive, mutagenic and teratogenic. It has a destructive effect on mucous membranes, the respiratory tract and skin. It is commonly used as an antiseptic and disinfectant. It is also used in the manufacture of cosmetics, drugs (e.g., aspirin), herbicides, synthetic resins, detergents and dyes. It can be found in the air in areas with heavy road traffic. The content of this compound in wastewater from the chemical and pharmaceutical industries is in the range of 20–70 mg·dm^{-3}, from coke production 800–1600 mg·dm^{-3}, and in urban wastewater mixed with industrial wastewater, it can reach 5–25 mg·dm^{-3} [Rutkowska-Narożniak 1997]. The permissible concentration of volatile phenols for treated industrial wastewater discharged to receiving waters is 0.1 mg·dm^{-3} [Sithole, Williams 1986]. Phenol removal from wastewater using traditional physicochemical methods (UV radiation, hydrogen peroxide or ozonation) is complicated and expensive. Biological treatment may provide an alternative [Teichmann *et al.* 2000; Pajor 2001].

Studying the effect of MF on the adaptation of microorganisms to growing in the presence of phenol or p-chlorophenol may assist in the development of alternative methods for the treatment of hardly degradable industrial wastewater. It is anticipated that the results obtained in this study will allow us to determine whether the application of an MF of 7 mT affects the proliferation of *S. cerevisiae* yeast in the presence of phenol or p-chlorophenol.

MATERIALS AND METHODS

EXPERIMENTAL SET-UP

A magnetostatic device producing a static MF of 7 mT was used for the tests. The magnetic circuit consisted of two blocks of magnets installed on the outer side of the poles, connected by MF expanders (Photo 1). With this setup, a constant, homogeneous MF was obtained, which was calibrated using a microteslometer for the experiment at 7 mT. Field homogeneity measurements were carried out on seven horizontal sections in the exposure zone. According to the histograms made in the Statistix 3.5 programme, the device provides a homogeneous field in the exposure zone between the poles, with a maximum deviation of ±10% from the mean value. The magnetic field direction is marked in Photo 1. The maximum field uniformity deviation is 2°.

Photo 1. A magnetostatic device generating a static magnetic field (photo: *A. Rutkowska-Narożniak* and *E. Pajor*)

MICROBIOLOGICAL ASSAYS

The yeast strain *Saccharomyces cerevisiae* used in this study was obtained from the collection of microorganisms of the Department of Biology of the Faculty of Building Services, Hydro and Environmental Engineering of the Warsaw University of Technology (Pol. Wydział Instalacji Budowlanych, Hydrotechniki i Inżynierii Środowiska Politechniki Warszawskiej).

Yeast cells were cultured in a YPG liquid medium containing peptone, glucose, and yeast extract [Grabińska-Łoniewska (ed.) 1999], and yeast abundance was determined using the Koch's pour-plating on a YPG agar medium.

PREPARATION OF THE CULTURE

A stock culture of *S. cerevisiae* was prepared in a YPG liquid medium as an inoculation for the tested yeast cultures. The test compounds phenol and p-chlorophenol were added at concentrations of 100 mg·dm^{-3}. Cultures were tested in six variants as follows:
- control culture on YPG medium (C),
- culture on YPG medium with phenol (P),
- culture on YPG medium with p-chlorophenol (p-chP),
- culture on YPG medium exposed to a static MF,
- culture on YPG medium exposed to a static MF with the addition of phenol (MF P),
- culture on YPG medium exposed to a static MF with the addition of p-chlorophenol (MF p-chP).

For each culture, baseline yeast cell counts were determined in duplicate at culture setup (time 0) and after 2, 3, 4, 6, 8, 10, 24, 28 and 48 h.

The culture plates were incubated at 28°C for four days. For each sampling time, the yeast count in 1 cm^3 of culture (in cfu·cm^{-3}) was calculated as the average of two replicates.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the following methods:
- descriptive statistics,
- Levene's test of equality of variances,
- two-way analysis of variances (ANOVA) – to determine the statistically significant differences in number of yeasts in measurements.

Statistical significance level was set at $\alpha = 0.05$. Analysis was performer using programming language and software R.

RESULTS

CHANGES IN THE ABUNDANCE OF *SACCHAROMYCES CEREVISIAE* IN THE PRESENCE OF PHENOL AND EXPOSED TO A STATIC MAGNETIC FIELD OF 7 mT

Studies on yeast growth in the control sample showed that their number increased during the experiment, reaching a value of four orders of magnitude higher than the initial amount and five orders of magnitude higher after 24 and 48 h, respectively. In the MF, these correlations were similar but higher than in the control sample, especially after 24 h of cultivation. The addition of phenol to the medium increased the number of microorganisms by five orders of magnitude after 24 h compared to the initial amount. In the MF, in the sample with the addition of P, intensive yeast growth was observed, almost twice as high as in the MF without phenol (Tab. 1, Fig. 1).

Table 1. Changes in the number of yeast *Saccharomyces cerevisiae* in the tested cultures during 24–48 h

Time (h)	Number of yeasts in culture (cfu·cm^{-3})					
	culture not exposed to MF			culture exposed to MF 7 mT		
	control	phenol	p-chloro-phenol	MF	phenol	p-chloro-phenol
0	4.2E+2	5.2E+2	5.5E+2	4.2E+2	5.2E+2	5.4E+2
2	4.8E+2	5.7E+2	4.9E+2	4.8E+2	5.3E+2	5.7E+2
4	1.2E+3	9.2E+2	8.0E+2	1.2E+2	1.3E+3	1.0E+3
6	3.4E+3	3.8E+3	3.7E+3	3.8E+3	3.9E+3	3.9E+3
8	5.4E+3	5.9E+3	5.8E+3	5.5E+3	9.0E+3	6.1E+3
10	1.8E+4	1.2E+4	1.5E+4	1.8E+4	2.4E+4	2.0E+4
24	3.6E+6	1.2E+7	4.6E+6	1.0E+7	2.0E+7	1.2E+7
28	2.3E+7	4.7E+7	2.9E+7	4.8E+7	7.5E+7	4.6E+7
48	4.5E+7	4.8E+7	8.0E+7	4.8E+7	8.6E+7	1.0E+8

Source: own study.

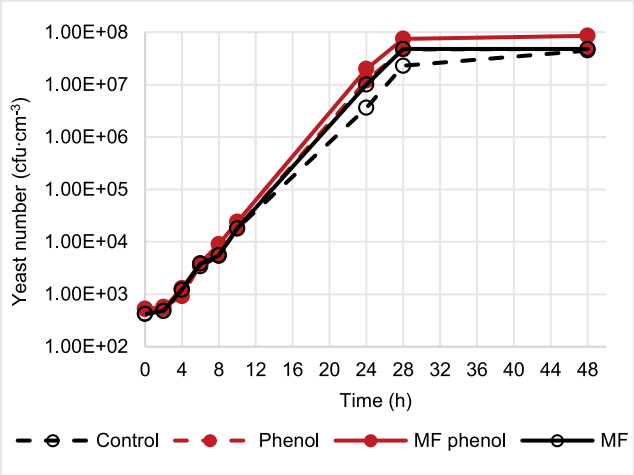

Fig. 1. Changes in the number of yeast *Saccharomyces cerevisiae* in cultures with phenol during 0–48 h; MF = magnetic field; source: own study

Results shown in the Table 1 were analysed using Levene's test. Equality of variances was confirmed for all data. The results indicated that phenol had no detrimental effect on yeast growth. The MF acted to increase the proliferation of *S. cerevisiae* both in the control sample (2.7-fold increase after 24 h) and in the presence of phenol (5.5-fold increase after 24 h and about 2-fold after 48 h compared to the control sample).

The interaction effect between repeated measurements (time) and the factor (phenol / MF phenol) was statistically significant, F (8, 32) = 76.1, p < 0.001. The obtained result indicates the significant difference in the increase in the number of yeasts in the cultures with phenol exposed to or not exposed to MF over time (Fig. 1). The strength of the observed effect turned out to be moderate, η^2 = 0.06 (Tab. 2).

Table 2. Results of two-way analysis of variances (ANOVA) of repeated measurements – phenol and MF phenol

Factor	*SS*	*df*	*MS*	*F*	*p*	η^2
Time	3.70E+16	8	4.62E+15	1115.7	<0.001	0.911
Time × factor	2.52E+15	8	3.15E+14	76.1	<0.001	0.062
Residual	1.33E+14	32	4.14E+12			

Explanations: MF = magnetic field, *SS* = sum of squares, *df* = degrees of freedom, *MS* = mean sum of squares, *F* = *F* statistics, *p* = significance level, η^2 = eta squared
Source: own study.

CHANGES IN THE ABUNDANCE OF *SACCHAROMYCES CEREVISIAE* IN THE PRESENCE OF P-CHLOROPHENOL AND EXPOSED TO A STATIC MAGNETIC FIELD OF 7 MT

Yeast development in the presence of p-chlorophenol was initially (for 2 h) inhibited, but then an increase in their abundance was observed, similarly to the growth of microorganisms in the control sample. It should be stressed that after 24 h, yeast growth was higher than that observed in the control sample and after 48 h, it reached its maximum of 8.0E+7 (about twice as high as in the control sample).

In the MF, no inhibition of yeast growth was observed in the medium with p-chP. After 24 h, the number of microorganisms increased by five orders of magnitude and after 48 h by six orders of magnitude compared to the initial amount.

The results indicated that the MF stimulated yeast proliferation in the presence of p-chP (Tab. 1, Fig. 2). It should be noted that changes in yeast abundance were similar in both the phenol and p-chlorophenol media.

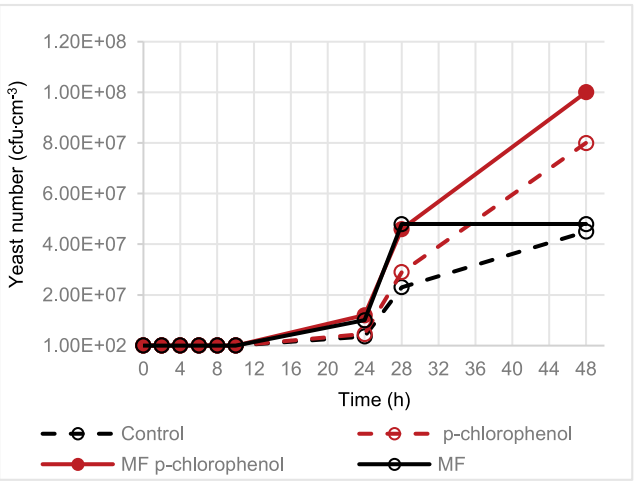

Fig. 2. Changes in the number of yeast *Saccharomyces cerevisiae* in cultures with p-chlorophenol during 0–48 h; source: own study

Effect of interaction between repeated measurements (time) and tested factors (Fig. 2) was statistically significant for:
- (p-chlorophenol / MF p-chlorophenol) F (8, 32) = 6.69, p <0.001; the strength of the observed effect turned out to be small, η^2 = 0.02 (Tab. 3);
- (control and p-chlorophenol), F (8, 32) = 12.8, p < 0.001; the strength of the observed effect turned out to be moderate, η^2 = 0.07 (Tab. 4);

Table 3. Results of two-way analysis of variances (ANOVA) of repeated measurements – p-chlorophenol and MF p-chlorophenol

Factor	*SS*	*df*	*MS*	*F*	*p*	η^2
Time	4.62E+16	8	5.78E+15	343.24	<0.001	0.962
Time × factor	9.00E+14	8	1.13E+14	6.69	<0.001	0.019
Residual	5.39E+14	32	1.68E+13			

Explanations: as in Tab. 2.
Source: own study.

Table 4. Results of two-way analysis of variances (ANOVA) of repeated measurements – control and p-chlorophenol

Factor	*SS*	*df*	*MS*	*F*	*p*	η^2
Time	2.19E+16	8	2.74E+15	177.9	< 0.001	0.901
Time × factor	1.58E+15	8	1.98E+14	12.8	< 0.001	0.065
Residual	4.93E+14	32	1.54E+13			

Explanations: as in Tab. 2.
Source: own study.

– (control and MF p-chlorophenol), $F(8, 32) = 157$, $p < 0.001$; the strength of the observed effect turned out to be moderate, $\eta^2 = 0.12$ (Tab. 5).

Table 5. Results of two-way analysis of variances (ANOVA) of repeated measurements – control and MF p-chlorophenol

Factor	*SS*	*df*	*MS*	*F*	*p*	η^2
Time	3.12E+16	8	3.90E+15	1089	< 0.001	0.840
Time × factor	4.49E+15	8	5.62E+14	157	< 0.001	0.121
Residual	1.14E+14	32	3.58E+12			

Explanations: as in Table 2.
Source: own study.

DISCUSSION

The obtained results indicate that the growth of *Saccharomyces cerevisiae* is stimulated by a magnetic field (MF) of 7 mT. The growth rate of yeast in the MF from the 4th hour of the study was higher than in cultures without the field. It was also observed that the presence of either phenol or p-chlorophenol at a concentration of 100 mg·dm^{-3} in the medium had a stimulating effect on yeast growth in the culture. These compounds were used as an additional carbon source. The slightly lower microbial abundance in cultures with p-chlorophenol compared to the cultures with phenol and controls may be due to the presence of chlorine as an additional substituent at the aromatic ring, which increases the toxicity of the compound. The greatest increase in *S. cerevisiae* abundance was observed in the culture with phenol that was exposed to an MF – from 4 to 28 h of cultivation. After 48 h of incubation, there was a decrease in yeast abundance in this culture, probably due to the depletion of food substrates and the accumulation of metabolic products. After 48 h of incubation, the highest abundance of the studied microorganisms was observed in the culture exposed to an MF with p-chlorophenol, and the lowest in the control culture (without phenol and p-chlorophenol and not exposed to an MF) – the difference was about 120%. For all cultures exposed to the field, it was shown that the growth rate of yeast in an MF was higher than that of their analogues cultured without an MF. Rutkowska-Narożniak [1997] also showed the effect of a 7-mT MF on increasing the growth rate of *Candida boidinii* yeast in the adaptation and logarithmic growth phases. Łebkowska *et al*. [2011; 2013] showed that a 7-mT MF has a positive effect on the biodegradation of refractory organic compounds and on the increase in the abundance and biomass of activated sludge microorganisms.

The results of studies on the effect of MF on microorganisms often contain incomparable data. The discrepancies in the research results obtained by different authors may be related to the differences in the MF induction values used in the experiments and large differences in the sensitivity of the tested species or strains of microorganisms. The effects of MF on organisms are known to vary non-linearly. Most studies show that a strong MF (>1 T) inhibits and a weak MF (of the order of a few militeslas) intensifies the physiological processes of organisms [Guevorkian, Valles 2006; Miyakoshi 2006; Zhang *et al*. 2007]. Some reports indicate that MF with an intensity of several hundred militeslas can also intensify biological processes. Křiklavová *et al*. [2014] showed that a 370-mT MF stimulates phenol oxidation by *Rhodococcus erythropolis* (by about 34%) and its growth (by about 28%), as well as shortening the adaptation and exponential phases and increasing bacterial respiration activity by about 10%. Similar results were obtained by Kthiri *et al*. [2019], who observed an increase in the viability of the yeast *Saccharomyces cerevisiae* under the influence of a 250-mT MF between 6 and 9 h, although a decrease in their abundance was shown up to 6 h. The effect of the MF depends on the experimental parameters, e.g., induction value, exposure time and the chemical composition of the surroundings [Guevorkian, Valles 2006; Miyakoshi 2006; Zhang *et al*. 2007].

Static MF have an effect on organisms, but the mechanism is not fully understood. It is accepted that an MF causes electrodynamic changes related to the Hall effect and Lorentz forces, influencing the magnetic spins of free radicals and other paramagnetic substances, along with the properties of liquid crystals present in cell membranes and hence transport functions. The movement of organisms in relation to magnetic force lines can trigger the induction of currents inside cells. Sadowski *et al*. [2007] confirmed the hypothesis of the influence of MF, among others, on cell transport functions. The cited authors showed that weak MF (2 mT) increases the permeability of fish eggshells (Atlantic salmon (*Salmo salar*), sea trout (*Salmo trutta*) and rainbow trout (*Oncorchynchus mykiss*)). Brysiewicz *et al*. [2017] and Brysiewicz and Formicki [2019] studied the effect of static magnetic field on melanophores in the sea trout (*Salmo trutta*), in the European whitefish (*Coregonus lavaretus*), and vendace (*Coregonus albula*). It was found that under low MF (1–5 mT) the number of melanophores was smaller, and the movement of melanin within the melanophores was visible as aggregation of pigment in the cells. Most probably, magnetite (Fe_3O_4) compounds, detected in many fish species, mainly migratory fishes such as trout, are responsible for such behaviour of melanophores.

The studies carried out in this paper do not offer the explanation for the increase in proliferation of the yeasts studied in the presence of P and p-chP, but show that the application of 7-mT MF has a beneficial effect on this process.

To better explain the mechanisms of MF effects on microorganisms, it would be worthwhile to apply molecular biology methods in future research.

CONCLUSIONS

This study has shown that a 7-mT static magnetic field (MF) stimulates the proliferation of *Sacharomyces cerevisiae* in cultures with the addition of phenol or p-chlorophenol. The experimental results indicate that weak MF of 7 mT can be used to increase the efficiency of phenol and p-chlorophenol removal from industrial wastewater.

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Rozporządzenie Ministra Gospodarki Morskiej i Żeglugi Śródlądowej z dnia 12 lipca 2019 r. w sprawie substancji szczególnie szkodliwych dla środowiska wodnego oraz warunków, jakie należy spełnić przy wprowadzaniu do wód lub do ziemi ścieków, a także przy odprowadzaniu wód opadowych lub roztopowych do wód lub do urządzeń wodnych [Ordinance of the Minister of Maritime Affairs and Inland Navigation of July 12, 2019 on substances particularly harmful to the aquatic environment and conditions to be met when discharging wastewater into waters or into the ground, as well as when discharging rainwater or

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