



Research articles

The effect of rotating magnetic field on bioethanol production by yeast strain modified by ferrimagnetic nanoparticles

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ABSTRACT

Bioethanol is a promising liquid biofuel, which can be produced from the wide range of biomass feedstock by the fermentation process with the *Saccharomyces cerevisiae* yeast. The application of the rotating magnetic field (RMF) is one of the possibilities to increase the efficiency of the process. Therefore, the magnetically-assisted bioreactor equipped with RMF generator was used to perform the ethanol fermentation process with the sugar-rich medium. Moreover, the yeast cells were modified by addition of Fe₃O₄ nanoparticles. The obtained data suggested that the stimulation of applied cells with RMF did increase the proliferation and the ethanol production process. Furthermore, the calculated maximum specific growth rate and the productivity coefficient showed RMF positive effect on this magnetically-assisted bioprocess. The stimulation was found as ruled by field frequency (connected with magnetic induction of RMF) and it was revealed that the process productivity was higher for experiments with modified cells and the growth rate was higher for the process without the addition of Fe₃O₄ nanoparticles.

1. Introduction

The enlarging world population and development of new technologies and processes cause the increasing energy consumption, which leads to finding new sources of fuels [1]. Nowadays, the improvement of renewable energy and biofuels is developed. The biofuel (mostly bioethanol and biodiesel) produced from a biomass can be promising in reaching the still raising energy needs and it may be considered as an alternative to the fossil fuels in a sustainable manner conserving biodiversity [2]. The bioethanol is a liquid biofuel, mainly produced from the wide range of biomass feedstock (e.g. sugar beet, sugarcane, corn, and molasses) [3]. The most preferred for the industrial-scale production of ethanol due to its high yield and productivity, osmotolerance, safety, low difficulty and cost of cultivation are yeast cells of *Saccharomyces cerevisiae* [4]. Moreover, *S. cerevisiae* can withstand a relatively high concentration of ethanol and sugar but also a low pH level culture medium with good viability [5].

The productivity is an important parameter in bioprocessing and many studies are conducted in order to find new methods to improve it, e.g. developing new modified strains, bioreactor construction involved agitation, aeration and control systems and optimization of already known methods [6]. One of the ways to modify the microorganism cell is the immobilization process, which provides the high cell density,

some protection of hard environmental conditions, and reduced risk of contamination and offers improved separation process of a cell from the product [7]. An alternative approach to increase productivity is the application of external electromagnetic field (EMF) or static magnetic field (SMF). Many researchers investigated the application of these types of fields to *S. cerevisiae* cultures. For example, the influence of electric field [8], SMF [9–12] and EMF [13,14] were analyzed. These reports proved that the biomass growth and ethanol content in the culture medium may be positively induced by the applied force field.

The special case of the EMF is a rotating magnetic field (RMF), which is created due to the superposition of EMFs generated by windings situated around the same axis and powered by a 3-phase alternate current. The electromagnetic force action, which is the result of interaction between induce currents and magnetic field, causes the movement of fluid or magnetic susceptible particles around its axis [6]. These particles may be treated as the dynamos, which increase the interfacial mass transfer between cell and fluid through the influence on the cell surface where stagnation zone commonly occurs. Moreover, the magnetic force generated by RMF may cause the movement of particles around the generator axis. Thus, it can be considered a non-invasive stirring device at the microscopic level as an alternative to mechanical impellers [15].

Our previous studies indicated that system assisted with the RMF

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could be used to the intensification of mass transfer [16] and the improvement of mixing process [17]. Furthermore, it was discovered that the RMF exposure can alter the proliferation and metabolic activity of certain bacteria strains [18] and mechanism of prophages induction [19]. Regarding previous studies and the literature survey, the knowledge about the RMF influence on cellular processes, especially on bioethanol production by *S. cerevisiae*, is still insufficient and needs to be improved. Therefore, the main objective of this work was to analyze the effect of RMF on the bioethanol production. For this purpose, the yeast strain ATCC 4098 was employed and in further steps, this strain was modified by synthesized Fe_3O_4 nanoparticles. The bioprocess was performed for sugar-rich medium (33%, w/w) to maximize the amount of produced bioethanol. This magnetically assisted bioprocess was characterized by means of the yeast cells growth rate, metabolic activities, bioethanol concentration, glucose uptake and fermentation process productivity.

2. Materials and methods

2.1. Experimental set-up

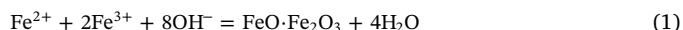
The experimental set-up equipped with the RMF generator is presented in Fig. 1.

The RMF was generated using 3-phase induction motor powered by the alternate current at 100 V and inverter controlling its frequency. A glass container filled with distilled water, the temperature of which was maintained at 28 °C, was placed inside the RMF generator. Inside the glass container, 15 mL plastic probes (CELLSTAR® CELLreactor™ Greiner, Germany) containing yeast suspension were placed and situated around the generator axis. The experiments were performed for continuous exposure up to 72 h for different RMF frequencies in the range of 10–50 Hz ($B_{\text{max}} = 16\text{--}18.5\text{ mT}$). The control probes were placed in the water bath at the same temperature (measured by precise thermometer ST-80, Termoprodukt, Poland) but without the influence of external electromagnetic field.

2.2. Materials

The yeast strain ATCC 4098 was employed to conduct the magnetically assisted fermentation process. The culture medium was created from a mixture of glucose (33%, w/w) and peptone (4.5%, w/w) dissolved in distilled water and then sterilized at 121 °C for 15 min. Composition of the culture medium was obtained after initial optimization performed by experiment design method, which aimed at maximizing the ethanol concentration after 72 h of cultivation.

The ferrimagnetic particles used in this study were synthesized by means of the co-precipitation method. For this purpose, the 0.1 M solutions of Mohr's salt (ammonium iron(II) sulfate) and ferric ammonium sulfate were mixed with distilled water and the 1 M ammonia solution was added in a ratio of 2:4:10:5. The main reaction is given by the following equation [20]



Obtained ferrimagnetic particles were washed few times to clean from the ammonia remains and dried. Those particles were analyzed using X-Ray Diffraction (XRD) method to verify its composition and size (Fig. 2). Detailed measurement procedure was described previously [40].

According to data library, obtained results indicated that the synthesized magnetite did not contain other iron compounds. The mean crystallite size was calculated by means of Scherrer method [21,22] and LaB_6 (SIGMA-ALDRICH, Germany) was used as a standard. The result showed that magnetite particles used in this study had 93 Å, thus their mean size was 9.3 nm.

The modification process was performed by adding 3 v/v% of water magnetite suspension to the yeast culture suspension at the same pH level (pH = 5.8) as the culture. Such formed mixture was vigorously shaken and then the flocculation effect was observed. The modified yeast cells were grown overnight in Sabouraud liquid medium to establish concentration about $3 \cdot 10^8$ CFU/mL (measured by microplate reader ELx800, Biotek, USA). Obtained yeast suspension was photographed under microscope (Fig. 3).

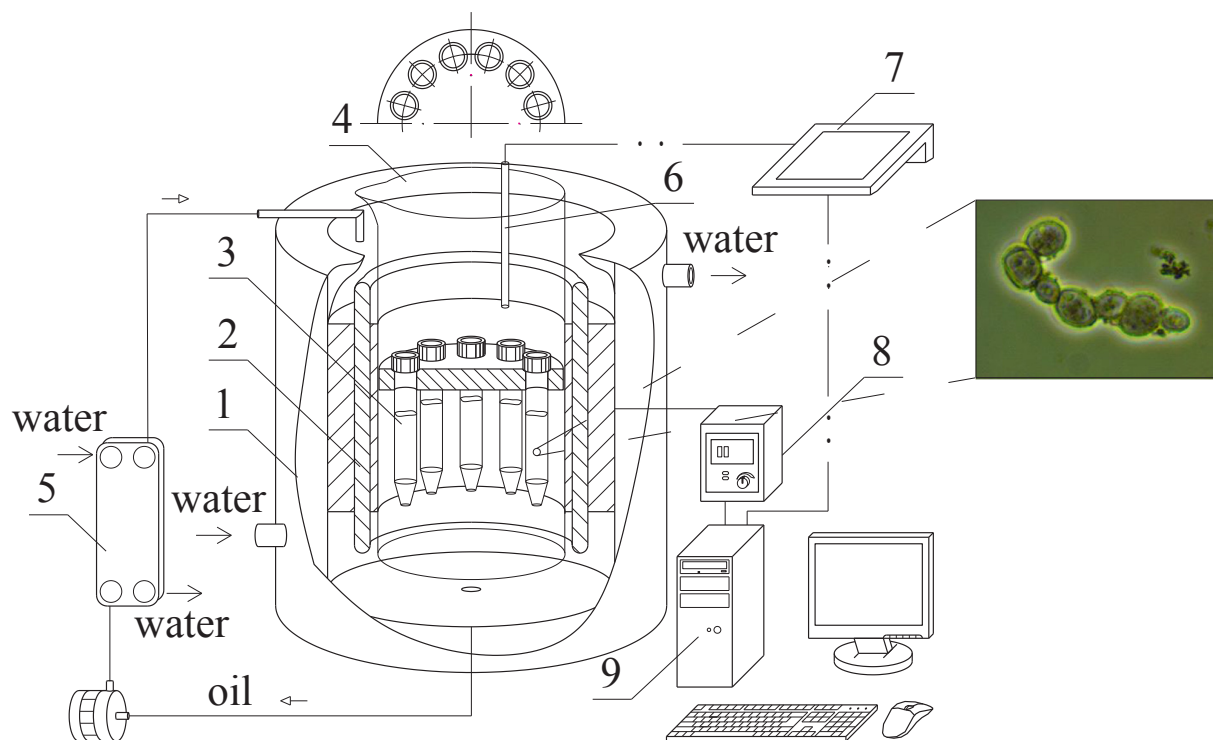


Fig. 1. The sketch of experimental system: 1 – tank, 2 – RMF generator, 3 – sample probes with cultures, 4 – glass container, 5 – plate heat exchanger, 6 – temperature probe, 7 – multifunctional meter, 8 – current inverter, 9 – PC.

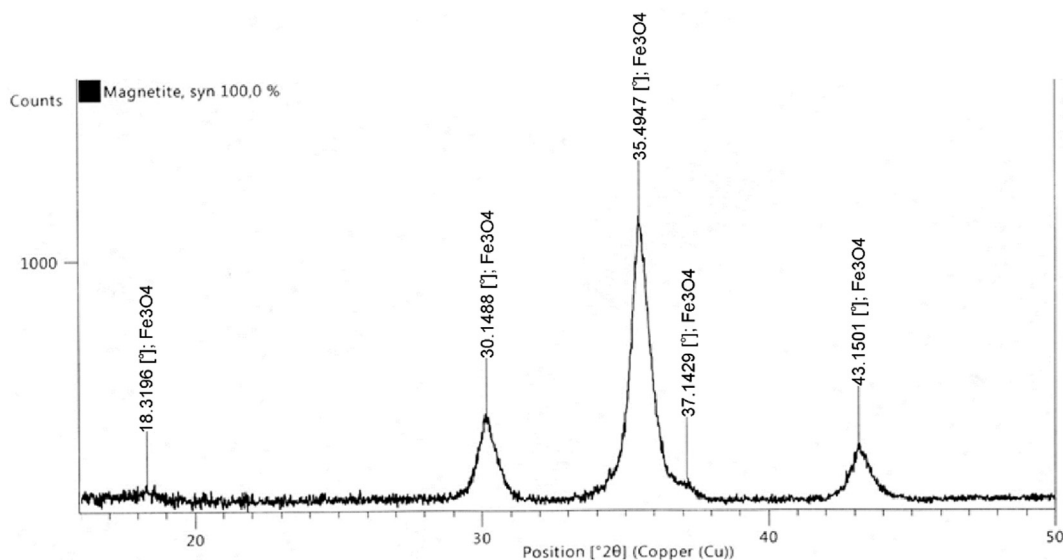


Fig. 2. Diffractogram of synthesized ferrimagnetic particles.



Fig. 3. Bright-field image of yeast cell modified with Fe_3O_4 particles and agglomerate of ferrimagnetic particles.

Fe_3O_4 nanoparticles are much smaller than the yeast cells and stick to the cell wall surface (Fig. 3, dark dots). Unbound nanoparticles might agglomerate (right-middle part of Fig. 3), although many will stick to the newly formed cells during cultivation. The resulting yeast suspension was tested using the permanent magnet. Completed experiment showed that the obtained microbiological material with the magnetic nanoparticles was magnetically sensitive. Thus, the application of magnetic field is also allowed to separate the immobilized cells from the suspension.

The obtained yeast suspension as the inoculum was transferred to 500 mL of sugar-rich medium (1% of inoculum, v/v) and this culture was mixed and split into plastic tubes. Half of the probes were exposed to the RMF for 72 h and the second half remained in the water bath for the same time. Samples were collected every six hours from both places in order to carry out the analysis.

2.3. Culture analysis procedure

The optical density, which is proportional to the biomass concentration [38], was measured by means of the spectrophotometric microplate reader (ELx800, Biotek, USA) for 96-well plate at 600 nm wavelength. The metabolic activity of the cell was calculated using AlamarBlue® procedure [23]. Results were expressed as the ratio

between the activity of exposed material and the control samples using following equation:

$$M_{AB} = \frac{(\varepsilon_{OX})_{\lambda_2} A_{\lambda_1} - (\varepsilon_{OX})_{\lambda_1} A_{\lambda_2}}{(\varepsilon_{OX})_{\lambda_2} A_{\lambda_1}^0 - (\varepsilon_{OX})_{\lambda_1} A_{\lambda_2}^0} \quad (2)$$

where ε_{OX} – molar extinction coefficient of the dye oxidized form, $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$; A – absorbance of the exposed sample; A^0 – absorbance of the control sample; λ_1, λ_2 – wavelengths (1–540 nm, 2 – 600 nm), nm.

After this procedure, the culture samples were centrifuged at 4000 rpm for 20 min (Centrifuge 5415D, Eppendorf, Germany) and the supernatant was collected. The glucose uptake was measured by the enzymatic protocol GOD-PAP using Glucose Assay Kit (Megazyme, USA). Concentration of ethanol was established using gas chromatograph (GC 2014, Shimadzu, Japan) equipped with the capillary column (Zebtron ZB-WAX Plus, Phenomenex, USA) and FID type detector working at 75 °C using 0.99999 pure hydrogen (Air Liquid, Poland) as the gas carrier. The injections were performed by chromatograph auto-sampler (AOC-20i, Shimadzu, Japan).

3. Results

The obtained results showed changes in the yeast growth under the action of rotating magnetic field in comparison with the control culture (see Fig. 4).

The process performed without Fe_3O_4 particles (Fig. 4a) and with modified cells (Fig. 4b) showed a stimulatory effect of the applied field on the biomass growth by 28% and 23% after 72 h, respectively. It was also found that changes in RMF frequency could influence the kinetics of the biomass growth (Fig. 5).

The stimulatory effect of RMF on the process without nanoparticles was maximized after 40–48 h. The application of this type of magnetic field allowed to increase the biomass production by up to 40% with an only small change in the RMF frequency (Fig. 5a). However, the addition of ferromagnetic nanoparticles moved the maximum point between 24 and 30 h of the bioprocess duration. The interaction of these particles and applied RMF had the stimulating effect for the process (up to 48%, but this effect has been decreased at the end even lower than for the first case). Nevertheless, it was revealed that the observed effect was much more sensitive to the RMF frequency changes (Fig. 5b) and the significant differences were observed by various frequencies of RMF.

Based on the obtained results (typical example is given in Fig. 4), the maximum specific growth rate μ , was calculated by means of the

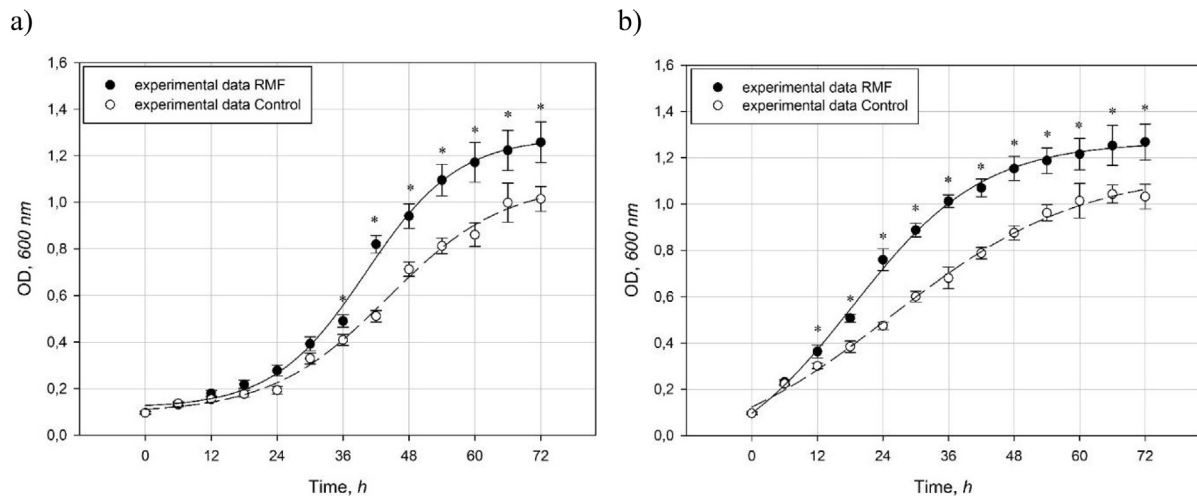


Fig. 4. Typical optical density changes with the process time: a) process without Fe_3O_4 , b) process with Fe_3O_4 for $f = 50$ Hz. Asterisk * means statistical difference for $P < 0.05$.

following equation [6]:

$$\mu = \frac{1}{X} \cdot \frac{dX}{dt} \Rightarrow \frac{dX}{X} = \mu dt \Rightarrow \int_{X_1}^{X_2} \frac{dX}{X} = \mu \int_{t_1}^{t_2} dt \Rightarrow \mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (3)$$

where X – biomass concentration, $\text{g} \cdot \text{dm}^{-3}$; t – time, s.

Values of the maximum specific growth rate were established based on previously obtained growth curves. Considering the log-growth phase and taking into assumption the cells proliferation for this phase as 100% [24], the μ value is the slope of the $\ln X(t)$ line [25].

Additionally, obtained values of the maximum specific growth rate, μ , were presented as the ratio between maximum specific growth rate for exposed culture (μ_{WPM}) and maximum specific growth rate for the control culture (μ_K):

$$\mu_r = \frac{\mu_{WPM}}{\mu_K} \quad (4)$$

The maximum specific growth rate is depended on the culture used, temperature, pH level, concentration of substrates and ionic forces [26]. In the present study, these parameters were equal at the starting point of the bioprocess, hence the only different parameter was the rotating magnetic field influence and its frequency changes.

The calculated μ_r values were correlated with the modified Reynolds numbers, which is proportional to the angular speed of the RMF. This

parameter is defined as follows [27]

$$\text{Re}_m = \omega_{WPM} l^2 \nu_m^{-1} \quad (5)$$

where ω_{WPM} – angular speed of the RMF defined as $2\pi f_{RMF}$, s^{-1} ; ν_m – viscosity, $\text{m}^2 \cdot \text{s}^{-1}$, l – linear dimension (in this case, this parameter was equal to diameter of RMF generator, $l = 0.15$ m), m.

Variations in the maximum specific growth rate in terms of modified Reynolds number for the bioprocess production of ethanol with and without magnetic nanoparticles are presented in Fig. 6.

Obtained results showed that the cells growth rate for exposed cultures increased for the process without nanoparticles and for modified cells up to 15.4% and 12%, respectively. It was observed that differences between these two types of the processes were slight. These differences were about 6% for the lowest frequency and about 3.4% for the highest frequency. However, the modified cell cultures were more sensitive to the RMF frequency and the growth rate increased with higher frequency values. In contrast, cultures without nanoparticles seemed to be less reactive to RMF frequency changes (Fig. 6).

In the case of tested process, the ethanol concentration in the culture medium was studied. Typical examples of the ethanol concentration in the probes were graphically presented in Fig. 7.

As it follows from data presented in Fig. 7, the ethanol production increased after 72 h of fermentation. In comparison to the control probe, this concentration was higher by 33% and 50% for the

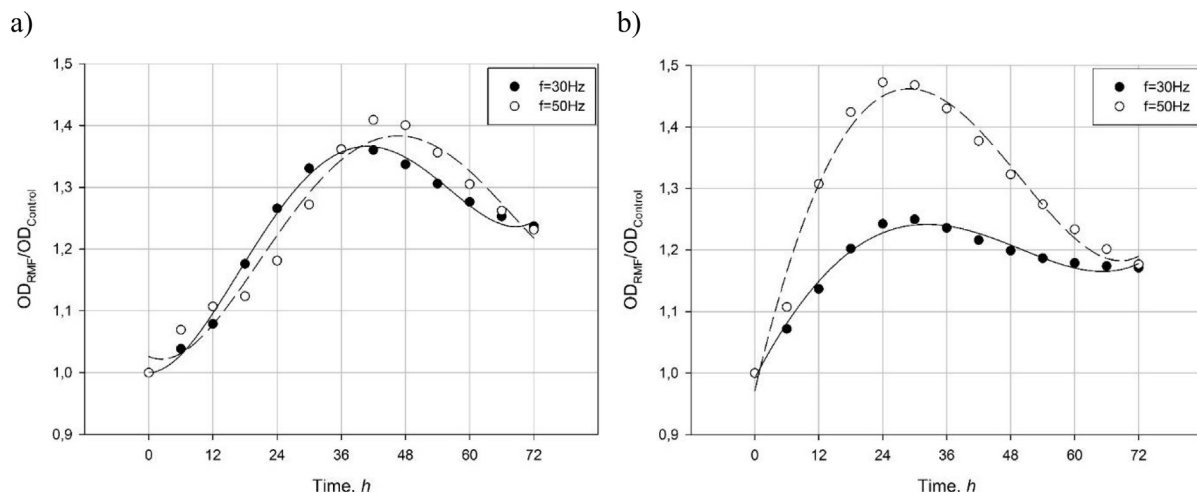


Fig. 5. Typical effect of different RMF frequency on the cell growth: a) process without Fe_3O_4 , b) process with Fe_3O_4 .

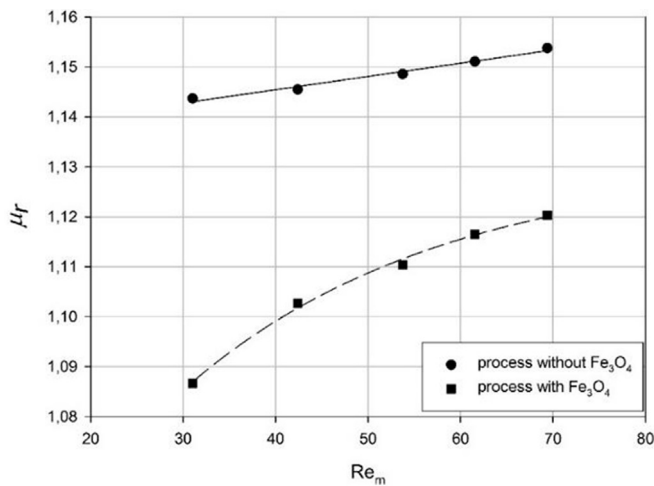


Fig. 6. Correlation between maximum specific growth ratio μ_r and magnetic Reynolds number.

bioprocess realized without nanoparticles and with the modified cells, respectively. The measured glucose concentrations also indicated the increase in sugar uptake from the medium. This suggested that the speed of metabolic conversion of glucose into ethanol was stimulated by RMF exposure in both cases in correlation to the control process.

Obtained results showed the influence of various RMF frequencies, which is illustrated in Fig. 8.

Various RMF frequencies had an effect on the fermentation process corresponding to the growth kinetics. It was observed that the process without nanoparticles was strongly depended on the applied frequency and when its value increased the maximum point, it occurred faster up to 18–24 h for $f = 50$ Hz and 36–42 h for $f = 30$ Hz giving the increased concentration by 210% and 160%, respectively (Fig. 8a). Addition of Fe_3O_4 nanoparticles caused the maximum point occurred faster (at 12 h), and it seemed to be not shifted with the frequency changes like its intensity (up to almost 260%, Fig. 8b). Furthermore, the RMF influence on the metabolic activity of yeast cells was studied (see Fig. 9).

As shown in Fig. 9, the RMF stimulated the metabolic activity for both types of experiments (with and without nanoparticles). At the beginning, the metabolic activity slightly decreased and it might be caused by the stress connected with the presence of magnetic field. It should be noticed that cells need time to adapt to the new environmental conditions. After the end of the process (duration time 72 h), the

increased metabolic activity values were very similar to the increased production of ethanol – values raised by 30% and 40%, respectively.

The productivity coefficient, X_{ps} , is defined as the ratio between the mass of produced ethanol (m_{prod}) and the product used in bioprocess (glucose, m_{subst}). This parameter may be used to characterize the efficiency of the fermentation process and was estimated at the end of the fermentation process (72 h):

$$X_{ps} = \frac{m_{prod}}{m_{subst}} \quad (6)$$

In the case of this experimental results, the ratio between the productivity coefficient for the exposed samples, $X_{ps, RMF}$ and this coefficient for the control process, $X_{ps, C}$, was defined as follows

$$X_{ps,r} = X_{ps, RMF} / X_{ps, C} \quad (7)$$

This relative ratio as the function of the magnetic Reynolds number is graphically presented in Fig. 10.

The obtained results indicated that the relative productivity coefficient increased with higher values of magnetic Reynolds number (proportional to the RMF frequency). For both types of experiment, $X_{ps,r}$ values indicated a stimulatory effect of RMF on the process efficiency, up to 23% and 42%, respectively. The presence of ferrimagnetic nanoparticles improved the productivity of fermentation process up to almost twice in comparison with the process without Fe_3O_4 particles. The immobilized cells fermentation was also more sensitive to the frequency changes in contrast to process without nanoparticles.

4. Discussion

The RMF induces the eddy currents in liquids characterized by relatively high electric conductivity (such as culture medium) [6,17]. These eddy currents can produce heat in the medium and cell cytoplasm [28]. Moreover, part of electromagnetic energy may be absorbed within ferromagnetic particles and locally heat up samples containing living cells, which alters the conditions of cell growth [29,30]. In the presence of non-homogenous electromagnetic field (e.g. RMF), the magnetite particles are attracted to the zone of maximum field intensity. In this case, the magnetic moments of magnetic-susceptible particles rotate to the minimum energy direction, which is parallel to the electromagnetic field lines. Such an interaction can alter the energy level of some molecules thus influence the physical properties of an exposed culture medium [31].

The eddy currents induced by RMF create a mixing effect at the microscale inside the liquid. The micro-mixing effect is produced due to

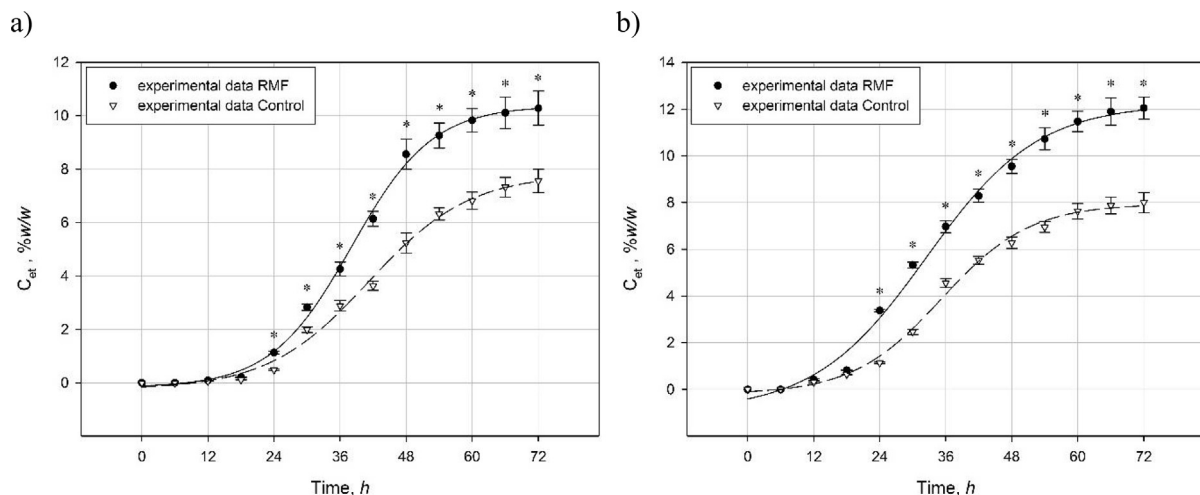


Fig. 7. Typical ethanol concentration changes in time for frequency of RMF equal to 50 Hz for bioprocess without Fe_3O_4 (a) and bioprocess with Fe_3O_4 (b). Asterisk * means statistical difference for $P < 0.05$.

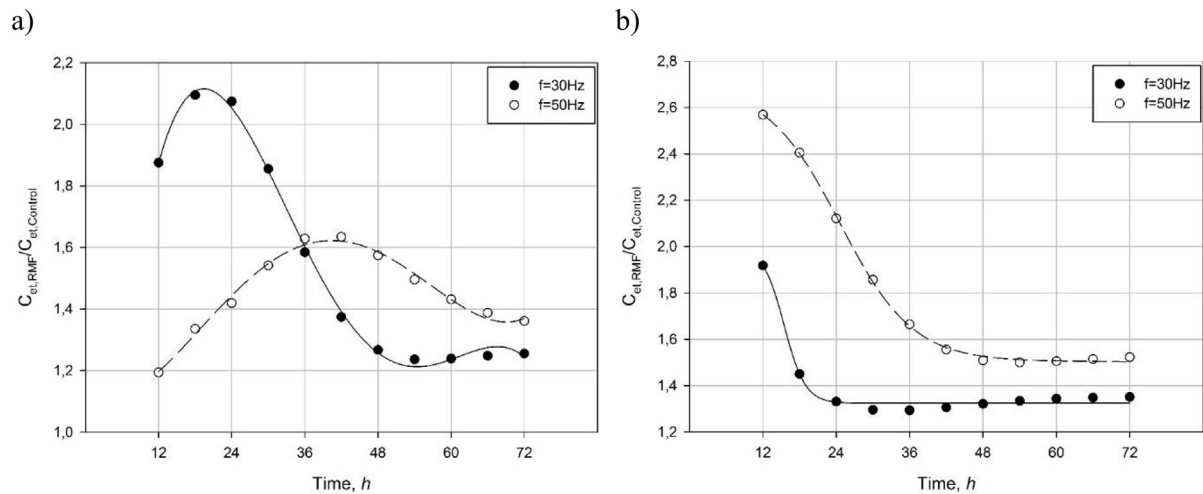


Fig. 8. Typical influence of the RMF different frequencies on the ethanol concentration: a) process without Fe_3O_4 , b) process with Fe_3O_4 .

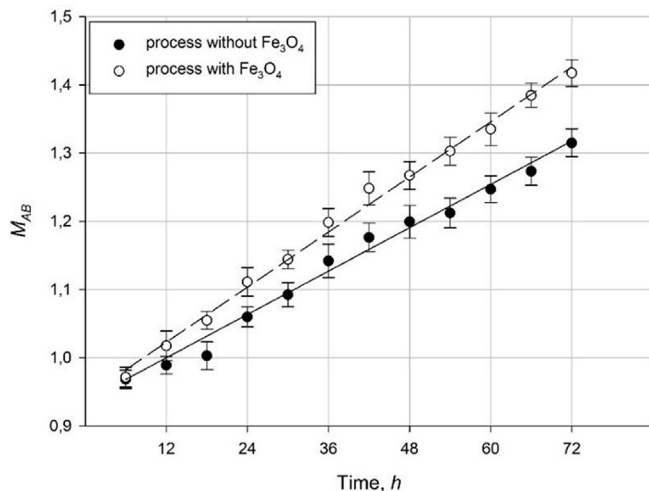


Fig. 9. Typical metabolic activity ratio changes with the process time ($f = 50\text{Hz}$).

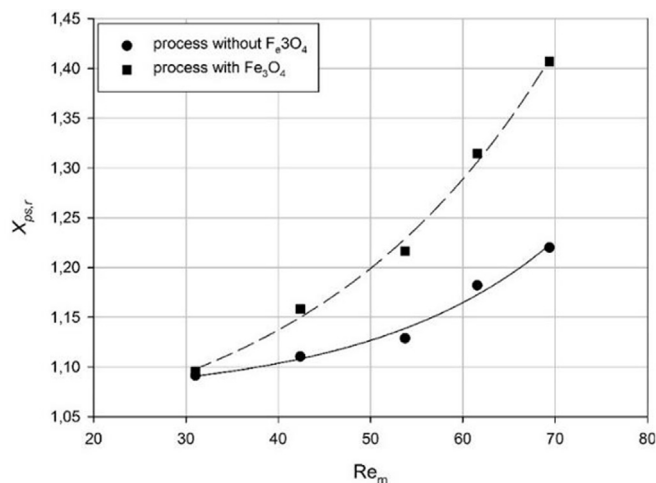


Fig. 10. Correlation between relative productivity coefficient ratio $X_{ps,r}$ as the function of the magnetic Reynolds number.

the fluid particles movement around their own axis [32], but also around the rotation axis of the magnetic field [33] (which is the same as the generator axis, see Fig. 11). This effect can create local changes in

ions position at the cell wall surface [32], which results in the increased concentration gradient. Thus, the RMF can enhance molecular transport and diffusion in the aqueous culture media characterized by relatively high electrical conductivity [34]. Obtained results, especially revealed stimulation of the metabolic activity (Fig. 9) and proliferation of the yeast cells (Fig. 4) suggests that RMF exposure could improve the cell transport, which affects the metabolic processes. It had been proven that micro-mixing effect intensifies the mass and heat transfer processes [35] like mechanic mixing; however, without creating high shearing stress values that can be very harmful to the yeast cells [36].

Moreover, the addition of ferromagnetic nanoparticles to the system with rotating magnetic field causes drop of the mixing time [37], which is one of the critical parameters for mixing efficiency. The mixing effect is strongly correlated with magnetic Reynolds number, which increases with the raise of the Reynolds number value [17]. This effect could be observed in Figs. 5 and 9, where changes in rotating magnetic field frequency strongly affect the process conducted with the addition of Fe_3O_4 nanoparticles. The stimulatory effect was highest for maximum frequency ($f = 50\text{Hz}$) and it has been found that the amount of produced ethanol and productivity of the process increased by 50% and 40% compared to the control process, respectively.

Previous research revealed that the RMF exposition could affect the gas-liquid mass transfer [16]. The paramagnetic particles of oxygen could be drawn in the direction of magnetic field gradient enhancing the mass transfer of oxygen e.g. to the cells. In case of the fermentation, increasing concentrations of products (CO_2 and ethanol) may act as inhibitors to yeast cells proliferation and limit the whole process [38]. The RMF could repel the diamagnetic particles of CO_2 along the field lines [32], thus decrease the local concentration of carbon dioxide near yeast cell and weakening the inhibitory effect. Moreover, the produced ethanol causes changes in the cell membrane permeability and deactivates enzymes inside cytosol, thus hindering the growth and metabolism of living cells. The application of magnetic field can improve the ethanol tolerance of the yeast cells [39]. Increased ethanol tolerance can allow obtaining a higher final concentration of biomass and ethanol during fermentation process [10], which could be observed in the presented study (see Fig. 7). The exposed cultures produced higher concentration of alcohol, up to 33% and 50% in comparison with the control process, which only confirmed this thesis.

5. Conclusion

Results presented in this study revealed that the RMF exposure can be successfully applied to the bioethanol fermentation process by *S. cerevisiae* yeast. It was observed that the presence of this field stimulated

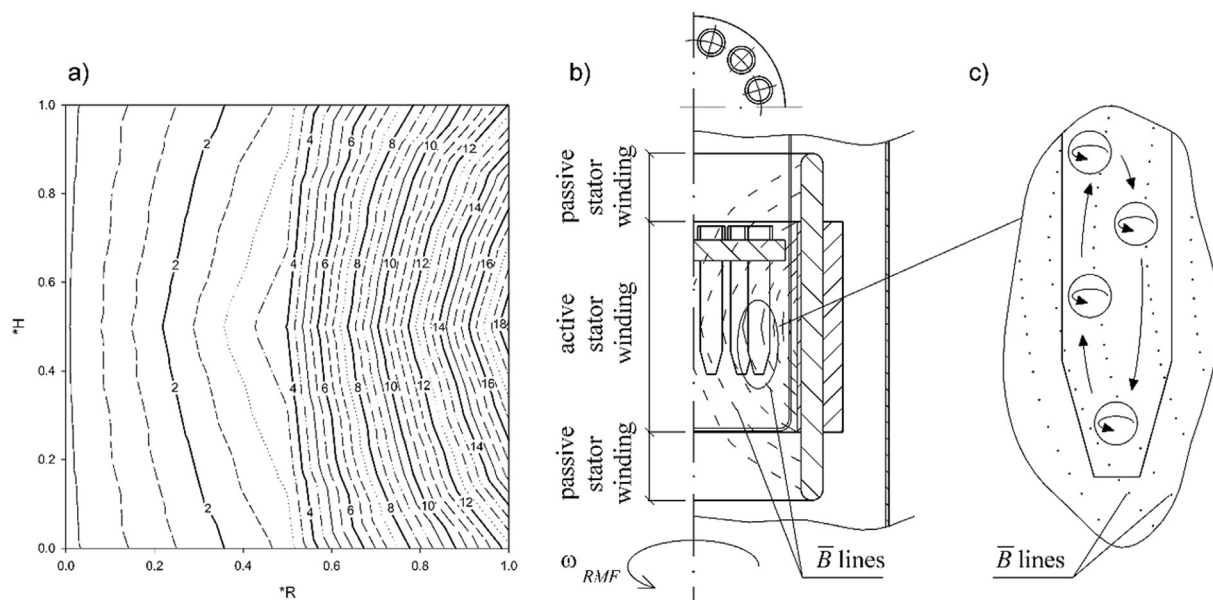


Fig. 11. Characteristics of rotating magnetic field applied: a) typical spatial distribution of magnetic induction ($f = 50$ Hz), b) schematic of probe situation inside the electromagnetic field, c) schematic of particles movement inside probe under the action of RMF exposure.

the cell growth and metabolic activity, increased amount of produced ethanol and improved the productivity of the whole process. The addition of Fe_3O_4 nanoparticles allowed to increase this positive effect by improving the micro-mixing effect of the RMF, which could be controlled by changes in the field frequency, but also gives an opportunity to use magnetic separation method to collect the biomass from medium and reuse it at the beginning of the new process. However, despite the obtained results, this study should be considered a preliminary one. The magnetically-assisted method of bioethanol production poses big promises, but it should be tested on a larger scale to meet the biofuel needs, thus the construction of bioreactor and its process parameters such as magnetic induction, frequency and exposure time should be analyzed and compared with the preliminary results.

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