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NADH oxidation in a microreactor catalysed by ADH immobilised on γ-Fe_2O_3 nanoparticles

Abstract: A new concept of nicotinamide adenine dinucleotide hydrate (NADH) oxidation which combines advantages of the microreactor technology with the advantages of magnetic nanoparticles (MNPs) application is developed. Acetaldehyde was used as a substrate for the NADH regeneration process while the reaction was performed in a batch reactor and in a microreactor using alcohol dehydrogenase (ADH)-loaded MNPs. Three different microreactor systems with MNPs were studied, two with stationary MNPs trapped on the inner surface of microchannel by permanent magnetic field and one where the MNPs actively moved across the channel (movement inside microchannel allowed by an oscillating magnetic field). In a reactor system with an oscillating magnetic field and an actively moving ADH-loaded MNPs 100% NADH conversion was achieved for residence time of just 2 min.

Keywords: alcohol dehydrogenase; coenzyme regeneration; enzyme immobilisation; microreactor; maghemite nanoparticle.

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1 Introduction

Immobilised enzymes are drawing significant attention for potential commercial applications as biocatalysts by reducing operational expenses and by increasing process utilisation of the enzymes. Typically, immobilised enzymes have greater thermal and operational stability at various pH values and ionic strengths. They are more resistant to denaturation than the soluble native form of the enzyme. Much effort has been devoted to the development of various carrier-bound immobilised enzymes, aiming to facilitate their use in continuous processes by overcoming the cost constraints of separation and recycling [1]. In recent years magnetic nanoparticles (MNPs) based on iron oxides, have attracted much interest thanks to their multifunctional properties, such as biocompatibility, superparamagnetism, small size and low toxicity [2]. Due to their high specific surface area and easy separation from the reaction medium by the use of a magnet, they have been employed in enzymatic catalysis applications [3, 4]. The most commonly employed magnetic support is magnetite (Fe_3O_4), then maghemite (γ-Fe_2O_3) and hematite (α-Fe_2O_3). Some other ferrites were also studied as support for immobilsation of proteins [5]. Comparing the maghemite and magnetite, some researchers like Kang et al. [6] stressed that those MNPs have a greater binding specificity than magnetite nanoparticles. Besides already mentioned MNPs advantages, another advantage is that they can be easily manipulated in a microsystem. From the early concepts in the late 1980s microreactor systems have not been given significant attention at universities, but also by industry. They offer many advantages for the performance of heat- and mass-transfer-limited reactions where the large gradients in concentration, pressure, density and temperature are achieved by shrinking the characteristic dimensions of a microreactor down to the micro scale [7]. Additionally, they are especially interesting for the optimisation of yield and selectivity of reactions since they provide the possibility of changing reaction conditions very easily compared to conventional reactors [8]. Some other great advantages are short residence time, better process control and new production concepts, smaller amount of reagents, catalyst and waste products compared to macro-scale, laminar flow, effective mixing [9]. All the advantages mentioned makes them a desirable tool for biotransformation reactions.

There have been many efforts to transform conventional biological work into a lab-on-a-chip combining microfluidics with nanotechnology [10]. Magnetic microparticles coated with biomolecules are often used as solid supports for biochemical reactions utilising high surface to volume ratios and other microsystem advantages [9]. In the microsystems, particles can be trapped without

physical barriers by simply stopping them in flow with an external magnet. They can be released on demand by removing the magnetic field [11]. Up to now, MNPs and magnetic force in combination with microfluidics have been used mostly to detect immunological reactions, DNA hibridisation and to separate blood cells [10], in proteomics etc. but rarely in biotransformations.

In one of our previous works [12–14] hexanol oxidation was performed in the microreactor using alcohol dehydrogenase as a biocatalyst. Obtained results ($X_{hexanol}$= 80%, τ=72 s, [13]) indicated that microreactors could be a good alternative to classical production processes (fermentation, extraction from plants and enzyme-catalysed reactions) [15–17]. Although the results were promising there are still some drawbacks in the developed system. One of the biggest was usage of the coenzyme NAD^+ that is essential for ADH functionality. High prices, the fact that it should be added in a stoichiometric amount and may not be replaced by more economical synthetic products are just some of the reasons why efficient coenzyme regeneration system is essential [18]. In order to solve this problem coenzyme regeneration with the same enzyme as in the main reaction (hexanol oxidation) was proposed using acetaldehyde as a substrate. Acetaldehyde was used because of its low price and the high specificity of ADH towards it [19–21]. Suspended and immobilised enzyme [22, 23] and suspended and immobilised permeabilised baker's yeast cells (as the source of ADH enzyme) were studied [24]. The best results were obtained by using suspended enzyme where 100% conversion of NADH was achieved for a resident time of just τ=0.8 s. In comparison, conversions that were obtained with immobilised enzyme were much lower (X=11.99%, τ=3.6 s). In the same time it was found that an immobilised system could be efficiently reused for several experiments. In our previous work immobilisation of ADH enzyme was performed directly on the microchannel wall according to the procedure described by Stojkovič and Žnidaršič-Plazl [25]. The immobilisation efficiency of just 12.52% was achieved. In order to increase immobilisation efficiency immobilisation of ADH was performed on magnetic nanoparticles and ADH-loaded magnetic nanoparticles were used for NAD^+ oxidation in a batch and in microreactor experiments.

The immobilisation of alcohol dehydrogenase on various supports has been investigated by different researches [26–28]. However, its complete immobilisation on MNPs was reported. The binding of ADH to the surface of superparamagnetic nanoparticles (Fe_3O_4) up to now has been performed by Liao and Chen [29] via carbodiimide activation where, compared to the free enzyme, the immobilised ADH retained 62% activity. In the first immobilisation of ADH on MNPs performed by Shinkai et al. [30] a residual activity of only 12% has been obtained. Recently, Goldberg et al. [31] employed ADH from *Rhodococcus ruber* (DSM 44541 overexpressed in *Escherichia coli*) for the immobilisation on porous glass beads, nanodiamonds and magnetic nanoparticles via glutaraldehyde cross-coupling. The immobilisation on the magnetic carriers had about 89% of yield, and obtained residual activity of about 49%. Li et al. [32] performed covalent immobilisation of ADH to Fe_3O_4 MNPs via glutaraldehyde coupling reaction and in their research immobilised ADH retained 48.77% of its initial activity. Although researchers claim that maghemite nanoparticles have a greater binding specificity than magnetite MNPs, to the best of our knowledge ADH immobilisation on maghemite nanoparticles was not studied.

In this work magnetic nanospheres of maghemite (γ-Fe_2O_3) were synthetised. Enzyme regeneration reaction was performed in a batch reactor and in a microreactor using ADH-loaded γ-Fe_2O_3 beads. Dynamic stability of immobilised nanoparticles and the possibility to reuse them was also investigated. The model prediction results, which could be used for further process optimisation, were verified on a set of independent experiments performed in a microreactor.

2 Materials and methods

2.1 Materials

2.1.1 Chemicals

Acetaldehyde was purchased from Fluka A.G. (Buchs, Switzerland). Ethanol, HCl, HCOOH, glycin and $Na_2P_2O_7{\cdot}10H_2O$ were purchased from Kemika (Zagreb, Croatia). NADH was purchased from Jülich Fine Chemicals (Jülich, Germany) and glutaraldehyde, $FeCl_3{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$, (3-aminopropyl)trietoxysilane (APTES) were from Sigma (St. Louis, MO, USA). Citric acid and glycerol were purchased from Gram-Mol (Zagreb, Croatia). Sodium silicate was purchased from Fisher Chemicals (Loughborough, UK) while ammonia solution (25% (w/w)) was from Carlo Erba (Milano, Italy). Methanol was purchased from J.T. Baker (Deventer, The Netherlands). Alcohol dehydrogenase from baker's yeast (1.1.1.1) with activity of *S.A.*=451 U/mg (where 1 U is the amount of enzyme that catalyses the conversion of 1 µmol of substrate per minute under standard conditions (pH=7, T=25°C)), was purchased from Sigma (Schnelldorf, Germany).

2.2 Methods

2.2.1 Preparation of nanoparticles

Magnetic nanoparticles of maghemite (γ-Fe_2O_3) were synthesised by co-precipitating Fe^{2+} and Fe^{3+} by NH_4OH and treating under hydrothermal conditions according to the procedure described by Šulek et al. [33, 34]. Briefly, the ferric and ferrous chlorides were dissolved in water. Chemical precipitation was achieved at 25°C under vigorous stirring by adding NH_4OH. During the reaction process, the pH was maintained at about 11. The precipitates were heated at 80°C for 30 min, and then washed several times with water and ethanol. The precipitate was extracted by a magnet and washed out with distilled water. Citric acid was added as primary surfactant into the mixture in order to obtain finely dispersed magnetic nanoparticles. According to the authors mentioned, the particles size that were obtained by this method were found to be in the range from 22.5 nm to 50.8 nm. In the next step surface functionalisation with silica and aminosilane (APTES) was performed.

2.2.2 Surface activation and enzyme immobilisation on magnetic nanoparticles

The surface of chemically modified maghemite nanoparticles was further subjected to activation with the crosslinker, glutaraldehyde followed by the enzyme immobilisation. Briefly, MNPs were washed with deionised water prior to exposure to 2% glutaraldehyde aqueous solution for 2 h. After the surface activation, MNPs were washed out with deionised water and the enzyme was introduced (γ_{ADH}=0.08 mg/cm^3) to attach during the 28 h. At those conditions the specific activity of immobilised ADH (0.08 mg enzyme immobilised on 5 mg Fe_2O_3) was determined to be *S.A.*=118±6 U/mg and immobilisation efficiency of η=84.97±3.67% was achieved. Compared to the native enzyme, the immobilised ADH retained 66.45±3.66% of initial activity.

2.2.3 Coenzyme regeneration

The possibility to use synthetised ADH-loaded γ-Fe_2O_3 nanoparticles for NADH oxidation was explored. Reaction was performed in a batch reactor (V=1.50 cm^3) and in a microreactor (V=0.27 cm^3). Initial concentration of NADH and acetaldehyde was kept constant for all experiments ($c_{i,NADH}$=5.5 mmol/dm^3, $c_{i,acetaldehyde}$=40 mmol/dm^3). All experiments were performed in glycine-pyrophosphate buffer, pH=9, T=25°C.

Batch experiments NADH oxidation was carried out in 1.5 cm^3 batch reactor on an IKA Vibrax Basic (IKA®-Werke GmbH & Co. KG, Staufen, Germany) shaker at 1000 rpm. Samples from the reactor (about 0.1 cm^3) were taken at regular time intervals. The concentrations of the NADH and ethanol were monitored using the methods described below.

Microreactor experiments The coenzyme and acetaldehyde were dissolved in a glycine-pyrophosphate buffer and fed into the PTFE (poly(tetrafluoroethylene)) tubular microreactor (length:diameter=330 mm:1 mm with the internal volume of 0.27 cm^3) using syringe pump (PHD 4400 Syringe Pump Series, Harvard Apparatus, Holliston, MA, USA) equipped with high pressure stainless steel syringes (8 cm^3, Harvard Apparatus, Holliston, MA, USA). For introducing magnetic nanoparticles into the microchannel a permanent magnet (Square magnet: length:width:depth=40:20:10 mm, H=0.38 T; Cylindrical magnet: length:diameter=40:10 mm, H=0.4 T, Artas d.o.o., Zagreb, Croatia) was used. The magnetic particles were introduced into the microchannel as a suspension of different concentrations ($\gamma_{nanoparticles}$=0.5–5 mg/cm^3, *S.A.*=118±6 U/mg$_{enzyme}$), and were attracted and held on one side of the channel by the magnetic force produced by the magnet.

Additionally, a system for magnetic field regulation was developed in order to utilise magnetic properties of MNPs (Figure 1). In this experiment I=0.36 A and U=5.5 V were used to drive electromagnet (H=1.73 T) with an oscillating magnetic field at a frequency of 0.5 Hz. During all experiments temperature change was monitored directly on the surface of a fully isolated coil. It was noticed that the temperature increased from just 0.2°C to 0.5°C/day and the heating was assumed to be negligible.

Developed system allowed particle movement inside microchannel without being washed out. Outflows from the microreactor containing reaction mixture components were collected in vials. All measurements were performed in triplicate and in the 95% confidence range the results showed no significant difference.

2.2.4 Analysis

The activity of suspended alcohol dehydrogenase and alcohol dehydrogenase immobilised on MNPs was measured using an enzymatic assay where the NADH concentration was determined by measuring the absorbance at 340 nm with a spectrophotometer (Shimadzu UV–1601, Kyoto, Japan) according to the procedure described elsewhere [35].

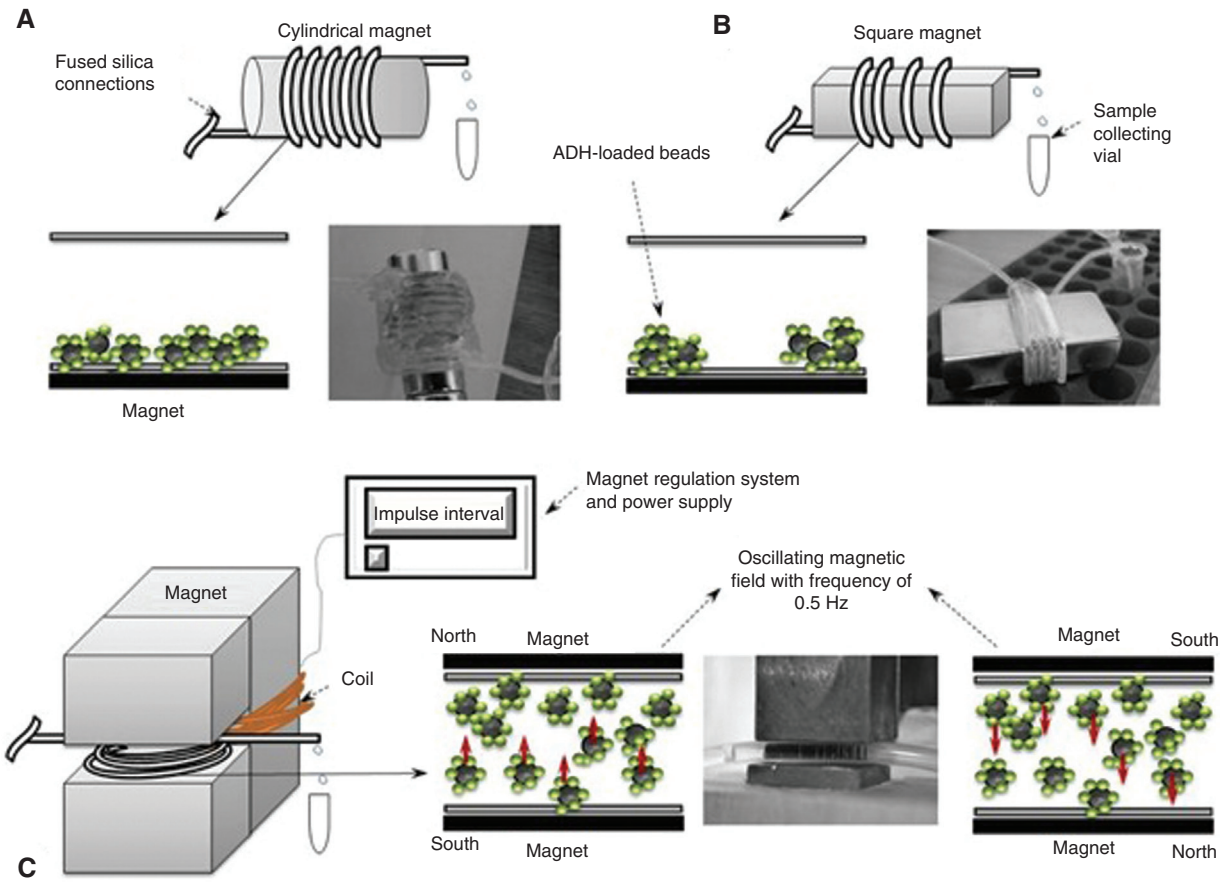

Figure 1 Experimental set-up with (A) cylindrical magnet, (B) square magnet and (C) system for magnetic field regulation.

Coenzyme concentration was determined by measuring the absorbance at 340 nm with a spectrophotometer (Shimadzu UV–1601, Kyoto, Japan) according to the procedure described elsewhere [35].

Ethanol concentration was determined using GC (Shimadzu GC-2014, Kyoto, Japan) with the flame ionisation detector. Polar column ZB-WAX (Phenomenex, Torrance, USA) and helium (as a gas carrier), were used. Concentration of acetaldehyde was calculated according to stoichiometric ratio and measured concentrations of ethanol and NADH [24].

All the measurements were performed in triplicate and in the 95% confidence range, the results showed no significant difference.

2.2.5 Mathematical model and data processing

The reaction rate of coenzyme regeneration was modelled with a double substrate Michaelis-Menten equation according to the kinetic measurements published elsewhere [22, 36].

Partial differential equations of the proposed model were solved by approximating microreactor as plug flow reactors in a steady-state [37].

Using the obtained parameters and according to the model equations [36]. "Episode" algorithm for a stiff system of differential equations, implemented in the "Scientist" software package (MicroMath Scientist®), was used for the simulations. Enzyme stability decay rate constant (k_d) was described by the first order kinetics.

3 Results and discussion

3.1 NADH oxidation in a batch reactor

As mentioned in the Introduction one of the biggest limitations and drawbacks of hexanol oxidation process catalysed by ADH is the usage of the coenzyme NAD+. Up to now several different systems for NADH oxidation, e.g., coenzyme regeneration processes, were investigated [22–24]. Although the best results were obtained when

suspended enzyme was used, previous results indicated that an overall process would be even more sustainable if an immobilised enzyme was applied. Up to now, direct covalent immobilisation on the microchannel surface was performed but the results were not satisfying because immobilisation efficiency obtained was too low (η=12.5%). According to the literature data [33] up to 80% of immobilisation efficiency could be obtained by immobilising the biocatalyst on the nanoparticle surface. After the optimisation of ADH immobilisation on magnetic nanoparticles (data not shown) specific activity of *S.A.*=118±6 U/mg and immobilisation efficiency of η=84.97%±3.67% were achieved. Compared to the native enzyme, the immobilised ADH retained 66.45±3.66% of initial activity.

However, acetaldehyde was used as a substrate for the coenzyme regeneration because of its low price and a high specificity of the ADH towards it. Batch experiments (V=1.5 cm^3) were carried out in the 0.75 mmol/dm^3 glycine-pyrophosphate buffer at T=25°C using ADH-loaded MNPs. Initial concentrations of NADH and acetaldehyde were kept constant for all experiments ($c_{i,NADH}$=5.5 mmol/dm^3, $c_{i,acetaldehyde}$=40 mmol/dm^3). Influence of enzyme activity, nanoparticle amount and storage time were investigated. Results are presented in Figure 2. The time to achieve maximal conversion (X=100%) was dependent on initial enzyme activity e.g., for the higher initial enzyme activities the process was faster (Figure 2A).

Additionally, the influence of initial concentration of loaded nanoparticles was also investigated (Figure 2B). It could be observed that decreasing the initial amount of nanoparticles the time for reaching maximal conversion is increasing and this dependency is almost linear.

Finally, the influence of enzyme storage time was also studied. NADH oxidation was first performed immediately after the immobilisation procedure. MNPs were then washed-out with the buffer re-suspended for storage in glycine-pyrophosphate buffer with ammonium sulphate in excess, c=2.4 mol/dm^3. Particles were reused

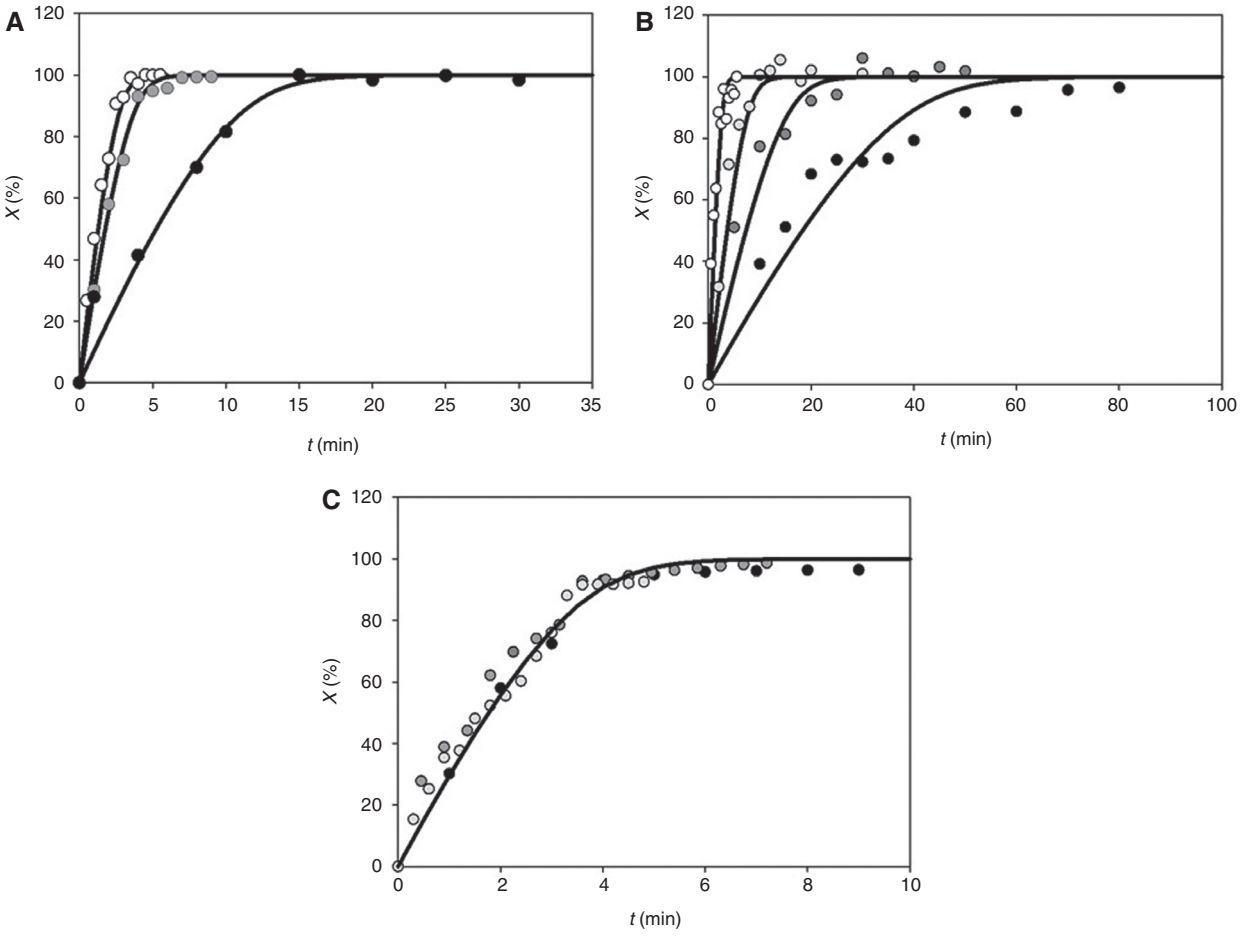

Figure 2 NADH coenzyme regeneration in a batch reactor (A) influence of enzyme activity (○ 118±6 U/mg, ● 65±2 U/mg, ●19±3 U/mg) (B) influence of nanoparticle amount (○ 5 mg, ● 2.5 mg, ● 1.25 mg, ● 0.5 mg, *S.A.*=118±6 U/mg) (C) influence of storage time (● 0 h, ● 96 h, ● 264 h) (T=25°C, $c_{i,acetaldehyde}$=40 mmol/dm^3, $c_{i,NADH}$=5,5 mmol/dm^3, — model).

after 96 h and 264 h of storage. Results are presented in Figure 2C. Coenzyme conversion of X=100% was achieved after 4 min. It was observed that there is practically no difference in experiments performed with particles used immediately after immobilisation procedure and stored particles. These results are in accordance with the results obtained in experiments where stability of immobilised ADH was studied (data not shown). Based on long-term stability of the stored enzyme without significant change in activity (~19 days) it is shown that the immobilised enzyme could be effectively reused in regeneration reaction for at least 11 days (Figure 2c).

Additionally, mathematical model of NADH oxidation in batch reactor [36] was used to simulate the process. A good agreement between the model and experimental results was obtained (Figure 2).

3.2 NADH oxidation in a microreactor

After batch experiments, NADH oxidation was performed in PTFE microreactor. ADH-loaded MNPs were injected into the PTFE microreactor using a syringe pump and retained in the microreactor utilising the magnetic force of the magnet. As mentioned, three different reactor systems were used in this research. Two of them were PTFE tube equipped with square and cylindrical permanent magnets (Figures 1A and 1B), and the third was the PTFE tube equipped with electromagnet with oscillating magnetic field developed to enable magnetic particles movement in a microreactor (Figure 1C).

When working with permanent magnets, the nanoparticles were attached just on one side of the channel (Figures 1A and 1B). When a cylindrical magnet was used nanoparticles were all homogeneously dispersed all along length of the channel. When working with a square magnet all particles were gathered on the side of the channel leaving "holes" in microreactor without any particles. During the process nanoparticles continued to move to the parts of the tube that was touching the edges of the square magnet where the magnetic force was the strongest. In that way, instead of having a homogenous nanoparticle field, approximately 20 agglomerates were formed along the tube. Additionally no adsorption of magnetic nanoparticles was observed on the inner surface of the microchannel except at the region where magnetic field was applied.

The particles in both microreactor configurations, equipped with square and cylindrical permanent magnets, placed on just one side of the reactor in several layers and the amount of the enzyme that was available to substrate, decreased from theoretical surface that 5 mg of NMPs have (A=0.32 m^2). Therefore an electromagnet with an oscillating magnetic field was developed. Using this concept it was possible to move actively or restrain the particles across the channel (Figure 1C). With this way beads cover the whole channel cross-section.

In experiments of NADH regeneration performed in PTFE microreactor the shape of the magnet has an influence on the reaction time for all three magnet configurations (Figure 3A). For the reactor system equipped with a permanent square magnet a 5-fold longer residence time (τ=10 min) was needed to achieve conversion of X=100%. In comparison, for the experiment performed with electromagnet maximal conversion of X=100% was achieved for residence time of just 2 min (Figure 3A).

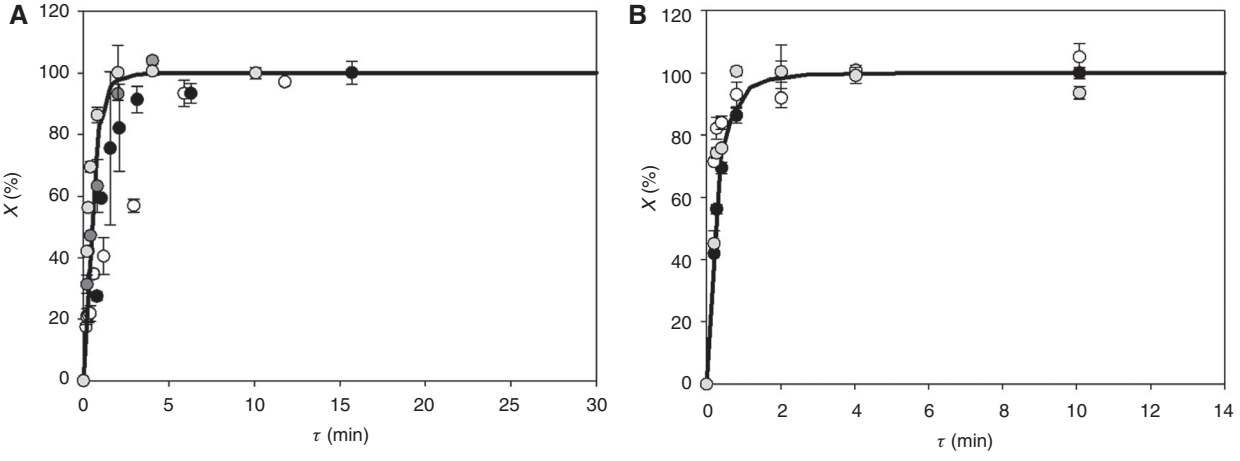

Figure 3 NADH coenzyme regeneration in a PTFE microreactor A) influence of magnetic type (○ square magnet, ● cylindrical magnet, electromagnet with ● stationary particles and ● oscillating particles) and B) influence of nanoparticle concentration (electromagnet with oscillating particles; ○ 5 mg, ● 2.5 mg, ● 0.5 mg, *S.A.*=118±6 U/mg, — model).

Unlike the case of the batch reactor, where the decrease of nanoparticle concentration had a negative effect on reaction time, the same effect was not noticed for the experiments performed in the microreactor (Figure 3B). As can be seen from the results shown in Figure 3b the same conversions were achieved for different concentrations of used nanoparticles while in all experiments performed available enzyme was probably in excess.

Previously developed mathematical model [22, 37] was used to describe the coenzyme regeneration process in a microreactor. As can be seen from Figure 3 a good agreement between the model simulation results and experimental results was obtained only for the system where an oscillating magnetic field was used. For all the other cases, when using a permanent magnet, the model did not show experimental data well. The reason for this was model assumption where the same amount of the enzyme was retained in the microchannel for all the systems. As previously mentioned in experiments performed with permanent magnets the nanoparticles were probably attached in several layers just on one side of the microreactor. In that way the amount of the enzyme that was available to the substrate decreased in comparison to the amount that was initially fed into a microreactor. Consequently, the developed mathematical model that did not predict distribution of nanoparticle was not able to show experimental results. In the experiment where the magnet with oscillating field was used the beads cover the whole channel cross-section and the entire enzyme amount initially fed into a microreactor became available to the substrate. Therefore a simple plug-flow kinetic model was sufficient to describe these experimental results quite well.

When working with the immobilised biocatalyst, another important factor was the long-term use of the biocatalyst. In order to determine the possibility of ADH-loaded MNPs long-term use, the NADH regeneration in a microreactor was performed for t=7 days at a total flow rate of Φ=5 mm^3/min. Enzyme operational stability decay rate constant (k_d) was described by the first order kinetics and it was determined to be 0.0127±0.0012 h^{-1}. As can be seen from the experimental results the system could be used for continuous operation but constant decrease of conversion was observed practically from the start of the experiment (Figure 4).

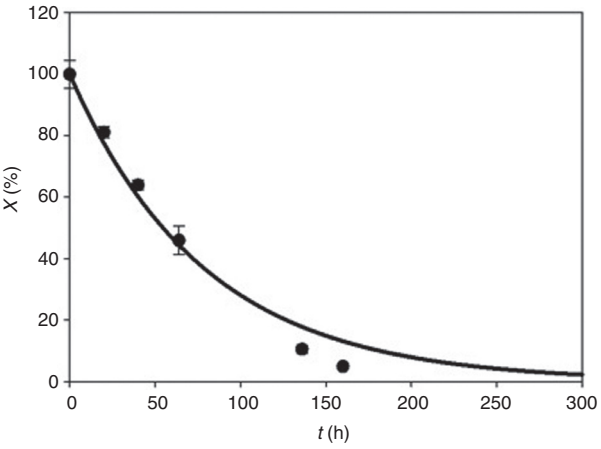

Figure 4 Stability of the tested system (T=25°C, $c_{i,acetaldehyde}$= 40 mmol/dm^3, $c_{i,NADH}$= 5.5 mmol/dm^3, substrate flow rate Φ=5 mm^3/min).

3.3 Comparison of NADH oxidation in different systems

When the results of NADH oxidation performed with ADH-loaded MNPs (0.08 mg enzyme immobilised on 5 mg Fe_2O_3 (*S.A.*=118±6 U /mg)) in the batch reactor were compared with the experiments performed in the PTFE tube microreactor (with the same amount of enzyme) with oscillating magnetic field, results were in favour of the microreactor system. 100% NADH conversion in a microreactor was achieved for a residence time of just 2 min, while with a batch reactor the same conversion was achieved for 4 min (Table 1).

When coenzyme regeneration was performed in PTFE microreactor using suspended enzyme (c_{enzyme}=0.1 mg/cm^3 (*S.A.*=261±9 U/mg), T=25°C, $c_{i,acetaldehyde}$=40 mmol/dm^3, $c_{i,NADH}$=5.5 mmol/dm^3) maximal conversion of X=94.36± 1.62% was achieved in shorter residence time (τ=0.76 min) [23].

Some additional comparisons of different NADH coenzyme regeneration systems in combination with a different type of used biocatalyst are presented in Table 1.

Although better results were obtained with suspended enzyme, reaction with immobilised enzyme is much more economical taking into consideration the costs of free enzyme solution that is necessary to feed into the reaction system continuously and with the conversions obtained. In order to keep maximal conversion (PTFE microreactor, X=100%, τ=0.76 min, $\Phi_{biocatalyst}$=300 mm^3/min) 43.2 mg/day (c_{enzyme}=0.1 mg/cm^3) of suspended biocatalyst should be consumed. In comparison, when working with immobilised enzyme, theoretically 0.08 mg [amount of enzyme that was used (optimised) for immobilsation on 5 mg of nanoparticles] of enzyme is necessary for the reaction. Additionally, taking into consideration the stability of the system when immobilised cells are used (7 days of continuous operation), the system with immobilised enzyme could be considered as sustainable despite the lower conversion.

Table 1 Comparison of NAD+ coenzyme regeneration within a microreactor and a batch reactor using suspended and immobilsed enzyme.

Type of biocatalyst	Type of reactor	τ (min)	X (%)	$A.V.$ (U/mg)
Suspended ADH [23]	Batch reactor (t=1 min)	–	100	261±9
	PTFE microreactor	0.76	94.36±1.62	252±5
ADH-loaded MNPs	Batch reactor (t=4 min)	–	100	112±4
	PTFE microreactor with:			
	Square magnet	10.00	100±1.21	124±7
	Cylindrical magnet	6.32	96.4±2.12	116±5
	Electromagnet	2.00	100±1.85	110±6

4 Conclusion

Enzyme immobilisation on MNPs opens up new possibilities for the biocatalysis as well as for the new applications of magnetic nanoparticles. Obtained results will be helpful for the practical application of ADH for a broad range of different reactions. Successful NADH oxidation was performed using ADH-loaded MNPs in different reactor systems, batch and microreactor. Comparing those two different systems, advantages of the micro continuous-flow process is more than obvious. Basically, taking into consideration microreactor advantages, the process intensification was made, actually, by maximisation. Maximising the mass and heat transfer, surface to volume ratio, process control, utilisation of chemicals and flexibility in a multiproduct environment a new concept in chemical engineering could be explored.

Utilising the advantages of magnetic nanoparticles such as biocompatibility, small size, low toxicity and, especially, superparamagnetisam, new paths have been opened, not only for proteomics, where they are already being exploited, but also in biotechnology and chemical engineering. Another great advantage of catalytic reactions performed with catalyst-loaded magnetic nanoparticles is the efficient and easy separation of catalyst particles from the reaction mixture. In addition, as demonstrated in this manuscript, a significant reduction of the catalyst amount used in a reaction can be achieved. In future work decrease in microchannel size and the influence on process intensification will be explored. Electrical switching of magnets could be interesting for the exchange of enzyme-charged material during long-term application of the flow-through reaction arrangement.

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