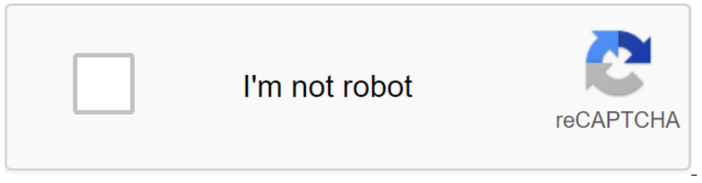

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Immobilization enzyme pdf

Enzymes immobilized in the beads of alginate gel Immobilized enzyme is an enzyme attached to an inert, insoluble material, such as calcium alginate (produced by the reaction of a mixture of sodium alginate solution and enzyme solution with calcium chloride). This can provide increased resistance to changes in conditions such as pH or temperature. It also allows enzymes to be held in place throughout the reaction, after which they are easily separated from the products and can be used again - a much more efficient process and therefore widely used in the industry for enzyme catalysis reactions. An alternative to immobilization of enzymes is the immobilization of whole cells. The commercial use of immobilized enzymes is very important for commercial use as they have many benefits for costs and reaction processes that include: Convenience: The minuscule amount of protein dissolves into the reaction, so that the work can be much easier. Upon completion, the reaction mixture usually contains only solvents and reactionary products. Economy: The immobilized enzyme is easily removed from the reaction, making it easy to recycle the biocatalist. This is especially useful in processes such as the production of lactose-free milk, as milk can be drained from a container, leaving the enzyme (Lactase) inside ready for the next batch. Stability: Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme. In the past, biological washing powders and detergents contained many proteases and lipases that broke dirt. However, when the cleaners contacted the human skin, they created an allergic reaction. That's why enzyme immobilization is important, not just economically. Enzyme immobilization There are various ways in which you can immobilize the enzyme: Affinity-tag binding: Enzymes can be immobilized to the surface, for example, in porous material, using non-covalent or covalent protein tags. This technology was created for the purpose of cleaning the protein. This method is generally accepted, and can be performed without first cleaning the enzyme with a clean drug as a result. Porous glass and its derivatives are used, where the porous surface can be adapted from the point of view of hydrophobic in accordance with the appropriate enzyme. Adsorption on glass, alginate or matrix: The enzyme is attached to the outside of the inert material. Overall, this method is the slowest among those listed here. Since asorptium is not a chemical reaction, the active location of the immobilized enzyme can be blocked by a matrix or beads, which significantly reduces the activity of the enzyme. Capture: The enzyme is trapped in insoluble beads or microspheres such as alginite Beads. However, these insoluble substances prevent the substrate from entering and leaving the products. Cross-bonding: Molecules of enzymes covalently bound are linked each other to create a matrix consisting of almost only an enzyme. The reaction ensures that the binding place does not cover the active activity of the enzyme, the activity of the enzyme is affected only by immobility. However, the inflexibility of covalent bonds excludes self-healing properties exhibited by chemo-asorbized self-assembly monolayers. Using a spacer molecule like poly (ethylene glycol) helps reduce the stericular interference of the substrate in this case. Covalent bond: Enzyme covalent is associated with insoluble support (e.g. silica gel or macroporous polymer beads with groups of epoxy). This approach provides the strongest interaction of the enzyme/support, and therefore the lowest protein leakage during catalysis. Random compared to the site of directed enzyme immobilization Numerous enzymes of biotechnological value were immobilized on various supports (inorganic, organic, composite and nanomaterials) through random multivolume attachments. However, immobilization by random chemical modifications leads to a heterogeneous protein population where more than one side chain (ameno, carboxil, thiol, etc.) present in proteins is associated with support for a potential decrease in activity due to the restriction of substrate access to an active area. In contrast, in the immobilization of an enzyme aimed at the site, support may be associated with one specific amino acid (usually N- or C-termini) in a protein molecule away from the active site. Thus, the maximum activity of enzymes is maintained due to the free access of the substrate to the active site. These strategies are mostly chemical, but may additionally require genetic and enzymatic methods to create functional groups (which are not present in the protein) to support and enzyme. The choice of SDCM method depends on many factors, such as the type of enzyme (less stable psychophilic, or more stable thermophilic homologous homolog, the stability of the pH enzyme, the presence of N- or C-termi to the reagent, the non-interference of the enzyme with the activity of the enzyme, the type of catalytic amino acid residues, the presence, price and ease of preparation of reagents. For example, generating additional interactive functions (alkin and azid) on the support and enzyme is one of the most convenient ways to immobilize enzymes with a chemical modification aimed at the site. Immobilization of substrate for enzymatic reactions Another widely used application of immobilization approach along with enzymes were enzyme reactions to immobilized substrates. This approach facilitates the analysis of enzyme activity and mimics the performance of enzymes, such as cell walls. References to Saunitsina, O.; Berillo, D.; Kirsebom, H.; Mattiasson, B. (2013). Cryostructured and cross-viable cells that form suitable for the use of bioreactors. Topics in catalysis. 57 (5): 339. 339. Aragao Buerner, R.; O.; Berillo, D.; Skapccha, N.; Mattiasson, B.; Kirsebom, H. (2014). Clostridium acetobutylicum DSM 792 immobilization as macro-positive units through cryogelation for butanol production. The process of biochemistry. 49: 10–18. doi:10.1016/j.procbio.2013.09.027. Wu, Hong; Liang, Yanpeng; Shi, Jiafu; Wang, Syoli; Yang, Dong; Jiang, Junyi (April 2013). The increased resistance of catalase is covalently immobilized in functional titanic submicrospheres. Materials science and engineering: C. 33 (3): 1438-1445. doi:10.1016/j.msec.2012.12.048. PMID 23827593. 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Advances in biotechnology. 37 (3): 357–381. doi:10.1016/j.biotechadv.2019.02.002. ISSN 0734-9750. PMID 30768953. Gray, C. J.; Weissenborn, M.J.; Eye, C. E.; Flich, S.L. (2013). Ensimatic reactions to immobilized substrates. Chemical Society Reviews. 42 (15): 6378–405. doi:10.1039/C3CS60018A. PMID 23579870. Volume 12, Issue 8 Enzyme Application is a great advantage against highly selective

reactions; However, this also includes problems due to their sensitivity. Immobilization offers one strategy to overcome these problems, allowing the stabilization of enzymes as well as retention. In this study, the most promising method of immobilization of the studied reaction system is covalent attachment to hydrophilic amine-functional media. To achieve this goal, a new method of preparation of silica particles has been developed, followed by amino acid functionalization to prepare spherical media for the immobilization of enzymes, in which high porousstas is produced on the basis of polymerization. Using these particles, the immobilization of alcohol dehydrogenase and dehydrogenase is implemented with residual activity of 70 and 80% after 12 consecutive batches, respectively. Two immobilized enzymes are used to reduce synnamy aldehyde with the regeneration of the sofactor in situ, receiving a conversion of 100% and 10 times more revs compared to a free enzyme. Biocatalytic processes are promising alternatives to conventional chemical reactions if they meet certain requirements, such as less by-products, less intermediate, environmentally friendly and sustainable reactions. In addition, the use of enzymes offers various benefits such as reactions at ambient temperature, pH and pressure, as well as high stereo and regioselectivities.1 Restrictions using these catalysts as the need for cofactors or recyclables can be overcome with effective cofactor regeneration and simplified downstream processing, which are key methods for developing biocatalytic processes. Thus, enzyme immobilization plays an important role because it provides for easier recovery and processing of these often economically intense catalysts, as well as possible stabilization of the reaction. Since there is no method of immobilization suitable for all biocatalists, it is necessary to consider the various factors and numerous procedures that establish a suitable method of immobilization for the enzyme and reaction system used.2 The variety of different immobilization methods can be divided into three main approaches: non-covalent binding, covalent binding and enzyme capture. Unculent binding through adsorption or ion interactions is a simple investment in the carrier; however, enzyme leaching is often a problem due to the weak interaction between the enzyme and the carrier. In contrast, In addition, the covalent immobilization on carriers is well known for the rigid fixation of the enzyme, which leads to very good residual activity, applying immobilized enzyme in successive batches.3 In addition, multi-post covalent attachment of the enzyme to the carrier allows the prevention of denature through deployment.4 Compared to covalent immobilization on the carrier, cross-binding, as a very simple method, implements a strong method of coerce. However, distortion of conformation and chemical modifications can affect the active center and are flawed in this method. Another approach is to capture enzymes. This method generates stable enzyme drugs with high immobilization, however, leaching and restriction of diffusion are reported to be the main problems using this technique.5 Other interesting methods are the formation of enzyme-inorganic crystal hybrid including enzyme-inorganic crystalline hybrid nanoflowers and enzyme-metal organic frame composites. These methods provide self-drill systems on one-step coau sediment precipitation complexity of the enzyme molecule and the metal component. However, these metals can influence the activity of enzymes as well as.6 However, not only the method itself, and the choice of support is important for the effectiveness of enzyme immobilization. Material properties, such as polarity, charge and dielectric constant, have a strong impact on activity and stability. On this basis, increased stability through the immobilization of enzymes allows inertia against reaction conditions and mechanical stability and, therefore, application in different types of reactors.7 In addition, the material used must exhibit appropriate biocompatibility and biodegradability, as well as a good regeneration capability for reuse of the carrier.8 An increasing number of immobilized enzymes are being used in the chemical industry, in connection with the simplification of biodegradation. For example, Merck has developed a new adorptively immobilized drug CalB with significantly increased stability compared to Novozym 435® for the production of obanakatib, a k.9 catheter inhibitor, unlike Tate and Lyle, immobilized epimerase on ion resin exchanges, carrying out the production of allulosulose sweetener in the reactor with a fixed bed. Since March 2017, they've been selling this calorie sweetener, Manufactured in their patented process.10 In the presented study, the immobilization of two commercially available enzymes, alcoholic dehydrogenase 97 L (ADH), expressed by CLECT, and dehydrogenase (FDH) from Candida boidinii (Sigma Aldrich), is under investigation for use in the model system of the reaction with the regeneration of sofactor in situ (Scheme 1). This system is of interest because it can be applied to synthesize flavors as well as flavors and serves as an alternative to chemically produced cinnamon derivatives. ADH reduces cynnamal aldehyde (1) to synnamy alcohol (2), while FDH regenerates nadh cofactor. Various immobilization methods have been tested to find a suitable method with high immobilization and activity output for use in this bi-ensmatic reaction system. ADH and FDH were immobilized using the method with the best results and characterized by surface area and recycling. Both immobilized enzymes were applied in bienzimatic reactions and compared to their free state. Reducing synnamy aldehyde (1) to synnamy alcohol (2) when regenerating in place of cofactor. (ADH - alcohol dehydrogenase, FDH - dehydrogenase) Screening of immobilization techniques was carried out using the reduction of sinenamyl aldehyde to the synnamyl alcohol catalyzed ADG to find a suitable technique. Methods investigated: covalent immobilization of hydrophobic and hydrophilic carriers, capture of alginite beads formation of cross-binding enzyme units (CLEAs). The researched methods of immobilization in relation to activity and immobilization, the results shown in Figure 1. The effect of immobilization on activity and immobilization. Highlighted in red: Synthesized silica particles. (MA - metacrylate, TP - Trisoperl®, capture. A-C - 0.05, 0.25, 0.5% chitosan, CLEA - cross-enzyme aggregates) Cond.: Immobilizations were carried out in accordance with the methods described using the Tris-HCl pH 8, 0.1 M buffer and phosphate buffer pH 8, 0.1 M, rt, cADH.0.5 M/ML. Activity: VH100 ml, cNADH-0.1 mM, cCinnamyl aldehyde-5 mM. The seizure was investigated using sodium alginate and chitosan for stabilization, based on studies by Chou and colleagues.11 The high yield of immobilization reported in the study was replicated in this screening. Lower concentrations of chitosan led to even higher immobilization and activity yields, 90 and 1% respectively (Figure 1, capture. A-C). In contrast, zhou et al. reported residual activity of 62%. However, the breakdown of the matrix was noted, leading to a loss of activity by 60% after 6 batches.11 To prepare CLEA, various precipitation methods were tested to find a suitable one (data not shown). The best results were obtained using 70% (w/v) ammonium sulfate and subsequent cross-link with glutarldehyde to achieve CLEAs. CLEAs achieved a high immobilization yield of 74% and the highest activity yield of 7.3% compared to all the methods studied in this study. However, the particles showed poor stability, abrasions and re-dissolution occurred during the measurement of activity. In contrast, Hu et al. reported that ADH CLEA residual activity with a higher yield of 41%.12 Commercially available hydrophobic and hydrophilic carriers from Purolithic® were tested with various pre-functional functionalizations, according to the supplier's method. Two epoxy-functional metacrylate carriers (ECR204F and ECR206F) and one with amine-functionalization (ECR309F) were used as hydrophophobic materials. All three carriers showed comparable low activity and low immobilization; however, using an amino acid-functional medium, the highest immobilization rate was achieved at 22%. Since the properties of the carrier can have a significant impact on the immobilization of the enzyme, hydrophilic media have also been studied. The use of this carrier showed 3 times higher yield of activity and, in addition, immobilization give twice as much. Comparable immobilization behavior for glass beads has already been described by Goldberg et al in our research team, which used TRISOPERL beads to immobilize ADH® residual activity of 45% was reported. Higher activity may be based on the enzyme and the reaction system, but also on particles with already opened epoxy groups and therefore less active functional groups for enzyme immobilization.13 For further research into the effects of buffer salts on immobilization, a phosphate buffer using hydrophilic carriers was tested.7b This resulted in an increase in immobilization yields from 42 to 55% and 3.4 times higher. In addition to conformal changes during covalent immobilization, Hydrophobic the surface of the enzyme has a significant effect on activity, which became apparent during the screening in this study.4 Cao suggested that immobilized enzymes are either active in aqueous or organic solvents, depending on the properties of the prop, for example, immobilization on hydrophilic carriers may be more active in aqueous media.14 Support, such as glass and polysaccharides, have the advantage of having the advantage of hydrophilic carriers. that these groups are poor leaving groups and need prior activation as opposed to e. g. the use of epoxy-functional hydrophobic carriers, where the opening of the ring can occur over time.15 Thus, several methods of functionalization of amine-functionalized media, such as with 3-amine-per-roppilityoxylan (APTES), vinyltriethoxysilan (VTES) or glutaraldelerid, are known to lead to different results of immobilization and activity.16 Summary, reaction system, and the nature of the enzyme used plays an important role in retention. For subsequent studies reducing the synnamyl aldehyde in a two-eshim reaction, CLEAs were not additionally evaluated, due to unstable aggregates. The absorbed enzyme in the alginate showed very low activity, possibly due to the limitation of diffusion, so the method was also not taken into account. The use of ADH covalently immobilized on hydrophobic media has resulted in the lowest immobilization and profitability of all procedures. Higher activity was caused by covalent immobilization through Schiff's basic formation on hydrophobic covalent supports using amine-functional media. The covalent immobilization of hydrophilic carriers showed a comparable high level of immobilization with significantly higher ADG activity compared to hydrophobic supports and capture, so this method was very promising for application in this process. Commercial hydrophilic silicon particles have shown the best results in immobilization screening, but these carriers are no longer on the market. Functionalized mesoporic silica has already been created for enzyme immobilization, offering pores with the appropriate pore size for enzyme diffusion and reactionary compounds, as well as reactionary compounds, as well as 17 Due to very promising results, the hydrophilic spherical silica beads were synthesized based on tetraethylortolicate condensate (TEOS) and functionalized according to W'rmeyer et al., shown in the 2.18 Functionalization of hydrophilic carriers with the subsequent immobilization of the enzyme. (TEOS - tetraethylotosis, APTES - 3'aminopropyltriethoxysilane, SiO2 - silicon, Glu - glutareddehyde, E - enzyme) For this TEOS was hydrolyzed in acidic conditions and subsequently polycondenced the basis for the high-resistant material. The synthesis of spherical particles was realized by resetting the solution into a column filled with a mixture of rapeseed oil/heptan/n-butylamine (0.81/0.16/0.03 (v/v)) and with 1 cm of heptal phase on top to reduce the surface voltage shown in the 3. The homogeneous oil/heptan mixture was important for slowly sinking particles to harden in the outer phase before reaching the bottom, avoiding deformations. After full condensation, the resulting spherical particles were washed and functionalized in two stages with APTES as a link and glutaldehyde (GA) as a specer. Subsequently, synthesized and functional particles were tested for enzyme immobilization. In the case of ADH, immobilization was achieved by 40% and the return on activity to 4%, marked in Figure 1 (red frame). Compared to recorded yields using hydrophilic carriers, the results were quite low in this study.13, 19 However, compared to immobilization on functional mesoporic silica, Described by Lei et al. with the release of immobilization 3% and immobilization methods described above, the immobilized enzyme on silica particles is in the middle range of immobilization yield and shows one of the highest yields of activity.17 Results are comparable to commercial hydrophilic carriers, representing a promising alternative to the enzyme used, so the method is of great interest for reducing cinnamyl alde.17 Set up to prepare silica particles. Thanks to the polycondenence, the carriers show a high porosity with a surface area of about 750 m2 and a pore diameter of 5 nm. These values were identified by measurements of isotherma asorption using the BET (Brunauer-Emmet-Teller) method. In Figure 2, non-functional particles were compared with functional (APTES and GA) and particles with immobilized ADH to see possible effects. A significant decrease in surface area was observed between non-functional and functional particles, which is the result of the binding of APTES and GA. A similar trend was reported by Gopinat and Sugunan, who investigated the asorptive immobilization of amylase in a vaccinated hydrophilic nature montmorillonite.20 They also reported a slight decrease in surface area by functionalizing with APTES and GA. With the immobilization of the enzyme a further reduction was measured by Gopinath and Sugunan, whereby it did not change significantly in the current study. A certain diameter of pores increases with functionalization and showed additional growth with the binding of the enzyme. This may be due to blocking smaller pores with APTES and GA, resulting in a shrinking surface. However, this effect did not occur in the Gopinath and Sugunan study. They also compared different concentrations of enzymes for immobilization and observed similar behaviors for both functionalization with APTES and GA. As the enzyme load increases on the montmorilite, they found a decrease in surface area. However, with the silica particles prepared two and four times a higher surface area than reported for Gopinath and Sugunan particles was received and half the diameter of the pore. In addition, BET self-assembly aspartague nanocolors studied by Wu et al. with 32 m2/g also in a much lower surface area and with 112.5 nm in 8 times higher pore size.21 Silica particles produced in our studies offer a high surface for multi-touch attachment during tape-based immobilization over other methods. BET measurements of synthesized particles of hydrophilic silica. (unfunt. - non-functional, funct. - functionalized with APTES and GA, ADH immo , functionalized with APTES, GA and immobilized ADH) Even if the stored activity is lower than for registered methods, increased stability and therefore recycling as the most interesting property of immobilized enzymes, in addition to its activities, have also been investigated with ADH. Twelve consecutive batches for ADH and FDH were conducted using the same immobilized enzyme drug, which were stored at room temperature in a buffer between them. The batches showed small fluctuations, but both enzymes were used with stored activity of 70% and 83% for ADH and FDH, respectively. The deviations of 12 batches using individual enzymes may be the result of different processing as well as deviations from the measurement itself, so reliable activity calculations were not possible for kinetics (cf. SI). Comparable high activity after 12 consecutive batches were obtained within one month without further loss in activity, which shows excellent suitability for use in targeted reactions (c. Figure 3). Recycling measurements for ADH (left) and FDH (right) are applied in their separate target response. Cond.: Phosphate buffer 0.1 M, pH 8, TK30 KK, B25 ml, mEnzime-2 g, cAldehyde-5 MM, cNa cNADH-0.1 mM, cNAD-0.1 mM, 360 nm. The main purpose of this study was to use an immobilized enzyme to reduce synnamyl aldehyde to synnamyl alcohol with in situ sofactor regeneration. The use of immobilized and free ADH/FDH, used in a two-esym reaction, is shown in Figure 4. In both reactions, 100% of the transformation of the substrate has been achieved, however, the progress of the reaction is rejected. It should be taken into account that the amount of enzyme for free reaction was calculated on the basis of theoretically immobilized amount on the media. A immobilized enzyme can show lower stored activity based on, for example, changes in enzyme conformation. Restricting diffusion plays an additional role for immobilized enzymes differentiated between internal and external diffusion restriction and can cause abnormalities seen in Figure 4. Studies of possible internal limitations of diffusion in the case of synthesized silica particles have revealed any significant limitations (c. SI). The final response system with the conditions of the process: Reducing the synnamy aldehyde to synnamyl alcohol using free and immobilized ADH and FDH. Cond.: Phosphate buffer 0.1 M, pH 8, TK30 KK, B25 ml, cAldehyde-3.5 mM, NASA formate-200 mM, cNADH-0.15 mM, (A) mfreeADH-2.8 mg, However, reactions using free and immobilized enzymes were performed in a packet reactor with an overhead stirrer, which can result in external restriction of diffusion by immovable enzymes. For further research, different reactors with increased flow through particles should be used to reduce possible external diffusion limitations and increase reaction rate for immobilized enzymes.22 Direct comparison of reduction of synnamyl aldehyde using a free and immobilized enzyme can be done using total turnover (TTN). This value describes the ratio of the converted substrate to the applied catalyst, and the calculated values for both reaction cascade enzymes are shown in Table 1. Based on the smaller amount of FDH used (m'0.38 mg) TTN is higher than for ADH (m 2.8 mg). For ADH and FDH, a significant increase in TTN after immobilization was achieved through successful use in 12 consecutive responses and despite the observed loss of activity caused by immobilization. ADH showed an 8-fold increase to 34.7 gzubstrata/gkat, while immobilized FDH showed a 10-fold increase to 303.1 gzbstrata/gkat. Since for both enzymes high conversion after 12 batches were obtained, TTN values may increase more if immobilized enzymes are applied in more consistent batch reactions. Table 1. Comparison of TN and TTN free and фермента. Фермент TN бесплатно «gsubstrate/gcat» TN обездвижен «gsubstrate/gcat» TTN TTN (gzobstrat/Gkat) ADH 4.3 4.3 34.7 FDH 30.4 30.4 303.1 TTN was calculated based on the conversion of the substrate and the amount of enzyme applied. The theoretical amount of immobilized enzyme and residual activity for 12 were used for immobilized enzymes. Package in recycling experiments. Tufvesson and his colleague described the permissible contribution to the cost of the catalyst used and the corresponding range of required performance for various uses, such as bulk chemicals or pharmaceuticals.23 The aromatic components studied in this study are possible cosmetic ingredients and belong to a group of specialized chemicals. When immobilizing FDH with TTN over 300 grobstrata/gcat values in the target area of specialized chemicals are included if the price of the catalyst is in the range of 100-500 euros/kg. However, the price of biocatase always depends on the volume of the market and the same immobilized enzymes can be used in the production of pharmaceutical and bulk chemicals, so that the production of the catalyst is common. The synthesis of synnamyl alcohol with the regeneration of the sofactor in situ was subsequently performed in 3 batches using the same immobilized enzymes. Figure 5 shows that conversions decrease with the number of games. This reduction in enzyme reprocessing compared to experiments in the processing of an individual immobilized enzyme (p. Figure 3) may be the result of the effect of a two-nzismic reaction on individual enzymes. For example, Candida boidinii's rapid deactivation of FDH in biotransformations is known and explored various methods of overcoming this deactivation, for example, through protein engineering or downstream processing.24 Repeated reduction of synnamy aldehyde to synnamyl alcohol using immobilized ADH and FDH. Cond.: Phosphate buffer 0.1 M, pH 8, TK30 KK, HC25 ml, cAldehyde-3.5 mM, cNa formate-200 mM, cNADH-0.15 mM, mimmo ADHH 2.8 mg, Mimmo FDH-0.38 mg. Another cause may be different diffusional behavior of individual components. For example, synnamyl alcohol provides a higher hydrophobic (logP-1.82) than the synnamil adde substrate (logP-2.00).1 Based on the different properties of the components, They can exhibit different interactions with the carrier as a result of various mass transport in, through and out of particle.7b However, recyclable enzymes used in reducing the synnamyl aldehyde with on-site cofactor regeneration needs more optimization to produce high conversion in successive batches of application of two-esizime reaction. This study screened various immobilization methods, which showed that hydrophilic media with amine-functionalization lead to high immobilization and activity for enzymes used. Method Method hydrophilic spherical particles were developed based on the polymerization reaction. Particle preparation can be easily expanded, which shows a high potential for commercial application and leads to spherical highly porous particles with a surface area of about 750 m2/g. Using these particles and additional functionality with APTES and glutareddehyde obtained by immobilization and activity gives after immobilization applied by ADH were comparable to commercially available particles. Immobilized ADH as well as FDH have been investigated regarding recycling behavior and have shown high stability on a developed carrier. Thus, residual activity of more than 80% can be found after 12 batches of particle storage in the buffer at room temperature. This highlights the very promising properties of these immobilized enzyme drugs, which have also been researched for cofactor-dependent reduction of synnamy aldehyde. With free and immobilized enzymes, 100% conversion was achieved, with different reaction rates. TTN used immobilized enzymes 10 times higher based on their recyclables, so this process is more economical than the use of free enzymes. However, the use of enzymes in successive batches of bienzimatic inactivation reaction occurs. Recycling of biocatalists is a key condition of industrial application, which leads to increased economic efficiency of large-scale processes. Prospective results of enzyme immobilization of individual enzymes in this study are based on efficient processing, which leads to a high amount of produced synnamy alcohol. At the same time, investments in used enzymes can be significantly reduced. Catalysts for research, alcohol dehydrogenase 97 L, was purchased from c'LEcta (Germany) and dehydrogenase for candida boidinii from Sigma Aldrich (Germany). Sodium formate, cynnamil aldehyde, butylamine, tetratyl-orthosilicate were bought from Sigma Aldrich (Germany), which resulted in NADH, NADH, sodium alginate, Chitosan, hydroic acid, glutaraldehyde, ethanol, heptan, potassium dihydrogenphosphate and dipotase hydrogen phosphate were purchased from For this immobilization was applied commercial resin ECR309F from Purolita® and the buffer Tris-HCl (0.1 M, 2 mM). The experiments were conducted at 25 degrees Celsius. First, the pitches are washed 3 times for equilibrium with the ratio of resin/buffer 1 : 1 (w/v). The carrier was then pre-functionalized with 2% (v/v) glutaraldehyde with a resin/solution ratio of 1 : 4 (w/v) for 60 minutes at 25 degrees Celsius. The resin was suspended in a ADH solution 0.5 mg/ml with a resin/solution ratio of 2.5 : 1 and stirred for 18 hours with an overhead mixer. the solution was filtered (0.45 microns) and rinsed 3 times with a buffer of 50 ml and with 50 ml 0.5 M. NaCl. EcR204F and ECR206F resins from Purolithic were used for this immobilization® and immobilization was made with the Tris-HCl buffer (0.1 M, 2 M MMG2, pH 7) at 25 degrees Celsius. Immobilization was carried out in the same way as immobilization on amine-functional media, but without glutaraldehyde. The resins were suspended directly into the enzyme solution and stirred for 18 hours with overhead stirrers and the solution was left after immobilization without stirring for 22 hours at room temperature. The resins are then filtered and washed by the buffer. Immobilization on hydrophilic carriers was carried out with TRISOPERL glass beads® (particle size 100-200 microns, The size of the pore 100.46 nm) using the Goldberg method, etc. 13,200 mg of beads were pre-activated in 10 ml 2.5% solution of glutaraldehyde, cooked with phosphate buffer (0.1 M, pH 7) under a vacuum (60 mbar) for 30 minutes, followed by 50 minutes under the environment. Thus, the color was changed from white to reddish. The beads washed 200 ml of deionized water to remove excess by a binder and dried at 65 degrees Celsius for 4 hours. Immobilization was carried out in a vacuum (60 mbar) at 4 degrees Celsius for 35 minutes and mixed further under ambient pressure at 4 degrees Celsius. Subsequently, the resins were filtered and rinsed 5 times with a phosphate buffer of 100 ml and hung in the glycine buffer 10 ml (0.1 m, pH 7.5) at 4 degrees Celsius. After 3 hours the filtered particles were washed 5 times with a phosphate buffer of 10 ml (0.1 m, pH 7.5). In addition, this method of immobilization was also carried out with the Tris-HCl buffer (0.1 M, 2 mM MKl2, pH 7). CLEAs were prepared in accordance with the protocol described by Hu et al. 12 First, THE ADH was besieged, adding 70% (w/v) ammonium sulfate to an enzyme solution in 1 ml. Solution incubated at 4 degrees Celsius for 2 hours after that the mixture was centrifuged at 13,000 per minute for 15 minutes at 4 degrees Celsius. The beleaguered enzyme was associated with a 2.5% (v/v) solution of glutaraldehyde with a ratio of 0.5 : 1 (w/w) glutaraldehyde/protein. The mixture was shaken for 90 minutes at 25 degrees Celsius and centrifuged subsequently at 5,000 rpm for 10 minutes. The solution over the pellets was decanted, and the pellets washed 3 times by a buffer of 5 ml. ADH was trapped according to the method described by Chou et al. 11 The different conditions used for capture are shown in table 2. Table 2. Conditions of immobilization for capture. Immobilization Time (h) cChitosan (w/v) cNa'alginate (w/v) cCaCl2 (w/v) 6 0.25 4 2 6 0.05 3 2 6 6 0.5 0.5 3 2 Sodium was added to 50 ml of deionized water and was shaken for 2 hours at 30 degrees Celsius to get a homogeneous solution. The ADH 0.5 mg/ml solution was dissolved in a tris buffer (0.1 M, 2 m MMgl2, pH 7) and mixed with equal volume of alginate solution at ambient temperature. As a therapeutic bath in the solution chitosan was prepared a solution of calcium chloride (CaCl2) at a rate of 2% (w/v), which dissipated into 5% acetic acid and mixed at 7000 rpm for 30 minutes. The ADH-alginat mixture was added to the CaCl2 bath with a 10 ml syringe and 18G needle. The beads were treated for 2 hours with a solution of 0.5% glutaraldehyde and finally washed with deionized water (3 x10 ml). To make silica particles mix 1 mole TEOS, 4 mole water, 2.4 moles of ethanol and 10.5 mole HCl was prepared and stirred for 30 minutes, then it was dumped into a mixture of canola seed oil, butyl amina and n'heptane (890 ml/178 ml/33 ml) through a syringe pump (60 ml, 10 ml/min) and prepared particles were treated overnight. For functionalization, 100g APTES (3 (Aminopropyl)triethoxysilane) were dissolved in 50 ml of ethanol and 10 grams of particles were incubated at 50 degrees Celsius for 48 hours. For cross-binding, the particles were treated with a 2.5% (v/v) glutaraldehyde solution for 1 hour at 30 degrees Celsius and then rinsed with water for 30 minutes to remove the residual glutaraldehyde. The enzyme immobilization into particles was carried out with a solution of 0.5 mg/ml of ADH dissolved in phosphate buffers (0.1 M, pH 8), as a result of which the particles were incubated at 2 hours at 25 degrees Celsius. They were subsequently rinsed with water to remove the unwashed enzyme. The yield of immobilization was determined spectrophototically in accordance with the pier protein scale. 10 MKL samples were mixed with 150 il Pierce mortar and incubated for 5 minutes. Subsequently, absorption was measured at 562 nm, and the concentration of protein was determined by calibrating the bovine serum albumin (BSA) as a standard. The effectiveness of immobilization was determined by subtracting the protein content of the solution after immobilization and all washing solutions from the ADH starter solution used for immobilization. The activity of the enzyme was determined on the basis of NADH, which was quantified spectrototometerally at 360 nm and 30 degrees Celsius. Yields were calculated on the basis of measured activity of immobilized protein and theoretical activity, which was possible on the basis of the yield of immobilization. Measurements of the specific surface area and diameter of the pores were made in accordance with Roman et al. 25 Samples were 24 hours at 348 K. The data were identified using the Brunauer-Emmett-Teller method (BET) and low-temperature analysis of nitrogen adsorion (quantarom Nova 3000e) between the p/p0 range 0.05-0.30.25 To measure the recycling of immobilized enzymes, a 3-shaped flask was used, and the solution was constantly mixed with overhead mixing. Reactions were carried out in 25 ml of phosphate buffer (0.1 M, pH 8) at 30 degrees Celsius using 2 grams of immobilized enzyme. The MEASUREMENT of ADH was carried out using 5 mm of synnamy aldehyde and 0.1 m of NADH, which resulted in FDH being investigated with 200 mm sodium formate and 0.1 mM of NADH. The conversions were measured spectrophototically based on the consumption of OR produced by NADH at a wavelength of 360 nm. To study on-site cofactor regeneration, both enzymes, ADH and FDH, were applied in their free and immobilized form. The reactions were carried out with the same setting that was used for recycling measurements. Both reactions were carried out in a 25 ml phosphate buffer (0.1 M, pH 8) at 30 degrees Celsius and with 3.5 mm of synnamyl-aldehyde, 100 m sodium and 0.15 mM NADH. 5 grams of FDH particles and 1 g of EDG particles were used for reactions with immobilized enzymes. Based on calculations for the immobilization of enzymes on media, 2.8 mg of ADH and 0.38 mg of FDH were used to react to free enzymes. To monitor the reaction, 500 samples of hl were taken, which were gently mixed with 500 p'hilen. After centrifuge, 300 Ls were measured using gas chromatography using the HP-5 column (Agilent Technologies, USA) and helium as a carrier gas. The temperature was increased from 120 degrees Celsius from 40 degrees Celsius to 160 degrees Celsius, holding for 2 minutes, and was increased again from 40 degrees Celsius/min 320 degrees Celsius carrying for 2 minutes. This work was supported by Deutsche Forschungsgemeinschaft (DFG, BU 3409/1-1 and DFG, WA 3957/1'1). We thank Purolite® carriers used for covalent immobilization, and Grit Brauckmann for their great lab work. The authors state that there is no conflict of interest. As a service to our authors and readers, this journal provides supporting information provided by the authors. Such content is reviewed and can be reorganized for online delivery, but not edited or typed. Technical support issues arising from supporting information (except for missing files) should be addressed to the authors. Please note that the publisher is not responsible for the content or functionality of any supporting information provided by the authors. 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