(54) Carrier for biocatalyst immobilization use, immobilized biocatalyst, and method for immobilizing biocatalyst

Träger für die Immobilisierung eines Biokatalysators, immobilisierter Biokatalysator, und Verfahren zur Immobilisierung eines Biokatalysators

Support utilisé pour l'immobilisation d'un biocatalyseur, biocatalyseur immobilisé, et méthode pour l'immobilisation d'un biocatalyseur

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(73) Proprietor: NITTO CHEMICAL INDUSTRY CO., LTD.
Tokyo (JP)

(72) Inventors:
- Doi, Toshiaki, c/o Nitto Chemical Ind. Co., Ltd.
  Tsurumi-ku, Yokohama-shi, Kanagawa (JP)

- Bamba, Hiroyasu,
  c/o Nitto Chemical Ind. Co., Ltd.
  Tsurumi-ku, Yokohama-shi, Kanagawa (JP)

- Murao, Kouzou, c/o Nitto Chemical Ind. Co., Ltd.
  Tsurumi-ku, Yokohama-shi, Kanagawa (JP)

(74) Representative:
Hansen, Bernd, Dr. Dipl.-Chem. et al
Hoffmann, Ette & Partner,
Patentanwälte,
Arabellastrasse 4
81925 München (DE)

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EP-A- 0 494 554
FR-A- 2 268 818
WO-A- 93/14133
US-A- 4 421 855

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Description

FIELD OF THE INVENTION

This invention relates to a carrier for use in the immobilization of biocatalysts, to an immobilized biocatalyst obtained by entrapping the biocatalyst in the carrier, and to a method for immobilizing the biocatalyst.

BACKGROUND OF THE INVENTION

When biocatalysts are used on an industrial scale, they are usually used in an immobilized form with the objectives of preventing elution of impurities from the biocatalyst, improving separability of the biocatalyst from the reaction product, improving applicability of the biocatalyst to repeated use, increasing enzymatic stability of the biocatalyst, and carrying out continuous operation of the production steps.

Immobilization of biocatalysts is effected by a carrier binding method, a cross-linking method, an entrapping immobilization method and the like (cf. T. Hattori and T. Frusaka, J. Biochem., vol. 48, pp. 831 (1960)), of which the entrapping immobilization method is most advantageous in that leakage of biocatalysts is small, and a decrease in the biocatalyst activity caused by the immobilization process is also small, because the biocatalyst and its carrier are not linked to each other. Furthermore, the method can be applied to the immobilization of a large variety of biocatalysts.

Examples of known carriers for use in the entrapping immobilization of biocatalysts include synthetic, high polymers such as polyacrylamide, polyvinyl alcohol, polyurethane, collagen, a photosetting resin and the like, and natural, high polymers such as carrageenan, alginic acid, agarose, starch, gelatin and the like (cf. U.S. Patent 4,526,867). In general, in comparison with the natural, high polymers, the synthetic, high polymers are high in strength, excellent in durability, and resistant to biodegradation. Of these, polyacrylamide is used most frequently because it is industrially inexpensive, has high polymer strength and causes less inactivation of biocatalysts at the time of polymerization (cf. U.S. Patent 4,421,855 and I. Chibata, T. Tosa and T. Sato, Appl. Microbiol., vol. 27, pp. 878 (1974)).

In addition, a process has been proposed in which acrylamide, a cationic ethylenic unsaturated monomer, and a water-soluble cross-linking monomer are subjected to copolymerization in order to reduce degree of swelling of a polyacrylamide base immobilized biocatalyst obtained by the entrapping immobilization method and to reduce inactivation of the catalyst at the time of the reaction (cf. JP-B-58-36078; the term "JP-B" as used herein means an "examined Japanese patent publication").

However, such polyacrylamide base immobilized biocatalysts, obtained by the entrapping immobilization method, are generally stored by soaking in an aqueous solution such as a buffer solution or the like, since their activities are apt to decrease when exposed to air oxidation or drying. When the storage is continued at room temperature for a prolonged period of time (for example, more than 1 month), the storing solution and the biocatalyst start to putrefy which causes generation of offensive odors from, and turbidity in, the storing solution and causes a decrease in the activity to such a level that it cannot be used as a catalyst. As a consequence, their storage is effected generally by putting them in a refrigerator or adding an antiseptic agent to the storing solution.

However, the cold storage method requires a huge cost for facilities, utilities and the like when biocatalysts are used in an industrially large quantity, and the other method, in which an antiseptic agent is added to the storing solution, causes permeation of the antiseptic agent itself into the immobilized biocatalyst, thereby adversely affecting the quality of products for the practical use.

It is important to overcome these problems especially in the case of the industrial application of immobilized biocatalysts.

SUMMARY OF THE INVENTION

The inventors of the present invention have conducted intensive studies on the development of a biocatalyst-immobilizing method which not only has the advantages of the prior art polyacrylamide base carriers but also is excellent in storage stability, and as a result, have found that the use of a specified copolymer, as a carrier, is markedly effective in overcoming the aforementioned problems. The present invention has been accomplished on the basis of this finding.

Accordingly, the present invention comprises a carrier for use in the immobilization of biocatalysts which is obtained by copolymerizing a first monomer represented by the following general formula (1) with a cationic acrylamide monomer and a water-soluble cross-linking monomer, both being capable of copolymerizing with the first monomer:
wherein each of \( R_1 \) and \( R_2 \) represents a methyl group or an ethyl group, an immobilized biocatalyst which is obtained by entrapping a biocatalyst in the carrier for biocatalyst immobilization use as well as a method for immobilizing a biocatalyst.

Other objects and advantages of the present invention will be made apparent as the description of the invention progresses.

**DETAILED DESCRIPTION OF THE INVENTION**

Illustrative examples of the compound represented by the aforementioned general formula (1) include N,N-dimethylacrylamide, N,N-diethylacrylamide and N-methyl-N-ethylacrylamide, which may be used alone or as a mixture of two or more compounds.

Illustrative examples of the cationic acrylamide monomer capable of copolymerizing with the monomer of general formula (1) include N,N-dialkylaminoalkyl methacrylamides, N,N-dialkylaminoalkylacrylamides and quaternary compounds thereof, such as N,N-dimethylaminopropylacrylamide, N,N-dimethyleniminopropyl methacrylamide, N,N-diethylaminopropyl methacrylamide, N,N-diethylaminopropylacrylamide and quaternary compounds thereof.

Illustrative examples of the water-soluble cross-linking monomer include N,N'-methylenebisacrylamide, N,N'-methylenebisacrymidamide, N,N'-(1,2-dihydroxyethylene)bisacrylamide, 1,3-di-acyramide methyl-2-imidazolidone, diacrylamide methylene urea, diacrylamide methyl ether, ethylene glycol diacrylate, ethylene glycol dimethacrylate, hexahydror-1,3,5-triaicyl-3-triazine, 2,2-bis(acylamide)acetic acid and the like. Of these, N,N'-methylenebisacrylamide, N,N'-methylenebisacrymidamide, N,N'-(1,2-dihydroxyethylene)bisacrylamide, 1,3-diacyramide methyl-2-imidazolidone and diacrylamide methylene urea are particularly preferred.

Based on the total amount of these monomers, the monomer of general formula (1) may be used in an amount of from about 70 to 99.8% by weight, preferably from 80 to 99% by weight, the cationic acrylamide monomer in an amount of from about 0.1 to 10% by weight, preferably from 0.5 to 10% by weight, and the water-soluble cross-linking monomer from about 0.1 to 20% by weight, preferably from 0.5 to 10% by weight.

If desired, other water-soluble monomers capable of copolymerizing with the monomer of general formula (1) may be used in an amount of about 0.01 to 10% by weight.

Examples of the biocatalyst to be immobilized by entrapping in the aforementioned immobilization carrier include enzymes, microorganisms, organella, animal and plant cells, and they may be used in the purified or disrupted forms, with no particular limitation in terms of their origin or form. For example, any genus of microorganism including bacteria, actinomycetes, yeasts, fungi and the like can be used, such as those belonging to the genera *Brevibacterium*, *Corynebacterium*, *Rhodococcus*, *Gordona*, *Vibrio*, *Nitrosomonas*, *Streptococcus*, *Lactobacillus*, *Bacillus*, *Azotobacter*, *Nocardia*, *Saccharomyces*, *Endomyces*, *Asperillus*, *Penicillium*, *Mucor*, *Rhizopus* and the like.

The immobilized biocatalyst of the present invention can be prepared, for example, by adding a mixture of the monomers to a suspension of a biocatalyst, further adding a commonly used polymerization initiator and accelerator, such as potassium persulfate and N,N,N',N'-tetramethylethylenediamine, to the suspension, and then incubating the resulting mixture at a pH value of from about 5 to 10, preferably from 6 to 8, at a temperature of from about 0 to 50°C, preferably from 0 to 35°C, for about 15 to 120 minutes, thereby effecting polymerization and gelation.

The biocatalyst content in the polymerized gel varies depending on the type, form and the like of each biocatalyst to be used, but the content may be in the range of generally from about 0.1 to 40% by weight, preferably from 1 to 20% by weight. The content of monomers in the polymerization reaction solution may be in the range of generally from about 2 to 30% by weight, preferably from 5 to 15% by weight.

These immobilized biocatalysts may be made into any shape such as granules, films, plates and the like.

The use of the biocatalyst immobilization carrier of the present invention renders possible the preparation of immobilized biocatalysts which are excellent in storage stability and simultaneously have the strength and the like advantages of the prior art high polymer base carriers, especially polyaeramide base carriers. The immobilized biocatalyst of the present invention does not putrefy even after one month of storage at ordinary temperature and therefore is extremely stable.

The following examples are provided to further illustrate the present invention. It is to be understood, however, that the examples are for the purpose of illustration only and are not intended as a definition of the limits of the present invention. The terms "part(s)" and "%" as used herein are based on weight, unless otherwise indicated.
EXAMPLE 1

*Rhodococcus rhodochrous* strain J-1 (FERM BP-1478) was aerobically cultured, and the resulting cells were washed and concentrated to prepare a cell suspension (15% as dry cells) for use in immobilization. 16 parts of 50 mM potassium phosphate buffer (pH 7.0, this is to be repeated in the following) and 10 parts of a monomer mixture solution composed of 92% N,N-diethylacrylamide, 3% N,N-dimethylaminopropylacrylamide and 5% N,N'-methylenebisacrylamide were added to 70 parts of the concentrated cell suspension cooled in an ice bath. The mixture was subsequently stirred in an ice bath to obtain a uniform suspension. 2 parts of 10% N,N,N',N'-tetramethylethylene diamine aqueous solution and 2 parts of 10% ammonium persulfate aqueous solution were added, followed by 1 hour of incubation at a temperature of 35°C or lower to effect polymerization and gelation. The thus obtained block of immobilized cells was cut into small particles and washed with water to be evaluated as a sample of immobilized cells.

When a 20 g portion of the thus prepared sample was soaked in 80 g of 0.5% sodium sulfate aqueous solution contained in a polyethylene bottle and stored at 30°C for one month after sealing the bottle, no changes in appearance were found and contamination of microorganisms was extremely low.

COMPARATIVE EXAMPLE 1

As a comparison, a sample of immobilized cells was prepared using an acrylamide base immobilization carrier, 1 part of 50 mM potassium phosphate buffer (pH 7.0) and 25 parts of a 40% monomer mixture aqueous solution composed of 92% acrylamide, 3% N,N-dimethylaminopropyl methacrylate and 5% N,N'-methylenebisacrylamide were added to 70 parts of the concentrated cell suspension. 2 parts of 10% N,N,N',N'-tetramethylethylene diamine aqueous solution and 2 parts of 10% ammonium persulfate aqueous solution were added to this mixture. Thereafter, polymerization and washing were carried out in the same manner as described in Example 1.

When the prepared sample was soaked in 0.5% sodium sulfate aqueous solution and stored at 30°C for one month in the same manner as described in Example 1, growth of a markedly large number of contaminated microorganisms and generation of a strong rotted odor as well as turbidity were observed.

EXAMPLES 2 TO 6 AND COMPARATIVE EXAMPLES 2 TO 6

Immobilized biocatalysts were prepared by changing types and concentrations of monomers and biocatalysts and evaluated, in the same manner as in Example 1 and Comparative Example 1. The results are shown in Table 1.

In this instance, the immobilized biocatalysts were stored at 30°C for one month by soaking them in 100 mM potassium phosphate buffer (pH 7) in the case of Examples 2 to 4 and Comparative Examples 2 to 4 or in 0.9% sodium chloride aqueous solution in the case of Examples 5 and 6 and Comparative Examples 5 and 6.

Putrefaction was judged based on the degree of odor generated from, and turbidity formed in, the soaking solution.

In the table, results of the evaluation are shown by 5 degrees where 0 means no putrefied odor or turbidity of the soaking solution and 4 means maximum odor or turbidity.
<table>
<thead>
<tr>
<th>Run No.</th>
<th>Monomers</th>
<th>Biocatalysts</th>
<th>Putrefaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name</td>
<td>Ratio (I)</td>
<td>Conc. (I)</td>
</tr>
<tr>
<td>5</td>
<td>N,N-Dimethylacrylamide</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N,N-Dimethylaminopropyl methacrylamide</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>N,N'-Methylenebis-acrylamide</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Acrylamide</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N,N-Dimethylaminopropyl methacrylate</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>N,N'-Methylenebis-acrylamide</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>N,N-Diethylacrylamide</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N,N-Dimethylaminopropyl methacrylamide quaternary compound</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>N,N'-Methylenebis-acrylamide</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Acrylamide</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N,N-Dimethylaminopropyl methacrylate</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>N,N'-Methylenebis-acrylamide</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>N,N-Diethylacrylamide</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N,N-Dimethylaminopropyl methacrylamide quaternary compound</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>N,N'-Methylenebis-acrylamide</td>
<td>6</td>
<td></td>
</tr>
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### Table 3 (cont’d)

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Monomers</th>
<th>Ratio</th>
<th>Conc.</th>
<th>Biocatalysts</th>
<th>Putrefaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(I)</td>
<td>(I)</td>
<td>Strain</td>
<td>Odor</td>
</tr>
<tr>
<td>Comp. Ex. 4</td>
<td>Acrylamide</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>N,N-Dimethylaminopropyl methacrylate</td>
<td>2</td>
<td>8</td>
<td><em>Gordona terrae</em> MA-1 (FERM BP-4535)</td>
<td>2</td>
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<tr>
<td></td>
<td>N,N'-Methylenebis-acylamide</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex. 5</td>
<td>N,N-Dimethylacrylamide</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N,N-Diethylacrylamide</td>
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<td></td>
<td><em>Nocardia</em> sp. N-775 (FERM BP-961)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N,N-Dimethylaminopropylacrylate</td>
<td>5</td>
<td>10</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>N,N'-Methylenebis-acylamide</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>N,N-Dimethylaminopropyl acrylate</td>
<td>5</td>
<td>10</td>
<td><em>Nocardia</em> sp. N-775 (FERM BP-961)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>N,N'-Methylenebis-acylamide</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex. 6</td>
<td>N,N-Diethylacrylamide</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N,N-Dimethylaminopropyl methacrylamide</td>
<td>3</td>
<td>8</td>
<td><em>Brevibacterium</em> sp. R-312 (FERM P-2722)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>N,N'-Methylenebis-acylamide</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comp. Ex. 6</td>
<td>Acrylamide</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>N,N-Dimethylaminopropyl methacrylate quaternary compound</td>
<td>3</td>
<td>8</td>
<td><em>Brevibacterium</em> sp. R-312 (FERM P-2722)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>N,N'-Methylenebis-acylamide</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Claims

1. A carrier for use in immobilizing biocatalysts, comprising the copolymer of a first monomer represented by the following general formula (1) with a cationic acrylamide monomer and a water-soluble cross-linking monomer, both being capable of copolymerizing with the first monomer:
2. An immobilized biocatalyst which is obtained by entrapping a biocatalyst in a carrier for biocatalyst immobilization, wherein said carrier is prepared by copolymerizing a first monomer represented by the following general formula (1) with a cationic acrylamide monomer and a water-soluble cross-linking monomer, both being capable of copolymerizing with the first monomer:

\[
\begin{align*}
\text{CH}_2 &= \text{CH} \\
\text{C} &\text{O} - \text{N} \arrows{R_1}
\end{align*}
\]

(1)

wherein each of \(R_1\) and \(R_2\) represents a methyl group or an ethyl group.

3. The immobilized biocatalyst according to claim 2, wherein said first monomer represented by the general formula (1) is N,N-dimethylacrylamide or N,N-diethylacrylamide.

4. The immobilized biocatalyst according to claim 2, wherein said cationic acrylamide monomer is selected from the group consisting of a N.N-dialkyldialkylaminoalkylacrylamide, a N,N-dialkyldialkylmethacrylamide, a quaternary compound of a N,N-dialkyldialkylaminoalkylacrylamide, and a quaternary compound of a N,N-dialkyldialkylmethacrylamide.

5. The immobilized biocatalyst according to claim 2, wherein said first monomer represented by the general formula (1) is used in an amount of from about 70 to 99.8% by weight, said cationic acrylamide monomer is used in an amount of from about 0.1 to 10% by weight and said water-soluble cross-linking monomer is used in an amount of from about 0.1 to 20% by weight, based on the total amount, by weight, of said monomers.

6. A method for immobilizing a biocatalyst comprising the steps of:

(i) creating a carrier by copolymerizing a first monomer represented by the following general formula (1) with a cationic acrylamide monomer and a water-soluble cross-linking monomer, both being capable of copolymerizing with the first monomer:

\[
\begin{align*}
\text{CH}_2 &= \text{CH} \\
\text{C} &\text{O} - \text{N} \arrows{R_1}
\end{align*}
\]

(1)

wherein each of \(R_1\) and \(R_2\) represents a methyl group or an ethyl group; and

(ii) entrapping a biocatalyst in said carrier formed in step (i).

7. The method of claim 6, wherein said first monomer represented by the general formula (1) is N,N-dimethylacrylamide or N,N-diethylacrylamide.

8. The method of claim 6, wherein said cationic acrylamide monomer is selected from the group consisting of a N, N-dialkyldialkylaminoalkylacrylamide, a N,N-dialkyldialkylmethacrylamide, a quaternary compound of a N,N-dialkyldialkylaminoalkylacrylamide, and a quaternary compound of a N,N-dialkyldialkylmethacrylamide.
9. The method of claim 6, wherein said first monomer represented by the general formula (1) is used in an amount of from about 70 to 99.8% by weight, said cationic acrylamide monomer is used in an amount of from about 0.1 to 10% by weight and said water-soluble cross-linking monomer is used in an amount of from about 0.1 to 20% by weight, based on the total amount, by weight, of said monomers.

**Patentansprüche**

1. Träger zur Verwendung beim immobilisieren von Biokatalysatoren, der das Copolymer eines ersten Monomeren, das durch die folgende allgemeine Formel (1) dargestellt wird, mit einem kationischen Acrylamid-Monomeren und einem wasserlöslichen vernetzenden Monomeren, die beide fähig sind, mit dem ersten Monomeren zu copolymerisieren, enthält:

   \[
   \begin{array}{c}
   \text{CH}_2 = \text{CH} \\
   \text{CO} - \text{N} - \text{R}_1 \\
   \text{R}_2
   \end{array}
   \]  \quad (1)

   worin \( \text{R}_1 \) und \( \text{R}_2 \) jeweils eine Methylgruppe oder eine Ethylgruppe darstellen.

2. Immobilisierter Biokatalysator, der durch Einschließen eines Katalysators in einen Träger zur Biokatalysator-Immobilisierung erhalten wird, wobei der Träger durch Copolymerisieren eines ersten Monomeren, das durch die folgende allgemeine Formel (1) dargestellt wird, mit einem kationischen Acrylamid-Monomeren und einem wasserlöslichen vernetzenden Monomeren, die beide fähig sind, mit dem ersten Monomeren zu copolymerisieren, hergestellt wird:

   \[
   \begin{array}{c}
   \text{CH}_2 = \text{CH} \\
   \text{CO} - \text{N} - \text{R}_1 \\
   \text{R}_2
   \end{array}
   \]  \quad (1)

   worin \( \text{R}_1 \) und \( \text{R}_2 \) jeweils eine Methylgruppe oder eine Ethylgruppe darstellen.

3. Immobilisierter Biokatalysator nach Anspruch 2, in dem das erste Monomer, das durch die allgemeinen Formel (1) dargestellt wird, N.N-Dimethylacrylamid oder N.N-Diethylacrylamid ist.


5. Immobilisierter Biokatalysator nach Anspruch 2, in dem das erste Monomer, das durch die allgemeine Formel (1) dargestellt wird, in einer Menge von etwa 70 bis 99.8 Gew.-% verwendet wird, das kationische Acrylamid-Monomer in einer Menge von etwa 0.1 bis 10 Gew.-% verwendet wird und das wasserlösliche vernetzende Monomer in einer Menge von etwa 0.1 bis 20 Gew.-% verwendet wird, jeweils bezogen auf die gesamte Gewichtsmenge dieser Monomeren.

6. Verfahren zur Immobilisierung eines Biokatalysators, das die folgenden Schritte umfaßt:

   (i) Schaffen eines Trägers durch Copolymerisieren eines ersten Monomeren, das durch die folgende allgemeine Formel (1) dargestellt wird, mit einem kationischen Acrylamid-Monomeren und einem wasserlöslichen vernetzenden Monomeren, die beide fähig sind, mit dem ersten Monomer zu copolymerisieren:
worin $R_1$ und $R_2$ jeweils eine Methylgruppe oder eine Ethylgruppe darstellen; und

(ii) Einschließen eines Bickatalysators in den in Schritt (i) gebildeten Träger.

7. Verfahren nach Anspruch 6, in dem das erste Monomer, das durch die allgemeine Formel (1) dargestellt wird, N, N-Dimethylacrylamid oder N,N-Diethylacrylamid ist.


9. Verfahren nach Anspruch 6, in dem das erste Monomer, das durch die allgemeine Formel (1) dargestellt wird, in einer Menge von etwa 70 bis 99,8 Gew.-% verwendet wird, das kationische Acrylamid-Monomer in einer Menge von etwa 0,1 bis 10 Gew.-% verwendet wird und das wasserlösliche vernetzende Monomer in einer Menge von etwa 0,1 bis 20 Gew.-% verwendet wird, jeweils bezogen auf die gesamte Gewichtsmenge dieser Monomeren.

**Revendictions**

1. Support pour une utilisation dans l'immobilisation de biocatalyseurs, comprenant le copolymère d'un premier monomère représenté par la formule générale suivante (1) avec un monomère d'acrylamide cationique et un monomère de réticulation hydrosoluble, les deux étant capables d'une copolymérisation avec le premier monomère:

   \[ C\overset{\text{H}}{\text{H}} = C\overset{\text{H}}{\text{H}} \quad \quad \quad \overset{\text{O}}{\text{N}} \quad \overset{\text{R}_1}{\text{R}_2} \quad \quad \quad (1) \]

dans lequel $R_1$ et $R_2$ représentent chacun un groupe méthyle ou un groupe éthyle.

2. Biocatalyseur immobilisé obtenu par piégeage d'un biocatalyseur dans un support pour immobilisation de biocatalyseur, dans lequel ledit support est préparé par copolymérisation d'un premier monomère représenté par la formule générale suivante (1) avec un monomère d'acrylamide cationique et un monomère de réticulation hydrosoluble, les deux étant capables d'une copolymérisation avec le premier monomère:

   \[ C\overset{\text{H}}{\text{H}} = C\overset{\text{H}}{\text{H}} \quad \quad \quad \overset{\text{O}}{\text{N}} \quad \overset{\text{R}_1}{\text{R}_2} \quad \quad \quad (1) \]

dans lequel $R_1$ et $R_2$ représentent chacun un groupe méthyle ou un groupe éthyle.

3. Biocatalyseur immobilisé selon la revendication 2, dans lequel ledit premier monomère représenté par la formule générale (1) est le N,N-diméthylacrylamide ou le N,N-diéthylacrylamide.
4. Biocatalyseur immobilisé selon la revendication 2, dans lequel ledit monomère d’acrylamide cationique est choisi dans le groupe constitué par un N,N-dialkylaminoalkylacrylamide, un N,N-dialkylaminoalkylméthacrylamide, un composé quaternaire d’un N,N-dialkylaminoalkylacrylamide et un composé quaternaire d’un N,N-dialkylaminoalkylméthacrylamide.

5. Biocatalyseur immobilisé selon la revendication 2, dans lequel ledit premier monomère représenté par la formule générale (1) est utilisé en une quantité comprise entre environ 70 à 99,8% en poids, ledit monomère d’acrylamide cationique est utilisé en une quantité comprise entre environ 0,1 à 10% en poids et ledit monomère de réticulation hydrosoluble est utilisé en une quantité comprise entre environ 0,1 et 20% en poids, par rapport à la quantité totale en poids desdits monomères.

6. Méthode pour l’immobilisation d’un biocatalyseur comprenant les étapes de:

(i) création d’un support par copolymérisation d’un premier monomère représenté par la formule générale suivante (1) avec un monomère d’acrylamide cationique et un monomère de réticulation hydrosoluble, les deux étant capables d’une copolymérisation avec le premier monomère:

\[
\begin{align*}
\text{C} & \text{H}_2 \text{N}^+ \text{R}_1 \text{R}_2 \\
\text{C} & \text{O} \text{N}^+ \text{R}_1 \text{R}_2 \\
\end{align*}
\]

(1)

(dans lequel \(R_1\) et \(R_2\) représentent chacun un groupe méthyle ou un groupe éthyle; et

(ii) piégeage d’un biocatalyseur dans ledit support formé à l’étape (i).

7. Méthode selon la revendication 6, dans laquelle ledit premier monomère représenté par la formule générale (1) est le N,N-diméthylacrylamide ou le N,N-diéthylacrylamide.

8. Méthode selon la revendication 6, dans laquelle ledit monomère d’acrylamide cationique est choisi dans le groupe constitué par un N,N-dialkylaminoalkylacrylamide, un N,N-dialkylaminoalkylméthacrylamide, un composé quaternaire d’un N,N-dialkylaminoalkylacrylamide et un composé quaternaire d’un N,N-dialkylaminoalkylméthacrylamide.

9. Méthode selon la revendication 6, dans laquelle ledit premier monomère représenté par la formule générale (1) est utilisé en une quantité comprise entre environ 70 à 99,8% en poids, ledit monomère d’acrylamide cationique est utilisé en une quantité comprise entre environ 0,1 à 10% en poids poids et ledit monomère de réticulation hydrosoluble est utilisé en une quantité comprise entre environ 0,1 et 20% en poids, par rapport à la quantité totale en poids desdits monomères.