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(54) **ENZYME CATALYST PREPARATION METHOD FOR ENZYME BIOFUEL CELL.**

(57) The present invention discloses an enzyme catalyst preparation method for enzyme biofuel cell, comprising the steps of S1, taking graphene oxide GO aqueous dispersion, adding NaOH and sodium chloroacetate, and ultrasonic reaction in a water bath; neutralising it with dilute HCl, and then adding manganese acetate solution by stirring and ultrasonic dispersion treatment, and The mixture was prepared, S2, and to S1 in the mixture was washed with deionised water, and then freeze-dried to obtain the surface carboxyl group-activated graphene oxide powder. By using graphene as an enzyme immobilisation carrier, the enzyme catalytic efficacy can be enhanced through synergistic catalysis, and the enzyme catalyst produced has high activity and stability.

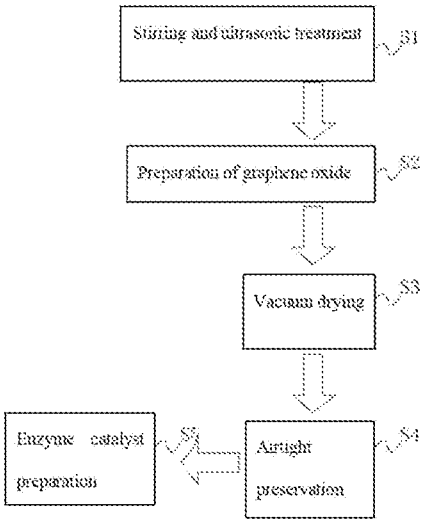

Fig. 1

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ENZYME CATALYST PREPARATION METHOD FOR ENZYME BIOFUEL CELL

TECHNICAL FIELD

The present invention relates to the technical field of enzyme biofuel cell, specifically an enzyme catalyst preparation method for enzyme biofuel cell.

BACKGROUND

An enzyme biofuel cell is a special type of fuel cell that uses enzymes as catalysts to oxidise its fuel rather than precious metals. Enzyme biofuel cells, although currently limited to research facilities, are widely acclaimed for their relatively inexpensive components and fuels as well as potential power source for bionic implants.

Enzymatic biofuel cells work on the same principle as all fuel cells: a catalyst is used to separate the electrons from the parent molecule and force them through a wire that bypasses an electrolyte barrier in order to generate an electric current. What makes enzymatic biofuel cells different from more traditional fuel cells is the catalyst they use and the fuel they accept. Most fuel cells use metals such as platinum and nickel as catalysts, whereas enzyme biofuel cells use enzymes from living cells, and enzyme catalysts prepared in the prior art for enzyme biofuel cells are generally active and unstable, and the process is complex and costly to use, and therefore the present invention proposes an enzyme catalyst preparation method for enzyme biofuel cell to solve the above mentioned problems.

SUMMARY

For the deficiencies of the prior art, the present invention provides an enzyme catalyst preparation method for enzyme biofuel cell, which solves the above mentioned problems of the background art.

In order to achieve the above purpose, the present invention is realised by the following technical solution: an enzyme catalyst preparation method for enzyme biofuel cell, specifically comprising the following steps:

S1, stirring and ultrasonic treatment: take a certain amount of graphene oxide GO aqueous dispersion, add an appropriate amount of NaOH and sodium chloroacetate, and ultrasonicate the reaction in a water bath for a certain period of time; neutralise it with dilute HCl, and then add manganese acetate solution through the reactor after stirring and processing in ultrasonic dispersion, and then leave it to make a mixture;

S2, the preparation of graphene oxide: the mixture in step S1 is then washed with deionised water to neutral, and then freeze-dried to obtain the surface carboxyl group activated graphene oxide powder.

S3, vacuum drying: the graphene oxide powder in step S2 is subjected to vacuum drying treatment through a vacuum dryer;

S4, airtight preservation: airtight preservation of the dried graphene oxide powder by means of a sealed bag;

S5, enzyme catalyst preparation: then add graphene oxide powder to Tris-HCl buffer, ultrasonic dispersion for a certain period of time, add a certain volume of 50% glutaraldehyde solution to the above solution, stirring at room temperature for 30 min, the resulting precipitate was washed with deionised water, ethanol, and then the precipitate separated to dry, the resulting precipitate was washed with deionised water at 500-600°C The enzyme catalyst was produced after carbonisation under the condition.

Further, said standing in said step S1 is standing at 10 to 20°C for 24h.

Further, said stirring in said step S1 is reacted for 12±2h using a microwave reactor heated to 260±30°C.

Further, the concentration of dilute HCL in said step S1 was 0.1-0.2 mol/L.

Further, the frequency of ultrasonic dispersion in said step S5 is 2000 KHz.

Further, the temperature of the vacuum drying treatment in said step S3 is 100°C and the treatment time is 1-2h.

Further, the concentration of the aqueous manganese acetate solution in said step S1 is 0.3-0.6 mol/L.

BENEFICIAL EFFECTS

The present invention provides an enzyme catalyst preparation method for enzyme biofuel cell. with the following beneficial effects compared with the prior art:

The enzyme catalyst preparation method for enzyme biofuel cell can enhance the enzyme catalytic efficacy by using graphene as an enzyme immobilisation carrier through synergistic catalytic effect, and the enzyme catalyst produced has high activity and stability, and the process is simple, the use cost is low, and industrial production is utilised.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a flow chart of the preparation process of the enzyme catalyst of the present invention;

FIG. 2 shows a chart comparing the market enzyme catalyst of the present invention with the embodiments;

FIG. 3 shows a table diagram comparing Examples 1-3 of the present invention.

DETAILED DESCRIPTION

The technical solutions in the embodiments of the present invention will be described clearly and completely in the following in conjunction with the accompanying drawings in the embodiments of the present invention, and it is obvious that the described embodiments are only a part of the embodiments of the present invention and not all of the embodiments. Based on the embodiments in the present invention, all other embodiments obtained by a person of ordinary skill in the art without making creative labour fall within the scope of protection of the present invention.

Referring to FIG. 1, the present invention provides a technical solution:

Example 1

An enzyme catalyst preparation method for enzyme biofuel cell, specifically comprising the following steps:

S1, stirring and ultrasonic treatment: take a certain amount of 100 ml of graphene oxide GO aqueous dispersion, add 5 g of NaOH and 5 g of sodium chloroacetate, and ultrasonicate the reaction for a certain period of time in a water bath; neutralise the reaction with dilute HCl, and then add manganese acetate solution by stirring and treating it through a reactor before ultrasonically dispersing it, and then leave it to stand for 24h at 10°C to make the mixture;

S2, graphene oxide preparation: the mixture in step S1 is then washed with deionised water to neutral, and then freeze-dried to obtain the surface carboxyl group activated graphene oxide powder.

S3, vacuum drying: the graphene oxide powder in step S2 is subjected to vacuum drying treatment at a temperature of 100°C for 1h through a vacuum dryer;

S4, airtight preservation: airtight preservation of the dried graphene oxide powder through a sealed bag;

S5, enzyme catalyst preparation: then graphene oxide powder was added to Tris-HCl buffer 0.07 mol/L, pH 7.0), ultrasonically dispersed for 30 min, a certain volume of 50% glutaraldehyde

solution was added to the above solution, and stirred for 30 min at room temperature, and the resulting precipitate was washed with deionised water and ethanol, and then the separated precipitate was dried, and the resulting precipitate was washed with deionised water carbonised at 500°C to produce the enzyme catalyst.

Step S1 in the case of stirring using a microwave reactor was heated to 260 ± 30 °C under the reaction of 12 ± 2h.

The concentration of dilute HCL in step S1 was 0.1 mol/L.

The frequency of ultrasonic dispersion in Step S5 was 2000 KHz.

The temperature of the vacuum drying treatment in Step S3 was 100°C, and the treatment time was 1 h. The concentration of manganese acetate aqueous solution in Step S1 was 0.3 mol/L.

Example 2

An enzyme catalyst preparation method for enzyme biofuel cell, specifically comprising the following steps:

S1, stirring and ultrasonic treatment: take a certain amount of 100 ml of graphene oxide GO aqueous dispersion, add 5 g of NaOH and 5 g of sodium chloroacetate, and ultrasonicate the reaction for a certain period of time in a water bath; neutralise it with dilute HCl, and then add manganese acetate solution through the reactor after stirring and treatment in the ultrasonic dispersion, and then the mixture was made by static standing for 24 h at 15 °C;

S2, graphene oxide preparation: the mixture in step S1 is then washed with deionised water to neutral, and then freeze-dried to obtain the surface carboxyl group activated graphene oxide powder.

S3, vacuum drying: the graphene oxide powder in step S2 is subjected to vacuum drying treatment for 1.5h at a temperature of 100°C by a vacuum dryer;

S4, airtight preservation: airtight preservation of the dried graphene oxide powder through a sealed bag;

S5, enzyme catalyst preparation: then graphene oxide powder was added to Tris-HCl buffer 0.07 mol/L, pH 7.0), ultrasonically dispersed for 30 min, a certain volume of 50% glutaraldehyde solution was added to the above solution, and stirred for 30 min at room temperature, and the resulting precipitate was washed with deionised water and ethanol, and then the precipitate isolated was dried, and the resulting precipitate was washed with deionised water carbonised at

550°C to produce the enzyme catalyst.

Step S1 in the case of stirring using a microwave reactor was heated to 260 ± 30 °C under the reaction of 12 ± 2h.

The concentration of dilute HCL in step S1 was 0.15 mol/L.

The frequency of ultrasonic dispersion in Step S5 was 2000 KHz.

The temperature of the vacuum drying treatment in Step S3 was 100°C, and the treatment time was 1.5 h. The concentration of manganese acetate aqueous solution in Step S1 was 0.45 mol/L.

Example 3

An enzyme catalyst preparation method for enzyme biofuel cell, specifically comprising the following steps:

S1, stirring and ultrasonic treatment: take a certain amount of 100 ml of graphene oxide GO aqueous dispersion, add 5 g of NaOH and 5 g of sodium chloroacetate, and ultrasonicate the reaction for a certain period of time in a water bath; neutralise it with dilute HCl, and then add the manganese acetate solution through the reactor with stirring and treatment before ultrasonically dispersing it, and then leave it to stand for 24h at 20°C to make the mixture;

S2, graphene oxide preparation: the mixture in step S1 is then washed with deionised water to neutral, and then freeze-dried to obtain the surface carboxyl group activated graphene oxide powder.

S3, vacuum drying: the graphene oxide powder in step S2 is subjected to vacuum drying treatment at a temperature of 100°C for 2h through a vacuum dryer;

S4, airtight preservation: airtight preservation of the dried graphene oxide powder through a sealed bag;

S5, enzyme catalyst preparation: then graphene oxide powder was added to Tris-HCl buffer 0.07 mol/L, pH 7.0), ultrasonically dispersed for 30 min, a certain volume of 50% glutaraldehyde solution was added to the above solution, and stirred for 30 min at room temperature, and the resulting precipitate was washed with deionised water and ethanol, and then the precipitate isolated was dried, and the resulting precipitate was washed with deionised water carbonised at 600°C to produce the enzyme catalyst.

Step S1 in the case of stirring using a microwave reactor was heated to 260 ± 30 °C under the reaction of 12 ± 2h.

The concentration of dilute HCL in step S1 was 0.2 mol/L.

The frequency of ultrasonic dispersion in Step S5 was 2000 KHz.

The temperature of vacuum drying treatment in Step S3 was 100°C, and the treatment time was 2 h. The concentration of manganese acetate aqueous solution in Step S1 was 0.6 mol/L.

Comparison experiment

An enzyme catalyst manufacturer, respectively, selected the enzyme catalyst of the preparation process in Examples 1-3 and the market enzyme catalyst for activity and stability comparison experiments, as can be seen from Fig. 2, the enzyme catalyst of the preparation process in Examples 1-3 has an activity of 0.94, a stability of 0.85, and the market enzyme catalyst has an activity of 0.80, a stability of 0.6, which shows that the enzyme catalyst of the invention is much better than the market enzyme catalyst activity and stability. Far better than the market enzyme catalyst activity and stability, as can be seen from Figure 3, the enzyme catalyst prepared in Example 2 has the highest enzyme catalyst activity and stability, and is the preferred solution; the remaining two are available.

Meanwhile, the contents not described in detail in this specification belong to the prior art known to the technicians in the field.

It is to be noted that in this paper, relationship terms such as first and second are used only to distinguish one entity or operation from another, and do not necessarily require or imply that any such actual relationship or order exists between these entities or operations. Further, the terms "including", "comprising", or any other variant thereof, are intended to cover non-exclusive inclusion, such that a process, method, article, or apparatus comprising a set of elements includes not only those elements, but also other elements not expressly listed, or other elements that are not expressly listed for the purpose of such a process, method, article or apparatus. elements, or also includes elements that are inherent to such process, method, article, or apparatus.

Although embodiments of the present invention have been shown and described, it will be appreciated by those of ordinary skill in the art that a variety of changes, modifications, substitutions, and variations may be made to these embodiments without departing from the principle and spirit of the present invention, and that the scope of the present invention is limited by the appended claims and their equivalents.

CLAIMS

1. Enzyme catalyst preparation method for enzyme biofuel cell, characterised in that: it specifically includes the following steps:

S1, stirring ultrasonic treatment: take a certain amount of graphene oxide GO aqueous dispersion, add an appropriate amount of NaOH and sodium chloroacetate, water bath ultrasonic reaction for a certain period of time; neutralisation with dilute HCl, and then add manganese acetate solution through the reactor after stirring treatment in the ultrasonic dispersion, and then static to produce the mixture;

S2, graphene oxide preparation: the mixture in step S1 is then washed with deionised water to neutral, and then freeze-dried to obtain the surface carboxyl group activated graphene oxide powder;

S3, vacuum drying: the graphene oxide powder in step S2 is subjected to vacuum drying treatment through a vacuum dryer;

S4, airtight preservation: airtight preservation of the dried graphene oxide powder by means of a sealed bag;

S5, enzyme catalyst preparation: then add graphene oxide powder to Tris-HCl buffer, ultrasonic dispersion for a certain period of time, add a certain volume of 50% glutaraldehyde solution to the above solution, stirring at room temperature for 30 min, the resulting precipitate was washed with deionised water, ethanol, and then the precipitate separated to dry, the resulting precipitate was washed with deionised water at 500-600°C The enzyme catalyst was produced after carbonisation under conditions.

2. Enzyme catalyst preparation method for enzyme biofuel cell according to claim 1, characterised in that: said standing in said step S1 is standing at 10～20°C for 24h.

3. Enzyme catalyst preparation method for enzyme biofuel cell according to claim 1, characterised in that: said stirring in said step S1 is reacted for 12±2h using a microwave reactor heated to 260±30°C.

4. Enzyme catalyst preparation method for enzyme biofuel cell according to claim 1, characterised in that: the concentration of dilute HCL in said step S1 is 0.1-0.2 mol/L.

5. Enzyme catalyst preparation method for enzyme biofuel cell according to claim 1,

characterised in that the frequency of ultrasonic dispersion in said step S5 is 2000 KHz.

6. Enzyme catalyst preparation method for enzyme biofuel cell according to claim 1, characterised in that the temperature of the vacuum drying treatment in said step S3 is 100°C and the treatment time is 1-2h.

7. Enzyme catalyst preparation method for enzyme biofuel cell according to claim 1, characterised in that the concentration of manganese acetate aqueous solution in said step S1 is 0.3-0.6 mol/L.

REVENDICATIONS

1. Méthode de préparation d'un catalyseur enzymatique pour une cellule de biocarburant enzymatique, caractérisée par le fait qu'elle comprend spécifiquement les étapes suivantes :

S1, traitement ultrasonique par agitation : prendre une certaine quantité de dispersion aqueuse d'oxyde de graphène GO, ajouter une quantité appropriée de NaOH et de chloroacétate de sodium, réaction ultrasonique au bain-marie pendant un certain temps ; neutralisation avec du HCl dilué, puis ajouter une solution d'acétate de manganèse à travers le réacteur après un traitement par agitation dans la dispersion ultrasonique, puis statique pour produire le mélange ;

S2, préparation de l'oxyde de graphène : le mélange de l'étape S1 est ensuite lavé avec de l'eau désionisée jusqu'à neutralité, puis lyophilisé pour obtenir la poudre d'oxyde de graphène activée par le groupe carboxyle en surface ;

S3, séchage sous vide : la poudre d'oxyde de graphène de l'étape S2 est soumise à un traitement de séchage sous vide dans un séchoir à vide ;

S4, conservation hermétique : conservation hermétique de la poudre d'oxyde de graphène séchée au moyen d'un sac scellé ;

S5, préparation du catalyseur enzymatique : ajouter la poudre d'oxyde de graphène au tampon Tris-HCl, dispersion ultrasonique pendant un certain temps, ajouter un certain volume de solution de glutaraldéhyde à 50% à la solution ci-dessus, agitation à température ambiante pendant 30 min, le précipité résultant a été lavé avec de l'eau désionisée, de l'éthanol, puis le précipité séparé pour sécher, le précipité résultant a été lavé avec de l'eau désionisée à 500-600°C Le catalyseur enzymatique a été produit après la carbonisation dans les conditions.

2. Méthode de préparation du catalyseur enzymatique pour la pile à biocarburant enzymatique selon la revendication 1, caractérisée en ce que : ledit repos dans ladite étape S1 est un repos à 10～20°C pendant 24h.

3. Méthode de préparation du catalyseur enzymatique pour cellule de biocarburant enzymatique selon la revendication 1, caractérisée en ce que : ladite agitation dans ladite étape S1 est mise en réaction pendant 12±2h à l'aide d'un réacteur à micro-ondes chauffé à 260±30°C.

4. Méthode de préparation du catalyseur enzymatique pour la cellule de biocarburant enzymatique selon la revendication 1, caractérisée en ce que : la concentration de HCL dilué

dans ladite étape S1 est de 0,1-0,2 mol/L.

5. Méthode de préparation du catalyseur enzymatique pour la cellule de biocarburant enzymatique selon la revendication 1, caractérisée en ce que la fréquence de la dispersion ultrasonique dans ladite étape S5 est de 2000 KHz.

6. Méthode de préparation du catalyseur enzymatique pour la cellule de biocarburant enzymatique selon la revendication 1, caractérisée en ce que la température du traitement de séchage sous vide dans ladite étape S3 est de 100°C et la durée du traitement est de 1-2h.

7. Méthode de préparation du catalyseur enzymatique pour la cellule de biocarburant enzymatique selon la revendication 1, caractérisée en ce que la concentration de la solution aqueuse d'acétate de manganèse dans ladite étape S1 est de 0,3-0,6 mol/L.

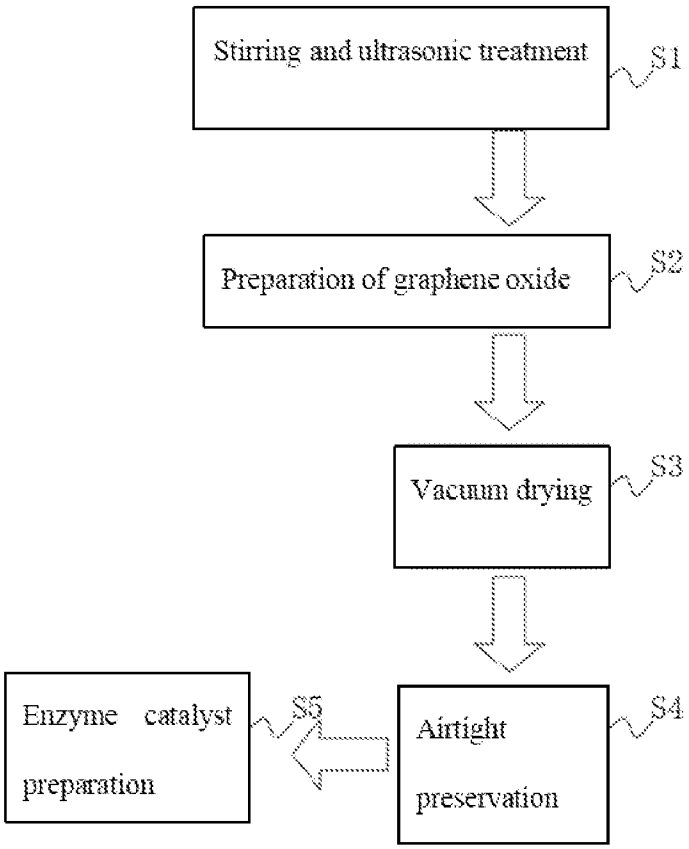
Stirring and ultrasonic treatment
S1
Preparation of graphene oxide
S2
Vacuum drying
S3
Airtight preservation
S4
Enzyme catalyst preparation
S5

Fig. 1

Statistical Projects / Statistical Objects	Activity	Stability
Example 1-3	0.94	0.85
Market enzyme catalyst	0.80	0.6

Fig. 2

Statistical Projects / Statistical Objects	Activity	Stability
Example 1	0.94	0.85
Example 2	0.95	0.86
Example 3	0.93	0.84

Fig. 3