

Method For Enzymatically Producing High-Activity Bio-organic Fertilizer

Technical Field

The invention relates to the technical field of biological fermentation, in particular to a method for enzymatically producing high-activity bio-organic fertilizer.

Background Art

Bio-organic fertilizer is a kind of fertilizer with both microbial fertilizer and organic fertilizer effects, which is composed of specific functional microorganisms and organic materials mainly sourced from animal and plant residues (such as livestock and poultry manure, crop straw, etc.) and undergoes harmless treatment and decomposing. Its characteristic is to add again (secondary addition) the functional microorganisms into high-quality organic fertilizer (after decomposing). The main functions of bio-organic fertilizer include the followings: increasing crop yield, improving crop quality, improving soil fertility, improving soil micro-ecosystem, and reducing the occurrence of plant diseases and insect pests. The executive standard of bio-organic fertilizer is NY884-2012, and its core indicator is the number of effective viable bacteria ≥ 20 million cfu/g. The technical solutions in the prior art mostly adopt the tank-type stacking fermentation method.

The technical solutions in the prior art mainly have the following problems:

1. The fermentation substrate is limited. The bacteria and technology

used are mainly designed for livestock and poultry manure, crop stalks and agricultural organic waste substances, etc., and cannot effectively deal with collagen, keratin and other complex conformational polymer proteins.

2. The functional microorganisms act slowly. The functional microorganisms that are added twice are mostly dormant bodies such as brood cells or spores. Because they are mainly heterotrophic bacteria, they are difficult to be activated after being applied to the soil, resulting in their quite slow action.

3. The cost is higher. The bio-organic fertilizers are generally produced by the ready-to-use microbial agents , so the cost and time burden of purchasing the microbial agents are relatively heavy. Taking the annual production of 10,000 tons of bio-organic fertilizer as an example, the microbial agents alone needs about 1.5 million yuan (RMB).

Summary of the Invention

In order to solve the above-mentioned problems in the prior art, the present invention provides a method for enzymatically producing high-activity bio-organic fertilizer, comprising the following steps:

(1) Pretreatment of waste protein substances: adding protease to the waste protein substances to give the fermentation materials;

(2) Activation of fermentation strains: activating *Bacillus subtilis*, lactic acid bacteria and *Trichoderma* respectively and combining them; and the number of effective viable bacteria after the combination is ≥ 40 million cfu/g;

(3) Fermentation: mixing the fermentation materials obtained in step

(1) with the activated bacteria obtained in step (2), and carrying out the fermentation; when the temperature is below 40°C, carrying out the second addition of bacteria.

In a specific embodiment of the present invention, the waste protein substances refer to the materials containing high molecular proteins with complex conformations such as collagen and keratin, for example, broken cowhide, cow hair, cow hoof nails, waste meat residue and keratin. The waste protein substances can be cut and crushed before use.

In a specific embodiment of the present invention, before adding the protease in step (1), a fermentation substrate is added; the fermentation substrate is one or more selected from the group consisting of edible fungus chaff, rice bran, grain chaff, peanut bran, soybean meal, sugarcane straw powder and mushroom residue; preferably is one or more selected from the group consisting of edible fungus chaff, rice bran, peanut bran and soybean meal.

Preferably, the mass ratio of the waste protein substances to the fermentation substrate(s) is 1:(1~3), preferably is 1:(1.2~2.5), further preferably is 1:(1.5~2), more preferably is 1:2.

In a specific embodiment of the present invention, the protease is alkaline protease.

In a specific embodiment of the present invention, the addition amount of the protease in step (1) is 0.08% to 0.15% of the mass of the waste protein substances, preferably is 0.10% to 0.12%, more preferably is 0.10%. Preferably, while adding the protease, dry casing powder can also be added, and the addition amount of dry casing powder is 3% to 6% of the mass of the waste protein substances, preferably is 5%.

In a specific embodiment of the present invention, before adding protease in step (1), the waste protein substances are boiled with alkali; preferably, the mass concentration of the alkali is 0.03% to 0.08%, preferably is 0.05% to 0.08%, more preferably is 0.05%; further preferably, the boiling time is 25 to 40 minutes, preferably is 30 to 35 minutes, more preferably is 30 minutes. Preferably, before adding the protease, the waste protein substances are subjected to a high-temperature drum for primary dissolution, and then alkali is added to boil.

In a specific embodiment of the present invention, the mass ratio of carbon to nitrogen in the fermentation materials is (20~30):1, preferably is (25~28):1, more preferably is 25:1.

In a specific embodiment of the present invention, the moisture content can be controlled by the combination matching of materials or fermentation substrates, in order to better adjust the carbon-nitrogen ratio required by the fermentation of the bacteria. Preferably, the moisture content of the fermentation materials is 58% to 68%, more preferably is 60% to 65%.

In a specific embodiment of the present invention, in step (2), *Bacillus subtilis* is proliferated with NB culture medium, lactic acid bacteria are proliferated with MRS culture medium, and then respectively added to the combination of bran and soybean meal to expand and activate. *Trichoderma* is added to the combination of bran and soybean meal to activate.

In the step of activation of fermentation strains, *Bacillus subtilis* and lactic acid bacteria are respectively proliferated with culture medium, and then added to the composition of bran and soybean meal for expansion

and activation. In this way, such liquid-solid two-phase fermentation method can be adapted to activate the strains, which can obtain a multiplier effect.

In a specific embodiment of the present invention, the moisture content of the composition of bran and soybean meal is 50% to 70%, and the mass ratio of bran to soybean meal is (8~10):1.

In a specific embodiment of the present invention, in the second addition of bacteria in step (3) is carried out by, adding one or more bacteria selected from the group consisting of *Bacillus subtilis*, *Bacillus mucilaginosus*, *Bacillus amyloliquefaciens*, *Streptomyces fradiae* and photosynthetic bacteria; preferably, *Bacillus subtilis*, *Bacillus mucilaginosus*, *Bacillus amyloliquefaciens* and *Streptomyces fradiae* can be added after activation with NB culture medium, and photosynthetic bacteria can be sprayed directly; more preferably, all the above strains are added after activation.

In the step of second addition of bacteria, parts or all of the activated beneficial microorganisms are added to make the effects of biological activity of the fertilizer quick and lasting.

The NB culture medium and MRS culture medium used in the present invention are culture mediums commonly used in the field, and both are commercially available.

Another object of the present invention is to provide a high-activity bio-organic fertilizer produced by the method according to the present invention.

The co-action of enzymes and microorganisms is adopted in the technical solutions of the present invention, that is, the enzymatic

biotechnology is adopted to effectively degrade the polymer proteins with complex conformations such as collagen and keratin.

In the technical solutions of the present invention, the protein waste substances is pretreated by enzymatic hydrolysis, and the activated microbial strains are added, which shortens the fermentation time and improves the fermentation efficiency.

The present invention has at least one of the following beneficial effects:

1. High activity. The number of effective viable bacteria can reach 40 million cfu/g, which is far higher than the basic requirement of the NY884-2012 standard of 20 million cfu/g.

2. Good fertilizer efficiency. The vegetative mass and the dormant body of the effective live bacteria are mixed. After being applied to the soil, the vegetative mass can take effect immediately, and the dormant body can play a long-term effect at a later stage.

3. Low cost. The use of enzymatic biotechnology can save about 10 days of fermentation time, and the use of activated bacteria can save more than half of the cost of commercial microbial agents.

4. The production environment is friendly. Adding lactic acid bacteria, photosynthetic bacteria, etc. in the fermentation process can effectively degrade ammonia nitrogen and hydrogen sulfide, which is environmentally friendly.

Brief Description of the Drawings

Figure 1 is a flow chart of the method for enzymatically producing high-activity bio-organic fertilizer of the present invention.

Best Mode of the Invention

The present invention is further illustrated by the following examples. It should be understood that the preparation methods described in the examples are only used to illustrate the present invention, and is not intent to limit the present invention. Any simple improvement of the preparation method of the invention under the premise of the conception of the invention falls within the scope of the protection required by the claims.

Refer to Figure 1 for the flowchart of the following examples.

Example 1

Taking the production of high-activity bio-organic fertilizer by processing 20 tons of waste protein substances per day as an example:

1. Pretreatment of the waste protein substances: 20 tons of the waste protein substances (broken cowhide, cow hair, cow hoof nails) were cut and crushed into 1cm to 2cm, and put in a plastic tank, added 0.05% NaOH solution and boiled for 30 minutes, and then mixed with 30 tons of edible fungus chaff and 10 tons of rice bran, and added 20 kg of alkaline protease.

2. Adjustment of the moisture content: the moisture content of the pretreated materials in step 1 was adjusted to 60% with edible fungus chaff to obtain the fermentation materials. The carbon to nitrogen ratio in the fermentation materials was 25:1.

3. Activation of fermentation strains: 10 g of *Bacillus subtilis* were proliferated with 5,000 mL NB culture medium and 10 g of lactic acid bacteria were proliferated with 5,000 mL MRS culture medium, and then respectively added to 50 kg of combination of bran and soybean meal (9:1) with 60% moisture content for expansion to obtain 100 kg of

activated bacteria; 1 kg of *Trichoderma* was added to 20 kg of combination of bran and soybean meal (9:1) with 60% moisture content to activate; and the activated bacteria were combined with the number of effective viable bacteria ≥ 40 million cfu/g.

4. Pile fermentation: the fermentation materials were mixed with the activated strains evenly, then a pile was built with a flat pile height of 1.2 meters and a width of 2.5 meters, timely turning over to make the temperature not exceed 70 °C; after 7 days, the temperature began to drop slowly and when the temperature dropped to about 40°C, the second addition of bacteria was carried out, i.e., *Bacillus subtilis*, *Bacillus mucilaginosus*, *Bacillus amyloliquefaciens* and *Streptomyces fradiae* were added after activation with NB culture medium, and photosynthetic bacteria were sprayed directly.

5. Processing after being decomposed. The fermentation products were dried at low temperature (below 40°C) to moisture content $\leq 30\%$, crushed, and packed after passing the inspection. Finally, 50 tons of high-activity bio-organic fertilizer was obtained.

Example 2

Taking the production of high-activity bio-organic fertilizer by processing 25 tons of waste protein substances per day as an example:

1. Pretreatment of the waste protein substances: 25 tons of the waste protein substances (broken cowhide, cow hair, cow hoof nails) were cut and crushed into 2cm, and put in a plastic tank, added 0.06% NaOH solution and boiled for 35 minutes, and then mixed with 30 tons of edible fungus chaff and 7.5 tons of grain chaff, and added 30 kg of alkaline protease.

2. Adjustment of the moisture content: the moisture content of the

pretreated materials in step 1 was adjusted to 65% with grain chaff to obtain the fermentation materials. The carbon to nitrogen ratio in the fermentation materials was 23:1.

3. Activation of fermentation strains: 12 g of *Bacillus subtilis* were proliferated with 5,000 mL NB culture medium and 12 g of lactic acid bacteria were proliferated with 5,000 mL MRS culture medium, and then respectively added to 50 kg of combination of bran and soybean meal (8:1) with 65% moisture content for expansion to obtain 100 kg of activated bacteria; 1.2 kg of *Trichoderma* was added to 20 kg of combination of bran and soybean meal (8:1) with 65% moisture content to activate; and the activated bacteria were combined with the number of effective viable bacteria ≥ 42 million cfu/g.

4. Pile fermentation: the fermentation materials were mixed with the activated strains evenly, then a pile was built with a flat pile height of 1.5 meters and a width of 2.5 meters, timely turning over to make the temperature not exceed 70 °C; after 7 days, the temperature began to drop slowly and when the temperature dropped to about 40°C, the second addition of bacteria was carried out, i.e., *Bacillus subtilis*, *Bacillus mucilaginosus*, *Bacillus amyloliquefaciens* and *Streptomyces fradiae* were added after activation with NB culture medium, and photosynthetic bacteria were sprayed directly.

5. Processing after being decomposed. The fermentation products were dried at low temperature (below 40°C) to moisture content $\leq 30\%$, crushed, and packed after passing the inspection. Finally, 55 tons of high-activity bio-organic fertilizer was obtained.

Example 3

Taking the production of high-activity bio-organic fertilizer by

processing 15 tons of waste protein substances per day as an example:

1. Pretreatment of the waste protein substances: 15 tons of the waste protein substances (broken cowhide, cow hair, cow hoof nails) were cut and crushed into 1 cm, and put in a plastic tank, added 0.03% NaOH solution and boiled for 30 minutes, and then mixed with 10 tons of grain chaff and 8 tons of mushroom residue, and added 12 kg of alkaline protease.

2. Adjustment of the moisture content: the moisture content of the pretreated materials in step 1 was adjusted to 58% with edible fungus chaff to obtain the fermentation materials. The carbon to nitrogen ratio in the fermentation materials was 28:1.

3. Activation of fermentation strains: 10 g of *Bacillus subtilis* were proliferated with 5,000 mL NB culture medium and 10 g of lactic acid bacteria were proliferated with 5,000 mL MRS culture medium, and then respectively added to 50 kg of combination of bran and soybean meal (10:1) with 50% moisture content for expansion to obtain 100 kg of activated bacteria; 1 kg of *Trichoderma* was added to 20 kg of combination of bran and soybean meal (10:1) with 50% moisture content to activate; and the activated bacteria were combined with the number of effective viable bacteria ≥ 40 million cfu/g.

4. Pile fermentation: the fermentation materials were mixed with the activated strains evenly, then a pile was built with a flat pile height of 1.2 meters and a width of 2.5 meters, timely turning over to make the temperature not exceed 70 °C; after 7 days, the temperature began to drop slowly and when the temperature dropped to about 40°C, the second addition of bacteria was carried out, i.e., *Bacillus subtilis*, *Bacillus mucilaginosus*, *Bacillus amyloliquefaciens* and *Streptomyces fradiae* were

added after activation with NB culture medium, and photosynthetic bacteria were sprayed directly.

5. Processing after being decomposed. The fermentation products were dried at low temperature (below 40°C) to moisture content $\leq 30\%$, crushed, and packed after passing the inspection. Finally, 28 tons of high-activity bio-organic fertilizer was obtained.

Example 4

Taking the production of high-activity bio-organic fertilizer by processing 30 tons of waste protein substances per day as an example:

1. Pretreatment of the waste protein substances: 30 tons of the waste protein substances (broken cowhide, cow hair, cow hoof nails) were cut and crushed into 2cm, and put in a plastic tank, added 0.08% NaOH solution and boiled for 40 minutes, and then mixed with 40 tons of edible fungus chaff and 20 tons of rice bran, and added 45 kg of alkaline protease.

2. Adjustment of the moisture content: the moisture content of the pretreated materials in step 1 was adjusted to 68% with edible fungus chaff to obtain the fermentation materials. The carbon to nitrogen ratio in the fermentation materials was 20:1.

3. Activation of fermentation strains: 15 g of *Bacillus subtilis* were proliferated with 5,000 mL NB culture medium and 15 g of lactic acid bacteria were proliferated with 5,000 mL MRS culture medium, and then respectively added to 50 kg of combination of bran and soybean meal (9:1) with 60% moisture content for expansion to obtain 100 kg of activated bacteria; 1.5 kg of *Trichoderma* was added to 20 kg of combination of bran and soybean meal (9:1) with 60% moisture content to activate; and the activated bacteria were combined with the number of

effective viable bacteria ≥ 45 million cfu/g.

4. Pile fermentation: the fermentation materials were mixed with the activated strains evenly, then a pile was built with a flat pile height of 1.5 meters and a width of 3 meters, timely turning over to make the temperature not exceed 70 °C; after 7 days, the temperature began to drop slowly and when the temperature dropped to about 40°C, the second addition of bacteria was carried out, i.e., *Bacillus subtilis*, *Bacillus mucilaginosus*, *Bacillus amyloliquefaciens* and *Streptomyces fradiae* were added after activation with NB culture medium, and photosynthetic bacteria were sprayed directly.

5. Processing after being decomposed. The fermentation products were dried at low temperature (below 40°C) to moisture content $\leq 30\%$, crushed, and packed after passing the inspection. Finally, 80 tons of high-activity bio-organic fertilizer was obtained.

Example 5

Taking the production of high-activity bio-organic fertilizer by processing 20 tons of waste protein substances per day as an example, the operation method is the same as that in Example 1, except that step (1) is carried out as follows: 20 tons of the waste protein substances (cow hair, cow hoof nails, waste meat residue) was cut and crushed into 1cm to 2cm, and put in a high temperature drum for primary dissolution, added 0.05% NaOH solution and boiled for 30 minutes, then mixed with 30 tons of edible fungus chaff and 10 tons of rice bran, and added 20 kg of alkaline protease and 1 ton of dry casing powder, adjusting the pH to not higher than 8.5.

Experimental example 1

The experiment was carried out on radishes with farm organic

fertilizer, and the administration amount of fertilizer was 500 kg/mu. The yield of radish was 5,000 kg/mu.

The bio-organic fertilizer of Example 1 according to the present invention was tested on radishes, and the administration amount of fertilizer was 350 kg/mu. The yield of radish was more than 5,000 kg/mu, and the quality and weight were both better than that of the farm organic fertilizer.

Experimental example 2

The experiment was carried out on Chinese cabbage with farm organic fertilizer, and the administration amount of fertilizer was 400 kg/mu. The yield of Chinese cabbage was 2,500 kg/mu.

The bio-organic fertilizer of Example 2 according to the present invention was tested on Chinese cabbage, and the administration amount of fertilizer was 300 kg/mu. The yield of Chinese cabbage reached 3,500 kg/mu, and the quality and weight were both better than that of the farm organic fertilizer.

Experimental example 3

The experiment was carried out on Chinese chives with farm organic fertilizer, and the administration amount of fertilizer was 500 kg/mu. The yield of Chinese chives was 3,500 kg/mu.

The bio-organic fertilizer of Example 3 according to the present invention was tested on Chinese chives, and the administration amount of fertilizer was 400 kg/mu. The yield of Chinese chives was 4,500 kg/mu, and the quality and weight were both better than that of the farm organic fertilizer.

Experimental example 4

The experiment was carried out on swamp cabbages with farm organic fertilizer, and the administration amount of fertilizer was 500 kg/mu. The yield of swamp cabbages was 3,500 kg/mu.

The bio-organic fertilizer of Example 4 according to the present invention was tested on swamp cabbages, and the administration amount of fertilizer was 400 kg/mu. The yield of swamp cabbages reached 4,500 kg/mu, and the quality and weight were both better than that of the farm organic fertilizer.

Experimental example 5

The experiment was carried out on Chinese wax gourd with farm organic fertilizer, and the administration amount of fertilizer was 500 kg/mu. The yield of Chinese wax gourd was 5,000 kg/mu.

The bio-organic fertilizer of Example 5 according to the present invention was tested on Chinese wax gourd, and the administration amount of fertilizer applied was 400 kg/mu. The yield of Chinese wax gourd was more than 5,000 kg/mu, and the quality and weight were both better than that of the farm organic fertilizer.

The above test results are summarized in Table 1.

Table 1. The comparative test results of the bio-organic fertilizer of the present invention and the farm organic fertilizer

Variety	Farm organic fertilizer		The bio-organic fertilizer of the present invention	
	Administration amount (kg/mu)	Yield (kg/mu)	Administration amount (kg/mu)	Yield (kg/mu)
Radishes	500	5000	350	5500

Chinese cabbage	400	2500	300	3500
Chinese chives	500	3500	400	4500
Swamp cabbages	500	3500	400	4500
Chinese wax gourd	500	5000	400	5500

It can be seen from the results in Table 1 that compared with the farm organic fertilizer, the administration amount of the bio-organic fertilizer of the present invention per mu can be reduced by 20%-30%, and the yield per mu can be increased by 10%-40%. For agricultural production, the method according to the invention may save costs, increase yield, and have very considerable economic benefits.

The preferred embodiments of the present invention are described in detail above. However, the present invention is not limited to the specific details in the above-mentioned embodiments. Within the scope of the technical concept of the present invention, various simple modifications can be made to the technical solution of the present invention. These simple modifications all belong to the protection scope of the present invention.

In addition, it should be noted that the various specific technical features described in the above-mentioned specific embodiments can be combined in any suitable manner without contradiction. In order to avoid unnecessary repetition, the combination method of the present invention will not be explained separately.

In addition, various different embodiments of the present invention can also be combined arbitrarily, as long as they do not violate the idea of the present invention, they should also be regarded as the content

disclosed in the present invention.

CLAIMS

What is claimed is:

1. A method for enzymatically producing high-activity bio-organic fertilizer, comprising the following steps:

(1) Pretreatment of waste protein substances: adding protease to the waste protein substances to give the fermentation materials;

(2) Activation of fermentation strains: activating *Bacillus subtilis*, lactic acid bacteria and *Trichoderma* respectively and combining them; and the number of effective viable bacteria after the combination is ≥ 40 million cfu/g;

(3) Fermentation: mixing the fermentation materials obtained in step (1) with the activated bacteria obtained in step (2), and carrying out the fermentation; when the temperature is below 40°C, carrying out the second addition of bacteria.

2. The method according to claim 1, wherein before adding the protease in step (1), a fermentation substrate is added; the fermentation substrate is one or more selected from the group consisting of edible fungus chaff, rice bran, grain chaff, peanut bran, soybean meal, sugarcane straw powder and mushroom residue; preferably is one or more selected from the group consisting of edible fungus chaff, rice bran, peanut bran and soybean meal;

preferably, the mass ratio of the waste protein substances to the fermentation substrate(s) is 1:(1~3); more preferably is 1:(1.2~2.5).

3. The method according to claim 1, wherein the protease is an alkaline protease.

4. The method according to any one of claims 1 to 3, wherein the addition amount of the protease in step (1) is 0.08% to 0.15% of the mass

of the waste protein substances, preferably is 0.10% to 0.12%.

5. The method according to any one of claims 1 to 3, wherein before adding protease in step (1), the waste protein substances are boiled with alkali; preferably, the mass concentration of the alkali is 0.03% to 0.08%, more preferably is 0.05% to 0.08%; further preferably, the boiling time is 25 to 40 minutes, more preferably is 30 to 35 minutes.

6. The method according to any one of claims 1 to 3, wherein the mass ratio of carbon to nitrogen in the fermentation materials is (20~30):1, preferably is (25~28):1, more preferably is 25:1; further preferably, the moisture content of the fermentation materials is 58% to 68%, more preferably is 60% to 65%.

7. The method according to any one of claims 1 to 3, wherein in step (2), *Bacillus subtilis* is proliferated with NB culture medium, lactic acid bacteria are proliferated with MRS culture medium, and then respectively added to the composition of bran and soybean meal to expand and activate; and *Trichoderma* is added to the combination of bran and soybean meal to activate.

8. The method according to claim 7, wherein the moisture content of the composition of bran and soybean meal is 50% to 70%, and the mass ratio of bran to soybean meal is (8~10):1.

9. The method according to any one of claims 1 to 3, wherein the second addition of bacteria in step (3) is carried out by adding one or more bacteria selected from the group consisting of *Bacillus subtilis*, *Bacillus mucilaginosus*, *Bacillus amyloliquefaciens*, *Streptomyces fradiae* and photosynthetic bacteria; preferably, *Bacillus subtilis*, *Bacillus mucilaginosus*, *Bacillus amyloliquefaciens* and *Streptomyces fradiae* can be added after activation with NB culture medium, and photosynthetic

bacteria can be sprayed directly.

10. A high-activity bio-organic fertilizer produced by the method according to any one of claims 1 to 9.