SUMMARY

[005a] In a first aspect of the present disclosure, there is provided a method for recovering polyhydroxyalkanoates from a biomass of microorganism cells containing intracellular polyhydroxyalkanoates, the method comprising the steps of:

enzymatically treating the microorganism cells of the biomass by:

(a) cleaving polynucleotide chains from the microorganism cells by addition of an endonuclease to the biomass;

(b) lysing the microorganism cells in the biomass by addition of a lysing agent to the biomass so as to disrupt cell walls of the microorganism cells and release the intracellular polyhydroxyalkanoates from the microorganism cells; and

(c) degrading proteins from the microorganism cells by addition of a peptidase to the biomass; and

separating polyhydroxyalkanoates from cellular debris of the microorganism cells,

wherein the endonuclease is added to the biomass prior to addition of the lysing agent to the biomass and the lysing agent is added to the biomass prior to addition of the peptidase to the biomass, and

wherein the method is carried out without the use of organic solvents in the cleaving, lysing, and degrading steps.

[006] In a first example, the present disclosure provides a method for recovering polyhydroxyalkanoates from a biomass of microorganism cells containing intracellular polyhydroxyalkanoates. According to one example, the method includes a step of enzymatically treating the microorganism cells of the biomass by: (a) cleaving polynucleotide chains from the microorganism cells by addition of an endonuclease to the biomass; (b) lysing the microorganism cells in the biomass by addition of a lysing agent to the biomass so as to disrupt cell walls of the microorganism cells and release the intracellular polyhydroxyalkanoates from the microorganism cells; and (c) degrading proteins from the microorganism cells by addition of a peptidase, to the biomass. Thereafter, the polyhydroxyalkanoates are separated from cellular debris of the microorganism cells. Importantly, according to the present disclosure, this method is carried out without the use of organic solvents in the cleaving, lysing, and degrading steps.

[007] According to certain examples, the endonuclease is preferably a deoxyribonuclease. In certain other examples, the endonuclease is preferably a ribonuclease.

[008] In some examples, the lysing agent preferably includes an agent selected from the group consisting of lysozyme, bromelain, papain, trypsin, and mixtures thereof. More preferably, the lysing agent comprises lysozyme. The lysing agent is preferably present in an amount from about 0.01 mg/L to about 8 g/L, preferably from about 0.01 mg/L to about 2 g/L, based on the volume of the biomass. Moreover, in certain examples, the lysing agent preferably also includes a non-ionic surfactant and a detergent.

[009] In some instances, the lysing step is preferably carried out at a temperature from about 20 ℃ to about 70 ℃ for a period of time from about 20 minutes to about 24 hours.

[010] In certain examples, the peptidase preferably includes a protease selected from the group consisting of a serine protease, a neutral metalloproteinase, a cysteine protease, and mixtures thereof. Also, according to certain examples, the protein degradation step is preferably carried out at a temperature from about 30 ℃ to about 65 ℃ for a period of time from about 20 minutes to about 24 hours.

[011] In certain examples, the microorganism cells are preferably bacterial cells. More preferably, the microorganism cells comprise at least one bacterial species selected from the group consisting of the genera *Ralstonia*, *Bacillus*, *Escherchia*, *Cupriavidus*, *Alcaligenes*, *Wausteria*, *Aeromonas*, and *Pseudomonas*. A particularly preferred species is *E. coli*.

[012] In some instances, the method also includes an additional step, prior to the enzymatic treatment, of inactivating the microorganism cells in the biomass. This inactivation may be carried out in various ways. For instance, in one example, the cells may be inactivated by exposing the cells to electromagnetic radiation. Alternatively, in another example, the cells may be inactivated by exposing the cells to infrared radiation energy in an amount sufficient to heat the biomass to a temperature of at least 50 ℃ for a period of time of at least 2 minutes.

[013] In a third example, the cells may be inactivated by injecting steam into the biomass. In still another example, the cells may be inactivated by either: (a) adding one or more acids to the biomass in amount sufficient to establish a biomass pH which is less than about 6.0; or (b) adding one or more bases to the biomass in amount sufficient to establish a biomass pH which is greater than about 8.0; or (c) high pressure (shear) homogenization of the biomass.

[014] In some examples, the method also includes an additional step, of bleaching the polyhydroxyalkanoates from the microorganism cells by addition of an oxidizing agent (preferably an oxidizing bleach) to the biomass.

[015] According to certain examples, the step of separating the polyhydroxyalkanoates from the cellular debris is preferably carried out by filtration or centrifugation.

[016] In certain examples a detergent is also preferably added to the biomass mixture prior to, during, or immediately after the protein degrading step. The detergent may be ionic or nonionic. In some instances, an anionic detergent may be preferred.

[017] In some examples, the endonuclease is preferably added to the biomass prior to addition of the lysing agent to the biomass. In other examples, the endonuclease and the lysing agent are preferably added to the biomass at the same time.

[018] Also, in certain examples, the lysing agent is preferably added to the biomass prior to addition of the peptidase to the biomass. In other examples, the lysing agent and the peptidase are preferably added to the biomass at the same time.

WHAT IS CLAIMED IS

Claim 1. A method for recovering polyhydroxyalkanoates from a biomass of microorganism cells containing intracellular polyhydroxyalkanoates, the method comprising the steps of:

enzymatically treating the microorganism cells of the biomass by:

(a) cleaving polynucleotide chains from the microorganism cells by addition of an endonuclease to the biomass;

(b) lysing the microorganism cells in the biomass by addition of a lysing agent to the biomass so as to disrupt cell walls of the microorganism cells and release the intracellular polyhydroxyalkanoates from the microorganism cells; and

(c) degrading proteins from the microorganism cells by addition of a peptidase to the biomass; and

separating polyhydroxyalkanoates from cellular debris of the microorganism cells,

wherein the endonuclease is added to the biomass prior to addition of the lysing agent to the biomass and the lysing agent is added to the biomass prior to addition of the peptidase to the biomass, and

wherein the method is carried out without the use of organic solvents in the cleaving, lysing, and degrading steps.

Claim 2. The method of Claim 1, wherein the endonuclease comprises a deoxyribonuclease.

Claim 3. The method of Claim 1, wherein the endonuclease comprises a ribonuclease.

Claim 4. The method of Claim 1, wherein the lysing agent comprises an agent selected from the group consisting of lysozyme, bromelain, papain, trypsin, and mixtures thereof, in an amount from 0.01 mg/L to 8 g/L, based on the volume of the biomass.

Claim 5. The method of Claim 4, wherein the lysing agent further comprises a non-ionic surfactant and a detergent.

Claim 6. The method of Claim 1, wherein the lysing step is carried out at a temperature from 20 ℃ to 70 ℃ for a period of time from 20 minutes to 24 hours.

Claim 7. The method of Claim 1, wherein the peptidase comprises a protease selected from the group consisting of a serine protease, a neutral metalloproteinase, a cysteine protease, and mixtures thereof.

Claim 8. The method of Claim 1, wherein the protein degradation step is carried out at a temperature from 30 ℃ to 65 ℃ for a period of time from 20 minutes to 24 hours.

Claim 9. The method of Claim 1, wherein the microorganism cells are bacterial cells.

Claim 10. The method of Claim 1, further comprising, prior to the cleaving step, a step of inactivating the microorganism cells in the biomass by exposing the cells to electromagnetic radiation.

Claim 11. The method of Claim 1, further comprising, prior to the cleaving step, a step of inactivating the microorganism cells in the biomass by exposing the cells to infrared radiation energy in an amount sufficient to heat the biomass to a temperature of at least 50 ℃ for a period of time of at least 2 minutes.

Claim 12. The method of Claim 1, further comprising, prior to the cleaving step, a step of inactivating the microorganism cells in the biomass by injecting steam into the biomass.

Claim 13. The method of Claim 1, further comprising, prior to the cleaving step, a step of inactivating the microorganism cells in the biomass by either: (a) adding one or more acids to the biomass in amount sufficient to establish a biomass pH which is less than 6.0; or (b) adding one or more bases to the biomass in amount sufficient to establish a biomass pH which is greater than 8.0.

Claim 14. The method of Claim 1, further comprising, a step of bleaching the polyhydroxyalkanoates from the microorganism cells by addition of an oxidizing agent to the biomass.

Claim 15. The method of Claim 1, wherein the separating of the polyhydroxyalkanoates from the cellular debris is carried out by filtration or centrifugation.

Claim 16. The method of Claim 1, further comprising, addition of a detergent to the biomass mixture prior to, during, or following the protein degrading step.