

ORIGINAL

METHOD FOR PRODUCING BUTANOL USING EXTRACTIVE FERMENTATION WITH OSMOLYTE ADDITION

Abstract of the Invention

A method is provided for producing butanol through microbial fermentation, in which the butanol product is removed during the fermentation by extraction into a water-immiscible organic extractant in the presence of at least one osmolyte at a concentration at least sufficient to increase the butanol partition coefficient relative to that in the presence of the osmolyte concentration of the basal fermentation medium and of an optional fermentable carbon source. The osmolyte may comprise a monosaccharide, a disaccharide, glycerol, sugarcane juice, molasses, polyethylene glycol, dextran, high fructose corn syrup, corn mash, starch, cellulose, and combinations thereof. Also provided is a method and composition for recovering butanol from a fermentation medium.

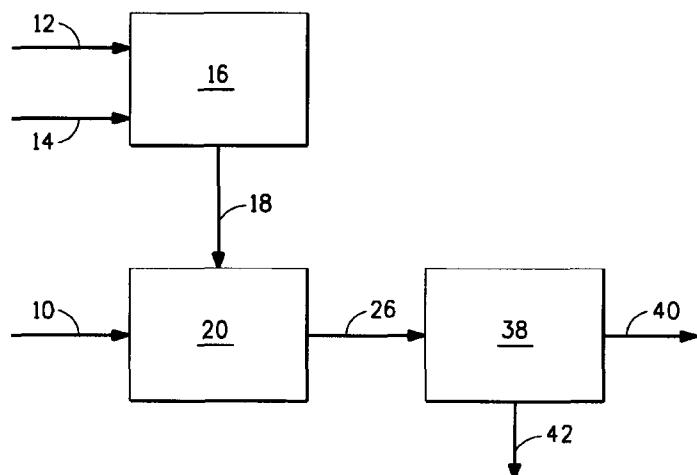


FIG. 1

We Claim:

1. A method for recovering butanol from a fermentation medium, the method comprising:
 - a) providing a fermentation medium comprising butanol, water, at least one osmolyte at a concentration at least sufficient to increase the butanol partition coefficient relative to that in the presence of the osmolyte concentration of the basal fermentation medium and of an optional fermentable carbon source, and a genetically modified microorganism that produces butanol from at least one fermentable carbon source;
 - b) contacting the fermentation medium with i) a first water-immiscible organic extractant selected from the group consisting of C₁₂ to C₂₂ fatty alcohols, C₁₂ to C₂₂ fatty acids, esters of C₁₂ to C₂₂ fatty acids, C₁₂ to C₂₂ fatty aldehydes, C₁₂ to C₂₂ fatty amides, and mixtures thereof, and optionally ii) a second water-immiscible organic extractant selected from the group consisting of C₇ to C₂₂ fatty alcohols, C₇ to C₂₂ fatty acids, esters of C₇ to C₂₂ fatty acids, C₇ to C₂₂ fatty aldehydes, C₇ to C₂₂ fatty amides and mixtures thereof to form a two-phase mixture comprising an aqueous phase and a butanol-containing organic phase; and
 - c) optionally, recovering the butanol from the butanol-containing organic phase to produce recovered butanol.
2. The method of claim 1, wherein a portion of the butanol is concurrently removed from the fermentation medium by a process comprising the steps of:
 - a) stripping butanol from the fermentation medium with a gas to form a butanol-containing gas phase; and
 - b) recovering butanol from the butanol-containing gas phase.

3. The method of claim 1, wherein the osmolyte is added to the fermentation medium, to the first extractant, to the optional second extractant, or to combinations thereof.
4. The method of claim 1, wherein the osmolyte comprises a monosaccharide, a disaccharide, glycerol, sugarcane juice, molasses, polyethylene glycol, dextran, high fructose corn syrup, corn mash, starch, cellulose, and combinations thereof.
5. The method of claim 1, wherein the osmolyte comprises a monosaccharide selected from the group consisting of sucrose, fructose, glucose, and combinations thereof.
6. The method of claim 1, wherein the osmolyte is selected from the group consisting of polyethylene glycol, dextran, corn mash, starch, cellulose, and combinations thereof.
7. The method of claim 1, wherein the genetically modified microorganism is selected from the group consisting of bacteria, cyanobacteria, filamentous fungi, and yeasts.
8. The method of claim 7 wherein the bacteria are selected from the group consisting of *Zymomonas*, *Escherichia*, *Salmonella*, *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Alcaligenes*, *Klebsiella*, *Paenibacillus*, *Arthrobacter*, *Corynebacterium*, and *Brevibacterium*.
9. The method of claim 7 wherein the yeast is selected from the group consisting of *Pichia*, *Candida*, *Hansenula*, *Kluyveromyces*, *Issatchenka*, and *Saccharomyces*.
10. The method of claim 1, wherein the first extractant is selected from the group consisting of oleyl alcohol, behenyl alcohol, cetyl alcohol, lauryl

alcohol, myristyl alcohol, stearyl alcohol, oleic acid, lauric acid, myristic acid, stearic acid, methyl myristate, methyl oleate, lauric aldehyde, 1-dodecanol, and a combination of these.

11. The method of claim 1, wherein the first extractant comprises oleyl alcohol.

12. The method of claim 1, wherein the second extractant is selected from the group consisting of 1-nananol, 1-decanol, 1-undecanol, 2-undecanol, 1-nananal, and a combination of these.

13. The method of claim 1, wherein the butanol is 1-butanol.

14. The method of claim 1, wherein the butanol is 2-butanol.

15. The method of claim 1, wherein the butanol is isobutanol.

16. The method of claim 1, wherein the fermentation medium further comprises ethanol, and the butanol-containing organic phase contains ethanol.

17. The method of claim 1 wherein the genetically modified microorganism comprises a modification which inactivates a competing pathway for carbon flow.

18. The method of claim 1 wherein the genetically modified microorganism does not produce acetone.

19. A method for the production of butanol comprising:

a) providing a genetically modified microorganism that produces butanol from at least one fermentable carbon source;

b) growing the microorganism in a biphasic fermentation medium comprising an aqueous phase and i) a first water-immiscible organic extractant selected from the group consisting of C₁₂ to C₂₂ fatty alcohols, C₁₂ to C₂₂ fatty acids, esters of C₁₂ to C₂₂ fatty acids, C₁₂ to C₂₂ fatty aldehydes, C₁₂ to C₂₂ fatty amides, and mixtures thereof, and optionally ii) a second water-immiscible organic extractant selected from the group consisting of C₇ to C₂₂ alcohols, C₇ to C₂₂ carboxylic acids, esters of C₇ to C₂₂ carboxylic acids, C₇ to C₂₂ aldehydes, C₇ to C₂₂ amides and mixtures thereof, wherein the biphasic fermentation medium further comprises at least one osmolyte at a concentration at least sufficient to increase the butanol partition coefficient relative to that in the presence of the osmolyte concentration of the basal fermentation medium and of an optional fermentable carbon source, for a time sufficient to allow extraction of the butanol into the organic extractant to form a butanol-containing organic phase;

c) separating the butanol-containing organic phase from the aqueous phase; and

d) optionally, recovering the butanol from the butanol-containing organic phase to produce recovered butanol.

20. The method of claim 19, wherein the osmolyte is added to the aqueous phase during the growth phase of the microorganism, to the aqueous phase during the butanol production phase, to the aqueous phase when the butanol concentration in the aqueous phase is inhibitory, to the first extractant, to the optional second extractant, or to combinations thereof.

21. The method of claim 20, wherein the osmolyte is obtained from a fermentation carbohydrate substrate.

22. A method for the production of butanol comprising:

- a) providing a genetically modified microorganism that produces butanol from a fermentation medium comprising at least one fermentable carbon source;
- b) growing the microorganism in a fermentation medium wherein the microorganism produces the butanol into the fermentation medium to produce a butanol-containing fermentation medium;
- c) adding at least one osmolyte to the fermentation medium to provide the osmolyte at a concentration at least sufficient to increase the butanol partition coefficient relative to that in the presence of the osmolyte concentration of the basal fermentation medium and of an optional fermentable carbon source;
- d) contacting at least a portion of the butanol-containing fermentation medium with i) a first water-immiscible organic extractant selected from the group consisting of C₁₂ to C₂₂ fatty alcohols, C₁₂ to C₂₂ fatty acids, esters of C₁₂ to C₂₂ fatty acids, C₁₂ to C₂₂ fatty aldehydes, C₁₂ to C₂₂ fatty amides and mixtures thereof, and optionally ii) a second water-immiscible organic extractant selected from the group consisting of C₇ to C₂₂ alcohols, C₇ to C₂₂ carboxylic acids, esters of C₇ to C₂₂ carboxylic acids, C₇ to C₂₂ aldehydes, C₇ to C₂₂ amides and mixtures thereof, to form a two-phase mixture comprising an aqueous phase and a butanol-containing organic phase;
- e) separating the butanol-containing organic phase from the aqueous phase;
- f) optionally, recovering the butanol from the butanol-containing organic phase; and
- g) optionally, returning at least a portion of the aqueous phase to the fermentation medium.

23. The method of claim 22, wherein the osmolyte is added to the fermentation medium in step (c) when the microorganism growth phase slows.

24. The method of claim 22, wherein the osmolyte is added to the fermentation medium in step (c) when the butanol production phase is complete.

25. The method of any one of claims 1, 19 or 22, wherein said at least one fermentable carbon source is present in the fermentation medium and comprises renewable carbon from agricultural feedstocks, algae, cellulose, hemicellulose, lignocellulose, or any combination thereof.

26. A composition comprising

- (a) a fermentation medium comprising butanol, water, at least one osmolyte at a concentration at least sufficient to increase the butanol partition coefficient relative to that in the presence of the osmolyte concentration of the basal fermentation medium and of an optional fermentable carbon source, and a genetically modified microorganism that produces butanol from at least one fermentable carbon source;
- b) a first water-immiscible organic extractant selected from the group consisting of C₁₂ to C₂₂ fatty alcohols, C₁₂ to C₂₂ fatty acids, esters of C₁₂ to C₂₂ fatty acids, C₁₂ to C₂₂ fatty aldehydes, C₁₂ to C₂₂ fatty amides and mixtures thereof; and
- c) optionally a second water-immiscible organic extractant selected from the group consisting of C₇ to C₂₂ fatty alcohols, C₇ to C₂₂ fatty acids, esters of C₇ to C₂₂ fatty acids, C₇ to C₂₂ fatty aldehydes, C₁₂ to C₂₂ fatty amides and mixtures thereof;

wherein a two-phase mixture comprising an aqueous phase and a butanol-containing organic phase may form and whereby butanol may be separated from the fermentation medium of (a).

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