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(54) **Title:** METHODS AND COMPOSITIONS FOR BIOMETHANE PRODUCTION.

(57) **Abstract:** Methods of processing municipal solid wastes (MSW) are provided whereby concurrent enzymatic hydrolysis and microbial fermentation of wastes results in liquefaction of biodegradable components as well as accumulation of microbial metabolites. Liquefied biodegradable components are then separated from nondegradable solids to produce a bioliquid characterized in comprising a large percentage of dissolved solids of which a large fraction comprises some combination of acetate, ethanol, butyrate, lactate, formate or propionate. This bioliquid is, itself, a novel biomethane substrate composition, which permits very rapid conversion to biomethane. Methods of biomethane production are further provided using this bioliquid and using other biomethane substrate compositions produced by concurrent enzymatic hydrolysis and microbial fermentation of organic materials.

## AMENDED CLAIMS

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1. A method of producing biomethane comprising the steps of
  - (i). providing a liquid biomethane substrate obtained by a process of concurrent enzymatic hydrolysis using cellulase activity and microbial fermentation of municipal solid waste (MSW) within a temperature range between 35 and 75 degrees C and at a pH less than 6.0, wherein concurrent microbial fermentation is accomplished by inoculation using one or more species of lactic acid bacteria, acetate-producing bacteria, butyrate-producing bacteria, propionate-producing bacteria, or ethanol-producing bacteria, in which biomethane substrate at least 40% by weight of the non-water content exists as dissolved volatile solids, which dissolved volatile solids comprise at least 25% by weight of any combination of acetate, butyrate, ethanol, formate, lactate and/or propionate,
  - (ii). transferring the liquid substrate into an anaerobic digestion system, followed by
  - (iii). conducting anaerobic digestion of the liquid substrate to produce biomethane.
2. The method of claim 1 wherein enzymatic hydrolysis and microbial fermentation are conducted within the temperature range 45-50 degrees C.
3. The method of claim 1 wherein at least 40% by weight of the dissolved volatile solids of the biomethane substrate comprises lactate.
4. The method of claim 1 wherein the liquid biomethane substrate comprises a total solids content of at least 8%.
5. The method of claim 1 wherein the liquid biomethane substrate comprises a dissolved methane content at 25 degrees C of less than 15 mg/L.
6. A method of producing biomethane comprising the steps of
  - (i). providing a liquid biomethane substrate obtained by a process of concurrent enzymatic hydrolysis using cellulase activity and microbial fermentation of hydrothermally pretreated lignocellulosic biomass within a temperature range between 35 and 75 degrees C and at a pH less than 6.0, wherein concurrent microbial fermentation is accomplished by

inoculation using one or more species of lactic acid bacteria, acetate-producing bacteria, butyrate-producing bacteria, propionate-producing bacteria, or ethanol-producing bacteria, in which biomethane substrate at least 40% by weight of the non-water content exists as dissolved volatile solids, which dissolved volatile solids comprise at least 25% by weight of any combination of acetate, butyrate, ethanol, formate, lactate and/or propionate,

(ii). transferring the liquid substrate into an anaerobic digestion system, followed by

(iii). conducting anaerobic digestion of the liquid substrate to produce biomethane.

7. The method of claim 6 wherein enzymatic hydrolysis and microbial fermentation are conducted within the temperature range 45-50 degrees C.

8. The method of claim 6 wherein at least 40% by weight of the dissolved volatile solids of the biomethane substrate comprises lactate.

9. The method of claim 6 wherein the liquid biomethane substrate comprises a total solid content of at least 8%.

10. The method of claim 6 wherein the liquid biomethane substrate comprises a dissolved methane content at 25 degrees C of less than 15 mg/L.

11. A liquid biomethane substrate obtained by a process of concurrent enzymatic hydrolysis using cellulase activity and microbial fermentation of municipal solid waste (MSW) within a temperature range between 35 and 75 degrees C and at a pH less than 6.0, wherein concurrent microbial fermentation is accomplished by inoculation using one or more species of lactic acid bacteria, acetate-producing bacteria, butyrate-producing bacteria, propionate-producing bacteria, or ethanol-producing bacteria, in which biomethane substrate at least 40% by weight of the non-water content exists as dissolved volatile solids, which dissolved volatile solids comprise at least 25% by weight of any combination of acetate, butyrate, ethanol, formate, lactate and/or propionate.

12. The biomethane substrate of claim 11 wherein enzymatic hydrolysis and microbial fermentation are conducted within the temperature range 45-50 degrees C.

13. The biomethane substrate of claim 11 wherein at least 40% by weight of the dissolved volatile solids comprises lactate.
14. The biomethane substrate of claim 11 comprising a total solids content of at least 8%.
15. The biomethane substrate of claim 11 comprising a dissolved methane content at 25 degrees C of less than 15 mg/L.
16. A liquid biomethane substrate obtained by a process of concurrent enzymatic hydrolysis using cellulase activity and microbial fermentation of hydrothermally pretreated lignocellulosic biomass within a temperature range between 35 and 75 degrees C and at a pH less than 6.0, wherein concurrent microbial fermentation is accomplished by inoculation using one or more species of lactic acid bacteria, acetate-producing bacteria, butyrate-producing bacteria, propionate-producing bacteria, or ethanol-producing bacteria, in which biomethane substrate at least 40% by weight of the non-water content exists as dissolved volatile solids, which dissolved volatile solids comprise at least 25% by weight of any combination of acetate, butyrate, ethanol, formate, lactate and/or propionate
17. The biomethane substrate of claim 16 wherein enzymatic hydrolysis and microbial fermentation are conducted within the temperature range 45-50 degrees C.
18. The biomethane substrate of claim 16 wherein at least 40% by weight of the dissolved volatile solids comprises lactate.
19. The biomethane substrate of claim 16 comprising a total solids content of at least 8%.
20. The biomethane substrate of claim 16 comprising a dissolved methane content at 25 degrees C of less than 15 mg/L.