Bioresources and Bioprocessing

RESEARCH Open Access

Evaluation of the biomethanation potential of enriched methanogenic cultures on gelatin

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Abstract

Purpose: Biomethane is an environment-friendly, economic, and alternative energy resource for a clean and green future. In the present study, we have evaluated the biomethanation potential of acetate-utilizing methanogenic culture (AUMC) and gelatin-enriched mixed culture (GEMC) with *Clostridium acetobutylicum* NCIM 2841 (GEMC-CA.) on gelatin as a sole carbon and nitrogen source.

Methods: We conducted experiments for examining the specific-methanogenic activity of these cultures in the metabolic assay media containing 1% gelatin. The produced methane and consumed gelatin were quantified by standard experimental methods. Exchange metabolites produced by these cultures were qualitatively analyzed by mass spectrometry.

Results: Results of our study show that the growth-associated amino acid catabolism partially or completely supported the methanogenesis of these defined cultures. AUMC and GEMC found to be suitable for enhanced methanogenic activity on gelatin but a rapid degradation of amino acid was attributed by GEMC-CA. The ammonia released from these cultures was directly proportional to gelatin degradation. Mass spectral data analysis identified some key exchange metabolites from acidogenic culture and methanogenic culture for confirming the growth-associated methanogenesis.

Conclusion: The biomethanation potential of these cultures on gelatin is coupled with the Stickland reactions-directed methanogenesis in a syntrophic manner. The present study provides the importance for the development of a starter culture for the biomethanation of protein-based industrial wastes in effective ways.

Keywords: Amino acid catabolism, Methanogenesis, Methanogenic culture, Gelatin, Anaerobic digestion, Defined culture

Introduction

Anaerobic digestion is a complex microbial metabolic process that can be carried out by different syntrophic organisms. It has been an economically attractive and alternative process for the treatment of different types of industrial wastes (Wu et al. 2017; Liu and Liao 2018; Perisin and Sund 2018). Lignocellulosic wastes are widely used as the raw materials for industrial production of organic solvents, organic acids, and biofuel. However, a large amount of energy consumption is needed for the pre-treatment of them in harsh conditions (Balan 2014). Lipid-based wastes are not being economical to make biofuel apart from biodiesel (Vlysidis et al. 2011). Anaerobic digestion of these wastes is a complex biogenic process and requires more retention time for biomethane production, but anaerobic bacteria can rapidly degrade the protein-based wastes.

The protein-based industry (food, meat, leather, fish, bone, etc.) typically produces wastewater containing a huge quantity of proteins and amino acids. Anaerobic digestion of these wastes has been employed successfully for industrial biomethane production. During the

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extraction and processing of them, a large quantity of gelatin is derived from the cleavage of collagen abundantly present in these wastes. Gelatin is noticeably a pure protein consisting of glycine (21.4%), proline (12.4%), hydroxyproline (11.9%), glutamate (10%), alanine (8.9%), arginine (7.8%), and aspartic acid (6.0%) as the major amino acids (Eastoe 1995). Tryptophan and cysteine are absent and methionine is only present at a relatively low level. Hence, gelatin is considered as a model protein substrate for the biomethanation of protein-based industrial wastes due to amino acids-coupled methanogenesis and enriched contents of glycine, alanine, and proline (Chellapandi et al. 2008, 2010; Chellapandi and Uma 2012a, b).

Acidogenic bacteria (*Clostridiaceae* family) are second trophic microorganisms in the anaerobic digestion process. These microorganisms degrade the gelatin into amino acids and small peptides. Furthermore, electron donor amino acids are oxidatively deaminated to volatile carboxylic acids (acetate or pyruvic acid) via the Stickland reactions (Stickland 1934; Nisman 1954). The produced acetate can be served as a carbon source for the growth and methanogenesis of the methanogenic bacteria. However, complete degradation of gelatin and efficient methanogenesis is achieved only in the presence of required species in defined methanogenic culture.

Several anaerobic mixed cultures have been developed for the biomethanation of various protein-based wastes (Ollivier et al. 1986; Vieira et al. 2001; Chellapandi et al. 2008, 2010; Chellapandi and Uma 2012a, b). A mixed culture of amino acid degraders and hydrogenotrophic methanogens has been developed for the rapid degradation of gelatin and methanogenesis (Jain and Zeikus 1989). Sasaki et al. (2011) have developed thermophilic cultures for effective degradation of casein, gelatin, and bovine serum albumin syntrophically in a methanogenic environment. Nevertheless, only a limited endeavor has been made on their metabolic stability and consistency to methane production from proteins.

In this perspective, our study was aimed to evaluate the biomethanation potential of AUMC, GEMC, and GEMC-CA growing on gelatin as a sole carbon source in batch culture. A methanogenic culture understudied would have more industrial utility as a starter culture for the biomethanation of protein-based wastewater.

Materials and methods

Growth and maintenance of *C. acetobutylicum*

A frozen culture of *C. acetobutylicum* NCIM 2841 strain was procured from the National Collection of Industrial Microorganisms, India. Clostridial spores were inoculated in 100 Ml cooked meat medium containing the composition (g/L) of beef extract, 45; dextrose, 2.0; protease peptone, 20; NaCl, 5.0; and then incubated at 37 °C in an anaerobic condition. It was maintained in the cooked meat agar medium until the next use.

Preparation of anaerobic seed culture

The anaerobically digested slurry was collected from a functional biogas plant (3 m^3) at the Biogas Research Centre, Gujarat Vidyapith, Sadra, India. It was filtered through a muslin cloth and centrifuged at 5000 rpm for 10 min by a bench top centrifuge. One volume of the collected supernatant was diluted with three volumes of dilution medium (g/L) containing $NaHCO_3$, 5.0; $NaCO_3$, 10; and two or three drops of resazurin (0.001%, w/v). It was pre-activated for 24 h at 37 °C before inoculation.

Enrichment technique for methanogens

Methanogens were enriched and cultivated by the anaerobic technique described previously (Wolfe 2011). Enrichment of methanogens was carried out in a medium containing the composition (g/L): NH_4Cl, 1.0; NaCl, 0.6; $NaHCO_3$, 5.0; KH_2PO_4, 0.3; K_2HPO_4, 0.3; $MgCl_2{\cdot}6H_2O$, 0.16; $CaCl_2{\cdot}2H_2O$, 0.009; and resazurin 0.001% solution, 1.0 mL. A solution of the following vitamins (10 mg/L): *p*-aminobenzoic acid, nicotinic acid, calcium pantothenate, pyridoxine, riboflavin, thiamine and 5 mg each of biotin, folic acid, α-lipoic acid, and cyanocobalamine B12 were prepared and 10 mL of this solution added to the above medium. A solution of trace minerals (g/L) was prepared and 10 mL of this solution mixed with 1 L of that medium: trisodium nitrilotriacetic acid, 1.5; $Fe(NH_4)_2(SO_4)_2$, 0.8; $NaSeO_3$, 0.2; $CoCl_2{\cdot}6H_2O$, 0.1; $MnSO_4{\cdot}H_2O$, 0.1; $Na_2MoO_4{\cdot}2H_2O$, 0.1; $NaWO_4{\cdot}2H_2O$, 0.1; $ZnSO_4{\cdot}7H_2O$, 0.1; $NiCl_2{\cdot}6H_2O$, 0.1; H_3BO_3, 0.01; and $CuSO_4{\cdot}5H_2O$, 0.01. The pH of the medium was adjusted to 7.4 with 0.1 M KOH. Cysteine-HCl and $Na_2S{\cdot}9H_2O$ were added separately to this medium. After that, 100 mM sodium acetate for acetate-utilizing methanogens and 1% (w/v) gelatin for gelatin-enriched cultures were added separately in the enrichment media. The pre-activated seed culture (5%, v/v) was inoculated to initiate the enrichment of methanogens and then incubated at 37 °C under N_2 headspace. Acetate was replenished everyday until the culture reached a late log phase.

Assay for specific methanogenic activity

Experiments were done in the anaerobic serum vials of 132 mL with a working volume of 50 mL phosphate-buffered basal (PBB) medium (Moench and Zeikus 1983). The PBB medium is composed (g/L) of KH_2PO_4, 1.5; $K_2HPO_4{\cdot}3H_2O$ 2.9; $MgCl_2{\cdot}6H_2O$, 0.2; $CaCl_2{\cdot}2H_2O$, 0.1;

NaCl, 0.9; NH_4Cl, 1.0; resazurin, 0.001% solution, 1.0 ml; trace mineral solution, 10 mL; vitamin solution (μg/mL): biotin, 10; pantothenate (calcium), 25; lipoic acid, 25; folic acid, 10; thiamine, 25; riboflavin, 25; pyridoxine HCl, 50; cyanocobalamine B12, 0.05; nicotinic acid, 25 and *p*-amino benzoic acid, 25. Trace mineral solution has consisted of (g/L) trisodium nitrilotriacetic acid, 12.8; Na_2SeO_3, 0.02; $CoCl_2{\cdot}6H_2O$, 0.16; $Na_2MoO_4{\cdot}2H_2O$, 0.01; H_3BO_3, 0.1; $FeSO_4$, 0.1; $MnCl_2$, 0.1; $ZnCl_2$, 0.1; $CuCl_2$, 0.02 and $NiSO_4$, 2.6 mg.

After the proper enrichment, methanogenic culture (5%, v/v) was inoculated into 50 mL of the PBS medium containing 100 mM sodium acetate as an initial growth substrate. It was incubated at 37 °C until the methane production and depletion of acetate cease. After addition of 2–3 drops of titanium (III)-nitrilotriacetate reductant, the gas phase of a serum bottle was changed with N_2 by evacuation and flushing. Sterilized gelatin at 1% (w/v) was added along with the overgrown culture of *C. acetobutylicum* and the time course was followed by incubation at 37 °C. A serum vial containing the same assay medium without gelatin was served as a negative control. The gas phase was replaced with N_2 gas daily. Gas production was measured using an air-tight syringe. After the incubation, the entire content of the digested liquid was centrifuged at 5000 rpm for 15 min and the supernatant used for determination of pH, volatile fatty acids, and other metabolites.

Analytical techniques

Gas samples were collected from the head phase of each vial and immediately analyzed for methane content using a Gas chromatograph equipped with thermal conductivity detector (Perkin-Elmer Autosystem) as outlined previously (Chellapandi and Uma 2012a, b). One microlitre of diluted biogas sample was injected into a packed 2-m Porapak T steel column using an air-tight syringe. Nitrogen was used as a carrier gas at the flow rate of 30 mL/min. The oven, injection, and detector temperatures were adjusted to 50, 70, and 150 °C, respectively. Gelatin concentration was analyzed by using a modified Bradford dye-binding protein assay (Noble and Bailey 2009) and then calibrated with a gelatin standard. The concentration of extracellular and intracellular amino acids was calorimetrically determined with ninhydrin reagent by measuring optical density at 570 nm (Moore and Stein 1954) using glycine and alanine as standards. The liberated ammonia during gelatin degradation was estimated by spectrophotometer using sodium nitroprusside reagent at 625 nm and then calibrated with ammonium sulfate standard (Chaney and Marbach 1962).

GC–MS/MS analysis

The exchange metabolites of these cultures were detected from the digested fluid by GC–MS/MS (Perkin-Elmer Autosystem with turbo mass) with PE-5MS column (30 m; 250 micron internal diameter; 0.25 micron thickness) (Halket et al. 2005). Helium was used as a carrier gas. The oven temperature was adjusted to 80 °C for 5 min and raised to 280 °C with the rate of 10 °C/min and of 15-min hold time. Both injection and electron impact source temperatures were set at 250 °C. Split ratio was adjusted to be 1:60 and mass ranged between 20 and 650 atomic mass units. The volatile fatty acids and hydrocarbons were extracted in dichloromethane, concentrated, and then injected 1 μL sample for analysis. Metabolites present in the samples were identified from mass–ionization ratio obtained in GC–MS/MS peeks using the National Institute of Standards and Technology 11 and National Bureau of Standards Mass Spectral Library.

Results and discussion

Effect of incubation time on microbial growth

In this study, gelatin was used as a model protein substrate to find out amino acid catabolism-coupled methanogenesis of AUMC and GEMC in batch digestion (Fig. 1a). It has shown that a maximum biomass yield was attained after 11 days of incubation. GEMC-CA has shown a maximum growth compared to GEMC and AUMC. It may be resulted due to the overgrowth of *C. acetobutylicum* in the enriched culture and its growth might be supported by gelatin in the minimal medium (Masion et al. 1987). It indicated that gelatin was a good growth substrate for these cultures similar to a mixed defined triculture of methanogens (Ollivier et al. 1986).

Effect of incubation time on a methanogenic activity

A considerable methanogenic potential was achieved after 4–6 days of incubation and then gradually declined (Fig. 1b). AUMC was shown a relative methanogenic activity on gelatin as it has more potential populations of acidogenic bacteria. GEMC-CA did not favor the methanogenesis compared to GEMC and AUMC due to rapid degradation of gelatin by *C. acetobutylicum*. The methanogenic potential of these cultures was faintly more in the presence of acetate than gelatin used alone. Some syntrophic bacteria in the AUMC and GEMC may have an impending metabolic activity and metabolite pairing (exchange) for the bioconversion of gelatin to methane. Perhaps, it could be improved for defined cultures by enhancing the growth population of methanogens or increasing acclimatization period on gelatin (Patel and Chellapandi 2009).

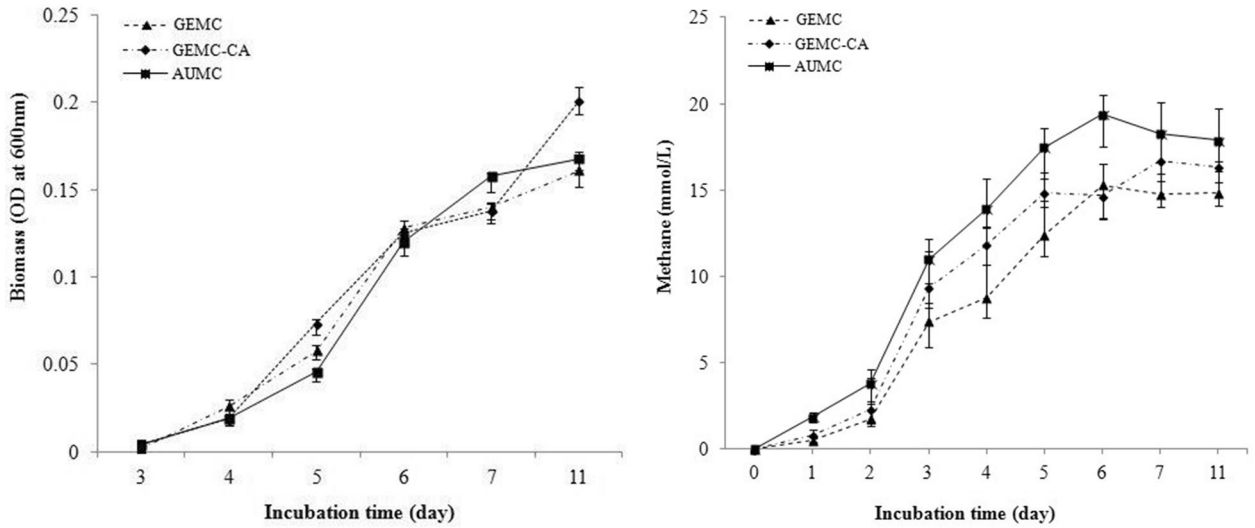

Fig. 1 Effect of incubation time on the growth (left) and methanogenic activity (right) of enriched methanogenic cultures on gelatin in anaerobic batch digestion (*AUMC* acetate-utilizing methanogenic culture, *GEMC* gelatin-enriched mixed culture, *GEMC-CA* gelatin-enriched mixed culture with *Clostridium acetobutylicum* NCIM 2841)

Effect of incubation time on gelatin degradation

The degradation of gelatin was started after 1 day and then gradually increased by the growth period (Fig. 2a). A constant rate of gelatin degradation was observed when GEMC used as a starter culture. GEMC-CA was initially supported for the gelatin degradation and after that its consumption rate was decreased. It described the relative fraction of proteolytic acidogenic bacteria in GEMC and AUMC responsible for the efficient degradation of gelatin during the anaerobic digestion process. The rate of gelatin degradation attained by GEMC was better than AUMC owing to enriched populations of gelatin-degrading bacteria as described earlier (Orlygsson et al. 1994; Fonknechten et al. 2010).

Effect of incubation time on amino acids assimilation

Glycine and alanine from gelatin were gradually metabolized and then transformed into methane in these

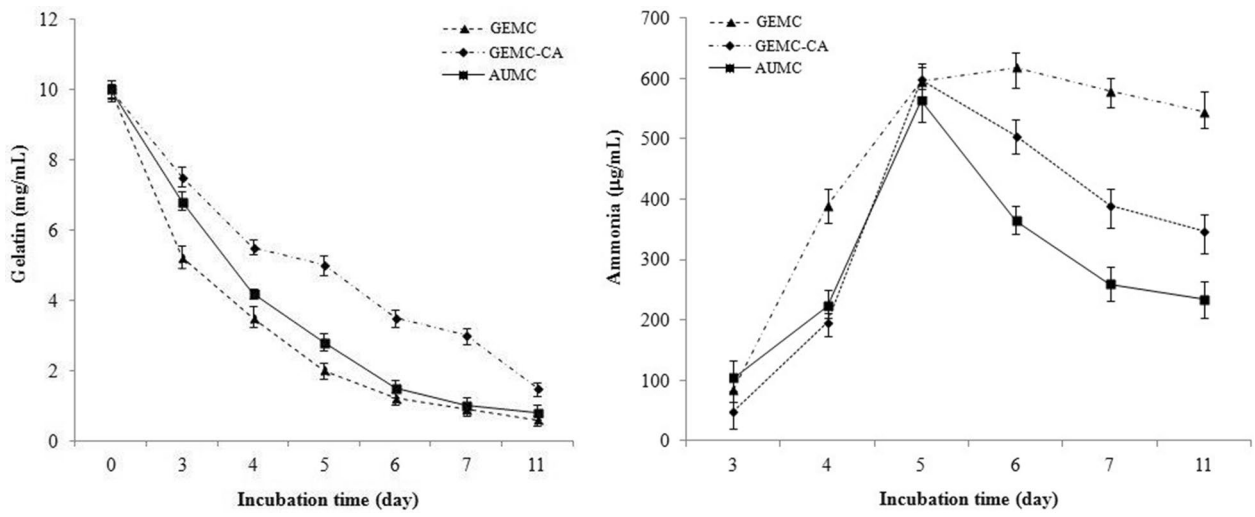

Fig. 2 Effect of incubation time on the degradation of gelatin (left) and liberation of ammonia (right) by enriched methanogenic cultures in anaerobic batch digestion (*AUMC* acetate-utilizing methanogenic culture, *GEMC* gelatin-enriched mixed culture, *GEMC-CA* gelatin-enriched mixed culture with *Clostridium acetobutylicum* NCIM 2841)

cultures. Alanine was excreted in the exponential phase but disappears slowly in the stationary growth (Additional file 1: Figure S1). It pointed out that extracellular glycine and alanine consumed more rapidly than intracellular glycine and alanine. The interspecies hydrogen transfer is necessary to accomplish amino acid metabolism (Nagase and Matsuo 1982; Orlygsson et al. 1994; 1995; Hartwich et al. 2012). Amino acid degradation was also enhanced by the accumulation of hydrogen gas in head phase. The intracellular catabolism of glycine and alanine in AUMC was slightly higher than GEMC. Catabolism of intracellular glycine and alanine was delayed by GEMC for oxidative deamination. It may be resulted due to rapid assimilation of such metabolites to its cellular energetic metabolism (Orlygsson et al. 1994, 1995). A significant quantity of proline is not detected in the fermentation media as a lack/low activity of proline dehydrogenase present in CAC or other acidogenic bacteria (Jain and Zeikus 1989). It pinpointed the need of another bacterial species to catabolize the proline for a complete transformation of gelatin to methane.

Effect of incubation time on ammonia liberation

Ammonia was liberated after 3 days of digestion, raised as a maximum of 5 days, and then suddenly decreased during gelatin degradation (Fig. 2b). It found a similar metabolic pattern to release ammonia from gelatin from GEMC and AUMC. Ammonia was steadily liberated at a log phase of GEMC and then slowly declined. It pointed out that increasing concentration of ammonia was directly proportional to an amino acid or gelatin degradation. This suggested that a methanogenic culture was actively maintained by accumulated ammonia (alkaline buffer), which was agreed to the earliest investigations (Masion et al. 1987; Orlygsson et al. 1994, 1995; Fonknechten et al. 2010).

Effect of incubation time on exchange metabolites

Using GC–MS/MS data, we have identified extracellular metabolites produced by these cultures (Fig. 3). Apart from methane and CO_2 formation, acetamide, ethanol, ethylamine, and acetaldehyde were found as exchange metabolites of acidogenic bacteria, when GEMC was grown on gelatin. It also reported that either acidogenic bacteria or methanogens have secreted tyrosine and methionine from their cellular metabolism. Methanogenic substrates such as formate, propionate, and acetate were identified from the digested fluid. It represented that these metabolites supported the growth-associated methanogenesis in a methanogenic environment. Butyric acid, ethanol, propanol, butanol, and acetaldehyde were end products of this digestion that may be produced by CAC. It indicated that acidogenesis and solventogenesis are active in acidogenic bacteria or CAC when gelatin is used as a growth substrate for these cultures in anaerobic condition.

The proposed pathway for Stickland reactions-coupled methanogenesis

Acidogenic bacteria can degrade the gelatin into amino acids, which are further transformed into precursors requiring for methanogenesis in two ways: (1) pairs of

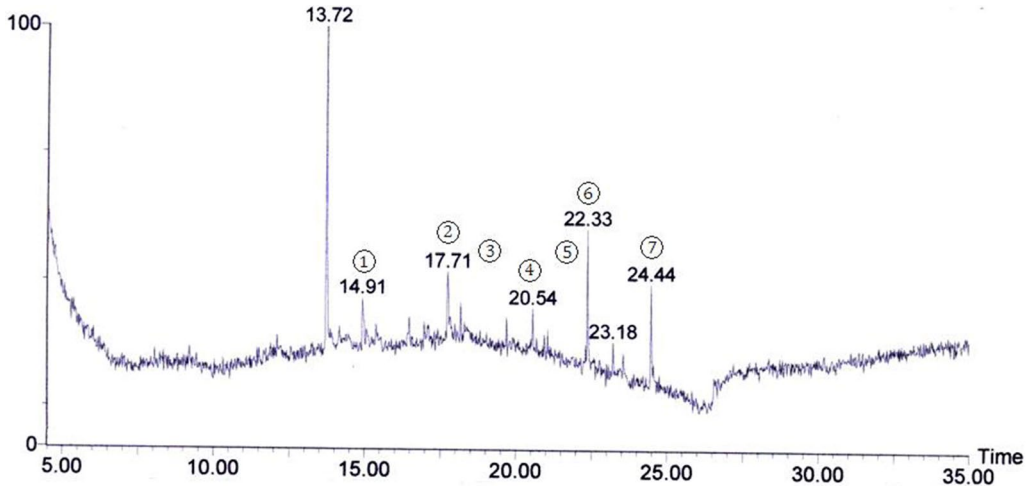

Fig. 3 GC–MS/MS profiling for the identification major exchange metabolites in anaerobically digested fluid after 11 days of incubation of enriched methanogenic culture on gelatin. 1—acetamide, acetaldehyde; 2—acetic acid, formic acid; 3—succinic acid, L-tyrosine, butanedioic acid, ethylamine, L-methionine, propanedioic acid, 2-pentanoic acid, 2-propionic acid; 4—butanoic acid; 5—1-propene, 3-butene; 6—*n*-butanol, 1-propanol; 7—propanoic acid

amino acids can be catabolized via the Stickland reactions and (2) single amino acids can be fermented in a process that requires the presence of hydrogen-utilizing methanogens (Vieira et al. 2001; Stickland 1934; Nisman 1954; Ollivier et al. 1986). Glycine/alanine or ornithine/proline or ornithine/lysine was used as an amino acid pair in the Stickland reactions of many *Clostridia* (Nagase and Matsuo 1982; Orlygsson et al. 1995; Fonknechten et al. 2010; Hartwich et al. 2012). However, uncoupled degradation of amino acids (10% of total amino acids) only occurs in mixed culture if there was a shortage of amino acids as electron acceptors (Orlygsson et al. 1994). Based on the growth of kinetic and GC–MS/MS data, we proposed the amino acid-catabolism-directed methanogenesis in a methanogenic culture (Fig. 4). In the proposed system, the presence of glycine reductase has reduced glycine alone to acetate with the release of ammonia via intermediate acetyl phosphate. Amino acid dehydrogenase and glycine reductase could be active in these methanogenic cultures during anaerobic digestion of gelatin. Clostridiale family has developed the specific pathways to degrade amino acids by fermentation processes (Nagase and Matsuo 1982; Fonknechten et al. 2010; Sangavai and Chellapandi 2017). Acetate probably arises from the oxidation of amino acids such as alanine or the reduction of glycine similar to the earlier work (Tang et al. 2005). Methanogenesis of these cultures depends on the growth environment where acetate is not necessarily a preferred substrate for all methanogenic bacteria. After depleting the acetate, acetoclastic or hydrogenotrophic methanogens in these cultures consume other methanogenic substrates such as propionic acid, pyruvic acid, hydrogen, and carbon dioxide produced during anaerobic fermentation of gelatin (Ramasamy and Pullammanmappallil 2001). AUMC and GEMC consisted of a considerable population of acidogenic bacteria but have no balanced tropical anaerobic bacteria.

Conclusions

Studying the growth of physiology and metabolites sharing is essential for evaluation of the biomethanation potential of defined methanogenic cultures on gelatin in batch digestion. Biosynthesis of methane can be directed partially or

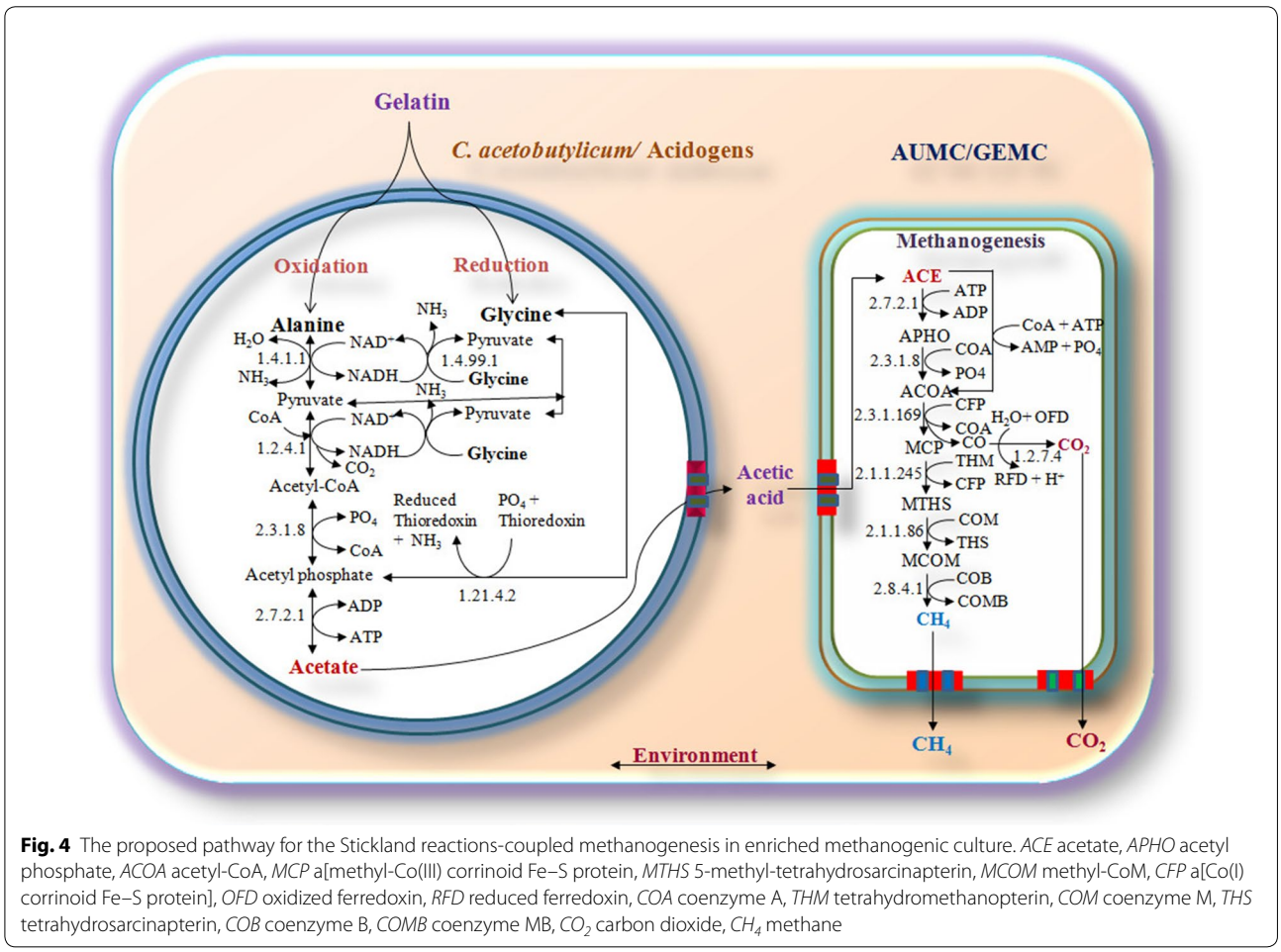

Fig. 4 The proposed pathway for the Stickland reactions-coupled methanogenesis in enriched methanogenic culture. *ACE* acetate, *APHO* acetyl phosphate, *ACOA* acetyl-CoA, *MCP* a[methyl-Co(III) corrinoid Fe–S protein, *MTHS* 5-methyl-tetrahydrosarcinapterin, *MCOM* methyl-CoM, *CFP* a[Co(I) corrinoid Fe–S protein], *OFD* oxidized ferredoxin, *RFD* reduced ferredoxin, *COA* coenzyme A, *THM* tetrahydromethanopterin, *COM* coenzyme M, *THS* tetrahydrosarcinapterin, *COB* coenzyme B, *COMB* coenzyme MB, CO_2 carbon dioxide, CH_4 methane

completely by growth-associated catabolism of amino acids in methanogenic cultures during anaerobic digestion of gelatin. The methanogenic activity of these cultures is enhanced with suitable amino acid pairs despite single amino acids. The biomethanation of these cultures on gelatin depends on the metabolic rate of the Stickland reactions-coupled methanogenesis and availability of methanogenic precursors. Conceivably, the present methanogenic cultures are suitable candidates for the bioconversion of protein-based wastes to methane in anaerobic batch digesters. The metabolic and growth stability of these enriched cultures should be further evaluated by biochemical and metabolic kinetic experiments. Such data would provide the importance to understand the metabolic behaviors of syntrophic parterres in the future.

Additional file

Additional file 1: Figure S1. Effect of incubation time on concentration of intracellular (left) and extracellular (right) glycine and alanine by enriched methanogenic cultures in anaerobic batch digestion (AUMC: Acetate-utilizing methanogenic culture; GEMC: Gelatin enriched mixed culture; GEMC-CA: Gelatin enriched mixed culture with *Clostridium acetobutylicum* NCIM 2841).

Abbreviations

AUMC: acetate-utilizing methanogenic culture; GEMC: gelatin-enriched mixed culture; GEMC-CA: gelatin-enriched mixed culture with *Clostridium acetobutylicum* NCIM 2841; CAC: *Clostridium acetobutylicum* NCIM 2841; GC–MS/MS: gas chromatography–mass spectrometry/mass spectrometry.

Authors' contributions

SPC and CP conceived the study. CP made contributions to the design of the experiments, the acquisition of data, the analysis and interpretation of data, and contributed to the manuscript writing. SC BM, AKP, PKP, and PHP conceived and organized the study and helped to draft the manuscript, and have revised the manuscript. All authors read and approved the final manuscript.

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Acknowledgements

The authors take immense pleasure in thanking the Higher Authority of Gujarat Vidyapith, Ahmadabad, India for providing host to carry out this investigation.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Consent for publication

All authors approved the consent for publishing the manuscript to bioresources and bioprocessing.

Ethics approval and consent to participate

Not applicable.

Funding

The corresponding author would like to thank the Tamil Nadu State Council for Science and Technology (TNSCST/YSFS/VR/3/2011-12/1912), India, for young scientists visiting fellowship.

Publisher's Note

Received: 17 February 2019 Accepted: 8 April 2019
Published online: 20 April 2019

References

Balan V (2014) Current challenges in commercially producing biofuels from lignocellulosic biomass. ISRN Biotechnol. https://doi.org/10.1155/2014/463074

Chaney AL, Marbach P (1962) Modified reagents for determination of urea and ammonia. Modified reagents for determination of urea and ammonia. Clin Chem 8:130–132

Chellapandi P, Uma L (2012a) Co-digestion of ossein factory waste for methane production in batch. Elixer Biotechnol 42:6383–6385

Chellapandi P, Uma L (2012b) Evaluation of methanogenic activity of biogas plant slurry on ossein factory wastes. J Environ Sci Health 54:10–13

Chellapandi P, Prabaharan D, Uma L (2008) A preliminary study on co-digestion of ossein factory waste for methane production. Eur Asia J BioSci 2:110–114

Chellapandi P, Prabaharan D, Uma L (2010) Evaluation of methanogenic activity of biogas plant slurry for monitoring anaerobic co-digestion of ossein factory waste and cyanobacterial biomass. Appl Biochem Biotechnol 162:534–535

Eastoe JE (1995) The amino acid composition of mammalian collagen and gelatin. Biochem J 61:589–600

Fonknechten N, Chaussonnerie S, Tricot S, Lajus A, Andreesen JR, Perchat N, Pelletier E, Gouyvenoux M, Barbe MV, Salanoubat M, Le Paslier D, Weissenbach J, Cohen GN, Kreimeyer A (2010) *Clostridium sticklandii*, a specialist in amino acid degradation: revisiting its metabolism through its genome sequence. BMC Genom 11:555

Halket JM, Waterman D, Przyborowska AM, Patel RKP, Fraser PD, Bramley PM (2005) Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. J Exp Bot 56:219–243

Hartwich K, Poehlein A, Daniel R (2012) The purine-utilizing bacterium *Clostridium acidurici* 9a: a genome-guided metabolic reconsideration. PLoS ONE 7:e51662

Jain MK, Zeikus JG (1989) Bioconversion of gelatin to methane by a co-culture of *Clostridium collagenovorans* and *Methanosarcina barkeri*. Appl Environ Microbiol 55:366–371

Liu WY, Liao B (2018) Anaerobic co-digestion of vegetable and fruit market waste in LBR+ CSTR two-stage process for waste reduction and biogas production. Appl Biochem Biotechnol. https://doi.org/10.1007/s12010-018-2910-4

Masion E, Amine J, Marczak R (1987) Influence of amino acid supplements on the metabolism of *Clostridium acetobutylicum*. FEMS Microbiol Ecol 43:269–274

Moench TT, Zeikus JG (1983) Nutritional growth requirements for *Butyribacterium methylotrophicum* on single carbon substrates and glucose. Curr Opin Microbiol 9:151–154

Moore S, Stein WH (1954) A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J Biol Chem 211:907–913

Nagase M, Matsuo T (1982) Interactions between amino-acid degrading bacteria and methanogenic bacteria in anaerobic digestion. Biotechnol Bioeng 24:2227–2239

Nisman B (1954) The Stickland reaction. Bacteriol Rev 18:16–42

Noble JE, Bailey MJA (2009) Quantitation of protein. Methods Enzymol 463:73–95

Ollivier B, Smiti N, Mah RA, Garcia JL (1986) Thermophilic methanogenesis from gelatin by a mixed defined bacterial culture. Appl Microbiol Biotechnol 24:79–83

Orlygsson J, Houwen FP, Svensson BH (1994) Influence of hydrogenothrophic methane formation on the thermophilic anaerobic degradation of protein and amino acids. FEMS Microbiol Ecol 13:327–334

Orlygsson J, Houwen FP, Svensson BH (1995) Thermophilic anaerobic amino acid degradation: deamination rates and end-product formation. Appl Microbiol Biotechnol 43:235–241

Patel CN, Chellapandi P (2009) Anaerobic digestion of cotton seed cake using developed mixed consortia. Electron J Environ Agric Food Chem 8:333–344

Perisin MA, Sund CJ (2018) Human gut microbe co-cultures have greater potential than monocultures for food waste remediation to commodity chemicals. Sci Rep 8:15594

Ramasamy IR, Pullammanmappallil PC (2001) Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry. Biodegradation 12:247–257

Sangavai C, Chellapandi P (2017) Amino acid catabolism-directed biofuel production in *Clostridium sticklandii*: an insight into model-driven systems engineering. Biotechnol Rep 16:32–43

Sasaki K, Morita M, Sasaki D, Nagaoka J, Matsumoto N, Ohmura N, Shinozaki H (2011) Syntrophic degradation of proteinaceous materials by the thermophilic strains *Coprothermobacter proteolyticus* and *Methanothermobacter thermautotrophicus*. J Biosci Bioeng 112:469–472

Stickland LH (1934) Studies in the metabolism of the strict anaerobes (genus Clostridium): the chemical reactions by which *Clostridium sporogenes* obtains its energy. Biochem J 28:1746–1759

Tang Y, Shigematsu T, Morimura S, Kida KJ (2005) Microbial community analysis of mesophilic anaerobic protein degradation process using bovine serum albumin (BSA)-fed continuous cultivation. J Biosci Bioeng 99:150–164

Vieira AM, Bergamasco R, Gimenes ML, Nakamura CV, Dias B, Filho P (2001) Microbial populations of up-flow anaerobic sludge blanket reactor treating waste water from a gelatin industry. Environ Technol 22:1477–1485

Vlysidis A, Binns M, Webb C, Theodoropoulos C (2011) A techno-economic analysis of biodiesel biorefineries: assessment of integrated designs for the co-production of fuels and chemicals. Energy 36:4671–4683

Wolfe RS (2011) Techniques for cultivating methanogens. Methods Enzymol 494:1–21

Wu J, Cao Z, Hu Y, Wang X, Wang G, Zuo J, Wang K, Qian Y (2017) Microbial insight into a pilot-scale enhanced two-stage high-solid anaerobic digestion system treating waste activated sludge. Int J Environ Res Public Health 14:1483