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Evaluation of support matrices for immobilization of anaerobic consortia for efficient carbon cycling in waste regeneration

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Abstract

Efficient metabolism of fatty acids during anaerobic waste digestion requires development of consortia that include "fatty acid consuming H_2 producing bacteria" and methanogenic bacteria. The objective of this research was to optimize methanogenesis from fatty acids by evaluating a variety of support matrices for use in maintaining efficient syntrophic–methanogenic consortia. Tested matrices included clays (montmorillonite and bentonite), glass beads (106 and 425–600 μm), microcarriers (cytopore, cytodex, cytoline, and cultispher; conventionally employed for cultivation of mammalian cell lines), BioSep beads (powdered activated carbon), and membranes (hydrophilic; nylon, polysulfone, and hydrophobic; teflon, polypropylene). Data obtained from headspace methane (CH_4) analyses as an indicator of anaerobic carbon cycling efficiency indicated that material surface properties were important in maintenance and functioning of the anaerobic consortia. Cytoline yielded significantly higher CH_4 than other matrices as early as in the first week of incubation. 16S rRNA gene sequence analysis from crushed cytoline matrix showed the presence of *Syntrophomonas* spp. (butyrate oxidizing syntrophs) and *Syntrophobacter* spp. (propionate oxidizing syntrophs), with *Methanosaeta* spp. (acetate utilizing methanogen), and *Methanospirillum* spp. (hydrogen utilizing methanogen) cells. It is likely that the more hydrophobic surfaces provided a suitable surface for adherence of cells of syntrophic–methanogenic consortia. Cytoline also appeared to protect entrapped consortia from air, resulting in rapid methanogenesis after aerial exposure. Our study suggests that support matrices can be used in anaerobic digestors, pre-seeded with immobilized or entrapped consortia on support matrices, and may be of value as inoculant-adsorbents to rapidly initiate or recover proper system functioning following perturbation.

Keywords: Support matrices; Immobilization; Wastewater; Syntrophic bacteria; Methanogens

Anaerobic wastewater treatment systems are used for treating a wide range of industrial effluents, including those containing toxic or inhibitory compounds [1,2]. The process is also used for treatment of domestic wastewater and is commonly applied for purification of industrial wastewaters with high organic matter content. Aerobic digestors are in wider use than anaerobic systems, even though they require higher amounts of capital resources and skilled manpower. In aerobic processes, approximately 67% of the organic matter is converted to cell biomass, which has significant running costs due to daily sludge production requiring further treatment and safe disposal. In contrast to this, anaerobic digestion converts approximately 3% of the wastewater organic matter to cell biomass with much of the remaining carbon being converted to CH_4 and CO_2 gases as stable-end products. In anaerobic systems, biological sludge production is much lower than in aerobic systems, further reducing the costs of treatment and disposal [3,4]. Finally, CH_4 can be utilized as a value-added product for generation of energy [2].

A complex interdependent community comprised of hydrolytic, fermentative, syntrophic, homoacetogenic, and methanogenic microorganisms recycles carbon in anaerobic waste reclamation systems [1,5]. Generation

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of CH_4 depends on at least three interacting groups of microbes. Cellulolytic and fermentative bacteria decompose cellulose and other complex biomolecules to alcohols and fatty acids (e.g., propionate, butyrate), CO_2, and H_2. Interspecies H_2 or formate transfer between syntrophic bacteria and methanogens further converts alcohols and fatty acids into acetate, CO_2, and H_2, which serve as carbon and electron donors for methanogens to form CH_4 [1,5,6]. Fatty acids formed in these series of reactions by primary fermentors are potent thermodynamic inhibitors and require coordinated consumption by syntrophic bacteria for optimum growth [1,5,6].

Immobilization of microbial consortia is important for optimum functioning of anaerobic treatment systems, such that concentrations of intermediates are sufficiently low for efficient carbon cycling. It has been reported that a distance of less than 1 μm between syntrophic bacteria and methanogens is a prerequisite for oxidation of fatty acids, allowing transfer of reducing equivalents to methanogens [5]. Hydrogen and acetate levels should be below threshold limits so that these reactions are exergonic and proceed to completion [7,8]. Poor coupling of syntrophs with methanogens results in accumulation of H_2 and acetate, and reaction(s) become endergonic. H_2 and acetate may accumulate, which decreases pH and leads to collapse of the system [1,9,10].

The addition of support materials, such as sepiolite, to fluidized-bed anaerobic digesters has been shown to enhance CH_4 production by potentially increased colonization by syntrophic microbiota [11–13]. The objective of this study was to evaluate a variety of support matrices to immobilize fatty acid oxidizing-hydrogen producing syntrophic bacteria and methanogens, which may expedite carbon cycling. A range of entrapment matrices and biofilm supports are available for use in such applications [11–15]. However, to our knowledge, there are no reports that have assessed effects of support matrices on rates of fatty acid metabolism to CH_4.

An additional objective was to investigate the effect of immobilization of syntrophic–methanogenic consortia and their use in perturbed conditions. Problems may arise during storage and transport, including loss of activity by key bacterial groups in the starting sludge [9,16]. The existing microbial community may not be adapted to conditions in a new reactor or the presence of toxic compounds [17]. Therefore, use of active consortia immobilized in suitable matrices may be useful for rapid startup or recovery of reactor functioning.

Materials and methods

Consortia isolation, microcosm construction, and growth conditions. An anaerobic consortium (termed as F1 consortium) was isolated from eutrophic soils from the Florida Everglades [18]. Serum bottles containing 25 ml of anaerobically prepared basal carbonate yeast extract trypticase medium (BCYT) [19] were crimped using rubber septa with aluminum seals (Bellco Glass, Vineland, N.J.). Fatty acids (20 mM propionate or butyrate) were added from anaerobically prepared stock solutions, resazurin (0.1%) was added as redox indicator and reduced with cysteine–sulfide solution as previously described [18]. F1 consortia were added to a final volume of 5% by nitrogen-flushed syringes to avoid aerial contact and incubated at 30 °C in the dark for syntrophic–methanogenic associations to develop. Subsequent enrichments were transferred to BCT medium (basal carbonate trypticase without yeast-extract) to discourage background growth of primary fermentors. Standard anaerobic culturing techniques were followed [20].

To test the tolerance of the consortium bound to different matrices on exposure to air, all microcosms containing each of 13 matrices were spiked with propionate and butyrate. Methanogenesis and depletion of fatty acids were followed until CH_4 production and fatty acid depletion (data not shown) stabilized. This was typically within 2 weeks. Support matrices were harvested by opening the anaerobic vials, exposing them to air, and transferring into fresh medium containing fatty acids.

Cell immobilization/entrapment matrices. Support matrices were prepared according to the individual matrix and their respective surface areas, and added into 25 ml BCYT medium. Propionate and butyrate well-grown F1 consortium was centrifuged in an anaerobic glove box and concentrated 5 times. Five percent of this concentrated cell-suspension was then added into each anaerobic vial (OD_{600} was kept constant at 0.3).

Four types of microcarriers were tested as supports and were prepared according to the manufacturer's instructions (Pharmacia Biotechnology, St. Albans, UK), washed twice in phosphate-buffered saline (150 mM sodium phosphate; 150 mM NaCl, pH 7.2), and added in the medium. Microcarriers (initially used at 3.8 g cytoline 2; 75 mg cytopore 2; 720 mg cytodex 3, and 25 mg cultispher each per 25 ml), as previously described [21], were sterilized by autoclaving. BioSep beads consist of powdered activated carbon (PAC) and are 3–4 mm in diameter. These consist of 25-wt% polymers and 75-wt% PAC [22,23], and were pretreated by incubating at 300 °C for 3 h and 1 g was added. Six filtration membranes circles, 25 mm in diameter, were introduced into each serum bottle prior to inoculation (hydrophobic membranes, polypropylene and teflon; hydrophilic membranes, nylon and polysulfone). Additionally, fatty acids were spiked onto the surface of hydrophilic membranes, allowed to dry, and introduced into anaerobic bottles under anaerobic conditions. Two different sizes of glass beads (106 and 425–600 μm) 600 and 50 mg of clays bentonite (average pore size > 2 μm; Sigma, St. Louis, MO) and magnesium montmorillonite (SM1200, 200 mesh; GSA Resources, Cortario, Arizona) were also tested. All support matrices were saturated with BCYT medium as outlined above and tested for methanogenesis.

Biomass measurements. Bacterial biomass on the support materials was quantified by a modification of the Biuret method for protein determination [21]. Every week one serum bottle from each support matrix was sacrificed and the matrix was placed in a test tube with 1 ml of 3 M NaOH. Samples were boiled for 10 min and left to cool for 25 min followed by the addition of $CuSO_4 \cdot 5\ H_2O$ solution (2.5% w/v) and incubated at room temperature for 5 min. Centrifuged microbial protein concentrations were determined by a total protein estimation kit according to the enclosed instructions (Sigma, St. Louis, MO). Absorbance of the resulting samples was read at 725 nm, and concentrations of protein were determined by comparison with bovine serum albumin standards against blanks prepared with distilled water. Analyses were conducted in triplicate.

Analytical methods. Differential interference contrast (DIC) microscopy was conducted with a short distance condenser and 100× objective in a Nikon OPTIPHOT (Nippon-Tokyo, Japan) biological microscope as described [18].

Fatty acids were quantified by HPLC equipped with a UV detector at 210 nm (Waters, Milford, MA) as outlined earlier [18]. Headspace pressures were measured prior to gas analysis using a digital pressure