Methane from biomass and waste - a program review*

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Introduction

Biologically produced energy sources have been identified as attractive alternatives to imported oil whenever supplies were seriously threatened. The oil embargo of 1973 and the subsequent oil shortfall caused by the Iranian conflict led to the establishment and growth of new energy conservation and renewable energy programmes in the United States. By 1980 the annual budget for the USDOE (US Department Of Energy's) Biomass Energy Systems programme had reached $ 56 million [1]. At that time it was estimated that the total annual expenditure for all federal biomass energy programmes was about $ 200 million. These programmes involved other USDOE divisions such as the Basic Bioenergy Sciences, Ecological Research and Industrial Programs, and other agencies such as the USDA (US Department of Agriculture), and the USEPA (US Environmental Protection Agency). Private sector sponsors initiating programmes included the GRI (Gas Research Institute), the EPRI (Electric Power Research Institute), and various industrial firms (e.g. oil, automotive, food processing, etc.).

Early results suggested that wastes and residues were inadequate to substantially impact the energy needs of a developed country. For example, one of the most abundant resources in the U S, MSW (municipal solid waste), represents a total of about 2 quads (1 quad = 10^{15} Btu, 1 Btu = 1055.06 joules), if all of it could be converted to useful energy, in comparison to the annual energy consumption of near 80 quads [2]. Further, seasonal availability, storage and convertibility problems represents additional constraints upon waste utilization for energy.

The selection of plant feedstocks for energy purposes must be based on economic considerations since the value of methane is low compared to other reduced forms of carbon. Economic and energetic calculations for utilizing domestic crops showed them to be unfavourable. A set of criteria, therefore, was defined for use in the development of biomass crops for energy production [3]. Methane yield is the most important economic parameter in the fermentation of a specific feedstock. Two basic approaches to increasing yield are: (1) controlling plant composition, and (2) improving digestion systems.

With regard to convertibility, it was learnt that conversion technologies developed for waste treatment or for high-value products were not competitive for energy production. In anaerobic digestion of biomass for energy production, high conversion efficiency is required for economic feasibility. Biologically sensitive steps, which are manageable in waste treatment, become major stumbling-blocks, being negatively affected by high loading rates. The difficulty of obtaining a complete characterization of the reactions involved in biomass-to-methane conversion forces consideration of novel approaches to an understanding of the roles of different

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biological mechanisms and of the possibilities for packaging appropriate biological material with optimal engineering designs. Therefore, based on the concept of a multiphase anaerobic digestion system, the authors' research programme was structured to address both the engineering and the biological advances necessary for economical 'methane from biomass' systems [4].

The initial strategy was to build an operating multiphase system as a research tool for addressing engineering and biological considerations integrated into a systems model for gauging sensitivities and charting research progress. Unfortunately, aid for bioenergy research in the U S began to decline in 1981, and after the reduction of crude oil prices in 1986, support dropped precipitously. By 1989, for example, the USDOE's Biofuels and Municipal Waste Division (formerly Biomass Energy Systems) support had dwindled to less than $ 15 million annually, the USDA had closed its Southern Agricultural Energy Center and scaled back other programmes, EPRI had terminated its bioenergy programme and GRI was considering redirection of its biomass-to-methane supply programme.

About the time these actions occurred, the accumulating data on acid rain and global climate change, and the need for clean-burning octane enhancers, less volatile and higher-oxygen fuels focused new attention on bioenergy fuels which are environmentally benign. Hence, in 1990, USDOE biofuels research enjoyed a modest budget increase. With the realization that bioconversion technologies that produce energy could also help solve MSW and many industrial waste processing problems, even more attention is being given to 'methane from waste'. For example, the interim report for the new U S National Energy Strategy [5] favourably considers bioenergy, and policy-makers are calling for more public support for research and development. Further, the private sector is also responding (namely, EPRI has reinstated a programme on biomass and waste to energy). With continued instability in the Middle-East, even greater attention is being given to renewable energy. Methane from biomass and waste programmes should be prominent in newly expanded projects. The programme summarized below, conducted during the past decade at the IFAS (Institute of Food and Agrcultural Sciences), University of Florida with co-funding from GRI, possesses elements common to various other programmes conducted in the U S for a variety of sponsors.

Feedstock development

Waste and residues

Until now the most abundant and sustainable biomass feedstock for energy production has been wood and wood waste, with 155.5×10^6 Mg used in the U S in 1983, and a projected consumption of 254×10^6 Mg by the year 2000. This represents about 2.5 to 3 quads, or just less than 4% of the U S energy use, but it places wood use for energy in excess of that for lumber or pulp and paper use [6]. Current usage represents 1.5% of the above-ground biomass. Unfortunately, wood is not amenable to methane fermentation with currently available technology. An inventory of agricultural crop residues [7] revealed that total U S crops represented an annual potential of 425×10^6 Mg, with an estimated 20-50% being capturable for biomass feedstock. These residues came mainly from small grains, corn and grain sorghum which are also difficult to convert to methane using conventional biogasification systems [8].

Animal manure in the U S is estimated at 101×10^6 Mg (dry) of which about 50% is recoverable [9]. While successful on-farm methane production and utilization systems have been demonstrated [10], general use of animal manures for methane has not evolved. Other resources, although not quantified, include food, beverage, and industrial wastes such as the alcohol fermentation waste processed to produce biogas for on-site utilization in the system described by Szendrey and Dorion [11]. Sewage sludge is also stabilized by anaerobic digestion at most sewage treatment plants with on-site biogas use. MSW represents about 160×10^6 Mg annually in the U S, of which about 60% is biodegradable. While one methane-from-MSW

plant operated in Florida [12], and a novel experimental system processing MSW is now being tested at the University of Florida [13], currently no MSW is being routinely digested anaerobically to methane in the U S. However, while statistics are not available on the quantities, considerable volume of methane is being recovered from landfilled MSW [10].

Energy crops

Energy crops show considerable potential for supplementing waste resources to sustain methane production in significant quantities. The projected quantity of biomass involves assumptions concerning land availability and production rates of convertible biomass at affordable costs. In the U S land should not be a constraint according to an analysis of land availability [14] which considers underutilized lands, factors relating to surpluses of domestic crops, and the potential for biotechnology applications to reduce the land requirement for current food/feed crops (as did fertilizers, pesticides and machinery in the forties and fifties).

A number of programmes nationwide have been initiated to address the question of production rates of convertible biomass. Programmes of the USDOE [2] have mainly targeted woody, herbaceous and aquatic crops, with liquid fuels as the major product goal. The IFAS [15] co-funded a project with GRI which has focused on energy crop development based on the potential for methane production [14]. The initial strategy was to evaluate each major class of plants to determine its potential to produce significant quantities of convertible biomass.

Growing crops for their methane commodity value is a relatively new concept; however, the process of crop development is well-established. The process involves species selection and evaluation of their potential to produce acceptable yields of convertible biomass with modest production inputs for the targeted environments. About 350 species/cultivars/ varieties in over 100 genera were screened through this process and the most promising ones selected for further evaluation and improvement. Because of declining support and the focus on more basic, longer-term research, target or model species were chosen. Plants were grouped into marine, freshwater aquatic, roots and tuberous, woody, and herbaceous crops.

Marine plants

The initial goal was to build upon the previous research on *Macrocystis* (giant kelp), *Laminaria*, and other species in the U S Northeast, Southwest, and Hawaii from programmes sponsored by GRI, the Solar Energy Research Institute, the National Science Foundation, and others [15]. After screening several species, intensive research was conducted with *Gracilaria* sp. because cultivation could take place in shallow tidal areas (< 1.5m depths). Simulating water exchanges in nature by tidal flushings, possible yields (all yields reported are dry weights) were estimated to reach 25 Mg ha^{-1} yr^{-1} based on raceway experiments [16]. Later, the emphasis was shifted to *Sargassum*, a floating marine plant, because expensive field support systems could be avoided. While yield studies similar to those conducted with *Macrocystis* and *Gracilaria* have not been done, experiments completed with *Sargassum* indicated that yields were comparable to *Macrocystis*, although lower than *Gracilaria*.

Before this research was closed out, information was compiled on nutrition and photosynthetic/respiration responses to salinity and temperature changes, and it was determined that there was considerable intrageneric variation and that a large genetic base existed for plant-breeding research.

Freshwater aquatic

Many aquatic species - submerged, emergent and floating - were screened, especially for growth in naturally eutrophic waters or waters enriched by human

activity. The most productive were *Eichhornia crassipes* (water hyacinth), *Pistia* sp. and *Hydrocotyle* [17]. Biomass yields of these floating species were greater than the yields by emergent plants such as *Typha* sp. (cattail), *Scirpas* sp. and *Phragmites* sp. In other environments, *Typha* and *Phragmites* have produced impressive yields. Under non-limiting conditions, a remarkable annual dry yield of 234 Mg ha^{-1} for *Eichhornia* was demonstrated. The responsiveness of this plant to production inputs is illustrated by a 2.5 times increase in growth rate when the CO_2 in the air was enriched. Typical biomass yields of *Eichhornia* were from 50-65 Mg ha^{-1} yr^{-1} when grown in amended or unaltered drainage water or eutrophic lake water.

Hydrocotyle is more tolerant to cold temperatures than *Eichhornia*. Therefore, the most promising production systems include these two species in sequence so that growth is sustained throughout the year. To achieve high levels of productivity, nutrients, plant density and harvesting practices have to be managed. Because of the productivity of these systems, the deliberate cultivation of *Eichhornia* and associated species in biomass farms to renovate nutrient-enriched wastes is attracting more attention. The potential to generate methane from biomass enhances the viability of such systems.

Root and tuberous crops

Numerous cultivars in 16 genera were field tested at research sites throughout Florida. Sweet potato (*Ipomoea batata*) and several of the cruciferous crops showed the most promise for biomass production [18]. Sweet potato yields were as high as 28.5 Mg ha^{-1} during production periods of 130-160 days. Cassava (*Manihot esculenta*) and taro (*Colocasia esculenta*), other spring/summer production crops, gave lower yields but could provide crops in environments (e.g. taro on flooded soils) not adapted to higher yielding species. During cool seasons, crucifer (*Brassica* sp.) and beet (*Beta* sp.) varieties produced up to 11 Mg ha^{-1}. Some of these mature in 80-100 days, making it possible to grow two crops in one year. Combining sweet potato and turnips (*Brassica rapa*), yields of 40 Mg ha^{-1} were possible in one year. Problems with these species relate to storage since they are perishable, and to the high cost of propagating some of the more productive crops (e.g. sweet potato) which do not produce seed.

Woody crops

Seventy-three species of woody plants were screened for productivity in field plots statewide [19]. Among these were five *Eucalyptus* species and sixty-two sources of *Leucaena leucocephala*, two of the most promising genera, along with *Pinus* sp. and *Sapium sebiferum*. Maximum yields established on excellent agricultural soils were 23 Mg ha^{-1} for *Eucalyptus* and 37 Mg ha^{-1} for *Leucaena*. Average yields of 10 Mg ha^{-1} (*Eucalyptus*) and 25 Mg ha^{-1} (*Leucaena*) were recorded for trees grown in 2 or 3 year rotations at densities ranging from 2000 to 5000 trees ha^{-1}. Higher densities resulted in maximum yields, but planting costs were excessive. The optimum yield rotations for these species resulted in biomass not convertible to methane with current technology. Co-operative research with others working with 'woodgrass' plantings (> 10 000 stems ha^{-1}, annual harvests) suggested that, with a specific inoculum for woody biomass, acceptable methane yields may be possible [20]. Until conversion advances are realized, woody species are being considered for other biofuels, as is the case for USDOE programmes.

Herbaceous crops

Hydrocarbon producing plants such as *Euphorbia* sp.and *Aesclepias* sp. were initially included in evaluations because of the potential to use non-biological means to produce methane from the biomass hydrocarbons. However, yield studies were not encouraging [21] so work with hydrocarbon plants was discontinued.

Many other non-conventional herbaceous plant species were evaluated [22] with some, such as *Eupatorium, Sida* and *Amaranthus*, showing reasonable annual yields (18-44 Mg ha^{-1}). Similarly, many species and varieties of the *Gramineae,* or grass family, were field tested in diverse environments both in this programme and in those of the USDOE. Because of the outstanding performance of *Pennisetum* sp., *Saccharum* sp., and *Sorghum* sp., these were chosen for concentrated research and development as methane feedstocks [23]. All three have the efficient C_4 metabolic system and grow especially well at the high growing temperatures in temperate and subtropical climates. *Pennisetum* and *Saccharum* are perennials that grow up to 9 months of the year in Florida. The superior *Pennisetum* (napier or elephant grass) and *Saccharum* (sugar cane) selections produced annual yields between 40-60 Mg ha^{-1} while only the best *Sorghum* yields approached the low end of this range [23]. *Pennisetum purpureum* (PI 300086) and *Saccharum spontaneum* (LP 79-1002) are especially productive selections that come closest to meeting the several criteria outlined for biomass crops [3]. However, these perennials show sensitivity to multiple or early fall harvests through reduced yield and reduced cold tolerance.

Yields along an environmental gradient were evaluated by growing two of the most promising *Pennisetums, Saccharums* and *Sorghums* in field tests ranging from a sub-tropical site in southern Florida to a reasonably cold temperate site in north Alabama [24]. The perennials were all winter-killed at the Muscle Shoals Alabama site. *Pennisetum* (N51) and *Saccharum* (LP 79-1002) maintained excellent stands and high yields at all the remaining sites (Auburn, Alabama and Jay, Quincey, Gainesville and Ona, Florida). Highest yields (30-47 Mg ha^{-1} yr^{-1}) were at the southernmost site (Ona, Florida) with average yields over all Florida sites being 32.7 Mg ha^{-1} yr^{-1} (Prine, 1990, unpublished data, University of Florida, Gainesville). *Sorghum* sp. proved to be highly susceptible to drought, with half the yield of the perennial grasses.

Research has been concentrated on these promising grass species because the BIOMET systems model, developed by this programme [25], showed that cost per cubic metre of methane declined little in production systems after yields exceeded 20 Mg ha^{-1} yr^{-1}. With *Pennisetum* and *Saccharum* easily exceeding this threshold, research was focused on improving the convertibility of the biomass to methane, even if total yield is reduced. This is being approached by conventional breeding to change the physiology of the plant to produce larger portions of the more convertible components [26] and through cultural (e.g., fertility level) and management practices (e.g., harvesting frequency) that affect methane yield [27].

Biotechnology applications

Two of the most promising biomass crops (*Ipomoea batata* - sweet potato, and *Pennisetum purpureum* - napiergrass) are reproduced vegetatively. To reduce the area (cost) for producing propagative material, research to tissue culture the two species was initiated. Sweet potato was tissue cultured for the first time in this programme and then used as a model for artificial seed development [28]. Shoot apical meristem dome explants produced embryogenic callus on agar medium containing auxin with frequencies greater than 90%. The clonal plantlets from callus were then embedded in various gels for fluid 'seeding' in the field. To improve survivability, Hung et al. [29] demonstrated that inoculating sweet potato with vesicular arbuscular mycorrhizal fungi improved phosphorus uptake and growth.

Napiergrass and other *Gramineae* species were also successfully regenerated through tissue culture, with the plants produced being true to type when compared to vegetative plantlets from the same source [30]. In addition, the early growth rate of tissue-culture-derived plants was greater than the growth rate of vegetative propagules. Another approach to lower propagation cost was to cross *Pennisetum* and obtain hexaploids capable of producing seed [31]. Recent work has focused on isolation

of protoplasts and obtaining protoplast fusion as a way of genetically improving vegetatively propagated species, and introducing foreign genes into protoplast and obtaining their expression [32]. Both electroporation and the biolistic methods of obtaining transgenic plants have shown promise. RFLP (restriction fragment length polymorphic) markers are being developed for the purpose of mapping the napiergrass genome and locating genes important in biomass production and methane bioconversion. These markers are being used to assist in genetic studies and in genetic improvement of *Pennisetum* biomass lines [33,34]. Genetic linkage relationships have been determined between plant traits, including stem and leaf composition traits, and RFLP markers (Smith R L, unpublished data, University of Florida, Gainesville).

Success with attempts to apply contemporary biotechnological techniques to biomass crops is encouraging. These techniques, combined with a growing body of data from this programme on plant characteristics relating to methane yields and rates, suggest that expeditious progress on genetic improvement of biomass crops as methane feedstocks should be possible.

Anaerobic digestion process development

Background

Anaerobic digestion consists of a complex series of reactions which are catalysed by a mixed group of microorganisms. In these reactions organic matter is converted in a stepwise fashion to methane and carbon dioxide. Polymers such as cellulose, hemicellulose, pectin and starch are hydrolysed to oligomers or monomers, which are then metabolised by fermentative bacteria with the production of hydrogen (H_2), carbon dioxide (CO_2), and volatile organic acids. The volatile organic acids other than acetate are converted to methanogenic precursors (H_2, CO_2 and acetate) by the hydrogen-producing, organic acid oxidizers. Finally, the methanogenic bacteria produce methane from acetate or H_2-CO_2.

Each biodegradable substrate which enters an anaerobic digester, and each extracellular intermediate produced from a substrate, creates a potential carbon and energy source to support growth of a physiological unique microflora. The more specialized the substrate, the more defined is the particular microflora which develops. Less specialized substrates, such as sugars, may support a more varied catabolic population. Under steady-state conditions, the dominant organisms are normally the ones whose characteristics are most favoured by the environment (ability to use substrate at lower concentrations, ability to excrete metabolic products rapidly, more efficient energy production, faster growth rate, etc.). If well-adapted organisms are not initially dominant, they soon become so. In mixed-culture systems such as digesters, high populations of an organism which is not normally dominant may sometimes be desired. This could be achieved by additions of desired non-dominant organisms, or some other control technique.

The formation of methane from biomass, therefore, involves many intermediates and the interaction of many microbial species. Acids believed to be the predominant precursors of methane are carbonic acid, acetic acid, propionic acid and butyric acid. The effects of these acids on the overall fermentation have not been elucidated, although they (with the exception of carbonic acid) have long been recognized as inhibitors of microbial growth. It has been speculated that short-chain organic acids inhibit the conversion of organic matter to methane by some mechanism which is pH independent [35]. The nature of this mechanism is obscure. High concentrations of propionic acid appear to be more toxic for anaerobic digestion than other acids [36]. Indeed, the metabolism of this acid is the most difficult parameter to control during the start-up of napiergrass-fed digesters (Wilkie A C, unpublished data, University of Florida, Gainesville).

In the course of the fermentation of organic material, the rate of acid formation may exceed the rate of dissimilation, in which case the concentration of the acids

continues to increase and the fermention ultimately ceases. This fact led to the development of two-phase anaerobic digestion systems in which the acid-forming phase is separated from the methane-forming phase. A rational approach to the optimal regulation of the overall process requires an understanding of the interplay between the production and dissimilation of organic acids and the formation of acid precursors, leading to a perception of the critical reactions and their rates. However, the regulation and control of metabolic processes are difficult to define in specific terms, mainly because the regulatory processes occur at the cellular level, with strong interactions between the different microbial species.

Conceptual issues

Developments in reactor design and operation have established anaerobic digestion as an accepted process for industrial, agricultural and municipal wastewater treatment. There is a wide variety of currently functioning, single-stage anaerobic digesters that are operational currently. Viewed from an engineering perspective, these include continuously stirred-tank reactors with and without recycle, upflow and downflow anaerobic filters, downflow stationary fixed-film reactors, anaerobic expanded-bed and fluidized-bed reactors, upflow anaerobic sludge blanket reactors and landfills [8]. There are almost limitless variations in the operational regimes employed for these single-stage digesters. However, an examination of existing processes for low- to high- solids fermentation [37-42] led to the conclusion that none of the many digester designs in contemporary use were suited to the goal of continuous production of commercial quantities of pipeline-quality methane gas. Both engineering and biological principles were addressed in the consideration of this analysis. The engineering aspects of digester function, e.g. temperature, pH, substrate feed rates, and monitoring of biogas production and composition, could be reasonably well controlled. Innovative approaches, such as real-time adaptive control systems, can further enhance the control of these parameters that are closely linked to bioprocesses [43,44]. By contrast, biological process control--in the sense of monitoring and adjusting the process to allow biological difficulties to be anticipated and controlled--had received little attention and there was insufficient information available to allow standards for the control and regulation of the biological parameters to be formulated.

The rumen fermentation suggests that biomass fermentation in more than one phase is biologically attractive. Detailed examination of ruminant physiology reveals the presence of at least two phases: (1) an anaerobic phase, in which organic acids are formed in the rumen, and (2) an aerobic phase, in which organic acids are metabolized in the animal tissue. The anaerobic phase is stratified into a high-solids subphase and a liquid subphase [45]. Metabolic activity and concentrations of intermediates are greater in the high-solids than in the liquid subphase. Such a multiphase digestion system mush have desirable lasting qualities since it has been maintained in Nature for millenia.

The many different physiological groups of bacteria, whose activities contribute to the overall process, could best be optimized relative to their individual metabolic rates if they could be separated into a number of different digestion phases. In two-phase anaerobic digestion processes, a higher overall reaction rate is achieved by optimizing two major phases. However, the process can be conceptualized as consisting of multiple phases, each characterized by the predominance of unique microbial communities. An engineering design based on this ecological concept would provide the best possible environment for the rapid growth and metabolic activities of specific physiological groups of bacteria. This multiphase digestion of biomass would require the construction of a number of different fermenters, each of which could be individually controlled relative to both engineering and biological operating parameters.

The optimum number of phases for biomass conversion has not been established. In multiphase digestion of biomass, the number of phases could be quite large to allow regulation and control of such parameters as temperature, pH, microbial life cycles and population shifts, osmotic changes, oxidation-reduction potentials, added oxidants, introduction of unique microorganisms, and production and removal of inhibitory levels of organic acids. While the advantages of multiphase over single-stage anaerobic digestion have not been demonstrated at the commercial level, the authors' research has focused on an intense study of microbial ecology relevant to the development of commercial multiphase digesters.

A 'multiphase anaerobic digestion concept' was thus envisaged [4,46] to serve as a conceptual basis for development of an anaerobic digester design that could optimally convert biomass to methane with maximum yield and minimal cost. Methanogenesis and other phases of the anaerobic digestion process should be amenable to improvement by application of current biotechnological and genetic engineering techniques. The strategy, therefore, was to exploit biotechnology in the development of a cost-optimized process.

Operational sensitivities

In the context of a biomass fermentation system for the production of pipeline quantities of methane, interacting factors which impact directly on projected methane production costs include mass transfer, heat transfer and rate control. Mass transfer is dealt with mainly by digester design and other engineering requirements. Materials handling of feedstock biomass could be minimized in a multiphase digestion system in which the different phases are created by altering the microbiology of the fermentation, at time intervals designed to optimize different reactions for maximum fermentation productivity.

Heat transfer is another major cost factor. The potential for producing heat and enhancing the conversion of large recalcitrant organic polymers, by addition of air, was considered and then abandoned because of difficulty in distributing a relatively insoluble gas (O_2) in the liquid fraction of the digesters, and the added complications of nitrogen, which would then have to be eliminated from the biogas. In contrast to oxygen, nitrate is water soluble and would not introduce nitrogen gas into the digester. Nitrate reduction by a carbohydrate substrate will, theoretically, produce more heat per electron pair than reduction of oxygen. This makes nitrate amendment potentially attractive for temperature regulation. Nitrate was therefore selected as an alternative to air and large amounts were successfully added to laboratory-scale napiergrass digesters. Nitrate was quantitatively reduced to ammonia and there was no inhibition of the fermentation from the ammonia produced [47]. The demonstration of nitrate conversion to ammonia suggests that nitrate could be economically retained in a recycle process (using conventional methods for the conversion of ammonia to nitrate) and while also enhancing the nitrogen nutrition of a multiphase digestion process. Further, nitrate was shown to be preferentially reduced in the presence of sulphate and nitrate, which suggests that nitrate amendment may offer possibilities for the regulation and control of inhibitory sulphide concentrations [47].

Rate control affects a number of important aspects of the anaerobic digestion process. Rate control may be used to keep the various components of the fermentation in balance, to maximize the overall rate of the fermentation (thus reducing fermenter volume) and to alter the end-products, producing biogas with maximum methane content. Overall rate control was subdivided, for experimental purposes, into the biological sub-areas of hydrolysis and organic acid metabolism.

Multiphase pilot unit

As a point of departure, a two-phase anaerobic digestion system for the conversion of napiergrass to methane was constructed. The first phase, the LB (Leaching-Bed) phase, in which organic acids are formed, contains the plant material. The methane-forming second phase, the PB (Packed-Bed) phase, receives liquid effluent from the first phase. The complete LB-PB system consists of four 300-litre LB reactors in parallel, coupled to a 600-litre upflow PB reactor. The number of components in such a system could be much larger, and would be determined by both engineering and biological considerations. Different reactions predominate in the packed-bed as compared to the LB reactors [4]. During normal operation of the LB, the most significant reactions are the conversion of biomass to soluble intermediates and volatile fatty acids whereas, in the PB, the most significant reactions are the conversion of soluble intermediates to volatile fatty acids and thence to methane and carbon dioxide. Each of the four LB reactors is operated as a batch reactor with respect to the solid phase and as a continuous flow reactor with respect to the fluid phase, except when charging with solids. The LB reactors are charged at different times so that the fluid flow to the PB reactor can be a mixture of streams from the LB rectors, each having a different composition and concentration. This dampens the concentration of volatile fatty acids in the influent to the packed bed, thus permitting it to operate in a more steady-state fashion.

There are several variables for the system that can be manipulated, such as (1) make-up water, to replace losses and control salt content and alkalinity; (2) waste sludge from the PB reactor; (3) recycle around the PB reactor, and (4) recycle around the LB reactors and, perhaps, around each individual LB reactor. Selecting the optimal operational strategy is, therefore, difficult.

Three possible reasons for recycling around the LB reactors are: (1) to bring the high volatile fatty acid effluent stream from the LB rectors in to contact with the low volatile fatty acid effluent from the PB reactor, thus lowering the pH and releasing CO_2 with a possible reduction in gas-scrubbing costs, (2) to maintain a high concentration of volatile fatty acids in the LBs for minimization of methane production in the LBs, and (3) inoculation of freshly charged LB reactors with appropriate microorganisms,

Proper adjustment of the ratio of recycle around the LB reactors to that around the reactor system, as well as recycle around each LB reactor, could also prove useful in avoiding inhibition of hydrolysis by high volatile fatty acid concentrations, if this proved to be a problem.

Specifications of the appropriate reactions, stoichiometric relations, and kinetics for the LB and PB reactors were used to prepare material balances, with the resultant set of equations comprising a generalized mathematical model for the process [48]. A computer-based simulation package was developed for iterative modelling of the system. Preliminary simulations for partial model validation, sensitivity analyses and exploration of the effects of the recycle streams on overall system performance have been reported [49]. The mathematical model developed for the LB-PB process can be considered a first approximation, requiring further refinement based on observed experimental data and more precise definition of the parameters used in the model equations, which will emanate from fundamental research being undertaken to elucidate the biological processes involved in the various phases of biomass conversion to methane.

A variation of the multiphase digestion concept, referred to as the SEBAC (sequential batch anaerobic composting process), has been developed at the University of Florida for conversion of the organic fraction of MSW to methane and has been evaluated at pilot scale [13,50]. The SEBAC process employs three reactors moved sequentially through three stages (new, mature and aged) for enhanced conversion of MSW to methane. In stage 1 (new), the coarsely-shredded organic fraction of MSW is packed into a reactor and inoculated by recycling leachate from the

already active stage 3 (aged) reactor. During start-up, leachate recycle removes inhibitory organic acid levels which are converted to methane in the stage 3 reactor. When the fermentation is balanced, the stage 1 reactor is operated in batch mode, corresponding to stage 2 (mature) of the process. Simultaneously, the current stage 2 reactor becomes the stage 3 reactor and serves as an inoculum for the next stage 1 reactor as well as an active methanogenic stage for conversion of the organic acids which accumulate during start-up in stage 1 [13,51]. Using this operational design, which could be conducted in reactors or controlled landfill cells, about 50% conversion of the organic fraction of MSW to methane and carbon dioxide is possible in 21-30 days with a methane yield of 0.2 L per g volatile solids (dry organic matter) per day [13].

Biochemical evaluation of the fermentation process

Historically, anaerobic digestion as an industrial process has been utilized in waste treatment under conditions not impacted by a need for rapid rates as in the conversion of plant biomass. Under such circumstances digester failure can usually be avoided by the simple expediency of reducing the feeding rate. However, as loading rates are increased, biological activities in digesters are stressed towards failure. It should be possible to continually operate digesters at a maximum stable rate for substrate conversion and methane production, if the methodology can be developed to quantitatively determine key parameters that are predictive of digester stress and impending fermentation failure.

The main objective of biological process control is to achieve a microbial population capable of responding rapidly to increased loading so that perturbations can be minimized. Knowledge of the metabolism of the organisms involved in methanogenesis and of factors determining the behaviour of these microorganisms is essential. This will provide a basis for improvements in the biotechnology of the process in terms of control and predictive methodology [51]. Major biological sensitivities applicable to the optimization of anaerobic digestion at the commercial scale have, therefore, addressed. The quantitative definition of the following fundamental biological processes should enable anaerobic digesters, in general, to be operated at maximum stable conversion efficiencies and methane productivities.

Probes

The term "probes" is used to mean analytical technology capable of evaluating molecular aspects of digester contents, to ascertain whether or not the digestion process is functioning optimally, and can continue to function optimally in the absence of future perturbations.

Pyridine nucleotides and factor 420

Direct measurement of pyridine nucleotide oxidation and reduction was selected for examination [52] because the pyridine nucleotides are directly involved with many metabolic reactions, catalysing one, two, or four electron transfers, depending on the specific reaction and the microbial species. The expectation is that changes in the relative concentrations of the reduced and oxidized form of the coenzyme should reflect changes in the metabolic rate of the fementation. A fluorescence probe designed to measure F_{420}, found only in methanogens, has also been explored [52], but both require additional research to determine their applicability.

Lipid analysis

Analysis of bacterial lipid in digesters appears to hold promise as an analytical procedure to monitor the health and well-being of anaerobic digesters at the commercial scale [53]. Analytical techniques have been developed which can precisely measure the specific lipids present in a complex ecosystem. Since specific lipids

are accurate signatures for specific bacteria, quantitative analyses of lipids can monitor the relative abundance of different microbial communities and, in turn, the health of the fermentation occurring in a reactor. In addition, the lipid composition of bacterial species seems to vary under conditions of stress. Calculations based on total extractable lipid phosphate showed a direct correlation with total bacterial number counts, suggesting that it should be possible to use an automated lipid analysis for continuous monitoring of viable microbial biomass in a fermentation system[54].

Antigenic analysis

The methanogenic bacterial population of an anaerobic digester carries out the terminal reaction of biomass conversion to methane. The overall process, therefore,is dependent on a large stable population of methanogenic bacteria. Direct and rapid determination of the overall methanogenic population in a specific digester at a particular time should provide the base information needed to manipulate methanogenic populations and to manipulate target populations of methanogens. Complete population descriptions are difficult, if not impossible, to determine quantitatively, because the predominating methanogenic bacteria present in samples overgrow other species present in smaller numbers. This problem is avoided by identifying the methanogens by their antigenic fingerprints. Results obtained, using this technique in the analysis of digesters, showed that there was considerable diversity in the methanogenic populations in different digesters [55,56]. Antibody probes now have been developed for 31 reference methanogens, which extends the application of the technique to a variety of environments [56].

Microbial degradation of plant structural polymers

Enzyme interrelationships

A wide spectrum of enzymes is recognized as functional in the degradation of plant material. Many of these enzymes are regulated in their synthesis by, as yet, poorly understood interactions between different physiological group of microbes. A striking example of this phenomenon is the effect of methanogenic bacteria on the synthesis of anaerobic fungal enzymes. Xylanosis has recently been shown to be markedly stimulated when anaerobic fungi were grown in association with *Methanobrevibacter smithii* [58]. Previously, it has been found that the rate and extent of cellulolysis by a *Neocallimastix frontalis* isolate was stimulated by growth with *Methanobrevibacter ruminantium* [59] and that straw solubilization by *Neocallimastix* spp. and *Piromonas* spp. was enhanced in methanogenic co-cultures [60]. The reasons for the stimulation of fungal activities by methanogens are likely to be complex and to involve several factors. Further studies are needed to determine the factors controlling these interspecies interactions.

Key enzyme systems were selected for evaluation on the assumption that an understanding of the structure and function of key enzymes would provide insights for enhancement of biodegradation of large polymers in commercial-scale digesters of the future. Procedures have been developed to separate many of the enzymes involved. Enzymes selected for study include phosphatases, cellulases, hemicellulases, xylanases, pectinases and hydrogenases, each with unique potential contributions to the improvement of the anaerobic digestion process.

Regulation of cell wall depolymerization

Actions of pectinases, cellulases and hemicellulases are preceded by an initial unmasking of the cell-wall components of plants being fermented. It was speculated that the presence of phosphocholine diesters may contribute to the inhibition of these enzymatic activities. Phosphatases and phosphodiesterases were suggested for potential use as a pretreatment to stimulate the depolymerization of plant cell wall polysaccharides and thus enhance the overall conversion rate of biomass (napiergrass was the test species) to methane (Gander J E, personal com-

munication, University of Florida). Phosphocholine phosphodiesterase should unmask plant sugar polymers substituted with phosphocholine diesters, allowing resistant polysaccharides to become susceptible to appropriate depolymerases. A phosphocholine phosphodiesterase has been purified to homogeneity and conditions for stabilization of this enzyme have been obtained.

Cellulose degradation

Once the polymers become unmasked, the goal is to develop a database which would be useful in enhancing methane formation from napier grass, by developing new insights into the structure and functions of key enzymes prominent in the depolymerization of large polymers. One such group of enzymes is the cellulases. Cellulase enzymes from digester anaerobes and from aerobic fungi were studied both from the standpoint of structure and function. The tight binding of extracellular cellulase enzymes, labelled with gold, to plant cell walls permits the ultrastructural location of cellulosic material in plant biomass [61]. This technique is useful in studies of cellulose degradation and cellulose deposition and in the studies of the interaction of cellulose with other wall components. Research results have established that fungal cellulase can enhance the anaerobic conversion of biomass to methane. The cellobiohydrolase II of the aerobic fungus *Trichoderma reesei* was found to act synergistically with the cellulases of three anaerobic cellulolytic isolates from biomass digesters [62].

Three protein components from a cellulose-grown culture of *Clostridium aldrichii*, which was isolated from a poplar-fed digester [63], were identified and partially characterized. There are two putative endoglucanases (with isoelectric points at 3.9 and 4.3, respectively) which cleave both barley glucan and CM-cellulose, in a manner yet to be elucidated (Brown R D, personal communication, University of Florida). Recently, studies on eight site-mutagenized cellobiohydrolases (also called exoglucanases) from *T. reesei* showed the amino acid residue ASP 175 to be essential for catalytic activity and several residues in this region of the enzyme structure have been implicated in binding to cellulose [64].

Pectin degradation

Pectins in the plant cell wall can be depolymerized by both eliminases and hydrolases. The action of these enzymes has the effect of unmasking other polymers such as cellulose and hemicellulose, thus enhancing the susceptibility of these substances to the action of hydrolytic enzymes. These enzymes also contribute to the overall fermentation by producing depolymerization products which contribute to the total methane yield. *Erwinia chrysanthemi* enzymes have been isolated and examined [65]. This phytopathogen elaborates a battery of four or more pectate lyases, with pI values ranging from 4.2 to 9.6. These enzymes display unique mechanisms in the depolymerization of pectins, ranging from predominantly endolytic to exolytic, which collectively contribute to the maceration of plant material. In contrast, bacteria associated with a stable environment, e.g. *Lachnospira multiparus* in the bovine rumen, *Clostridium populeti* from a poplar-fed methane digester, or bacteria associated with healthy tissues of the brown algae *Sargassum*, secrete acidic pectate or alginate lyases with exolytic/endolytic depolymerization mechanisms [66-68]. Through the secretion of different polyuronate lyases, these bacteria should provide a useful genetic resource for the directed depolymerization and solubilization of plant tissue.

Xylan degradation

Polymers of xylan are second only to cellulose in natural abundance and represent an integral component of plant cell wall structure. The depolymerization and subsequent metabolism of this material contributes appreciably to the methane yield.

Butyrivibrio fibrisolvens plays a major role in the degradation of xylan in the rumen of cattle and was found to be one of the most abundant xylan-degrading organisms present in the napiergrass-fed laboratory-scale anaerobic digesters [69]. Consequently, the enzymology and genetics of xylan degradation were examined using *Butyrivibrio fibrisolvens* as a model organism.

Xylan is composed of mixtures of sugars, and multiple enzymes are required for its degradation. The genes encoding these enzymes are co-ordinately regulated and appear to be located adjacent to each other on a cloned DNA fragment [70, 71]. Development of genetic exchange systems and shuttle vectors to carry modified genes between *B. fibrisolvens* and *Escherichia coli* is ongoing, providing a potential mechanism to improve the production of key enzymes needed for the optimal degradation of plant structural polymers (Ingram L O, personal communication, University of Florida).

Methane potential from biomass

The amount of methane that can be obtained from a given amount of plant material by anaerobic fermentation is difficult to assess because it is a function of the many factors influencing the anaerobic digestion of organic matter. Plant composition (and methane production) is affected by variety, age, anatomy, agronomic practices, *inter alia* [72]. Thus, a database ranking potential plant feedstocks, based on methane yield, could prove useful.

Fermentability of different feedstocks was investigated using the biochemical methane potential assay [73]. The methane yields varied for the four major resource groups, freshwater aquatics, forage and grasses, roots and tubers, and marine species [4]. The yield from marine species was lower than from any other resource group; the highest yields were obtained from root crops, followed by forage and grasses, and freshwater aquatics, respectively [4,27]. After extending these assays to additional species, plant parts, growth conditions, harvesting sequences and storage procedures, the ultimate fermentability is generally in the range of 40 to 70% (corresponding to a methane yield of 0.2 to 0.4 litre/g volatile solids) for most lignocellulosics, being inversely related to the lignin content [74]. The goal is to use this extensive database to develop a model to relate fermentability to chemical composition. With knowledge of these relationships and those that affect composition, methane yield from biomass becomes more predictable and manageable.

Culture collection and electron microscopy

Numerous new bacterial species have been isolated at the University of Florida, the University of California at Los Angeles and at the Oregon Graduate Institute of Science and Technology, to assist in the development of a strong fundamental base for biomass-to-methane research. A key achievement of the culture work conducted in the GRI-IFAS programme was the study of *Methanosarcina mazei*, which was isolated in pure culture by Mah [75]. This organism, which has a complex life cycle and a unique growth pattern, plays a major role in the metabolism of acetic acid during the conversion of biomass to methane [76-78]. A culture collection was established which includes these isolates, other important methanogens, and anaerobes that interact with methanogens during the biomass conversion. Over 85 methanogenic species and strains are currently deposited in the collection along with other associated organisms.

Methanogenic bacteria were examined at the ultrastructural level to provide a database for the identification of these organisms in mixed cultures, and to visualize and identify the cellular sites of methane formation in the complex environment of an anaerobic digester [79,81].

Organic acid metabolism

Theoretical modelling has been used to characterize the inhibitory effects of organic acids on the anaerobic fermentation process. However, no data are available to evaluate the effects of the rates of formation or the concentration of these acids on the rate and stability of anaerobic digestion of biomass in a single-stage process. Emphasis in this programme has recently focused on propionic acid and it has been shown that a stable fermentation of biomass can be maintained at elevated propionate concentrations [82]. This suggests that, if other factors could be regulated to accommodate the fermentation, it should be possible to operate a phase of a multiphase digestion system at high concentrations of organic acids.

Wilkie has shown that the addition of specific micronutrients has a stimulatory influence on the catabolism of organic acids in napiergrass-fed digesters [82]. In four separate experiments, the concentrations of acetic, propionic and butyric acids decreased below the detection limits of 0.1 mM for all three acids, and volumetric methane productivity increased to levels theoretically anticipated on the basis of complete catabolism of the organic acids present in the mixed-liquor, after micronutrient addition [83]. The results obtained permit quantitative examination of the effects on the fermentation profile of a variety of parameters, such as organic acid concentrations, organic loading rates and pH. Subsequent to micronutrient supplementation, it was possible through increased loading rates to further increase volumetric methane productivities five-fold, while still maintaining complete methanogenic conversion of the volatile fatty acids generated (Wilkie A C, unpublished data, University of Florida, Gainesville).

The economic implication of these findings is important since trace elements would be a simple, low-cost nutritional additive for biomass fermentations which are micronutrient-limited. The micronutrient composition of energy crops should, therefore, be determined relative to their utilization as feedstocks for methane production. Alteration of a fermentation profile by manipulation of micronutrients may prove a useful tool in the regulation of specific aspects of multiphase digestion systems.

These results have led to a new experimental design which facilitates the examination of the effects of intermediate metabolites on the overall reaction sequences occurring in the conversion of biomass to methane. This experimental design enables the measurement of fermentation parameters in digesters infused with specific extracellular intermediates and the comparison to like parameter measurements in non-infused control digesters. The role of propionic acid, for example, in the regulation of biomass conversion to methane has been particularly enigmatic. Using this basic experimental design, it was shown that methane formation in mixed cultures infused with propionic acid is only 87% of the stoichiometric methane yield anticipated based on the operating parameters and methane yields of control cultures [84]. The inhibition observed is not a function of the concentration of hydrogen or propionic acid, pH, or the capacity of the microflora to produce methane, from acetic acid or molecular hydrogen. Unless proven otherwise, it is concluded that the bacteria which metabolize propionic acid produce some factor that is inhibitory to the initial phases of the conversion of complex organic material to methane [84].

Concluding remarks

As the results of the GRI-IFAS Biomass to Methane programme indicate, there has been substantial progress in recent years in the development of basic understanding about the growth of plants as an energy feedstock and the process of converting biomass into an alternate energy form. The time has come to construct and operate a biomass-to-energy system to produce methane, with a production area and conversion device of sufficient scale to assure that results obtained would be applicable to commercialization. The system constructed should be flexible to allow testing

of a wide variety of plant species and conversion processes, and should serve as a focal point for specific research and development studies from the molecular level to the systems level. It should have sub-systems, not only to deal with an alternate energy product, but also, to deal with the numerous environmental problems associated with energy production and industrial waste streams. Success in such an undertaking would provide major economic and environmental benefits.

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