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Methane Fermentation of Woody Biomass*

Charles E. Turick, Michael W. Peck,‡ David P. Chynoweth, Douglas E. Jerger§

Agricultural Engineering Department, University of Florida, Gainesville, Florida 32611, USA

Edwin H. White

College of Environmental Science and Forestry, State University of New York, Syracuse, New York 13210, USA

Louis Zsuffa & W. Andy Kenney

Faculty of Forestry, University of Toronto, Ontario, Canada MS5 1Al

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Abstract

*Woody biomass has been previously considered to be highly refractile to anaerobic digestion without extensive pretreatment. However, this study has demonstrated that high rates and ultimate methane yields may be obtained in batch assays without pretreatment, other than some particle size reduction. In a survey of 32 woody samples (chiefly willow and poplar species grown in a wood–grass system) significant yields of methane (≥ 0·25 liters g^{-1} volatile solids added) were obtained from 19 of the samples, while the yield from 3 of the samples (*Salix eriocephala, S. exigua *and* Platanus occidentalis*) exceeded 0·30 liters g^{-1} volatile solids added. The commercial production of energy through the biological gasification of woody biomass may therefore represent a viable alternative for energy production in the United States. An interesting feature of this study was the biphasic nature of methane production from two-thirds of the samples tested.*

Key words: Poplar, willow, anaerobic digestion, methane, wood, biological gasification.

INTRODUCTION

The requirement for alternative sources of energy has led to an examination of commercial methane production from woody biomass through anaerobic digestion (biological gasification). Although thermal gasification is considered a more feasible process for methane production from wood in some circumstances, biological gasification has several advantages: (1) the natural moisture of the wood does not reduce the efficiency of gasification; (2) the product gas has few components (methane, carbon dioxide and traces of hydrogen sulfide) and a high heating value, and thus burns more efficiently than the product from thermal gasification, requiring less upgrading to produce pipeline quality gas; (3) oxygen is not required; (4) the process is economic at a variety of scales; and (5) a potentially valuable product (lignin) remains undegraded in a relatively pure form. With intensive culture of rapid-growing, high methane-yielding clones, woody biomass could make a significant contribution to the energy requirements of the United States (Ranney & Cushman, 1980; Jerger *et al.*, 1982; Chynoweth & Jerger, 1985; Chynoweth *et al.*, 1985; Klass, 1987; White *et al.*, 1989). However, doubts over woody biomass biodegradability (Wilson & Pigden, 1964; Baker *et al.*, 1975; Bjorndal *et al.*, 1987) have led to the development of many pretreatment procedures designed to disrupt the lignocellulosic complex so

*Florida Agricultural Experimental Station, Journal Series No. R-00734.

‡To whom correspondence should be addressed at: Genetics and Microbiology Department, AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich, Norfolk NR4 7UA, UK

§Present address: O.H. Materials Corp., Findlay, Ohio, USA.

that cellulose and xylans may be more fully and rapidly utilized (Jerger *et al.*, 1982; Chynoweth & Jerger, 1985; Lin *et al.*, 1985; Tsao, 1987). In earlier studies, however, the authors achieved a substantial conversion of some hardwoods to methane using the biochemical methane potential (BMP) assay (Jerger *et al.*, 1982; Chynoweth & Jerger, 1985; Chynoweth *et al.*, 1985), a finding confirmed recently with a *Populus* species (Tong *et al.*, 1990). This assay provides a measure of biomass biodegradability without recourse to lengthy digester studies.

This paper describes a study of the suitability of 32 woody biomass species and clones as a commercial source of methane using the BMP assay, concentrating on species of *Salix* (willow) and *Populus* (poplar) grown according to the wood-grass concept (Mitchell *et al.*, 1988; White *et al.*, 1989; Abrahamson *et al.*, 1990). In this system trees are grown at a high density and coppiced each year to give an annual energy crop harvest. Studies have concentrated on willow and poplar species as they have given high coppiced biomass yields (Mitchell *et al.*, 1988; White *et al.*, 1989) and a limited number of tested samples have demonstrated a substantial conversion to methane (Jerger *et al.*, 1982; Chynoweth & Jerger, 1985; Tong *et al.*, 1990).

MATERIALS AND METHODS

Biomass samples

Woody biomass samples were principally obtained from the Gas Research Institute/New York State Energy and Research Development Authority/New York Gas Group (GRI/NYSERDA/NYGAS) growth program, with additional samples kindly provided by J. Ranney (Oak Ridge National Laboratory) and R. Bush (Northwest Missouri State University). Species, genotypes, and clone designations are detailed in Table 1. All willow and poplar samples were harvested in the winter of 1986, 1987 or 1988 and consisted of one-year-old coppiced wood (except where indicated — Table 2). The black locust, cottonwood, sycamore and sweetgum samples were not coppiced. Upon receipt, cut, chilled, entire wood samples (stem and bark) were shredded (Black and Decker shredder, model no. 8501) and ground through an Urschell extrusion mill with a 0·8-mm millhead (Comptrol 3600) for particle size reduction. Each ground wood sample was mixed to facilitate homogeneity and stored in plastic containers at −20°C. Total solids (TS) and volatile solids (VS) were determined (APHA, 1985) prior to easy assay.

Biochemical methane potential assay

Methane production rate constants and ultimate methane yields were determined employing batch assays: these contained biomass samples (at 2 g VS liter^{-1}), inoculum and nutrients in a system which was carbon limited (Owen *et al.*, 1979). The medium for each BMP assay included the addition of salts, vitamins, micronutrients and buffer (Owen *et al.*, 1979), with trace amounts of additional minerals (0·19 mg liter^{-1} H_2WO_4, 4·05 mg liter^{-1} $NiCl_2.6H_2O$). An inoculum concentration of 20% (v/v) and an inoculum-to-substrate ratio of 2:1 (as volatile solids) gave the maximum methane production rate (Hashimoto, 1989; Jerger, D. E. & Turick, C. E., 1987, unpublished data) and were used for each assay. A 5-liter, continuously stirred tank reactor, operated in a stable manner, served as the source of inoculum. The reactor was maintained at 35°C with a 20-day hydraulic retention time and a loading rate of 1·6 g VS liter^{-1} day^{-1}, and was fed once per day with primary sludge obtained from Gainesville municipal sewage treatment plant. Reactor effluent was anaerobically transferred as inoculum to the medium, and stirred constantly during further anaerobic transfer into assay bottles. Biomass samples were added to 250-ml serum bottles and gassed out with anaerobic nitrogen/carbon dioxide (70:30, v/v). A volume of 100 ml of medium (including inoculum) was dispensed into each serum bottle. Solids samples (TS and VS) were taken periodically throughout the transfer process and analyzed to ensure that a consistent inoculum was delivered to all bottles. Capped bottles were stored inverted and maintained at 35°C throughout each study. Inoculum and Avicel cellulose (FMC Corporation, Pine Brook, New Jersey, USA) controls were tested concurrently in each assay. Biomass samples and controls (tested in duplicate or triplicate) were analyzed periodically for gas production. Assays were carried out for 60–100 days and were terminated when gas production became negligible. Upon termination, pH measurements were taken of all bottles to ensure that imbalanced conditions had not led to the cessation of gas production (pH measurements ranged from 6·8 to 7·1). Total and volatile solids analyses of aliquots from each bottle were determined for effluent solids balances (Jerger *et al.*, 1982) when each assay was

Table 1. Woody biomass species used in biochemical methane potential assays

Species name	*Clone designations*	*Common name*	*Sample source*[a]	*Year*[b]
Salix eriocephala	163, 183, 242	Pussy willow	LZ	1988
S. lucida	1251, 2242, 3274	Shining willow	LZ	1988
S. exigua	2164, 2181, 3181	Coyote willow	LZ	1988
S. viminalis	SV1	Common osier	EW	1988
S. alba var *sanguinea*	SA2	White willow	EW	1988
S. alba	SA22	White willow	EW	1988
S. purpurea	SP3	Purple osier	EW	1988
S. purpurea	SP3	Purple osier	EW	1986
S. amygdaloides	SAM3	Peachleaf willow	EW	1988
S. amygdaloides	SAM5	Peachleaf willow	EW	1986
S. hastata	SH3	Halberd-leaved willow	EW	1988
S. hastata	SH2	Halberd-leaved willow	EW	1986
Populus nigra × *P. maximowiczii*	NM5	Hybrid poplar	EW	1987
P. × *euramericana*	DN30 cv. 'Canada Blanc'	Hybrid poplar	EW	1987
P. × *euramericana*	DN33 cv. 'Virginia Nancy'	Hybrid poplar	EW	1987
P. deltoides		Eastern cottonwood	JR	1988
Populus sp.		Hybrid poplar	RB	1986
Platanus occidentalis		Sycamore	JR	1988
Robinia pseudoacacia		Black locust	JR	1988
Liquidambar styraciflua		Sweetgum	JR	1988

[a] *Sources:*
LZ = L. Zsuffa, University of Toronto.
EW = E. White, State University of New York, Syracuse.
JR = J. Ranney, Dept. of Energy, Oak Ridge.
RB = R. Bush, Northwest Missouri State University.

[b] *Harvest year.*
1986 harvested in winter of 1985–86.
1987 harvested in winter of 1986–87.
1988 harvested in winter of 1987–88.

completed, and the balances ranged from 87 to 106%.

Methane yields and production rates

Gas production was determined with a glass syringe, using the volume displacement technique (Miller & Wolin, 1974). The methane content of the biogas was determined on a gas chromatograph as described previously (Peck & Chynoweth, 1990). Methane volumes were corrected by subtracting the mean methane volume of the inoculum control and were converted to standard temperature and pressure (Chynoweth *et al.*, 1981). Methane yields were calculated based on the initial volatile solid concentration of sample in each bottle.

The ultimate methane yield (B_0) (liters g^{-1} VS) and the methane production rate constant (k) (day^{-1}) of each replicate were calculated using least squares fit of the data (Statgraphics version 1.1, STSC Incorporated, Rockville, Maryland, USA) to a first-order kinetic model as described by the following equation (with B = methane yield (liters g^{-1} VS) at time t (day)):

$$B = B_0(1 - e^{-kt}) \quad (1)$$

For each assay bottle it was assessed whether methane production was biphasic or monophasic based on the best fit (using least squares). Biphasic methane production curves are recorded as two separate curves, with two rates and ultimate yields reported separately. In this case the secondary methane yield represents the overall methane yield (i.e. sum of both the first and second phases).

RESULTS AND DISCUSSION

BMP (biochemical methane potential) assays have been conducted with 32 samples from 15 woody biomass species. Approximately a third of the samples tested gave monophasic curves of methane production (Fig. 1, Table 2), with the remainder giving biphasic curves of methane production (Fig. 2, Table 2). Initial rates for the

Table 2. Ultimate methane yields and methane production rate constants of woody biomass samples

Wood species	*Ultimate methane yield (liters g^{-1} VS added)*		*Methane production rate constant (day^{-1})*		*No. of replicate*
	Primary yield[f]	*Secondary yield*[f]	*Primary rate*[f]	*Secondary rate*[f]	
Cellulose (control)	0·39 (0·05)		0·18 (0·02)		8
S. eriocephala (163) U[a]	0·29 (0·01)		0·02 (<0·01)		2
S. eriocephala (183) U	0·31 (0·01)		0·03 (<0·01)		2
S. eriocephala (242) U	0·27 (0·01)		0·02 (<0·01)		2
S. lucida (1251) U	0·29 (<0·01)		0·02 (<0·01)		2
S. lucida (2244) U	0·27 (0·01)		0·02 (<0·01)		2
S. lucida (3274) U	0·27 (<0·01)		0·03 (<0·01)		2
S. exigua (2164) U	0·27 (<0·01)		0·01 (<0·01)		2
S. exigua (2181) U	0·28 (0·01)		0·01 (<0·01)		2
S. exigua (3181) U	0·31 (<0·01)		0·01 (0·01)		2
S. viminalis (SV1) F	0·10 (0·01)	0·24 (<0·01)	0·09[g] (0·01)	0·14 (0·03)	2
S. viminalis (SV1) U	0·12 (0·01)	0·22 (<0·01)	0·07[g] (0·01)	0·31 (0·06)	2
S. alba var *sanguinea* (SA2) F	0·12 (0·01)	0·18 (0·02)	0·15[g] (0·05)	0·16 (0·01)	2
S. alba (SA22) F	0·12 (0·01)	0·19 (0·02)	0·11[g] (<0·01)	0·10 (0·01)	2
S. purpurea (SP3) F-1988	0·14 (0·01)		0·12 (<0·01)		2
S. purpurea (SP3) U-1988	0·12 (0·01)	0·16 (0·02)	0·11[g] (0·03)	0·11 (0·03)	2
S. purpurea (SP3)-1986	0·23 (0·01)		0·04 (<0·01)		3
S. amygdaloides (SAM3) F	0·12 (<0·01)	0·25 (<0·01)	0·18[h] (<0·01)	0·01 (0·01)	2
S. amygdaloides (SAM5)	0·21 (0·02)		0·04 (<0·01)		3
S. hastata (SH3) F	0·10 (0·01)	0·27 (0·01)	0·12[h] (0·13)	0·02 (0·01)	2
S. hastata (SH2)	0·23 (0·01)		0·04 (<0·01)		3
P. nigra × *P. maximowiczii*[b,c]	0·11 (0·01)	0·28 (<0·01)	0·17[i] (0·02)	0·08 (<0·01)	3
P. nigra × *P. maximowiczii*[b,d]	0·13 (0·01)	0·28 (0·02)	0·16[j] (0·02)	0·10 (0·02)	3
P. nigra × *P. maximowiczii*[b,e]	0·13 (<0·01)	0·29 (0·01)	0·19[j] (0·01)	0·01 (0·01)	2
P. × *euramericana* (DN30)[c]	0·13 (<0·01)	0·28 (<0·01)	0·19[j] (0·03)	0·10 (<0·01)	3
P. × *euramericana* (DN30)[d]	0·11 (0·01)	0·24 (0·01)	0·18[j] (0·05)	0·11 (0·03)	3
P. × *euramericana* (DN33)[c]	0·12 (<0·01)	0·28 (<0·01)	0·17[j] (<0·01)	0·10 (<0·01)	3
P. × *euramericana* (DN33)[d]	0·13 (<0·01)	0·26 (<0·01)	0·16[j] (<0·01)	0·10 (<0·01)	3
Populus sp.	0·08 (<0·01)	0·27 (0·04)	0·22[k] (0·01)	0·03 (0·01)	3
P. deltoides	0·08 (<0·01)	0·22 (0·02)	0·23[l] (0·10)	0·06 (0·03)	2
Pl. occidentalis	0·06 (0·02)	0·32 (0·01)	0·21[m] (0·33)	0·03 (0·05)	2
R. pseudoacacia	0·06 (<0·01)	0·24 (<0·01)	0·25[n] (0·04)	0·05 (0·01)	2
L. styraciflua	0·05 (<0·01)	0·21 (<0·01)	0·12[j] (<0·01)	0·04 (0·01)	2

[a]Clone designations appear in parentheses with harvested year designated for *S. purpurea* and F = fertilized treatment, U = unfertilized treatment.
[b]Clone NM5.
[c]First year growth.
[d]Second year growth.
[e]Third year growth.
[f]Values in parentheses = standard deviation.
[g]Duration of primary methane production 48 days.
[h]Duration of primary methane production 25 days.
[i]Duration of primary methane production 21 days.
[j]Duration of primary methane production 12 days.
[k]Duration of primary methane production 14 days.
[l]Duration of primary methane production 8 days.
[m]Duration of primary methane production 11 days.
[n]Duration of primary methane production 15 days.

biphasic curves persisted for 8–48 days (Table 2, Fig. 2). The secondary phase of biphasic curves led to a significant ($P < 0{\cdot}01$, as determined by a paired-difference test (Snedecor & Cochran, 1967)) increase in the ultimate methane yield, with an average methane yield of 0·10 liter g^{-1} VS for phase one, and an average overall methane yield of 0·25 liters g^{-1} VS. By the same criterion the average rate for phase one (0·17 day^{-1}) was significantly higher ($P < 0{\cdot}01$) than the average rate for phase two (0·08 day^{-1}). An exception to this is one sample of *S. viminalis* SV1, which as a consequence of a sudden production of biogas between days 48 and 60, has a secondary rate

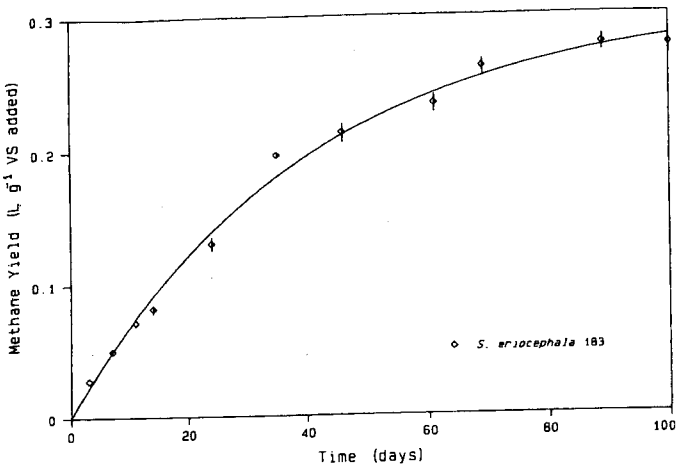

Fig. 1. Monophasic methane production curve from the batch anaerobic fermentation of *Salix eriocephala* 183.

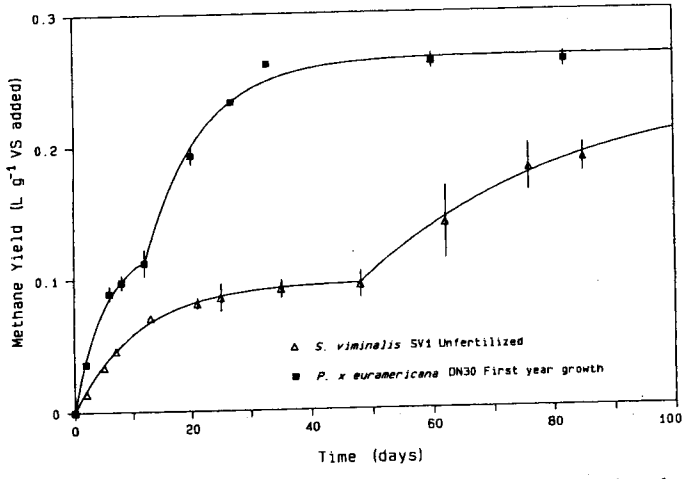

Fig. 2. Biphasic methane production curves from the batch anaerobic fermentation of coppiced woods.

superior to the primary rate. Biphasic methane production from woody biomass conversion has not been reported previously and is a subject of present investigation. The initial, rapid degradation of nonstructural carbohydrates and other compounds may account for the primary phase, while secondary methane production curves may be due to a shift in the microbial population toward organisms selected for their ability to degrade less accessible structural polymers found in the lignocellulosic matrix. An alternative explanation suggests that plant-associated toxins may be important in restricting methane production.

An ultimate methane yield of 0·39 liters g^{-1} VS was obtained from the cellulose control and is similar to that reported recently (Tong *et al.*, 1990). Substantial ultimate methane yields ($\geq$ 0·25 liters g^{-1} VS) were recorded for 19 out of the 32 wood samples examined (Table 2), with yields in excess of 0·30 liters g^{-1} VS obtained with three clones (Table 2). Clones of *S. eriocephala*, *S. lucida* and *S. exigua* gave consistently high methane yields, with lower yields recorded for *S. alba*. Variation amongst the clones of all species was discernably lower than that between species (Table 2). These methane yields are comparable with those obtained in preliminary studies for *Platanus occidentalis* (0·32 liters g^{-1} VS added), *Populus sp.* (0·32 liters g^{-1} VS added) and *Populus deltoides* (0·22 liters g^{-1} VS added) although no rate constants were recorded (Jerger *et al.*, 1982; Chynoweth & Jerger, 1985). A final methane yield of 0·29 liters g^{-1} VS (rate constant 0·08 day^{-1}) has also been recently reported for *Populus* sp. (Tong *et al.*, 1990). The ultimate methane yields achieved in this study indicate that in many cases over two-thirds of the wood solids have been degraded anaerobically. A theoretical maximum methane yield of 0·44 liters g^{-1} VS being anticipated (for hybrid poplar) from a chemical oxygen demand (COD) of 1·26 g COD g^{-1} VS (since 0·35 liters CH_4 g^{-1} COD), and assuming that 10% of the degraded solids are synthesized into bacterial cells (Tong *et al.*, 1990). However, studies employing the in-vitro organic matter digestibility (IVOMD) assay obtained only a minimal bioconversion of wood subjected only to particle size reduction (Wilson & Pigden, 1964; Baker *et al.*, 1975; Bjorndal *et al.*, 1987). This is presumably a consequence of the short incubation period involved ($<$100 h) with an assay developed principally for use with ruminant feed forage crops and other non-woody biomass. The BMP assay is therefore the preferred procedure with which to determine the methane potential of woody biomass; although, due to the duration of initial rates of methanogenesis from biphasic curves (Fig. 2, Table 2), BMP assays conducted for less than 50 days may also underestimate ultimate methane yields.

An explanation of the different biodegradability of woody biomass species merits further consideration. Previously, *Pinus taeda* (loblolly pine), *Eucalyptus* sp. (eucalyptus) and *Abies concolor* (white fir) were found to be poorly degraded (Jerger *et al.*, 1982; Chynoweth & Jerger, 1985; Tong *et al.*, 1990). This study has identified small differences between woody biomass species that may be substantially converted to methane. It has been proposed that many factors may influence

the anaerobic biodegradability of lignocellulosic materials, including: relative lignin, α-cellulose and hemicellulose content; proportion of structural and non-structural carbohydrates; cellulose crystallinity; nutrient availability; pore volume; degree of association between lignin and carbohydrates; particle size; wood-to-bark ratio; and extractive and toxic components (Cowling, 1975; Chandler *et al.*, 1980; Jerger *et al.*, 1982; Lin *et al.*, 1985; Mitchell *et al.*, 1988; Kenney *et al.*, 1990; Tong *et al.*, 1990). However, although many of these factors are, at present, only poorly understood and have yet to be systematically quantified, the lignin content has received much attention and is believed to be one of the more important factors. Lignin is a heterogeneous polymer composed of three *p*-hydroxy cinnamyl alcohols, and its composition, structure and linkages to holocellulose vary significantly between species (Fengel & Wegener, 1984). It is not appreciably degraded anaerobically, and is also responsible for shielding some holocellulose from degradation. Strong ($r^2 = 0{\cdot}94$) (Chandler *et al.*, 1980) and weak ($r^2 = 0{\cdot}59$–$0{\cdot}82$) (Tong *et al.*, 1990) correlation coefficients have been obtained inversely relating the lignin content to the rate and extent of biomass conversion, indicating the importance of other chemical and physical parameters. The selection of woods with a low lignin content may therefore represent a desirable goal for plant geneticists. However, until factors limiting the rate and extent of wood bioconversion are fully understood, it remains prudent to assess wood biodegradation using the relatively simple procedure adopted here.

The ultimate methane yield and methane production rate constant of clones were statistically similar for each willow species ($P < 0{\cdot}10$), except for the ultimate yields for the two *S. hastata* clones (which were also harvested in different seasons), and the rate for *S. eriocephala* 163 which was significantly lower ($P < 0{\cdot}10$) than the rate from the other two clones of this species. It is anticipated that through genetic breeding and the testing of new clones with the BMP assay, it will be possible to select for clones with even higher methane yields and methane production rates. This potential for improving poplar and willow through genetic research has been discussed previously (Mitchell *et al.*, 1988; White *et al.*, 1989). No significant effect ($P < 0{\cdot}10$) of fertilizer application was identified with *S. viminalis* SV1 or *S. purpurea* SP3. However, the sample of *S. purpurea* SP3 harvested in 1986 gave a significantly ($P < 0{\cdot}10$) higher ultimate methane yield but lower methane production rate than material harvested at a similar age in 1988. Thus, genetic differences and environmental growth conditions may influence the biochemical methane potential of woody material. A comparison of harvest age revealed that *P.* × *euramericana* clones DN30 and DN33 gave significantly ($P < 0{\cdot}05$ and $0{\cdot}025$ respectively) higher ultimate methane yields when taken after the first year compared with the second year of coppiced growth (Table 2). However, no significant differences were discernable between any of the rates, or in samples of *P. nigra* × *P. maximowiczii* harvested after the first, second, or third season of coppiced growth. A more rapid production of methane and higher methane yield have been reported previously with 6-month-old hybrid poplar (wood–grass) compared with two- and three-year old (not coppiced) hybrid poplar (Chynoweth & Jerger, 1985). The effect of harvest age on biodegradability is a subject meriting further attention as an accurate prediction of process economics needs to be based upon the quality (and quantity) obtained by coppicing over perhaps a 20-year period. The effect of clonal differences, environmental growth conditions (including fertilizer application, irrigation, pest protection and site selection), year of harvest, and harvest age on the biochemical methane potential of woody biomass, all need to be examined in considerable detail so that the wood selected for commercial biogasification is of the highest quality. Furthermore, this information needs to be related to growth data to ensure maximum biomass productivity. The effect of these factors on other measures of wood quality pertinent to energy production (e.g. ratio of wood-to-bark, moisture content, chemical composition) has been recently reviewed by Kenney *et al.* (1990).

Willow and poplar clones grown in a wood–grass system may be substantially converted to methane without recourse to pretreatment (other than some particle size reduction) and represent an excellent choice for commercial methane production. The species and clones selected for further study should be based on rates and ultimate methane yields obtained in batch assays such as those reported here, in addition to growth data derived elsewhere (Mitchell *et al.*, 1988; White *et al.*, 1989). The biological conversion of wood to methane is likely to represent a suitable commercial route for energy production in the United States, particularly with wood grown

through short rotation intensive culture. Growth reports have suggested that willow species may be particularly well suited for this purpose (White *et al.*, 1989), and this assertion is further supported by the high methane yields obtained in the present study. The selection and utilization of rapidly converted and high methane yielding clones, together with the optimization of growth conditions, should improve the economics of the biological gasification process by permitting a reduction in digester size.

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