and utilization of organic material. Often, biomass for fuels must compete with important alternative uses. The impact of biomass for fuels on food, feed and fiber prices is not fully known. And the need to return organic material to the soil for erosion control and organic matter maintenance continues to be of concern. Also competition between food crops and fuel production from biomass is an unresolved issue and will need a great deal more attention[24]. Certainly, a net energy gain from biomass fuels relative to the petroleum input is essential for a successful biomass fuels program. However, an overall net energy gain may not be necessary in the short run if a low quality bulky fuel is upgraded to a high quality clean burning fuel, especially a high energy density fuel to power existing mobile vehicles.

Finally, a word on the autonomy of a fuels program. Of course, economics drives our free enterprise system. Although our economic system is already highly distorted by regulations, subsidies and tax incentives, a biomass fuels program should eventually stand on its own. Temporary subsidies and incentives may be justifiable to promote development of such a program due to the high risks and uncertainties involved.

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Ethanol from cellulose

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Summary. An excess of organic waste, containing up to 60% cellulose and hemicellulose is produced worldwide. The conversion of this cellulosic material to ethanol is discussed: The two-step process consisting of a hydrolysis step to glucose and the subsequent fermentation by yeasts; and the one-step process, a fermentation of the cellulose by the anaerobic thermophile *Clostridium thermocellum*, or by a thermophilic, anaerobic, defined mixed culture. The use of the latter seems to be very feasible. To achieve an economic process, it is suggested to combine this approach with a thermophilic fermentation of the effluent and/or stillage obtained to produce methane.

Ethanol for technical and industrial purposes has been in use for only 100 years. Presently, there is an increasing demand for ethanol for fuel and feedstock chemicals. The petrochemical sources are very limited, and thus, the cost of oil is increasing continuously. After 50 years of producing ethanol mainly from petrochemical sources, the conversion of biomass to ethanol has become interesting again. Cellulose and hemicellulose are potentially important substrates for such processes. This is mainly due to the fact that cellulose is abundant, renewable and, at present, poorly utilized[1–7].

It has been calculated[1–3], that a total of 85×10^9 tons of cellulose and hemicellulose are produced annually in the world; of this figure, land plants account for 20 tons produced per capita each year. Many microorganisms degrade these polymers aerobically or anaerobically. Human beings and higher animals cannot degrade cellulose, except in commensalism with microorganisms, e.g. bacteria in the rumen of cattle or in the gut of termites[8]. Only small amounts, about 2%, of the annual cellulose production is decomposed by human beings through burning or industrial processes. Most of the harvested cellulose becomes waste or parts of agricultural and food wastes, municipal and industrial wastes and urban refuses containing more than 40% of paper and paper products. The amount of the various kinds of waste produced is increasing world-wide.

About 22% of the landmass of the globe is covered by large forests. With present wood harvesting methods, about 40% of the organic material is left as waste in the forest[9] and normally is decomposed by microorganisms. Most of it is aerobically mineralized to carbon dioxide and water, and a smaller amount is anaerobically degraded to alcohols, fatty acids, carbon dioxide and molecular hydrogen. In addition, on a world-wide basis, about 1.3×10^9 tons of cellulose and hemicellulose are produced annually as waste from grain (straw), cotton, bamboo etc. About the same amount of cellulosic waste results from printed paper and paper products. The cellulose content of the waste produced annually in the USA (to take an example from an industrialized country) is summarized in the table. According to statistics, an American citizen produces about 2.2 tons of liquid and solid cellulosic waste in 1 year. The following calculation may illustrate the theoretical potential of a bioconversion of waste to ethanol: assuming that at least 1 mole ethanol per mole glucose equivalent of the cellulose can be formed from the 2.2 tons of cellulosic waste, the tremendous amount of 630 kg or 768 l absolute ethanol per citizen and year could be produced. The cost of raw materials is still a controversial point when analyzing the problems in the bioconversion of cellulosic material to ethanol but the long term availability of large quantities of cellulosic biomass, required for the successful production of liquid fuel, should not be a substantial problem. Many of the potential raw materials for ethanol fermentation can be obtained at practically zero cost: lowgrade wood, waste from processing of wood and pulp, agricultural waste from corn, grain and sorghum, or used newspapers and governmental papers out of date. Waste materials have no significant value; however, they probably will receive new values when suitable methods to convert them into useful products are applied. Considerable expense is involved in the collection and in the transportation of the wastes or the biomass. Consequently, industrial companies and communities will be better off if they are able to treat their waste at the location of production. Fermentation processes are now required that work economically in small units without high investment costs and without highly trained man-power. Unfortunately, realization of such projects is being strongly hindered by alcohol legislation and in those industrialized countries which are producing such copious amounts of waste. It seems easier and cheaper to dump the waste.

And yet, the primary goal at present should be the conversion of cellulosic waste into useful products in order to stop pollution of our environment. Profit-making should be only a secondary concern. The conversion of waste to ethanol can be an effective way in fighting pollution since ethanol is a clean energy source and our present level of biotechnology should now enable us to intelligently utilize cellulose, hemicellulose and waste material.

The bioconversion to ethanol has not yet been studied with normal waste under technical conditions as has been done for the conversion to methane or SCP. Under laboratory conditions, new processes have been developed and new prospective bacteria have been isolated. Now the conversion of normal waste resources has to be studied in pilot plants from the point of view of economy.

Presently, there are 2 major ways of producing ethanol from cellulose.

Annual production of solid cellulosic waste in the USA

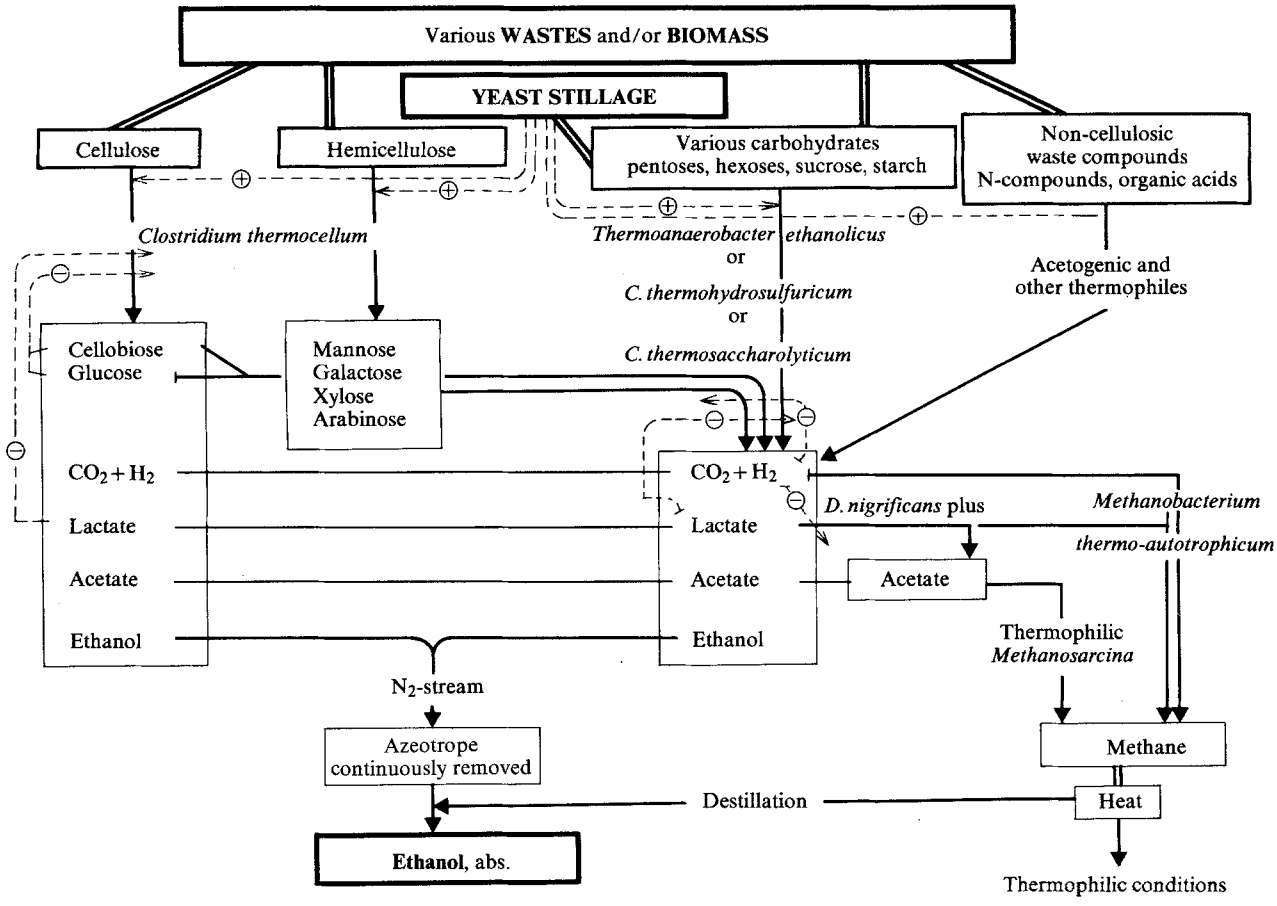
Waste type	Waste per year (tons $\times 10^6$)	Assumed cellulose content (%)	(tons $\times 10^6$)
Agricultural and food wastes	400	60	240
Manure	200	50	100
Urban refuse	150	45	67.5
Logging and other wood wastes	60	55	33
Industrial wastes	45	33.3	15
Municipal sewage solids	15	33.3	5
Miscellaneous organic waste	70	25	17.5
Total:	940		478

[a] Based on values from Humphrey et al.[10].

a) The two-step process: cellulose is converted enzymatically or by a treatment with chemicals to glucose which in the 2nd step is fermented to ethanol by yeasts.
b) The one-step process: cellulose is degraded anaerobically to ethanol by the cellulolytic thermophilic *Clostridium thermocellum* or by defined anaerobic thermophilic recombined cultures consisting of cellulolytic and glycolytic bacteria.
Both methods can be applied to the various cellulose sources, to the cellulosic wastes, or to cellulosic material specially grown for the bioconversion into methane and/or ethanol.

The two-step process

Presently, *Saccharomyces* strains are being used in the conventional ethanol fermentation. These yeasts are not able to hydrolyze cellulose; consequently, the biopolymers have first to be hydrolyzed to glucose. Several processes are known using either a treatment with chemicals or with cellulase preparations.
The chemical processes require first a milling to obtain suitable particle sizes. The hydrolysis is performed either with alcali, acids, or organic solvents. For the alcaline treatment the cellulose is incubated with 1–2% sodium hydroxide or with NH_3[11] for 24 h at room temperature or with 4% sodium hydroxide at 80–100 °C and 200–300 atm for some minutes[12,13]. A short incubation time with 0.5 N sulfuric acid at 120 °C leads to the hydrolysis of the hemicellulose only, whereas the hydrolysis of the cellulose requires a treatment over several hours[14]. None of these methods gives a quantitative reaction, and concomitantly a partial decomposition of the sugars obtained occurs. An almost quantitative hydrolysis is obtained with a treatment of a mixture of 5% cadmiumoxide with 28% ethylendiamine in H_2O[15]. This and the other



Ethanol production from cellulosic wastes and biomass by thermophilic anaerobes. The various wastes and the biomass contain cellulose, hemicellulose, various carbohydrates as sugars, cellobiose, starch etc. and many N-compounds, fatty acids. etc. These different components are degraded by the various bacteria as indicated. The stillage of yeast ethanol fermentation is added to promote the degradation (– – ⊕ – → –). Several products obtained during the conversion are inhibitory (– – ⊖ – → –) and thus they have to be kept low in the fermentation broth through utilization by other bacteria. Some of the by-products (e.g. acetate and H_2/CO_2) can be directly converted to methane, whereas lactate has to be degraded to the methanogenic substrate acetate. This reaction is carried out by *Desulfotomaculum nigrificans* producing acetate, carbondioxide and H_2S. In the absence of high concentrations of sulfate and in the presence of *M. thermoautotrophicum* CH_4 is formed instead of H_2S. The combining of the cellulose degradation to ethanol with the methane fermentation helps a) to avoid product inhibition as far as possible, b) to diminish the overall fermentation residue and c) to obtain a cheap and clean energy source for heat production required for the elevated temperature of the fermentation vessel and for the ethanol distillation. Ethanol is removed from the fermentation vessel by a low stream of oxygen-free N_2.

methods, however, have the disadvantage that the employed chemicals are strong pollutants or even strong poisons. Therefore they have to be removed from the sugars before the fermentation process can be started; thus, the conversion of the organic wastes leads to anorganic wastes and pollutants. This is unfortunate as the pollutive effect of these chemicals is less reversible than that of the organic material.

A better method is the enzymatic hydrolysis. Although this process is more expensive, the cellulose is almost quantitatively hydrolyzed to glucose and no caramelization products are formed. Recently, Reese and Mandels at the Army Natick Research and Development Command (USA) obtained suitable enzyme preparations with high catalytic activities[16,17]. However, there is still a demand for cheaper enzymes of a better quality. The desired enzymes should have a lower product inhibition and a higher stability. Especially the enzyme preparations for the conversion of cellulose dispersed in other wastes need a high stability against inactivation through proteases, heavy metals and elevated temperatures. The stability of the single components of the cellulase complex varies considerably under hydrolysis conditions e.g. pH 4.8, 50 °C and 24 h[16]. For instance, merthiolate and other Hg-compounds are extremely potent inhibitors of the cellobiohydrolase (over 60% inhibition) whereas most of the various endoglucanases are less affected. Shaking and mixing generally decrease the cellulose degradation rates and also the enzyme recovery[16]. The use of *Trichoderma viride, T. reesi* or similar cellulases should be economical for processes using relatively pure cellulose. From waste with a low cellulose content, the enzyme is difficult to recover or is not reusable due to its inactivation. Another main disadvantage of this two-step process is the inability of the ethanol-producing yeasts to utilize the pentoses derived from hemicellulose[18]. For the enzymatic hydrolysis of straw and of waste consisting of wood and bark, pre-treatment with pressurized water steam[35] seems very promising. This method does not lead to severe pollution; the hemicellulosic part is extracted with water. This steaming technique and the organo-solvent process[36] are gaining more and more importance for various strategies converting material containing lignocellulose. The links between lignin and (hemi)cellulose have to be destroyed, otherwise the enzymes have only limited access to the cellulosic part. Such pre-treatment of lignocellulosic biomass is also necessary for the one-step process.

The one-step process

There are several bacteria which can hydrolyze cellulose. None of the mesophilic cellulolytic anaerobes known produce ethanol as the main fermentation product. The ability to produce ethanol from various sugars is widespread among bacteria; however, the production of ethanol as the sole fermentation product is relatively rare (for distribution of ethanol production among microorganisms see Wiegel[19] and Lorry[20]). Among the ethanol producing organisms there are several thermophilic (T_{opt} above 42 °C and T_{max} above 50 °C) and extreme thermophilic (T_{opt} above 65 °C and T_{max} above 70 °C) anaerobes[19]. Thermophilic processes are much more suited for the industrial production of ethanol than are the mesophilic ones[19,21]. Some of the advantages are: fast degradation rates, a relatively cheap fermentation process since heating a fermentation vessel is easier than cooling it, less danger of contamination, no growth of pathogenic microorganisms - most of them are destroyed by the elevated temperature, cooling is necessary for mesophilic processes due to the production of heat through microbial degradation of the biomass and mixing the fermentation broth. Several thermophilic microorganisms can degrade cellulose: *Actinomyces, Sporocytophaga* species, fungi and clostridia. Examples of the thermophilic cellulolytic fungi are *Chaetomium thermophile var. disstum* and *Talarmyces mersonii.* Some of the strains exhibit a very high cellulase activity[22,23]. However, there are several disadvantages to the fungi: they grow slowly, many produce antibiotics or substances which are poisonous for animals and humans, and ethanol - if it is at all produced - is only a minor product. As is the case with the fungi, the potential of the thermophilic actinomyces *Thermomonospore curvata* and related species is very low for the ethanol production. Recently, Belamy[24] described thermophilic *Sporocytophaga* species with a high cellulase activity. They seem obligate syntrophic with other glycolytic bacteria, similar to an extreme thermophilic *Clostridium (C. caldocellum)*[25]. All these thermophiles might be useful in developing stable enzyme preparations to be used in the two-step procedure, but they do not produce high amounts of ethanol. Only the thermophilic *Clostridium thermocellum (C. thermocellulaseum)* produces up to 1 mole ethanol per mole glucose equivalent of the cellulose degraded[18,19].

Thus, the direct conversion of cellulose to ethanol is possible and has been subject to study by several groups[18,26,27]. Although very useful mutants of *C. thermocellum* with low product inhibition have been obtained[18,26], the yield has not exceeded significantly 1 mole ethanol per mole glucose equivalent. Recently Wang and co-workers[18,26] started to use co-cultures with *Clostridium thermosaccharolyticum.* This glucolytic bacterium has some useful properties. Contrary to the cellulose degrader, it utilizes starch directly and converts pentoses to ethanol, too. Pentoses will always be present in the fermentation broth of cellulosic material due to the hydrolysis of hemicellulose by the cellulase of *C. thermocellum,* which utilizes only slowly the pentoses. However, *C. thermosaccharolyticum*

produces ethanol only under special conditions, vegetative cells normally form butyrate[28-31]. The yield of ethanol is not higher than 1 mole per mole of glucose utilized, neither in pure nor in co-culture with *C. thermocellum*, so far.

Two other bacteria, extremely thermophilic, seem more suitable for such a co-culture: *Clostridium thermohydrosulfuricum* and the recently described *Thermoanaerobacter ethanolicus*[30,32]. *C. thermohydrosulfuricum* produces up to 1.6 mole ethanol per mole glucose, if the pH-value of the culture shifts from about pH 7.5 to below 6.9 during the fermentation[31]. *T. ethanolicus* ferments glucose and pentoses up to 1.9 mole ethanol per mole sugar. In addition, both organisms utilize starch, cellobiose, various hexoses and the various pentoses derived from hemicellulose hydrolysis. With all the substrates, ethanol is the main fermentation product. The cellobiose concentration in the cellulolytic co-culture may play an important role due to regulatory effects on the cellulase activity. A pH-shift is not required for high ethanol production with *T. ethanolicus*. Thus this bacterium presently seems to be an ideal organism for co-cultures with thermophilic, cellulose degrading bacteria[19,30,32]. It produces ethanol between pH 4.4 and 9.8; the pH optimum for growth and the ethanol production rate is between 5.8 and 8.5. This unusually broad pH-optimum suits it for an industrial application especially in combinations with cellulolytic co-cultures since in these cultures the pH-value always drops due to the concomitant production of lactate and acetate. The temperature range for growth and ethanol production is from 38 to 78 °C. The ethanol yield does not change drastically with the fermentation temperature. Strain JW 200 has been proved to adapt easily to higher ethanol (8%) concentrations. More than 1.4 mole ethanol per mole glucose equivalent of the cellulose degraded was obtained in a co-culture with *C. thermocellum* JW 20[33]. Both organisms need yeast extract for growth and for a high ethanol yielding fermentation. This requirement can easily be fulfilled by the addition of the stillage from yeast ethanol fermentation. Presently this stillage is used as animal feed, either directly or after an enrichment with protein, using pentose utilizing yeasts; the distillers' solubles are often converted to fodder by concentration. Since *Thermoanaerobacter* converts pentoses and starch directly to ethanol, the additional stillage would also increase the fermentable C-source and thus the ethanol yield.

The application of the extreme thermophilic cellulolytic and glycolytic bacteria in co-cultures makes possible a continuous fermentation process from cellulose to ethanol. Both organisms have a temperature optimum of about 68 °C, but still exhibit a rapid metabolic activity at 72/74 °C. The boiling point of the ethanol-water azeotrope is 78.2 °C. Subsequently, only an oxygenfree stream of N_2 or a very low vacuum is necessary to separate the ethanol from the fermentation broth. The removal of ethanol from the fermentation broth through extraction procedures with other solvents to save destillation energy, as proposed by Dellweg and Misselhorn[34], does not seem feasible for thermophilic processes using waste materials. The fermentation broth is already at an elevated temperature and other interfering materials, possibly poisonous, might also be extracted from the wastes. Many of the energy cost tied to the thermophilic ethanol fermentation process could be recovered by producing biogas from the remaining residues (distillers' wastes).

Even at high conversion ratios of the cellulose and other additional carbohydrates to ethanol, the outflow or the stillage still has a high content of proteins, volatile fatty acids, lactate and other organic compounds. Many of these are methanogenic substrates or could be converted into the same by other thermophiles. One example is the production of methane and acetate by a co-culture of *Desulfotomaculum nigrificans* (lactate to acetate, CO_2 and 2 H_2) and *Methanobacterium thermoautotrophicum* (CO_2 and 4 H_2 to methane). The second product of this co-culture, acetate, can be converted to methane by a thermophilic *Methanosarcina* spec. as isolated by Schobert (Jülich, West Germany; pers. commun.). From the higher volatile fatty acids, methanogenic acetate can be produced by acetogenic bacteria. In addition, many amino acids can be converted to acetate, CO_2 and H_2 by not yet identified thermophiles (unpublished results). The weight yield of methane generation can be about 0.23 g per g total solids in a conventional ethanol stillage, which contains up to 20% solids[5]. A direct thermophilic fermentation of the distillation residues (outflow of continous culture) to methane, would lead to a clean energy source and would diminish the waste production. The obtained methane could be used for heating the fermentation vessels, if neccessary, for the distillation process or for drying the distillation waste. If the latter one contains higher amounts of lignin, the dried residues may also be used for heating processes. (Dry lignin has up to 3500 kJ/kg).

The combined process of the ethanol and methane fermentation by thermophilic organisms is summarized in the figure. It should be possible to increase the net energy conversion rate of cellulose and biomass far above 50%[5,3].

Conclusions. Cellulose, obtained either as waste or as specially grown biomass, can be efficiently converted to ethanol by thermophilic, anaerobic co-cultures *(C. thermocellum* and *T. ethanolicus).* In addition to defined mixed cultures containing acetogenic and methanogenic thermophiles, a minimum of residual waste can be obtained. The direct microbial conver-

sion of cellulose to ethanol has many advantages over the two-step process consisting of a yeast fermentation after the cellulose is hydrolyzed by chemical treatments or by fungal cellulase preparations. Much more work has to be done, including studies with actual waste under technical conditions, before a final judgement can be made. But the direct process seems one of the most promising alternatives for ethanol production from cellulosic waste.

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Degradation of cellulose

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Microorganisms degrading cellulosic materials

One of nature's most important biological processes is the degradation of lignocellulosic materials into carbon dioxide, water and humic substances. Different kinds of microorganisms are involved in the process of degrading woody materials, but it is mainly a task for fungi. Bacteria are considered to have only a limited capability of wood degradation. The strong wood-degrading effect that fungi have has to do, in part, with the organization of their hyphae which gives the organisms a capacity for penetration.

Different types of fungi give rise to different types of wood rot. One normally distinguishes between soft-rot, brown-rot and white-rot fungi[1]. Fungi from the first 2 groups mainly attack the polysaccharides of wood and other lignocellulosic materials while the white-rotters also are capable of a substantial attack on the lignin. The degradation of the different compounds in lignocellulosic materials is catalyzed by enzymes produced by the respective microorganisms. Knowledge of these reactions may be of importance for the conversion of biomass into chemicals and fuels.

The enzyme mechanisms for cellulose degradation by fungi are known in great detail and will be summarized below. The corresponding enzyme mechanisms for lignin degradation are less known and will not be subject to description in this article.

Enzyme mechanisms involved in cellulose degradation

The enzyme mechanisms involved in cellulose degradation have been particularly well studied in 2 fungi,