Journal of Environmental Protection and Ecology 11, No 1, 209–216 (2010)

ALTERNATIVE BIOTECHNOLOGICAL SOLUTIONS FOR PAPER AND MILL PLANTS SOLID WASTES REUSING

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Abstract. The waste deposition law (1999/31/EC Directive 95/2005 MEWM Order) prohibits land filling with pulp and paper industry solid wastes with organic content and imposes their end treatment. This fact leads to the necessity of finding new uses for residual materials that can be re-used. The paper is a brief review of recent literature on the possibilities of advanced biotechnological solutions to produce alternative energy sources as methane, hydrogen, ethanol and valuable organic products as acetic acid, lactic acid, from pulp and paper solid wastes. The paper also includes the preliminary experimental results obtained for biogas production from paper and mill organic sludge in combination with fermented municipal sludge and cattle manure as inoculum.

Keywords: paper and mill, solid wastes, biotechnological solutions, alternative energy sources.

AIMS AND BACKGROUND

European Waste Framework Directive – 2000/532/EC classifies paper and mill industry waste under the code EWL 03, on a list with 20 indexing positions. These industrial wastes are among those which by volume and pollution potential rise important problems at the EU level. In USA, paper and mill industry is considered the third industrial polluter. Currently, an important proportion of the solid waste stream is dumped in sites which do not meet actual sanitary landfill design standards and environmental regulations.

Therefore, the objective of this work was to assess the biotechnological solutions that use paper and mill plants solid wastes as raw material for the production of alternative energy source: biogas, bioethanol, hydrogen, or valuable organic products as acetic acid or lactic acid. So far, the biogas producing possibility was experimented with promising results.

In recent years, effective utilisation of lignocellulosic resources has received considerable attention from the starting point of environmental protection. However, large quantities of lignocellulosic such as woody wastes from building materi-

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als, sawdust, waste paper, bark are still incinerated or discarded in the environment. Since high water content usually makes incineraion of sludges uneconomic and new waste laws restrict landfilling of organic wastes, new methods for sludge handling were studied. Although thermal processes may be applied to recover energy from wastes, a more practical approach to pulp-mill waste conversion is to apply anaerobic digestion processes to recover waste as methane, ethanol or other valuable products[1].

Cellulose is considered to be the rate-limiting substrate in anaerobic degradation of solid wastes. The anaerobic degradation of cellulose is a microbial mediated process which is important in a range of economically relevant processes: for example anaerobic waste treatment and the breakdown of feeds in the rumen of herbivores such as sheep and cattle[2]. Cellulose is a polymer made up of glucose units composed in a complex structural arrangement and associated with other polymers such as hemicelluloses and lignin[3]. Before cellulose can be fermented in energy-providing reactions by microorganisms it must be solubilised so that the monomers can be taken up across the cell walls of the fermentative microorganisms and transformed into valuable products. It is the solubilisation step that makes the breakdown of cellulose slower than that of more readily degradable substrates such as sugars, starches, fats and proteins. It is commonly accepted that rates of cellulose solubilisation in the rumen are faster than those found in landfills or anaerobic digesters. However, comparisons of rates of cellulose solubilisation from literature are complicated by several factors because a wide range of different reactor designs and operational conditions are used in reactor studies[2].

There are mainly two systems to obtain microbial hydrogen production, namely photochemical and fermentative. The first one consists in producing by means of photosynthetic microorganisms such as algae and photosynthetic bacteria[4]. The second one is carried out by fermentative hydrogen-producing microorganisms, such as facultative anaerobes and obligate anaerobes. Reported studies on microbial hydrogen production have been conducted mostly using pure cultures, either natural or genetically modified[5]. Hydrogen is a key intermediate in the anaerobic degradation of organic compounds and may be recovered from wastewater or solid waste using mixed cultures. In these studies, hydrogen production resulted from the inhibition of methane fermentation. On the other hand, solid substrate anaerobic digestion (SSAD) has been shown to be an effective way of reclaiming paper mill sludge as well as obtaining methane as a fuel and soil amender or protein enrichments from the digested solids. Yet, methane or carbon dioxide combustion products are known to be greenhouse gases. Hydrogen might be produced from paper wastes using microorganisms from SSAD, suppressing the activity of hydrogenotrophic methanogens with specific and non-specific inhibitors such as 2-bromoethanesulfonate (BES) or acetylene[6].

Lignocellulosic biomass can be utilised to produce ethanol, a promising alternative energy source for the limited crude oil. There are mainly two processes involved in the conversion: hydrolysis of cellulose in the lignocellulosic biomass to produce reducing sugars, and fermentation of the sugars to ethanol. The cost of ethanol production from lignocellulosic materials is relatively high based on current technologies, and the main challenges are the low yield and high cost of the hydrolysis process[7]. Considerable research efforts have been made to improve the hydrolysis of lignocellulosic materials. Pretreatment of lignocellulosic materials to remove lignin and hemicellulose can significantly enhance the hydrolysis of cellulose. Optimisation of the cellulase enzymes and the enzyme loading can also improve the hydrolysis. Simultaneous saccharification and fermentation effectively removes glucose, which is an inhibitor to cellulase activity, thus increasing the yield and rate of cellulose hydrolysis and, respectively, the ethanol production rate[5].

Acetic acid is an important feedstock for various industrial products and also used in food and pharmaceutical industries. Acetic acid is produced by chemosynthesis from butane, methanol and other petroleum products and also by microbial fermentation. Due to depleting natural resources, more emphasis is given to fermentative production of acetic acid using cheap and abundantly available biomass as substrate[8]. The conversion of cellulosic biomass to acetic acid is mainly carried out either by the conventional multi-step approach or by a single-step process[9]. The conventional multi-step process includes acid or enzymatic hydrolysis of the substrate, followed by yeast fermentation and oxidation to acetic acid by *Acetobacter* sp. Acid hydrolysis is hindered mainly by glucose yields and corrosion of the equipment. Enzymatic hydrolysis, which usually employs cellulases from *Trichoderma reesei*, achieves higher substrate conversion yields, but its production is very expensive. Therefore, direct conversion of cellulosic biomass to acetic acid by a single fermenting organism is economical, which eliminates the need for separate fermentors. In this direction, there are numerous reports on isolation of various cellulolytic mesophilic, anaerobic and acetogenic *Clostridium* spp. from faecal droppings of various herbivorous animals and birds[8].

Venkatesh (1997) reported that to effectively apply the simultaneous sacharification and fermentation method to produce substances fermented from glucose, several conditions should be satisfied. The final product inhibition on the cellulase system should be less than that of glucose or cellobiose. The conditions, such as pH and temperature for the fermentation should be compatible with the conditions for cellulase enzymatic hydrolysis[10]. It has been reported[10] that lactic, acetic, citric and succinic acids scarcely inhibit the cellulase system. Therefore, SSF can be efficiently used to convert cellulose to these organic acids, if the microorganisms which produce these acids are compatible with the cellulase enzyme system[10].

EXPERIMENTAL

The experiments for biogas production using paper-mill solid waste were performed in a single-stage thermophilic conditions using a 4l laboratory bioreactor Biostat A plus.

Fig. 1. Experimental setup for biogas production

The digester was maintained anaerobic, mechanically stirred at 150 rpm, pH controlled (pH=7), at thermophilic (50^{o}C) temperature based on the reports that better gasification rates of paper-mill sludges were achieved by thermophilic organisms.

The thermophilic culture was developed from a mesophilic inoculum obtained from a municipal sludge digester.

The bioreactor was fed with pulp-mill sludge mixed with municipal organic sludge in a volume ratio of 1:1. The digester operated in batch mode, monitoring dry substances, volatile substances, biogas production and nutrients content (N, P and K).

RESULTS AND DISCUSSION

Initially, the bioreactor was feed with pulp-mill sludge mixed with municipal organic sludge in volume ratio of 1:1, but after two days of operating, the biogas production ceased, indicating the fact that the fermentable substrate was consumed and no cellulose hydrolysis occurred.

In order to inoculate the bioreactor with cellulase producing microorganisms, we added ruminant manure which has high cellulosollytic activity.

Therefore, 200 g of cattle manure were added after the 6th day and the 21st day of operation. After the inoculation with cattle manure, a progressive improvement

of organic material digestion rate and biogas production is observed as presented in Table 1, indicating that this inoculum produces cellulase enzymes that partially hydrolysate the cellulosic material.

Table 1. Physicochemical analysis of initial and fermented sludge samples

Crt. No	Parameter	M. U.	Sludge samples					
			PS*	PS+MS**	FS_6	FS_{13}	FS_{21}	FS_{28}
1	D.S.	%	9.86	4.15	2.92	1.79	1.84	1.20
2	V. S.	% d.s.	58.69	63	61	59.82	52.33	50.99
3	organic carbon	% d.s.	26.57	29.53	9.002	27.67	30.34	31.20
4	K	mg/kg d.s.	400	570	496	3360	3369	4749
5	P_{tot}	mg/kg d.s.	2038.50	3533.60	132.19	254.20	213	322.50
6	TKN	mg/kg d.s.	69416.80	40414.30	55267.20	49271.30	25260.80	35933.30
7	Ca	mg/kg d.s.	2140	884	175.90	216	150.34	225.83
8	SO_4^{2-}	mg/kg d.s.	5961.25	5250.45	7261.87	8460.89	117.29	76.03
9	SO_3^{2-}	mg/kg d.s.	< 0.01	< 0.01	< 0.01	0.996	4.16	65
10	S_2O_3	mg/kg d.s.	6.08	51.26	251	466.57	< 0.01	< 0.01
11	S^{2-}	mg/kg d.s.	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
12	biogas	exp. days			1–5	6–12	13–20	21–28
		biogas (ml)	–	–	950	1550	750	1250

*PS – paper – mill sludge; **PS+MS = paper-mill sludge+municipal sludge 1:1 volume ratio; *FS_x – fermented sludge after *x* days.

The results obtained prove that after 28 days of digestion at 50°C, the lignocellulosic waste is stabilised and has a high residual nutrients content (N, P, and K compounds) which makes it suitable to be used as agriculture fertiliser after dewatering/composting.

Potential pathogen bacteria such as total coliforms, fecal coliforms and fecal streptococcus are absent after 28-day digestion period (Table 2) meaning that the digested sludge can be used, without any health risk, as agricultural fertiliser.

Table 2. Microbiological characterisation of initial and digested sludge samples

Crt. No	Parameter	M.U.	PS+MS	FS_6	FS_{13}	FS_{21}	FS_{28}
1	Total mesophile	CFU/g.d.s.	59.9×10^6	32.5×10^6	24.8×10^6	105.9×10^6	18.3×10^6
2	Total coliforms	CFU/g.d.s.	14.1×10^3	9.6×10^3	7.8×10^3	–	–
3	Fecal coliforms	CFU/g.d.s.	–	–	–	–	–
4	Fecal streptococus	CFU/g.d.s.	218	508	2.5×10^3	3.2×10^3	–

Anaerobic fermentation is an extremely complex process, gathering a great number of reversible/irreversible, consecutive, parallel, autocatalytic chemical

and biochemical reactions. An exact mathematical model of the process, even if it could be conceived, would contain a large number of parameters that would need a considerable amount of experimental data.

The common technique is to consider a global, irreversible kinetic as:

[fermentable organic components] → [biogas]

Considering this global kinetic process, the expression of reaction velocity is given by the following equation:

$$v_r = -\,dC_{sv}/dt = kC_{sv}.$$

where C_{sv} represents the concentration of volatile substances in sludge (determined experimentally). The negative sign in front of the derivate shows that the concentration decreases in time.

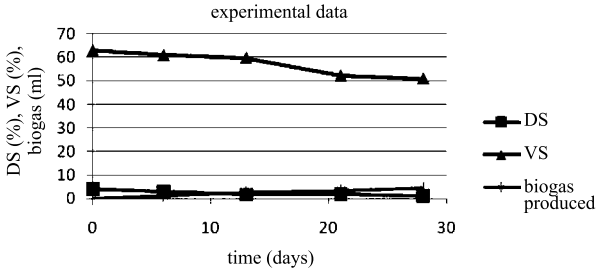

Fig. 2. Evolution, in time, of anaerobic digestion process parameters

The hypothesis for which the kinetic model is integrated are the following:

– the anaerobic digester is a batch reactor;

– the Reynolds criterion associated to the system has the value of 16300, which indicates a strongly turbulent hydrodynamic regime;

– the reaction space can be considered, in this conditions perfectly mixed;

– other process parameters are considered constant during experiment (pH, temperature).

In these conditions, for a certain temperature, the differential model of the reactor is given by a differential equation with separable variables which are integrated as follows:

$$\int_{C_{sv}^0}^{C_{sv}} \frac{dC_{sv}}{C_{sv}} = -k\int_0^t dt\ .$$

After integration and introduction of integration limits in the primitive expression is obtained:

$$C_{sv} = C_{sv}^{\ 0}\exp(-kt).$$

The superscript '0' indicates the initial value of volatile substances concentration in the system. For the determination of the rate constant k, the Lasdon gradient reduction algorithm was used, on the basis of the least square estimator.

In Table 3 are presented the measured values of volatile substances concentration and the calculated values for the same parameter, using the proposed kinetic model with the deduced value for kinetic constant k=0.00738 $(day)^{-1}$.

Table 3. Measured and calculated data using the proposed kinetic model for VS

Time (days)	VS (%)	$VS_{calc.}$ (%)	Error (%)
0	63	63	0
6	61	60.27	1.20
13	59.82	57.23	4.32
21	52.33	53.95	–3.10
28	50.99	51.23	–0.48

The adequacy of the proposed kinetic model is illustrated by the diagram in Fig. 3, where the line represents the calculated values while the isolated points represents the measured values for the concentration f volatile substances.

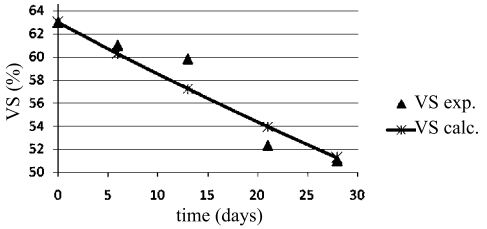

Fig. 3. Measured and calculated data for VS. Velocity constant: k=0.00738 day

CONCLUSIONS

Among the reviewed alternative biotechnological solution for pulp-mill solid waste reusing, the most promising seems to be the ones for methane and for ethanol production. They both seem promising, even if there is a major difference between the existing technologies (biogas production has an important industrial experience while bioethanol production is still in laboratory experiments phase).

The experimental results showed that paper-mill wastes can be used for biogas production by thermophilic (50°C), anaerobic digestion at a retention time of 28 days. Even if biogas production is considerable lower than in the case of anaerobic digestion of municipal sludge, it is still sufficient for biogas installation maintenance.

Potential pathogen bacteria such as total coliforms, fecal coliforms and fecal streptococcus are absent after 28-day digestion period, meaning that the digested sludge can be used, without any health risk, as agricultural fertiliser

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Received 5 March 2008
Revised 29 April 2008