

Bioplastic production using wood mill effluents as feedstock

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Water Science & Technology, Vol 63 No 6 pp 1196–1202 IWA Publishing 2011

doi:10.2166/wst.2011.358

Abstract

Fibreboard production is one of the most important industrial activities in Galicia (Spain). Great amounts of wastewater are generated, with properties depending on the type of wood, treatment process, final product and water reusing, among others. These effluents are characterized by a high chemical oxygen demand, low pH and nutrients limitation. Although anaerobic digestion is one of the most suitable processes for the treatment, lately bioplastics production (mainly polyhydroxyalkanoates) from wastewaters with mixed cultures is being evaluated. Substrate requirements for these processes consist of high organic matter content and low nutrient concentration. Therefore, wood mill effluents could be a suitable feedstock. In this work, the possibility of producing bioplastics from wood mill effluents is evaluated. First, wood mill effluent was converted to volatile fatty acids in an acidogenic reactor operated at two different hydraulic retention times of 1 and 1.5 d. The acidification percentage obtained was 37% and 42%, respectively. Then, aerobic batch assays were performed using fermented wood mill effluents obtained at different hydraulic retention times. Assays were developed using different cultures as inoculums. The maximum storage yield of 0.57 Cmmol/Cmmol was obtained when the culture was enriched on a synthetic media.

Keywords: acidogenic fermentation, bioplastics, polyhydroxyalkanoates, sequencing batch reactor, volatile fatty acids, wood mill effluent.

Introduction

Fibreboard production is one of the most important industrial activities in Galicia (Spain) in which great amounts of wastewater are generated producing a negative environmental impact. The properties of these wastewaters depend on type of wood, treatment process, final product, water reusing, etc. Nevertheless, these effluents are characterized by high chemical oxygen demand (COD) content, low pH values and nutrients scarcity. Anaerobic digestion is one of the most used processes to treat them. It works without oxygen supply, the amount of sludge generated is lower than in aerobic processes and it produces biogas, which can be used as fuel. The high COD and the low nutrient contents present in this kind of water makes it also feasible substrate to obtain polyhydroxyalkanoates (PHA).

PHA are polyesters of various hydroxyalkanoates which have plastic properties. These PHA are synthesised by bacteria as energy and carbon storage materials. Hence, they are completely biodegradable by microorganisms. Due to this properties PHA could be good substitutes of petroleum derived plastics.

Although PHA are already industrially produced their applications are limited to niche applications, and they are not yet considered as a commodity material because their production cost are still much higher than for synthetic polymers. Industrial production of PHA is based on the use of pure cultures and pure substrates. The price of PHA is highly dependent on substrate costs (Lee 1996). One way to reduce PHA price would be to use mixed cultures and cheap substrates (preferably waste organic carbon).

Recently, the use of wastewater as substrate and mixed cultures on PHA production has been reported in several research works (Dionisi et al. 2005; Albuquerque et al. 2007; Coats et al. 2007; Salmiati et al. 2007; Bengtsson et al. 2008a). Many studies use a synthetic VFA mixture in the experimental system. Surthridge et al. (2009) are working on PHA production from pulp and paper wastestreams. Bengtsson et al. (2008a, b) are also studying PHA production with different wastewaters, including paper mill effluents. However, paper mill wastewaters contain contaminants resulting from bleaching processes which are not present in wood mill effluents. Therefore, their characteristics could be different.

In this work, the possibility of using wood mill effluents as feedstock to produce PHA is suggested as it is a large source of organic carbon and has a low nutrient content. In order to evaluate the possibility of using this kind of waste, an acidogenic reactor was operated at two different hydraulic retention times (HRT) to produce VFA. The effluent derived from the acidogenic reactor was used as substrate in several aerobic batch assays where the feasibility of producing PHA was tested. In order to determinate the PHA production potential of the sludge enriched in PHA accumulating bacteria present in our laboratory at this moment, several assays were developed with two different cultures; one acclimated to fermented brewery wastewater and another one enriched on a synthetic medium containing a mixture of VFA as substrate.

Materials and Methods

Influent wastewater

The acidogenic reactor was fed with water collected from a wood industry producing medium density fibreboard (MDF). This mill uses pine and black poplar as raw material for its process, with a proportion of 80% and 20%, respectively. The type of wood used in the fibreboard production process affects the composition of the final effluent and thus, the biodegradability of the wastewater. The VFA proportions obtained at the end of the fermentation could depend on the type of wood.

Wastewater treatment plant of this mill consists of two units: flotation, anthracite filter, cartridge filters and reverse osmosis. During two months, samples have been collected weekly in different points of the treatment plant: raw waste-water, flotation outlet and reverse osmosis outlet (final effluent). General parameters of wastewater characterization were measured in each point, giving the average results shown in Table 1. Water collected from flotation outlet was the influent used for acidogenic fermentation. The COD in this stream consisted of 30% total sugars, 25% VFA and 10% ethanol, the remaining COD was of a more complex composition.

Parameter	Units	Sample point		
		1	2	3
COD _T	mg/L	42,582	11,110	1,193
COD _S	mg/L	14,767	10,540	1,132
COD _S /COD _T		0.37	0.96	0.96
BOD ₅	mg/L	6,422	3,922	220
BOD ₅ /COD _T		0.16	0.36	0.21
TSS	mg/L	13,874	60	4
VSS	mg/L	13,491	53	3
NH ₄ ⁺	mg/L	18.2	2.3	2.7
TKN	mg/L	241	109	11
pH		4.6	5.8	4.9
Alkalinity	mg/L	170	753	67

Experimental set-up

Acidogenic fermentation

Wood mill effluent fermentation was carried out in a continuous stirred tank reactor (CSTR) with a useful volume of 1.6 L and a settler of 0.8 L. Since there was no possibility of obtaining anaerobic sludge from the wood industry, the reactor was inoculated with biomass from the anaerobic reactor of a brewery industry wastewater treatment plant in Galicia (Spain) at concentration of 4 g VSS (volatile suspended solids)/L and kept at 30°C. The reactor was operated at two different hydraulic retention times (HRT) of 1 d and 1.5 d resulting in average organic loading rates (OLR) of 5.6 and 2.9 g COD/L*d, respectively. pH was kept at 5.5 by addition of NaOH 2 M solution. These conditions were close in agreement with results obtained in previous experiments (unpublished results).

PHA producing biomass enrichment

Two different cultures were used in this study. The first one was enriched in a 1 L-SBR (sequencing batch reactor) fed with fermented brewery wastewater (culture 1) at an OLR of 34 Cmmol/L*cycle and the second one was got in a 1.5 L-SBR fed with synthetic media containing acetic, propionic, butyric and n-valeric acids as substrate at an OLR of 27 Cmmol/L*cycle (culture 2). The initial inoculum used to seed both reactors was the same. Both SBR were operated under aerobic dynamic feeding (ADF) conditions. Operational conditions fixed in both reactors were the same: SBR cycle of 12 hours, HRT of 1 d and solids retention time (SRT) of 7 d. Air was supplied at a flow rate of 1 vvm. pH and temperature in the reactors were not controlled, but they were

monitored. Nitrogen was added in a concentration of 1.4 Nmmol/L. Table 2 shown the main operational parameters applied in the enrichment of the aerobic SBR.

Table 2: Operational parameters applied during the PHA producing biomass enrichment.

Culture	Operational parameters									VFA percentage			
	VRx	HRT	vvm	pH	T	X ₀	C ₀	N	C/N	HAc	HPr	HBu	HnVa
1	1	1	1	nc	nc	97	34	1.34	26	61	9	18	12
2	1.5	1	1	nc	nc	128	27	1	27	69	10	9	12

(VRx: reactor volume; vvm: flow air rate; X₀: initial active biomass; nc: not controlled)

Aerobic batch assays

Aerobic batch assays were performed with the sludge from the enrichment reactors previously described. Assays using fermented wood mill effluent and synthetic media were performed in a 0.6 L vessel connected to a respirometer. Operational conditions were close to the ones in the SBR.

Analytical procedures

Total suspended solids (TSS), VSS, biochemical oxygen demand (BOD) and total Kjeldahl nitrogen (TKN) were measured as described in Standard Methods (APHA 1998). Total and soluble chemical oxygen demand (COD_T and COD_S, respectively) were evaluated by a method consisting in the acid digestion of the sample with dichromate at 150°C. Ammonium and phosphate concentrations were determined by using colorimetric methods.

Volatile fatty acids (VFA) (acetate, propionate, butyrate, i-valerate and n-valerate) and ethanol concentrations were determined by high performance liquid chromatography (HPLC) using a Hewlett Packard chromatograph equipped with a Supelcogel C-610 column using an ultraviolet (UV) detector and a RI detector, respectively. Phosphoric acid 0.1% was used as mobile phase, with a flow of 0.5 ml/min.

The column was kept at 30°C. The wavelength for detection was set at 210 nm. VFA concentrations were calculated using a calibration curve ranging from 25 to 3000 mg/L.

PHA were determined by gas chromatography (GC) following the method described by Braunegg et al. (1978), modified by Comeau et al. (1988) and Satoh et al. (1992). The method consists on the rupture of cell membranes and structures. Then, the PHA chains are hydrolysed and the monomers are methylated. The methylated monomers are extracted with chloroform, injecting the organic phase in a liquid-gas chromatograph. A calibration curve with P(HB- HV) standard (88%/12%) corrected by an internal standard (heptadecane) was used to determinate hydroxybutyrate (HB) and hydroxyvalerate (HV) concentrations.

Calculations

Acidogenic fermentation

The total VFA concentration corresponds to the sum of acetate, propionate, butyrate, i-valerate and n-valerate concentrations. The percentage of organic matter conversion into VFA was calculated as (mg COD-VFA in the effluent/mg COD in the influent)*100. The acidogenic activity was considered as mg of VFA in the effluent per mg of biomass in the reactor (both in C mg) and per liter.

Aerobic batch assay

Sludge PHA content was determined as a fraction of VSS on a mass basis (%PHA = PHA*100/VSS; PHA and VSS concentration in g/L). Biomass concentration is the sum of PHA content and active biomass (SSV= PHA + X; where X represents the active biomass). Active biomass was calculated considering all ammonia was used for growth. The PHA concentration corresponds to the sum of HB and HV monomers. PHA production (Y_{STO}) on substrate consumed was calculated as the ratio between the overall amount of PHA and the total amount of substrate consumed. The specific HA production (Δf_{HA}) was determined by subtracting the initial specific HA concentration from the final specific HA concentration (Cmmol HA/Cmmol X).

Results

Acidogenic fermentation

Experiments were carried out at different HRT (1 and 1.5 d). VFA effluent concentration was quite similar for the two HRT tested. However, the VFA composition was different, with an increase of long-chain VFA concentration (HBu and HVal) at the higher HRT applied (Table 3). Acidification percentage was 37% at 1 d and 42% at 1.5 d. Bengtsson et al. (2008a) obtained maximal acidification of 75% at HRT of 1 d using a paper mill effluent, but this is slightly different of wood mill effluent, which could influence the conversion obtained.

The nature of the organic matter present in wood mill effluent might not be easily transformed into VFA. Total COD is composed in a 65% by the sum of VFA, sugars and ethanol, which could be easily transformed by acidogenic fermentation. Thus, other operation conditions need to be tested in order to improve the acidification of the wood mill effluent, at least until a 65% of acidification percentage. Still, the other 35% of total COD, in accordance with the BOD/COD ratio, could be formed by hardly degradable compounds, which usually appear in this kind of streams, so a pre-treatment could be needed in order to improve acidification above 65%.

Table 3 : Ammonia, COD₀ (COD in the inlet), COD_e (COD in the effluent), VFA and individual acids concentration (mg/L) in fermented wood mill effluent and acidification percentage at two different HRT

HRT	VFA	N-NH ₄ ⁺	COD ₀	COD _e	HAc	HPr	HBu	HVal	%VFA
1.0 d	1731	25	5611	4594	1161	486	28	0	37
1.5 d	1422	2	4276	3299	725	551	97	43	42

PHA production

Two different aerobic cultures were used to seed the PHA production from fermented wood waste, one enriched on brewery wastewater (culture 1) and the second one enriched in a synthetic medium (culture 2). Figure 1 shows a typical cycle of the SBRs where the biomass was enriched for PHA accumulating bacteria.

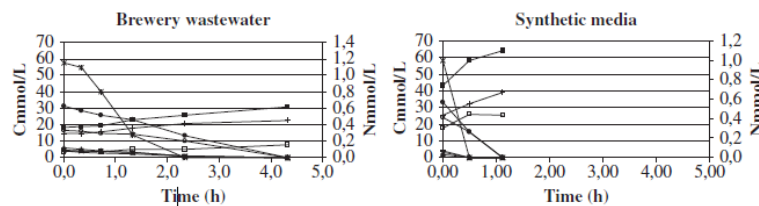


Figure 1 | Typical profiles of concentration during a SBR cycle. VFA (●), HAc (○), PrH (◊), BuH (▲), nValH (×), HB (+), HV (□), HA (■) and N (*).

Both aerobic cultures used in these assays were previously submitted to feast/famine conditions and operated during several months in order to select a biomass with high storage capacity. The operational parameters applied in both reactors were the same. The systems containing these cultures were stable over time, with a similar storage yield and HA content in both (Y_{STO} of 0.35 and 0.40 Cmmol/Cmmol and HA content of 28% and 36% in culture 1 and culture 2, respectively) (Figure 2a and b). However, it is worth remarking that the sludge fed with brewery wastewater presented lower substrate uptake rate, giving a longer feast period (Figure 3). Despite of the different acclimation times, both reactors had achieved the stationary state which was characterized by a constant biomass concentration, similar feast times and similar variation in pH and DO (dissolved oxygen) values.

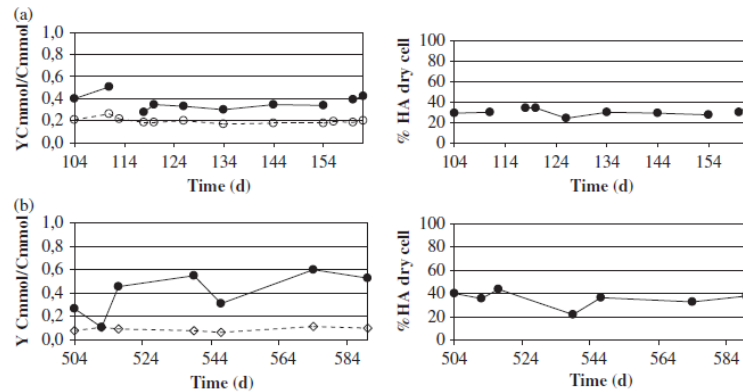


Figure 2: Y_{GROTWH} (J), Y_{STO} (K) and %HA (K) obtained during de period of time where the cultures were used to do the assays. Figure (a) is related to culture 1 and figure (b) is associated to culture 2.

In order to determine the viability of using wood mill effluents as substrate in a PHA production process several batch assays were developed. Two assays (assays I and II) were performed with the acidogenic effluents obtained at HRT of 1 and 1.5 d, respectively and sludge acclimated to brewery wastewater (culture 1) obtaining similar storage yields (Y_{STO}) and polymer composition (Table 4 and Figure 4). The slightly higher Y_{STO} obtained in assay II could be explained by the lower nitrogen concentration in acidogenic effluent. As known, low nitrogen concentrations limit growth capacity resulting in more carbon directed to the storage process. Several authors (Serafim et al. (2004), Albuquerque et al. (2007), Kumar et al. (2004), Punrattanasin et al. (2006) and Bengtsson et al. (2008b)) observed an increase in HA accumulation when nutrients are limited in the media.

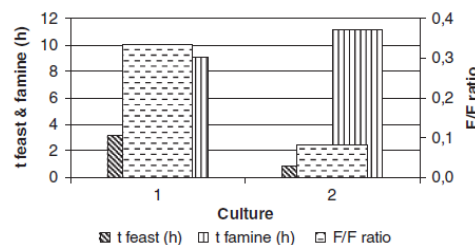


Figure 3: Feast and famine time and feast/famine ratio observed in the operation of the SBR containing the cultures used in this study.

Though VFA composition from the acidogenic effluents attained at different HRTs (Table 1) was different, the polymer composition derived from the batch assays was quite similar, with a high HB monomer content (HB:HV 80:20). In both cases acetic acid was the main acid and the

valeric and butyric acid contribution was insignificant. Furthermore, other non-VFA contributors for PHA storage could enhance the HB monomer production.

A third assay (assay III) using the same sludge but feeding synthetic media with the same VFA concentration than acidogenic effluent was carried out to study the possible effect of the fermented wood mill effluent on the PHA formation. The results derived from this assay were quite similar to those obtained with real wastewater (Table 4 and Figure 4), therefore it was concluded that substrate inhibition did not take place. Previous works with real wastewater reported in literature mention the possibility of inhibition when a real substrate is used, resulting in lower HA contents than those with a synthetic substrate (Albuquerque et al. 2007). However, in this case the lower HA content achieved are not consequence of a substrate inhibition. As shown Table 4, the specific substrate uptake was higher in the assay developed with real wastewater than that attained in the assay made with synthetic media.

Table 4 : Results derived from the batch assays

Assay	Substrate	C0	N0	C/N	HAc	HPr	HBu	HnVal	-qS	qP	-qN	%HA	Y _{STO}	Polymer	%HB	%HV
I	Wood ww	44.2	2.71	16	68	32	0	0	0.063	0.012	0.010	22	0.23	P(HB:HV)	83	17
II	Wood ww	48.3	0.46	105	47	38	7	8	0.151	0.040	0.009	27	0.35	P(HB:HV)	81	19
III	Synthetic	46.8	0.54	87	45	37	8	10	0.070	0.011	0.004	31	0.27	P(HB:HV)	71	29
IV	Wood ww	44.7	0.45	99	50	39	6	5	0.199	0.034	0.009	29	0.57	P(HB:HV)	46	54

(C₀: initial substrate concentration as Cmmol/L; N₀: initial nitrogen concentration as Nmmol/L; Y_{STO}: storage yield (Cmmol/Cmmol); -q_S: specific substrate uptake rate (Cmmol/Cmmol/h); q_P: specific polymer production rate (Cmmol/Cmmol/h); -q_N: specific nitrogen uptake rate (Nmmol/Cmmol/h)).

The higher Y_{STO} attained in the assay developed with real substrate (assay II) with regard to the assay made with synthetic media (assay III) (Table 4, Figure 4) could be explained by the presence of compounds different from VFA, like alcohols or other simple molecules present in acidogenic effluent, which are contributing to PHA accumulation. This hypothesis is supported by the polymer composition obtained in the assays carried out with real wastewater and the assay with synthetic medium. As shown Table 4, the HB content is higher in the polymer attained with real wastewater (close to 80%) than in that attained with synthetic media (70%). Carucci et al. (2010) also observed an increase on the ratio between the PHB formed and the VFA consumed (0.74 vs 0.55 COD/COD) when they used real wastewater as substrate instead of acetate. They explained this fact assuming that PHB was also stored from other substrates present in the wastewater.

Finally, in order to determine the effect of the biomass enrichment step on PHA accumulation, other assay (assay IV) was performed using a different inoculum; biomass from a SBR reactor fed with synthetic media (culture 2). As shown in Table 4 and Figure 4 a higher Y_{STO} was obtained (0.57 Cmmol/Cmmol) which indicates that the biomass from the reactor fed with brewery wastewater had lower capacity to accumulate PHA. As mentioned before, although both cultures were enriched under the same operational parameters, the feast time observed in SBR fed with brewery wastewater was longer than that observed in the SBR fed with synthetic media (Figure 2). The longer feast time in the enrichment step using brewery wastewater can influence the storage capacity of the sludge. A relative short feast phase and long famine period is more selective for PHA accumulating bacteria (Johnson et al. 2009). The feast/famine ratio (F/F ratio) is known to affect the sludge storage capacity. If famine stage is not long enough to ensure an internal limitation the culture will adapted to grow instead of storage internal reserves when an

external substrate is added. Dionisi et al. (2006) observed a loss of the PHA accumulation capacity of the sludge when OLR higher than 25 g COD/ L*d were fed to the aerobic reactor. They concluded that at the highest OLR tested (25 and 31.25 g COD/L*d) the extent of feast and famine conditions was not enough to select for microorganism with high storage rates and, on the contrary, microorganisms showed a growth response. Albuquerque et al. (2010) also attained higher PHA storage when the F/F imposed in the reactor was low, 0.22 instead of 0.53. They remark the importance of controlling this parameter as higher feast phases could cause the selective pressure for PHA storage decreasing. Villano et al. (2010) also observed a decreasing storage ability of the biomass at increasing OLR indicating that biomass performance in batch was dependent on its previous cultivation conditions in the SBR.

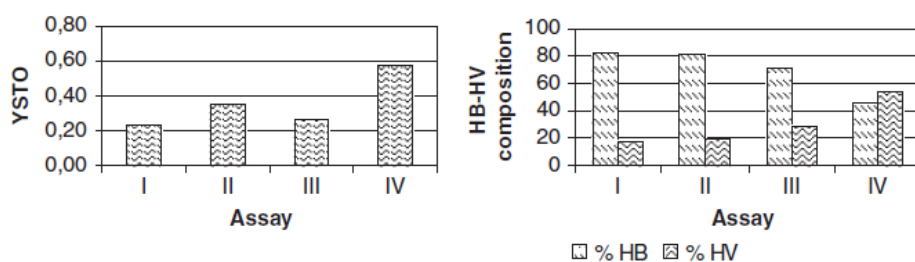


Figure 4: Storage yield (Y_{STO}) and polymer composition obtained during the different batch assays.

Conclusions

The main conclusions from this work are listed hereafter.

Acidogenic fermentation of a wood mill effluent was carried out at pH 5.5 and 30°C and at HRT of 1.0 and 1.5 d resulting in two different OLR (5.6 and 2.9 g COD/L*d). Reactor performance was stable during the operation time, obtained similar acidification percentage (37 and 42%) for the two different HRT applied. However, VFA composition was different, being observed an increase of the long-chain VFA concentration at the higher HRT (1.5 d) tested. Conversion of organic matter into VFA could be improved trying different operational conditions in the acidogenic reactor.

In order to determine the feasibility of using this stream as a substrate for PHA production aerobic batch assays were performed using fermented wood mill effluents obtained at different HRT. The assays were also developed with two different cultures: the first one previously enriched in a SBR fed with fermented brewery wastewater and the second one enriched in a SBR fed with synthetic substrate containing acetate, propionate, butyrate and n-valerate as substrate. Low Y_{STO} were attained when the culture enriched in brewery wastewater was used (0.27 and 0.35 Cmmol/Cmmol), being observed the lowest value when nitrogen was present in the media at high concentration (0.22 Cmmol/Cmmol).

However, a Y_{STO} of 0.57 was achieved when the culture enriched in synthetic media was used. The longer feast time in the enrichment step observed in the SBR fed with brewery wastewater could influence the storage capacity of the sludge, being more competitive those cultures enriched in long SBR cycles where the feast time is shorter like the sludge obtained in the SBR fed with synthetic media.

Wood mill effluent is a good carbon source for PHA production.

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