CHEMICAL ENGINEERING TRANSACTIONS

VOL. 49, 2016

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Enrico Bardone, Marco Bravi, Tajalli Keshavarz

ISBN 978-88-95608-40-2; **ISSN** 2283-9216

DOI: 10.3303/CET1649022

Microbiological Valorisation of Bio-composites Based on Polylactic Acid and Wood Fibres

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The use of wood fibres for production of bio-based composites has attracted interest in various application sectors ranging from packaging to automotive components and in other high value applications. In the course of the present research activity, several bio-based composites were developed using wood fibres with a compostable polymeric matrix such as polylactic acid (PLA) and a flexible biodegradable polymer such as poly(butylene adipate-co-terephthalate) (PBAT). The developed materials were used for the manufacture of several prototypes for food packaging (trays, boxes for refrigerated or frozen fish, egg box), agricultural applications (pots and yarns), automotive components (spoiler and seats) as well as containers for cosmetics and chemicals. Biodegradability and compostability are desired properties, allowing bio-recycling as end of life scenario, mainly for materials used in food packaging and agricultural applications. Thus, they may be recycled at the end of their life time service producing compost as a value-added by-product. Composting is the main option for bio-recycling but also other valuable pathways can be pursued. Because lignocellulose is one of the components of developed materials, several by-products such as enzymes, reducing sugars, proteins, amino acids, carbohydrates, organic acids, etc. may be obtained from the bio-composites produced. Alternatively, the bio-composites can be also used for the production of yeast biomass. This is important as another recyclability way of the new produced materials. In the present research the bio-composites produced were investigated as substrates for the production of the methylotrophic yeast Pichia pastoris, a potential source of single-cell protein (SCP), β-carotene, and Rhodotorula sp. as potential source of food and feed grade colorant. This is another more valuable alternative to the composting considering also that composting cannot be used to dispose of large quantities of bio-plastics, and in the future it will become more and more important to find alternative routes of valorisation for bio-plastics disposal.

1. Introduction

Lignocellulosic biomass is mostly discarded in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries. Due to their abundance and renewability, there has been a great deal of interest in utilizing it for the production of bio-composites and for recovery of many value-added products (Angelini 2015, Chiellini 2009, Cinelli 2006, Pandey et al., 2000; Das and Singh, 2004; Mtui, 2009,). The use of wood fibres for production of bio-based composites has attracted interest of various application sectors ranging from packaging to automotive components and other high value applications. Composting is the main option for bio-recycling at the end of life cycle of bio-composites but also other valuable pathways can be pursued.

Enzymes reducing sugars, furfural, ethanol, protein and amino acids, carbohydrates, lipids, organic acids, phenols, activated carbon, degradable plastic composites, cosmetics, bio-sorbent, resins, medicines, foods and feeds, methane, bio-pesticides, bio-promoters, secondary metabolites, surfactants, fertilizer and other miscellaneous products are included among the main recovery products from biomasses (Tengerdy and Szakacs, 2003;Taherzadeh and Karimi, 2008; Demirbas, 2008; Sánchez, 2009).

Please cite this article as: Seggiani M., Cinelli P., Geicu M., Popa M.E., Puccini M., Lazzeri A., 2016, Microbiological valorisation of bio-composites based on polylactic acid and wood fibers, Chemical Engineering Transactions, 49, 127-132 DOI: 10.3303/CET1649022

In the course of the present research activity, several new composites were developed whose main novelty was the use of wood fibres with biodegradable polymeric matrices (Rapa 2011, Seggiani 2015). The bio-based composites were addressed with compostable polymeric matrices based on polylactic acid (PLA) and poly(butylene adipate-co-terephthalate) (PBAT). Both PLA and PBAT are biodegradable polymers. They are thermoplastics which can be processed using most conventional polymer processing methods. PLA is high in strength and modulus but brittle while PBAT is flexible and tough. In view of their complementary properties, blending PLA with PBAT becomes a natural choice to improve PLA properties without compromising its biodegradability (Jiang 2006).The developed bio-composites were used for the manufacture of several prototypes for food packaging (trays, boxes for refrigerated or frozen fish, egg box), agricultural applications (pots and yarns) as well as containers for cosmetics and chemicals.

Biodegradability and compostability were desired properties, mainly for materials planned for food packaging and agricultural applications. Thus they may be recycled at the end of their life time service producing compost as a value-added by-product. In the case of other materials such as those for chemical container, maintenance of functionality without deterioration during service lifetime was put before biodegradability/compostability criteria. On the other side, as soon as materials developed exceed its lifetime, they will be considered and treated as waste. Composting is a good choice for materials susceptible of this treatment. However, other treatments are available that would recover many value-added products from these wastes. In this work, a microbiological valorisation process of the materials developed were performed, concerning the production of yeast biomass (single cell proteins and β-carotene) on hydrolysates derived from the developed composites.

2. Experimental

2.1 Materials

Commercial PLA was supplied from Natureworks®, USA (Natureworks PLA 2003D) and PBAT (Ecoflex®) from BASF® (Germany) with melting point of 110-120 °C, density of 1.25-1.27 g/cm^3, and MW=131440 Da. Both polymers were supplied in pellet form and used as received. Wood fibres were supplied by Rettenmaier & Söhne (Germany) (type EFC100), with 6.7 aspect ratio. Formulations were prepared with weight PLA/PBAT ratios of 50/50 and 20/80 and wood fibre content of 15 and 20%.

2.2 Composite production and characterization

PLA, the fillers and Ecoflex (PBAT) were processed with a MiniLab II Haake Rheomex CTW 5 conical twin-screw extruder (Thermo Scientific Haake GmbH, Karlsruhe, Germany) at a screw rate of 80 rpm/min and a cycle time of 30 seconds, in the temperature range from 180 to 200 °C depending on the material, and transferred to a Haake MiniJet II mini injection moulder (Thermo Scientific Haake GmbH, Karlsruhe, Germany) to produce Haake 3 type specimens used for the mechanical tests. Tensile tests were performed at room temperature, at a crosshead speed of 10 mm/min, by means of an Instron 4302 universal testing machine (Canton MA, USA) equipped with a 10 kN load cell and interfaced with a computer running the Testworks 4.0 software (MTS Systems Corporation, Eden Prairie MN, USA).

2.3 Microbiological valorisation

Valorisation tests have been performed using 2 strains of yeasts *Pichia pastoris* and *Rhodotorula sp*. in order to obtain, respectively, a high production of single cell protein (*Pichia pastoris)* for animal feed and a valuable compound producer *Rhodotorula sp.* for β carotene from hydrolysates obtained from the developed materials.

Pichia pastoris and Rhodotorula are yeasts that can grow to very high cell densities in various inexpensive culture media; it is useful when trying to produce large quantities of protein (single cell protein-SCP) without expensive equipment; the proteins could be used as additives in animal feed.

The bio-composite materials as granules or end products were grinded and different treatments were applied in order to hydrolyse the substrate and to make it available to the yeasts. Three different hydrolysis methods were tested. In one (I) of these methods the sample was mixed with distilled water and autoclaved for 2 h at 121 ºC. After this physical pre-treatment the sample was mixed with H_2SO_4 2%. In the second and the third hydrolysis method the sample was mixed with NaOH 1% and H_2O_2 0.3 % (II), respectively, with distilled water and H_2SO_4 2% (III) and autoclaved for 2 h at 121 ºC. Then, all the samples were incubated for 20 h at 55 °C under agitation at 250 rpm. pH was corrected to 4.6 and at the end all the samples were filtered and centrifuged.

The H_2SO_4 and NaOH hydrolysis with thermal treatment is based on reduction reaction of 3.5 dinitro-salicilic (DNS) acid by – COH reducing groups from glucides. 2 mL of supernatant was mixed with 3 mL DNS; the samples were covered and then incubated for 15 min on water bath at boiling point. After cooling, they were measured at spectrophotometer at 640 nm in comparison with distilled water. Etalon curve was realized with 0.1% glucose aqueous solution prepared in distilled water. It was measured the extinction depending on glucose concentration.

Optical density (OD) values measured at spectrophotometer were transformed with the help of etalon curve in mg glucose: Glucose concentration (%) = mg glucose of sample – mg glucose of control/ 2 x 10.

The substrate needed for yeast growth was obtained via hydrolysis of materials in combination with thermal treatments and additional ingredients, such as 0.17 % Yeast Nitrogen Base and 0.5 % $(NH_4)_2SO_4$ added into solution. The substrates were sterilized for 15 min at 121 °C, cooled and inoculated with the yeasts strains *Pichia pastoris* and *Rhodotorula sp.* A control sample was prepared in the same way, but without microorganisms inoculation. A common yeasts growth culture media, YPG (Yeast Peptone Glucose), was used in experiments in order to compare the microorganisms growth on the different substrates.

In order to analyse the microorganism growth, OD determinations at 640 nm for viability test were done every 24 h, during a 96 h period. After incubation at 26 °C for 96 h, wet and dry biomass were also determined as weight percentage.

3. Results and Discussion

3.1 Mechanical characterization of the biocomposites

The tensile properties of the different developed composites are reported in Table 1. As shown, with the increase in PBAT content (50-80 wt %), the blend showed decreased tensile strength and modulus; however, elongation and toughness were dramatically increased. With the addition of PBAT, the failure mode changed from brittle fracture of the neat PLA to ductile fracture of the blend as demonstrated by tensile test. Consequently, the composites based on PLA/Ecoflex 20/80 matrix presented higher values of elongation at break than composites based on 50/50 matrix, and, of course, lower values of tensile strength and modulus. Thus the PLA/Ecoflex ratio of the biodegradable matrix can be tuned in dependence of the technical parameters envisaged to meet the technical requirements for packaging and agriculture applications.

In all formulations, elongation at break is consistently reduced by the addition of fibres but tensile strength and modulus were maintained and in some formulations moderately increased.

The formulations with the best mechanical performances, such as PLA/Ecoflex 50/50 with 15 wt % wood fibre and PLA/Ecoflex 20/80 with 20 wt% wood fibre, were selected for the industrial production of prototypes such

Table 1: Comparison of mechanical properties of the PLA and composites based on PLA/Ecoflex 50/50 and PLA/Ecoflex 20/80 as continuous matrix.

Sample	Yield stress (MPa)	Young's Modulus (GPa)	Elongation at break (%)
PLA	86.4	4.4	5
PLA/Ecoflex 50/50 (w/w)	40.2	2.2	40
PLA/Ecoflex 50/50 (w/w) + 15 wt% wood fibre	44.7	2.5	13
PLA/Ecoflex 50/50 (w/w) + 20 wt% wood fibre	42.7	2.8	6
PLA/Ecoflex 20/80 (w/w)	24.6	0.5	193
PLA/Ecoflex 20/80 (w/w) + 15 wt% wood fibre	24.3	0.6	70
PLA/Ecoflex 20/80 (w/w) + 20 wt% wood fibre	29.3	0.7	19

as pots and tomato yarns (Fig.1). These prototypes were positively validated in agriculture applications (Rapa et al., 2011). These formulations in pellet form were used for the valorisation tests as substrate for *P. pastoris* SCP production.

(a) (b)

Figure 1: Pictures of tomato yarn–round shape (a) PLA/Ecoflex 50/50 and 15 wt% wood fiber and (b) PLA/Ecoflex 20/80 and 20 wt% wood fiber

3.2 Microbiological valorisation

The results for the glucose and nitrogen content after acidic and alkaline hydrolysis of the biocomposite materials are presented in Table 2.

Table 2: Amount of glucose and nitrogen obtained by acid and alkaline hydrolysis

N	Sample	Hydrolysis method	Glucose (mg/100g)	N composition (%)
1	PLA/Ecoflex 20/80 and 20 wt% wood fibre	I	2.20	0.106
2	PLA/Ecoflex 20/80 and 20 wt% wood fibre	II	1.54	0.087
3	PLA/Ecoflex 20/80 and 20 wt% wood fibre	III	10.86	0.117
4	PLA/Ecoflex 50/50 and 15 wt% wood fibre	I	1.40	0.101
5	PLA/Ecoflex 50/50 and 15 wt% wood fibre	II	0.64	0.053
6	PLA/Ecoflex 50/50 and 15 wt% wood fibre	III	8.26	0.120

The alkaline hydrolysis (II method) combined with thermal treatment provided the lowest quantities of glucose and nitrogen from the bio-composite materials tested. The highest amount of glucose and nitrogen were obtained after sulfuric acid hydrolysis (III method) combined with thermal treatment for both bio-composite material samples. The quantity of glucose obtained was used as carbon source for the growth yeasts substrate. The quantity of nitrogen obtained after hydrolysis was insufficient, so 0.17% Yeast Nitrogen Base and 0.5% $(NH_4)_2H_2SO_4$ were added into substrate solution in order to assure the requested nitrogen amount for yeasts growth. The results obtained show that the sample PLA/Ecoflex 20/80 and 20 wt% wood fibre provided a larger amount of glucose than the other sample (PLA/Ecoflex 50/50 and 15 wt% wood fibres) for all hydrolysis methods applied.

The results for yeast viability and yeasts biomass production obtained for the different substrates are presented in Table 3. Results show that OD values for *Pichia pastoris* are the highest compared with *Rhodotorula sp.* In accordance with OD results, wet and dry biomass productions are higher for *Pichia pastoris* in comparison with those for *Rhodotorula sp.*

The most promising results of glucose production are those obtained by acidic hydrolysis in combination with alkaline one.

Table 3: Viability and yeasts biomass production for the different substrates obtained after acidic and alkaline hydrolysis

N	Sample	Hydrolysis method	Yeast strain inoculated	initial OD	OD after 96 h from inoculation	wet biomass (%)	dry biomass (%)
1	PLA/Ecoflex 20/80 20 wt% wood fibre	I	-	0.063	0.068	0.810	0.007
2		I	*Rhodo.sp.*	0.074	0.092	0.920	0.029
3		I	*Pichia pas.*	0.094	0.289	0.710	0.010
4		II	-	0.067	0.068	0.540	0
5		II	*Rhodo.sp.*	0.091	0.100	0.495	0.0015
6		II	*Pichia pas.*	0.115	0.353	0.495	0.0015
7		III	-	0.217	0.238	1.355	0.040
8		III	*Rhodo.sp.*	0.262	0.293	0.650	0.0085
9		III	*Pichia pas.*	0.272	0.456	0.635	0.030
10	PLA/Ecoflex 50/50 15 wt% wood fibre	I	-	0.053	0.062	0.685	0.035
11		I	*Rhodo.sp.*	0.077	0.100	0.575	0.015
12		I	*Pichia pas.*	0.105	0.312	1.235	0.055
13		II	-	0.025	0.035	0.535	0.010
14		II	*Rhodo.sp.*	0.057	0.066	0.540	0.015
15		II	*Pichia pas.*	0.073	0.297	1.795	0.010
16		III	-	0.198	0.201	1.325	0.050
17		III	*Rhodo.sp.*	0.212	0.248	2.390	0.110
18		III	*Pichia pas.*	0.236	0.389	1.435	0.055
19	YPG growing media	-	-	0.059	0.059	0.960	0.020
20		-	*Rhodo.sp.*	0.071	0.072	0.995	0
21		-	*Pichia pas.*	0.085	0.782	2.145	0.90

4. Conclusions

Bio-composites were prepared with natural lignocellulosic fibre and PLA/Ecoflex based matrix up to 20 wt% loading of wood fibre. Tuning the ratio of PLA/Ecoflex as well as the content of wood fibre allows tuning mechanical properties of the composites.

Finally the best and cheaper hydrolysis process for the tested materials and also the best and cheaper medium for yeast growing were established at lab scale. The highest amount of yeast biomass has been obtained for the compound PLA/Ecoflex 50/50 and 15 wt% wood fibres hydrolysed using H_2SO_4 and high temperature treatment. This result is correlated with the highest glucose yield obtained after hydrolysis. Among *Rhodotorula sp.* and *Pichia pastoris*, the amount of biomass was in general bit higher for the *Pichia pastoris*, but no significant differences were noticed in the most cases. Consequently, the results obtained at lab scale showed that the microbiological valorisation of the developed bio-composites may represent a promising and valuable alternative to their composting.

Acknowledgements

The EC contribution granted in the FP7 Project FORBIOPLAST. GA 212239, Forest Resource Sustainability through Bio-based-Composite Development is acknowledged.

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