Lactic acid production from unmatured banana peel and flesh through simultaneous saccharification and fermentation

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Abstract

The aim of this study was to establish a process of lactic acid (LA) production from two different kinds of african organic waste i.e. peel and flesh of un-matured banana by using as model strain *Lactobacillus bp Pentosus* AH 239. The bioconversion of glucose contained in the biomass to LA was performed following the Simultaneous Saccharification and Fermentation (SSF) process. The Separated Hydrolysis and Fermentation (SHF) was also applied in this study to compare the efficiency of both process. The results showed that the enzymatic hydrolysis yield was significantly improved in case of SSF recording a rate of hydrolysis in the range of 82%-90% against 52%-61% under SHF conditions. The results showed also that SSF give more efficient lactic acid production with a yield above of 90%, and a high concentration up to 50 g/L. Due to its performance, the SSF process for the lactic acid production could be an important way of bioconversion for lignocellulosic residues in Africa. The optimization of this process need to be adapted for African context and for its development on an industrial scale.

Keywords: Organic waste, SSF process, SHF process, Lactic acid, *Lactobacillus bp Pentosus* AH 239, Banana peel, Banana flesh.

Résumé

Le but de cette étude est d'établir un processus de production d'acide lactique (LA) à partir de deux différents types de déchets organiques en Afrique, à savoir la pelure de bananes non-matures en utilisant comme souche modèle *Lactobacillus pb pentosus* AH 239. La bioconversion du glucose contenu dans la biomasse d'acide lactique (LA) a été réalisée après le processus de saccharification et fermentation simultanées (SSF). L'Hydrolyse séparé et la fermentation (SHF) ont été également appliqués dans cette étude pour comparer l'efficacité des deux processus. Les résultats ont montré que le rendement de l'hydrolyse enzymatique a été significativement amélioré en cas d'un SSF enregistrant un taux d'hydrolyse dans l'intervalle de 82%-90% contre 52%-61% dans les conditions SHF. Les résultats montrent également que la SSF donne une production plus efficace d'acide lactique avec un rendement supérieur à 90%, et une concentration élevée jusqu'à 50 g/L. En raison de sa performance, le processus de SSF pour la production d'acide lactique peut être un moyen important de bioconversion de résidus lignocellulosiques en Afrique. L'optimisation de ce processus doit être adaptée au contexte africain et pour son développement à l'échelle industrielle.

Mots clés: Déchets organiques, Processus SSF, Processus SHF, Acide lactique, *Lactobacillus bp Pentosus* AH 239, Pelure de banane.

INTRODUCTION

The organic biomass, such as crop residues or by-products from food industry, could be considered as an important carbon source for the production of other value-added products (biogas, ethanol, biofertilizer, lactic acid and amino acid). Several techniques for valorization and bioconversion were developed to optimize the conversion of biomass to new products generated. This article focuses on optimizing the conversion of agricultural biomass for the production of lactic acid.

Thus, lactic acid (LA) has an important interest in the industry for its different properties (Abdel-Rahman et *al*, 2011). Its use range across several industry sectors as food preservative and acidulate in dairy products, beverages, confectionery. The lactic acid properties are also of interest in non-food industries, especially the textile, pharmaceutical and cosmetic products (Singhvi et *al*, 2010). The demand for this organic acid increases more and more in the world, given that it can be used as feedstock for the production of poly-lactic acid (PLA). This polymer is a biodegradable compound widely used in surgical sutures, orthopedic implants, drug delivery systems and disposable consumer products (Adnan and Tan, 2007).

The production of lactic acid can be performed in two ways: by chemical synthesis or by fermentation (Abdel-Rahman et *al*, 2011). Chemical synthesis is based on petrochemical resources and has the disadvantage of giving rise to a racemic mixture of DL-lactic acid (Hofvendahl and Hahn-Hägerdal, 2000). As for the second lane of production, it corresponds to lactic fermentation based on a biomass carbon source; this path is more advantageous for its low energy consumption and its pure LA production by selecting the appropriate lactic acid bacteria (LAB) strain (Ilmen et *al*., 2007; Pandey et *al*., 2001).

Several types of organic raw materials can be introduced into the lactic acid fermentation process. These raw materials must be cheap and in abundant amounts to be competitive on an industrial scale (Rojan et *al*, 2009). Other criteria may be decisive in choosing the biomass to use biomass namely low content in contaminants and toxic substances, the use of pretreatment, formation of other products that LA and availability throughout the year (Wee *et al*., 2006; John *et al*., 2007).

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Crop residues and food processing by-products are material very rich in carbohydrate favorable to conversion by LAB (John *et al.*, 2007). These residues are distinguished according to their nature and their composition into two biomass categories: lignocellulosic materials and starchy materials.

The lignocellulosic materials are composed of cellulose, hemicellulose and lignin. The cellulose is a glucose polymer mainly found in the cellulosic membrane where it is associated with lignin polymers. This assembly forms a compact, almost impermeable structure. The proportion of these components varies from plant species hence the interest of selecting materials which have proportions giving rise the better yield of lactic acid. In this sense, many lignocellulosic residues have been investigated as raw materials for LA production: e.g. corncob (Miura *et al*., 2004), wood (Woiciechowski et *al*. 1999), wastepaper (Park et *al*., 1998).

As for the starchy materials, they are made up of starch grain (D-glucopyranose). These glucose molecules are found in the form of linear structure consisting of a chain of D-glucose residues with glycosidic bonds. Fermentation tests were carried out on starchy residues have allows to obtain high yields of lactic acid namely wheat starch (Hofvendahl et *al*., 1999), barley (Javanainen and Linko, 1996), cassava bagasse (Rojan et *al*., 2005; John et *al*, 2006a, b, c), corn starch (Rojan et *al*., 2005;.John et *al*, 2006a, b, c).

The bioconversion process of these two categories of biomass involves transformation of complex carbohydrate to monomer sugar as glucose, xylose and arabinose. These simple sugars can be assimilated by the majority of lactic bacteria and then converting these monomers to lactic acid depending on their metabolism. In general, we can summarize the steps of bioconversion of lignocellulosic and starchy materials to the following three steps:

Pre-treatment: This operation facilitates enzymatic attack by increasing surface action. The pre-treatment is especially necessary in the case of cellulose because of the need to remove the blocking protection due for the lignin winding around the cellulose. To remove these obstacles, high pressure methods, high temperature, or extreme acidic or alkaline conditions are generally applied (Sun and Cheng, 2002). However, these conditions lead to a number of inhibitors that can subsequently affect the effectiveness of two-step enzymatic hydrolysis and fermentation (Palmqvist and Hahn-Hägerdal, 2000).

Enzymatic hydrolysis: consists in the decomposition of polymers such as cellulose and starch into easily usable monomeric sugars during the fermentation. Cellulose hydrolysis requires the simultaneous action of β1,4 glucane hydrolases enzymes which are of three types: endocellulases (cut within the chains at the amorphous areas), exoglucanases (cleave by induction and release cellobiose) and exoglycosidases (from cellobiose will give glucose molecules). Furthermore, the enzymatic hydrolysis of starch can be accomplished by the use of two enzymes: The α-amylase (an endohydrolase which specifically cleaves the α1,4 glycosidic bonds by releasing variable size of maltodextrins) and amyloglucosidase (enzyme which hydrolyzes α1,6 and α 1.4 bonds and lead to glucose molecules).

Fermentation: this step allows the conversion of simple sugars to lactic acid with the use of LAB. The choice of bacterial strain depends on their ability to assimilate hexoses or pentoses, the homogeneity of the final product (homo-fermentative or hetero-fermentative) and the fermentation productivity. Examples for strains investigated may be mentioned for their ability fermentative: *Lb. brevis* (Guo *et al*., 2010), *Lb. delbrueckii* NCIM 2025 (John et *al*., 2006), *Lb. lactis* RM 2-24 (Singhvi *et al*., 2010), *Lb. pentosus* ATCC 8041 (Bustos et *al*., 2004).

The production of lactic acid can be performed in two type of fermentation process: hydrolysis and fermentation process separate (SHF) and simultaneous saccharification and fermentation (SSF). The SHF is a process wherein the two steps of enzymatic hydrolysis and fermentation are separate (Abdel-Rahman *et al*, 2011). Although SSF has the advantage that both steps occurring in their optimal conditions (pH and temperature), this process exhibit the disadvantages of the inhibitory effect of the sugars released on hydrolysis yield and the need to add more enzymes to ensure the continuity of hydrolysis (Jeffries and Jin, 2000).

However, the SSF allows reducing the time of LA production by performing the enzymatic hydrolysis and fermentation simultaneously. In addition, the SSF offers the advantage of reducing the inhibitory effect of the released sugars, increasing the productivity, improving the efficiency of hydrolysis and LA production (Hofvendahl and Hahn-Hägerdal, 2000;John et *al*., 2006c; Linko and Ja vanainen, 1996; Sun and Cheng, 2002).

In this study, it was considered the objective of optimizing the LA production under SSF conditions using banana peels and flesh. It particularly seeks to evaluate the performances of SSF compared to SHF using the LAB strain *Lb. pentosus* 239 AH.

MATERIALS AND METHODS

Materials

Raw material

The lignocellulosic and starch material studied in this experiment is banana (peel and flesh). Un-matured banana samples Cavendish (Musa spp.) were purchased from a local fruit market in Menofia Governorate in Egypt. The banana samples were washed and separated into, namely, peel of un-matured banana (UBP) and flesh of un-matured banana (UBF), followed by drying and milling. The milled samples were stored in sealed plastic bags at room temperature.

The composition of this biomass was described in Table 1.

Model strain

Lactobacillus pentosus AH 239 strain was obtained from Technical University of Denmark (DTU) and stored by stab cultures in MRS Broth containing: Peptone (1%), Meat extract (0.8%), Yeast extract (0.4%), Dipotassium hydrogen phosphate (0.2%), Sodium acetate trihydrate (0.5%), Triamonium citrate (0.2%), Magnesium sulphate heptahydrate (0.02%), Manganous sulphate tetrahydrate (0.005%) and Glucose (2%). The seed culture of *Lb. pentosus* was grown at 40°C for 20 h in a 10-mL flask containing MRS Broth.

Enzymes

Enzymes used in this study are from Novozymes A/S:

- α-amylase (NS22176)
- Amylo-glucosidase (NS22180)
- Cellulase mix (NS81016)
- Beta-glucanase (NS82213)

Methods

Biomass chemicals analysis

Dry Matter content determination

Dry Matter contents (DM) of all the samples tested were measured according to the protocol A0001 from Enzyme Lab of DTI (Denmark), in principle by weighing the samples before and after overnight drying at 105 °C in oven.

Ash content determination

Ash contents of samples were measured according to the protocol A0002 from Enzyme Lab of DTI (Denmark), in principle by weighing the samples before and after ashing at 550 °C for two hours in Muffle Furnace.

Carbohydrate characterization

The carbohydrate composition of the samples were determined following the protocol A0003 from Enzyme Lab of DTI (Denmark), in principle of releasing the monomer sugars by two steps acid hydrolysis and quantify the released sugars by HPLC analysis. Samples were first made soluble in 72% (w/w) H_2SO_4 at 30°C for 60 minutes and then hydrolyzed in 4% (w/w) H_2SO_4 at 121°C for 60 minutes. Klason lignin was determined as the ash free residue after hydrolysis.

Fermentation

SHF conditions

Enzymatic hydrolysis of the substrates was conducted in 100 ml flasks containing 100 ml of sterilized milli-Q water with 10% (w/v) of substrate (banana flesh and wet-milled banana peel). The pH of slurries were checked and adjusted to 5.5-6.0. The slurries were then added by 0.1% (v/w-biomass) of α-amylase NS22176 and heated to 95°C for 60 min. Then,0.2% (v/w-biomass) amylo-glucosidase NS22180 was added and the mash was incubated at 60 ° C with magnetic stirring at the speed of 100 rpm for two hours. After that, the pH were checked and adjusted to pH 5. After adding 2.5% (v/w-biomass) cellulases mixture NS81016 and 0.8% (v/w biomass) beta-glucanase NS81223,the slurries were incubated under 45 °C for 48 hours.

The fermentation was performed in a Biostat Q fermentor with a volume of 1 liter. The hydrolyzate was added in a ratio of 10% v/v in 500 ml MRS Broth medium. The incubation was started after strain inoculation (*Lb. Pentosus* AH 239) for a period of 20 hours in the following conditions: pH = 5.5, T = 40 ° C and stirring at 75 rpm.

SSF conditions

SSF tests were carried out in the fermentor biostat Q having a volume of 1 liter. Fermentors containing substrates (10% w/v) and 500 ml of MRS Broth and α-amylase enzyme NS22176 (0.1% v/w-biomass) are autoclaved for 121 ° C for 20 min. After sterilization of the medium, the enzymatic hydrolysis of the starch was activated and performed by adding amylo-glucosidase NS22180 in the ratio of 0.2% enzyme v/w-biomass at 55 ° C and pH of 5.5 for 2 hours.

The culture of *Lb. pentosus* AH 239 was inoculated with adding 2.5% (v/w-biomass) of cellulases mixture NS81016 and 0.8% (v/w biomass) beta-glucanase NS81223. Incubation have remained for 40 hours under temperature of 40 ° C and agitation of 100 rpm. The pH was controlled at 5.5 automatically.

Samples analysis

All samples taken from were measured by a high performance liquid chromatography (HPLC) to quantify lactic acid, glucose, ethanol, formate and acetate. The system used is characterized by a refractive index detector equipped with an Aminex HPX-87H column (Bio-Rad Laboratories Ltd., USA) running at 63°C with 5 mM H_2SO_4 as eluent with a flow rate of 0.6 ml/min.

Calculation

The theoretical Lactic acid production (100% efficiency) is determined by:

- LA theoretical (g/g DM) = 1 x g total glucose/g DM
- The real lactic acid production determined by HPLC and gravimetric analysis can be expressed in the following ways:

1. g LA real/g DM
2. g LA real/g total glucose
3. g LA real/L

The Conversion efficiency equals to:

Conversion (%)=LA real(g/g DM)/LA theoretical (g/g DM)

Table 1: Characterization of unmatured banana (peel and flesh)

	DM%	Ash (%DM)	Lignin (%DM)	Glucose (%DM)	Xylose (%DM)	Arabinose (%DM)
Un-matured banana peel	90.5	20.5	15.0	34.5	9.9	4.0
Un-matured banana flesh	88.7	5.4	3.3	82.2	7.2	0.5

RESULTS AND DISCUSSION

Fermentation SHF

The SHF conditions allow the separation of enzymatic hydrolysis step from the fermentation step. The HPLC results showed that concentrations of glucose released after enzymes application are approximately 3 g/l and 34 g/l respectively for banana peel and banana flesh (see Table 2).

The hydrolysis efficiency observed varies between 52% and 61%. These relatively low yields reflect the inhibitory effect of monosaccharides released on the action of hydrolyzing enzymes. This finding is one of the most important disadvantages of the SHF process.

The fermentation was conducted based on the hydrolyzate obtained (Figure 3 and 4) from the first step. The ratio studied in our case for hydrolyzate addition in medium (MRS Broth) was set at 10% (v/v). Accordingly, and given the efficiency of hydrolysis that recorded relatively low rates, the concentration of glucose in the fermentation medium is quite low, especially for banana peels.

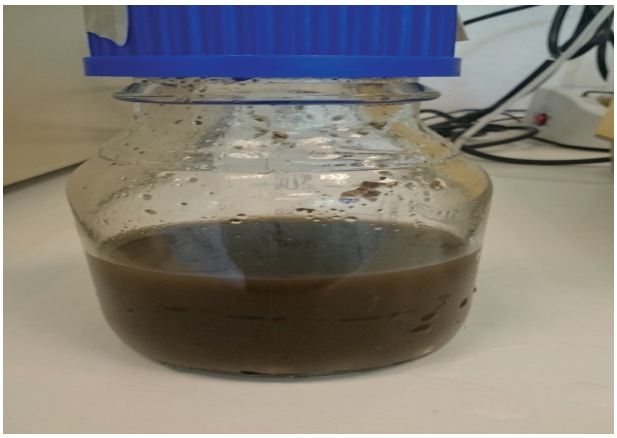

Figure 1: Banana peel hydrolyzed in 100 ml flask

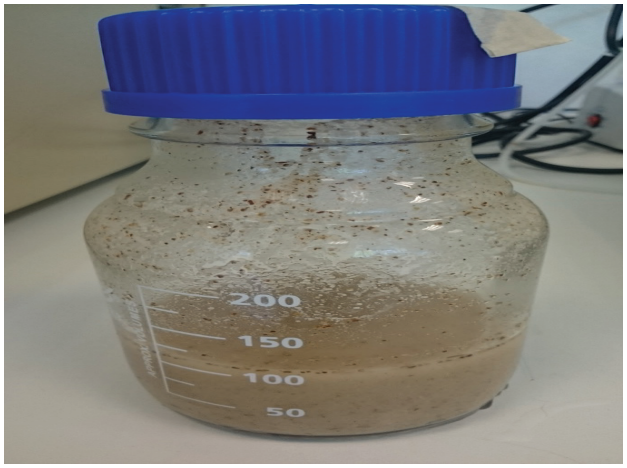

Figure 2:Banana flesh hydrolyzed in 100 ml flask

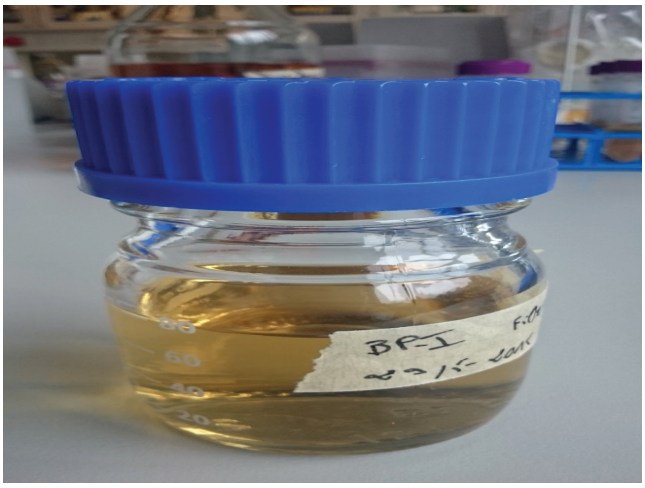

Figure 3: Filtered hydrolyzate of banana peel

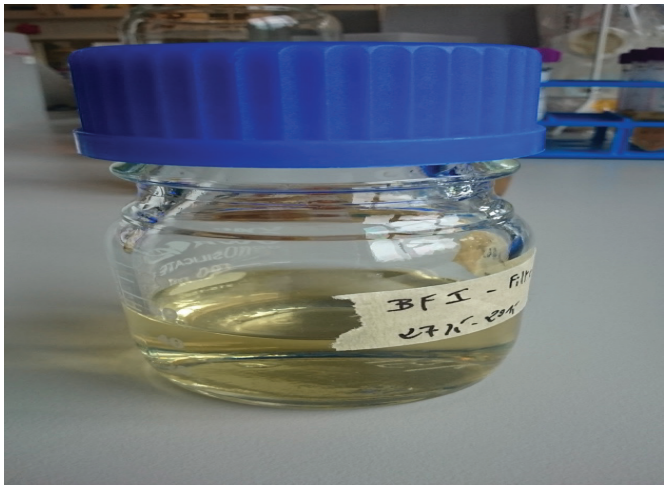

Figure 4:Filtered hydrolyzate of banana flesh

In fact, Figures 5 and 6 shows that all the glucose released by hydrolysis was consumed by the LAB strain designed for LA production. The final concentration of lactic acid was 1.27 g/l banana peel and 2.81 g/l for banana flesh. Based on the theoretical conversion rate which is 1g LA per 1 g glucose, LA concentrations obtained confirm that the strain *Lb. Pentosus* AH 239 has the ability to convert other simple sugars other than glucose such xylose. Indeed, the starting concentrations of glucose were lower than the levels of lactic acid at the end of incubation.

Furthermore, it is noted that the HPLC results showed that the concentrations of ethanol, formate and acetate remained constant along the incubation period to the initial values that reflects that this strain has a homo-fermentative metabolism.

Table 2: Results of enzymatic hydrolysis of unmatured banana (peel and flesh)

Sample	ID	DM%	Glucose (%DM)	Weigh of sample (g)	Volume (ml)	Final concentration (g/l)	Efficiency (%)
Un-matured banana peel	BP I	91%	35%	1,973	120	3,11	61%
	BP II	91%	35%	2,083	120	3,05	56%
Un-matured banana flesh	BF I	89%	82%	10,021	120	35,45	58%
	BF II	89%	82%	10,083	120	31,97	52%

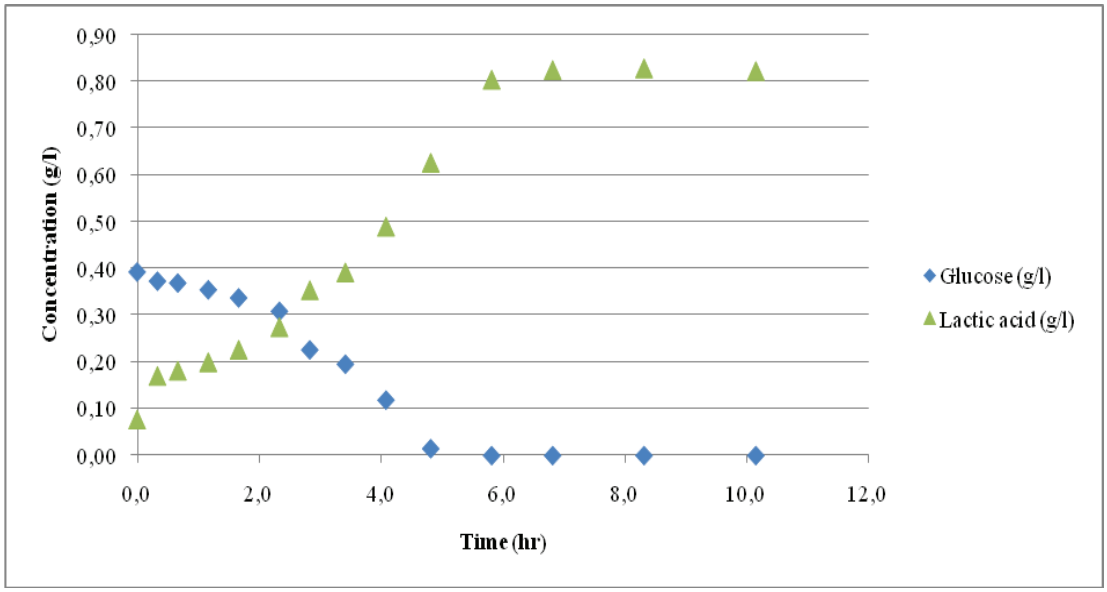

Figure 5: SHF of banana peel hydrolyzed in MRS Broth by Lb. Pentosus AH 239 (pH = 5.5, T= 40°C and 75 rpm)

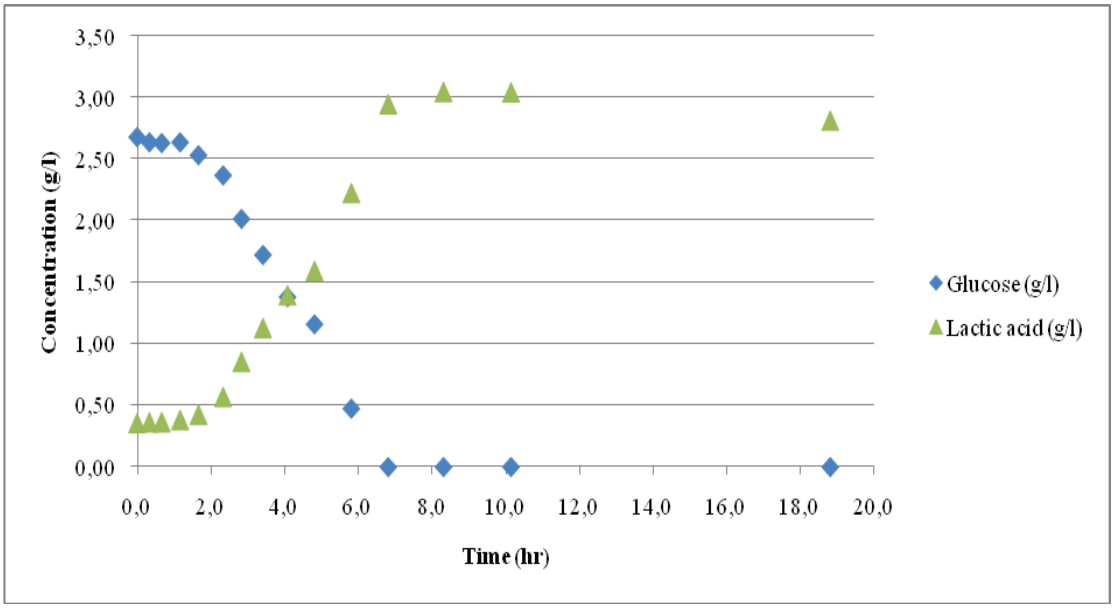

Figure 6: SHF of banana flesh hydrolyzed in MRS Broth by Lb. Pentosus AH 239 (pH = 5.5, T= 40°C and 75 rpm)

Figure 7: SSF using banana peel and flesh by Lb. pentosus AH239 on fermentor Biostat Q – 1L

Table 3: Results of enzymatic hydrolysis of unmatured banana (peel and flesh)

Sample	ID	DM%	Glucose (%DM)	Weigh of sample (g)	Volume (ml)	Final concentration (g/l)	Efficiency (%)
Un-matured banana peel	BP I	91%	35%	12,38	710	4,73	87%
	BP II	91%	35%	12,25	710	4,75	88%
Un-matured banana flesh	BF I	89%	82%	50	520	63,23	90%
	BF II	89%	82%	50	550	54,22	82%

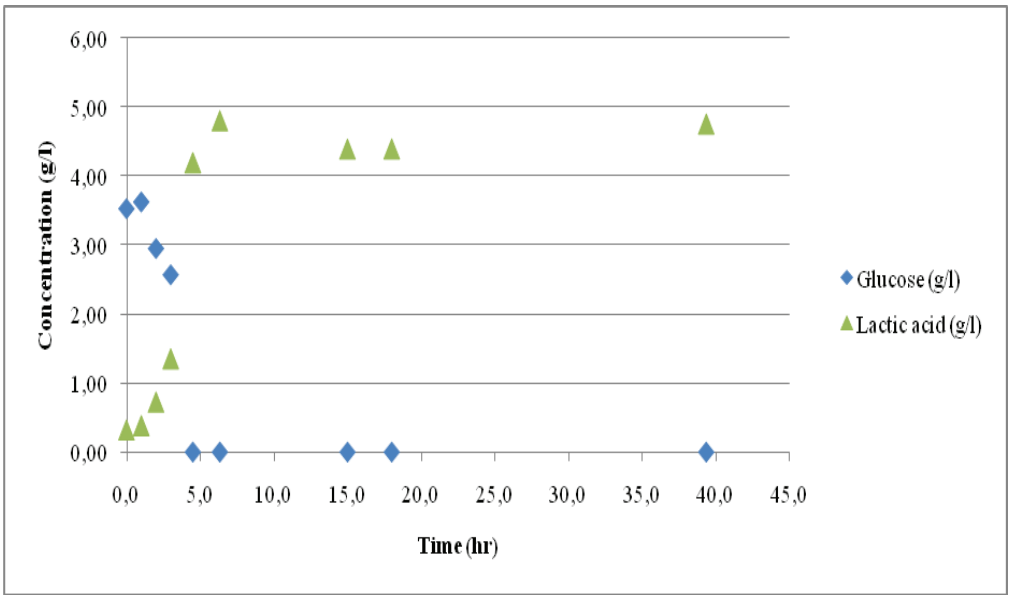

Figure 8: SSF of banana peel hydrolyzed in MRS Broth by Lb. Pentosus AH 239 (pH = 5.5, T= 40°C and 100 rpm)

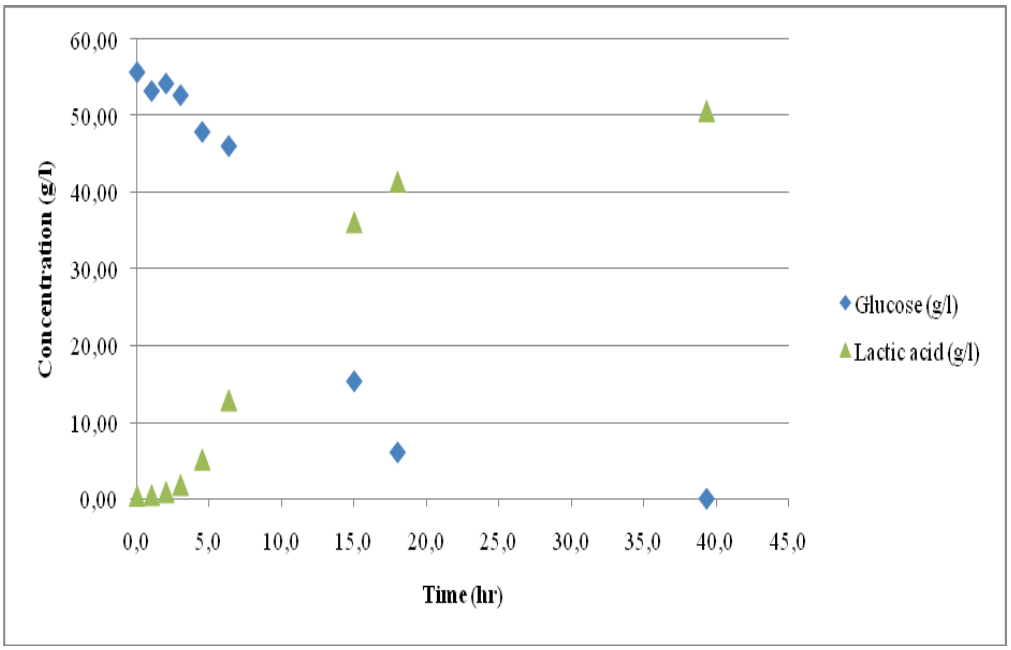

Figure 9: SSF of banana peel hydrolyzed in MRS Broth by Lb. Pentosus AH 239 (pH = 5.5, T= 40°C and 100 rpm)

Fermentation SSF

Under the conditions of SSF, the enzymatic hydrolysis results, which takes place concurrently with the fermentation step, indicate efficiencies that are much higher than that of SHF. Indeed, the recorded hydrolysis yields are between 82% and 90% respectively for bananas peel and flesh (Table 2). These results confirm that the inhibitory effect of glucose on enzyme action is reduced in the case of SSF that give a higher hydrolysis rate.

Figures 8 and 9 show the results of lactic acid production in the conditions of SSF by *Lb. Pentosus* AH 239. According to these figures, we can see that the glucose concentration becomes almost zero after 5 hours of incubation in the case of banana peel, whereas the glucose is completely consumed after 25 hours of incubation in the case of banana flesh. This can be explained by the initial glucose content between these two substrates which is lower in the peels (4 g/l) and higher in the flesh (56 g/l). Thus, the consumption time is longer for banana flesh.

Concerning the production of lactic acid, the final concentrations of LA are reached 4.8 g/l and 50 g/l respectively for banana peels and flesh. The conversion rate calculated based on the theoretical concentration of lactic acid is 130% for bananas peels and 91% for bananas flesh.

The rate of 130% observed for the peels can be explained by the fact that other sugars such as xylose were used by *Lb. Pentosus* during the fermentation. This is confirmed by the composition of banana peel, which contain less glucose and more xylose and arabinose relative to the flesh.

CONCLUSIONS

Using SSF Process for LA production based on lignocellulosic and starchy substrate gives several advantages compared to SHF with two separate stages of hydrolysis and fermentation. Indeed, the results obtained in this study confirm that the efficiency of enzymatic hydrolysis and lactic acid productivity is much better in the case of SSF. These performances are assets that can promote the adoption of this biotechnology to industrial scale for the production of lactic acid with interesting costs.

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