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Agricultural biowaste as resources for fodder yeast additives development

GABRIELA BAHRIM*
**University "Dunărea de Jos" Faculty of Food Science and Engineering, Bioengineering Department, Str.Domnească No. 111, Galaţi, Romania*

Abstract

Currently, the production of SCP (Single Cell Protein) is based on renewable resources and its interest is also kept as a means of conferring value on waste materials. Yeast selected strains included within the Candida genus (C. arboreea, C. tropicalis, C. utilis and others) can use, as a major source of carbon, the sugars from agricultural waste hydrolyse and generate, under aerobic cultivation conditions, high yield coefficients of biomass. The study is important for the biotechnologies that are trying to capitalise some agricultural residues and transform them into SCP.

Keywords**:** *Candida* sp., fodder yeast, agricultural residues, bioremediation.

Introduction

Life is associated with waste production and the exploitation of these materials as renewable resources for bioproduct development could be a major challenge for biotechnology [7].

Agroindustrial residues are generated annually and their use as raw material in technological processes for obtaining products of high added value is gaining interest.

Plant cell walls, the major reservoir of fixed carbon in nature contains three major polymers: cellulose, hemicellulose (noncellulosic polysaccharides including xylans, mannans, and glucans) and lignin (a complex polyphenolic structure) [1,2]. Xylans are found mainly in secondary walls of plants and can represent up to 35% of the total dry weight in certain plants. Xylan is a complex polysaccharide consisting of a backbone of D-1,4-linked xylopyranoside units substituted with acetyl, glucuronosyl, and arabinosyl side chains.

Bioconversion processes have been developed for the utilization of renewable resources to produce useful chemicals and feed stocks. The use of renewable resources and, in particular, of hemicellulosic biomass, has broad environmental implications in today's world. These materials, especially, agroindustrial residues are rich sources of carbohydrates that can be converted by biotechnological means into products of high value. In order to release the sugars from the hemicellulosic fraction, hydrolysis is carried out as the first stage of fermentative processes employing vegetal biomass as the substrate.

However, during hydrolysis, significant amounts of chemical compounds are produced, and for their removal the hydrolysate must be treated before the bioconversion process. After treatment, the hydrolysate can be fermented under selected environmental conditions. Different *Candida* species (*C. maltosa, C. tropicalis*, and *C. utilis*) are currently used in industry for the production of single-cell protein (SCP) and ethanol from steamed hemicellulose [3].

Interest in the production of microbial biomass as food and fodder is intensified nowadays. The most important features resulting from the research on SCP concerned the following topics:

- diversity of the substrates used and their catabolism (renewable substrate);
- breeding and genetic improvement of a great variety of micro-organisms.

Enzymatic hydrolysis of hemicellulosic materials constituted the object of some optimisation studies and can lead to high yields of xylose. This makes obtaining microbial protein rich biomass from vegetal wastes a very productive process.

A large number of studies describe the potential of yeasts included within the *Candida* genus (*C. arboreea, C. tropicalis, C. utilis* and others) to metabolize the xylose in aerobiosis with protein-rich biomass accumulations. This is a process of manifold industrial applications to obtain microbial proteins.

Previous investigations have already shown the potential of some selected *Candida* strains to grow on media with xylose as the only source of carbon [4, 5, 6].

Starting from these results, the current study intends to highlight the potential of selected yeasts to develop on simple media, where the carbon sources are exclusively represented by simple glucides obtained by chemical and enzymatic hydrolysis of some agricultural wastes.

Material and Methods

Micro-organisms, media and cultural conditions. The experiments made use of five yeast strains from the *Candida* species: *C. robusta* MIUG 3.2, *C. robusta* MIUG 3.3, *C. tropicalis* MIUG 3.4, *C. utilis* MIUG 3.5, from Galati University Collection of Microorganims (acronym MIUG), and a new strain *Candida antartica* isolated from polar soils - East Antarctica, the Lasermann Hills Progress Station region (coded 1P).

The basic media for cultivation were made by acid or enzyme hydrolyses of some vegetal wastes: wheat and barley straws, corn cobs, sawdust and milling residues (remnants of corn, soy, barley and oat) used as fodder.

After the hydrolysis and separation of solid fractions, to obtain fermentative media, nitrogen and mineral sources (0.95% complex manure, 0.7% ammonia sulphide and 0.06% magnesium sulphide) were added to the extract.

The efficiency of using liquid media to grow yeasts was evaluated by determining the dry biomass yield obtained by inoculating yeasts cells in sterile liquid medium (10^6 cells·cm^{-3} fermentative medium) with pH=5.5 and submerged cultivation on a rotary shaker at 230 rot·min^{-1}, during 72 hrs, at 25ºC.

Analytical methods. The cell biomass concentration was measured gravimetrically after centrifuging 10 cm^3 of sample, washing the cells with distilled water and then drying them at 105°C until a constant weight was achieved.

The Shaffer-Somogyi method was used to determine the reducing sugars concentration from the hydrolysed, cultural media or in the supernatant after biomass separation.

Results and discussions

To obtain hydrolyses different treatment schemes were applied depending on the vegetal wastes composition (Figure 1, Figure 2).

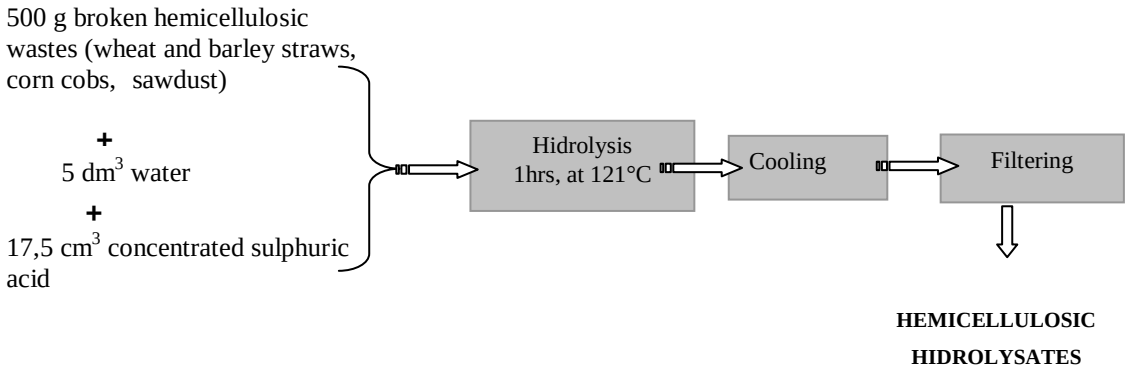

Figure 1. Acid hydrolysis of the hemicellulosic wastes

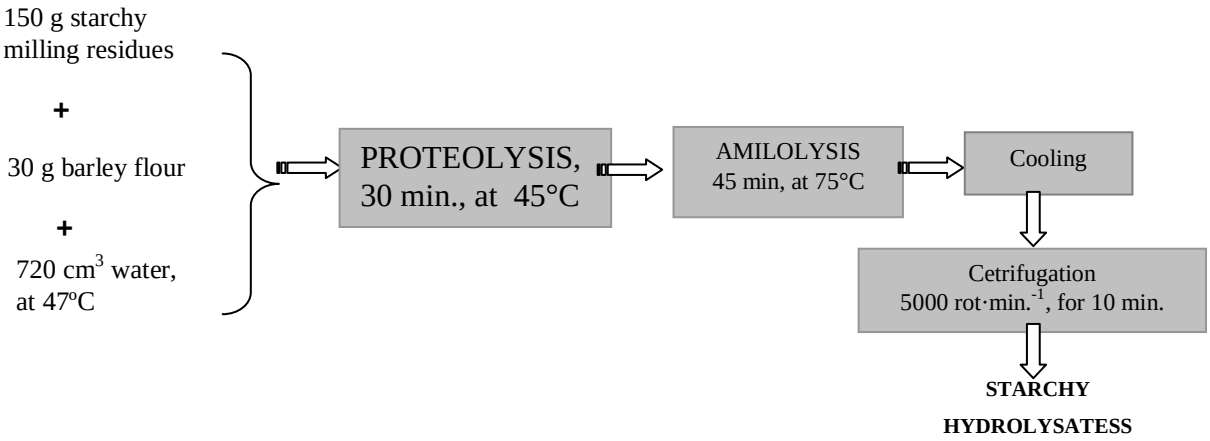

Figure 2. Enzymatic hydrolysis of the starchy milling resıdues

Evaluation of yeast growth on hemicellulosic hydrolysate

The hydrolysis of wheat and barley straws, corn cobs, sawdust, was chemically performed at high temperature (121ºC). The content of reducing glucides from the hydrolysed thus obtained was determined by the Shaffer-Somogyi method. The results are given in **Table 1**.

From the results achieved, it is found a qualitative and quantitative variation of the fermentative glucide spectrum depending on the raw material subject of hydrolysis. The hydrolysed obtained from corn cobs is characterized by the largest amount of glucose and pentose while the sawdust features the smallest content of reducing glucides.

The yeasts growth potential was evaluated by overall dry weight of biomass with respect to 100 cm^3 liquid (**Figure 3**).

Table 1. The content of reducing glucides from the hemicellulosic acid hydrolysate

Vegetal wastes	Reducing glucides, g %			
	Glucose	Arabinose	Xylose	Total
Wheat straws	0.509	0.639	0.645	1.793
Barley straws	0.661	0.799	0.807	2.264
Corn cobs	0.769	0.780	0.993	2.569
Sawdust	0.276	0.389	0.403	1.068

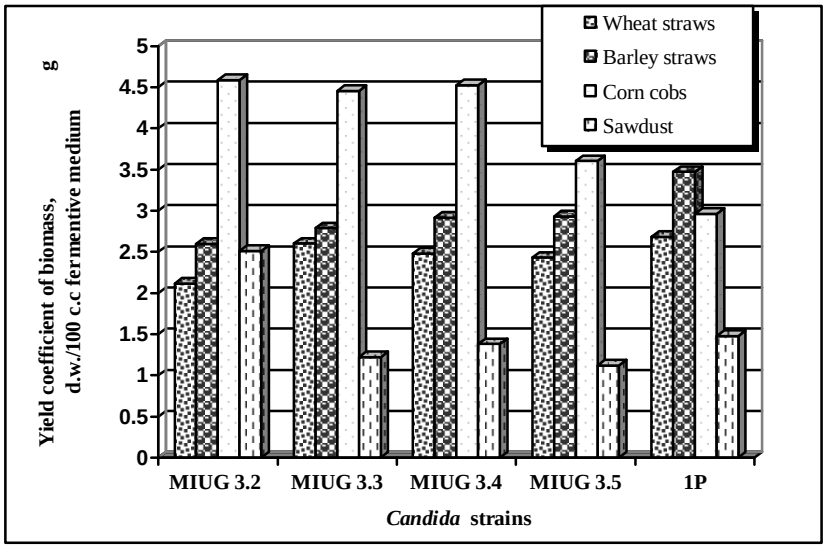

Figure 3. *Candida* sp.strains potential of growing on hemicellulosic hydrolysates

From the data presented in **Figure 3** the following conclusions are drawn:

Biomass yield coefficients (Y_x) are affected by the nature and concentration of carbon source in fermentative medium.

- Yeast behaviour is differentiated by growing on hydrolysate from various vegetal sources.
- The yeasts *C. arborea*, *C. tropicalis* and *C.utilis* are more suitable to grow on corn cobs hydrolysate closely followed by those obtained from barley straws.
- Strain *C. antarctica* 1P isolated from polar soil develops best on barley straws and corn cobs hydrolysed.
- Except for the strain *C. robusta* MIUG 3.2, which grew on hydrolysed sawdust similar to that made from corn cobs, all the other types of strains featured a much lower yield.

Evaluation of yeast growth on starchy milling residues

For the release of soluble substances, easy to assimilate by the yeast cells, the starchy residues (remnants of corn, soy, barley and oat) have been hydrolysed by acid and enzymes. Due to the complex composition of the extract, it was rather difficult to characterize the content of the hydrolysed in carbon and nitrogen compounds.

When the mixture has undergone a chemical hydrolysis similar to that of the hemicellulosic wastes, the yield coefficient of biomass varied as presented in **Figure 4**.

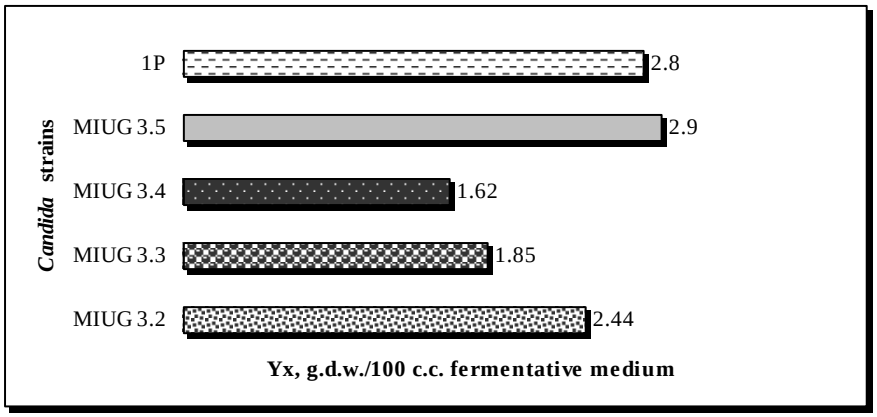

Figure 4. Biomass accumulation by cultivation on starchy residues hydrolyses

Under these growth conditions, the best yield coefficient of biomass was achieved by the strains *C. utilis* MIUG 3.5, *C. antarctica* 1P and *C. robusta* MIUG 3.2. Except for the strain from polar soils, the biomass yield decreased for all the other yeasts as compared with the best variant of the previous experiment represented by the basic medium on hydrolysed corn cobs.

Taking into account that the yeasts tested do not produce extra cellular amylases and proteases capable to provide substrate hydrolysis, for a better use of the raw material, an enzymatic hydrolysis scheme was designed to use fine malt flour as source of enzymes. The hydrolysed element was obtained according to similar principles applied in brewing at ratio malt flour: starchy mixture 1 : 5.

The results obtained are given in **Figure 5**. After 5 days of submerged growing, at 25 ºC and 230 rot/min on liquid medium based on vegetal extract, the best yield coefficient of biomass was achieved with increments within 1.5 and 2.6 times as compared with the best previous variants, namely the hydrolysed corn cobs acid.

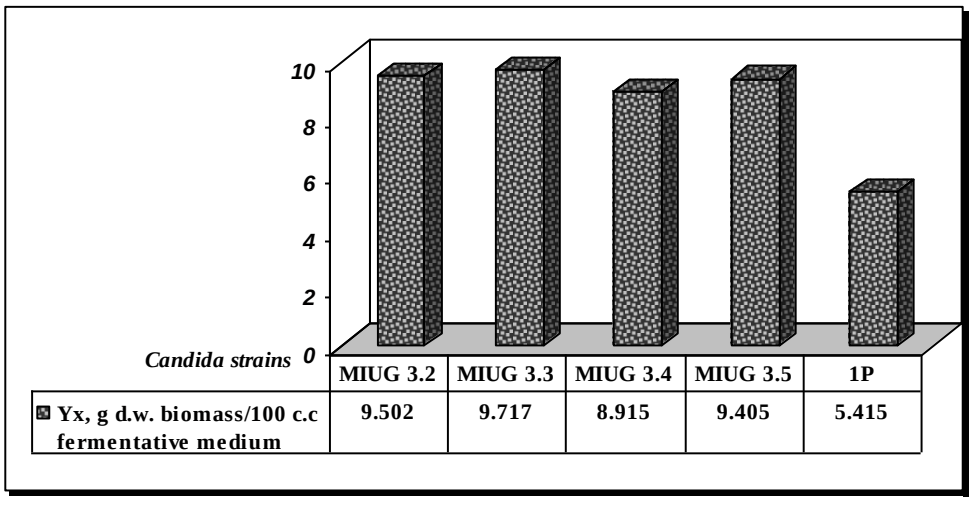

Figure 5. Potential of the *Candida* strains to grow on liquid medium based on enzymatically hydrolysed starchy milling residues mixtures

In this case too, for equal inoculum concentrations ($A_{600\ nm} \cong 1.04$), higher yield coefficients of biomass can be reached with *C. robusta* MIUG 3.2, *C. robusta* MIUG 3.3 and *C.utilis* MIUG 3.5 strains. With *C. antarctica* 1P, however, the previous performance is no longer present. As a matter of fact, it was previously shown that this yeast is able to develop quite well on xylose media as major carbon source [5].

Conclusions

The current study highlighted the availability of some selected *Candida* strains to grow on hydrolysed vegetal wastes, without any additions of carbon or nitrogen organic sources.

The study is important for the disposal of vegetal and hemicellulosic wastes for the purpose of obtaining protein-rich biomass with wide applications in development of industrial biotechnologies and environment protection.

Preliminary results have made it possible to point out to a number of trends, such as:

- Optimisation of hydrolysis processes to increase the concentration of easily assimilated compounds.
- Evaluation of the main kinetic parameters, which characterize the yeasts growth and their approach in terms of physical/chemical factors affecting the yeast growth and biomass accumulation.
- Evaluation and characterization of the biomass protein content.
- The importance of studying each type of waste related to the yield coefficient of biomass and protein content.
- The optimisation of the cultivation by improving the fermentative medium composition with new sources without affecting the economic efficiency of the process.
- For a better use of the fermented medium as fodder based on saccharifyed starch mixture, without losses of substrate, the adoption of the solid-state fermentation seems the best choice. Recent investigations have shown good ability for the yeasts to grow in solid-state fermentation system on enzymatically hydrolysed starchy residues mixtures.

In the quest for new sources of energy and food, given the demographic boom, the possibility of converting vegetal residues into fuel or proteins represents a priority on the agenda of many companies and organizations. An additional advantage of this approach is that an ecological disposal of these wastes is also assured.

Acknowledgements

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