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Research Article

Screening of Some Culture Media Compositions for Obtaining the Optimal Yeast Strains Development on Different Natural Sources of Wood Wastes (Wood Hydrolyzed)

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Abstract

The main purpose of our work was the superior use of the paper and cellulose industry's wastes by using them as substrate in an optimized culture media formula for the yeasts strains development.

The best results were obtained on culture media with 35% wood hydrolyzed and also with well determined concentrations of sugar beet molasses and mineral supplements.

The yeasts growth were verified by optical density (determination meaning the cells number which are present in a well determined liquid volume), the reducing sugar's consumption and by the Ph dynamics. The results obtained with these yeasts strains selected from different natural sources and named D4 and D5, were compared with those obtained by using identified yeasts strains kept in the microbial collection of our institute, such as *Candida robusta* and *Candida tropicalis.*

Our conclusion indicates that different types of hydrolyzed wood wastes can be recycled by microbiological ways, so that by so called "Green technologies" which seems to act friendly to our environment, while one of our main tasks was to use materials and technologies as natural as possible.

Keywords: Yeasts; Hydrolyzed Wood; Media Composition

Abbreviations

D1- D24: Indicators For The New Yeast Strains, Isolated From Dried Beech Wood Chips And Verified On Culture Media With Hydrolyzed Wood Wastes, Before Their Identification; CRS: Abbreviation For *Candida Robusta*; CT: Abbreviation For *Candida Tropicalis*; 1A, 2B, 3C: Indicators For Different Culture Media Variants, Tested For Obtaining The Best Yeast Biomass Development.

Introduction

There are a lot of studies around the world carried out in order to obtain better wood hydrolysates starting from industrial or agricultural wood wastes, who can then be used in industry, for Ethanol or fodder yeasts production or in construction.

Some of these studies were oriented to study only the improvement of hydrolysis parameters [1-5]. without involving the microbial additional activity during the process, mostly while the conversion starts from some preparations already pretreated.

On the other hand, there are many research actions that take into consideration the support of some microbial strains, such as the fungi Phanerochete chrysosporium, Trichoderma reesei, Aspergillus niger and the bacteria Azotobacter chroococcum MTCC 3853 and Bacillus cereus MTCC 4079 [6], heterotrophic bacteria, fungi and actinomycetes [7], ligninolytic, xylanolytic fungi and cellulolytic bacteria isolated from soil (8), a microbial consortium containing Proteobacteria, Paenibacillus and Pseudomonas [9], Paenibacillus lautus strain BHU3 [10], Streptomyces flavogriseus AE64X and AE63X – isolated from Eucalyptus camaldulensis and Populus nigra producing cellulase and xylanase [11], Aspergillus tubingensis NKBP-55 [12], a mixed culture of two yeast strains, MK-157 and MK-118 [13], Lichtheimia ramosa [14] or a microbial enzyme cocktails for the conversion of polysaccharides of plant origin into fermentable sugars [15].

This paper presents a set of our studies concerning the conversion of some paper and cellulose industry's wastes by selecting a proper culture media composition, requested for the further development of some useful yeast strains.

Materials and Methods

The yeast strains isolated from industrial hydrolyzed wood wastes and presenting a better development after our first screening realized on solid media (D4 and D5) were then tested on more variants of fermentation media.

The results obtained with these yeasts strains selected from different natural sources were compared with those obtained by using identified yeasts strains kept in the microbial collection of our institute, such as Candida robusta and Candida tropicalis.

The best results were considered those with a higher consumption of the reducing sugar and a higher level of the optical density after 48 hours of the bioprocess.

The selected fermentation media (named 1A) contained:

- KH_2PO_4 2 g/L
- $(NH_4)_2SO_4$ 2 g/L,
- KCl 1 g/L
- sugar beet molasses (25% reducing sugar) 8 g/L,
- Mg SO_4, 0,5 g/L
- hydrolyzed wood 350 mL,
- 0,1 mL of each 10% solution of $ZnCl_2$, $MnSO_4$ and $FeSO_4$
- distilled water add to 1 L.

The flasks were sterilized for 30 minutes at 110 °C each of them containing 100 or 50 mL fermentation media with pH 4,5.

Other tested variants of the fermentation media (named 2B and 3C) were realized with the same initial composition, but by supplementing the wood hydrolyzed with a higher concentration of molasses or with a higher concentration of salts.

The yeasts strains isolated from different natural sources were marked at the beginning only with indicators, such as D4 and D5 and their activity was compared with that of different yeasts strains previously identified and included in our institute's microbial collection.

By using this method and some other characters, our selected yeasts strains could belong mostly to the species *Candida robusta* and *Saccharomyces cerevisiae.*

Results and Discussions

Media variant's Name	1A	2B	3C
KH_2PO_4	0	0	2 g
$(NH_4)_3PO_4$	2 g	4 g	0
$(NH_4)_2SO_4$	2 g	4 g	2 g
KCl	1 g	2 g	1 g
sugar beet molasses (25 % reducing sugar)	8 g	16 g	8 g
Mg SO_4	0,5 g	0,5 g	0,5 g
$ZnCl_2$	0,1 mL from 10 % solution	0,1 mL from 10% solution	0,1 mL from 10% solution
$MnSO_4$	0,1 mL from 10 % solution	0,1 mL from 10% solution	0,1 mL from 10% solution
$FeSO_4$	0,1 mL from 10 % solution	0,1 mL from 10% solution	0,1 mL from 10% solution
Wood hydrolyzed	350 mL	350 mL	350 mL
Current water	Ad 1 L	Ad 1 L	Ad 1 L
pH (corrected with 40 % sol. NaOH	4.5	4.5	4.5

Table 1: Composition of the fermentation media (total reducing sugar of wood hydrolyzed 24 g/L).

Strain	Media variant	0 h			24 h			48 h		
		pH	Sugar %	DO (optical density)	pH	Sugar %	DO (optical density)	pH	Sugar %	DO (optical density)
CRS	1A	4,5	0,5	7,5	4,5	1,8	12,5	4,2	0,599	8,25
CT	1A	4,5	0,3	5,6	4,5	2,0	12,5	4.0	0,909	2,25
D4	1A	4,5	1,6	6,8	4,5	2.0	11,8	4,2	0,599	7,37
D5	1A	4,5	1,4	9,3	4.2	2,4	19,37	4,2	0,444	6,62

Table 2: Yeasts development on the "1 A variant" culture media.

The fermentation Indicators:

Inoculum with pH 5,0 for all yeast strains, Inoculum age- 24 h, 6 ml inoculum for 100 ml media.

Strain	Media variant	0 h			24 h			48 h		
		pH	Sugar %	DO (optical density)	pH	Sugar %	DO (optical density)	pH	Sugar %	DO (optical density)
CRS	2B	4,5	3,0	10,6	4,5	0,6	12,0	4,2	0,537	5,5
CT	2B	4,5	1,5	7,5	4,2	2,0	18,87	4.0	0,909	8,87
D4	2B	4,5	3,2	7,5	4,5	0,6	9,3	4,2	0,506	9,0
D5	2B	4,5	1,6	10,7	4,2	1,8	18,12	4,2	0,413	6,75

Table 3: Yeasts development on the "2 B variant" culture media.

The fermentation Indicators:

Inoculum with pH 5,0 for all yeast strains, Inoculum age- 24 h, 6 ml inoculum for 100 ml media.

Strain	Media variant	0 h			24 h			48 h		
		pH	Sugar %	DO (optical density)	pH	Sugar %	DO (optical density)	pH	Sugar %	DO (optical density)
CRS	3C	4,5	1,6	10,6	4,2	0,5	11,2	4,2	0,909	8,12
CT	3C	4,5	1,5	8,1	4,2	2,2	16,2	4.0	0,940	1,88
D4	3C	4,5	1,4	6,8	4,5	1,4	21,87	4,2	0,384	6,75
D5	3C	4,5	1,6	6,8	4,5	0,5	9,3	4,2	0,351	7,25

Table 4: Yeasts development on the "3 C variant" culture media.

The fermentation Indicators:

Inoculum with pH 5,0 for all yeast strains, Inoculum age- 24 h, 6 ml inoculum for 100 ml media.

Conclusions

The comparison of the new isolated yeasts strains development with the other ones (taken from authorized microbial collections) and some other methods usually applied for yeasts identification allowed us to found that the new isolated strains belong to the species *Candida robusta* and *Saccharomyces cerevisiae*. Different types of hydrolyzed wood wastes can be recycled by microbiological ways, so that by so called "Green technologies" which seems to act friendly to our environment, while one of our main issue was to use the local materials (mostly Pine wood and Beech wood) and technologies as natural as possible.

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