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Research Article

Emphasizing the Efficiency of a Cellulolytic Preparation on the Hydrolyzed Wood Wastes

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Abstract

According to the literature data, the cellulose, the most abundant organic molecule on Earth, is found mainly as a structural component of plants and algal cell walls, it is also produced by some animals such as tunicates, and it can be produced also by several bacteria, by a natural pathway or by biotechnology. Due to this situation, there are many studies focused on the use of this kind of renewable source and having as their issues the preparation of different valuable products, such as sugars, ethanol. different chemicals, the whole yeasts biomass valorization with agricultural applications or for biofuels development.

Our issue of this present study was to verify if we can avoid the microbial additional activity during the process, mostly while the conversion starts from some materials already pretreated, such as the paper and cellulose industry's wastes, by using them as substrate in an optimized culture media formula, for the further yeasts strains development.

Keywords: Yeasts; Wood; Cellulases; Hydrolyzed; Wastes

Abbreviations

CA: Candida robusta; D 21, D 25: Indicators for the New Yeast Strains of Saccharomyces cerevisiae

Introduction

According to the literature data, the cellulose, the most abundant organic molecule on Earth, is found mainly as a structural component of plants and algal cell walls, it is also produced by some animals such as tunicates, and it can be produced also by several bacteria, by a natural pathway or by biotechnology [3,12]. Due to this situation, there are many studies focused on the use of this kind of renewable source and having as their issues the preparation of different valuable products, such as sugars [3,7,8,10], ethanol [4,9,14], different chemicals [10,11,15], the whole yeasts biomass valorization with agricultural applications [2,13] or for biofuels development [1,4,5,6,13,15].

The usually applied methods in order to realize the hydrolysis of the lignocellulosic substrates until fermentable sugars are involving some first steps of chemical and hydrothermal pretreatment, followed sometimes by an enzymatic hydrolysis [3,10]. However these biotechnological pathways must be established according to many factors, such as the geographical origin of the raw material, the nature of the substrate (directly woody biomass, industrial wastes or agricultural residues) the nature of the enzymatic complex (depending on the applied microbial strains) and the final task of the studies able to ensure the economic viability of bioconversion [4,10].

Our issue of this present study was to verify if we can avoid the microbial additional activity during the process, mostly while the conversion starts from some materials already pretreated, such as the paper and cellulose industry's wastes, by using them as substrate in an optimized culture media formula, for the further yeasts strains development.

Materials and Methods

The samples used as researches' substrate were taken from the paper and cellulose industry's wastes and they were represented by dried beech wood chips, wet beech wood chips and wet and fine beech wood chips, containing 24 - 25 g/L reducing sugar all of them.

The optimal results concerning the culture media composition were obtained with 35% vegetable wastes hydrolyzed, sugar beet molasses, mineral supplements and a pH correction at 5,0 - 6,0 and they were presented within a previous paper [16].

The enzymatic preparation used for our researches was represented by a commercial product.

The yeasts strains isolated during our first studies from different natural sources were marked at the beginning only with indicators, such as D1-D25, CA-C3, etc. depending on their harvest area, and their activity was compared with that of different yeasts strains previously identified and included in the microbial collection kept inside our institute.

By using this method and some other characters, our previously selected yeasts strains could belong mostly to the species Candida robusta and Saccharomyces cerevisiae [16].

The yeasts growth were verified by the optical density evolution (determination meaning the cells number which are present in a well determined liquid volume), the reducing sugar's consumption, the Ph dynamics and the yeasts dry weight increase.

Results and Discussion

A set of 4 series of fermentation processes were carried out at a laboratory scale, by using 3 previously selected yeast strains (CA, D-21, D-25) and were verified on a process duration of 66 hours.

The obtained results are presented by the table 1-3, each of them being realized for all 3 yeasts strains and according to the evolution of a fermentation parameter (pH, sugar concentration and optical density).

Figure 1: The evolution of the yeasts dry weight.

The final levels of the yeast dry weight were presented also separately by the figure 1.

Our obtained results indicate that the enzymatic supplement containing cellulases did not have an important role related to the yeasts growth, which means that in the case of using as substrate some industrial wood wastes, the chemical and hydrothermal pretreatment involved during the usual industrial production and supplemented with other soft pretreatment methods used at a laboratory scale were enough in order to hydrolyze the lignocellulosic material until significant levels of reducing sugars concentration and yeast dry weight.

No flask	Strain	Culture media	0h	24h		42h		66h	
			pH	pH	pH correction with H_2SO_4	pH	pH after correction	pH	Dry weight g/L.
1	CA	E (with enzyme and pH correction)	6,0	7,5	With 1 drop	7,5	5,0	5,0	8,80
2	CA	Without E	6,0	7,0	0	8,0	No pH correction	8,0	8,82
3	CA-2	Without E	6,0	7,0	0	8,0	No pH correction	8,0	8,81
4	CA-3	Without E	6,0	7,0	0	8,0	No pH correction	8,0	8,82
5	D21	E (with enzyme and pH correction)	6,0	7,3	With 1 drop	7,5	6,0	6,0	8,52
6	D21	Without E	6,0	7,0	0	8,0	No pH correction	6.0	8,18

7	D21-2	Without E	6,0	7,0	0	8,0	No pH correction	6.0	8,20
8	D21-3	Without E	6,0	7,0	0	8,0	No pH correction	6.0	8,23
9	D-25	E (with enzyme and pH correction)	6,0	7,3	With 1 drop	7,5	5,0	5.0	9,71
10	D-25	Without E	6,0	7,0	0	8,0	No pH correction	5,0	10,75
11	D-25-2	Without E	6,0	7,0	0	8,0	No pH correction	5,0	10,77
12	D-25-3	Without E	6,0	7,0	0	8,0	No pH correction	5,0	10,74

Table 1: The pH evolution in g case of the additional enzymic preparation on the yeasts development on wood hydrolyzed.

The fermentation Indicators:

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

Initial sugar level: 0,847% at media without enzyme, 1,126% for media supplemented with enzyme.

Initial dry weight: 1,645 g/L.

No flask	Strain	Culture media	0h	24h		42h	66h	
			Sugar %	Sugar %	pH correction with H_2SO_4	Sugar %	Sugar %	Dry weight g/L.
1	CA	E (with enzyme and pH correction)	0,8	0,21	With 1 drop	0,21	0,18	8,80
2	CA	Without E	0,3	0,27	0	0,27	0,24	8,82
3	CA-2	Without E	0,3	0,27	0	0,27	0,24	8,81
4	CA-3	Without E	0,3	0,27	0	0,27	0,24	8,82
5	D21	E (with enzyme and pH correction)	0,8	0,12	With 1 drop	0,12	0,09	8,52
6	D21	Without E	0,3	0,21	0	0,21	0,21	8,18
7	D21-2	Without E	0,3	0,21	0	0,21	0,21	8,20
8	D21-3	Without E	0,3	0,21	0	0,21	0,21	8,23
9	D-25	E (with enzyme and pH correction)	0,8	0,21	With 1 drop	0,18	0,15	9,71
10	D-25	Without E	0,3	0,27	0	0,15	0,12	10,75
11	D-25-2	Without E	0,3	0,27	0	0,15	0,12	10,77
12	D-25-3	Without E	0,3	0,27	0	0,15	0,12	10,74

Table 2: The evolution of sugar concentration in the case of the additional enzymic preparation on the yeasts development on wood hydrolyzed.

The fermentation Indicators:

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

Initial sugar level: 0,847% at media without enzyme, 1,126% for media supplemented with enzyme.

Initial dry weight: 1,645 g/L.

No flask	Strain	Culture media	0h	24h		42h	66h	
			DO	DO	pH correction with H_2SO_4	DO	DO	Dry weight g/L.
1	CA	E (with enzyme and pH correction)	2,3	9,25	With 1 drop	9.25	9,5	8,80
2	CA	Without E	1,37	9,37	0	9.25	9,5	8,82
3	CA-2	Without E	1,37	9,37	0	9.25	9,5	8,81
4	CA-3	Without E	1,37	9,37	0	9.25	9,5	8,82
5	D21	E (with enzyme and pH correction)	2,37	9,37	With 1 drop	9,37	9,5	8,52
6	D21	Without E	1,3	11,0	0	9,87	9,4	8,18
7	D21-2	Without E	1,3	11,0	0	9,87	9,4	8,20
8	D21-3	Without E	1,3	11,0	0	9,87	9,4	8,23
9	D-25	E (with enzyme and pH correction)	2,3	10,25	With 1 drop	8,75	11,0	9,71
10	D-25	Without E	1,3	8,0	0	10,7	10,25	10,75
11	D-25-2	Without E	1,3	8,0	0	10,7	10,25	10,77
12	D-25-3	Without E	1,3	8,0	0	10,7	10,25	10,74

Table 3: The DO (Optical density) evolution in the case of the additional enzymic preparation on the yeasts development on wood l.

The fermentation Indicators:

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

Initial sugar level: 0,847% at media without enzyme, 1,126% for media supplemented with enzyme.

Initial dry weight: 1,645 g/L.

Conclusion

- The main purpose of our work was the valorization of the paper and cellulose industry's wastes by using them as substrate for the yeasts strains development.
- This paper presents only the results obtained by using the yeasts strains new selected by our previously researches.
- The enzymatic supplement containing cellulases did not have an important role related to the yeasts growth, which means that in the case of using as substrate some industrial wood wastes, the thermo- chemical pretreatment involved during the usual industrial production and some other soft pretreatment methods used at a laboratory scale were enough in order to hydrolyze the lignocellulosic material until a significant reducing sugar concentration.
- 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.
- The obtained yeasts biomass can be further used as fodder yeasts, for Ethanol obtaining, for biofuels production or as a component of different nutritional supplements.

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