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Fungal protein enrichment of residual liquor from a sugar-cane waste *Saccharum officinarum.*

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RESUMEN. Enriquecimiento proteíco fúngico del licor residual de la caña de azúcar. Se estudió el uso del licor residual del bagazo de caña de azúcar *(Saccharum officinarum)* como fuente de proteínas. Se obtuvo el bagazo en las fábricas de azúcar de la Región y el hongo utilizado fué el *Aspergillus niger* IZ-9. Se sometió el licor a análisis físico-quimicos y en el producto final se determinaron el nitrógeno total y los aminoácidos. Para el crecimiento, mantenimiento y almacenaje del cultivo se utilizó el medio sintético de Pontecorvo modificado por Christias et al. La significancia estadística de los resultados fué determinada por el análisis de variancia y los valores promedios comparados por la Prueba de Tukey. Se observó que el mencionado licor puede ser utilizado como medio de cultivo para la producción de biomasa a partir del *A. niger* IZ-9, siendo la mayor producción obtenida cúando 100% del medio sintético fué reemplazado por el licor tratado balanceado con sales y carbono y pH ajustado a 5,0. Los niveles más elevados de aminoácidos fueron encontrados en el medio que contenía 40% del licor tratado con balanceamiento de sales y carbono y pH 5,0. A excepción de la metionina, aminoácido limitante, la proteina del *Aspergillus niger* parece proveer todos los aminoácidos esenciales a la dieta y por lo tanto puede ser considerada de buen valor biológico.

SUMMARY. The use of residual liquor obtained from cane-thrash, a sugar-cane waste *Saccharum officinarum*, lineo, as a protein source was studied. The cane-thrash was avaiable in local sugar-mills and the fungus utilized, the *Aspergilus niger* IZ-9, was provided by the University of São Paulo and derived from a strain un der the number NRRL-337. The liquor was previously hydrolyzed and physicochemical analyses were performed. Total nitrogen and amino acid contents were measured in the final product. The synthetic medium of Pontecorvo et al (6) modified by Christias et al (7), was used for growth, maintenance and storage of the strain. The analysis of variance was used to determine the statistical significance of all results. Mean values were compared by the Tukey's test. According to our data, the residual liquor from the prehydrolyzed cane-thrash can be used as a medium for biomass production from the *A. niger* IZ-9 and that the highest biomas production was obtained when the synthetic medium was substituted for 100% treated liquor added with balanced salts and carbon, and pH adjusted to 5,0. The highest amino acid values were detected in the *A. Niger* grown in the medium containing 40% treated liquor added with balanced salts and carbon, with pH adjustment to 5.0 with exception of methionine, this fungal protein seems to provide all essential amino acids in a diet, and could certainly be considered of good biological value.

INTRODUCTION

The utilization of waste materials as a mean of alleviating world food shortage and energy reserves and, at the same time, contributing to the solution of environmental pollution problems caused by these wastes is being examined. Environmental pollution is closely associated with industrialization and a proportion between pollution and economic growth rates seems to occur. This is atributed to the inadequate disposal practices for wastes by industries. Only 5% of the annual production of nutrients on land is used directly as food by man; the rest (95%) can be utilized by bioconversion systems making use of microorganisms for single cell protein (SCP) production(1). Fungi seem superior to bacteria and actinomycetes for producing biomas with less denaturated proteins. Filamentous fungi, especually *Aspergillus niger*, sp *Chaetomium* sp and *Trichoderma* sp, are the most used to convert industrial wastes into fungal protein. (2). A variety of agroindustrial by-products and cellulose wastes is avaliable in Brazil. Sugar-cane, for example, which results from production of carburetted alcohol. It is estimated that about 33% of the bagasse surplus, except that used for distillery furnaces, is avaliable for alternative uses. (3). The purpose of this study was to investigate the potential utilization of a sugar-cane waste (a liquor resulting from sugar-cane processing) for protein production to alleviate nutritional problems and contribute to pollution control.

MATERIAL AND METHODS

The liquor was obtained from the sugar-cane bagasse *(Saccharum officinarum, Lineo)*. The bagasse, available in local sugar-mills, is the squashed chopped fibre left

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behind after sugar is extracted from the cane and initially contains 50% water and 3-5% sugar.

Aspergillus niger IZ-09, provided by the Zimotecnic Institute of the Department of Rural Technology, University of São Paulo, and derived from the strain NRRL-337, was the fungus used.

Untreated liquor: Five hundred grams of air-dried and ground bagasse was mixtured with water (bagasse/water ratio was 1:10 (Kg/1) and kept in an Autoclave Reg-Med AU/EA 20 at 170ºC for 30 min. Then the resulting liquor was collected through a stainless steel water-cooling coil.

Treated liquor: The liquor was gently heated for removal of volatile compounds which inhibits microbial growth. Volume decreased because of heating and distilled water was addes to the liquor to complete the initial volume.

Physicochemical analyses were performed according to the methods described elsewhere. (4,5)

Paper chromatography was used for qualitative and quantitative determinations of liquor sugars. A n-butanol/glacial acetic acid/water solvent mixture (4:1:0.5 V/V) was used to compare results.

Values for liquor sugars were used to estimate sugar carbon sources in both liquors for balancing the carbon used as a growth culture medium for *A. niger*.

The synthetic medium by Pontecorvo et al, (6) modified by Christias et al., (7) was used for maintenance and storage of the strain, culture growth and biomass production. The composition of the medium was as follows:

Glucose	10 g
Sodium nitrate	6 g
Dihydrogenated potassium phosphate	1.52 g
Potassium chloride	0.52 g
Heptahydrated magnesium sulphate	0.52 g
Peptone	2.0 g
Yeast extract	1.0 g
Acid casein	3.0 g
Hydrolyzed nucleic acid	0.5 g
Distilled water	1,000 ml
Agar	20g (solid medium)
pH	5.0 g

Autoclave sterilizaton (121.5ºC) for 15 min was used.

The fungus was isolated from aerial conidia on inclined agarcontaining flasks an incubated al 30ºC for 6-8 days. The culture was transferred into a Petri dish and after 6-8 days 5mm agar discs were cut from the periphery of the young culture for inoculation. The fungus grew in a complete liquid medium (used as standars) in 250ml Erlenmeyer flaks containing 62.5 ml of the medium. Media were innoculated with 5 agar discs and incubated in an oscillatory shaker at approximately 30ºC for 72 hours. At the end of the incubation period, the liquid medium was filtered though a qualitative filter paper. Mycelia were weighed dried in an 100ºC oven with air circulation for 24 hours. Six experiments were performed. Carbon and salt levels, as well as pH were similar to those of the synthetic medium. The medium was substituted for either treated or untreated liquor at different concentrations. Substitution levels varied from % to 100%. Total nitrogen was measured by the micro-Kjeldahl method (9) and the crude protein content of the mycelium was calculated by the factor 6.25 x N.(4). Amino acids were measured in a Beckman auto-analyzer reaction with ninhydrine. (16)

The analysis of variance was used at random to determine the statistical significance. Mean value were compared by the Tukey's test. (8)

RESULTS AND DISCUSSION

Physicochemical, sugar content and carbon source data for both liquors and substitution of the synthetic medium for both liquors (V/V) used as carbon sources are presented in Tables 1,2,3 and 4, respectively.

The biomass production depended on the liquor amounts added to the synthetic medium, especially when the treated liquor was used.

There were significant differences between the fungal growth in the tow liquors. As expected, production was higher in the treated liquord because furfural, which was present in the liquor (2.25 Kg/100ml), had been removed by thermal treatment. *A.niger* growth occurred even when 100% of the synthetic medium was substituted for the treated liquor to which balanced salts and carbon were added and with adjusted pH. (Table 5)

In our experiment, pH played and important role in biomass production. This finding does not agree with Agnihotri's data, (10) who did not observe pH effects on

FUNGAL PROTEIN ENRICHMENT OF RESIDUAL LIQUOR FROM A SUGAR-CANE WASTE *(Saccharum officinarum)*.

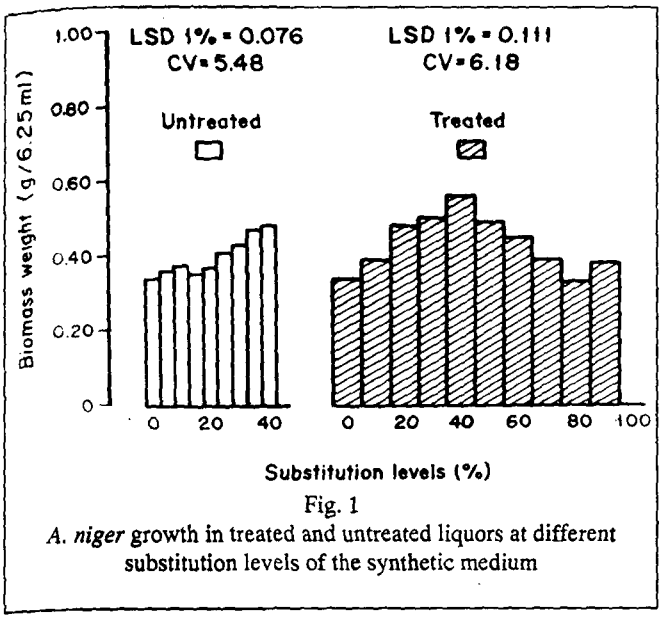

Fig. 1

A. niger growth in treated and untreated liquors at different substitution levels of the synthetic medium

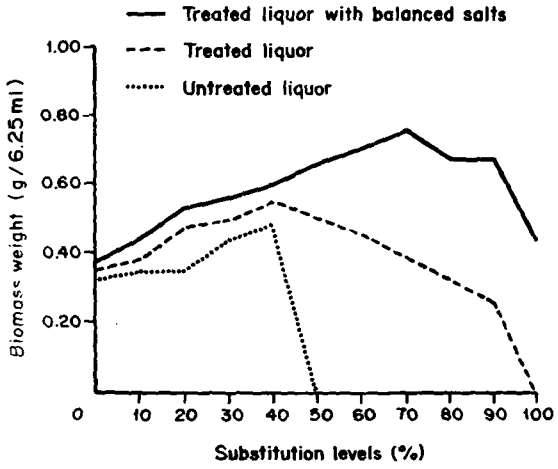

Fig. 2

Comparisons among *A. niger* grown in treated and untreated liquors and in treated added with blanced salts

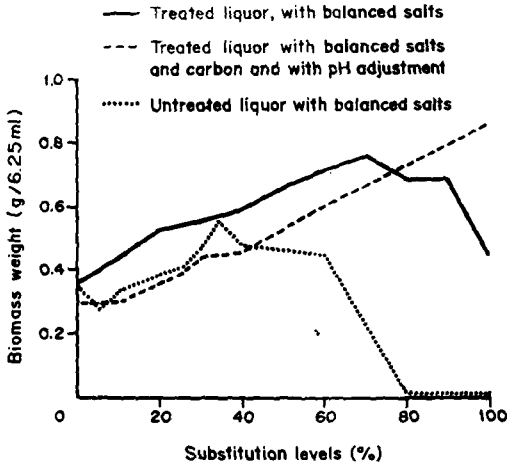

Fig. 3

A. niger growth in treated and untreated liquors added with balanced salts and with and without pH addjustment

biomass production from *Aspergillus* sp. There was no correlations between the amount (0.85g/6.25ml) and the protein content (29.69%) of the biomass when the 100% substitution level was used, which agrees with Mohyuddin et al. (11) findings in *Aspergillus* flavus. (Table 6)

A significant difference was noted between biomas production in both liquors (Fig.1) Salts and pH were the main growth-limiting factor for A. niger. (Figs. 2 and 3).

The brute protein content of biomass (29.69% or 4.06g protein/l liquor), was higher than that found by Menezes et al., (12) who used *Myrothecium verrucaria* grown in milled and screended bagasse (7.7% protein or 0.30g protein/l). (Table 7)

There was no positive correlations between the amino acid profile and the total protein content (2.59g/l). The highest values for amino acid were found in the biomass produced in the treated liquor (substitution level 40%),

with exception of alanine and glycine values which were lower than those of the standard medium. (Table 7 and 8)

The essential amino acid contents were compared with those from the biomass produced by other microorganisms and with those of the egg. (Table 9) Methionine was the limiting amino acid.

CONCLUSIONS

- The residual liquor from sugar-cane bagasse can be used as a medium for biomass production from *Aspergillus niger* IZ-9
- The highest biomass production was obtained when the synthetic medium was substituted for 100% treated liquor added with balanced salts and carbon and with pH adjusted to 5.0

TABLE 1
PHYSICOCHEMICAL ANALYSES OF THE LIQUORS

Analyses	Treated Liquor	Untreated Liquor
Physical analyses		
Specific weight (20/20 °C)	1,010	1,008
Density (g/cm³)	1,004	1,004
Solid concentration (g/l)	28,712	29,494
Chemical analyses		
pH	3,48	3,43
Ash on dry matter (%)	3,357	3,262
Furfural level (g/l)	0,192	2,256
Pentose (g/l)	24,81	20,72
Pentose (%)	86,42	70,25
Carbon (%)	1,08	1,24
Nitrogen (%)	1,01	1,01
Posphate (%)	0,002	0,001
Potassium (%)	0,030	0,032

TABLE 2
SUGAR VALUES OF THE LIQUORS

Sugars	Treated Liquor		Untreated Liquor	
	Concentration (g/l)	Sugars (%)	Concentration (g/l)	Sugars (%)
Saccharose	0,25	10,6	0,29	14,3
Glucose	0,31	13,1	0,30	14,8
Fructose and arabinose a/	0,15	6,4	0,09	4,4
Xylose	0,69	29,2	0,44	21,7
Polysaccharides	0,80	33,9	0,75	36,9
Oligosaccharides	0,16	6,8	0,16	7,9
TOTAL	2,36	100,0	2,03	100,0

a/ Fructose and arabinose spots did not separate at analysis

TABLE 3
CARBON SOURCES OF LIQUOR SUGARS

Sugars	MW	Carbon Liquor (g/l)		
		Sugars (%)	Treated	Untreated
Saccharose ($C_{12}H_{22}O_{11}$)	342	42,1	1,05	1,22
Glucose ($C_6H_{12}O_6$)	180	40,0	1,24	1,20
Fructose ($C_6H_{12}O_6$) and Arabinose ($C_5H_{10}O_5$)	180 150	40,0	0,60	0,36
Xylose ($C_5H_{10}O_5$)	150	40,0	2,76	1,76
Polysaccharides $(C_5H_8O_4)^n$	132	45,4	3,63	3,40
Oligosaccharides $(C_6H_{10}O_5)^n$	162	44,0	0,71	0,71
Total			9,99	8,65

TABLE 4
SUBSTITUTION OF THE SYNTHETIC MEDIUM FOR TREATED AND UNTREATED LIQUORS (V/V USED AS CARBON SOURCES, WITH UNCHANGED AND INCREASING LEVELS OF CARBON, WITHOUT CHANGES IN THE SALT LEVELS, WITH AND WITHOUT pH ADJUSTMENTS. a/

Substitution Levels b/ (%)	Total Carbon (mg)	Carbon Glucose (mg)	Carbon Liquor Sugars (mg)	Liquor Volume (ml)
0	250	250,00	-	-
5	250	218,75	31,25	3,12
10	250	187,50	62,50	6,25
15	250	156,25	93,75	9,37
20	250	125,00	125,00	12,50
25	250	93,75	156,25	15,60
30	250	62,50	187,50	18,75
35	250	31,25	218,75	21,90
40	250	-	250,00	25,00
60	375	-	375,00	37,50
80	500	-	500,00	50,00
100c/	625	-	625,00	62,50

a/ pH adjustment to 5.0 by adding NaOH (0.01N)
b/ Quantities per total volume (62.5 ml)
c/ Salts and glucose were dissolved en distilled water used complete volume (62.5 ml) prior to addition. At the 100% substitution level, the liquor solution was used instead of water

TABLE 5
BIOMASS PRODUCTION (g/6.25 ml) FROM *(Aspergillus niger)* GROWN IN DIFFERENT MEDIA

Liquors	Substitution Levels (%)							
	0	10	20	30	40	60	80	100
Treated liquor								
S	0,34	0,39	0,48	0,50	0,56	0,45	0,33	0
Salts	0,39	0,44	0,53	0,55	0,59	0,71	0,68	0,44
C and salts	0,34	0,32	0,30	0,35	0,40	0,56	0,61	a/
C, salts, pH	0,30	0,29	0,34	0,39	0,45	0,61	0,72	0,85
Untreated liquor								
S	0,34	0,37	0,37	0,43	0,48	0	0	0
C, salts, pH	0,34	0,32	0,38	0,45	0,48	0,46	0	0
C.V. (%)	1,36	1,76	2,01	2,58	1,64	0,48	0,48	1,80

S = Substitution of the synthetic medium for the liquors
C = Carbon source
pH ajdjusted to 5.0
a/= At the 100% substitution level, salts and carbon were not added to the medium

TABLE 6
PROTEIN PRODUCTION (%) FROM *(A. niger)* GROWN IN TREATED AND UNTREATED LIQUORS

Substitution Levels (%)	Treated Liquor			Untreated Liquor		
	S	Salts, C	Salts, C	Salts, pH	S	C, Salts, pH
0	31,27ab	31,27d	31,27c	31,27bc	31,27c	31,27bc
10	36,19a	37,79b	35,40abc	33,42ab	33,07c	30,96bc
20	35,62a	36,81b	34,38bc	33,35bc	38,48b	31,39abc
30	29,31b	36,63b	37,92a	33,41b	38,04b	32,50zbc
40	29,44b	36,48b	35,79abc	36,20a	45,23a	35,85ab
60	31,79ab	34,19c	37,21ab	32,24bc	0d	0d
80	33,33ab	33,79c	35,86abc	29,63c	0d	0d
100	0c	44,13a	a/	29,69c	0d	0d

a/ At the 100% substitution level, salts and carbon were not added to the medium

TABLE 7
TOTAL PROTEIN PRODUCTION (g/l) FROM *(A. niger)* GROWN IN TREATED AND UNTREATED LIQUORS

Substitution Levels (%)	Treated Liquor			Untreated Liquor		
	S	Salts, C	Salts,C	Salts, pH	S	C,Salts,pH
0	1,82cd	1,94e	1,74c	1,55e	1,74e	1,71c
10	2,24bc	2,64cd	1,68c	1,52e	1,94d	2,20c
20	2,78a	3,10cd	1,67c	1,83e	2,30c	2,68bc
30	2,34b	3,24bc	2,21bc	2,3dc	2,60b	3,39ab
40	2,66ab	3,46abc	2,28cd	2,59cd	3,47a	3,92a
60	2,27b	3,88a	2,72b	3,13bc	0	3,90a
80	1,76d	3,66ab	3,53a	3,57ab	0	0d
100	0e	3,08cd	a/	4,06	0	0d
C.V. (%)	7,57	6,24	13,53	7,63	5,02	12,45

a/ At the 100% substitution level, salts and carbon were not added to the medium

TABLE 8
AMINO ACID COMPOSITION (g/100g PROTEIN) OF THE *(A. niger)* MYCELIUM
GROWN IN TREATED AND UNTREATED LIQUORS COMPARED
WITH THAT OF THE STANDARD MEDIUM

Amino Acid	Substitution Level (%)				Standard medium[a]
	Treated		Untreated		
	(100)	(40)	(30)	(40)	
Lysine	2,58	6,52	5,37	4,95	4,08
Histidine	1,50	2,65	2,16	1,85	1,50
Arginine	3,87	6,88	5,70	5,01	3,87
Aspartictic acid	7,33	11,23	8,98	10,31	8,15
Threonine	3,10	5,14	4,31	4,68	3,87
Serine	2,79	4,18	3,53	3,89	3,62
Glutamic acid	11,46	18,90	13,78	15,87	17,39
Proline	4,56	6,18	4,91	4,82	5,28
Glycine	6,92	7,42	7,82	7,48	7,59
Alanine	5,34	8,63	7,02	7,51	9,13
Valine	4,81	7,59	6,20	7,20	6,00
Methionine	0,17	0,34	0,17	0,21	-
Isoleucine	3,55	5,33	4,40	5,00	4,03
Leucine	5,80	9,17	7,46	8,40	7,10
Tyrosine	1,09	4,07	2,53	2,84	1,19
Phenylalanine	3,44	5,73	4,70	5,26	2,54

a Christias et al. (7)

TABLE 9
COMPARISONS AMONG THE ESSENTIAL AMINO ACIDS FROM THE A. niger *CANDIDA UTILIS, BACILLUS SP*, AND EGG.

Essential Amino acids	g/100 PROTEIN			
	C.Utilis a/	*Bacillus* b/	*A, Niger*c/	Egg d/
Isoleucine	7,9	6,1	5,3	6,8
Leucine	7,5	8,9	9,2	9,0
Lysine	8,7	6,9	6,5	6,3
Methionine	1,8	2,7	0,3	3,1
Phenylalanine	5,1	5,6	5,7	6,0
Threonine	5,5	4,3	5,1	5,0
Tryptophan	1,4	n.m	n.m	1,7
Valine	6,3	6,7	7,6	7,4

a/ According to InskeepP et al (13)
b/ According to Mateles et al (14)
c/ *A. niger* grown in the 40 % treated liquor added with balanced carbon and salts and pH adjustment.
d/ According to Burton (15)
n.m = non - measured.

- There was no correlations between mycelia weight and total protein production

- Methionine was the limiting amino acid of biomass

- The highest amino acid values were detected in the *A. niger* grown in the medium containing 40% treated liquor added with balanced salts and carbon, with pH adjusted to 5,6; and

- With exception of methionine, this fungal protein can provide all essential amino acids in a diet and can be considered of good biological value.

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